NEUROIMAGING PATTERNS OF STRUCTURAL BRAIN ANOMALIES AND ASSOCIATED FACTORS IN CHILDREN WITH SPINA BIFIDA ATTENDING AT MUHIMBILI ORTHOPAEDIC INSTITUTE AND MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA

UMMEHANI MUSTAALI DOSSAJEE, MD

Masters in Medicine (Radiology) Dissertation
Muhimbili University of Health and Allied Sciences.

October, 2024

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF RADIOLOGY AND IMAGING

NEUROIMAGING PATTERNS OF STRUCTURAL BRAIN ANOMALIES AND ASSOCIATED FACTORS IN CHILDREN WITH SPINA BIFIDA ATTENDING AT MUHIMBILI ORTHOPAEDIC INSTITUTE AND MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA

By:

UMMEHANI MUSTAALI DOSSAJEE, MD

HD/MUH/T.235/2021

A Dissertation submitted in (Partial) fulfilment of the Requirements of the degree of Masters in Medicine (Radiology) at Muhimbili University of

Health and Allied Sciences.

October, 2024

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled "**Neuroimaging patterns of structural brain anomalies and associated factors in children with Spina Bifida attending at Muhimbili Orthopedic Institute and Muhimbili National Hospital, Dar-Es-Salaam, Tanzania***".* In (Partial) Fulfillment of the requirements for the degree of Master of Medicine (Radiology) of Muhimbili University of Health and Allied Sciences.

_____

Dr. Mboka Jacob MD, MMed, PhD-Neuroimaging
Supervisor

_____

Date

_____

Dr. Mary Kamuzora, MD, MMed (Radiology)
Co-Supervisor

_____

Date

_____

Dr. Hamisi K. Shabani, MD, MMed (Neurosurgery)
Co-Supervisor

_____

Date

DECLARATION AND COPYRIGHT

I, **Ummehani Mustaali Dossajee** declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature.....Date ……

This dissertation is a copyright material protected under the Berne Convention, The copyright Act 1999 and other international and national enactments, in that behalf, an intellectual property. It may not be reproduced by any means, in full or part, except for short extracts in fair dealing; for research or private study, critical scholarly review or discourse with an acknowledgement, without written permission from the Directorate of Postgraduate Studies, on behalf of both the author and Muhimbili University of Health and Allied Sciences.

AKNOWLEDGEMENT

First and foremost, I would like to thank Allah for giving me the strength, capability and ability to be able to complete this work.

I would like to thank my supervisors Dr. Mboka Jacob, Dr. Mary Kamuzora and Dr. Hamisi K. Shabani for their unconditional support, guidance encouragement and motivation. All of this helped to bring this dissertation to completion. The same appreciation goes to all the radiologists in the Radiology department (MUHAS).

Sincere thanks to all the specialists and radiographers, in the Radiology departments at MNH and MOI for their support during dissertation development to completion.

I would like to express my gratitude to the MUHAS management through the Director of Postgraduate Studies who granted permission for this study to be conducted.

Special thanks to my family specifically my beloved parents Mr. and Mrs. Mustaali Dossajee, parents-in-law Mr. Mrs. Hassanali Mohamedali, and my husband Mr. Mohsin Mohamedali for their understanding patience, prayers and unconditional support they have provided me throughout this period.

Heartfelt gratitude is extended to my siblings and their spouses for their support and love.

DEDICATION

This work is dedicated to:

My beloved parents Mr. and Mrs. Mustaali Dossajee for their love, encouragement, prayers, care and support.

My beloved husband for his understanding and unwavering support throughout this period.

TABLE OF CONTENTS

CERTIFICATION i
DECLARATION AND COPYRIGHT ii
AKNOWLEDGEMENT iii
DEDICATION iv
LIST OF TABLES viii
LIST OF FIGURES ix
LIST OF ABBREVIATIONS x
DEFINITION OF TERMS xi
ABSTRACT xii
CHAPTER ONE 1
1.0 INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 2
1.2.1 Conceptual framework 3
1.3 Rationale of the Study 5
1.4 Research question 5
1.5 Study objectives 6
1.5.1 Broad objective 6
1.5.2 Specific objectives 6
CHAPTER TWO 7
2.0 LITERATURE REVIEW 7
2.1 Neuroimaging findings in children with spina bifida 7
2.2 Factors associated with patterns of spina bifida 8
2.3 Correlation between brain MRI and ETV findings in children with spina bifida 9
CHAPTER THREE 11
3.0 METHODOLOGY 11

3.1 Study design 11
3.2 Study area 11
3.3 Study population 12
3.4 Sample size 12
3.5 Sampling method 14
3.5.1 Inclusion criteria 14
3.5.2 Exclusion criteria 14
3.6 Variables 14
3.6.1 Dependent variables 14
3.6.2 Independent variables 16
3.7 Data collection 16
3.7.1 Imaging parameters 17
3.7.2 Image interpretation 17
3.7.3 Reliability 18
3.8 Data analysis 18
3.8.1 Ethical issues 19
CHAPTER FOUR 20
4.0 RESULTS 20
4.1.1 Maternal and child characteristics 20
4.1.2 Spine MRI findings 22
4.1.3 Brain MRI findings 24
4.1.4 Relationship between brain MRI findings and SB patterns 28
4.1.5 Factors associated with spina bifida patterns 31
4.1.6 Association between ETV findings and brain MRI findings 35
CHAPTER FIVE 37
5.0 DISCUSSION 37
5.1 Limitations 40
CHAPTER SIX 41
6.0 CONCLUSION AND RECOMMENDATIONS 41

6.1 Conclusion 41
6.2 Recommendations 41
REFERENCES 42
APPENDICES 48
Appendix I: Data collection template 48
Appendix II: Consent form (English version) 53
Appendix III: Consent form (Swahili version) 56
Appendix IV: Ethical clearance 59
Appendix V: Permission letter 61

LIST OF TABLES

Table 1: Sample size calculator module for cross-sectional studies 12
Table 2: Child demographic characteristics of the study participants (N=90).....21
Table 3: Maternal characteristics and their distribution (N=90) 22
Table 4: Spine MRI findings in children with spina bifida (N=90) 24
Table 5: Frequency and percentage of brain MRI findings, overall and according to SBA subtype 29
Table 6: Frequency and percentage of brain anomalies, overall and according to SB lesion level.....30
Table 7: Frequency and percentage of brain anomalies, overall and according to SB defect span 31
Table 8: Association between maternal/child characteristics and spina bifida defect span ...33
Table 9: Association between maternal/child characteristics and spina bifida lesion level...35
Table 10: Association between ETV findings and brain MRI findings (Abnormal T2/FLAIR SI and intraventricular septations 36

LIST OF FIGURES

Figure 1: Schematic diagram of SB subtypes 15
Figure 2: Study flow chart for inclusion of participants 20
Figure 3: Spine MRI images showing spina bifida aperta subtypes. 23
Figure 4: Brain MRI findings in children with spina bifida.....25
Figure 5: Brain MRI images showing Chiari types.....26
Figure 6: Subependymal grey matter heterotopia.. 27
Figure 7:Intraventricular spetations.....28

LIST OF ABBREVIATIONS

CNS:	Central Nervous System
CSP:	Cavum Septum Pellucidum
ETV:	Endoscopic Third Ventriculostomy
MMed:	Masters of Medicine
MNH:	Muhimbili National Hospital
MOI:	Muhimbili Orthopaedic Institute
MRI:	Magnetic Resonance Imaging
MSc:	Masters of Science
MUHAS:	Muhimbili University of Health and Allied Sciences
SB:	Spina Bifida
SBA:	Spina Bifida Aperta
SBO:	Spina Bifida Occulta
SPSS:	Statistical Package for Social Sciences

DEFINITION OF TERMS

Endoscopic Third Ventriculostomy (ETV): A surgical procedure for the treatment of hydrocephalus in which an opening is created in the floor of the third ventricle using an endoscope placed within the ventricular system through a burr hole (1)

Hydrocephalus: An active distension of the ventricular system of the brain related to the inadequate passage of CSF from its point of production within the ventricular system to its point of absorption into the systemic circulation (2)

Magnetic Resonance Imaging (MRI): A medical imaging technique that uses a magnetic field and computer-generated radio waves to create detailed images of the organs and tissues in your body (3)

Spina bifida: A type of neural tube defect that occurs as a result of incomplete closure of the neural tube during the fourth week of embryonic development (4)

ABSTRACT

Background: Spina bifida (SB) is a neural tube defect that occurs as a result of incomplete closure of the neural tube during the fourth week of embryonic development. It is the most common and most complex non-fatal neurological birth defect. This can result in a range of physical and neurological impairments. Despite its prevalence and impact, there is little data on SB neuroimaging pattern and associated factors.

Objective: The main aim of this study was to explore spina bifida patterns, brain anomalies and associated factors.

Methodology: From June 2022 to February 2024, an extensive cross-sectional study was conducted at Muhimbili Orthopaedic Institute and Muhimbili National Hospital-Upanga. Maternal-child clinical data, intra-operative Endoscopic Third Ventriculostomy (ETV), and brain and spine MRI data of 90 children with spina bifida were collected and analysed using SPSS version 26. The analysis employed descriptive statistics, binary, and multinomial logistic regression, with a significance level set at p-value < 0.05.

Results: 60% of the study participants were males. The majority of the study participants (75.6%) were neonates, with median age of 9.5 days and interquartile range of 4.5 days. 27.8% (25/90) were born with a low birth weight (BW < 2500g). A total of 17 (18.9%) study participants are the fourth or older child in their family. All children had SB aperta, with most having myelomeningocele (86.7%). Chiari malformation (93.3%) and hydrocephalus (88.9%) were the most prevalent brain anomalies. Children with upper SB lesion level and larger defect span had higher occurrence of abnormal corpus-callosum, cavum-septum-pellucidum and cerebellum (p-value< 0.05). Male sex (OR 5.8; 95%CI 1.0 – 33.1; p-value 0.049) and maternal anaemia (OR 24.0; 95%CI 4.5 – 128.3; p-value < 0.001) were associated with higher odds for having an upper SB lesion level and larger defect span. The presence of intraventricular septations on MRI correlated well with turbid CSF on ETV (p-value 0.045).

Conclusion:

There was significant diversity in associated brain anomalies among spina bifida patterns. Higher occurrence of anomalies is noted with upper SB lesion level and larger defect span. Maternal anaemia and male sex are independent risk factors for severe spina bifida. Brain MRI may help to predict intra-operative ETV findings.

Recommendations:

Findings from this study highlight the importance of detailed CNS evaluation in children with spina bifida. The association of severe forms of spina bifida with maternal anaemia underscores the importance of pre-conceptual folic acid in the prevention of neural tube defect in Tanzania. Further studies on the cognitive and functional outcome of children with spina bifida are recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

With a global incidence of around 0.5 per 1000 pregnancies, spina bifida is the most common neurological birth defect in humans (5). It is often classified as the most complex non-fatal central nervous system (CNS) birth defect (6) with a one-year survival rate of infants estimated at 90.8% (7). A recent meta-analysis study shows a pooled prevalence of spina bifida among newborns in Africa to be 1.3 per 1000 births, with the prevalence in Tanzania being 2.6 per 1000 births (4). Another study done in Dar-es-salaam, Tanzania reported the prevalence of spina bifida to be 3.7 per 1000 births (8).

Spina bifida is a type of neural tube defect that occurs as a result of incomplete closure of the neural tube during the fourth week of embryonic development (4). It occurs in various grades of severity with a spectrum of clinical symptoms depending on the level and type of spina bifida. (6)

After fertilization, neurulation begins on day 18 and lasts until day 28 of the embryo's life, very early during pregnancy by when most mothers barely know that they have conceived already. Numerous important cellular activities take place from week 6 until mid-gestation, including neurogenesis and cell differentiation. Although brain development continues after birth, the fetal brain is more susceptible to stimuli and damage due to rapid growth and the complex brain development process. (38)

There are several classifications of spina bifida. A recent study aimed to classify spina bifida based on histological findings which can also be applied to diagnostic imaging. This includes spina bifida occulta (the closed type) and three spina bifida aperta (the open type) subtypes which are meningocele, myelomeningocele, and myeloschisis (9).

Several studies have demonstrated that spina bifida translates into substantially altered brain development in various structural and functional domains (10–13). Marked heterogeneity exists among individuals with spina bifida in terms of brain development. These differences are significantly associated with spina bifida lesion level and type. While most patients with spina bifida aperta have some form of brain anomaly, these are uncommon in patients with spina bifida occulta (9,12,14,15).

Primary central nervous malformations such as anomalous development of the skull, corpus callosum agenesis or dysgenesis, posterior fossa, cerebellar, cerebral cortex, and brainstem anomalies are all a part of the prolonged disruption of neuro embryogenesis that characterizes spina bifida (5,9,10,12,14). It is however unclear as to whether these malformations occur in parallel or if they have a temporal relationship (5).

Hydrocephalus is the most common brain abnormality seen in patients with spina bifida (5). While some brain anomalies like Chiari II malformation are unique to individuals with myelomeningocele, others such as corpus callosum and posterior fossa abnormalities are non-specific (16).

Various studies have shown that individuals with spina bifida exhibit varied levels of neuropsychological and cognitive deficits which are a function of the underlying developmental brain anomalies. (10,16–20).

