COMPARISON OF ULTRASONOGRAPHY AND AMNIOTIC FLUID CYTOLOGY IN SCREENING FOR SUSPECTED NEURAL TUBE DEFECTS IN INTRAUTERINE FETAL DEMISE AT KENYATTA NATIONAL HOSPITAL

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DECLARATION

I attest that this research dissertation is my original work under the guidance of the supervisors listed below and has not been presented to the University of Nairobi or any other learning institution.

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DEDICATION

I dedicate this work to all the mothers who participated in the research despite the loss and pain of having intrauterine fetal deaths/ stillbirths. You made this research come to reality at Kenyatta National Hospital.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AFP	Alpha-fetoproteins
ALT	Alanine transaminase
ANC	Antenatal care
CDC	Centres for Disease Control and Prevention
CHAMPS	Child Health and Mortality Prevention Surveillance
CNS	Central nervous system
DAB	Diaminobenzidine /(chromogen)
2DUS	2 Dimension Ultrasound scan
3DUS	3 Dimension Ultrasound scan
DPX	Distyrene (polystyrene), plasticizer (tricresyl phosphate) & xylene.
EDTA	Ethylenediaminetetraacetic acid
GFAP	Glial fibrillary acidic protein
IUFD	Intrauterine fetal demise
ICC	Immunocytochemistry
IHC	Immunohistochemistry
KNH	Kenyatta National Hospital
LMIC	Low- and middle-income countries
MDGs	Millennium Development Goals
MRI	Magnetic Resonance Imaging
NSE	Neuron- specific enolase

NTDs	Neural tube defects
PBS	Phosphate buffered saline
PMMR	Post-mortem MRI (Magnetic Resonance Imaging)
PMUS	Post-mortem ultrasound
PPEs	Personal protective equipment
UN	United Nations
UoN	University of Nairobi
SOPs	Standard operating procedures
SYN	Synaptophysin
WHO	World Health Organisation

WORKING DEFINITIONS

Intrauterine fetal demise – This is the death of a fetus that occurs in the uterus or during delivery after 20 gestation weeks of pregnancy completion. It is also known as a stillbirth.

Reduced fetal movements – This is reduced muscular movements/ activities by the fetus in the uterus.

Neural tube defects – These are a group of congenital disabilities/ congenital abnormalities that involve the brain and spinal cord.

Clinical suspicion (intrauterine) of Neural tube defects – This is a working hypothesis about a patient's diagnosis tested by performing targeted tests to reach a definitive diagnosis. In this case, the patient is the gravid mother who has clinical suspicion of a fetus with a neural tube defect.

ABSTRACT

Background: Neural tube defects are a cause of intrauterine fetal demise or stillbirth globally. Ultrasonography screening for NTDs in IUFDs poses limitations as non-diagnostic sonogram yields have been shown from studies among macerated IUFDs in screening for brain and heart defects. Amniotic fluid cytology is inexpensive, rapid, and complements ultrasonograms on screening for open NTDs and would improve the sensitivity and specificity of screening. The amniocentesis procedure is safe and feasible, carrying low risks or complications for mothers. Studies have been performed on intrauterine fetal demise in various populations, but few in Kenya. There are limited facts on the frequency of neural tube defects in Kenya and studies have recommended more studies and surveillance monitoring systems.

Objective: To detect exfoliated neural cells from suspected amniotic fluid smears, to identify positive IHC-stained amniotic fluid cellblocks as confirmatory for open NTDs, and to compare suspected fetal anatomy sonograms for NTDs with their amniotic fluid cytology results.

Methodology: This was a cross-sectional descriptive study. Ultrasonograms and amniotic fluid samples were collected from 77 pregnant women with confirmed IUFDs over four months (Dec 2021 – Mar 2022) by purposive sampling method. Clinical and socio-demographic data was collected using a structured questionnaire. Amniotic fluid samples were processed using manual liquid-based cytology, routine staining, and immunohistochemistry staining. Kappa statistical analysis and cross-tabulation were used to check the level of agreement and significance between the diagnostic methods respectively. Results were presented as tables, figures, bar graphs, and pie charts.

Results: A total of 77 gravid mothers with IUFDs were recruited and out of these, 26 had a complete dataset. Multiple imputation analysis was done to replace the missing data with substitute values to retain information about the dataset. Out of the 77 ultrasonograms and amniotic fluid samples, 20 ultrasonographic scans showed abnormal fetal anatomy of the IUFDs and 29 amniotic fluid samples were positive for neural tube defects respectively. The prevalence of neural tube defects among IUFDs was 26%. There was a significant difference between ultrasonography and amniotic fluid cytology ($p = 0.003$), and a comparability of 0.82 kappa statistic.

Conclusion: The study provides essential information comparison between ultrasonography with amniotic fluid cytology for screening pregnant women with IUFDs. The data showed a significant difference between ultrasonography and amniotic fluid cytology screening for NTDs, with cytology performing better. Amniotic fluid cytology in this study complements ultrasonography in the detection of neural tube defects in IUFDs.

1.0 INTRODUCTION

Neural tube defects affect the development of the human central nervous system and are the second most common congenital anomalies(1). These defects can be fatal or lead to paralysis which is determined by the degree and location of the lesion. The common forms of these anomalies are anencephaly, spina bifida, and encephalocele(2).

Information on mortality among babies concerning neural tube defects and the associated factors in low and middle-income countries is scarce due to the lack of surveillance systems on birth defects, registration of deaths, and linkage among them(3).

The total burden estimation of neural tube defects in low- and middle-income countries on all pregnancies as live births, stillbirths, and terminations is 2.55/1000 births (IQR = 1.56 – 3.91). For anencephaly, spina bifida and encephalocele defects account for 1.03/1000 (IQR = 0.67 – 1.60), 1.04/1000 (IQR = 0.67 – 2.48) and 0.21/1000 (IQR = 0.16 – 0.28) burden respectively. (4).

Neural tube defects are classified as open and closed defects. The intrauterine fetal demise (IUFD) with open neural tube defects exfoliates cells from the lesion and is accompanied by elevated amniotic fluid alpha-fetoprotein (AFP) and acetylcholinesterase levels (AChE). Closed neural tube defect cases have normal levels of alpha-fetoprotein but show atypical features on sonographic scans(5,6,7). Amniotic fluid alpha-fetoproteins and acetylcholinesterase raised levels have also been shown to be due to other medical conditions and blood-contaminated samples(8). The levels can be relied upon with other combinations of tests such as sonograms and cytogenetics, which are a disadvantage in cost-effectiveness(9,10).

The AFP levels change throughout the gestation period. Internal quality control purposes for interpretation of AFP levels rely on maternal weight, age, race, gestation period, and the values converted to multiple medians (MoM) for specific geographical regions posing difficulty in standardization and comparisons(11).

Developed countries have protocol standards for diagnostic evaluation of intrauterine fetal demise cases to determine causes and reduce unexplained cases. Protocol standards involve the systematic collection of information with laboratory tests from in-utero to post-partum(12). However, the laboratory tests pose financial constraints, and sometimes not all are done due to parents' requests.

The majority of studies focus on the prevalence of neural tube defects among live births. However, there is a possibility that these defects could be more numerous in stillbirths and miscarriages than in live births(13). Therefore, investigating the causes of IUFD is crucial because it helps counsel the parents, and investigations provide answers to possibilities of recurrence in subsequent pregnancies, appropriate preventive measures where applicable and closure(14).

1.1 Rationale

Neural tube defects are among the causes of intrauterine fetal demise or stillbirths. The prevalence of neural tube defects among cases of intrauterine fetal death is unknown. The studies that have been done mainly focus on live births and exclude stillbirths, miscarriages, and terminated pregnancies.

The common forms of these defects are encephalocele, spina bifida, and anencephaly, which result in various complications. Anencephaly leads to fatalities, encephalocele causes seizures, varying degrees of motor and vision impairments and delays in development, and varying degrees of paralysis due to spina bifida. People born with these defects face stigmatization which leads to emotional, social, and economic distress to their families(2).

The gold standard ultrasonography method has been shown to pose limitations in screening for congenital defects as studies have shown non-diagnostic identification of brain and heart defects by sonography on macerated IUFD cases(20,21,22).

The use of amniotic fluid cytology as an additional screening tool for open NTDs is demonstrated in studies where it complements sonographic scans in intrauterine investigations and is advantageous in rapidness and inexpensive(17,18). The amniocentesis procedure is safe and feasible, carrying low risks or complications for mothers(20). Amniotic fluid is an untapped resource that holds massive information about the foetus, unlike cultural beliefs and religious practices that cause grieving parents to bury or refuse post-mortem (invasive) examinations of their baby due to disfigurement. Screening for congenital disabilities such as neural tube defects is vital for the health of mothers and children(21).

1.2 Research questions

How do ultrasonography and amniotic fluid cytology compare in screening for suspected neural tube defects in intrauterine fetal demise at Kenyatta National Hospital?

1.3 Objectives

1.3.1 Broad objective

To compare ultrasonography and amniotic fluid cytology in screening for suspected neural tube defects in intrauterine fetal demise.

1.3.2 Specific objectives

1. To detect cytopathological exfoliated neural-appearing cells from suspected amniotic fluid-stained smears.
2. To identify the amniotic fluid cellblock sections that are positive by immunohistochemistry staining as a confirmatory for open NTDs.
3. To compare suspected fetal anatomy sonograms for NTDs with their amniotic fluid cytology results.

2.0 LITERATURE REVIEW

2.1 Background

Neural tube defects occur at the third to fourth weeks of gestation, whereby the embryo's neural tube fails to close, affecting the central nervous system. The most common forms of neural tube defects are spina bifida, encephalocele and anencephaly(2).

The statistical actual number of congenital anomalies globally may be greater than the values shown for they do not often consider stillbirths and terminated pregnancies(22). The Millennium Development Goals (MDGs) did not consider stillbirths, and the UN or the global burden of disease do not pursue them as their focus is only on live births. In 2009, international attention on stillbirths was low despite estimates showing 2.6 million having an uncertainty range of 2.1 – 3.8 million stillbirths(23).

