UNIVERSITY OF WITWATERSRAND
FACULTY OF HEALTH SCIENCES
SCHOOL OF MEDICINE

EFFICACY OF CEREBROSPINAL FLUID ANALYSIS IN THE DIAGNOSIS OF PRIMARY PAEDIATRIC BRAIN TUMOURS AT TWO TERTIARY NEUROSURGICAL UNITS IN JOHANNESBURG

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A RESEARCH REPORT SUBMITTED TO THE FACULTY OF HEALTH SCIENCES, UNIVERSITY OF THE WITWATERSRAND, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE IN THE DIVISION OF NEUROSURGERY, JOHANNESBURG, 2024

DECLARATION

I, Dr Gugu Ndziba, declare that this research report is my work. It is being submitted for the degree of Master of Medicine (Neurosurgery) at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other University.

gndziba
_____

(Signature)

_____24th____ day of ____February___ 2025.

DEDICATION

To my parents, Daliwonga and Nonzwakazi Ndziba, and my siblings, Mkhuseli, Zizile, and Yongama for their love and support.

ABSTRACT

INTRODUCTION

Paediatric brain tumours are the most common type of solid childhood tumours and are a leading cause of morbidity and mortality. To date, the diagnosis of these tumours is dependent on histopathological analysis of tumour specimens. This may lead to delays in diagnosing and managing these children and poses an even more significant challenge in diagnosing tumours located in surgically unfavourable locations such as the brainstem.

In recent years, growing interest in genetic and molecular profiling of tumours has led to renewed interest in CSF analysis for diagnosing primary paediatric brain tumours. Analysis of CSF is an important adjunctive diagnostic tool that will help facilitate a less invasive, and timeous diagnosis of paediatric brain tumours.

AIM

This study aims to describe changes in the cerebrospinal fluid of children with primary brain tumours and assess the value of CSF cytology in this population.

METHODS

The records of children who presented with primary brain tumours and who met the study criteria were reviewed for the following information: demographics, CSF microscopy, and chemistry results, CSF cytology results, and histopathological results.

RESULTS

- Fifty-eight children presented with primary brain tumours during the study period.
- Thirty-eight children met the study criteria.
- A female predominance was observed (58%).
- Most of the population was in the 4-10 age group (57,9%).
- Eleven histological tumour types were identified.
- The most common tumour location was the posterior fossa.
- The most common tumour type in the cohort was ependymomas (26,3%), closely followed by medulloblastomas (21%) and astrocytomas (10,5%).
- In CSF microscopy, the only cell type observed was erythrocytes.
- A statistically significant correlation existed between the WHO tumour grade and CSF protein levels.
- There was no correlation between the CSF glucose and the tumour grade.
- CSF cytology had a specificity of 100% and a very low sensitivity of 14%.

CONCLUSION

Although a significant correlation between the WHO tumour grade and CSF protein levels was demonstrated in the study, the analysis of CSF microscopy, chemistry, and cytology did not prove effective in diagnosing primary paediatric brain tumours. CSF cytology in the cohort had a high specificity of 100%, while the sensitivity was much lower than most comparative studies (14%), with a negative predictive value of only twelve percent.

ACKNOWLEDGMENTS

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I also wish to express my gratitude to the University of Witwatersrand, Chris Hani Baragwanath Academic Hospital, and Nelson Mandela Children's Hospital for allowing me to conduct my research.

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H: NHLS letter.

LIST OF ABBREVIATIONS

AQP4:	Aquaporin-4
CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
CHBAH:	Chris Hani Baragwanath Academic Hospital
CNS:	Central Nervous System
CSF:	Cerebrospinal Fluid
DNA:	Deoxyribonucleic acid
EVD:	External Ventricular Drain
GBM:	Glioblastoma
ICP:	Intracranial pressure
MCS:	Microscopy, Culture and Sensitivity
NHLS:	National Health Laboratory Services

NMCH: Nelson Mandela Children’s Hospital

SEGA: Subependymal Giant Cell Astrocytoma

VPS: Ventriculoperitoneal Shunt

CHAPTER 1

1.0 INTRODUCTION

Paediatric brain tumours are the most common type of solid childhood cancer. They are a leading cause of morbidity and mortality in this population. [1–3] In the past few decades, advances in neuroimaging, and neuro-oncology have radically changed the management of children with brain tumours, thus, improving their survival. [3] However, the diagnosis of brain tumours is still highly dependent on the histopathological analysis of tumour specimens. [3] In recent years, growing interest in genetic and molecular profiling of tumours has led to renewed interest in CSF analysis for diagnosing primary paediatric brain tumours. This is known as central nervous system (CNS) liquid biopsies. [3–5] Liquid biopsies facilitate less invasive and timeous diagnosis of brain tumours, allowing for monitoring of response to therapy and assist with early detection of recurrent tumours. [2,5]

This study focuses on analyzing CSF microscopy, chemistry, and cytology in diagnosing primary paediatric brain tumours. Globally, there is a paucity of literature on variations of CSF cells and chemistry in different paediatric brain tumours. Contrary to this, extensive research has been conducted on CSF for the diagnosis of neurological conditions such as CNS infections, inflammatory and autoimmune conditions such as Guillain-Barrè, multiple sclerosis. [6,7]

1.1 PROBLEM STATEMENT

Traditionally, the diagnosis of CNS tumours has been based on imaging and histopathological analysis of tumour specimens. In resource-constrained countries like South Africa, this often leads to delays in diagnosing and managing children with brain tumours. For example, in Chris Hani Baragwanath Hospital, a histopathological report can take two to four weeks.

1.2 JUSTIFICATION

In recent years, analysis of CSF specimens in children with primary brain tumours has been an area of research interest. [2] CSF represents the tumour microenvironment due to proximity and is easily accessible by minimally invasive methods. [2] The use of liquid biopsies to help diagnose primary CNS tumours can supplement the traditional methods and facilitate a less invasive, timeous diagnosis and monitoring of paediatric brain tumours. [5]

1.3 RESEARCH QUESTION

What is the efficacy of CSF analysis in diagnosing paediatric brain tumours?

1.4 AIM

To determine the efficacy of CSF analysis in diagnosing paediatric brain tumours.

1.5 OBJECTIVES.

1. To describe changes in the composition of CSF (microscopy and chemistry) in paediatric patients with brain tumours.
2. To compare differences in the composition of CSF (microscopy and chemistry) and cytological diagnostic yield of CSF collected at different sites, namely lumbar, cisternal, and intraventricular.
3. To determine the value of CSF cytoanalysis in diagnosing paediatric brain tumours by analysing the rate of positive CSF cytology and comparing it to the rate of positive histopathology.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 CEREBROSPINAL FLUID PHYSIOLOGY.

CSF is an ultrafiltrate of plasma. [7–9] 80% of CSF is produced by the choroid plexus in the lateral, third, and fourth ventricles, while the ependymal lining of the ventricular system produces the remaining 20%. [2,7–9] However, more recent studies have challenged this dogma, proposing numerous other theories. Zhang et al. propose that interstitial fluid transfer produced CSF in the subarachnoid space. [10] This theory states that differences in the hydrostatic pressure in the capillaries found in the subarachnoid space and those occurring in the brain result in CSF formation through ultrafiltration. [10] This fluid transfer is mediated by aquaporin-4 (AQP4), an abundant water channel protein in the brain. [10]

CSF is produced at a rate of 0.3ml/min. [2,7,8] The total CSF volume in the CNS at an instant is 150mls. [2,6] In a day, approximately 450-750mls of CSF is produced. [2,7,8] CSF consists of cellular and non-cellular components. [7] In a standard CSF sample the cellular components include 0-5 lymphocytes with no polymorphs or erythrocytes. [7] The non-cellular components consist of proteins in low concentrations, glucose at a concentration of 60-80% of blood glucose concentration, and electrolytes such as sodium, potassium, chloride, and calcium. [7] CSF has three main functions in the CNS: it plays a protective role by offering a cushioning effect to the CNS. Secondly, it has a metabolic role; it helps in the transportation and excretion of toxic metabolites, and lastly, it plays a role in the autoregulation and maintenance of normal intracranial pressures (ICP). [7]

CSF composition varies with age and site. [7] In the pediatric population, a decline in CSF protein levels has been observed in the first few months of life as the blood-CSF barrier matures. [7,11] Similarly, a decline in the lymphocytic count has been observed from infancy to adulthood. [7] In addition, the composition of CSF in the ventricles differs slightly from that found in the lumbar region. [7] CSF protein in the lumbar region is slightly higher than the ventricular system. [7]

2.2 CHANGES IN CSF CHEMISTRY IN BRAIN TUMOURS.

There is a lack of literature on the changes in the cellular components of CSF in patients with brain tumours. However, some research has been conducted on the changes in CSF protein levels. [12] Proteins play a pivotal role in cell function and structure.[12] In the past few years, proteomic profiling has been an area of research interest. [2,3] CSF proteome has been shown to represent the proteome of cerebral tissue. [13] In neurosurgery, CSF protein analysis has played a crucial role in the diagnosing and managing children with germ cell tumours.[2]