This study aims at assessing structural brain anomalies in children with spina bifida in our setting, their relationship with spina bifida lesion level and type, and various maternal characteristics that may determine the risk of certain anomalies. It will form a foundation for future studies that will look into clinical outcomes in children with spina bifida.

1.2 Problem statement

Despite a relatively high (4) and increasing (21) prevalence of spina bifida in Tanzania, little emphasis is given to understanding the associated developmental anomalies which are

exhibited by children with spina bifida, which have an immense impact on their lives in terms of sensory, motor and cognitive domains.

Due to associated challenges especially in ambulation, and the strengths in language and social abilities that many individuals with spina bifida exhibit, the general notion of spina bifida as an orthopaedic condition in our communities persists (6). This disregards the "unseen" problems in terms of a spectrum of CNS impairments that these individuals exhibit which may be a direct function of the underlying associated structural brain anomalies.

Structural brain anomalies are best detected by MRI, an imaging technology that is currently available in our setting. There is a paucity of published data about radiological findings of the CNS in children with spina bifida in Tanzania. The effect of various maternal characteristics on the burden of spina bifida and the associated anomalies is also not well known.

Management of children with spina bifida in our country is undertaken in many forms including neurosurgical, physiotherapy, and occupational therapy. Spina bifida repairs and Endoscopic Third Ventriculostomies (ETVs) for patients with hydrocephalus are also commonly performed at MOI. A better understanding of associated brain anomalies and various maternal factors that influence these is required to guide interventions and predict the clinical prognosis of these children.

1.2.1 Conceptual framework

Figure 1 below, illustrates the conceptual framework that was used to conduct this study. The study is intended to establish neuroimaging findings of structural brain anomalies and associated factors in children with spina bifida.

The dependent variables included various SB patterns and structural brain anomalies which were studied using brain and spine MRI findings respectively.

Independent variables included various child demographics and maternal characteristics. Intra-operative findings of a subset of study participants who underwent ETV as a treatment for hydrocephalus were also included as independent variables.

The association between the independent and dependent variables was assessed. The correlation between brain MRI and ETV findings was also assessed (as shown by the arrows).

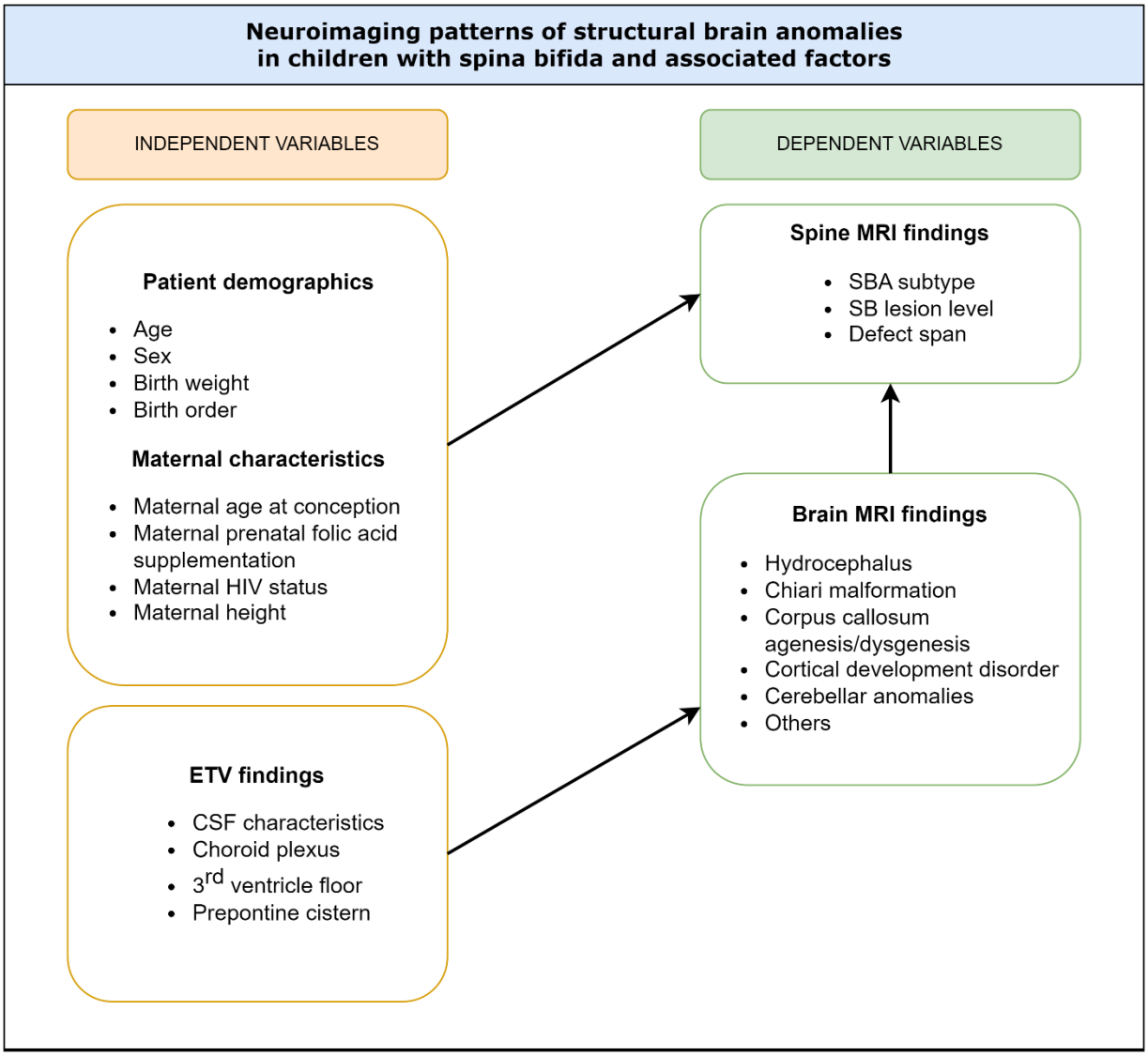


Figure 1: Conceptual framework

1.3 Rationale of the Study

This study provides baseline information on the magnitude and variability of neuroimaging findings in children with spina bifida in Tanzania.

Radiological identification of these anomalies not only provide insight to clinicians, neurosurgeons, and rehabilitative health care providers but also aids in better planning interventions and predicting prognosis based on the individual patient's brain findings. Radiological findings are correlated with specific anatomical findings as seen during the ETV procedure which some of the children with hydrocephalus had undergone. These will aid to identify various predictors of surgical complications such as infection.

It also helps to guide pre-natal counselling as many cases of spina bifida are diagnosed during antenatal ultrasound scans. Parents can be made aware of possibilities of other associated brain anomalies beforehand to plan on management and supportive requirements for when the child is born.

Moreover, a focus on specific maternal characteristics that may influence the risks of such anomalies helps policymakers and healthcare planners to modify policies and plan community-based interventions in our country. Interventions in the fields of education, sports, and the workplace can be undertaken to better accommodate and improve the quality of life of individuals with spina bifida.

The study forms a foundation for future studies that will look deeper into various functional outcomes such as neuropsychological behaviour, and sensory, motor, and cognitive domains.

1.4 Research question

i. What are the neuroimaging patterns of structural brain anomalies and associated factors in children with spina bifida attending MOI and MNH in Dar-es-salaam, Tanzania?

ii. Is there a relationship between brain MRI findings and pattern of spina bifida?

iii. What are the factors associated with SB patterns as seen on spine MRIs of children with SB attending MOI and MNH?

iv. Is there a correlation between brain MRI and ETV findings of children with SB attending MOI and MNH?

1.5 Study objectives

1.5.1 Broad objective

To determine neuroimaging patterns of structural brain anomalies and associated factors in children with spina bifida (SB) attending MOI and MNH from June 2022 to February 2024, Dar-es-salaam, Tanzania

1.5.2 Specific objectives

1. To characterize neuroimaging and ETV findings of children with SB attending at MOI and MNH from June 2022 to February 2024
2. To determine the relationship between brain MRI findings and SB patterns as seen on spine MRI of children with SB attending MOI and MNH from June 2022 to February 2024
3. To determine the factors associated with SB patterns as seen on spine MRI of children with SB attending MOI and MNH from June 2022 to February 2024
4. To assess the correlation between brain MRI and ETV findings of children with SB attending MOI and MNH from June 2022 to February 2024

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Neuroimaging findings in children with spina bifida

Spina bifida is the most common neural tube defect, and is often regarded as the most complex non-fatal CNS anomaly (6). Globally, 5 out of 10,000 children are born with spina bifida every year (5). A meta-analysis study conducted in Africa in 2020, shows a pooled prevalence of spina bifida among newborns to be 1.3 per 1000 births (4).

Despite various strategies in place to combat the problem, another meta-analysis study has shown that the rate of neural tube defects amongst which spina bifida was the majority, has increased by 4% from the year 1983 to 2018 in East African countries. (21)

An estimated 1 in every 500 children is born with spina bifida in Tanzania. This number is nearly four times that of the global prevalence of spina bifida (4).

There are various classifications of spina bifida. In this study, we will use the system proposed by K. Schindelmann et al (9) which classifies spina bifida into spina bifida occulta and spina bifida aperta. SBA is further classified into 3 subtypes; meningocele, myelomeningocele and myeloschisis. This classification is simpler and can be used from both radiological and neuropathological perspectives. Precise classification of these anomalies is important for neurosurgical considerations and prediction of clinical outcomes of these children. (9).

Due to a large variation in the classification of spina bifida aperta in literature, it is difficult to assess the prevalence of its subtypes. Of the specified subtypes of spina bifida aperta by K. Schindelmann (9), 55% of the cases were myeloschisis, 41% were myelomeningocele and the remaining comprised meningoceles.

Spina bifida lesion level has been shown to have an impact on the severity and number of brain anomalies (14,22). A higher spine lesion level is a predictor of more severe brain anomalies. Children with higher spinal lesions had significantly higher frequency for

anomalies of the tectum, pons, posterior fossa, falx, cerebellar hemispheres and splenium of corpus callosum compared to children with lower spinal lesions(14).

Failure of proper neural tube closure subsequently leads to altered development of the brain and spinal cord. Studies have shown that almost all children with spina bifida aperta and rarely spina bifida occulta have some form of structural brain anomaly (5,23). These include abnormal development of the skull as well as various supra and infratentorial regions of the brain (15).

Hydrocephalus is the most common abnormality found in children with SBA (5). This is followed by various grades of Chiari malformation type II which is shown to be highly associated with myelomeningocele (15). Other non-specific but commonly occurring anomalies include corpus callosum agenesis or dysgenesis (11), posterior fossa anomalies such as reduction in volume (22), gyration abnormalities like polymicrogyria and stenogyra and tectal beaking (5).

There is a paucity of data that describes the patterns of structural brain anomalies in children with spina bifida in our setting. This study aims to bridge this gap.

2.2 Factors associated with patterns of spina bifida

Environmental factors, especially during the peri-conceptional and prenatal period have an important role in the development of the fetal nervous system (24).

One could speculate several reasons for the increase in the rate of spina bifida in East Africa compared to declining rates in the developed world (21). Evidence from decades ago (25) and recent studies (26,27) have shown that peri-conception folic acid supplementation markedly decreases the risk of neural tube defects including spina bifida. However, there is still a lack of widely established practices such as folic acid fortification of foods in developing countries (21).

Peri-conceptional folic acid supplementation has also been shown to reduce the severity of spina bifida in terms of lesion level. Pre and post-fortification studies have shown a significant decrease in cervical and thoracic spina bifida lesions. (28,29)

Studies have shown a higher prevalence of spina bifida in children born to mothers who were exposed to anti-retroviral drugs during the time of conception (32,33). The prevalence of HIV in Tanzania is 5.1% in people aged 15-49 years. Quite a significant difference is noted when stratified by sex. 6.2% of women aged 15-49 were found to be HIV positive, compared to only 3.8% of men in the same age group (30). According to the recent National guideline aimed at preventing vertical transmission of HIV, all mothers are encouraged to take lifelong ARVs once diagnosed at ANC (31).

Pre-pregnancy maternal weight and height is other characteristics shown by studies to impact the likelihood of having a child with spina bifida. Obese women have significantly higher odds up to 3.5, of having a child with spina bifida compared to non-obese women (32–34). A recent study even suggests using maternal weight as an additional marker in screening for spina bifida during the first trimester (35).

A study done in France has found that the maternal nutrition status is a significant modifiable factor for fetal neurodevelopment. The epigenetic alteration of fetal genes is regulated by the macro and micronutrient status of the mother. These effects continue throughout the pregnancy, starting from implantation and extending to later stages in terms of nutrients transfer through the placenta to provide the fetus with the building blocks for growth (24).

Whilst many studies show the association between various factors and the occurrence of spina bifida, little is known whether these factors also play a role in determining the occurrence of structural brain anomalies in these children.

2.3 Correlation between brain MRI and ETV findings in children with spina bifida

ETV is a minimally invasive endoscopic surgical procedure to treat hydrocephalus where a small surgical slit is made at the floor of the 3rd ventricle to allow CSF to flow. It is widely

used as an alternative for shunt placement and in some cases as a first-line treatment for obstructive hydrocephalus especially in patients with myelomeningocele (36,37).