On the global burden of neural tube defects, prevalence estimates may not include the defects in stillbirths and pregnancy terminations due to a lack of data collection, even in the presence of surveillance systems causing miscalculation of global prevalence(24). The underestimate of the total Neural tube defects burden has been due to numerous studies excluding stillbirths and elective termination of pregnancies due to fetal anomalies(4,26,27,28).

2.2 Epidemiology of IUFDs related neural tube defects

The second most common congenital anomaly that affects the development of the central nervous system in humans is neural tube defects. They are divided into two main subgroups open and closed neural tube defects(1). The most common forms of these congenital malformations are anencephaly, spina bifida and encephalocele(2,3,27).

The prevalence of neural tube defects varies globally and is high in countries that do not have compulsory food fortification programs. The Global prevalence of anencephaly and spina bifida is approximately 18.6 per 10,000 live births and approximately 14.2 per 10,000 live births in sub-Saharan Africa and 13.1 per 10,000 live births in Southeast Asia(3,26). In East Africa, the prevalence of neural tube defects is approximately 13 per 10,000 live births(2).

A surveillance network called the Child Health and Mortality Prevention Surveillance (CHAMPS) was established in seven African countries to determine the cause of death among stillbirths, infants and children under 5 years. Out of these, four countries Kenya, Ethiopia,

South Africa, and Bangladesh have recently reported data on anencephaly and spina bifida prevalence that is hospital-based surveillance. Ethiopia was found to have the highest pooled prevalence of 63 NTDs per 10,000 live births(3).

2.3 Pathology of IUFDs related neural tube defects

Neural tube defects occur due to failure or disruption at various stages of the neurulation process that leads to central nervous system development. The neurulation process occurs in the third and fourth weeks of the embryo gestation(3,5,29,30). These NTDs differ depending on the lesion's extent and localization(29).

Anencephaly neural tube defect occurs when the neural tube rostral end fails to close with partial absence of the brain and cranial vault and leads to prenatal fatality(5,30,31). Spina bifida occurs when the primary distal neuropore fails to close and reopens again(30,32). The herniation or protrusion of intracranial structures through a defect in the skull is the neural tube defect called encephalocele(29).

2.3.1 Cytopathology of IUFDs related neural tube defects

The sub-classification of neural tube defects is closed and open defects. The open neural tube defects such as anencephaly and forms of spina bifida, exfoliate neural-appearing cells into the amniotic fluid(5,17,33). The cytopathological neural-appearing cells amongst the other cells such as large foamy macrophages, urothelial cells, squamous cells, amnion cells, and mature, nucleated red blood cells are significant in amniotic fluid cytology analysis for open neural tube defect identification among the IUFD cases(5,17).

Immunohistochemistry staining technique of amniotic fluid cellblock sections with immuno-stains such as neuron-specific enolase (NSE) and synaptophysin (SYN), is useful in the cytopathologic neural-appearing cells of neural origin confirmation. A study showed the demonstration of the neural cells with the aid of an immunocytochemistry special staining technique that was performed on amniotic fluid smears(18). The cytoplasm of the neural-appearing cells is stained by the cytoplasmic immuno-stains that are identified microscopically.

2.4 Imaging and limitations in diagnosis of IUFDs related neural tube defects

The development of non-invasive post-mortem ultrasound (PMUS) and post-mortem MRI (PMMR) for fetal and neonatal examinations was led by a reduced number of parental consent for invasive autopsies(15). PMUS consumes less time at approximately 20 minutes giving

images of diagnostic quality that are accurately comparable to the PMMR which takes up to 90 minutes.

Studies have shown post-mortem ultrasound fails at diagnostic accuracy when the fetus is macerated mostly of the brain and heart organs(20,21,22). Fetal maceration is a term used when the fetus dies in the uterus/womb and changes with skin/ peeling of the skin, and the collapse of the fetal skull and the tissues take place. This process stops once the fetus is delivered. The changes are associated with fetus death of at least 6 – 8 hours(33).

A study conducted showed a diagnostic accuracy of ultrasonography and magnetic resonance imaging (MRI) in the detection of fetal anomalies that MRI was more sensitive than ultrasonography by 88.9%, 3-dimensional Ultrasound scan (3D US) by 66.7%, and 2-dimensional Ultrasound scan (2D US) by 72.2% respectively for CNS defects(34). Ultrasonography image quality was reportedly poor with decreased amniotic fluid and excessive bone shadowing in the 2016 study (34).

2.5 Comparison of imaging and cytopathology IUFDs related NTDs

A study showed cytopathological neural cells present in the aspirated amniotic fluid of suspected fetuses with open neural tube defects that were confirmed by sonographic imaging and after termination of the pregnancies. The neural-epithelial cells were demonstrated by immunocytochemistry special staining technique using NSE and SYN immunostains. The study showed proof of the developmental changes of an exencephaly to an anencephaly NTDs(32).

3.0 METHODOLOGY

3.1 Study design

Cross-sectional descriptive study.

3.2 Study site

The study site was Accidents and Casualty, the Radiology Department, the Obstetric ultrasound sections in Room 33 and Room C, and the labour ward.

3.3 Study population

The study participants were gravid mothers with confirmed intrauterine fetal demise and suspected cases of neural tube defects and had given consent. The participants were from the labour ward, obstetric ultrasound section – room 33 and C at KNH.

3.4 Inclusion/ exclusion criteria

3.4.1 Inclusion criteria

1. The gravid mothers had IUFDs with suspected NTD cases.
2. The gestation of the gravid mothers was 20 weeks and above.
3. The participants had given informed consent.

3.4.2 Exclusion criteria

1. The mothers had rhesus blood group negative due to the risk of rhesus-positive blood immune response sensitization that leads to rhesus disease.
2. Mothers had multiple gestation pregnancies because of the higher rate of stillbirths.
3. The mothers had IUFD of known causes and not NTDs, such as severe maternal infections such as malaria, syphilis, cytomegalovirus, toxoplasmosis, and listeriosis. Rhesus disease, Placental pathology, Fetal growth restriction and umbilical cord complications were also causes of IUFDs.

3.5 Sample size determination

The prevalence of neural tube defects among IUFDs /stillbirths alone at Kenyatta National Hospital and Kenya is unknown. A conservative estimate of 50%, considering a confidence interval of 95%, was used to estimate prevalence(35). The sample size was calculated as below:

$$n_{\circ} = \frac{Z^2 P (1 - P)}{e^2}$$

$$n_{\circ} = \frac{1.96^2 \times 0.5 \times 0.5}{0.05^2}$$

$$n_{\circ} = \frac{0.9604}{0.0025}$$

$n_{\circ} = 384.16$

$n_{\circ}$ = Calculated sample size

P = Expected prevalence of proportion expected with desired characteristics = 50%
Q =1-P
Z = is the value from the standard normal distribution reflecting the confidence level that will be used (e.g., Z = 1.96 for 95%)
e = Precision/ Allowable Error (0.05)

The value of ($n_{\circ}$) was then adjusted to account for the size of the target population. The new sample size (n) is calculated below:

$$n = \frac{n_{\circ}}{1 + \frac{n_{\circ} - 1}{N}}$$

$$n = \frac{384}{1 + \frac{384 - 1}{96}}$$

n = 76.96033 Rounded off to 77.

Where: N = The target population of gravid mothers with IUFDs in the labour ward is approximately 96.

$N_{\circ}$ = Calculated sample size.

N = New sample size.

3.6 Sampling method

The purposive sampling method was used to recruit study participants based on the knowledge of the population as the rare cohort of interest.

3.7 Recruitment and consenting procedure

A sonographer performed obstetric ultrasound scans on gravid mothers who had presented request forms querying the absence of fetal movements. On confirmation of an IUFD, the gravid mothers returned the results to the doctor, who explained the scan results. On notification, counselling was offered to gravid mothers by the research counsellor, and the research assistant recruited participants, obtained informed consent (Appendix I and II), and administered the study questionnaire (Appendix III). The process of recruitment and consenting was sensitive due to the grieving mothers. The recruited were those who had understood the information concerning the study and had given consent. The work plan of the study is shown below (Figure 1).

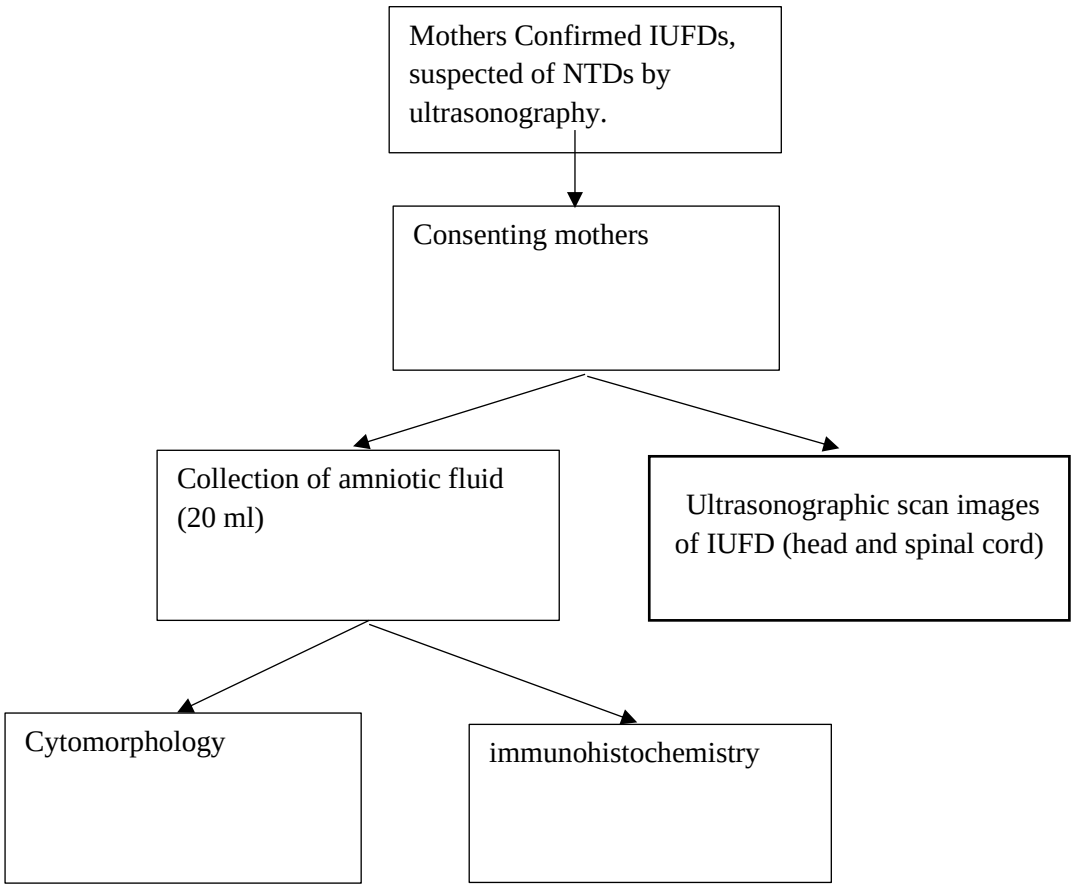

Figure 1: Work plan of the study

3.8 Sample collection

20 ml amniotic fluid samples were collected. Subdivision of the amniotic fluid samples into two for:

- Amniotic fluid cytology.
- Immunohistochemistry.