A study by Al-Dalean et al. detected increased CSF protein levels in patients with brain tumours. [12] This study further found a statistically significant increase in the total protein of patients with malignant tumours compared to benign tumours.[12] This increase in CSF protein could be explained by increased capillary permeability resulting in increased blood-brain barrier permeability.[12]

Furthermore, Whithin et al. conducted an experimental study on laboratory rodent models, and discovered an increase in protein levels of laboratory rats with gliomas.[14] This increase was observed as early as 30 days of tumorigenesis, and this was much earlier than the radiological changes only observed after 90 days.[14] CSF proteins commonly elevated in brain tumours include glial fibrillary acidic protein (GFAP), elevated in patients with glioblastomas (GBM), tenascin, which is often elevated in astrocytic tumours, osteopontin (OPN), and matrix metalloproteinases (MMPs) have also been elevated in gliomas.[15]

Bruschi et al. analyzed the CSF proteome of 29 children diagnosed with brain tumours and 17 children were in the control group.[3] A total of 1526 and 1598 proteins were identified in each group, respectively.[3] Approximately, 1335 (74,6%) proteins overlapped in the two groups, with only 191 and 263 being exclusive to each condition, respectively. [3] From the study, the authors could differentiate tumour samples from non-tumour samples. [3] The main proteins used to distinguish between the two were TAF15, found to be higher in the non-tumour samples, and S100, which was higher in the tumour samples. [3] Furthermore, they could discern two major tumour groups in this population, namely pilocytic astrocytomas and medulloblastomas. [3] Similarly, a study by Kwaja et al. concluded that CSF proteomic analysis could be used to differentiate between benign and malignant CNS tumours. [16]

2.3 CSF CYTOANALYSIS IN PAEDIATRIC BRAIN TUMOURS.

CSF cytology, in conjunction with neuraxis MR imaging, remains the gold standard for early diagnosis of the leptomeningeal spread of brain tumours. [2,17,18] In the paediatric

population, about 20%-30% of primary CNS tumours exhibit leptomeningeal spread, compared to the 2% observed in adults.[17] Leptomeningeal spread is often observed in high-grade embryonal tumours such as medulloblastomas. [19]The presence of leptomeningeal disease at diagnosis results in a poorer prognosis for the patient. [18] CSF cytoanalysis has dual benefits in the management of CNS tumours.[17] It offers a diagnostic value and monitoring of response to intrathecal chemotherapy.[17]

Preparation of CSF for cytoanalysis involves collecting approximately 10,5ml of CSF and examining the specimen under microscopy to look for malignant cells. [2] Determining the site of CSF sampling continues to pose a great challenge.[20] CSF samples can be obtained intraoperatively via cisternal drainage, ventriculostomy or lumbar puncture at 2-3 weeks post operatively.[2,19,20]

A study conducted by Souweidane et al. on CSF sampling in children with posterior fossa tumours found that variability in sampling site, CSF volume, and interval time between surgery and lumbar puncture could affect the reliability of CSF cytoanalysis often leading to underestimation of leptomeningeal disease.[20] Discordant rates of approximately 20% were observed in the study due to the variability of CSF sampling sites.[20] They recommended Intraoperative CSF sampling due to closer proximity to the tumour location.[20]

Another study by Terterov et al. conducted in Los Angeles Children's Hospital, looked at the value of CSF analysis in the staging of medulloblastomas, supratentorial primitive neuroectodermal tumours, and ependymomas and found a discordance percentage of 18% in patients with a positive intracranial CSF and negative lumbar

CSF cytology. [18] Although intracranial CSF sampling is easily accessible at the time of surgery or via ventriculostomy, it may yield high false-positive results due to the sloughing of tumour cells.[18] The authors also demonstrated a discordance of 17% when comparing positive neuraxis MR imaging versus negative lumbar CSF cytology.[18]

Damiani et al. analyzed the CSF of 70 paediatric patients who presented with medulloblastomas and observed that cell clustering with nuclear molding was the key cytomorphologic change in leptomeningeal metastasis.[17]

Although CSF cytoanalysis is essential, it does have limitations. While it has a high specificity of >95%, the sensitivity is often <50%.[2,21] To mitigate this problem, obtaining a specimen of more than 10,5mls has been recommended. [2] Another limitation is the intermittent shedding of tumour cells in the CSF which may lead to false-negative results as high as 20%. [2] To avoid this problem in post-operative lumbar specimens, Gajjar et al. recommend that the specimen only be collected 2-3 weeks post-operatively.[19] Lastly, standardized techniques are lacking in examining cytology specimens. CSF cytoanalysis involves the identification of pathological cells by GIEMSA stain, and it is dependent on the pathologists to make the differentiation between benign and malignant cells. [2] The morphologic resemblance of ependymal cells and malignant cells, and the smaller quantity of cells to be examined further results in false-positive specimens.[19]

A useful adjunct in CSF cytology is CSF cytometry analysis.[2] CSF cytometry provides information about the proteins expressed on the surface of the cells.[2] In conjunction

with cytology, it offers higher sensitivity[2] and can be used to analyze smaller CSF specimens with lower cell counts.[2]

CHAPTER 3

3.0 METHODS AND MATERIALS

3.1 STUDY DESIGN

The study was a prospective, descriptive cohort study. It was carried out over 16 months. The study was conducted after ethics clearance was granted.

3.2 STUDY SITE

The study was conducted in two paediatric neurosurgical units in Gauteng, Chris Hani Baragwanath Academic Hospital (CHBAH) and Nelson Mandela Children's Hospital (NMCH). The first hospital, CHBAH, is an academic hospital located in the Southwestern Townships of Johannesburg (SOWETO), South Africa. This hospital is affiliated with the University of Witwatersrand. CHBAH is the largest hospital in South Africa and the third largest in the world with approximately 3200 beds. The hospital has a drainage area that covers three metropolitan municipalities in Gauteng and goes as far as the Northwest province.

The second hospital, Nelson Mandela Children's Hospital (NMCH) is in Parktown, Johannesburg, adjacent to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), and the University of Witwatersrand Medical School. It is a 200-bedded, supra-regional, specialist paediatrics facility with a vast drainage area. It is also affiliated with Witwatersrand University. It has taken over the paediatric neurosurgical workload from CMJAH.

3.3 STUDY POPULATION

All consecutive paediatric patients, aged 14 years and younger, radiologically diagnosed with a primary brain tumour during the study period, at the two participating sites, CHBAH and NMCH, were invited to participate. An age limit of 14 years was decided upon because in the two hospitals, paediatric admissions only include patients between the ages of 0 to 14 years.

3.3.1 EXCLUSION CRITERIA

The following patients were excluded from the study:

- Patients with recurrent brain tumours.
- Patients with previous radiation to the brain.
- Patients with previous CNS infections.
- Patients with missing or incomplete clinical, radiological, cytological, or histopathology data.

3.5 SAMPLE SIZE AND SAMPLING METHOD

For a population of patients with paediatric brain tumours, a sample size of 19 is sufficient to estimate a parameter of interest with 95% confidence and a margin of error of 5%. Consecutive sampling was used to include all accessible participants in the study.

3.6 DATA SOURCES

Five data sources were used namely, direct interviews with patients, patient files, data from the cytology, histopathology, and radiology department. Clinical data was retrieved from patient files. Cytology and histopathology data was retrieved from the National Health Laboratory Services (NHLS) electronic system. Radiological data was retrieved from the radiology department via their Picture and Archiving and Communication System (PACS).

3.7 DATA COLLECTION

When the patients and their parents/next-of-kin presented to either of the two hospitals, the patients were screened to see if they met the inclusion criteria. The parents/next of kin were invited to participate in the study. When they agreed to participate, informed consent was obtained. Assent was also signed by individuals above the age of 12 years.

Data was collected systematically using a data collection tool (Appendix A) which was developed based on study objectives.

The variables collected included:

- Demographic data: Age, sex, ethnicity

- Clinical data: Glasgow Coma Scale (GCS): Presentation – incidental, disturbance of consciousness, seizure disorder, focal signs: cranial nerves, long tracts, cerebellar signs.
- Radiological data: Hydrocephalus – yes/no, Mass Effect – yes/no, Tumor location – supratentorial/infratentorial, Tumour size, leptomeningeal enhancement – yes/no, proximity of tumour to ventricular system.
- Site of CSF Collection – CSF is collected as part of routine management in children with paediatric brain tumours. 10 mls of CSF was collected intraoperatively via cisternal aspiration or via a lumbar drain. For children requiring external ventricular drains for raised intracranial pressure, CSF was collected pre-operatively via external ventricular drain.
- CSF – Glucose, protein, Cells – Lymphocytes, Erythrocytes, Polymorphs
- Cytological data – Positive/Negative
- Histopathological Data – Medulloblastomas, ependymomas, gliomas, pineal region tumours, craniopharyngiomas, and choroid plexus papillomas.