The use of endoscopy allows for real-time exploration and visualization of the intracranial anatomy. In addition, it also provides specific important details such as CSF characteristics, the presence of debris in case of infection, and the flow of CSF through the stoma (38). Such details cannot be fully ascertained by existing imaging modalities.

The use of endoscopy provides an excellent opportunity to explore real-time intra-cranial anatomy. It also enables to collect of CSF samples for analysis and culture in case of suspicion for infection.

Intracranial anatomy observed during ETV can be used to predict various congenital brain anomalies that these children may have. Brain mantle thickness, septum pellucidum, Foramen of Monro and Aqueduct of Sylvius are some of the structures that can be studied during the procedure.

Choroid plexus and pre-pontine scarring and deposits on the 3rd ventricle floor are some findings that can easily be seen during ETV and are limited on brain MRI. These have potential implications on the patient's post-op management and potential for complications.

On the other hand, pre-operative imaging provides information on the likelihood of success of the ETV procedure by providing an initial anatomical assessment of the ventricular system. (36)

Since ETV is mainly a therapeutic procedure and not used primarily for diagnosis, there is a paucity of literature in determining the relationship between the widely used brain MRI findings with those of Intra-operative ETV findings.

This study aimed to establish various factors that may be associated with specific spina bifida patterns as well as groundwork data on these findings and may be able to provide insight into the interdependent role of imaging and endoscopy.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

The study was a hospital-based cross-sectional descriptive study where primary data about maternal and child demographics was collected via questionnaires and secondary data such as medical records, brain and spine MRI findings, and ETV findings undertaken from June 2022 to February 2024 was retrospectively reviewed according to the prepared checklist.

3.2 Study area

The study was conducted at the Radiology departments of Muhimbili National Hospital (MNH) and Muhimbili Orthopedic Institute (MOI). These are tertiary hospitals within the Dar-es-salaam region and cater for the eastern zone of Tanzania. They also serve as National Referral Hospitals for neuro-surgical cases. Children with spina bifida are referred from various regions in Tanzania to these hospitals for spina bifida repair and/or management of hydrocephalus.

Some of these children were born at MNH to mothers in whom a prenatal diagnosis of spina bifida was made at primary health facilities by ultrasound and were referred to MNH for delivery at a more resourceful centre with better NICU care.

The Radiology departments at both hospitals provide both diagnostic and interventional radiology services. The radiology team includes full-time employed radiographers, radiologists, subspecialty radiologists including Neuroradiologists and Interventional radiologists and other supportive and administrative staff. Masters of Medicine residents and Masters of Science fellows play an important role as part of the daily workforce.

Available imaging modalities include ultrasonography, plain radiography, CT and MRI.

3.3 Study population

The study included all children with spina bifida who attended MNH and MOI from June 2022 to February 2024 and a subset of those who underwent ETV at MOI.

After receiving ethical clearance and permission to conduct the study from both MOI and MNH, study participants were sought through hospital records such as NICU register, paediatric clinics and theatre registers.

Their guardians were contacted for consent to participate in the study and primary data was be collected from them through interviews. Their brain and spine MRI images were retrieved from hospital imaging databases such as PACS and ClearCanvas.

3.4 Sample size

Sample size calculator module for cross-sectional studies from https://openepi.com/ (39) was used as shown by table 1 below:

Table 1: Sample size calculator module for cross-sectional studies

Sample Size: Cross-Sectional, Cohort, & Randomized Clinical Trials	
Two-sided significance level(1-alpha):	95
Power (1-beta, % chance of detecting):	80
Ratio of sample size, Unexposed/Exposed:	1
Percent of Unexposed with Outcome:	26
Percent of Exposed with Outcome:	6
Odds Ratio:	0.18
Risk/Prevalence Ratio:	0.23
Risk/Prevalence difference:	-20
	Kelsey
Sample Size - Exposed	53
Sample Size-Nonexposed	53
Total sample size:	106

The module above used the formula by Kelsey et al (40) as shown below:

The sample size formula for the method described in Kelsey et. al. is:

$$n_1 = \frac{\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 \bar{p}\bar{q}(r+1)}{r(p_1 - p_2)^2}$$

and

$$n_2 = r\,n_1$$

where

n_1 = number of exposed

n_2 = number of unexposed

$Z_{\alpha/2}$ = standard normal deviate for two-tailed test based on alpha level (relates to the confidence interval level)

Z_{β} = standard normal deviate for one-tailed test based on beta level (relates to the power level)

r = ratio of unexposed to exposed

p_1 = proportion of exposed with disease and $q_1 = 1-p_1$

p_2 = proportion of unexposed with disease and $q_2 = 1-p_2$

$\bar{p} = \frac{p_1 + rp_2}{r+1}$ and $\bar{q} = 1 - \bar{p}$

The following values were assumed:

Confidence level: 95%

Power of study: 80%

The ratio of sample: Unexposed/Exposed = 1

The proportion of exposed and unexposed with the desired outcome i.e., 6% and 26% are taken from a study done to determine possible differences in the prevalence of upper lesion level of spina bifida between a correct folic acid supplementation (exposed) group and an incorrect folic acid supplementation (unexposed) group. (29)

A contingency of 10% was set to cater for patients who may be excluded due to missing brain and spine MRI images.

Thus,

N = 106 + (10% x 106)

= 117

The adjusted sample size is **117 patients**.

3.5 Sampling method

A convenient non-probability sampling method was used. Children with spina bifida who met the inclusion criteria within the study period were included in the study until the sample size was reached.

3.5.1 Inclusion criteria

Children under 18 years of age with spina bifida who attended MOI or MNH, had done brain and spine MRIs, and a subset of those who underwent ETV for hydrocephalus management from June 2022 – February 2024.

3.5.2 Exclusion criteria

Patients with missing required data, specifically Brain and Spine MRI images were excluded

3.6 Variables

3.6.1 Dependent variables

Dependent variables included parameters from brain and spine MRI findings as listed below:

- Brain MRI findings:
    - Hydrocephalus: was diagnosed when the patient had enlarged ventricles, with Evan's index >0.3
    - Chiari malformation: cerebellar tonsillar herniation of ≥5mm beyond the plane of the foramen magnum
    - Tectal beaking: elongated inferior colliculus pointing posteriorly
    - Corpus callosum abnormalities: Abnormal corpus callosum includes either complete agenesis or dysgenesis of rostrum, genu, body and/or splenium.
    - Cerebellum abnormalities: include dysplasia or hypoplasia of the cerebellum

- Malformation of cortical development: includes grey matter heterotopia or polymicrogyria in this study sample
- Abnormal Cavum Septum pellucidum (CSP): Includes either absent, or abnormally shaped CSP

- Spine MRI findings:
  - SB type: Occulta: presence of covering membrane; Aperta: absence of covering membrane
  - SBA subtype: As classified by Schindelmann K. (9) , illustrated in Figure 1 below.

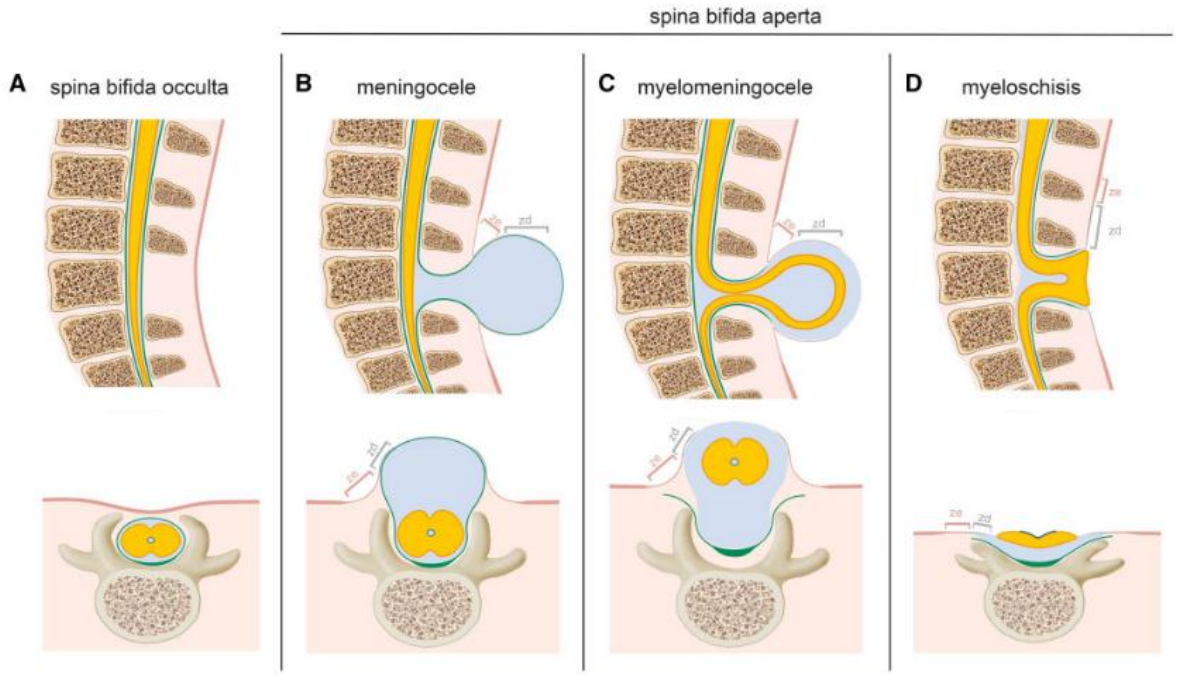


Figure 1: Schematic diagram of SB subtypes; sagittal plane on top and axial plane below (A) Spina bifida occulta (B) Meningocele (C) Myelomeningocele (D) Myeloschisis (9)

- SB lesion level: Lesion level was assigned according to the highest vertebral level affected in a midsagittal image of the spine. The last bright disk space of

the lumbosacral spine was found to consistently be L5–S1, and this was used as the frame of reference for evaluating lesion level.

- SBA lesion level according to the highest vertebral level affected, categorized as upper (T12 and above) and lower (L1 and below) lesion levels; and SB defect span which denotes the number of vertebral segments affected, categorized as ≤ 3, 4 – 6, or ≥ 7 vertebral segments.(41)
- Defect segment span: Segment span was defined as the number of vertebral levels affected—that is, a lesion that extended from L5 through S5 spanned six segments.
- Other findings such as kyphosis, syrinx, and tethered cord were diagnosed accordingly.

3.6.2 Independent variables

Independent variables include maternal and child demographic characteristics and Intra-operative ETV findings as listed below:

- Child demographics: Age, Sex, Birth weight and Birth order
- Maternal characteristics: Maternal age at conception (<35 years; ≥35 years) , Maternal prenatal folic acid supplementation, Maternal HIV status and ARV use, Maternal height (<150cm; ≥150cm).
- Intra-operative ETV findings: CSF characteristics, Choroid plexus, 3rd ventricle floor, and Prepontine cistern

3.7 Data collection

Brain and spine MRI images were retrieved from the available patient database from the two hospitals. Data collection was done using a structured template (Appendix I) that was filled by the PI. Patient demographic information and ETV findings were obtained from the patient's hospital file records.

The data collection template includes four main parts.

- Part One: Maternal child characteristics
- Part Two: Spine MRI findings
- Part Three: Brain MRI findings
- Part four: ETV findings

Data was collected using Kobo Toolbox data collection software, in which the data collection template was coded and deployed on mobile phones. Data collection was assisted by trained research assistants who were granted access to Kobo Toolbox software.

3.7.1 Imaging parameters

Patients enrolled in the study underwent brain and spine MRI at the radiology department of MOI and MNH using 1.5 T Avanto and 3 T Spectra SIEMENS MAGNETOM A Tim + Dot system MRI scanners respectively. Imaging was performed by qualified radiographers fully employed in both hospitals.

Brain MRI images in the standard T1WI, T2WI, FLAIR and DWI sequences in axial, coronal and sagittal planes were studied. Spine MRI images in T1WI and T2WI sequences obtained in axial and sagittal planes were studied.

The chloral-hydrate syrup was used for sedation of children undergoing brain and spine MRI at both MNH and MOI. It was given at 50mg/kg, 30-40 min prior to examination.

3.7.2 Image interpretation

Image interpretation was done by the PI and consensus attained from one radiologist with at least 5 years of experience. In case of disagreement between the two observers, another opinion was sought from another radiologist with more experience. Data was entered using the data collection tool after consensus was reached.

3.7.3 Reliability

Inter-rater reliability in terms of consistency in imaging findings was based on 10% of the sample size which is 11 patients. Measures of each finding were compared to and reported using Kappa statistics.

3.8 Data analysis

Objective 1: To characterise neuroimaging and ETV findings of children with SB attending MOI and MNH

Descriptive statistics are used to summarize the data for the brain MRI, spine MRI and ETV findings of the study participants. Frequencies and percentages have been used to present all categorical variables. The central tendency and measures of variability such as mean, median, standard deviation, and range are used for continuous variables such as age.

Objective 2: To determine the factors the relationship between brain MRI findings and SB patterns in children with SB attending MOI and MNH

Chi square test, and where applicable, Fisher's exact test is used to explore the relationship between various brain MRI findings and SB patterns.

Objective 3: To determine the factors associated with SB patterns as seen on spine MRI of children with SB attending MOI and MNH

Binary and multinomial logistic regression is used to determine the association of maternal/child factors with SB lesion level and defect span respectively.