3.8.1 Sample collection procedure

The study participants/ patients were counselled by the research counsellor. In addition, the COVID-19 safety measures observed were social distancing and hand sanitization, and the study participants received a change of face mask to ensure they were comfortable before sample collection.

Collection of amniotic fluid samples by transabdominal amniocentesis procedure(36):

1. Study participants lay on their backs and their abdomens exposed on the examination couch.

2. The healthcare provider applied gel to the abdomens and using an ultrasound transducer aided by high-frequency sound waves showed the fetus's position on a monitor.
3. The intervention radiologist performed ultrasound scans to determine the fetus' exact location in the uterus. Scanning images of the head and spinal cord were held stationary by the machine, and photographs were taken by an Android phone/tablet for research analysis.
4. An antiseptic was applied on to the abdomens of the participants by the intervention radiologist.
5. Guided by an ultrasound transducer, the healthcare provider inserted a G20 or G22 (gauge size 0.9mm) needle attached to a 20 ml syringe through the abdominal wall and into the uterus.
6. The amniotic fluid was aspirated into the syringe to acquire 20 ml, and the needles were withdrawn carefully from the puncture sites (Participants may have felt a stinging sensation).
7. The samples in the syringes were dispensed into sample containers and in biohazard bags for transportation to the laboratory for analysis.
8. The participants were observed for some time, allowed to dress up, escorted back to the labour ward for hospital procedures/ management, and avoided any strenuous activity for the rest of the day.

3.8.2 Sample transportation

The specimens were transported to the UoN Immunohistochemistry and Molecular Pathology laboratory for the Human Pathology Department for subdivision. One part was for amniotic fluid cytology, and the remaining part was for cellblock preparations and immunohistochemistry staining.

3.9 Specimen analysis

3.9.1 Amniotic fluid preparation for cytomorphology

The cytocentrifuge used instruments to concentrate cells in amniotic fluid specimens onto microscope slides for staining and microscopic examination. The instruments were mega funnels, centrifuge and charged microscope slides.

Manual liquid-based cytology was performed after sample collection by pipetting 1 ml of the amniotic fluid into a mega funnel (cytology funnel). The sample was centrifuged by a cytocentrifuge at 2000 revolutions for 5 minutes, and thin deposits of cells were automatically deposited onto charged slides. The slides were immediately and gently flooded with 95% ethanol (ethyl alcohol) fixative. The routine Papanicolaou staining procedure was performed (Appendix IVa).

3.9.2 Cell block preparation – Plasma thrombin procedure

(Appendix Ivb).

3.9.3 Tissue sectioning and slide preparation procedure

(Appendix Ivc).

3.9.4 H & E (Haematoxylin and Eosin) staining procedure

(Appendix Ivd)

3.9.5 Immunohistochemistry staining procedure

(Appendix Ive)

3.10 Quality assurance for sample analysis

Pre-analysis: The amniotic fluid samples were ensured to be well labelled, corresponding to the unique study identification that had been assigned to participants and recorded on a study data booklet. The processing items were ensured as well as personal protective equipment (PPEs).

Analysis: The Amniotic fluid samples were immediately processed by manual liquid-based cytology to prevent the degeneration of cells. The smears were stained using Papanicolaou stains, cell block sections stained with H & E stain, and Immunohistochemistry staining following the standard operating procedures (SOPs).

Post analysis: All contaminated items were properly disposed and analytical results were recorded in the study data booklet.

3.11 Data management

Samples were allocated unique study ID numbers for identification and linkage with participants socio-demographic and clinical characteristics. Research study participants data and results were entered into an MS Excel spreadsheet and secured with a password.

3.12 Data analysis

The research data was from photographed ultrasound scans, laboratory test results from study samples, and the participants socio-demographic and clinical characteristics were collected using structured questionnaires.

The independent variables were socio-demographic and clinical characteristics study data that included age, gestation period, weight, height, blood glucose levels, hypertension, history of congenital defects/ previous multiple stillbirths, smoking, epilepsy/ exposure to anticonvulsant medications e.g., folate antimetabolites, valproate, and Vit B12/Folic acid uptake. Dependent variables were amniotic fluid cytology results, immunohistochemistry (IHC), and ultrasonographic scan images.
The data was cleaned and coded, then exported into SPSS Analytical software version 23 for analysis. Multiple imputation analysis was done to replace the missing data with substitute values to retain the information of the 51 datasets out of the 77 ultrasonograms and amniotic fluid samples. Interrater kappa statistics were to show the strength between the methods and descriptive statistics were presented as tables, and figures in the form of bar graphs and pie charts.

The study objectives in the detection of cytopathological exfoliated neural appearing cells from suspected amniotic fluid-stained smears and identification of the amniotic fluid cellblock sections that are positive by immunohistochemistry staining as a confirmatory for open NTDs and ultrasonography scans were analysed by conducting multiple imputations for substitute values of 51 datasets that had missing data to retain the dataset information. 26 participants had a complete dataset. A table was prepared showing the results and findings of the methods used.

On comparison of suspected fetal anatomy sonograms for NTDs with their amniotic fluid cytology results were analysed by cross-tabulation to obtain the p-value on significance and a 2 by 2 table that showed the sensitivity and specificity of amniotic fluid cytology. The kappa statistic followed showing the comparability of the diagnostic methods.

Cross-tabulations were each done with ultrasonography for the rest of the variables to get the respective p-values.

3.13 Ethical considerations

The research acquired approval from Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KHN-UoN ERC) as P111/02/2021, the Director of Reproductive Services, and the Research Programs Office at KNH.

The COVID-19 safety measures observed were social distancing and hand sanitization, and the study participants received a change of face mask to ensure they were comfortable before sample collection.

An intervention radiologist conducted the amniocentesis procedure to mitigate risks to the study participants. The risks for amniocentesis were(36,37,38):

- The transmission of infection from mother to fetus such as HIV, toxoplasmosis, and Hepatitis C was not likely for the demise of the fetus had already occurred.
- The procedure was conducted at the second trimester which carried a 0.1 – 0.3% risk of miscarriage and the death of the fetus had already occurred.
- There was no needle injury to the fetus for the demise of the fetus had already occurred.
- There were no risks of amniotic fluid leakage for the demise of the fetus had already occurred and upon confirmation, pregnancy induction for delivery occurred thereafter.
- There was no maternal rhesus-negative blood sensitization by the fetus rhesus-positive blood for the study excluded these mothers.
- There was scarce to no trigger for uterine infection by the amniocentesis procedure.

The study participants, may have felt a stinging sensation at the puncture site after the procedure.

Study participants were required to have signed informed consent forms before enrolment in the study. No consequence occurred on voluntary withdrawal at any point of the investigation by the research participant. There was termination of amniocentesis when the study participant experienced any discomfort.

Study participants were assigned study ID numbers to protect their details and identity, and these were only known by the principal investigator. The research data for the study participants security, was aided by the use of passwords to the computer folders.

3.14 Variables

3.14.1 Independent variables

The socio-demographic and clinical characteristics study data included age, Gestation period, Weight, Height, Blood glucose levels, Hypertension, History of congenital defects/ previous multiple stillbirths, smoking, Epilepsy/ exposure to anticonvulsant medications e.g., folate antimetabolites, valproate, and Vit B12/Folic acid uptake.

3.14.2 Dependent variables

Amniotic fluid cytology results, Immunohistochemistry (IHC), and ultrasonographic scan images.

4.0 RESULTS

4.1 Social-demographic and clinical characteristics of the study participants

A total of 77 gravid women with confirmed intrauterine fetal demise (IUFD) were recruited from the Labour Ward at Kenyatta National Hospital. Out of these, 26 had a complete dataset. Multiple imputation analysis was done to replace the missing data with substitute values to retain information about the datasets.

Social-demographic and clinical characteristics showed that the majority of the study participants were between (40%, 31 – 35) age group, 57% were between gestation 31 – 40 weeks and a majority reported previous cases of stillbirths (60%, 46/77). Most of the study participants (74%, 57/77) had no history of pre-gestational diabetes and congenital disabilities (69%, 53/77). Nearly half (47%) of the study population was hypertensive and 69% (53/77) were on Vitamin B12/ Folic supplements during the pregnancy term (Table 1).

Table 1: Socio-demographic and clinical characteristics (n=77)

Characteristics	**Categories of the study variables/ characteristics**	**n (%)**
Age	20 – 25 26 – 30 31 – 35 36 – 45	6 (7.8) 20 (26) 40 (51.9) 11 (14.3)
Gestation period (weeks)	20 – 30 31 – 40	33 (42.9) 44 (57.1)
Previous stillbirth	None Stillbirths	31 (40.3) 46 (59.7)
BMI	Normal Overweight Obese	15 (19.5) 40 (51.9) 22 (28.6)
Pre-gestational Diabetes	None Diabetic	57 (74) 20 (26)
Hypertension	None Hypertensive	41 (53.2) 36 (46.8)
History of congenital defects	None Defects	53 (68.8) 24 (31.2)

Vit B12/ Folic acid supplements	Yes	53 (68.8)
	No	24 (31.2)

The study participant's BMI, hypertension status, uptake of supplements, and pre-gestational diabetes were not associated with IUFD presentation ($p>0.05$). However, a history of congenital defects, either self or among relatives was significantly associated with IUFD based on sonographic scans ($p=0.007$) (Table 2).