3.8 DATA MANAGEMENT

The data was stored in a secure environment and only the primary investigator had access to the data. Data was collected on data sheets and patients were anonymized. Data was exported into Excel (Microsoft, Redmond, USA). The data was then exported from Excel into STATA Version 16 (Stata Corp, Ltd, Texas, USA) and statistical analysis was done with the aid of a statistician.

3.9 DATA ANALYSIS

Categorical variables such as gender were described as frequency, percentages, or charts, while nominally distributed continuous variables such as age were reported as mean ± standard deviation. Non-nominally distributed variables were presented as median (interquartile range). The association between two categorical variables was determined using Pearson's Chi-square test and logistic regression at 5% level of significance. The association between one numeric and one categorical variable was determined using a T- test or ANOVA at a 5% level of significance. The association between two numeric variables was determined using Spearman correlation test at a 5% level of significance.

Table 3.9: Data analysis

Objective	**Variables**	**Data analysis**
1. To describe CSF composition changes in paediatric brain tumours.	Categorical variables. CSF chemistry CSF microscopy	Frequencies, percentages or charts

2. To compare differences in composition of CSF collected at different sites.	Categorical variables. Site of CSF collection: Lumbar: Intraventricular: EVD, VPS	Frequencies, percentages, charts
3. To determine the value of CSF cytoanalysis in diagnosing paediatric brain tumours by analysing the rate of positive CSF cytology and comparing it to positive histopathology.	Categorical variables CYTOLOGY: positive/negative HISTOLOGY: Positive/negative	Frequency, percentages, charts T – test, Pearson Chi Square test, Spearmen Correlation

3.10 ETHICAL CONSIDERATIONS

- Approval to conduct the study was obtained from the Wits Human Research Ethics Committee. Reference: M230317.
- Permission to conduct the study was granted by the Chief Executive Officer at Chris Hani Baragwanath Academic Hospital and Nelson Mandela Children's Hospital.
- The study was registered on the National Health Research Database. NHRD Number: GP_202211_023.
- Informed Consent was obtained from all participants.

- Confidentiality was upheld and maintained using study participant numbers on the data sheets.
- Records were kept safe and private.
- There was no deviation from the standard of care because of the study as it is purely observational.

CHAPTER 4

4.0 RESULTS

4.1 INTRODUCTION

Fifty-eight children were diagnosed with primary brain tumours at Chris Hani Baragwanath Academic Hospital (n=21) and Nelson Mandela Children's Hospital (n=37) in a period of 16 months. Twenty patients were excluded from the study due to no records of CSF results on the NHLS system (n=15), and incomplete clinical records (n=5). A total of thirty-eight participants met the study criteria. Nelson Mandela Children's Hospital accounted for 68,4% (n=26) of the participants, while Chris Hani Baragwanath Academic Hospital accounted for 31.6% (n=12).

4.2 DEMOGRAPHICS OF STUDY PARTICIPANTS

The majority of the study participants were of African race (n= 35, 92.1%), followed by the mixed-race (n= 2, 5.3%), then, lastly, Caucasian race (n=1, 2.6%). The ratio of African to mixed-race participants was 17.5: 1, while for the African to Caucasian participants it was 35: 1.

Table 4.2: Demographics of the study participants.

Demographics	n	%
Race		
African	35	92.1
Mixed	2	5.3
Caucasian	1	2.6
Gender		
Males	16	42.1
Females	22	57.9

n= number; % = percentage

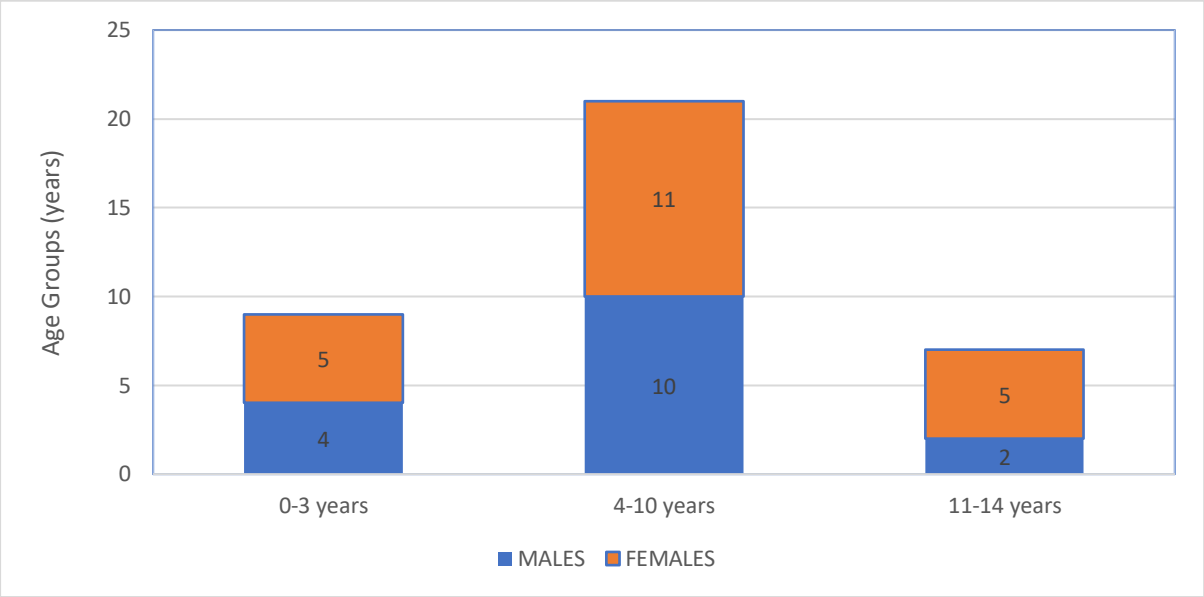
A female predominance of 58% was observed in the study, with males making up 42% of the study participants. The male-to-female ratio was 1: 1.4.

The mean age at presentation was 6.5 years, and the standard deviation was 3.4 years. The participant's age groups were categorically divided into the following age groups; 0-3 years (n=9, 23.7%), 4-10 years (n=22, 57.9%), and 11-14 years (n=7, 18.4%). The majority of the participants in the study were between the ages of 4-10 years. The youngest patient was 8 months, and the oldest patient was 14 years old.

Table 4.2.1: Age categories and frequency

Age (years)	Frequency (n)	Percentage (%)
0-3	9	23.7%
4-10	22	57.9%
11-14	7	18.4%

Figure 1: Male-to-female distribution in the different age categories.



4.3 PREVALENCE OF TUMOUR HISTOLOGICAL TYPES

A total of 11 different histological types were observed in this study. The most common tumour types were ependymomas (26,3%), with infratentorial ependymomas accounting for 18.4% (n=7) while supratentorial ependymomas accounted for 7.9% (n=3), closely followed by medulloblastomas at 21% (n=8), pilocytic astrocytomas 10.5% (n=4), glioblastomas 7.9% (n=3) and craniopharyngiomas 7.9% (n=3). Pineal region tumours, subependymal giant cell astrocytomas (SEGA's), choroid plexus tumours, oligodendrogliomas, ganglioglioma, and DIPG each accounted for 2.6% (n=1). Four histological specimens (10.5%) were reported as normal brain tissue.

Figure 2: Prevalence of tumour histological types

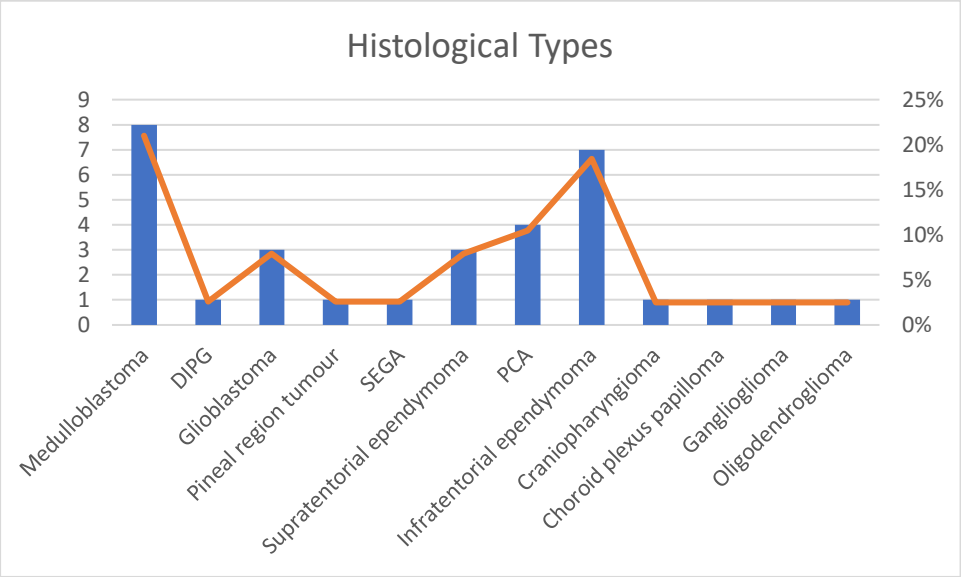


Table 4.3: Prevalence of Tumour Histological Types

HISTOLOGY	**n**	**%**
Medulloblastomas	8	21
Infratentorial Ependymomas	7	18,4
Pilocytic Astrocytomas	4	10,5
Supratentorial Ependymomas	3	7,9
Glioblastomas	3	7,9
Craniopharyngiomas	3	7,9
DIPG	1	2,6
Pineal Region tumours	1	2,6
SEGA	1	2,6
Choroid Plexus Papilloma	1	2,6
Ganglioglioma	1	2,6
Oligodendroglioma	1	2,6

n= number , %= Percentage

The posterior fossa was the most common location for paediatric brain tumours, accounting for 57.9% (n=22), while 42.1% (n=16) occurred in the supratentorial region. The most common tumour site in the posterior fossa was the 4th ventricle (50%), followed by the cerebellum (40,9%), and brainstem (9%). In the supratentorial region, the most common location was the parietal region, followed by the suprasellar region, temporal, frontal, lateral ventricles, and pineal region.