Objective 4: To assess the correlation between brain MRI and ETV findings of children with SB attending at MOI and MNH.

The chi-square test is used to explore the correlation between the brain MRI and ETV findings.

All data analysis was performed using IBM SPSS Statistics for Windows, Version 26.0, released in 2019.

3.8.1 Ethical issues

Permission to conduct the study at the Radiology departments at MOI and MNH, access to patients' files and images (brain and spine MRI) is sought from both hospital administrations. Anonymity has been maintained by using codes instead of patients' actual names and file numbers. All information is stored in a secure place and kept confidential.

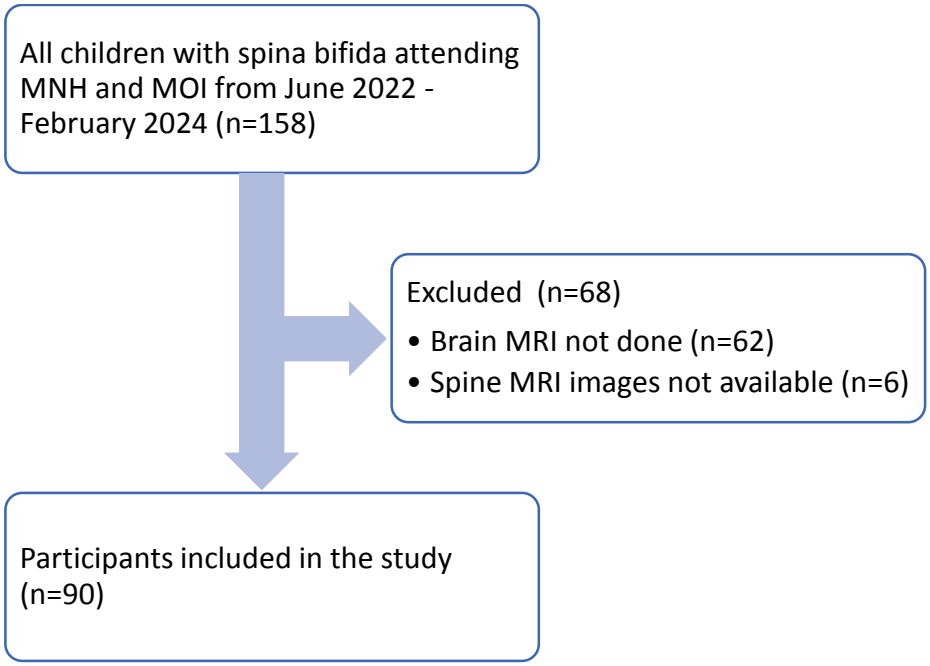
Informed consent in both verbal and written form was obtained from the guardian/parent of the study participants. English or Swahili language was used in attaining informed consent as per the language preference of the guardian/parent.

Ethical clearance has been obtained from the University Institution Review Board (MUHAS-IRB), with **Certificate No: 14879.**

CHAPTER FOUR

4.0 RESULTS

This study involved 90 children with spina bifida who met the inclusion criteria. 68 participants were excluded due to unavailability of either brain or spine MRI images, as illustrated in in Figure 2 below.



- *Figure 2: Study flow chart for inclusion of study participants who attended MNH and MOI from June 2022 - February 2024*

4.1.1 Maternal and child characteristics

Males constituted 60% of the study participants. The majority of the study participants (75.6%) were neonates. The median age was 9.5 days, with an interquartile range of 4.5 days. 27.8% (25/90) were born with a low birth weight (BW < 2500g). A total of 17 (18.9%) study participants are the fourth or older child in their family. These findings are presented in Table 2 below.

Table 2: Child demographic characteristics of the study participants (N=90)

Variable	Categories	Frequency (%)
Sex	Male	54 (60)
	Female	36 (40)
Age	< 1 month	68 (75.6)
	≥ 1 month	22 (24.4)
Birth weight	Normal (≥2500g)	65 (72.2)
	Low birth weight (<2500g)	25 (27.8)
Birth order	Lower birth order	73 (81.1)
	Higher birth order	17 (18.9)

Lower birth order: First, second or third; Higher birth order: Fourth or higher

Several demographic and health-related characteristics of the study participants' mothers were investigated, as indicated in Table 3.

Majority of the mothers were less than 35 years of age (79%). 14% were found to be of short stature, height less than 150cm. In terms of prenatal care, a substantial 72.2% of mothers reported using folic acid supplementation during pregnancy. Notably, all participants who used FA supplementation, initiated during pregnancy, with no reports of starting before pregnancy. Anaemia during pregnancy was found in 34.4%of the mothers. Four out of the ninety women were tested to be HIV positive, and all 4 had prenatal exposure to anti-retroviral drugs. None of the mothers had prenatal history of diabetes or hypertension.

Table 3: Maternal characteristics and their distribution (N=90)

Variable	Categories	Frequency (%)
Age	< 35 years	79 (87.8)
	≥ 35 years	11 (12.2)
Height	< 150cm	14 (15.6)
	≥ 150cm	76 (84.4)
Prenatal FA supplementation	Yes	65 (72.2)
	No	25 (28.8)
FA supplementation initiation	Before pregnancy	0
time	During pregnancy	65 (100) *N=65*
Anaemia during pregnancy	Yes	31 (34.4)
	No	59 (65.6)
Prenatal HIV status	Negative	86 (95.6)
	Positive	4 (4.4)

FA: Folic acid

4.1.2 Spine MRI findings

The frequency and distribution of spine MRI results among the study participants are presented in Table 4. All study participants had spina bifida aperta, of which 78 (86.7%) had myelomeningocele, followed by myeloschisis and meningocele as depicted in Figure 3 below.

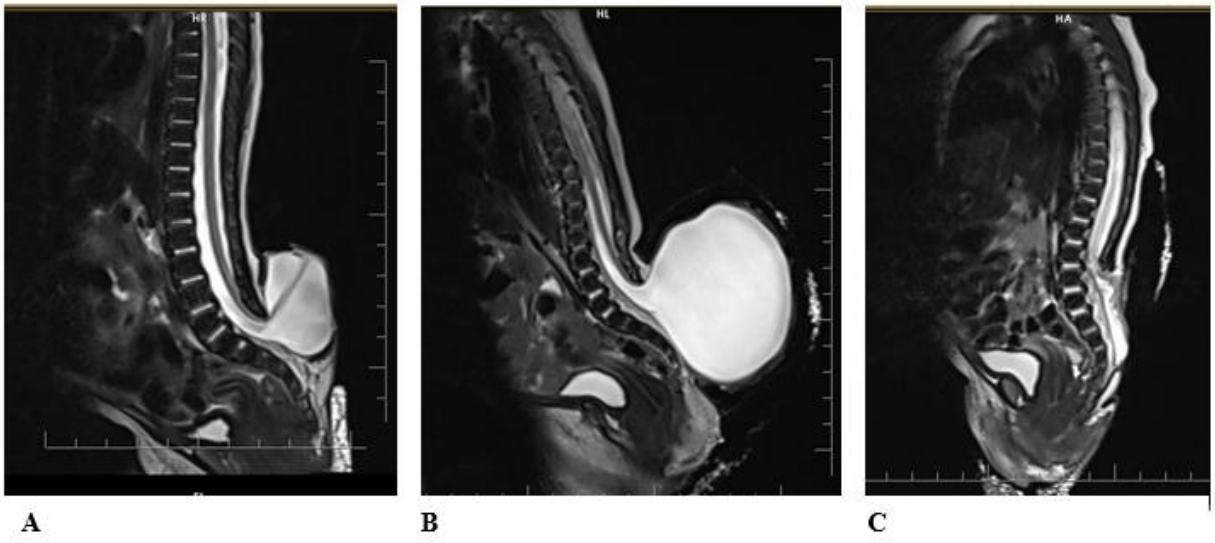


Figure 3: Sagittal spine MRI images of 3 study participants. (A)Myelomeningocele: 14-days old female, with a sacral cystic mass with nerve root within it. Tethered cord is also present. (B)Meningocele:10-days old female with a sacral cystic mass. No neural tissue seen within it. (C) Myeloschisis: 4-dasy old female, with a lumbar-sacral spine defect consisting of flattened plate-like mass of neural tissue with no overlying membranes.

A total of 15 children with SB, accounting for 16.7% of the sample, had upper lesions located at or above the T12 vertebra, with the maximum lesion observed at the C1 vertebral level. The majority (40/75) of those with lower lesions were located in the lower lumbar region (L3-L5).

The range of SB defect span shows significant variation, ranging from a minimum of 1 vertebra affected to a maximum of 13. These were classified into three distinct groups, as seen in Table 4. 20% exhibited abnormalities that affected seven or more vertebrae.

Several other findings were noted on spine MRI. Over 50% of the subjects exhibited tethered cord (Figure 3A), which was identified on sagittal spine MRI as the conus medullaris terminating below the L2 vertebral body.

19% of the children had syrinx, with 3 participants having extensive syringohydromelia extending from the cervical to lumbar spinal cord. 12% had lumbar kyphosis. Only one study participant had split cord (diastematomyelia).

Table 4: Spine MRI findings in children with spina bifida (N=90)

Variable	Categories	Frequency (%)
SBA subtype	Meningocele	2 (2.2)
	Myelomeningocele	78 (86.7)
	Myeloschisis	10 (11.1)
SB lesion level	Upper lesion	15 (16.7)
	Lower lesion	75 (83.3)
SB vertebral defect span	≤ 3 vertebrae	37 (41.1)
	4 – 6 vertebrae	35 (38.9)
	≥ 7 vertebrae	18 (20.0)
Other findings		
• Tethered cord	-	51 (56.7)
• Syrinx	-	19 (21.1)
• Lumbar kyphosis	-	12 (13.3)
• Split cord	-	1 (1.1)

Upper lesion: T12 and above; Lower lesion: L1 and below

4.1.3 Brain MRI findings

Brain MRI images of all participants were studied, which revealed a variety of structural abnormalities, results shown in Figure 4 below. Chiari malformation and hydrocephalus were the most prevalent anomalies at 93.3 and 88.9% respectively.

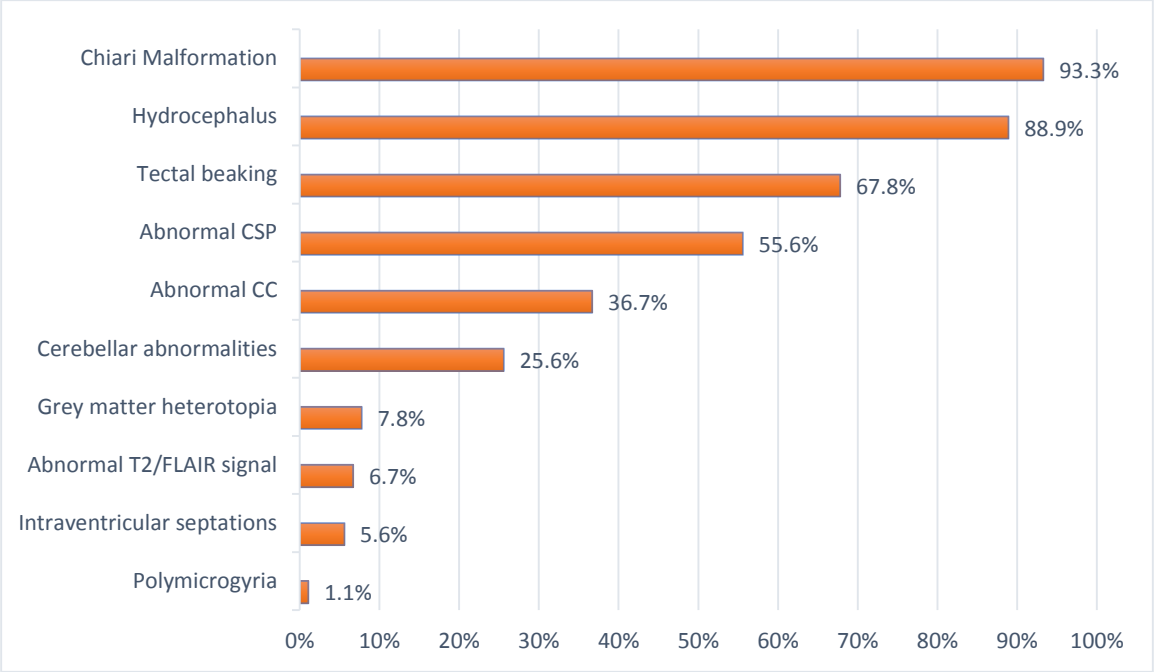


Figure 4: Brain MRI findings in children with spina bifida (N=90)

CC: Corpus callosum; CSP: Cavum septum pellucidum

Chiari type II malformation was the most common Chiari type (82/84) (Fig. 5A). 2 study participants exhibited a Chiari Type III malformation (Fig. 5B).