Table 2: Clinical characteristics of study population and ultrasonography findings in IUFDs (n = 77)

Characteristics	**Categories of the study variables/ characteristics**	**n**	**Ultrasonography**		**P value**
			Positive n (%)	**Negative n (%)**	
Pregestational Diabetes	None	57	14 (24.6%)	43 (75.4%)	0.633
	Diabetic	20	6 (30%)	14 (70%)	
BMI	Normal	15	3 (20%)	12 (80%)	
	Overweight	40	13 (32.5%)	27 (67.5%)	0.395
	Obese	22	4 (18.2%)	18 (81.8%)	
Hypertension	None	41	11 (26.8%)	30 (73.2%)	0.855
	Hypertensive	36	9 (25%)	27 (75%)	
History of Congenital Defects	None	53	9 (17%)	44 (83%)	**0.007***
	Defects	24	11 (45.8%)	13 (54.2%)	
Vit B12/ Folic acid Supplements	Yes	53	13 (24.5%)	40 (75.5%)	0.667
	No	24	7 (29.2%)	17 (70.8%)	

A total of 77 study participants were recruited into the study. 12 eligible participants declined to participate in the study while 18 participants were excluded from the study. A total of 145 gravid mothers with IUFDs attended the labour ward during the study period (Figure 2).

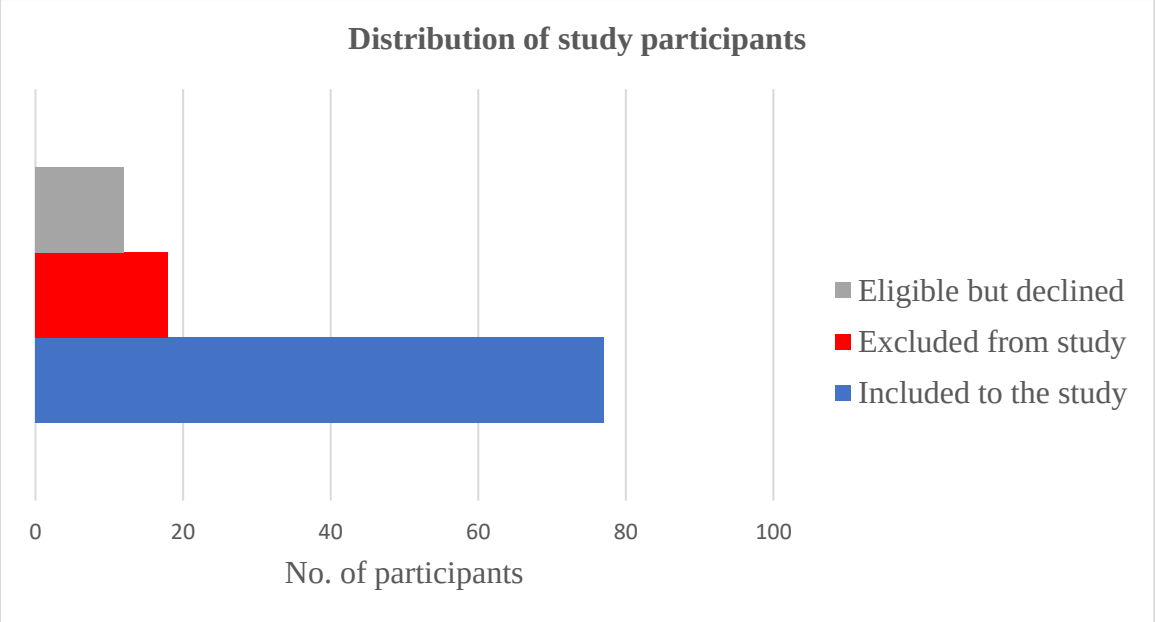

Figure 2: The distribution of study participants

The majority of the study participants 52% were overweight with 29% being obese with a median weight of 77 Kg (IQR 72 – 83) and a mean height of 162 cm (SD, 2.1) (Figure 2).

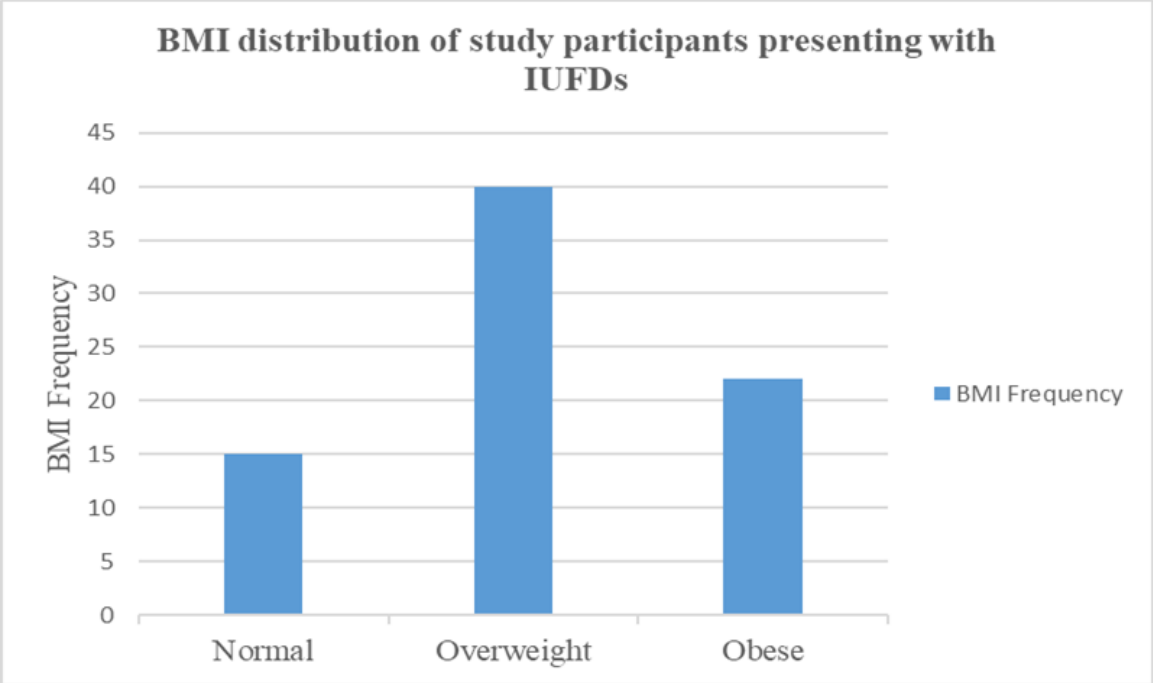

Figure 3: BMI distribution of the study participants presenting with IUFDs at Kenyatta National Hospital.

4.2 Study findings on the evaluation of screening method for NTDs among study participants presenting with IUFDs.

The Majority of the sonographic scans of the IUFDs were normal fetal anatomy of the central nervous system (74%). Sixty-two percent (48/77) of the study participants presented with normal cytomorphological smears prepared from the amniotic fluid samples (Table 3).

Table 3: Study findings of the evaluation methods for NTDs among study participants (n = 77).

Evaluation methods	**Results/Findings**	**n (%)**
Sonographic scans	Normal	57 (74)
	Abnormal	20 (26)
Amniotic fluid smears	Negative (Absence of neural-appearing cells)	48 (62.3)
	Positive (Presence of neural-appearing cells)	29 (37.7)
Amniotic fluid/ cellblock ICC/IHC	Negative (IHC with NSE Staining)	54 (70.1)
	Positive (IHC with NSE Staining)	23 (29.9)

4.3 Prevalence of neural tube defects among pregnant women with IUFDs.

Out of the 77 study participants, 26% (n = 20) showed the presence of neural tube defects (fetal anomalies) while 74% (n = 57) were normal fetal anatomy of the central nervous system (negative for NTDs) (Figure 3).

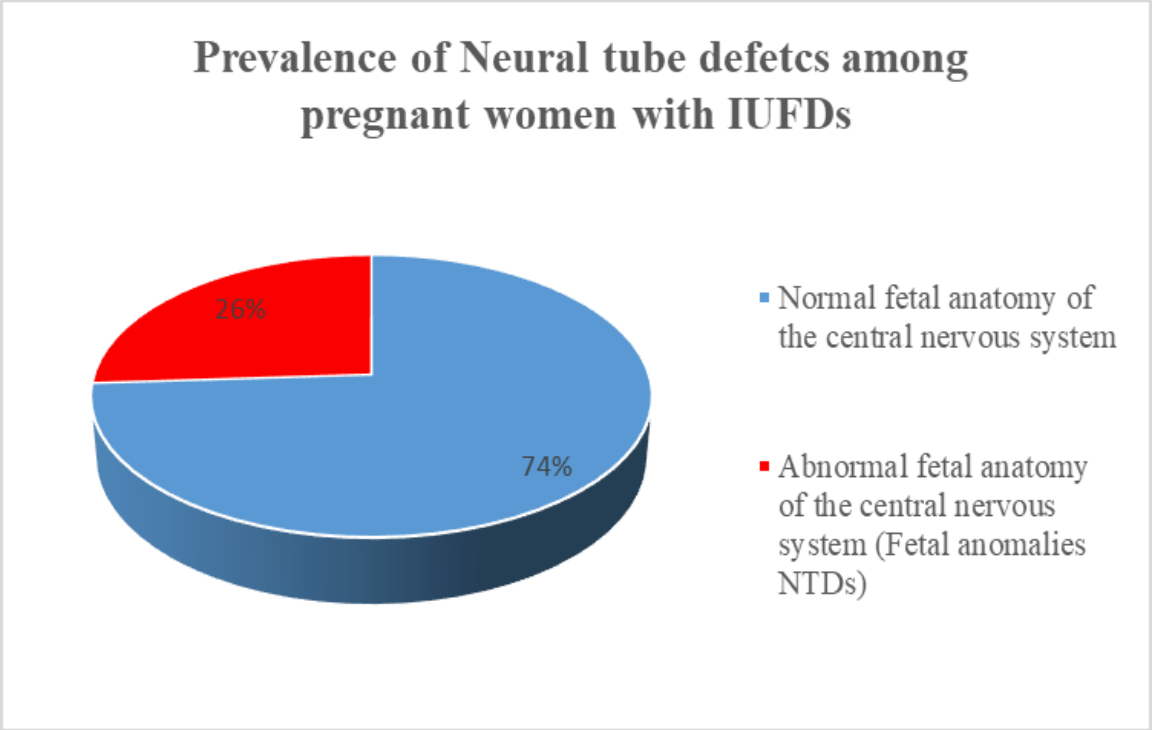

Figure 4: Prevalence of Neural tube defects among study participants with IUFDs

This study compared the gold standard ultrasonography with amniotic fluid cytology, in the screening of neural tube defects among the IUFDs/stillbirths. There was a significant difference between the two methods (p = 0.003). Amniotic fluid cytology test had a sensitivity (65%) and specificity (72%) in the detection of positive cases of NTDs among IUFDs/stillbirths; positive predictive value (PPV) (44.8%) and negative predictive value (NPV) (85.4%).

