CHAPTER ONE: INTRODUCTION

1.1 Introduction

George Papanicolaou (1883-1962) is generally credited with the use of cytopathological examination of cells for diagnosis of tumours. Fine Needle Aspiration Cytology (FNAC) involves subjecting appropriately stained aspirated cells to cytopathological examination where the cells are evaluated for morphological changes based on the appearance of individual cells or cell clusters[9]. Theoretically this procedure is supposed to diagnose most tumours with acceptable sensitivity and specificity.

Salivary gland tumours make up 10% of all head and neck tumours (Parotid gland tumours contribute 2-6.5%) and 3% of all tumours of the body[4, 9, 10, 11]. There are two types of salivary glands, major (Parotid, submandibular and lingual) and minor salivary glands[1]. The Parotid gland tumours are 10 times more common than submandibular tumours and 100 times more common than lingual tumours. 80% of Parotid tumours are benign[1].

The incidence of Parotid gland tumours is 1-2/100,000[1]. There is equal incidence between the sexes[1]. Risk factors for development of Parotid gland tumours include nutritional deficiencies, exposure to radiation, ultra-violet exposure, genetic predisposition and infection with Epstein-Barr virus[1].

Generally, it has been accepted among Head and Neck Surgeons that tumours of the Parotid gland, present management problems for various reasons[4, 11]. In the first instance, most surgeons believe that the presence of a Parotid gland tumour is an indication for removal[4,9,11]. Clinical diagnosis has been used to preoperatively diagnose and influence surgical management of a patient with a Parotid gland tumour. But it is difficult to distinguish clinically between inflammatory and neoplastic lesions, and between benign and malignant lesions for neoplastic lesions[1]. This is because inflammatory lesions are not subjected to surgery but conservative management (non-surgical treatment)[1, 4, 9]. Clinical diagnosis has resulted in over treatment of patients with inflammatory Parotid tumours which have been subjected to surgery and under treatment of patients with malignant Parotid lesions that have been treated by Parotid conserving surgery[4,9,11]. Clinical

diagnosis cannot decide the fate of the facial nerve, whether to excise or not. The excision of facial nerve with its consequence of facial nerve paralysis has resulted in increased morbidity[1,4,9,11].

It remains a challenge to obtain a preoperative tissue diagnosis of Parotid gland tumours because salivary gland tumours are not subject to conventional tissue diagnosis by core needle biopsy or incisional biopsy as these complicate into formation of salivary fistulae and implantation of malignant tumour cells[1, 4, 9, 10, 11,12].

Secondly, a preoperative diagnosis ensures planning of the surgical procedure that needs to be done (whether conservative surgery/partial Parotidectomy or total Parotidectomy and whether to do neck dissection or not in the case of malignant tumours)[1, 4, 9, 10, 11, 12]. However, lack of a preoperative diagnosis poses a challenge to appropriately counsel patients about the preoperative diagnosis, the planned surgical procedure to be done, and whether or not the facial nerve will be preserved[4].

Thirdly, an intraoperative diagnosis at the time of surgery by assessment of frozen sections is not readily available even in most developed centers and is also not available in Zambia[4,9,12].

In view of the above, FNAC has gained popularity among Head and Neck surgeons who have used this procedure to preoperatively diagnose Head and Neck Tumours[4, 9]. The application of FNAC for diagnosis of Parotid gland tumors has been an area that has been widely debated[4, 5, 9, 10, 11].

An audit of the Parotid gland surgeries was performed at UTH between August 2013 and September 2014 as indicated in Table 1. A total of 14 Parotid gland surgeries were done for Parotid gland tumours. 79% were superficial Parotidectomies and 21% were total Parotidectomies. None of the patients had a preoperative diagnosis to objectively ascertain the extent of the surgery to avoid over or under treatment. Only 14% had a post-operative diagnosis the rest had missing histopathology reports or missing excised Parotid samples in the UTH Pathology laboratory, either they were taken to other laboratories or the samples were lost. This supported the need to conduct our own study that could obtained a preoperative diagnosis of the Parotid gland tumours. Such a

preoperative diagnosis could determine whether surgical intervention was required or not and if it was required then it would guide the extent of surgery.

