DIAGNOSTIC ACCURACY OF CYTOLOGY SMEAR AND FROZEN SECTION IN GLIOMA

DR. SARAH BINTI ZULKARNAIN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (ANATOMICAL PATHOLOGY)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

WHO	: World Health Organization
CT	: Computed tomography
MRI	: Magnetic-resonance imaging
CNS	: Central nervous system
IDH	: Isocitrate dehydrogenase
H&E	: Haematoxylin and Eosin
USM	: Universiti Sains Malaysia
LIS	: Lab information system
SPSS	: Statistical Package for Social Sciences
CBTRUS	: The Central Brain Tumor Registry of the United States
DNOR	: Danish Neuro-Oncology Registry
PPV	: Positive Predictive Value
NPV	: Negative Predictive value
MGG	: May-Grünwald Giemsa
PAP	: Papanicolaou
HREC	: Human Research Ethics Committee
FISH	: Fluorescence In Situ Hybridization
PCR	: Polymerase Chain Reaction
IDH1/IDH2	: Isocitrate dehydrogenase 1/ Isocitrate dehydrogenase 2
ATRX	: Alpha thalassemia/mental retardation syndrome X-linked
RELA	: Reticuloendotheliosis viral oncogene homolog A
NOS	: Not otherwise specified
TSC1/TSC 2	: Tuberous sclerosis1/Tuberous sclerosis 2

GFAP	: Glial fibrillary acidic protein
APJCP	: Asian Pacific Journal of Cancer Prevention

ABSTRAK

Pengenalan: Glioma adalah kanser otak yang paling kerap berlaku dan diagnosis adalah berdasarkan ujian lumuran sitologi, hirisan beku dan histopatologi. Diagnosis patologi glioma semasa intraoperatif memainkan peranan yang penting dalam menentukan rawatan lanjut pesakit. Kajian ini bertujuan untuk menilai ketepatan penemuan dalam lumuran sitologi dan hirisan beku untuk mendiagnosis glioma setelah dibandingkan dengan hirisan parafin.

Metodologi: Satu kajian rentas telah dijalankan melibatkan 22 kes glioma yang didiagnosis ketika pembedahan dari Januari 2013 hingga Ogos 2019. Data klinikopatologi dikumpul melalui laporan patologi. Tisu-tisu yang terpilih telah diproses menggunakan teknik lumuran sitologi dan hirisan beku. Lebihan tisu kemudian diproses kepada hirisan parafin. Keputusan yang diberi sama ada gred rendah atau gred tinggi adalah berdasarkan bilangan sel, ciri pleomorfik nuklear, kiraan mitosis, percambahan salur darah dan nekrosis. Sensitiviti dan spesifisiti lumuran sitologi dan hirisan beku dikenalpasti setelah dibandingkan dengan hirisan parafin yang merupakan ujian standard emas. Ketepatan kedua-dua teknik dibandingkan menggunakan analisis statistik.

Keputusan: Secara keseluruhan, sensitiviti dan spesifisiti lumuran sitologi adalah 100% dan 76.9% sementara sensitiviti dan spesifisiti hirisan beku adalah 100% dan 84.6%. Tiada perbezaan yang signifikan untuk ketepatan mendiagnosis glioma semasa intraoperatif di antara lumuran sitologi dan hirisan beku ($p>0.05$).

Perbincangan: Spesifisiti yang sederhana bagi lumuran sitologi adalah disebabkan lumuran yang tebal dan signifikan artifak hancur manakala bagi hirisan beku, artifak kristal ais adalah penyebab utama. Ketepatan diagnostik bagi lumuran sitologi hampir sama dengan hirisan beku kerana lumuran sitologi juga mampu memberikan bilangan sel dan morfologi yang baik.

Kesimpulan: Lumuran sitologi boleh menjadi satu kaedah alternatif kepada hirisan beku. Lumuran sitologi merupakan kaedah yang pantas, kos yang rendah, menggunakan tisu yang sedikit dan kurang memerlukan kemahiran teknikal. Hasil kajian ini boleh dimanfaatkan oleh hospital atau pusat rawatan yang tidak mempunyai kemudahan hirisan beku.

ABSTRACT

Introduction: Glioma is the commonest primary malignant brain tumour and diagnosis is based on cytology smear, frozen section and histopathological examination. Intraoperative pathological diagnosis using either cytology smear, frozen section or combination of both, plays a crucial role in patient's future management and prognosis. This study aims to determine the accuracy of cytology smear and frozen section in glioma, and to compare the difference between both techniques.