The interrater reliability kappa statistic is 0.82 on comparability between ultrasonography and amniotic fluid cytology (Table 4).

Table 4: Comparison of ultrasonography with amniotic fluid cytology (n = 77)

Test		**Ultrasonography**		
		Abnormal	Normal	Total
Amniotic Cytology	Positive	13 (44.8%)	16 (55.2%)	29
	Negative	7 (14.6%)	41 (85.4%)	48
	Total	20	57	77

4.4 Amniotic fluid cytology photomicrograph results

Photomicrographs of amniotic fluid cytology – microscopic findings

Negative cytology/cellblock cytomorphological findings are squamous cells, amnion cells, urothelial cells from the fetus, and small macrophages (< 25µm) mature maternal and nucleated fetal red blood cells.

Positive cytology/cellblock cytomorphological features are neural-appearing cells (5 – 10µm), large foamy macrophages (20 – 40µm), squamous cells, amnion cells, urothelial cells from the fetus, mature maternal and nucleated fetal red blood cells.

On cytomorphology features:

(A) (B) The neuroepithelial cells (arrowheads) with scant cytoplasm, round dense nuclei and macrophages with vacuolated, moderate cytoplasm, and eccentric nuclei.

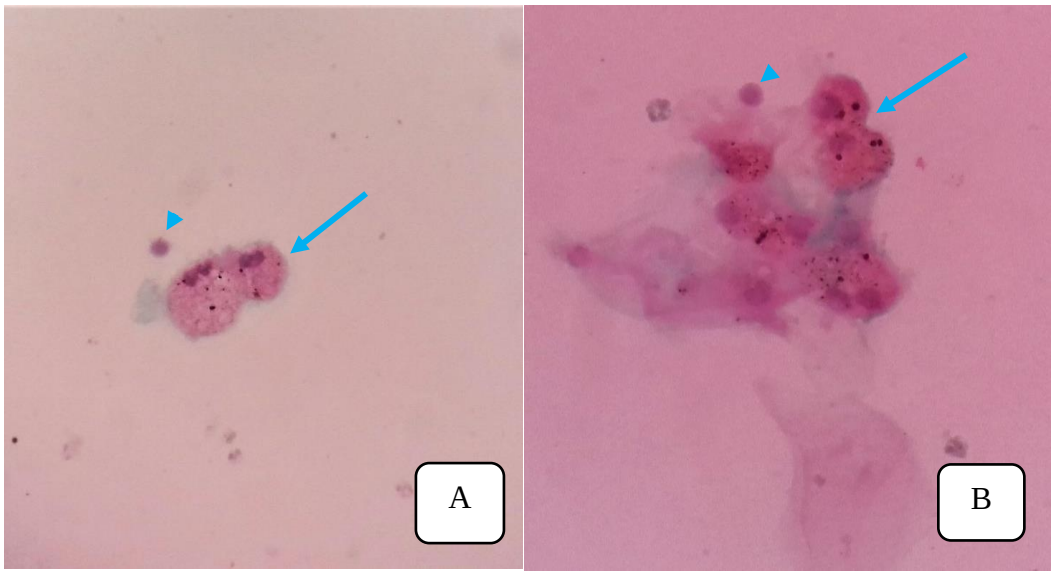

Figure 5: Amniotic fluid smears showing neural epithelial cells (arrowheads) and macrophages (arrows) (A) (B). I stained with Papanicolaou staining x40 magnification.

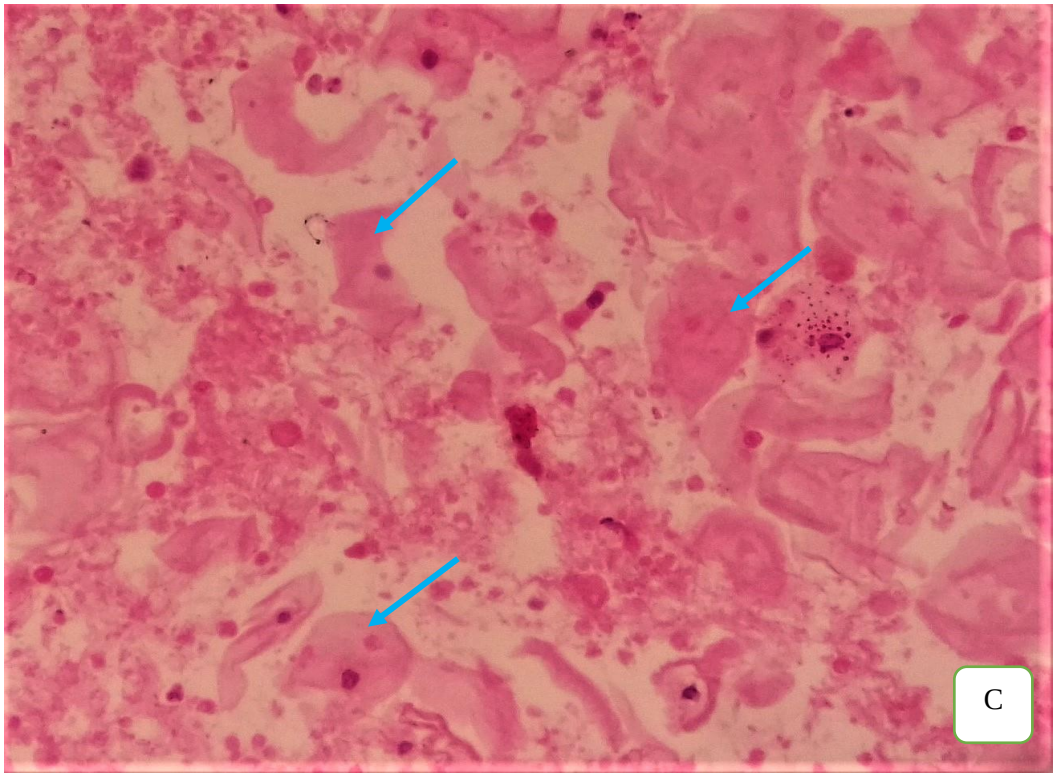

Figure 6: Amniotic fluid cellblock showing squamous epithelial cells (arrow) shedding from the fetus skin stained with H/E staining at x40 magnification (C).

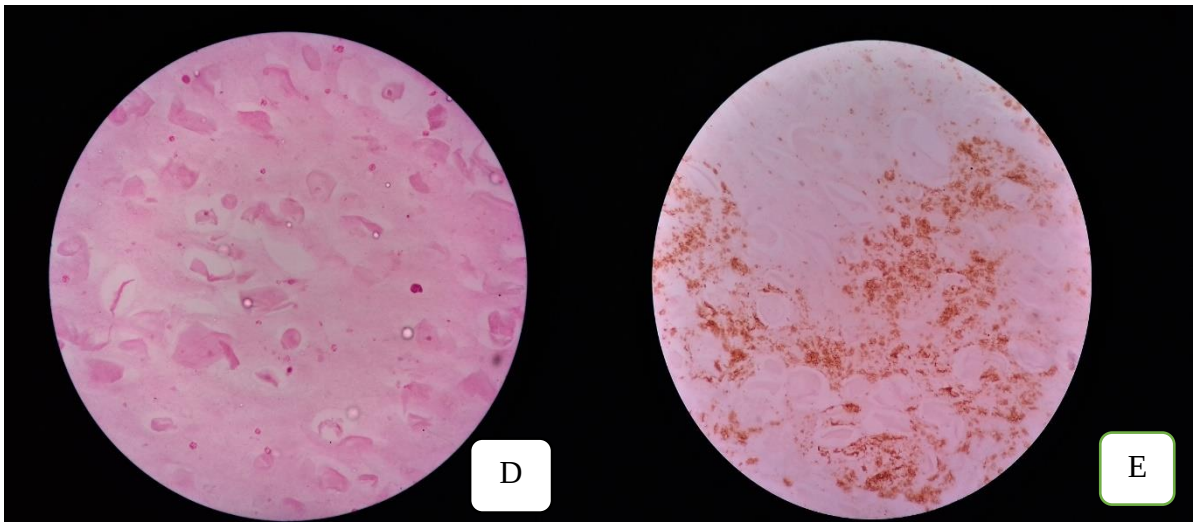

Figure 7: Amniotic fluid cellblocks at high magnification power showing region of interest H/E staining (D) and Neuron-Specific Enolase (NSE) Immunohistochemistry with positive staining at x40 magnification (E).

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study presents a survey on gravid mothers with intrauterine fetal demise (IUFDs) attending Kenyatta National Hospital. The study aimed to compare the ultrasonography and amniotic fluid cytology findings in the screening for neural tube defects in intrauterine fetal demise (IUFDs).

A total of 77 gravid women with confirmed intrauterine fetal demise (IUFD) were recruited from the Labour Ward at Kenyatta National Hospital. Out of these, 26 had a complete dataset. Multiple imputation analysis was done to replace the missing data with substitute value to retain information about the dataset. The study found 20 cases of neural tube defects. The prevalence of neural tube defects among pregnant women with intrauterine fetal demise in this study was 26%. Studies have not shown the prevalence of NTDs among intrauterine fetal demise cases.