Table 1**:** Audit of Parotid Surgeries done at UTH from August 2013 to September 2014

No.	Date	Clinical Diagnosis	Procedure	Firm	Histopathology
1	07.08.2013	lt. Parotid tumour	Superficial Parotidectomy	Green	Pleomorphic adenoma
2	26.06.2013	Parotid mass	Superficial Parotidectomy	Blue	?
3	08.07.2013	Rt Parotid swelling	Superficial Parotidectomy	Yellow	Pleomorphic adenoma
4	13.09.2013	Parotid mass	Superficial Parotidectomy	Yellow	?
5	17.09.2013	Parotid mass	Superficial Parotidectomy	Red	?
6	08.11.2013	Parotid mass	Superficial Parotidectomy	Yellow	?
7	03.02.2014	Parotid swelling	Superficial Parotidectomy	Red	?
8	18.03.2014	Parotid tumour	Superficial Parotidectomy	Red	?
9	06.06.2014	Parotid mass	Superficial Parotidectomy	white	?
10	26.07.2014	Parotid tumour	Total Parotidectomy	white	?
11	22.08.2014	Parotid tumour	Superficial Parotidectomy	Yellow	?
12	16.09.2014	Parotid mass	Superficial Parotidectomy	Red	?
13	18.09.2014	Parotid tumour	Total Parotidectomy	Yellow	?
14	08.09.2014	Parotid tumour	Total Parotidectomy	Yellow	?

This study evaluated the diagnostic accuracy of FNAC of Parotid gland tumours by correlating it with the histopathological diagnosis. Before this study was conducted, Clinical diagnosis was used to determine the need for surgery and extent of surgery when managing Parotid Tumours. At the

time of operation after obtaining informed consent, an FNAC sample of the Parotid gland tumour planned for excision was obtained while the patient was under anesthesia. The excised Parotid gland tissue was also collected after surgery. Cytopathological and histopathological examinations were performed by different pathologists to avoid bias. The cytopathological and histopathological diagnoses were evaluated for correlation. In this case histopathology was used as the control.

If the correlation between FNAC and histopathology was significant then a cytopathological preoperative diagnosis could start the initial treatment of patients with Parotid gland tumours and it could influence the management of patients with Parotid gland tumours. This would then avoid excess morbidity associate with over treatment of Parotid gland tumours.

1.2 Statement of the Problem

Management of a patient with a Parotid gland tumour is challenging to most clinicians and surgeons[1, 4, 9, 10, 11,12,13], and Zambian surgeons are not an exception to these challenges:

1. Lack of a preoperative diagnosis leads to clinically derived surgical decision making in management of most Parotid gland tumours. All the 14 patients who were operated at the University Teaching Hospital from September 2013 and September 2014, did not have a preoperative diagnosis. Only 2 had a histological postoperative diagnosis (Pathology Department and Clinic 4 Register). This observation was consistent with what other authors had reported that most surgeons use clinical diagnosis to manage Parotid gland tumours because they felt that presence of a Parotid gland tumour was an indication for removal[4,11]. The challenge in getting a tissue diagnosis preoperatively was because the Parotid gland was not amenable to conventional biopsy methods[4,9,10].
2. Lack of intraoperative diagnosis by frozen sections in Zambia.

The above challenges implied that the surgeon does not have a tissue diagnosis before or at the time of surgery and therefore relied on clinical diagnosis. Therefore, deciding which lesions needed to be removed and those to be managed conservatively was a challenge[1, 4]. The Parotid gland was not amenable to conventional methods of obtaining tissue biopsy such as core needle biopsy or incisional biopsy[1, 4, 6, 9, 10, 12]. The core biopsy and incisional biopsies when performed

on the Parotid gland tumours, they complicate into malignant tumour seeding or implantation and formation of salivary fistulae[4, 6, 9, 10].