Methods: A cross-sectional study was conducted involving 22 cases of glioma diagnosed intraoperatively from January 2013 until August 2019 in Hospital Universiti Sains Malaysia. The selected tissues were processed for cytology smear and frozen section. The remaining tissues were proceeded for paraffin section. The diagnosis was categorized as either low-grade or high-grade glioma based on cellularity, nuclear pleomorphism, mitotic count, microvascular proliferation and necrosis. The sensitivity and specificity of the frozen section and cytology smears were determined based on paraffin section being as the gold standard. The accuracy of both techniques was compared using statistical analysis.

Results: The overall sensitivity and specificity of cytology smear were 100% and 76.9%, respectively. Meanwhile, the sensitivity and specificity of frozen section were 100% and 84.6%. There was no significant difference in diagnostic accuracy between cytology smear and frozen section in glioma ($p>0.05$).

Discussions: The moderate specificity in the cytology smear was contributed by thickened smear and marked crush artefact. Meanwhile, ice crystal artefact led to moderate specificity in frozen section. The diagnostic accuracy of cytological smear was almost equal to that of frozen section in glioma as the former was also able to provide good cellularity and morphology on smear.

Conclusions: Cytology smear provides an alternative method for frozen section. Cytology smear is rapid, inexpensive, small amount of tissue requirement and less technical demand. This finding may benefit to the hospital or treatment centres where frozen section facility is unavailable.

CHAPTER 1 : INTRODUCTION

1.1 Epidemiology

Primary brain tumour is a heterogenous brain tumour arising from the intracranial and meningeal cells. According to the Central Brain Tumour Registry of the United States report, primary brain tumour account for 2% of total number of cancer cases with an overall annual incidence of 22 per 100 000 population. Among these, meningioma is the commonest benign lesion while glioma is the commonest malignant primary brain tumour. The latter is the most commonly diagnosed histologically in all age group. Gliomas account for 75% of malignant brain tumours, and more than half out of these are glioblastomas (Ostrom *et al.,* 2017). The anaplastic astrocytoma and glioblastoma are the commonest among the older age group (75-84 years old) however oligodendroglioma is common in people of middle age (35-44 years). On the other hand, the most common paediatric age group is between 1-4 years of age whereby pilocytic astrocytoma being the commonest type of glioma (Lapointe *et al.*, 2018). In other study, glioma accounts about 24.7% of all primary brain tumour (Gupta *et al.,* 2005).

In Malaysia, primary brain tumour is the 11th most common cancer in male, 2.5% of total cancer cases. In female, it is the 13th most common cancer that account for 1.9% from total cancer cases. It is slightly more predominant in male with highest incidence among the Chinese followed by Malay and Indian. Glioma accounts for 51% of total brain tumour in Malaysia and the most common subtype is also glioblastomas (Azizah *et al.,* 2016).

1.2 Gliomas

Glioma is the primary brain tumour arising from the glial tissue which include astrocytoma, oligodendroglioma, oligoastrocytoma or ependymoma. The diagnosis is based on clinical evaluation, neuroimaging modalities and histopathological assessment.

1.3 Clinical manifestations

The clinical manifestations of glioma may appear abrupt over days to weeks or progressively over months to years which depends on the location of the tumour and the speed of growth. More significant neurological deficits are observed if the tumour occur at the functional areas. For instance, in frontal lobe tumour, the patient may complain weakness or dysphasia whereas patient may have visual disturbance if the tumour involves the optic radiation. Cognitive dysfunction such as short-term memory deficit or behaviour changes might happen if the tumour is located at the prefrontal lobe, temporal lobe, or corpus callosum. Infratentorial tumours can manifest with cerebellar dysfunction, cranial-nerve palsies or long-tract signs. The patient can present with generalized symptoms such as seizure and symptoms of intracranial pressure. The latter includes headache worsening at night, blurring of vision, vomiting and sometimes diplopia (Louis *et al.,* 2016).

1.4 Tumour location and neuroimaging

Pilocytic astrocytoma, WHO grade I commonly occur in infratentorial region. Computed topography (CT) and magnetic resonance imaging (MRI) show well circumscribed lesion and two third of cases display cystic lesion with enhancing mural nodule. Focal contrast enhancement and occasional calcification may be visualised (Ullrich *et al.,* 2008, Louis *et al.,* 2016). Diffuse astrocytoma, WHO grade II and anaplastic astrocytoma, WHO grade III can develop at any central nervous system (CNS) region, preferably in the frontal lobe (Stockhammer *et al.,* 2012, Louis *et al.,* 2016). CT scan usually shows an ill-defined homogenous mass of low density for both lesions. In addition, partial contrast enhancement and perifocal oedema is visualized in anaplastic astrocytoma. The masses are hypodense at T1 and hyperdense at T2 on MRI (Ullrich *et al.,* 2008).