In this study, the amniotic fluid cytology showed the exfoliated cytopathological neural cells, large foamy macrophages and positive cellblock confirmation of open neural tube defects. This was comparable to other studies that showed neural cells (5 – 10μm in diameter) and large foamy macrophages (20 – 40μm in diameter) in cases with open neural tube defects(17,33). A study demonstrated using differential staining techniques the cytopathological neural cells and large macrophages from amniotic fluid samples in the diagnosis of neural tube defects(5). This study identified 38% of positive amniotic fluid cytology screening for NTDs among pregnant women with IUFDs.

The Immunohistochemistry (IHC) special staining technique for neuron-specific enolase (NSE), was used in the study as a confirmatory test for amniotic fluid cytology cellblock sections for open NTDs. A positive cellblock was stained with the immunostaining while negative cellblocks were not stained. This was comparable to similar studies that used the immuno-stains as neuron-specific enolase (NSE), synaptophysin (SYN), and glial fibrillary acidic protein (GFAP) as confirmatory tests in immunocytochemistry (ICC) staining(17,33).

The immunocytochemistry staining technique employs staining on prepared sample smears on microscope slides. For open neural tube defects, the smears stained positive with NSE, and SYN for the presence of the cytopathological neural cells and stained negative with GFAP stain (17,33). A study in the diagnosis of neural tube defects from amniotic fluid samples used

differential staining techniques such as Papanicolaou, hematoxylin-eosin, and Diff-Quik (modified Romanowsky stain) for demonstration of the cytopathological neural cells and large macrophages(5). This study identified 30% of positive amniotic fluid cytology cellblocks in the screening for NTDs among pregnant women with IUFDs Use of IHC increases the confidence of cytology as a screening test for open NTDs by identifying and confirming cells of neural origin in amniotic fluid.

On ultrasonography and amniotic fluid cytology comparison on the screening for NTDs among pregnant women with IUFDs, this study found a significant difference of p = 0.003. Ultrasonography identified 26% of abnormal scans for fetal anatomy of the central nervous system and amniotic fluid cytology 29% of positive NTD cases. The kappa statistic was 0.82 on comparability between ultrasonography and amniotic fluid cytology giving a near-perfect agreement between the two methods. There is limited data on the comparability of ultrasonography and cytology, though this study showed that amniotic fluid cytology had a positive predictive value of 44.8% and a negative predictive value of 85.4%.

In this study, the majority of the stillbirths were reported as macerated. A study was done to compare postmortem ultrasound and MRI of the whole body of the fetus on demonstration of maceration determining diagnostic yield. The study showed that out of 265 examinations by ultrasonography for the brain achieved 79.2% (210/265), and out of this 66 IUFDs/stillbirths achieved 51.5% (34/66). The study concluded that non-diagnostic results using postmortem ultrasound were due to fetal maceration for brain and heart anomalies(15). Other studies reported consistently the same results for non-diagnostic reports for the brain and cardiac anomalies as 18.6% (13/70) for the brain(39) and 18.7% (20/107) cases for the brain(17).

The history of congenital anomalies among the study participants showed p = 0.007, which may have been an associated risk among intrauterine fetal demise cases with NTDs.

5.2 Conclusion

The study showed the significance of amniotic fluid-stained smears in the detection of exfoliated cytopathological neural cells, which were confirmed by immunohistochemistry staining as open NTDs. The data showed that there is a significant difference between ultrasonography and amniotic fluid cytology in screening for neural tube defects and where cytology performed better. Amniotic fluid cytology in this study complements ultrasonography in the screening for open NTDs in pregnancy.

5.3 Recommendation

Amniotic fluid cytology is a useful screening test for NTDs and should be recommended in the clinical workup for pregnant women with IUFDs, and other superior screening tools such as MRI, could be used for the confirmation of closed NTDs.

5.4 Study limitations

Time factor – There was no control over the time of day patients were admitted with IUFD cases for they came at any time of the day and night. The patients were either induced for delivery or had delivered the stillbirths on checking them in the morning.

Complications – There were patients with complications such as antepartum haemorrhage - went to the theatre as an emergency, preterm premature rupture of membranes, ruptured uterus, eclampsia, born before arrival, maternal Covid -19, blood transfusion at the time of the study. This affected amniotic fluid sample collection together with the IUFD scans of the patients who had consented to the study.

Departmental (Radiology) availability– The intervention radiologists were available during the morning hours from 8 a.m. to noon. Afternoons and evenings were a challenging time to recruit participants and have them undergo a sonogram. Some participants gave up and returned to the labour ward due to queues.

COVID-19 Pandemic - Accrual of cases during the pandemic was an unforeseen challenge and KNH admissions dropped drastically due to among other factors lockdown and limitations in intercounty travel restrictions.

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APPENDICES

APPENDIX I: PATIENT CONSENT FORM

COMPARISON OF ULTRASONOGRAPHY AND AMNIOTIC FLUID CYTOLOGY ON SCREENING FOR SUSPECTED CASES OF NEURAL TUBE DEFECTS, RISK FACTORS AND PREVALENCE AMONG GRAVID MOTHERS WITH IUFDs AT KENYATTA NATIONAL HOSPITAL

Investigators

Investigator	Position	Institution
Ms Peris S. Anunda	Principal Investigator	UoN
Prof. L. Muchiri	Supervisor	UoN

Principal Investigator	KNH – UoN ERC Secretary
Contact: 0720-784312	Contact: 020-2726300 ext 44355/44102.

Investigator Statement

It is a kind request to be a participant in the research study. It is voluntary participation, and your refusal to participate in the study will not compromise the quality of care given to you by the hospital. You have a right not to participate or withdraw at any time from the study. The consent form intends to provide the information you require to decide whether to or not participate in the study. Carefully read this form. You may ask questions if there are any benefits, risks, your rights as a voluntary participant, or anything else about the research or that is stated on this form and not clear. On satisfactorily answered questions, you can decide whether you want to participate in the study or not. This process is called informed consent.

Background information

The most common congenital anomalies are Neural tube defects (NTDs) that affect the brain and spinal cord. The most common neural tube defects are spina bifida, anencephaly, and encephalocele in the third and fourth weeks of the gestation period. Whereby the embryo's neural tube fails to close. Complications associated with neural tube defects are hydrocephalus, developmental delays, seizures, varying degrees of paralysis, vision impairments, and anencephaly defect, which is fatal for the fetus born with a significant part of the brain, skull,

and scalp absent. In addition, people born with neural tube defects suffer stigmatization, lack acceptance from the community, and affect their families emotionally, economically, and socially.

Purpose of the research study

The study will assist us in finding out how frequent are intrauterine fetal demise cases caused by neural tube defects among gravid mothers attending Kenyatta National Hospital. If this is common, we will recommend amniotic fluid cytology screening to be part of antenatal care at the hospital.

Study procedure

If you agree to participate in this study, medical history information and a physical examination will be collected. The data will involve your age, history of current pregnancy, miscarriages, and stillbirths. An intervention radiologist will perform and print an ultrasound scan of the IUFD head and spine and perform an amniocentesis procedure to collect 20 ml of amniotic fluid for research. The information on the test results will be shared with you by the primary care physician.

Confidentiality

All obtained information from the research study will be strictly confidential and will only be shared with you by the primary care physician(s). Similarly, samples collected for the research will strictly be used for study purposes and destroyed after that.

Your refusal to participate in the study will not compromise the quality of care given to you by the hospital, and the research is on voluntary participation. You have a right not to participate or withdraw at any time from the study. Therefore, 77 mothers will take part in this study. You require a single visit for sample collection, and a second visit will be for your results. The results will be ready after one week.

Benefits and Risks to the participant

Benefits

- On postnatal clinic visits, the participant will be informed of the study results.
- The participant will not pay any medical laboratory charges for the study.
- Counselling is provided by a research counsellor.

- If the results are positive for the neural tube defect associated IUFD case, the results will be recorded in your file to share the information with you. The hospital will provide medical advice to prevent reoccurrence in the future.

Risks

During the amniocentesis procedure, the study participant may feel a stinging sensation for amniotic fluid sample collection at the puncture site.

The risks for amniocentesis are:

- Transmission of infection from mother to fetus such as HIV, toxoplasmosis, and Hepatitis C. In this study, the death of the fetus has already occurred.
- Miscarriage. Amniocentesis at the second trimester carries a 0.1 – 0.3% risk of miscarriage. However, in this study, the death of the fetus has already occurred.
- Needle injury to the fetus. The fetus may move in the path of the needle. In this study, the death of the fetus has already occurred.
- Amniotic fluid leakage. A small amount of amniotic fluid can leak through the vagina after the amniocentesis procedure and stops within a week. However, in the study, the death of the fetus occurred and once confirmed, the pregnancy was induced for delivery.
- Maternal rhesus negative blood sensitization by the fetus rhesus positive blood. In this study, there is the exclusion of rhesus-negative gravid mothers.
- Amniocentesis may trigger uterine infection though it is scarce.

There is no risk expected to occur to the fetus, for death has already happened.

The research study is approved by the Kenyatta National Hospital - University of Nairobi Ethics and Research Committee (KNH – UoN ERC).

If you have any queries /questions concerning study participant rights, you can call the principal investigator - Peris Anunda -Phone number 0720 784 312 or Prof. M. L Chindia, Secretary of KNH – UoN ERC – Phone number 020 – 2726300, Extension 44355 or 44102 – email: uonknh_erc@uonbi.ac.ke

Signature

Investigator…….

Date ……

Participant's statement

I ……. have been explained to and understood the purpose of the research study, procedures, benefits, and risks and given my consent to participate in the study.

…….

Participant's Signature/ Thumbprint.

APPENDIX II: FOMU YA RIDHAA YA KUSHIRIKI KATIKA UTAFITI

ULINGANISHO WA ULTRASONOGRAFIA NA AMNII MAJI CYTOLOGIA KWA UCHUNGUZI WA KESI ZA VISA VYA KASORO ZA NEURAL TUBE, VIPENGELE VYA HATARI NA MAAMBUKIZI MIONGONI MWA KINA MAMA WA UJAUZITO NA VIJUSI KATIKA HOSPITALI KUU YA KITAIFA YA KENYATTA

Watafiti

Mtafiti	**Cheo**	**Taasisi**
Peris S. Anunda	Mtafiti mkuu	UoN
Prof. L. Muchiri	Msimamizi	UoN

Mtafiti mkuu	**Katibu wa KNH – UoN ERC**
Namba ya rununu simu: 0720-784312	020-2726300 ext 44355/44102.