Clinical diagnosis could not accurately distinguish neoplastic from inflammatory lesions nor could it distinguish benign from malignant lesions[1, 4]. Consequences of clinical diagnosis were[4,9,10,11,16,17]:

1. Risky unnecessary surgeries were done on participants including those with inflammatory lesions.
2. Operating inflammatory lesions resulted in increased morbidity for participants, wastage of theatre resources and theatre time depriving deserving cases. Theatre time has been a rare commodity in our institutions that have long waiting theatre lists, especially at University Teaching Hospital were Phase 3 theatre lists are full by midyear in most General Surgery firms/units.
3. Increased morbidity of post Parotidectomy participants due to over treatment of inflammatory lesions which are subjected to surgery, benign tumours that are treated with total Parotidectomies with facial nerve excision and malignant tumours treated with superficial Parotidectomies.
4. Without a tissue diagnosis, it was difficult to counsel participants on prognosis preoperatively.
5. Clinical decision of excising a Parotid tumour without a preoperative tissue diagnosis not reliable to guide extent of tumour surgery. A tissue diagnosis is required before a tumour is excised.

The lack of examination of frozen sections has been a compound factor that has resulted in over treatment of participants[11, 16, 17].

Patient counseling was challenging because it was not clear whether the tumour a patient had was malignant or benign and whether after surgery they would have a facial nerve paralysis or not nor whether they would have to be re-operated because the Parotid gland tumour that was thought to be benign had turned out to be malignant on histopathology[4, 9, 10].

1.3 Study Justification

When dealing with a tumour of the Parotid gland, clinicians/surgeons were presented with a complex anatomy and pathology that made therapeutic management challenging[1, 2, 3, 4, 9]. The fact that the Parotid gland is divided by the facial nerve into a superficial and a deep lobe entails that this nerve is at high risk of injury during core needle biopsy and incisional biopsy and these two procedures also complicate into seeding of malignant cells (in the case of malignant tumours) and formation of salivary fistulae, but there is no evidence that FNAC causes these complications[6].

Clinical diagnosis results in over treatment of participants with inflammatory lesions (who do not need surgery and could be managed medically or conservatively) and benign lesions that had been treated as malignant tumours with total Parotidectomy with excision of the facial nerve resulting in facial nerve paralysis[4].

Clinical diagnosis has grave consequences[4]:

a) It does not rely on a tissue diagnosis.
b) It has resulted in increased postoperative morbidity due to Parotid surgery done for inflammatory lesion or facial nerve excision done in a benign Parotid gland tumour.

Studies have shown that FNAC has established a role in the diagnosis of Parotid gland tumours as indicated by Attilio et al[12], Choudhury et al[4] and Khandekar et al[10]. FNAC can distinguish Parotid gland cells from non-Parotid gland cells, it can distinguish inflammatory from neoplastic lesions and benign from malignant cells. However these studies had also noted that due to overlapping morphological features of Parotid gland tumours, FNAC had at times failed to provide a diagnosis and it had also failed to accurately diagnose malignant tumours[4, 9]. This had been a source of controversy for most clinicians and surgeons as to whether FNAC could be relied upon instead of examination of frozen sections during surgery[4, 9, 16, 17]. But again frozen sections are not commonly found in most centres and are not done in Zambia. Hence most patients had been operated without preoperative diagnosis, without proper counselling and they had increased morbidity associated with over treatment such as facial nerve palsy (3 of the 14 patients who had superficial Parotidectomy in 2011), and under treatment in cases where lesions that were thought to be benign

(2 readmitted for fungating tumours of the Parotid) were discovered malignant on histopathology[4, 9, 10].

Schmidt et al[18] have argued that FNAC does not accurately diagnose malignant tumours, however they also note that more studies have to be done on the use of FNAC in the diagnosis of Parotid gland tumours, adding to the wide debate on the usefulness of FNAC in diagnosis of Parotid gland tumours.