Table 4.3.1: Tumour Location

Tumour Location	**n**	**%**
Infratentorial	22	57,9
4th Ventricle	11	50
Cerebellum	9	40,9
Brainstem	2	9
Supratentorial	16	42,1
Suprasellar	5	31,3
Parietal	6	37,5
Temporal	1	6,3
Frontal	1	6,3
Lateral Ventricles	2	12,5
Pineal Region	1	6,3

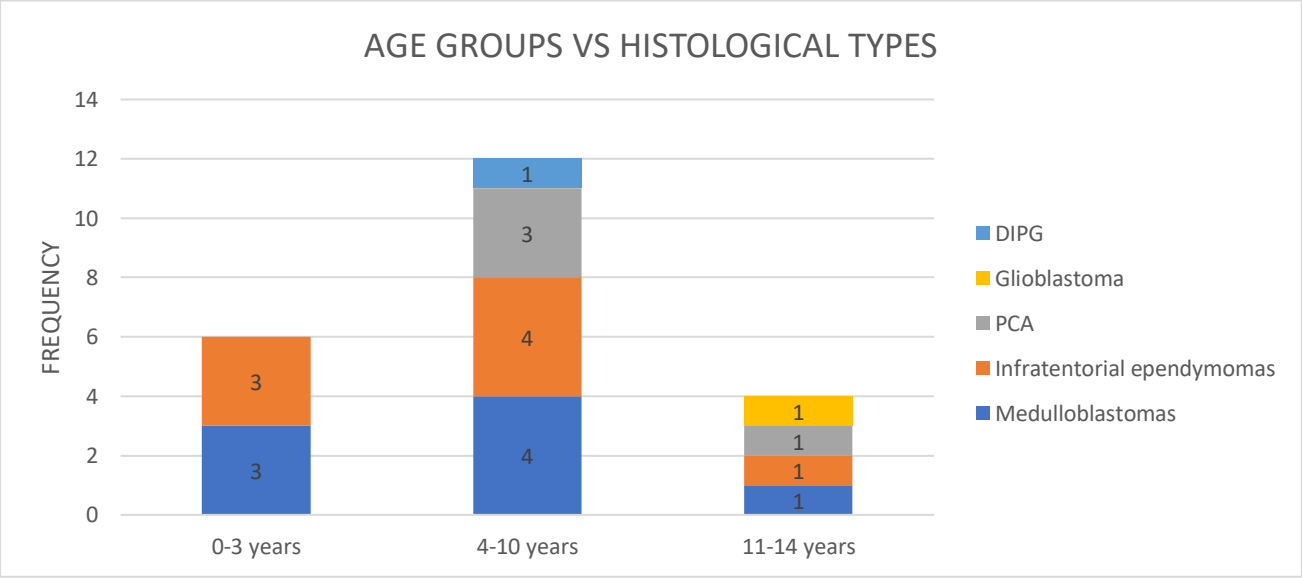
n = number , % = Percentage

Although not statistically significant (p=0,75), the majority of posterior fossa tumours were observed in the 4-10 age group (54,5%), followed by children in the 0-3 age group, (27,3%) and (18,2%) in the 11-14 age group.

The most commonly occurring posterior fossa tumours in the 0-3 age group were ependymomas (50%) and medulloblastomas (50%).

In the 4-10 age group, the most common posterior fossa tumour types were medulloblastomas (33,3%) and ependymomas (33,3%), followed by pilocytic astrocytomas (25,1%), then DIPGs at 8,3%.

Figure 3: Frequency of histological types in the different age groups.



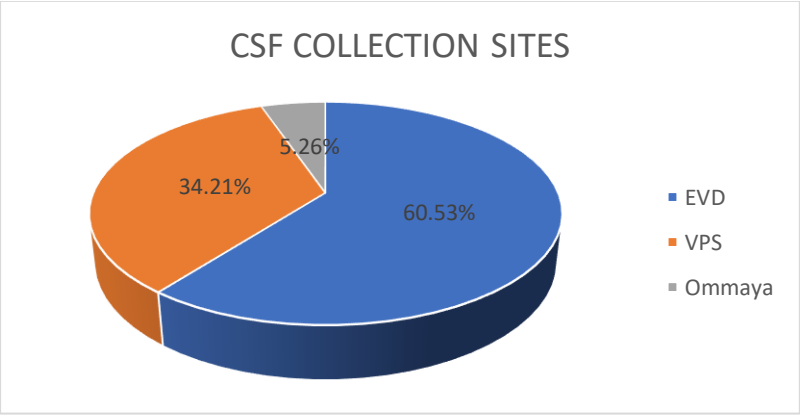
In the 11-14 year age group, four posterior fossa tumours were observed, namely, pilocytic astrocytoma, glioblastoma, medulloblastoma, and ependymoma.

The supratentorial region housed forty-two percent of tumours in the study. Twenty-one percent of these tumours were found in the 0-3 age group, while fifty percent occurred in the 4-10 age group and twenty-nine percent in the 11-14 age group. The supratentorial tumour types found in the 4-10 age group included; supratentorial ependymomas (28,6%), pineoblastomas (14,3%), craniopharyngiomas (28,6%), SEGAs (14,3%), and gangliogliomas (14,3%). In the 11-14 age group glioblastomas, pilocytic astrocytomas, and oligodedrogliomas were observed. While craniopharyngioma, supratentorial ependymoma and one choroid plexus tumour were observed in the 0-3 age group.

4.4 CSF COLLECTION AND ANALYSIS.

CSF was collected via external ventricular drain (60.5%), ventriculoperitoneal shunt (34.2%), and Ommaya reservoir (5.3%). None of the CSF was collected via a lumbar drain. Most of the CSF collection was performed intra-operatively. A total of 10-15mls of CSF was collected. The CSF collected was sent to the laboratory for microscopy, chemistry, and cytoanalysis.

Figure 4: CSF collection sites.

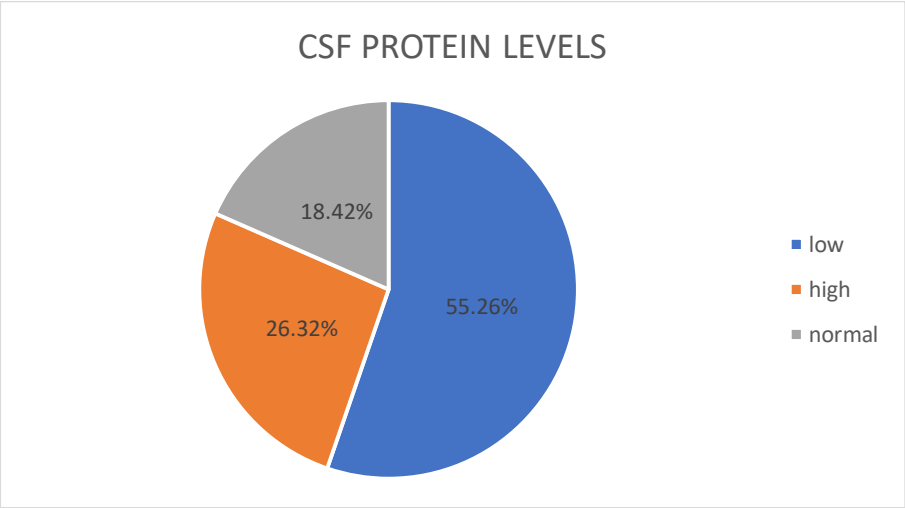


4.5 CHANGES IN CSF CHEMISTRY AND CELLS IN PAEDIATRIC BRAIN TUMOURS

The most common cell type, observed in 55.2% of the participants was erythrocytes. None of the specimens had polymorphs and lymphocytes. Erythrocytes were found in 57% of medulloblastomas, 42.9% of ependymomas, 33% of glioblastomas, and 25% of pilocytic astrocytomas. Although no statistically significant (p =0,49) correlation has been established between the presence of erythrocytes in CSF and the WHO tumour grade, 47.6% of WHO grade III-IV tumours demonstrated erythrocytes in the CSF, while only 33.3% of low-grade (WHO I-II) had erythrocytes.