Taarifa ya mtafiti

Hii ni kukuomba kwa ukarimu kuwa mshiriki katika utafiti. Huu ni ushiriki wa hiari na kukataa kwako kushiriki katika utafiti hautaathiri ubora wa huduma uliyopewa na hospitali. Una haki ya kutoshiriki au kujiondoa wakati wowote kutoka kwa utafiti. Fomu ya idhini inakusudia kutoa maelezo unayohitaji kusaidia katika kuamua ikiwa au kutoshiriki katika utafiti. Soma kwa makini fomu hii. Unaweza kuuliza maswali ikiwa kuna faida zozote, hatari, haki zako kama mshiriki wa hiari au kitu kingine chochote kuhusu utafiti au ambazo zimeelezwa kwenye fomu hii na sio wazi. Kwenye maswali yako ya kuridhisha, unaweza kuamua kama unataka kushiriki katika utafiti au la. Utaratibu huu unaitwa idhini ya taarifa.

Taarifa ya mandharinyuma

Kasoro ya viungo neural tube ni ugonjwa ambayo inaathiri akili na mgongo. Spina bifida, anencephaly na encephalocele ni aina ya neural tube kasoro kwamba kutokea wakati wa wiki ya tatu na ya nne ya kipindi cha ujauzito ambapo embrio ya neural tube inashindwa kufunga. Matatizo yanayohusiana na hydrocephalus ya neural dosari ni kama vile, ucheleweshaji wa ukuaji, kifafa, viwango tofauti vya kupooza, maono kuona na kasoro ya anencephaly ni mbaya ambapo kwa kijusi inaweza kuzaliwa na sehemu kubwa ya ubongo, fuvu la kichwani kukosekana. Watu wanaozaliwa na dosari ya neural wanakabiliwa na unyanyapazi, kukosa kukubalika kutoka kwa jamii na huathiri familia zao za kihisia, kiuchumi na kijamii.

Madhumuni ya utafiti

Utafiti huu utatusaidia kujua ni mara ngapi kesi za vijusi zinazosababishwa na kasoro za mirija ya neural miongoni mwa akina mama wanaohudhuria Hospitali ya Kitaifa ya Kenyatta. Ikiwa hii ni kawaida, tutapendekeza uchunguzi wa cytologia ya amnii kuwa sehemu ya utunzaji ufaao katika ujauzito hospitalini.

Utaratibu wa utafiti

Ikiwa unakubali kushiriki katika utafiti huu, habari ya historia ya matibabu itachukuliwa na uchunguzi wa kimwili kufanyika. Hii itahusisha umri wako, historia ya mimba ya sasa, mimba kuharibika kabla kuzaliwa. Mtaalamu radiologist atafanya tambazo la ultrasound scan na kuchapisha picha za kichwa na uti wa mgongo ya fetasi katika ujauzito wako na utaratibu amniocentesis itakuwa kukusanya mililita ishirini (20) ya amnii kwa ajili ya utafiti. Utapewa taarifa ya matokeo ya vipimo vya utafiti na daktari wa msingi wa huduma.

Usiri

Taarifa zote zitakapopatikana kutoka kwa utafiti zitakuwa siri sana na zitashirikiwa tu na daktari wa huduma ya msingi. Vivyo hivyo, sampuli zitakapokusanywa kutoka kwa utafiti zitatumika kwa madhumuni ya utafiti huu na kuharibiwa baadaye.

Kukataa kwako kushiriki katika utafiti hakutaathiri ubora wa utunzaji uliotolewa kwako na hospitali, utafiti upo kwenye ushiriki wa hiari. Una haki ya kushiriki au kukataa wakati wowote kutoka kwa utafiti. Akina mama sabini na saba (77) watashiriki katika utafiti huu. Ziara moja itahitajika kwa ajili ya ukusanyaji wa sampuli na ziara ya pili itakuwa ya matokeo yako. Matokeo yatakuwa tayari baada ya wiki moja.

Faida na hatari kwa mshiriki

Faida

- Katika ziara ya kliniki baada ya kuzaa, mshiriki atajulishwa kuhusu matokeo ya utafiti.
- Mshiriki hatalipa gharama zozote za maabara ya matibabu kwa ajili ya utafiti.
- Ushauri utatolewa na mshauri wa utafiti.
- Ikiwa matokeo yanaonyesha kasoro ya neural inayohusishwa na kesi ya kijusi, matokeo yatarekodiwa katika faili yako ili upewe habari. Ushauri itafanyika na

ushauri wa kimatibabu uliotolewa kwenu ili kusaidia kuzuia kesi ya tukio hilo baadaye.

Hatari

Kuhusu ushiriki kwa utafiti, wakati wa utaratibu wa amniocentesis, mshiriki wa utafiti anaweza kuhisi hisia za maumivu madogo sehemu pale sindano ilidungwa kwa ukusanyaji wa sampuli ya amnii.

Hatari za utaratibu wa amniocentesis ni:

- Maambukizi kutoka kwa mama hadi fetasi kama vile ukimwi, toxoplasmosis, Hepatitis C. Katika utafiti huu, kifo cha fetasi tayari kimetokea.
- Kuharibika kwa mimba. Amniocentesis katika trimester ya pili hubeba hatari ya 0.1 – 0.3% kuharibika kwa mimba. Katika utafiti huu, kifo cha fetasi tayari kimetokea.
- Fetasi kuumia kwa sindano. Fetasi inaweza kusonga katika njia ya sindano. Katika utafiti huu, kifo cha fetasi tayari kimetokea.
- Kuvuja kwa maji ya amnii. Kiasi kidogo cha amnii kinaweza kuvuja kupitia njia ya uke baada ya utaratibu wa amniocentesis na kuacha baada ya wiki moja. Katika kesi ya utafiti huu, kifo cha fetasi kimetokea na mara moja kinapothibitishwa, ujauzito hutayarishwa kujifungua.
- Damu ya mama rhesus hasi huhamasishawa na damu ya fetasi rhesus chanya. Katika utafiti huu, kina mama wa ujauzito ambao ni rhesus hasi wanatengwa na utafiti.
- Amniocentesis inaweza kusababisha maambukizi ya uterasi ingawa ni nadra sana.

Hakuna hatari inayotarajiwa kutokea kwa fetasi kwa sababu kifo tayari kimetokea.

Utafiti huu umeidhinishwa na Chuo Kikuu cha Nairobi na Bodi ya Mapitio ya Maadili ya Hospitali ya Kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi.

Ikiwa una swali lolote juu ya haki zako kama mshiriki wa utafiti, unaweza kupiga simu kwa mtafiti mkuu -Peris Anunda- namba ya simu (0720 784 312) ama Katibu wa KHN- UoN ERC -Namba ya simu 020-2726300 ext 44355/44102 – Barua pepe: uonknh_erc@uonbi.ac.ke

Sahihi

Mtafiti.....

Tarehe.....

Kauli ya mshiriki

Mimi..... nimeelezwa na kuelewa madhumuni ya utafiti, taratibu, faida na hatari na kutoa idhini yangu ya kushiriki katika utafiti.

.....

Saini ya mshiriki/chapa ya gumba.

APPENDIX III: QUESTIONNAIRE/ DODOSO

COMPARISON OF ULTRASONOGRAPHY AND AMNIOTIC FLUID CYTOLOGY ON SCREENING FOR SUSPECTED CASES OF NEURAL TUBE DEFECTS, RISK FACTORS AND PREVALENCE AMONG GRAVID MOTHERS WITH IUFDs AT KENYATTA NATIONAL HOSPITAL *ULINGANISHO WA ULTRASONOGRAFIA NA AMNII MAJI CYTOLOGIA KWA UCHUNGUZI WA KESI ZA VISA VYA KASORO ZA NEURAL TUBE, VIPENGELE VYA HATARI NA MAAMBUKIZI MIONGONI MWA KINA MAMA WA UJAUZITO NA VIJUSI KATIKA HOSPITALI KUU YA KITAIFA YA KENYATTA*

Participant Study Number / ***Namba ya mshiriki katika utafiti*** **_____**

Date of interview **/** *tarehe ya mahojiano* (Day/ Month/ Year) _ _/_ _/2020

Questions / *Maswali*

1. Age / *Umri wa mama* _____ (Years/ *Miaka*)
2. Estimated gestation weeks / *Ujauzito huu una wiki ngapi?* _____
3. Last menstrual period / kipindi cha mwisho cha hedhi?

4. The number of times pregnant / *Umeshika mimba mara ngapi?* _____
5. The number of live births / *Je, ni Watoto wangapi umejifungua wakiwa hai?* _____
6. Did you have any Number of stillbirths / *Je, ni Watoto wangapi umejifungua wakiwa wamefariki?* _____
7. Did you have multiple gestation pregnancies/ Je, umekuwa na mimba nyingi za ujauzito zikifwatana? _____
8. Any history of congenital disabilities in a previous pregnancy / Historia yoyote ya kasoro za ujauzito? _____
9. Do you have any close relatives with congenital disabilities in a previous pregnancy/ Je, una jamaa wa karibu ambao walikuwa na kasoro katika ujauzito uliopita?