Glioblastoma, IDH-mutant predominantly located in frontal region as well as basal ganglia and thalamus. The mass is usually large in size which composed of cystic and diffuse area with less perifocal oedema (Eoli *et al.*, 2007, Lai *et al.*, 2011). Glioblastoma, IDH-wild type in contrast is commonly localized in the subcortical white matter and deeper grey matter of the cerebrum. It appears irregular in shape on CT scan with hyperdense ring-shaped zone of contrast enhancement around the hypodense central area of necrosis (Louis *et al.*, 2016).

Oligodendroglioma, WHO grade II and anaplastic oligodendroglioma, WHO grade III have predilection in the white matter and cortex of the cerebrum which is most commonly at frontal lobe. The lesions presented with well-circumscribed hypodense and isodense mass with frequent calcification. Heterogenous patterns are visible as a result of secondary changes that include intratumoural haemorrhages, cystic degeneration and necrosis which can be visualized in anaplastic oligodendroglioma (Louis *et al.* 2016).

Majority of ependymoma located in the posterior fossa (60%) followed by supratentorial compartment and least in the spinal canal (10%). The mass is well-circumscribed with different degrees of contrast enhancement on Gadolinium-enhanced MRI. Heterogenous patterns as in anaplastic oligodendroglioma can be occasionally seen. Due to its location, the tumour can cause ventricular obstruction, brain stem displacement and hydrocephalus (Ullrich *et al.*, 2008, Louis *et al.*, 2016).

1.5 Role of intraoperative diagnosis

Although advancement of neuroimaging has been established, histopathological examination remains the gold standard in diagnosing glioma (Brat *et al.*, 2008).

The surgeon will request for intraoperative assessment to obtain rapid diagnosis. Intraoperative histopathological diagnosis plays a crucial role in guiding the clinician on optimization of surgical resection, confirmation of diagnostic tissue adequacy, the

therapeutic plan and predicting the prognosis of patient diagnosed with glioma. In rare occasion, this modality can detect the unexpected lesion which may not be capture during neuroimaging. The methods for intraoperative histological diagnosis constitute either frozen section, cytology smear or combination of both techniques.

Frozen section initially has been the established method worldwide since it was first introduced in 1891. Frozen section is a rapid technique and it provides good overall architectural characteristic. The major detriment of this pertaining technique is ice crystal artefact or freezing artefact which may hinder the true morphology of the tumour. Apart from that, it requires specific costly machine and skilful technician in which some centres are unable to provide them.

Cytology smear was eventually introduced and there was an increased usage of this technique for supplementing or replacing the use of frozen section technique (Jaiswal *et al.,* 2012). This is mainly due to rapid technique, lower expense, technically much easier and requires only a small piece of tissue ~1-2 mm^3 for each slide (Sharifabadi *et al.,* 2016). It also provides an alternative method when facilities for frozen section are limited (Ahmed *et al.,* 2014). Recently, there is frequent usage of streotactic biopsy procedure in which small brain tissue biopsy is taken with the aid of CT imaging technique. This procedure is frequently used for small or deep-seated intracranial tumour which is unsuitable for radical excision (Ostertag *et al.,* 1980, Jackson *et al.,* 2001). As this procedure was introduced, invasive craniotomy procedure can be avoided leading to reduce mortality and morbidity of patient. Subsequently, the tiny tissue produced from this procedure has led to more frequent usage of cytology smear technique.

Cytology smear technique comprises of crush smears and touch imprints. In crush preparation, the fresh tissue is gently crushed on a glass slide by a second slide held at right angle. Meanwhile, the fresh tissue is gently touched on a glass slide using a forcep

in touch imprint. Both air dried smears and 95% ethyl alcohol fixed imprints will be stained using hematoxylin and eosin (H&E) stains.

1.6 Hospital Universiti Sains Malaysia as centre of study

In Malaysia, brain tumour is primarily managed at the tertiary healthcare centre and teaching hospitals. Hospital Universiti Sains Malaysia (Hospital USM) is one of the teaching hospitals in Malaysia which act as a centre of excellence for East Coast Malaysia. The hospital is located in Kota Bharu, Kelantan. Kelantan is one of the main states in peninsular Malaysia. Majority of the population in Kelantan is Malay followed by the Chinese population and small proportion of Indian and Siamese population.

1.7 Rationale of Study

The neurosurgeons will send intraoperative small tissue samples for histopathological diagnosis using cytology smear and frozen section methods. Hence, scientific evidence is required for the diagnosis of brain tumour specifically glioma being the most common malignant brain tumour based on local study. To our knowledge, no local study comparing diagnostic accuracy of cytology smear and frozen section in glioma. We also would like to see whether there is significant difference between both techniques. This study is beneficial for centres that are lacking frozen section facilities whereby cytology smear can be applied as an alternative method to frozen section.