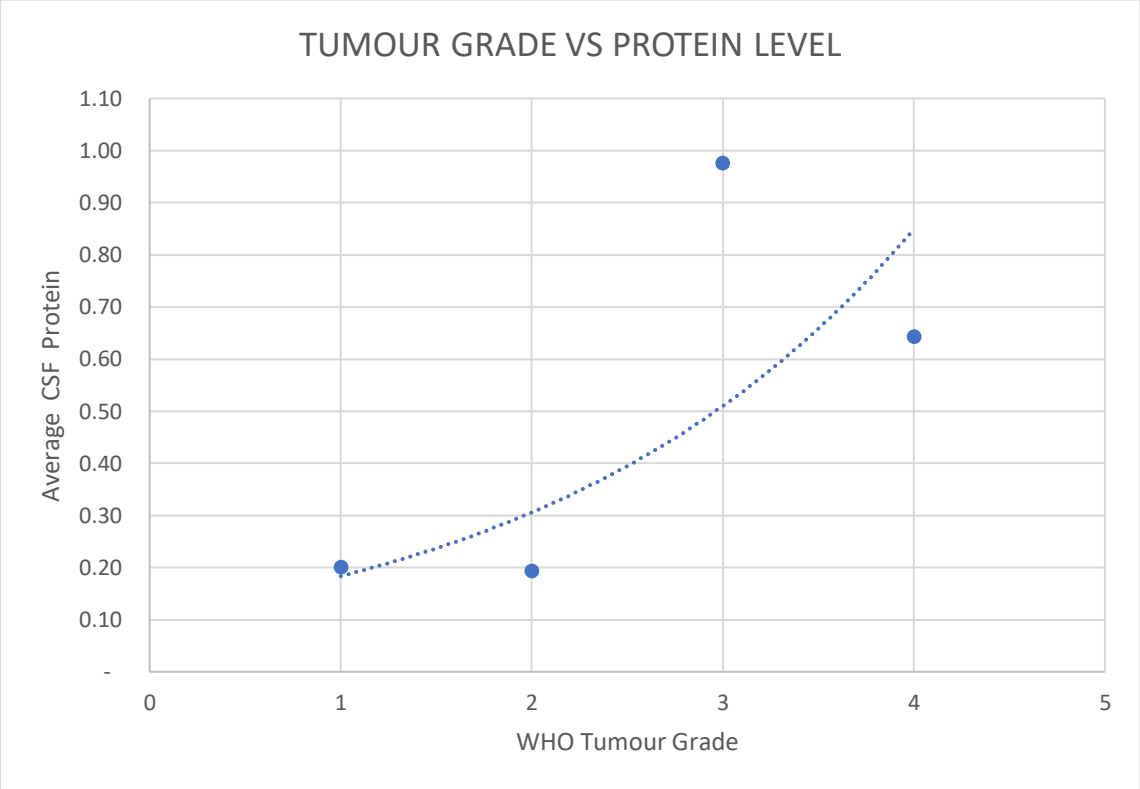
In CSF chemistry, CSF protein and glucose were analyzed. CSF protein of 0.18-0.45g/l was considered normal, whereas CSF protein of < 0.18g/l was considered low and CSF of > 0.45g/l was considered high. The protein levels were low in 55,2% of the specimens, within normal limits in 18.4% of patients, and high in 26.3% of specimens.

Figure 5: CSF protein levels



On average, high-grade tumours demonstrated higher protein levels than low-grade tumours, see Figure 6.

Figure 6: Tumour grade vs average protein level



Although more than fifty percent of CSF specimens in the cohort demonstrated low protein levels no significant correlation existed between low CSF protein and the tumour grade (p=0,06). There was, however, a statistically significant correlation between high CSF protein levels and tumour grade (p= 0,007). The majority of high-grade tumours had high protein levels. Elevated protein levels were identified in 100% of glioblastomas, 30% of ependymomas, and 6% of medulloblastomas.

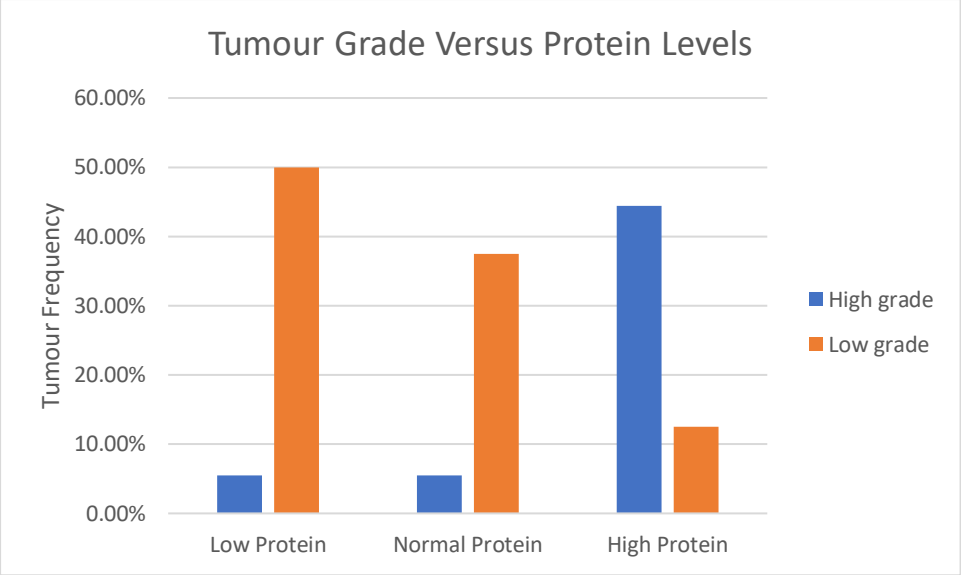


Figure 6: Tumour grade versus protein levels

CSF glucose is normally 60% of serum glucose. It was considered low when below 2.5 mmol/l, normal between 2.5- 3.5 mmol/l, and high when above 3.5 mmol/l. Only 7.8% of the specimens had low glucose, 26.3% of the patients had glucose within normal limits, and 60% had high glucose.

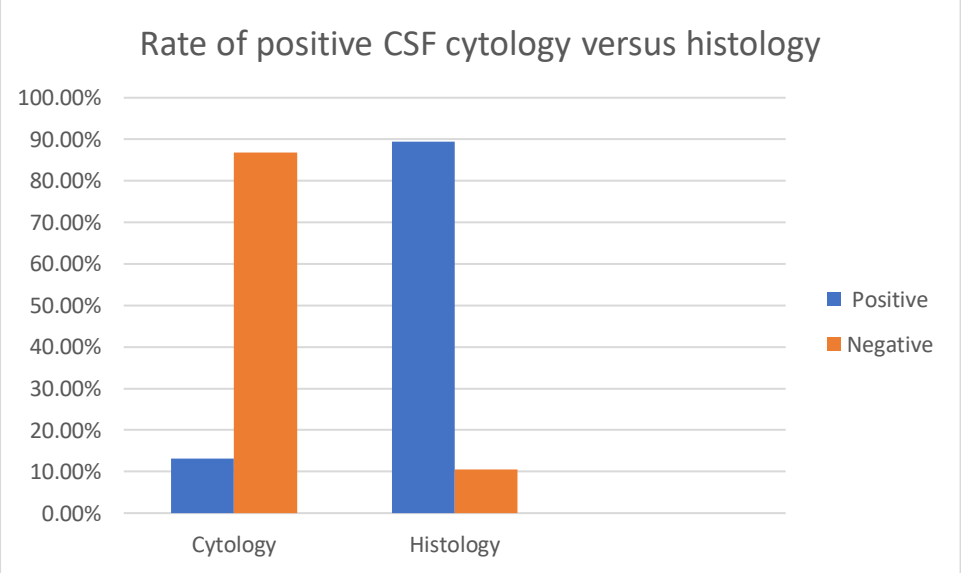
Only three tumour types demonstrated low glucose levels, these included; one glioblastoma, one grade II supratentorial ependymoma, and one ganglioglioma. Most of the specimens had high glucose levels. The high glucose subgroup comprised of a combination of low grade (43.5%), and high grade tumours (39%). The most common tumour type in this subgroup was medulloblastomas, followed by ependymomas, glioblastomas, craniopharyngiomas then lastly pilocytic astrocytomas. 80% of tumours with a normal glucose range were high grade tumours; these mainly comprised ependymomas, medulloblastomas, DIPGs, and glioblastomas. There was no statistically significant correlation between the CSF glucose level and tumour histology and WHO tumour staging.