   _____
10. Any history of multiple intrauterine fetal demises (IUFDs) / historia yoyote ya vijusi katika ujauzito? _____
11. Weight / *Uzito wako?* _____ (Kg/ *Kilo*)
12. Height / *Urefu wako?* _____ (Metres/ *Mita*)
13. Blood group / *Kundi la damu yako?* _____
14. Are you diabetic / *Je, ukona ugonjwa wa Kisukari?* Yes / No

If yes, what were the blood glucose levels/*kama ndio, kipimo cha Kisukari kilikuwa ngapi*? _____

15. Did you take folic/ Vitamin B12 supplements during the antenatal period / *Je, uliweza kuchukua folic/vitamini B12 virutubisho wakati wa kipindi cha ujauzito?* _____
16. Are you hypertensive (high blood pressure)/ ugonjwa wa shinikizo la damu?
17. Are you epileptic/Je uko na ugonjwa wa kifafa? Yes/ No _____
18. Any exposure to anticonvulsant medications, e.g. Valproate or other folate antimetabolites/ Je ushawahi kutumia dawa za kifafa? Yes/No _____
19. Do you smoke cigarettes/ Je unavuta sigara? Yes/No_____

APPENDIX IV: LABORATORY PROCEDURES

APPENDIX IVa: Papanicolaou staining procedure

1. Fix in 95% ethyl alcohol (ethanol) for 15 minutes.
2. Hydrate in decreasing concentrations of Ethanol as 80%, 70%, 50%, and ten dips each.
3. Rinse in tap water.
4. Stain in Harris Haematoxylin for 4 minutes.
5. Rinse in tap water.
6. Differentiate in two changes of 0.05% Acid alcohol, ten dips each.
7. Rinse in tap water.
8. Blue in Scotts tap water, ten dips each.
9. Dehydrate in 95% Ethanol, ten dips each.
10. Stain in OG-6 stain for 1.5 minutes.
11. Dehydrate in 95% Ethanol, ten dips each.
12. Stain in Eosin Azure (EA- 36) stain for 3 minutes.
13. Dehydrate in two changes of 95% Ethanol, ten dips each.
14. Dehydrate in two changes of Absolute Ethanol, ten dips each.
15. Clear in three changes of Xylene, ten dips each.
16. Mount in D.P.X.
17. Dry at room temperature and examine smears at 10X and 40X objectives microscopy.

APPENDIX IVb: Cell block preparation – Plasma thrombin procedure

Principle: to enmesh the cellular material into a clot.

1. Centrifuge samples at 3000 revolutions per minute (r.p.m) at 5 minutes.
2. Discard supernatant to have the sample sediment.
3. Add four drops of plasma followed by four drops of Thrombin to 1ml sample sediment.
4. Gently mix the contents.
5. Let the sediment stand for a few minutes.
6. Wrap clot masses in Whatman filter papers and place them into labelled tissue cassettes.
7. Fix in 10% neutral buffered formalin, overnight.
8. Dehydrate in ascending grades of Ethanol at 50%, 70%, and 80%, hourly intervals.
9. Clear in Chloroform, two changes.
10. Infiltration of molten paraffin wax, overnight.

11. Unwrap clot masses and place them in metal troughs (tissue tek).
12. Add molten paraffin wax.
13. Cool metal troughs on an ice bench.

APPENDIX IVc: Tissue sectioning and slide preparation procedure

1. Place cell blocks onto a microtome.
2. Section cell blocks to have tissue ribbons.
3. Place tissue ribbons onto the microscope slide using a camel hairbrush.
4. Remove creases by adding three drops of 70% alcohol.
5. Carefully dip slides into a water bath at 56ºC to suspend tissues, and remove creases and wax.
6. Carefully pick the tissues using charged slides.
7. Dry on a rack at room temperature.

APPENDIX IVd: H & E (Haematoxylin and Eosin) staining procedure

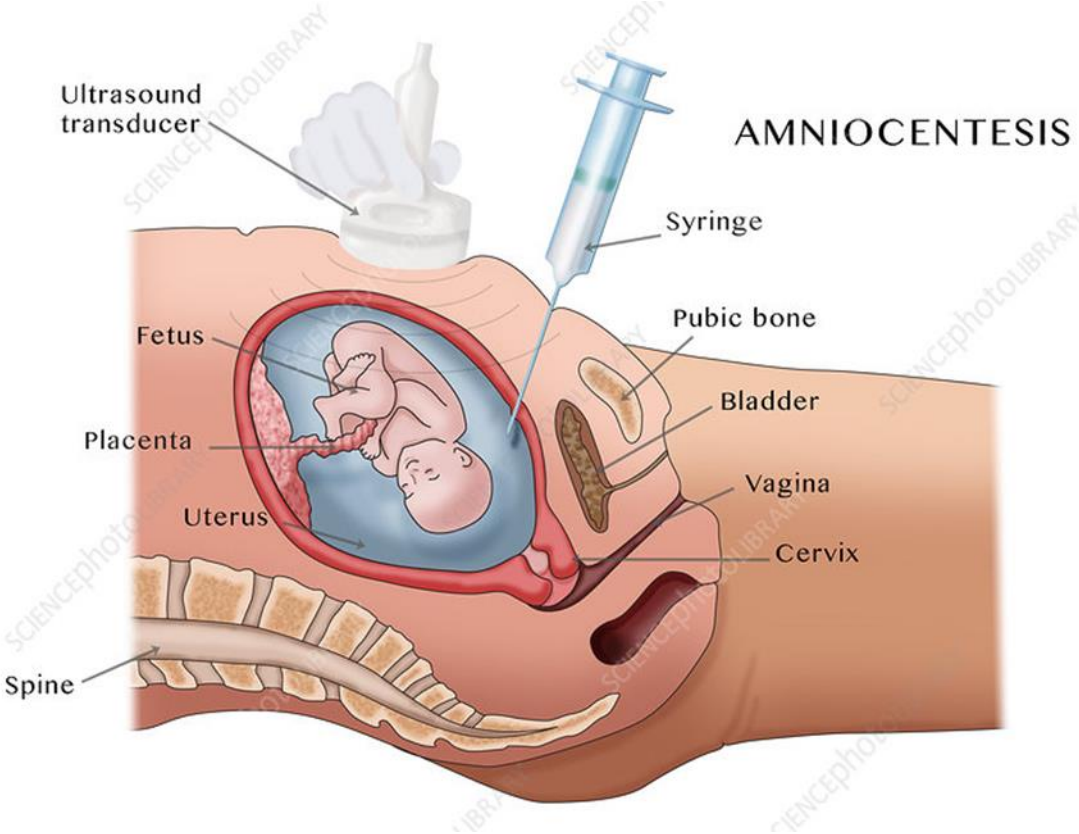
1. Fix in 95% ethyl alcohol (ethanol) for 15 minutes.
2. Hydrate in decreasing concentrations of Ethanol as 80%, 70%, 50%, and ten dips each.
3. Rinse in tap water.
4. Stain in Harris Haematoxylin for 4 minutes.
5. Rinse in tap water.
6. Differentiate in two changes of 0.05% Acid alcohol, ten dips each.
7. Rinse in tap water.
8. Blue in Scotts tap water, ten dips each.
9. Stain in 1% Eosin for 1 minute.
10. Dehydrate in two changes of 95% Ethanol, ten dips each.
11. Dehydrate in two changes of Absolute Ethanol, ten dips each.
12. Clear in three changes of Xylene, ten dips each.
13. Mount in D.P.X.
14. Dry at room temperature and examine smears at 10X and 40X objectives microscopy.

APPENDIX IVe: Immunohistochemistry staining procedure

1. Place slides in an oven at 37ºC overnight or on a hotplate at 100ºC for one hour.
2. Cool slides on a bench at room temperature.
3. Dewax in three changes of Xylene, 2 minutes each.

4. Hydrate in descending grades of alcohol as 80%, 70%, and 50%, for 2 minutes each.
5. Air dry the slides for a few seconds and outline tissue areas with a Nova pen.
6. Place slides in a coupling jar filled with antigen retrieval buffer (citric buffer pH 6.0 or EDTA buffer pH 8.0) as per the test kit.
7. Place the coupling jar in a vegetable steamer for 20 minutes, checking intervals of 5 minutes to refill the antigen retrieval buffer.
8. Cool slides out of coupling jar at room temperature for 10 minutes while rinsing with distilled water.
9. Place slides in a humid chamber.
10. Apply peroxidase block and leave for 5 minutes.
11. Rinse slides thoroughly with Tris/ PBS buffer.
12. Apply protein block and leave for 10 minutes.
13. Rinse slides thoroughly with Tris/ PBS buffer.
14. Apply primary antibody-neuron-specific enolase (NSE) and leave for 30 minutes.
15. Rinse slides thoroughly with Tris/ PBS buffer.
16. Apply post-primary conjugate/ secondary antibody and leave for 30 minutes.
17. Rinse slides thoroughly with Tris/ PBS buffer.
18. Apply Polymer and leave for 30 minutes.
19. Rinse slides thoroughly with Tris/ PBS buffer.
20. Apply chromogen DAB, and leave for 10 minutes.
21. Rinse slides thoroughly with Tris/ PBS buffer.
22. Counterstain with Haematoxylin stain for 2 minutes.
23. Rinse slides with distilled water.
24. Differentiate in 1% acid alcohol, for a few seconds.
25. Rinse slides in distilled water.
26. Blue in Scott's tap water, for a few seconds and rinse with distilled water.
27. Dehydrate in ascending grades of alcohol as 50%, 70%, 80%, for 2 minutes each.
28. Clear in three changes of Xylene, 2 minutes each.
29. Mount in D.P.X and leave to dry at room temperature.

APPENDIX V: DIAGRAM OF AMNIOCENTESIS PROCEDURE



An illustration of the amniocentesis procedure(40).

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Ref: KNH-ERC/A/344

30th September, 2021

Peris Stella Anunda
Reg. No. H56/12125/2018
Dept.of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 30 SEP 2021 QUALITY HEALTH CARE KNH/UoN-ERC P.O. Box 20723 - 00202, NAIROBI

Dear Peris

RESEARCH PROPOSAL: COMPARISON OF ULTRASONOGRAPHY AND AMNIOTIC FLUID CYTOLOGY IN SCREENING FOR SUSPECTED CASES OF NEURAL TUBE DEFECTS, RISK FACTORS AND PREVALENCE AMONG GRAVID MOTHERS WITH IUFDs AT KENYATTA NATIONAL HOSPITAL (P111/02/2021)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 30th September 2021 – 29th September 2022.

This approval is subject to compliance with the following requirements:

i. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
ii. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
iii. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from KNH- UoNERC for each batch of shipment.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).
vii. Submission of an executive summary report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L CHINDIA
SECRETARY, KNH- UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chair, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Human Pathology, UoN
Supervisors: Prof. Muchiri L.W. Dept. of Human Pathology, UoN
Ms. Josephine N. Rioki, Dept. of Human Pathology, UoN

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UNIVERSITY OF NAIROBI

SIGN: _____ DATE: 13TH DECEMBER 2023

DR. PETER.M. MATURI

CHAIRMAN DEPT. OF HUMAN PATHOLOGY

UNIVERSITY OF NAIROBI

SIGN: _____ DATE: 13/12/2023

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