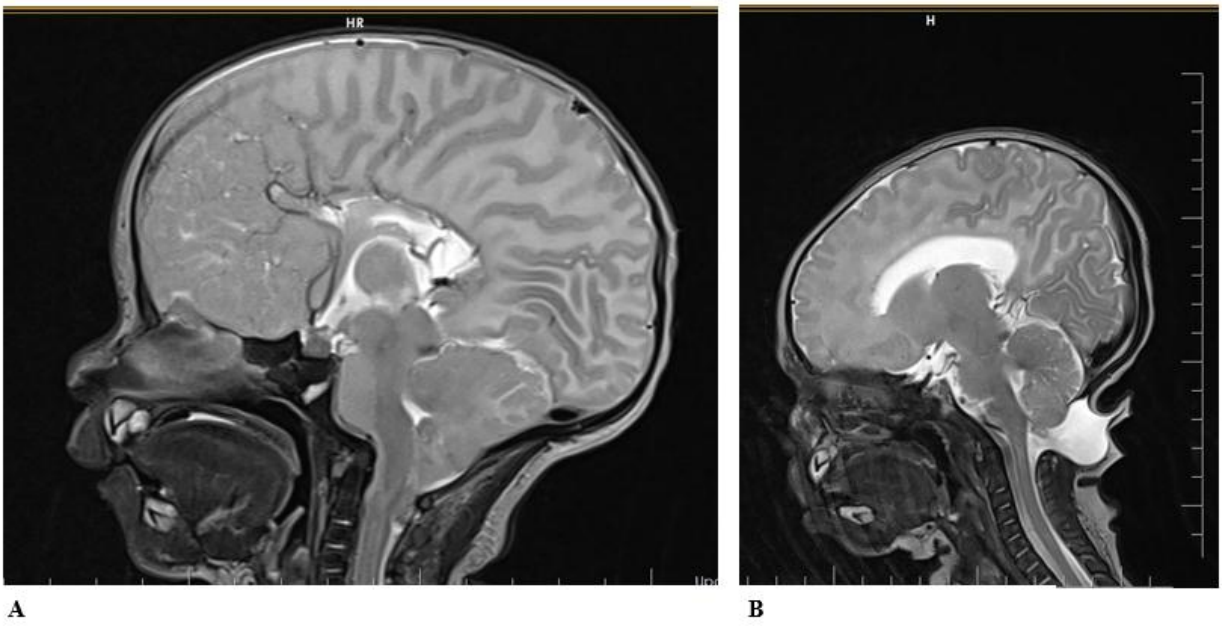


Figure 5(A and B): Sagittal T2W brain MRI images. (A) Chiari type II: 7-days old female with crowded posterior fossa, and cerebellar tonsillar herniation >5mm below the level of foramen magnum. CC dysgenesis of the body and splenium, with vertically oriented gyri is also noted. (B) Chiari type III: 30-days old male with a high cervical encephalocele with protrusion of meninges and cerebellar tissue.

Majority of those who had hydrocephalus, had non-communicating type, accompanied with aqueductal stenosis.

More than half of the study participants (50/90) had abnormal cavum septum pellucidum (CSP) which included those with either absent, or abnormal shaped (triangular or slit-like) CSP.

Cerebellar malformations other than Chiari were present in about a quarter of the study participants, which included aplasia, dysplasia or hypoplasia of the cerebellum.

Participants with abnormal corpus callosum (CC) included those with complete agenesis (4/90) and dysgenesis of either/or rostrum, genu, body or splenium (16/90) (Fig. 4A). Corpus

callosum anomalies could not be assessed in 11 participants due to severe hydrocephalus causing marked thinning if the brain mantle.

Malformation of cortical development, however less prevalent, were observed in 8/90 participants, of which 7 had grey matter heterotopia (Fig. 6) and only 1 with polymicrogyria.

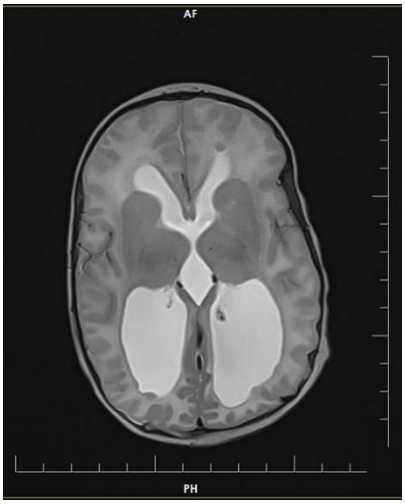


Figure 6: Subependymal grey matter heterotopia. Axial T2W brain MRI of 13-days old female showing enlarged bilateral lateral and third ventricles, with bilateral focal subependymal nodularity isointense to grey matter at the occipital horns of the lateral ventricles.

Abnormal periventricular T2/FLAIR high signal intensity was noted in 6/90 participants, denoting ventriculitis. Five out of ninety participants had intra-ventricular septations and

fluid-fluid level (CSF-debris) suggestive of pus or haemorrhage, within the lateral ventricles.

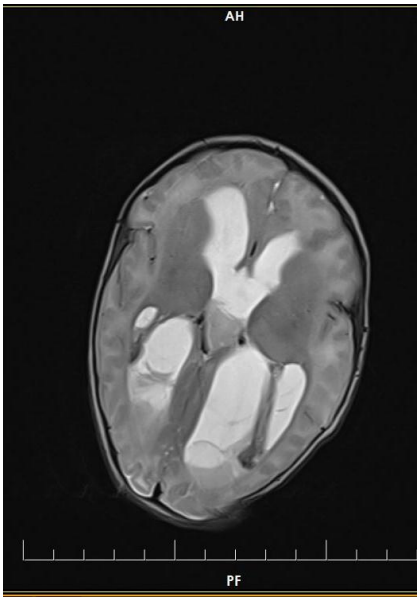


Figure 7: Axial brain T2W MRI image of a 28-days old female showing enlarged bilateral lateral and third ventricles, with CSF-debris levels noted within. Thick intraventricular septations are noted in the left lateral ventricle.

4.1.4 Relationship between brain MRI findings and SB patterns

Tables 5 – 7 below show the relationship between brain MRI findings and various SB patterns.

With regards to SBA subtype (Table 5), as expected, Chiari malformation was found to be more prevalent in children who had myelomeningocele as compared to the other subtypes (p-value 0.032). Other brain MRI findings showed no statistically significant heterogeneity amongst the SBA subtypes.

Table 5: Frequency and percentage of brain MRI findings, overall and according to SBA subtype

Brain MRI finding	All (N=90)	SBA subtype			p-value
		Meningocele (n=2)	Myelomeningocele (n=78)	Myeloschisis (n=10)	
Hydrocephalus	80 (88.9)	2 (100)	70 (89.7)	8 (80.0)	0.461
Chiari malformation	84 (93.3)	2 (100)	75 (96.2)	7 (70.0)	**0.032**
Tectal beaking	61 (67.8)	1 (50.0)	54 (69.2)	6 (60.0)	0.642
Abnormal cerebellum	23 (25.6)	1 (50.0)	18 (23.1)	4 (40.0)	0.229
Abnormal CC* (n=69)	33 (47.8)	1 (100.0)	29 (47.5)	3 (42.9)	0.846
Abnormal CSP	50 (55.6)	1 (50.0)	44 (56.4)	5 (50.0)	0.873
Grey matter heterotopia	7 (7.8)	0 (0)	7 (9.0)	0(0)	1.000
Polymicrogyria	1 (1.1)	0 (0)	1 (1.3)	0 (0)	1.000
Intraventricular septations	5 (5.6)	0 (0)	3 (3.8)	2 (20.0)	0.195
Abnormal T2/FLAIR signal intensity	6 (6.7)	0 (0)	5 (6.4)	1 (10.0)	0.587

p-values < 0.05 are regarded as being statistically significant and are bolded.
CC: Corpus callosum; CSP: Cavum septum pellucidum;
**In 21 patients, corpus callosum could not be assessed on brain MRI due to severe hydrocephalus; hence these are regarded as cases with missing values and are excluded.*

Significant variation is observed in the occurrence of brain anomalies according to spina bifida lesion level as shown in Table 6 below. Children with upper spinal lesions had higher frequencies of MRI-detected abnormalities involving major structures known to be implicated in spina bifida. The frequency was significantly greater for upper-than for lower-level lesions for abnormal cerebellum, corpus callosum, cavum septum pellucidum and polymicrogyria (p-values <0.001, 0.008, 0.001 and 0.025 respectively).

Chiari malformation, hydrocephalus and tectal beaking were highly frequent in both groups, but generally did not occur at a different rate.

Table 6: Frequency and percentage of brain anomalies, overall and according to SB lesion level

		SB lesion level		
Brain MRI finding	All (N=90)	Upper lesion level (n=15)	Lower lesion level (n=75)	p-value
Hydrocephalus	80 (88.9)	15 (100)	65 (86.7)	0.134
Chiari malformation	84 (93.3)	14 (93.3)	70 (93.3)	1.000
Tectal beaking	61 (67.8)	13 (86.7)	48 (64.0)	0.086
Abnormal cerebellum	23 (25.6)	11 (73.3)	12 (16.0)	**<0.001**
Abnormal CC* (N=69)	33 (47.8)	8 (88.9)	25 (41.7)	**0.008**
Abnormal CSP	50 (55.6)	14 (93.3)	36 (48.0)	**0.001**
Grey matter heterotopia	7 (7.8)	1 (6.7)	6 (8.0)	0.860
Polymicrogyria	1 (1.1)	1 (6.7)	0 (0)	**0.025**
Intraventricular septations	5 (5.6)	0 (0)	5 (6.7)	0.303
Abnormal T2/FLAIR signal intensity	6 (6.7)	1 (6.7)	5(6.7)	1.000

p-values < 0.05 are regarded as being statistically significant and are bolded.
CC: Corpus callosum; CSP: Cavum septum pellucidum;
**In 21 patients, corpus callosum could not be assessed on brain MRI due to severe hydrocephalus; hence these are regarded as cases with missing values and are excluded.*

Table 7 below shows the relationship between brain MRI findings and SB defect span. Children with spina bifida affecting a span of ≥7 vertebral segments had a generally higher occurrence of brain abnormalities. Tectal beaking, abnormal cerebellum, corpus callosum and cavum septum pellucidum were found to be significantly more prevalent in this group (p-

value 0.024, <0.001, 0.028 and <0.001 respectively) compared to children having smaller SB defect spans.

Table 7: Frequency and percentage of brain anomalies, overall and according to SB defect span

Brain MRI finding	All (N=90)	SB defect span			
		≤ 3 (n=37)	4-6 (n=35)	≥7 (n=18)	p-value
Hydrocephalus	80 (88.9)	31 (83.8)	31 (88.6)	18 (100)	0.199
Chiari malformation	84 (93.3)	34 (91.9)	33 (94.3)	17 (94.4)	0.900
Tectal beaking	61 (67.8)	22 (59.5)	22 (62.9)	17 (94.4)	**0.024**
Abnormal cerebellum	23 (25.6)	3 (8.1)	9 (25.7)	11 (61.1)	**<0.001**
Abnormal CC* (n=69)	33 (47.8)	10 (34.5)	14 (48.3)	9 (81.8)	**0.028**
Abnormal CSP	50 (55.6)	13 (35.1)	20 (57.1)	17 (94.4)	**<0.001**
Grey matter heterotopia	7 (7.8)	3 (8.1)	4 (11.4)	0(0)	0.337
Polymicrogyria	1 (1.1)	0 (0)	0 (0)	1 (5.6)	0.132
Intraventricular septations	5 (5.6)	1 (2.7)	3 (8.6)	1 (5.6)	0.554
Abnormal T2/FLAIR signal intensity	6 (6.7)	1 (2.7)	3 (8.6)	2 (11.1)	0.425

p-values < 0.05 are regarded as being statistically significant and are bolded.

CC: Corpus callosum; CSP: Cavum septum pellucidum;

**In 21 patients, corpus callosum could not be assessed on brain MRI due to severe hydrocephalus; hence these are regarded as cases with missing values and are excluded.*

4.1.5 Factors associated with spina bifida patterns

Spina bifida patterns were assessed in terms of defect span affected and level of the lesion (upper vs. lower). No significant differences were found between the spina bifida aperta subtypes in terms of associated factors.

Eight maternal and child factors were assessed in this study. These include; sex, birth weight and birth order of the study participant; and the age at conception, height, history of prenatal folic acid supplementation, HIV status and history of anaemia of their mothers.

Multinomial and binary logistic regression analysis was used to analyse the factors associated with severity of spina bifida in terms of defect span (Table 8) and level of lesion (Table 9) respectively. Maternal HIV status was not included in the regression analysis due to insufficient numbers.

Table 8 below shows the association of various maternal/child characteristics and spina bifida defect span with the reference group being cases with ≤ 3 vertebral levels affected. Findings are adjusted for spina bifida subtype.

Males were found to have more than 9 times higher odds of having a larger defect span (≥ 7) as compared to females. This association is statistically significant for the ≥ 7 vertebral levels category in comparison to ≤ 3 vertebral levels group (OR: 9.69, 95%CI: (1.76 – 53.28), p-value: 0.009).

Maternal anaemia is shown to be strongly correlated with the occurrence of larger defect span in patients with open spina bifida. Patients with mothers who had anaemia during pregnancy had 10 times higher odds to have a larger defect span (≥ 7 vertebral levels) as compared to those who did not have anaemia (OR:10.13, 95%CI (2.42 – 42.36), p-value 0.002).

Low birth weight and higher birth order were also shown to have increased odds (OR 3.28 and 2.17 respectively) of having defect span ≥ 7, but the association was not statistically significant.