4.6 HISTOPATHOLOGY VERSUS CYTOANALYSIS

Thirty-eight specimens were sent for histopathological analysis and cytoanalysis. Of the 38 specimens sent for histopathology, 89.5% had positive results. A total of 11 histological types were observed in this study, see Table 4,3. Ten percent (10.5%) of the specimens were reported as normal brain tissue. Normal brain tissue was reported in two tumours located in the cerebellum, one suprasellar region, and one parietal region.

The majority of tumours in the study were high-grade tumours. WHO IV tumours accounted for 38,2% of the tumours while WHO III tumours accounted for 14.7%. WHO II tumours coprised 20,6% of the cohort, and WHO I accounted for 26.5%.

Figure 7: Rate of positive CSF cytology versus positive histology.



In cytoanalysis, most of the specimens (86,8%) had negative cytological results, meaning no tumour or malignant cells were identified on the specimen. Only five

specimens (13,5%) demonstrated malignant cells on the CSF. Positive CSF cytology was only observed in high-grade tumours; three infratentorial ependymomas, and two medulloblastomas. Three of the above specimens had been collected intraoperatively via external ventricular drain, while two were collected via a ventriculoperitoneal shunt.

The sensitivity of the CSF cytology was 14,7%, while specificity was 100%. The positive predictive value of CSF cytoanalysis was 100%, while the negative predictive value was 12%.

CHAPTER 5

5.0 DISCUSSION

Brain tumours are the most common solid tumour in children and are a leading cause of morbidity and mortality in this population.[1–3] Advances in neuroimaging and neuro-oncology have led to early detection and improved the management of these tumours. However, confirmation of the diagnosis of primary paediatric brain tumours is highly dependent on histological tumour specimens obtained at surgery.[3] This often results in delays in diagnosing these tumours.

This study looked at the efficacy of CSF analysis in diagnosing paediatric brain tumours at two tertiary paediatric neurosurgical institutions in Johannesburg.

5.1 DEMOGRAPHICS

The mean age at presentation was 6.5 years with a standard deviation of 3,4. These results are similar to findings from a study conducted by Nkusi et al. at Chris Hani Baragwanath Academic Hospital 25 years ago. Comparative studies demonstrated a slightly higher mean age at presentation. [1,22,23] In the study, an increased tumour prevalence was observed in children aged 4-10 years. These findings were in keeping with results from studies by Mehta et al., Wilne et al., and Wei-yang et al.[22–24]

A female predominance was observed in the study, with females making up 58% of the population, while males made up 42%. The male-to-female ratio was 1: 1.4. This

distribution did not keep with global trends. In most similar studies, a male predominance was observed.[1,17,22–25]

The majority of the study participants were of African race (92%), followed by mixed race (5.3 %), and lastly, Caucasian race (2.6 %). The racial demographics in our country may explain the disparities observed in the different ethnic groups. They may also be attributed to the socioeconomic status amongst the ethnic groups, with the majority of African children seeking medical care in public hospitals as opposed to private hospitals.

5.2 PREVALENCE OF TUMOUR HISTOLOGICAL TYPES

A total of 11 different tumour histological types were identified in the study. The most common tumour types were ependymomas (26,3%), with infratentorial ependymomas accounting for 18.4% (n=7) while supratentorial ependymomas accounted for 7.9% (n=3), closely followed by medulloblastomas at 21% (n=8), then pilocytic astrocytomas (10.5%). Glioblastomas, and craniopharyngiomas each made up about 7.9% while SEGAs, pineal region tumours, choroid plexus tumours, oligodendrogliomas, and gangliogliomas each made up 2.6%. The most common tumour location was the posterior fossa (58%), with the 4th ventricle making up 29% of tumours in the region, followed by the cerebellum at 23%. Other studies identify the posterior fossa as the most common anatomical site for paediatric brain tumours.[1,22,23] Shah et al. and Mehta et al. cited the cerebellum as the most common location in the posterior fossa.

Although, the prevalence of tumours varies with the different age groups, the tumour frequency observed in the study was consistent with findings from similar studies. A study by Shah et al. found medulloblastomas to be the commonest paediatric brain tumours (22.4%), followed by pilocytic astrocytomas (18.4%), craniopharyngiomas (11.8), and then ependymomas at 6.6%. Another study by Mehta et al. found medulloblastomas to be the commonest tumours (33%), followed by ependymomas (16.1%), and the pilocytic astrocytomas at (14.9%).

The most commonly occurring tumours in the 0-3 age group were infratentorial ependymomas (50%), followed by medulloblastomas (33.3%) then choroid plexus papillomas (16.7%). In similar studies, this age group's most common tumour type was medulloblastomas, followed by ependymomas. [22,23]

In the 4-10 age group the most common tumour type was medulloblastomas (30.8%), followed by infratentorial ependymomas (23%), then lastly pilocytic astrocytomas (23%). These findings are inconsistent with similar studies, as astrocytic tumours are the most common in this age group.[1,22,23]

Lastly, in the 11-14 year age group only three posterior fossa tumours were observed, namely, glioblastoma, medulloblastoma and ependymoma.

5.3 CSF COLLECTION AND ANALYSIS

In the study, the most common method of CSF collection was via an external ventricular drain, a ventriculoperitoneal shunt, and an Ommaya reservoir. This was attributable to the fact that EVDs can be inserted pre-operatively for urgent CSF diversion. A third of the study participants required pre-operative CSF diversion. The CSF collected was sent to the laboratory for cells, chemistry, and cytoanalysis.

Lumbar CSF collection remains the gold standard for diagnosing leptomeningeal spread and monitoring treatment response. [16,20]Gajjar et al. observed a statistically significant difference in the number of malignant cells detected in lumbar CSF versus ventricular CSF. However, numerous authors have concluded that cisternal and ventricular CSF may be superior in detecting malignant cells. [18,21,26,27]

In this study, a comparison between CSF collected from the lateral ventricles versus CSF collected from the lumbar drain could not be made. This is because, in the study, none of the participants required lumbar drains. This may be attributed to the fact that more than half of the participants had posterior fossa tumours with obstructive hydrocephalus, requiring urgent CSF diversion via EVD or ventriculoperitoneal shunt. Furthermore, in our setting, the standard of care for posterior fossa tumours is lumbar CSF collection at 3-4 weeks post-surgery to assess for leptomeningeal spread and monitor treatment response.

5.4 CSF CELLS AND CHEMISTRY

Globally, there is a paucity of literature on the changes in CSF cells and chemistry in brain tumours. In this study, the most common cell type observed in CSF was erythrocytes in both benign and malignant tumours. Erythrocytes were mainly found in medulloblastomas, ependymomas, glioblastomas, and pilocytic astrocytomas. Lymphocytes and polymorphs were not identified.

A study conducted by Greer et al looked at the behavior of white blood cells in CSF samples of children diagnosed with brain tumours. The study identified lymphocytes as the most common cells type, seconded by monocytes. [28] The overall findings were that both high-grade tumours and low-grade tumours demonstrated low white blood cell counts, with almost no presence of macrophages. They concluded that these tumours induce an innate immune response in their microenvironment.[28]

A statistically significant correlation was established between tumour grade and protein levels (p=0,007). High-grade tumours had high CSF protein levels. There was no statistically significant correlation between the CSF glucose level and tumour histology and WHO tumour staging. These findings are consistent with findings from other similar studies. Al-Dalaen et al. observed a statistically significant increase in CSF protein levels of subjects with malignant tumours versus beinign tumours. Similar findings were reproduced by Kwaja et al. and Bruschi et al.

5.5 HISTOPATHOLOGY VERSUS CYTOANALYSIS

Ninety percent of the histopathological specimens had positive results. On the other hand, CSF cytology had a high negative rate (86,8%). In the study CSF cytology had a specificity of 100% and sensitivity of 14,7%. A positive predictive value (PPV) of 100% was observed with a negative predictive value of only 12%. These findings are consistent with findings from numerous similar studies. [1–3,12,17,18,20,29,30]

Souweidan et al. demonstrated a specificity of 100% with a sensitivity of 60-70% in CSF cytoanalysis; possible reasons for these results included variations in sampling site, CSF volume, and time between CSF collection and analysis. Low et al demonstrated a specificity of >95% with sensitivity < 50%.

In a study by Escudero et al. analyzing the subtypes and monitoring of medulloblastomas, thirteen CSF samples were examined for malignant cells and circulating tumour DNA (ctDNA), the results showed that none of the specimens possessed any malignant cells, while ctDNA was detected in 77% of the specimens.

5.6 LIMITATIONS

As the study population is small, the author is unable to draw generalized conclusions from the study.

Incomplete record keeping. 5 of the study participants were excluded due to incomplete records. Fifteen participants were excluded due to no CSF results on the NHLS system.

Only one CSF sample of 15 ml was collected per participant; this may have resulted in a poor yield of tumor cells in the samples. A few authors have reported that cytological yield increased with multiple samples.