CHAPTER 2 : OBJECTIVES OF THE STUDY

2.1 General objective

To study the accuracy of cytology smear and frozen section in glioma based on paraffin section

2.2 Specific objectives

1. To determine the sensitivity and specificity of cytology smear in glioma based on paraffin section
2. To determine the sensitivity and specificity of frozen section in glioma based on paraffin section
3. To determine the difference in the proportion of accuracy between cytology smear and frozen section in glioma based on paraffin section

2.3 Research hypothesis

1. Cytology smear has good specificity and sensitivity based on paraffin section
2. Frozen section has good specificity and sensitivity based on paraffin section
3. There is no significant difference in the accuracy of cytology smear and frozen section based on paraffin section

CHAPTER 3 : MANUSCRIPT

3.1 Title

DIAGNOSTIC ACCURACY OF CYTOLOGY SMEAR AND FROZEN SECTION IN GLIOMA

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3.2 Abstract

Introduction: Glioma is the commonest primary malignant brain tumour. Diagnosis is made based on cytology smear, frozen section and histopathological examination. Intraoperative pathological diagnosis using either cytology smear, frozen section or combination of both, plays a crucial role in patient's future management and prognosis. This study aims to determine the accuracy of cytology smear and frozen section in glioma, and to compare the difference between both techniques.

Methods: A cross-sectional study was conducted involving 22 cases of glioma diagnosed intraoperatively from January 2013 until August 2019 in Hospital Universiti Sains Malaysia. The selected tissues were processed for cytology smear and frozen section. The remaining tissues were proceeded for paraffin section. The diagnosis was categorized as either low-grade or high-grade glioma based on cellularity, nuclear pleomorphism, mitotic count, microvascular proliferation and necrosis. The sensitivity and specificity of frozen section and cytology smears were determined based on paraffin section being as the gold standard. The accuracy of both techniques was compared using statistical analysis.

Results: The overall sensitivity and specificity of cytology smear were 100% and 76.9%, respectively. Meanwhile, the sensitivity and specificity of frozen section were 100% and 84.6%. There was no significant difference in diagnostic accuracy between cytology smear and frozen section in glioma ($p>0.05$).

Conclusions: Cytology smears provides an alternative method for frozen section due to good cellularity and morphology on smear. Cytology smear is rapid, inexpensive, small amount of tissue requirement and less technical demand. This finding may benefit to the hospital or treatment centres where frozen section facility is unavailable.

Keywords: Diagnostic accuracy, cytology smear, frozen section, glioma

3.3 Introduction

Glioma is a central nervous system tumour arises in glial tissue and it can be astrocytoma, oligodendroglioma, oligoastrocytoma or ependymoma. Worldwide, glioma is the most common primary intracranial malignancy. Glioma accounts for 75% of malignant brain tumours, and more than half out of these are glioblastomas.[1] Intraoperative histopathological diagnosis plays a crucial role in optimizing the surgical procedure and determining the treatment plan of the patient. In certain situation, it can detect an unexpected lesion that cannot be determined by clinical or radiological imaging. Frozen section has been an established intraoperative histopathological evaluation worldwide since it was first introduced in 1891. Subsequently, cytology smear was introduced later and there was an increased usage of this technique for supplementing or replacing the use of frozen section technique.[2]

Cytology smear techniques include squash smears and touch imprints are rapid, less expensive, technically much easier and requires only a small piece of tissue for each slide with high percentage of accuracy between 84.9%-95.36%.[3-9] It also provides an alternative method when facilities for frozen section are limited.[10]

Frozen section technique is rapid and able to demonstrate the architectural characteristic however the major drawback of using this technique is freezing artefact. Besides that, this technique requires trained staff and an expensive equipment.

3.4 Methodology

3.4.1 Case and sample selection

This study was a cross-sectional study conducted at Hospital Universiti Sains Malaysia (USM) involving 22 cases of glioma diagnosed intraoperatively within period of 6 years and 7 months from January 2013 until August 2019. The retrospective samples were obtained from the archived Lab Information System (LIS). Frozen section, cytology

smear and paraffin slides were retrieved from the Hospital USM lab. The demographic data and neuroimaging findings were collected through patient's pathology reports.

For prospective samples, the samples were obtained from the surgeon intraoperatively. The selected tissues were processed for cytology smear and frozen section. The remaining tissues were proceeded with paraffin section. 22 cases fulfilled the inclusion criteria. Patients diagnosed with brain tumour other than glioma or unavailability of cytology smear and/or frozen section and/or paraffin section were excluded.