Maternal age ≥ 35 years, short stature and no history of prenatal folic acid supplementation were seen to have lesser odds of having either 4-6 or ≥ 7 vertebral levels affected than ≤ 3 vertebral levels. However, this association was not statistically significant.

Table 8: Association between maternal/child characteristics and spina bifida defect span

Factor		≤ 3 vertebral levels OR (95% CI) p value	4 – 6 vertebral levels OR (95% CI) p value	≥ 7 vertebral levels OR (95% CI) p value
Sex				
	Male	1.00 (Ref)	2.07 (0.74 – 5.77) 0.167	9.69 (1.76 – 53.28) **0.009**
	Female (Ref)	-	-	-
Birth weight				
	< 2500g	1.00 (Ref)	0.76 (0.22 – 2.63) 0.667	3.28 (0.71 – 14.53) 0.13
	≥ 2500g (Ref)	-	-	-
Birth order				
	Higher (≥ 4)	1.00 (Ref)	3.77 (0.88 – 16.08) 0.073	2.17 (0.28 – 16.79) 0.457
	Lower (< 4) (Ref)	-	-	-
Maternal age				
	≥ 35 years	1.00 (Ref)	0.47 (0.08 – 2.68) 0.392	0.76 (0.09 – 6.39) 0.802
	< 35 years (Ref)	-	-	-
Prenatal FA supplementation				
	No	1.00 (Ref)	0.99 (0.33 – 2.98) 0.981	0.57 (0.09 – 3.30) 0.533
	Yes (Ref)	-	-	-
Maternal height				
	< 150 cm	1.00 (Ref)	0.94 (0.24 – 3.63) 0.922	0.48 (0.07 – 3.50) 0.466
	≥ 150 cm (Ref)	-	-	-
Maternal anaemia				
	Yes	1.00 (Ref)	1.35 (0.44 – 4.16) 0.602	10.13 (2.42 – 42.36) **0.002**
	No (Ref)	-	-	-

p-values < 0.05 are regarded as being statistically significant and are bolded.
The ORs and 95%CIs were found by multinomial logistic regression comparing ≤ 3 vertebral levels cases as the reference group. OR: Odds ratio; CI: Confidence interval; Ref: Reference Findings are adjusted for spina bifida subtype

Table 9 below shows the results of binary logistic regression analysis to determine the association between maternal/child characteristics and spina bifida lesion level. Lesion level was assigned as the highest vertebral level affected as seen on sagittal T2W spine MRI. The levels were then categorized into upper (T12 and above) and lower lesions (L1 and below). Findings are interpreted with the lower lesion level group as the reference.

Male sex is significantly associated with increased likelihood of having an upper lesion level than female sex (OR: 5.79, 95%CI (1.01 – 33.14), p-value 0.049).

Moreover, participants with maternal history of anaemia during pregnancy were 24 times more likely to have an upper lesion as compared to those with no history of anaemia during pregnancy. This association is found to statistically significant (p-value <0.001)

Low birth weight and higher birth order were shown to have increased odds of having upper lesion level, however, the association was not statistically significant.

There was however a notable difference in percentages, where 24% of those who were born with a low birth weight had upper lesion level compared to 13.8% of those born with birth weight > 2500g.

Table 9: Association between maternal/child characteristics and spina bifida lesion level

Factor	Upper lesion level n (%)	Lower lesion level n (%)	OR (CI 95%)	p-value
Sex				
Male	12 (22.2)	42 (77.8)	5.79 (1.01 – 33.14)	**0.049**
Female	3 (8.3)	33 (91.7)	-	-
Birth weight				
< 2500g	6 (24.0)	19 (76.0)	1.88 (0.44 – 8.03)	0.394
≥ 2500g	9 (13.8)	56 (86.2)	-	-
Birth order				
High (≥ 4)	3 (17.6)	14 (82.4)	1.65 (0.23 – 11.86)	0.618
Low (< 4)	12 (16.4)	61 (83.6)	-	-
Maternal age				
≥ 35 years	2 (18.2)	9 (81.8)	0.55 (0.07 – 4.58)	0.581
< 35 years	13 (16.5)	66 (83.5)		
Prenatal FA supp.				
No	4 (16.0)	21 (84.0)	0.72 (0.13 – 4.12)	0.713
Yes	11 (16.7)	54 (83.1)		
Maternal height				
< 150 cm	2 (14.3)	12 (85.7)	0.66 (0.09 – 4.96)	0.688
≥ 150 cm	13 (17.1)	63 (82.9)		
Maternal anaemia				
Yes	13 (41.9)	18 (58.1)	24.02 (4.50 – 128.28)	**< 0.001**
No	2 (3.4)	57 (96.6)		
Maternal HIV status				
Positive	1 (25.0)	3 (75.0)	-	-
Negative	14 (16.3)	72 (83.7)		

p-values < 0.05 are regarded as being statistically significant and are bolded.
The ORs and 95%CIs were found by binary logistic regression comparing ≤ 3 vertebral levels cases as the reference group.
OR: Odds ratio; CI: Confidence interval
Upper lesion level: T12 and above; Lower lesion level: L1 and below
Findings are adjusted for spina bifida subtype

4.1.6 Association between ETV findings and brain MRI findings

ETV findings in this study provide an endoscopic lens to the brain structure, that may be complimentary to brain MRI findings. Since ETV is not a diagnostic imaging procedure, only clinically relevant findings and their corresponding imaging characteristics are studied.

A subset of 29 patients with spina bifida with hydrocephalus underwent ETV as a therapeutic procedure. Association between ETV findings and brain MRI findings specifically abnormal T2/FLAIR signal intensity and intraventricular septations are shown in Table 10 below.

Higher frequency of turbid/hemorrhagic CSF, scarred choroid plexus and prepontine cistern, and presence of pus at 3rd ventricular floor were noted in patients whose brain MRI showed abnormal periventricular T2/FLAIR high signal intensity and the same is observed in children with intraventricular septations as seen in their brain MRIs. However, the only association that was statistically significant was between CSF characteristic and intraventricular septations (p-value 0.045).

Table 10: Association between ETV findings and brain MRI findings (Abnormal T2/FLAIR SI and intraventricular septations

		Abnormal T2/FLAIR SI			Intraventricular septations		
ETV finding	All (N=90)	Present (n=5)	Absent (n=24)	p-value	Present (n=3)	Absent (n=26)	p-value
CSF: Turbid/hemorrhagic	10 (35)	3 (60)	7 (29)	0.306	3 (100)	8(31)	**0.045**
Scarred choroid plexus	13 (45)	4 (80)	9 (38)	0.144	3 (100)	10 (39)	0.078
Pus at 3rd ventricular floor	7 (24)	2 (40)	5 (21)	0.651	2 (67)	5 (19)	0.316
Scarred pre-pontine cistern	15 (52)	3 (60)	12 (50)	1.000	2 (67)	13 (50)	1.000

p-value < 0.05 is regarded as being statistically significant and is bolded.

SI: signal intensity

CHAPTER FIVE

5.0 DISCUSSION

In this baseline cross-sectional descriptive study, various parameters are studied with regards to neuroimaging findings and associated factors in children with spina bifida.

Our study sample consisted of 90 participants with spina bifida who had undergone both brain and spine MRI, with a subset of 29 participants who underwent ETV as a treatment for hydrocephalus.

All study participants in our sample had spina bifida aperta. This may have been due to the fact that most spina bifida occulta cases are much less severe and may be clinically indolent, and hence did not present to the hospital.

Myelomeningocele was the most common SBA subtype (86.7%). Similar findings have been observed by many studies such Schneider et al, with a frequency of 96% of myelomeningocele in their study (6,10,23,42) . However, another study done in 2021 found that myeloschisis occurred more frequently than myelomeningocele (31.1 vs 23.3% respectively). No such comparable study is done in our setting. Heterogeneity across different literatures is due to the many classifications of spina bifida aperta.

Twenty-five percent of the study participants had a higher lesion level denoting lesion level T12 and above. This is much higher than other what is observed in other studies. In the study conducted by Trigo et.al (42) , only 6.1% of the study participants had higher lesions. Shneider et.al found higher lesions to consist of only 11% of their study population(23). The increase occurrence of higher lesions in our sample may be attributed to the various maternal and child factors as discussed later.

All participants in our study had at-least one brain anomaly. Hydrocephalus and Chiari malformation were the most prevalent (88.9 and 93.3% respectively). Other non-specific but commonly occurring anomalies include abnormal corpus callosum (11), cerebellar anomalies, tectal beaking and malformations of cortical development which include grey matter

heterotopia and polymicrogyria are also frequent. (5,15,22,43). These are similar to our findings. Abnormal corpus callosum was found to be the most prevalent brain anomaly by Schneider et al. (23).

As anticipated, Chiari malformation was most prevalent in the myelomeningocele group. The difference in its occurrence as compared to other SBA subtypes is a known phenomenon. None of the other brain MRI findings, despite being frequent in all subtypes, were seen to have heterogeneity in occurrence. Similar findings are observed in other studies (23).

Our study suggests that spinal lesion level constitutes a visible source of diversity in terms of brain anomalies in children with spina bifida. Children with upper spinal lesions in our study had statistically significant higher frequencies of MRI-detected abnormalities involving major structures known to be implicated in spina bifida than for lower-level lesions. These anomalies include abnormal cerebellum, corpus callosum, cavum septum pellucidum and polymicrogyria. Similar findings are observed by Fletcher et al (14).

Moreover, children with larger defect spans (≥7) were seen to have higher frequency of brain anomalies. Studies done on the relationship between defect span and outcome of patients show much poorer outcome in terms of function in children with larger defect spans (41) .

A temporal relationship between the severity of spina bifida in terms of higher lesion level and larger defect span may be suggested. Further studies that may look into this causal association are required. This would help to predict whether early intrauterine SB repair will have any benefit to reducing the occurrence of associated brain anomalies.

Male sex and maternal history of anaemia during pregnancy were found to have higher odds of having an upper lesion with a larger defect span. Studies have shown varied distribution of spina bifida according to sex, with a slight female predilection (10).

In our study, male children had significantly higher odds of presenting with larger spina bifida defects (OR: 9.69, $p = 0.009$) and upper lesion levels (OR: 5.79, $p = 0.049$). Although SB does not have a well-documented sex predilection globally, some research suggests that male

sex may be associated with an increased risk of severe neurological conditions. Studies on other congenital malformations have also identified that male foetuses are more vulnerable to prenatal environmental stressors, including maternal undernutrition. This vulnerability might be due to differences in sex hormone regulation, fetal growth patterns, or genetic factors linked to the X chromosome.(44)

While the exact biological mechanisms remain unclear, the data from this study reinforce the notion that male sex could play a role in determining the severity of neural tube defects, warranting further investigation. Understanding how sex-specific factors contribute to the development and severity of spina bifida could lead to targeted interventions and improve clinical outcomes.

The association between anaemia and higher odds of having an upper lesion with larger defect span has not been quite studied. In our study, mothers with history of anaemia during pregnancy had 10 times higher odds of having a child with a large SB defect span and 24 times higher odds of having an upper SB lesion as compared to smaller defect span and lower lesion respectively.

Some studies have shown that low iron status is not an independent risk factor for the occurrence of spina bifida (45). But whether or not it could potentially play a role in occurrence of severe forms of spina bifida is not yet well studied. Our results could also be explained by a much holistic approach on undernutrition of these mothers. Further studies are required to assess the strength of this association.

Many studies look into Folic acid supplementation, which has been shown to be significantly associated with occurrence of spina bifida in the population, proven by large clinical trials and meta-analysis studies(21,32). In our study, folic acid supplementation was not found to associated with severe SB occurrence. However, since all of the mothers initiated FA supplementation during pregnancy, the results of this effect may not be reliable.

This study takes into account, through a different lens, ETV findings of children with spina bifida and hydrocephalus. Interestingly, higher frequency of turbid/hemorrhagic CSF, pus at

3rd ventricular floor, scarred choroid plexus and pre-pontine cisterns is noted especially in children with intraventricular septations and abnormal periventricular T2/FLAIR high signal intensity. These together are findings suggestive of possible ventriculitis or intraventricular haemorrhage in children with open spina bifida. Diagnosing this based on highly suspicious MRI features may aid in improving surgical outcomes in these children. The lack of statistical significance may be due to smaller number of children who underwent ETV.

5.1 Limitations

The study has its limitation pertaining to it being hospital-based and conducted at tertiary facilities. This may have led to bias as the sample may not represent the true population. Also, children with more severe forms of spina bifida are more likely to be referred to MNH and MOI as tertiary hospitals for management. This may have exaggerated the occurrence of prevalence of brain anomalies among these children.

Some patients did not have STIR sequence images for spine MRI, hence some prevalent forms of spinal dysraphisms such as lipomyelomeningocele, and other lipomatous lesions may have been missed.

Also, the minimum sample size of 117 participants as calculated was not reached, since patients had to be excluded from the study due to incomplete brain and spine MRI. This may have reduced the power of the study. To mitigate this, theoretically-strong indicators were measured.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

All study participants had spina bifida aperta, with myelomeningocele being the most common subtype. Male sex and maternal history of anaemia during pregnancy were significant predictors SB severity in terms of upper lesion level and larger defect span.

Chiari malformation and hydrocephalus were the most common brain anomalies found in this study sample. Children with upper lesion level and larger defect span had significantly higher number and frequency of brain anomalies compared to those with lower lesion and smaller defect spans.