No CSF was collected via the lumbar drain for comparison.

5.7 CONCLUSION

This study aimed to describe CSF changes in children with primary brain tumours, and to assess the efficacy of CSF cytoanalysis in diagnosing these tumours. The study cohort mainly consisted of children aged 4-10 years. Many of the participants were of African race. A female predominance was observed. Eleven histological tumour types were identified, with an overrepresentation of high-grade tumours. In CSF chemistry, the only prevalent cell was erythrocytes, no lymphocytes were identified. There was a statistically significant correlation between CSF protein and the histological grade of the tumour. High CSF protein levels were mostly found in

high grade tumours. Although CSF cytology specificity in the study was comparable to similar studies, CSF cytology sensitivity was very low.

5.8 RECOMMENDATIONS

- A larger cohort.
- Further studies on CSF proteomic analysis are needed.
- Further studies on circulating tumour DNA (ctDNA) which has been proven to be superior to CSF cytoanalysis.
- Follow-up studies with multiple CSF samples for better yield.

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APPENDICES

APPENDIX A

DATA COLLECTION TOOL.

Demographics

Study participant number:

Age:

Gender:

Ethnicity:

Clinical findings

Presenting complaint: headache, nausea and vomiting, seizures.

Duration of symptoms.

CNS exam: GCS. /15. PUPILS. FNS

Laboratory findings

BLOODS:

CSF: Specimen reference and date of collection:

MCS:

CELLS: Polymorphs: Erythrocytes: Lymphocytes.

Site of CSF collection: Cisternal. Lumbar. Intraventricular.

CSF Volume collected:

CYTOLOGY: Specimen reference

Results: Positive/ Negative

HISTOLOGY RESULTS: Specimen Reference

Results: Positive/ Negative

Imaging

CTB/MRI: Tumour location: supratentorial / infratentorial.

Intraventricular:

Drop mets:

Hydrocephalus:

Management

Surgical- craniotomy & tumour resection. VS Chemoradiation therapy.

Timing of CSF of collection: intra -op, post op- at how many weeks.

GLASGOW OUTCOME SCALE

1. Death.
2. Persistent vegetative state.
3. Severe disability, needing assistance with activities of daily living.
4. Moderate disability, no assistance with activities of living.

   Disabilities: varying degrees of dysphasia, hemiparesis, ataxia, intellectual and memory deficits, and personality changes.
5. Good recovery, resumption of normal activities, with/without minor neurologic or psychologic deficits.

APPENDIX B **CONSENT FORM**

Study title: Efficacy of CSF analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg.

I ……confirm that Dr G Ndziba has informed me about the nature of the study. I have also read / it was read to me, and I understood the information sheet.

I understand that my child's/ the patient's participation is voluntary and that I can withdraw at any moment without giving a reason and this will not affect my child's right to medical care.

I understand that the author and the supervisors will review my child's/ the patient's medical records. I also understand that a CSF specimen will be collected either via lumbar puncture, external ventricular drain or via cisterns during surgery. The CSF will be sent to the laboratory for analysis. My child's/ the patient's identity will remain confidential.

I consent to have my child's/ the patient's hospital records reviewed and to CSF collected for analysis.

Printed name of parent/ guardian/next of kin:

Signature date:

Printed name of the child date:

Printed name of researcher:

Signature date:

Researcher: DR G. Ndziba. Contact details: cell 076 901 7567/ e-mail: gndziba90@gmail.

Mr Rhulani Mkansi. Administrative Officer: Human Research Ethics Committee (Medical). Tel: 011 717-1234/2656/2700
E-mail: Rhulani.Mkansi@wits.ac.za or HREC-Medical.ResearchOffice@wits.ac.za

APPENDIX C. **ASSENT FORM**

Study title: Efficacy of CSF analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg.

I ……confirm that Dr G Ndziba has informed me about the nature of the study. I have also read / it was read to me, and I understood the information sheet.

I understand that my participation is voluntary and that I can withdraw at any moment without giving a reason and this will not affect my right to medical care.

I understand that the author and the supervisors will review my medical records. I also understand that a CSF specimen will be collected either via lumbar puncture, external ventricular drain or via cisterns during surgery. The CSF will be sent to the laboratory for analysis. My identity will remain confidential.

I agree to take part in the study. I consent to my records reviewed and to have CSF collected for analysis.

Print name of participant:

Signature date:

Print name of researcher:

Signature date:

Researcher: DR G. Ndziba. Contact details: cell 076 901 7567/ e-mail: gndziba90@gmail.

Mr Rhulani Mkansi. Administrative Officer: Human Research Ethics Committee (Medical). Tel: 011 717-1234/2656/2700

E-mail: Rhulani.Mkansi@wits.ac.za or HREC-Medical.ResearchOffice@wits.ac.za

APPENDIX D

PATIENT INFORMATION SHEET FOR PARENTS/GUARDIANS.

Study title: Efficacy of CSF analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg.

Investigator: Dr Gugu Ndziba.

Supervisors: Prof J. Ouma, Dr R. Khohomela.

Greetings

Good day, my name is Dr. Gugu Ndziba. I am a neurosurgical registrar at Chris Hani Baragwanath Hospital. I am doing a study on the benefit of CSF analysis in diagnosing children with brain tumours. CSF analysis means performing laboratory tests on the fluid that cushions the brain and spinal cord to help diagnose diseases found in this region. I am undertaking this study, in part, to obtain a M Med degree from Wits University.

Invitation to participate

Your child is invited to be part of a research study that will be investigating the benefit of CSF analysis in children that have brain tumours. The purpose of this Information Sheet is to give you information about the study so you can make an informed decision about whether or not you would agree to have your child participate. Should you require any translation, or interpretation of the Information Sheet please inform the investigator for assistance (contact details below).

Once you understand everything and if you agree to your child's participation in the study, you will be given a consent form to sign, and child will be given an assent form to sign. Your child may only participate if both of you agree.

What is the study about?

The study will be looking at the benefit of analyzing CSF in children that have brain tumours. A brain tumour is an abnormal growth of cells, it can either be a slow-growing tumour, known as a benign tumour, or a very fast-growing and aggressive tumour which is known as a malignant tumour. CSF is a clear fluid that surrounds and cushions the brain and the spinal cord. Because CSF is close to the brain, tumour cells may be found in the CSF, therefore, sending this fluid to the laboratory might make it easier and faster to diagnose these tumours.

How will the study be conducted?

Those who agree to participate in the study and meet the criteria will be enrolled on admission to the ward. During their hospital stay, 15 ml of CSF will be collected before the operation using a device called an external ventricular drain (EVD). CSF can also be collected in theatre using a device called the lumbar (lower back) drain or during the operation from the CSF that is cushioning the brain. An EVD is a small pipe (catheter) that is 3.3mm in diameter and 35cm in length. This pipe is inserted in the ventricles, which are small chambers deep in the brain where CSF is produced and stored. To gain access to these chambers, the surgeon drills a 1x1cm burhole on the skull, while the patient is sedated or asleep, and then inserts the EVD catheter into the ventricles. EVD insertion and CSF drainage are part of the routine management of children presenting with brain tumours and features of increased

pressure in the brain. This procedure can be performed in the emergency unit, in the ward under sedation, or in theatre while the patient is asleep. A lumbar drain is a catheter that is inserted in the subarachnoid space, which is a space where the CSF is contained. A needle is first inserted in the lumbar region then the catheter is put into the subarachnoid space. In children, this is performed in the theatre while the child is under general anesthesia. This allows for CSF drainage during brain tumour surgery to help reduce the pressure in the brain.

What will happen to me if I decide to let my child take part in the study?

If you agree to your child's participation in the study, you will be given a Consent Form to sign. We will review your child's file, ask you a few questions and do a clinical examination. During your hospital stay, CSF will be collected as described above. The results of your CSF analysis will be given to you at the outpatient clinic. Your child's participation in the study is voluntary and either of you may decide to discontinue at any point without giving a reason. Discontinuation will not affect your child's medical management. You child will not get any money for participating in the study and it will not cost anything either.

Confidentiality and Privacy.

The study participants' identity and personal information will be kept confidential in a secure database hosted by Wits University. Only the author and supervisors will have access to the information. The participants will be given a research identification number. The participants' identity will not be revealed in any presentation or publication.

What will happen to the results of the research?

The results of the study will be presented as an M Med degree, presented in publications or academic meetings. Any participant or parent/guardian may see a summary of the study on request, free of charge.

Contact details of the researcher.

If you have any questions regarding the study, please feel free to contact Dr G. Ndziba, cell: 0769017567, e-mail: gndziba90@gmail.com.

My supervisor: Dr R Khohomela, cell: 0826948622, e-mail: rambsta@gmal.com

Who has reviewed this study?