The diagnosis was categorized as either low grade or high grade glioma according to St. Ann-Mayo grading system that include cellularity, nuclear pleomorphism, mitotic count, microvascular proliferation and necrosis. WHO grade I and II were grouped as low grade glioma while WHO grade III and IV were consider as high grade glioma. The sensitivity and specificity of the cytology smear and frozen section were determined on paraffin section being as the gold standard. The accuracy of both techniques was compared using statistical analysis.

The sample size was calculated using the estimation of sensitivity and specificity sample size calculator by Dr Lin Naing @ Mohd Ayub Sadiq from School of Dental Sciences, USM. The estimated sample size to answer the objectives was 48 calculated based on the expected sensitivity and specificity were 100% and 86.21% respectively.[10] This study was approved by the Human Research Ethics Committee, USM with the reference USM/JEPeM/18010083.

3.4.2 Cytology smears

The cytology smear's methods applied were touch imprint and crush smear. In touch imprint, the fresh unfixed specimen was gently touched on a labelled glass slide using a forceps. Fresh unfixed specimen was gently crushed on a labelled glass slide by

a second slide held at right angle for crush smear. Both air dried smears and 95% ethyl alcohol fixed smears were stained using hematoxylin and eosin (H & E) stains.

3.4.3 Frozen section

The specimens were processed in cryostat machine at -22^{o} celcius to -26^{o} celcius. Initially, the specimens were described and measured. Then, they were placed on the chuck which has spread with cryo compound. The chuck was inserted to the location hole and oriented accordingly. Subsequently, the specimens were trimmed in order to obtain a plane parallel surface. Sectioning started at approximately 5-8 µm in thickness, then the sectioning thickness will be decrease to the required value. Finally, sectioned slides were stained using rapid H & E stain and for slide mounting. The remaining tissues were fixed in formalin for paraffin section preparation and subsequently stained for H and E stain.

3.4.4 Statistical analysis

The clinicopathological information were analysed using the descriptive statistic. The sensitivity, specificity, positive predictive value and negative predictive value were calculated using specific formula for each value. Mc Nemar test was used to determine the significant difference in the proportion of accuracy between cytology smear and frozen section in glioma from the same specimens using Statistical Package for Social Sciences (SPSS) software version 20.

3.5 Results

3.5.1 Clinicopathological data

The clinicopathological data for glioma is presented in Table 3.1. A total of 22 glioma cases were included in this study. The median age of glioma cases diagnosed in Hospital USM by cytology smear and frozen section was 31.7. The number of male and female patients diagnosed with glioma were almost equal, total of 12 female patients

(54.5%) and 10 male patients (45.5%). All the patients were Malay. The commonest presenting symptoms were headache whereby 54.5% of the glioma patients presented with this complaint followed by hemiplegia, 45.5%; vomiting, 22.7%; seizure,18.2% and 9.1% each for blurring of vision, speech disturbance, facial weakness and unsteady gait. The common site of glioma occurrence was frontal (40.9%) followed by temporal (9.1%), cerebellum (9.1%), basal ganglia (9.1%), thalamus (9.1%) with parietal, ventricular, pons, corpus callosum and pineal region that account for 4.5% each.

A total of 13 low grade glioma and 9 high-grade glioma cases were diagnosed from paraffin section. Meanwhile, 11 cases each for low grade and high grade glioma were diagnosed based from frozen section. Cytology smears diagnosed 10 cases of low grade glioma with 12 cases of high grade glioma. The morphologies of the examined slides are shown in Figure 3.1-3.6.

The distribution of glioma is depicted in table 3.2. Low and high grade glioma were frequently diagnosed after two decades of life with both grades having 8 out of 22 cases (36.4%). Low grade glioma was common among male (40.9%) in comparison with high grade glioma which was common in female (27.3%). Majority of the low grade and high grade glioma arise from the frontal region of the cerebral hemisphere (18.2 % in low grade and 22.7% in high grade glioma).

3.5.2 Sensitivity and specificity of cytology smear and frozen section

The overall sensitivity of cytology smears was 100% while only 10 out of 13 low-grade glioma cases were able to detect giving the overall specificity of 76.9%. The positive predictive value and negative predictive value for frozen section were 75% and 100%, respectively.

The overall sensitivity of frozen section was 100% where frozen section able to detect all 9 high grade glioma cases. Frozen section able to detect only 11 low grade

glioma cases from total of 13 low grade glioma cases giving the overall specificity of 84.6%. The positive predictive value and negative predictive value for frozen section were 81.8% and 100%, respectively. These findings are summarized in table 3.3.