Increased frequency of various abnormal ETV finings such as scarred choroid plexus and prepontine cistern, pus at 3rd floor of 3rd ventricle and turbid/hemorrhagic CSF are noted amongst patients who had abnormal T2/FLAIR signal intensity and intraventricular septations.

6.2 Recommendations

A larger multi-centre or population-based study is recommended to further assess the associations, so that they may be applicable to the general population.

This study shows marked heterogeneity in terms of association between brain anomalies and SB patterns. Further studies to assess cognitive and functional outcome may be of great benefit so as to be able to better plan a holistic approach to management of spina bifida.

Modifiable risk factor such as maternal anaemia has been shown to increase occurrence of more severe SB patterns. Further studies to assess the causal mechanism and to plan community-based interventions to decrease likelihood of severe SB cases is recommended.

REFERENCES

1. Yadav YR, Parihar V, Pande S, Namdev H, Agarwal M. Endoscopic third ventriculostomy. J Neurosci Rural Pract. 2012;3(02):163–73.

2. Rekate HL. A Contemporary Definition and Classification of Hydrocephalus. Vol. 16, Seminars in Pediatric Neurology. W.B. Saunders; 2009. p. 9–15.

3. Hashemi RH, Bradley WG, Lisanti CJ. MRI: the basics: The Basics. Lippincott Williams & Wilkins; 2012.

4. Oumer M, Taye M, Aragie H, Tazebew A. Prevalence of spina bifida among newborns in Africa: A systematic review and meta-analysis. Scientifica (Cairo). 2020;2020:14–8.

5. Paschereit F, Schindelmann KH, Hummel M, Schneider J, Stoltenburg-Didinger G, Kaindl AM. Cerebral Abnormalities in Spina Bifida: A Neuropathological Study. Pediatr Dev Pathol [Internet]. 2022 Mar 1 [cited 2022 Oct 12];25(2):107–23. Available from: https://journals.sagepub.com/doi/full/10.1177/10935266211040500

6. Fletcher JM, Brei TJ. Spina Bifida-A Multidisiplinary Perspective. Dev Disabil Res Rev. 2011;16(1):1–5.

7. Parker SE, Yazdy MM, Mitchell AA, Demmer LA, Werler MM. A description of spina bifida cases and co-occurring malformations, 1976-2011. Am J Med Genet Part A. 2014;164(2):432–40.

8. Kishimba RS, Mpembeni R, Mghamba JM, Goodman D, Valencia D. Birth prevalence of selected external structural birth defects at four hospitals in Dar es Salaam, Tanzania, 2011-2012. J Glob Health. 2015;5(2):1–6.

9. Schindelmann KH, Paschereit F, Steege A, Stoltenburg-Didinger G, Kaindl AM. Systematic classification of spina bifida. J Neuropathol Exp Neurol [Internet]. 2021

Mar 22 [cited 2022 Oct 12];80(4):294–305. Available from: https://academic.oup.com/jnen/article/80/4/294/6133912

10. Nagaraj UD, Bierbrauer KS, Stevenson CB, Peiro JL, Lim FY, Zhang B, et al. Myelomeningocele versus myelocele on fetal MR images: Are there differences in brain findings? Am J Roentgenol [Internet]. 2018 Oct 17 [cited 2022 Oct 12];211(6):1376–80. Available from: www.ajronline.org

11. Kawamura T, Nishio S, Morioka T, Fukui K. Callosal anomalies in patients with spinal dysraphism: Correlation of clinical and neuroimaging features with hemispheric abnormalities. Neurol Res. 2002;24(5):463–7.

12. Kunpalin Y, Deprest J, Papastefanou I, Bredaki E, Sacco A, Russo F, et al. Incidence and patterns of abnormal corpus callosum in fetuses with isolated spina bifida aperta. Prenat Diagn [Internet]. 2021 Jul 1 [cited 2022 Oct 12];41(8):957–64. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/pd.5945

13. Bell JE, Gordon A, Maloney AFJ. The Association of Hydrocephalus and Arnold-Chiari Malformation With Spina Bifida in the Fetus. Neuropathol Appl Neurobiol [Internet]. 1980 Jan 1 [cited 2022 Oct 12];6(1):29–39. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2990.1980.tb00201.x

14. Fletcher JM, Copeland K, Frederick JA, Blaser SE, Kramer LA, Northrup H, et al. Spinal lesion level in spina bifida: A source of neural and cognitive heterogeneity. J Neurosurg. 2005;102 PEDIAT(SUPPL. 3):268–79.

15. Juranek J, Salman MS. Anomalous development of brain structure and function in spina bifida myelomeningocele. Dev Disabil Res Rev. 2010;16(1):23–30.

16. Lindquist B, Jacobsson H, Strinnholm M, Peny-Dahlstrand M. A scoping review of cognition in spina bifida and its consequences for activity and participation throughout life. Acta Paediatr Int J Paediatr. 2022;111(9):1682–94.

17. Burro F, Cama A, Lertora V, Veneselli E, Rossetti S, Pezzuti L. Intellectual efficiency in children and adolescents with spina bifida myelomeningocele and shunted hydrocephalus. Dev Neuropsychol. 2018 Apr 3;43(3):198–206.

18. Hetherington R, Dennis M, Barnes M, Drake J, Gentili F. Functional outcome in young adults with spina bifida and hydrocephalus. Child's Nerv Syst. 2006 Feb;22(2):117–24.

19. Ramsundhar N, Donald K. An approach to the developmental and cognitive profile of the child with spina bifida. South African Med J. 2014;104(3):221.

20. Jenkinson MD, Campbell S, Hayhurst C, Clark S, Kandasamy J, Lee MK, et al. Cognitive and functional outcome in spina bifida-Chiari II malformation. Child's Nerv Syst. 2011 Jun;27(6):967–74.

21. Ssentongo P, Heilbrunn ES, Ssentongo AE, Ssenyonga LVN, Lekoubou A. Birth prevalence of neural tube defects in eastern Africa: a systematic review and meta-analysis. BMC Neurol [Internet]. 2022;22(1):1–11. Available from: https://doi.org/10.1186/s12883-022-02697-z

22. Sweeney KJ, Caird J, Taufiq Sattar M, Allcutt D, Crimmins D. Spinal level of myelomeningocele lesion as a contributing factor in posterior fossa volume, intracranial cerebellar volume, and cerebellar ectopia: Clinical article. J Neurosurg Pediatr. 2013;11(2):154–9.

23. Schneider J, Mohr N, Aliatakis N, Seidel U, John R, Promnitz G, et al. Brain malformations and cognitive performance in spina bifida. Dev Med Child Neurol. 2021 Mar 1;63(3):295–302.

24. Cortés-Albornoz MC, García-Guáqueta DP, Velez-Van-meerbeke A, Talero-Gutiérrez C. Maternal nutrition and neurodevelopment: A scoping review. Nutrients. 2021;13(10).

25. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. New Engl J Med. 1992;326(26):1832–5.

26. Parker SE, Yazdy MM, Mitchell AA, Demmer LA, Werler MM. A description of spina bifida cases and co-occurring malformations, 1976-2011. Am J Med Genet Part A [Internet]. 2014;164(2):432–40. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ajmg.a.36324

27. Williams J, Mai CT, Mulinare J. National Birth Defects Prevention Month and Folic Acid Awareness Week — January 2015 Updated Estimates of Neural Tube Defects Prevented by Mandatory Folic Acid Fortification — United States , 1995 – 2011. Vol. 64, Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report. 2015.

28. Mai CT, Evans J, Alverson CJ, Yue X, Flood T, Arnold K, et al. Changes in Spina Bifida Lesion Level after Folic Acid Fortification in the US. J Pediatr [Internet]. 2022;249:59-66.e1. Available from: https://doi.org/10.1016/j.jpeds.2022.06.023

29. Bergman JEH, Otten E, Verheij JBGM, de Walle HEK. Folic acid supplementation influences the distribution of neural tube defect subtypes: A registry-based study. Reprod Toxicol [Internet]. 2016;59:96–100. Available from: http://dx.doi.org/10.1016/j.reprotox.2015.11.007

30. Tanzania Commission for AIDS (TACAIDS), Zanzibar AIDS Commission (ZAC), National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS) and II 2013. Tanzania HIV/AIDS and Malaria Indicator Survey 2011-12 [Internet]. Vol. 3. 2013. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2214109X14703389%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3990612&tool=pmcentrez&rendertype=abstract%5Cnhttp://www.nejm.org/doi/abs/10.1056/NEJMe1410977%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed

31. MOHCDGEC: National AIDS Control Programme. National Guidelines for the Management of HIV and AIDS. Vol. 7th Ed., Development. 2019.

32. Zhang L, Zhang Y, Li Z, Ren A, Liu J, Ye R. Maternal periconceptional body mass index and risk for neural tube defects: results from a large cohort study in China. J Matern Neonatal Med [Internet]. 2021;34(2):274–80. Available from: https://doi.org/10.1080/14767058.2019.1606192

33. Watkins ML, Rasmussen SA, Honein MA, Botto LD, Moore CA. Maternal obesity and risk for birth defects. Pediatrics. 2003;111(5 II):1152–8.

34. Shaw GM, Todoroff K, Schaffer DM, Selvin S. Maternal height and prepregnancy body mass index as risk factors for selected congenital anomalies. Paediatr Perinat Epidemiol. 2000;14(3):234–9.

35. Arbuzova S, Nikolenko M, Cuckle H. Maternal weight as an additional first trimester spina bifida screening marker. J Matern Neonatal Med [Internet]. 2022;35(17):3353–8. Available from: https://doi.org/10.1080/14767058.2020.1818217

36. Beuriat PA, Szathmari A, Grassiot B, Plaisant F, Rousselle C, Mottolese C. Role of Endoscopic Third Ventriculostomy in the Management of Myelomeningocele-Related Hydrocephalus: A Retrospective Study in a Single French Institution. World Neurosurg [Internet]. 2016;87:484–93. Available from: http://dx.doi.org/10.1016/j.wneu.2015.07.071

37. El-Ghandour NMF, Salama MM, Ghoneim MA, Attia AM. Endoscopic third ventriculostomy for management of hydrocephalus associated with Chiari malformation type II in children. Child's Nerv Syst [Internet]. 2023;39(6):1565–71. Available from: https://doi.org/10.1007/s00381-023-05832-4

38. Elgamal EA, El-Dawlatly AA, Murshid WR, El-Watidy SMF, Jamjoom ZAAB. Endoscopic third ventriculostomy for hydrocephalus in children younger than 1 year

of age. Child's Nerv Syst. 2011;27(1):111–6.

39. Sullivan KM, Soe MM. Sample Size for a Cross-Sectional , Cohort , or Clinical Trial Studies Sample Size for Cross-Sectional & Cohort Studies & Clinical Trials. 2007;1–3.

40. Kelsey JL, Whittemore AS, Evans AS, Thompson WD. Methods in observational epidemiology. Vol. 10. Monographs in Epidemiology and; 1996.

41. Chao TT, Dashe JS, Adams RC, Keefover-Hicks A, McIntire DD, Twickler DM. Fetal spine findings on MRI and associated outcomes in children with open neural tube defects. Am J Roentgenol. 2011;197(5):956–61.

42. Trigo L, Eixarch E, Bottura I, Dalaqua M, Barbosa AA, De Catte L, et al. Prevalence of supratentorial anomalies assessed by magnetic resonance imaging in fetuses with open spina bifida. Ultrasound Obstet Gynecol. 2022 Jun 1;59(6):804–12.

43. Kunpalin Y, Richter J, Mufti N, Bosteels J, Ourselin S, De Coppi P, et al. Cranial findings detected by second-trimester ultrasound in fetuses with myelomeningocele: a systematic review. BJOG An Int J Obstet Gynaecol. 2021;128(2):366–74.