This study has been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Professor Paul Ruff, who may be contacted via any one member of the secretariat. The telephone numbers for the Committee secretariat are 011 717 2700/1234/2656/1252 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za, Rhulani.Mukansi@wits.ac.za, Mapula.Ramaila@wits.ac.za and Iain.Burns@wits.ac.za

Thank you for reading this Study Information Sheet.

September 2023

APPENDIX E

PATIENT INFORMATION SHEET FOR MINORS.

Study title: Efficacy of CSF analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg.

Investigator: Dr Gugu Ndziba.

Supervisors: Prof J. Ouma, Dr R. Khohomela.

Greetings

Good day, my name is Dr. Gugu Ndziba. I am a neurosurgical registrar at Chris Hani Baragwanath Hospital. I am doing a study on the benefit of CSF analysis in diagnosing children with brain tumours. CSF analysis means performing laboratory tests on the fluid that cushions the brain and spinal cord to help diagnose diseases found in this region. I am undertaking this study, in part, to obtain a M Med degree from Wits University.

Invitation to participate

You are invited to be part of a research study that will be investigating the benefit of CSF analysis in children that have brain tumours. The purpose of this Information Sheet is to give you information about the study so you can make an informed decision about whether you would like to participate. Should you require any translation, or interpretation of the Information Sheet please inform the investigator for assistance (contact details below).

Once you understand everything and if you agree to participate in the study, you will be given an assent form to sign, and your parents or guardian will be given a consent form to sign. You may only participate if both you and your parent or guardian agree.

What is the study about?

The study will be looking at the benefit of analyzing CSF in children that have brain tumours. A brain tumour is an abnormal growth of cells, it can either be a slow-growing tumour, known as a benign tumour, or a very fast-growing and aggressive tumour which is known as a malignant tumour. CSF is a clear fluid that surrounds and cushions the brain and the spinal cord. Because CSF is close to the brain, tumour cells may be found in the CSF, therefore, sending this fluid to the laboratory might make it easier and faster to diagnose these tumours.

How will the study be conducted?

Those who agree to participate in the study and meet the criteria will be enrolled on admission to the ward. During their hospital stay, 15 ml of CSF will be collected before the operation using a device called an external ventricular drain (EVD). CSF can also be collected in theatre using a device called the lumbar (lower back) drain or during the operation from the CSF that is cushioning the brain. An EVD is a small pipe (catheter) that is 3.3mm in diameter and 35cm in length. This pipe is inserted in the ventricles, which are small chambers deep in the brain where CSF is produced and stored. To gain access to these chambers, the surgeon drills a 1x1cm burhole on the skull, while the patient is sedated or asleep, and then inserts the EVD catheter into the ventricles. EVD insertion and CSF drainage are part of the routine

management of children presenting with brain tumours and features of increased pressure in the brain. This procedure can be performed in the emergency unit, in the ward under sedation, or in theatre while the patient is asleep. A lumbar drain is a catheter that is inserted in the subarachnoid space, which is a space where the CSF is contained. A needle is first inserted in the lumbar region then the catheter is put into the subarachnoid space. In children, this is performed in the theatre while the child is under general anesthesia. This allows for CSF drainage during brain tumour surgery to help reduce the pressure in the brain.

What will happen to me if I decide to take part in the study?

If you agree to take part in the study, you will be given an Assent Form to sign. We will review your file, ask you a few questions and do a clinical examination. During your hospital stay, CSF will be collected as described above. The results of your CSF analysis will be given to you at the outpatient clinic. Your participation in the study is voluntary, you are allowed to discontinue at any point without giving a reason. Your discontinuation will not affect your medical management. You will not get any money for participating in the study and it will not cost anything either.

Confidentiality and Privacy.

The study participants' identity and personal information will be kept confidential in a secure database hosted by Wits University. Only the author and supervisors will have access to the information. The participants will be given a research identification number. The participants' identity will not be revealed in any presentation or publication.

What will happen to the results of the research?

The results of the study will be presented as an M Med degree, presented in publications or academic meetings. Any participant or parent/guardian may see a summary of the study on request, free of charge.

Contact details of the researcher.

If you have any questions regarding the study, please feel free to contact Dr G. Ndziba, cell: 0769017567, e-mail: gndziba90@gmail.com.

My supervisor: Dr R Khohomela, cell: 0826948622, e-mail: rambsta@gmail.com

Who has reviewed this study?

This study has been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Professor Paul Ruff, who may be contacted via any one member of the secretariat. The telephone numbers for the Committee secretariat are 011 717 2700/1234/2656/1252 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za, Rhulani.Mukansi@wits.ac.za, Mapula.Ramaila@wits.ac.za and Iain.Burns@wits.ac.za

Thank you for reading this Study Information Sheet.

September 2023

APPENDIX F

The Superintendent

Chris Hani Baragwanath Academic Hospital

26 Chris Hani Road

Diepkloof

Dear Sir/Madam

I am Dr G. Ndziba, a registrar in the neurosurgical department at the University of Witwatersrand. With your permission, I would like to conduct a research project at CHBAH, titled: ***EFFICACY OF CEREBROSPINAL FLUID ANALYSIS IN THE DIAGNOSIS OF PRIMARY PAEDIATRIC BRAIN TUMOURS AT TWO TERTIARY NEUROSURGICAL UNITS IN JOHANNESBURG.***

This research is a requirement for the MMED degree at the university.

I hereby request your permission to use the CHBAH patient records, to review their imaging on PACS and check their results on the NHLS system. The participants will first be given an information sheet about the study. Once they have read, understood and agree to take part in the study, they will then be required to sign a consent form.

Attached to this letter are copies of protocol, patient information sheet and consent form. Data gathered from this study might prove beneficial in the future management of children suffering from the same condition.

Kind Regards

Dr G Ndziba

Neurosurgery registrar

APPENDIX G

The Superintendent

Nelson Mandala Children Hospital

6 Jubilee road

Parktown

Johannesburg

Dear Sir/Madam

I am Dr G. Ndziba, a registrar in the neurosurgical department at the University of Witwatersrand. With your permission, I would like to conduct a research project at NMCH, titled: ***EFFICACY OF CEREBROSPINAL FLUID ANALYSIS IN THE DIAGNOSIS OF PRIMARY PAEDIATRIC BRAIN TUMOURS AT TWO TERTIARY NEUROSURGICAL UNITS IN JOHANNESBURG.***

This research is a requirement for the MMED degree at the university.

I hereby request your permission to use the NMCH patient records, to review their imaging on PACS and check their results on the NHLS system. The participants will first be given an information sheet about the study. Once they have read, understood and agree to take part in the study, they will then be required to sign a consent form.

Attached to this letter are copies of protocol, patient information sheet and consent form. Data gathered from this study might prove beneficial in the future management of children suffering from the same condition.

Kind Regards

Dr G Ndziba

Neurosurgery registrar

APPENDIX H

LETTER TO HEAD OF NHLS AT CHBAH & NMCH.

Dear Sir/Madam

I am Dr G. Ndziba, a registrar in the neurosurgical department at the University of Witwatersrand. I am conducting a research project at CHBAH and NMCH, titled: ***EFFICACY OF CEREBROSPINAL FLUID ANALYSIS IN THE DIAGNOSIS OF PRIMARY PAEDIATRIC BRAIN TUMOURS AT TWO TERTIARY NEUROSURGICAL UNITS IN JOHANNESBURG.***

This research is a requirement for the MMED degree at the university.

I hereby request your permission to use the NHLS system to check and follow up results of my study participants. The participants will first be given an information sheet about the study. Once they have read, understood and agree to take part in the study, they will then be required to sign a consent form.

Attached to this letter are copies of protocol, patient information sheet and consent form. Data gathered from this study might prove to be beneficial in the future management of children suffering from the same condition.

Kind Regards

Dr G Ndziba

Neurosurgery registrar

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:

Dr G Ndziba
School of Clinical Medicine
Department of Surgery
Division of Neurosurgery
Medical School
University

E-mail: gndziba90@gmail.com

CC:

Supervisor: Professor J Ouma and Dr R Khohomela
<glioma42@gmail.com>
and <HREC-Medical Research Office@wits.ac.za>

FROM:

Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2023/09/06

REF: R14/49

PROTOCOL NO: **M230317** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Efficacy of cerebrospinal fluid analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R49 Dr G Ndziba

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230317

NAME:
(Principal Investigator) Dr G Ndziba

DEPARTMENT: School of Clinical Medicine
Department of Surgery
Division of Neurosurgery
Medical School
University

PROJECT TITLE: *Efficacy of cerebrospinal fluid analysis in the diagnosis of primary paediatric brain tumours at two tertiary neurosurgical units in Johannesburg*

DATE CONSIDERED: 2023/03/31

DECISION: Approved unconditionally

CONDITIONS:

NOTE: If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Professor J Ouma and Dr R Khohomela

APPROVED BY:

Professor P Ruff, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2023/09/06 **EXPIRY DATE:** 2028/09/05

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **March** and therefore reports and re-certification will be due in the month of **March** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

Date

University of Witwatersrand-1.docx

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