3.5.3 Difference in the proportion of accuracy between cytology smear and frozen section

There are 19 glioma cases that were correctly graded by cytology smear and frozen section. On the other hand, grading was inaccurate in 2 of the cases by both methods. There is only a glioma case that was correctly graded by frozen section in which cytology smear failed. Based on Mc Nemar test, there was no significant difference in the proportion of accuracy between cytology smear and frozen section, giving p value of >0.05 (table 3.4).

3.6 Discussion

Glioma is the commonest malignant brain tumour in United States and Malaysia.[1,11] Glioma also has been the predominant neoplastic lesion in most of the studies.[4,5,8,12-15] This tumour possesses distinct clinicopathological characteristic with regards to the age, sex and tumour location.

In our study, both genders were almost equally affected whereas according to the latest Malaysian National Cancer of registry from 2007-2011, brain tumour was predominant in male and it was the 11th commonest cancer in male compare to female .[11] The Central Brain Tumor Registry of the United States (CBTRUS) reported from 2010 to 2014, 42 % of all brain and other central nervous system (CNS) occur in male while slightly higher percentage was visualized in female, 57.9%. However, malignant brain tumour cases were common in male, 55.4% compared to females, 44.6% whereby glioma specifically glioblastoma being the commonest malignant brain tumour.[1] In Denmark, Danish Neuro-Oncology Registry (DNOR) 2009 to 2014 reported that there was slight predominant of glioma in male with overall male:female ratio of 3:2.[16]

Based on Malaysian National Cancer of Registry from 2007-2011, higher incidence rate of brain and nervous system cancer was observed in Chinese followed by Malay and Indian.[11] However, all the cases were from Malay population in our study. In term of ethnicity, the finding was representative of population in Kelantan which composed of 99% of Malay population followed by Chinese, Indian and minority of Siamese population.

In general, glioma can affect wide range of age which ranges from paediatric age group until older age group. In our study, the mean age group was 31.7. Majority of both low grade and high grade glioma occurred after two decades of life in our study. Older age group were observed in United States with median age of 59 years old.[1] All of our paediatric cases were diagnosed as low grade glioma in contrast with high grade glioma which only occurred at older age group in our study. These findings were agreed by other studies whereby Danish Neuro-oncology stated that the mean age increased with the tumour grading of glioma.[16] To be more specific on histological types related to age of diagnosis, pilocytic astrocytoma is common in younger age group with age adjusted incidence rate of 0.89 per 100 000 population and the incidence decrease as the age advanced.[1] On the other hand, glioblastoma is common among older age group and the incidence increase as the age recline.

The distribution of tumour site depends on the subtype of glioma. In general, malignant tumours occur at frontal (23.7%), temporal (17.4%), parietal (10.5%), and occipital (2.7%) of the cerebral hemispheres.[1] Similar location trends were observed in our study where frontal and temporal are the commonest tumour location. If we divide based on glioma subtype, high grade glioma is commonly situated at the frontal lobe. Some of the studies concluded Glioblastoma (IDH-mutant), WHO grade IV; anaplastic astrocytoma, WHO grade III and anaplastic oligodendroglioma, WHO grade III predominantly located in frontal region.[17,18]

The role of neurosurgical, neuroimaging and neuropathological experts are crucial in diagnosing and managing patient with glioma. The pathologist must be well-experienced and knowledgeable in handling glioma cases. Before treatment of patient is commenced, appropriate diagnosis must be made. The main role of pathologist is to provide the accurate diagnosis from the brain tissue sent by the neurosurgeon, as histological examination remained the gold standard for diagnosis of glioma.[19] Certain indications need immediate intraoperative diagnosis by the pathologist. The main goal for intraoperative diagnosis of glioma is to plan the best treatment for the patient.

Other reasons include guidance for the surgeon on the extent of surgery, to determine the adequacy of tissue sample especially in cases where the tumour contain extensive areas of necrosis, to differentiate between neoplastic and nonneoplastic lesion or low grade and high grade glioma especially in situation whereby neuroimaging unable to give conclusive diagnosis as well as in situation where discrepancy of clinical presentation and neuroimaging.[3,7,20]

The laboratory techniques used during intraoperative diagnosis are either cytology smears, frozen section or both depends on the availability of resources. Thus, determination of diagnostic accuracy of both techniques in glioma, understanding the strength and limitation of each in addition to clinicopathology characteristic are of major importance to ensure the best and optimum management of patient with glioma.

Accuracy depends on complete clinical and radiological findings, sampling, technical performance and competency of the pathologist.[9,21-23]

Frozen section has been the established and preferred modalities for intraoperative diagnosis of glioma. Histology architectural detail and cytomorphology are best visualized by frozen section. The major drawback of frozen section is freezing artefact. On the other hand, cytology smear is rapid, low-cost, effortless, does not required

experienced staff and expensive equipment. This technique is very useful when limited tissue sample is provided by the neurosurgeon especially in the recent advancement of stereotactic biopsy. Thus, more tissue is available for further paraffin section.