44. Brokowski C AM. HHS Public Access. Physiol Behav. 2019;176(5):139–48.

45. Molloy AM, Einri CN, Jain D, Laird E, Fan R, Wang Y, et al. Is low iron status a risk factor for neural tube defects? Birth Defects Res Part A Clin Mol Teratol [Internet]. 2014 Feb 18;100(2):100–6. Available from: https://onlinelibrary.wiley.com/doi/10.1002/bdra.23223

APPENDICES

Appendix I: Data collection template

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE – DEPARTMENT OF RADIOLOGY AND IMAGING

P.O.BOX 65001, DAR-ES-SALAAM, TANZANIA

Study participant ID No: _____

PI/Interviewee Initials: _____

Date: _____

PART ONE: MATERNAL AND CHILD DEMOGRAPHIC CHARACTERISTICS		
A: Child demographics		
1.	Age	_____
2.	Sex	_____
3.	Birth weight	1. <2500g 2. ≥2500g
4.	Birth order	_____
B: Maternal characteristics		
5.	a: Place of residence - Region	_____
	b: Urban vs Rural	1. Urban 2. Rural
6.	Age at the conception of the index child	_____
7.	a: Prenatal folic acid supplementation	1. Yes 2. No
	If yes the above;	1. Started before conception 2. Started during 1st trimester

	b: Timing of commencing folic acid supplementation	3. Started during 2nd trimester 4. Started during 3rd trimester
8.	a: Maternal prenatal HIV status	1. Positive 2. Negative
	If Positive in an above; b: ARV use	1. Yes 2. No
	If Yes in b above: c: ARV drug combination	_____
9.	a: Maternal height	_____
	b: Maternal weight before conception of the index child	_____
	c: Maternal BMI before conception of the index child	1. BMI <30kg/m^2 2. BMI ≥30kg/m^2
PART TWO: SPINE MRI FINDINGS		
10.	Spina bifida type	1. Occulta 2. Aperta
11.	If Aperta in Qn. 10 above, Spina bifida aperta subtype	1. Meningocele 2. Myelomeningocele 3. Myeloschisis
12	Spina bifida lesion level	1. Cervical 2. Thoracic 3. Lumbar 4. Sacral
13.	Spina bifida cyst/defect area	_____
14.	Any other findings	_____ _____

PART THREE: BRAIN MRI FINDINGS		
15.	Skull	1. Normal 2. Microcephaly 3. Macrocephaly
16.	Brain mantle	1. Normal 2. Thin
18.	a. Cortical development	1. Normal 2. Abnormal
	If abnormal in the above: b: Malformation of cortical development	1. Schizencephaly 2. Lissencephaly 3. Polymicrogyria 4. Other
19.	a: Hydrocephalus	1. Present 2. Absent
	If Present in an above: b: Type of hydrocephalus	1. Communicating 2. Non-communicating
20.	Aqueduct of Sylvius	1. Open 2. Closed
21.	Cavum septum pellucidum	1. Present 2. Absent
22.	Foramen of Monro	1. Normal 2. Narrow 3. Wide
23.	Choroid plexus	1. Normal 2. Abnormal
24.	a. Corpus callosum	1. Normal 2. Agenesis

		3. Dysgenesis
	If Dysgenesis in the above; b: Part of abnormal Corpus Callosum	1. Splenium 2. Body 3. Genu 4. Rostrum
25.	a. Chiari malformation	1. Present 2. Absent
	If Present in an above: b: Type of Chiari malformation	1. Chiari I 2. Chiari II 3. Chiari III 4. Chiari IV
26.	Dandy Walker malformation	1. Present 2. Absent
27.	Cerebellum	1. Normal 2. Hypoplasia 3. Dysplasia
28.	Thalamic band	1. Present 2. Absent
29.	Tectal beaking	1. Present 2. Absent
30.	Any other abnormality	_____ _____
	If hydrocephalus is present in 19 and above,	
31	Was ETV done?	1. Yes 2. No
	If yes in Qn 31 above,	

	Proceed to Part Four: ETV findings	
PART FOUR: ETV FINDINGS		
32.	CSF	1. Clear 2. Haemorrhagic
33.	Brain mantle thickness	1. Normal 2. Thin
34.	Septum pellucidum	1. Intact 2. Not intact
35.	Foramen of Monro	1. Normal 2. Narrow 3. Wide
36.	Choroid plexus	1. Normal 2. Scarred
37.	3rd ventricle floor	1. Clear 2. Pus 3. Yellow deposits 4. Thalamic adhesions/bands
38.	Aqueduct of Sylvius	1. Open 2. Closed
39.	Prepontine cistern	1. Clean 2. Scarred

Appendix II: Consent form (English version)

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF RESEARCH AND PUBLICATIONS
P.O.BOX 65001, DAR-ES-SALAAM, TANZANIA

Study participant ID No: _____

Introduction:

My name is Dr. Ummehani Mustaali Dossajee. I am researching to determine the Neuroimaging findings of structural brain anomalies and associated factors in children with spina bifida attending Muhimbili National Hospital and Muhimbili Orthopedic Institute.

Purpose of the study:

This study has the purpose of the partial fulfilment for attaining of the degree of Masters of Medicine in Radiology from Muhimbili University of Health and Allied Sciences. Moreover, the study aims at adding more knowledge in the neuroradiology field in terms of a better understanding of spina bifida-associated brain anomalies and various maternal factors that influence these to guide interventions and improve the clinical outcome of these children.

Participant involvement:

This consent form is to establish your willingness to allow the PI access and use of medical and imaging records of your child, and also answer specific questions about your child and your pre-conception and ante-natal history.

Once you agree for you and your child to be involved in the study and this informed consent form has been signed, a series of questions will be asked. Medical and imaging findings will be sought discreetly.

Confidentiality:

Information obtained from you and your child's medical records will be kept confidential. Interviews will be done as discreetly as the environment allows. No name will appear on any document of the study and identification numbers shall be used instead.

Participant rights:

The decision for participating in the study is voluntary. Refusal or withdrawal from the study will not interfere with your management at the hospital and no penalty will be given.

Benefits:

Your participation in this study will help us to determine neuroimaging patterns of structural brain anomalies in children with spina bifida in our setting. It will also provide insight into specific maternal factors that influence these anomalies. Collectively, this study will form the basis for prenatal counselling, management of children with spina bifida and guide interventions.

Risks:

Your participation in this study would not compromise your/your child's disease outcome nor influence the health service provision at the hospital. Also, personal medical information will not be disclosed to the public and will be kept confidential except to those involved in undertaking the study.

Who to contact:

If you have any questions about this study, you can contact the following:

Dr. Ummehani Mustaali Dossajee: Principal Investigator

Mobile No: +255-765-867087; Email: ummehanimustaali@gmail.com

Dr. Mboka Jacob: Main supervisor and Head of Department of Radiology and Imaging at MUHAS

Mobile No: +255-768-517877; Email: mbokajacob@gmail.com

Dr. Nahya Salim Masoud: Chairperson – MUHAS Research Ethics Committee

Mobile No: +255-713-250074

I ……. have read and understood the contents of this form. I have agreed/not agreed to participate in this study.

Signature of Participant ……. Date……..……

Signature of Principal Investigator ……. Date…….

Appendix III: Consent form (Swahili version)

CHUO KIKUU CHA AFYA NA SAYANSI VISHIRIKA MUHIMBILI
KURUGENZI YA UTAFITI NA MACHAPISHO
S.L.P 65001, DARESALAAM, TANZANIA

FOMU YA RIDHAA

Nambari ya kitambulisho cha mshiriki wa utafiti: _____

Utangulizi:

Naitwa Dr Ummehani Mustaali Dossajee. Ninafanya utafiti ili kubaini matokeo ya vipimo kwa jaili ya kubaini upungufu katika muundo wa ubongo kwa kuzaliwa kwa watoto wenye "spina bifida" wanaohudhuria Hospitali ya Taifa Muhimbili na Taasisi ya Mifupa ya Muhimbili.

Madhumuni ya utafiti:

Utafiti huu una madhumuni ya utimilifu wa sehemu ya kupata Shahada ya Uzamili ya Tiba katika Radiolojia kutoka Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili. Zaidi ya hayo, utafiti huo unalenga kuongeza ujuzi zaidi katika nyanja ya neuroradiolojia katika suala la uelewaji bora wa matatizo ya ubongo yanayohusiana na spina bifida na mambo mbalimbali ya uzazi ambayo huathiri haya ili kuongoza hatua za matibabu na kuboresha maisha ya watoto hawa.

Ushiriki wa mshiriki:

Fomu hii ya idhini ni kuthibitisha utayari wako wa kuruhusu PI kufikia na kutumia rekodi za matibabu na picha za mtoto wako, na pia kujibu maswali mahususi kuhusu mtoto wako na historia yako ya kabla ya mimba kutungwa na kabla ya kuzaa.

Pindi tu unapokubali wewe na mtoto wako kuhusika katika utafiti na fomu hii ya idhini iliyo na taarifa imetiwa sahihi, maswali yataulizwa kutoka kwenye dodoso lililoandaliwa. Majibu ya vipimo vya MRI na nyaraka za kitabibu zitachunguzwa kwa busara.

Usiri:

Taarifa zitakozopatikana kutoka kwako na rekodi za matibabu za mtoto wako zitawekwa kwa siri. Mahojiano yatafanywa kwa busara kadri mazingira yanavyoruhusu. Hakuna jina litakaloonekana kwenye hati yoyote ya utafiti na nambari za utambulisho zitatumika badala yake.

Haki za washiriki:

Uamuzi wa kushiriki katika utafiti ni wa hiari. Kukataa au kujiondoa kutoka kwa utafiti hakutaingilia upataji wa huduma hapa hospitalini na hakuna adhabu itakayotolewa.

Faida:

Kushiriki kwako katika utafiti huu kutatusaidia kubainisha hitilafu za miundo ya ubongo kwa watoto waliozaliwa na spina bifida katika mazingira yetu. Pia itatoa ufahamu kuhusu vipengele mahususi vya uzazi vinavyoathiri hitilafu hizi. Kwa pamoja, utafiti huu utakuwa msingi wa ushauri nasaha kabla ya kuzaa, matibabu kwa watoto walio na spina bifida, na kuongoza afua za jamii.

Hatari:

Kushiriki kwako katika utafiti huu hakutaathiri matokeo ya ugonjwa wako/wa mtoto wako wala kuathiri utoaji wa huduma za afya hospitalini. Pia, taarifa za kibinafsi za matibabu hazitafichuliwa kwa umma na zitawekwa siri kila wakati isipokuwa kwa wale wanaohusika katika kufanya utafiti.

Nani wa kuwasiliana naye:

Ikiwa una maswali yoyote kuhusu utafiti huu, unaweza kuwasiliana na wafuatao:

Dk. Ummehani Mustaali Dossajee: Mpelelezi Mkuu

Nambari ya Simu: +255-765-867087; Barua pepe: ummehanimustaali@gmail.com

Dk. Mboka Jacob: Msimamizi Mkuu wa Utafiti na Mkuu wa Idara ya Radiolojia, MUHAS.

Nambari ya Simu: +255-768-517877; Barua pepe: mbokajacob@gmail.com

Dk. Nahya Salim Masoud: Mwenyekiti – Kamati ya Maadili ya Utafiti ya MUHAS

Namba ya simu: +255-713-250074

Mimi ……. nimesoma na kuelewa yaliyomo katika fomu hii. Nimekubali/sijakubali kushiriki katika utafiti huu.

Saini ya Mshiriki ……. Tarehe……..……

Saini ya Mpelelezi Mkuu ……. Tarehe……

Appendix IV: Ethical clearance

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

OFFICE OF THE DIRECTOR – RESEARCH, PUBLICATIONS AND INNOVATION

Ref. No.DA.282/298/01.C/1820 Date: 24/07/2023

MUHAS-REC-07-2023-1820
UMMEHANI MUSTAALI DOSSAJEE
SCHOOL OF MEDICINE
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: NEUROIMAGING PATTERNS OF STRUCTURAL BRAIN ANOMALIES AND ASSOCIATED FACTORS IN CHILDREN WITH SPINA BIFIDA ATTENDING AT MUHIMBILI ORTHOPAEDIC INSTITUTE AND MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA

Reference is made to the above heading,

I am pleased to inform you that the Chairperson has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 24/07/2023
EXPIRATION DATE OF APPROVAL: 24/07/2024

STUDY DESCRIPTION:

Purpose:

The purpose of this cross-sectional study is to determine neuroimaging patterns of structural brain anomalies and associated factors in children with spina bifida (SB) attending at MOI and MNH from Jan 2017 – Dec 2022, Dar-es-salaam, Tanzania
The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014879.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain material transfer agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country,
8. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
9. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
10. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Dr. Nahya Salim Masoud,

Chairperson, MUHAS Research and Ethics Committee

Muhimbili University of Health and Allied Sciences
DIRECTOR
Research, Publications & Innovations
Box 65001

Appendix V: Permission letter

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

In reply please quote;

Ref. No.: MNH/CRTCU/Perm/2023/462 **Date: 14th August, 2023**

Head of Department,
- Imaging Diagnostic Section
- Medical Records

Muhimbili National Hospital

RE: PERMISSION TO COLLECT DATA AT MNH.

Name of Student	**Ummehani Mustaali Dossajee**
Title	**"Neuroimaging Patterns of Structural Brain Anomalies and Associated Factors in Children with Sina Bifida Attending at MNH and MOI".**
Institution	Muhimbili University of Health and Allied Sciences
Supervisors	Dr. Mboka Jacob (MUHAS)
Co - Supervisor	Dr. Mary Kamuzora (MNH)
Period	14th August, 2023 to 14th December, 2023

Approval has been granted to the above mentioned student to collect data at MNH. **A final study report should be lodged with Clinical Research, Training and Consultancy Unit.** Dissemination of study findings to MNH stakeholders shall be overseen by Dr.Mary Kamuzora (MNH) Co – supervisor.

Kindly ensure that the student abide to the ethical principles and other conditions of the research approval.

Sincerely,

HEAD, TEACHING, RESEARCH & CONSULTANCY UNIT * MUHIMBILI NATIONAL HOSPITAL * P.O. Box 65000 DAR ES SALAAM

Dr. Jessie Mbwambo
Head of Clinical Research, Training and Consultancy Unit

c.c DCSS, DICT
c.c Dr. Mary Kamuzora
c.c **Ummehani Mustaali Dossajee**

Upanga West, Kalenga Street, Plot No. 10480/3, P.O. BOX 65000, Dar es Salaam, Tanzania.
Telephone: +255-22-2151367-9, Telephone: +255-22-2151351-2
Email: info@mnh.or.tz, Website: www.mnh.or.tz