It said that cytology smear and frozen section complement each other as each technique overcome the disadvantages of the other technique. Cytology smears are best in diagnosing glioma due to its soft consistency and high water content.[8] As high water content can cause marked freezing artefact in frozen section that may destroy the architecture and cytomorphology of the tumour. Moreover, cytology smear can be smeared easily and displayed good cytoarchitectural detail. Cytology smear visualized better tumour matrix specifically glial fibrillary background where gliomas will give cotton-like appearance. It can differentiate whether the matrix is reactive process or high grade glioma based on the quality of the fibrillary filament. Fine filament is present in reactive process while coarse filament as in high grade glioma.[24]

One of the features in pilocytic astrocytoma which is Rosenthal fibers are well appreciated in cytology smear.[18] Other than that, the nuclear and cytoplasmic details are better observed by cytology smear.[22] The procedure involving the cytology smear maintained the nuclear and cytoplasmic characteristic of the tumour cells. Unlike in frozen section where freezing artefacts will cause crenated nuclei.[24] This will lead to difficulties in making final diagnosis as nuclear characteristic is hardly appreciated.

Cytology smear techniques include touch imprint and crush smear. From our study, touch imprint is very helpful in high cellularity tumour as crush smear introduced marked crush artefact which hinder the true cytoarchitectural details. The thickness of the smear produced was even and the architectural details such as rosettes formation, Rosenthal fibres and microcystic areas were maintained. These architectures may be demolished if high pressure is applied in crushed smear. These findings are supported by

some studies.[3,6] Touch imprint is useful in cases of lymphoma and metastatic adenocarcinoma.[7] However, touch imprint is not recommended for low cellularity tumour as this will lead to low-cellular yield which cause limited availability of the sample for analysis.

We graded the glioma based on St. Anne-Mayo, 4-tiered grading system that includes tumour cellularity, presence of nuclear atypia, endothelial proliferation, increase mitosis and necrosis. This grading system are widely used in most of the studies.[19,25] In low grade glioma, the cellularity is low and the cells display uniform nuclei. Minimal anisocytosis is acceptable which can be observed in some cases. In our study, vascular proliferation may be visualized in low grade glioma but the size of the vessels is small. None of low grade glioma in our study exhibit vascular proliferation of large in size which is observed in high grade glioma case. Occasional mitosis (not more than 1) is allowed specifically in grade II.[19]

In contrast, high grade glioma required at least 2 or more features to diagnose.[20] The cellularity is mostly high with significant nuclear atypia. The criteria of nuclear atypia include nuclear pleomorphism, enlarged hyperchromatic nuclei, coarse chromatin and some cells exhibit prominent nucleoli. Multiple large vessels proliferation, increase in mitosis and necrosis are sometimes visualized. In our study, all high grade glioma cases showed high cellularity and marked nuclear atypia. Majority of the cases contained multiple large vessels proliferation while three of the cases displayed prominent necrosis. Mitosis is hardly identified in which only one case noted to have mitosis.

In our current study, the overall diagnostic accuracy of cytology smear was 86.3%. The result is comparable with other studies with the accuracy ranging from 84.9-91.25%.[5,8,9,26] 19 out of 22 cases were correctly graded. 3 of the cases were overgraded as high grade glioma whereby the paraffin section revealed as low grade glioma. This

happened because of marked crushed artefact in crushed smear which demonstrates anisonucleosis and due to thickened smear, that was subsequently misinterpreted as high cellularity. The limitation of crushed smear and experienced the same problem in a case of low grade oligoastrocytoma which was over diagnosed as high grade glioma.[7] This highlighted the importance of good smearing technique to overcome the shortcomings of crushed smear.

One of the cases was overgraded as high grade glioma due to misinterpretation of vascular proliferation. This situation is significantly relevant in a case of oligodendroglioma where vascular proliferation is one of the characteristics.[8] In addition, vessels proliferation is common in tumour to provide adequate nutrient for the tumour growth. On this basis, only large vessels proliferation is considered for high grade glioma as proven in our study whereby majority of high grade glioma exhibit large vessels proliferation.

In most studies, pathologist encountered some difficulties in grading glioma where it was known for its heterogeneity element.[5,8,12] Different tumour site may reflect different characteristic. To our knowledge, cytology smear consumes small tissue sample that has raised the issue of correct and adequate tissue sampling by the neurosurgeon. Misdiagnosed occurred when wrong tissue site was sampled that do not reflect the true behaviour of the glioma. We encountered a problem in diagnosing one of the tissue samples. The sample that we received was only red blood cells. Good communication between the pathologist and neurosurgeon are mandatory to establish the accurate diagnosis. It is advisable for the pathologist to ask for further sample if the sample is not representative or inadequate. The sample must be adequate and representative for the pathologist to make the diagnosis confidently.

The overall diagnostic accuracy of frozen section in our study was 90.9% supported by other previous studies which were 86.8%-95%.[5,8,26] There were 2 out of 22 cases overgraded as high-grade glioma. This was attributed by the well-known freezing artefact in frozen section. The nuclear pleomorphism and nuclear membrane irregularities caused by freezing artefact were misinterpreted. Some of the other cases encountered difficulties during analysis due to similar reason. Mitosis were also difficult to identify as the cytomorphology of the tumour cells were hampered. Setting the optimal temperature of the machine able to reduce the artefact provided the machine is in good condition. Features include necrosis and microvascular proliferation able to aid the pathologist in grading the glioma if other features were difficult to interpret. Study done by Obeidat et al.[23] experienced similar problem and concluded that these features were reliable to overcome the problem.

Moreover, the tissue thickness and staining quality play roles in making the diagnosis. They can affect the cellularity and cytoarchitectural assessment.[27] Two of the cases were regard as high cellularity due to thickened section. The cytoarchitectural detail was difficult to interpret as the cells overlapped with each other and the tissue were folded. Nuclear hyperchromasia was not well visualized when the cells were understained. This signified the important of competent lab personnel in handling the specimen to produce good quality slides. Sampling adequacy, quality slides preparation, heterogenicity of glioma, pathologist expertise, and communication between pathologists and surgeons are also the major contributing factors in determining the accuracy of frozen section in glioma.

Based on our study, there was no significant difference in diagnostic accuracy of cytology smear and frozen section as both techniques revealed comparable and high diagnostic accuracy. Other studies also conclude the same findings.[7,10,25,28]

This provide an alternative solution to the centres that are lack frozen section facilities and skills.

3.7 Conclusion and limitation

Intraoperative diagnosis of glioma plays a major role in determining the patient management intraoperative and postoperatively. Diagnosis is made by mean of frozen section, cytology smear or both techniques.

Frozen section technique was the established and commonly used technique in most centres. Architectural characteristic and cytomorphology were best demonstrate by frozen section. However, the main limitation was ice crystal or freezing artefact that obscure the true architectural detail that eventually causing misinterpretation. Besides that, this technique demanded well-trained staff and expensive machine.

Cytology smear was introduced later and widely used as the technique is rapid, inexpensive, small tissue requirement and less technical demand when comparing with the frozen section. Cytology smear is recommended for glioma due to soft-nature of the tumour. The cytomorphology as well as architectural detail were better portrayed by cytology smear.

Based on our study, there was no significant difference in the diagnostic accuracy of cytology smear and frozen section techniques. Both techniques showed high percentage of accuracy and sensitivity. This concluded that cytology smear can be as an alternative method to frozen section. This finding may benefit to the centres where frozen section facilities and skills are unavailable.

We found that cytology smear and frozen section were complimentary to each other. This finding was significant when either frozen section or cytology smear failed to demonstrate the architectural detail and cytomorphology of glioma. It is recommended to

use both techniques to improve the diagnostic accuracy if facilities and skills are available.

In addition, complete clinical history, neuroimaging information, adequate sampling by the surgeon, good technical skills and trained neuropathology will help in achieving accurate and rapid diagnosis of glioma. Good communication between the surgeon and pathologist also plays a crucial in management of the patient.

Our main limitation in this study was sample size. Total of 18 cases were not included due to unavailability of the cytology smears, frozen section and/or paraffin slides. Paraffin slide was unavailable in two of the cases due to tissue exhaustion. Some of the cases planned for intraoperative diagnosis were cancelled and some had proceeded for the procedure however unfortunately the diagnosis made was not glioma. We suggest expanding this study to other teaching and government hospitals in Malaysia in order to obtain bigger sample size.

3.8 References

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3.9 Tables

Table 3.1 Clinicopathological distributions of the glioma cases (n=22)

Variables	Mean (SD)	N (%)
Age		
0 to 14 years old		6 (27.3)
15 to 19 years old	31.7 (19.1)	0 (0.0)
20 to 60 years old		16 (72.7)
Gender		
Male		12 (54.5)
Female		10 (45.5)
Race		
Malay		22 (100)
Others		0 (0.0)
Clinical Symptoms		
Headache		12 (54.5)
Hemiplagia		10 (45.5)
Vomiting		5 (22.7)
Seizure		4 (18.2)
Blurring of vision		2 (9.1)
Speech disturbance		2 (9.1)
Facial weakness		2 (9.1)
Unsteady gait		2 (9.1)