Application of FNAC in the diagnosis of Parotid gland tumours has pitfalls which are the indications for more research that needs to be done:

a) Failure to diagnose a Parotid tumour at times due to overlapping morphological features of salivary glands[4,17]. Not two Parotid adenomas look the same histologically.
b) Low sensitivity to diagnose malignant tumours[4,9]. Some authors have maintained that the current studies and literature on the application of FNAC in the diagnosis of Parotid tumours shows FNAC cannot accurately diagnose malignant tumours[17,18].
c) Most surgeons are opposed to FNAC preferring frozen sections[4,9,16,17,18].

At University Teaching Hospital 14 patients who had Parotid gland surgery between 2013 and 2014 did not have a tissue preoperative diagnosis and only 28% had postoperative histopathology results. Seventy-two percent (72%) have been lost to follow-up. For the 72% of patients, it could not be objectively determined if they had over or under-treatment.

Choudhury et al[4], advise clinicians to be cautious on the use of their study results and other statistics from other studies done in other institutions because each institution should audit itself and perform research to verify their results considering several variables such as competency of the cytopathologist and the clinician's technique of collecting the aspirated cells. These variables are institutional based and are not the same from one institution to the next[4]. There is need to establish whether in Zambia we could sufficiently depend on the FNAC to have enough sensitivity and specificity to influence management of Parotid gland tumours. The treatment of Parotid gland tumours by clinical diagnosis can result in increased morbidity due to over or under treatment of

patients and wastage of theatre resources and deprivation of theatre time to deserving cases. This study will respond to these controversies.

This study aimed to evaluate the diagnostic accuracy of FNAC by correlating it with histopathology at four major hospitals in Zambia because Parotid tumours are rare with prevalence of 2-6.5% according to studies done elsewhere but this is undetermined in our setting but Parotid tumours are rare tumours in Zambia. If indeed a correlation existed then FNAC would help avoid over treatment of Parotid gland tumours by avoiding unnecessary surgery and enable the surgeon to appropriately counsel patients for surgery, plan for the surgery and perform an optimum procedure for the patient's diagnosis.

1.4 Research Question

Was there a correlation between FNA cytopathology and histopathology in the diagnosis of Parotid tumours?

1.5 Hypothesis (Alternative Hypothesis)

There is a significant correlation of sensitivity and specificity between FNAC and histopathology for the diagnosis of Parotid gland tumours.

1.6 Objectives

1.6.1 General Objective

To determine the correlation of FNAC and histopathology in the diagnosis of Parotid tumors at University Teaching Hospital in Lusaka, Chipata Central Hospital in Chipata, Livingstone Central Hospital in Livingstone and Ndola Teaching Hospital in Ndola, Zambia.

1.6.2 Specific Objectives

i. To correlate FNAC and Histopathology findings.
ii. To determine the cytopathological findings of Parotid gland tumours by FNAC.
iii. To establish the histopathological findings of excised Parotid gland tumours.

iv. To determine the sensitivity and specificity of FNAC in the diagnosis of Parotid gland tumours.
v. To calculate the positive and negative predictive values of FNAC in the diagnosis of Parotid gland tumours.
vi. To calculate the likelihood ratio of FNAC.
vii. To establish the usefulness of FNAC in the management of Parotid tumours

1.7 Organisation of Dissertation

This study is divided into preliminaries and chapters which include the following:
Preliminaries includes the title page; copyright; declaration; dedication; acknowledgements; table of contents, list of tables and figures; appendices and abbreviations.

Chapter one describes the introduction, statement of the problem, study justification and objectives.

Chapter two deals with literature review and explains the application of fine needle aspiration cytology in the management of Parotid gland tumours in surgery.

Chapter three provides the study conceptual framework, research methodology, ethical consideration and research limitations.

Chapter four gives the study results with use of tables and figures to interpret the data.

Chapter five discusses results of the study comparing study results with other regional and international studies on application of fine needle aspiration cytology in management of parotid gland tumours

Chapter six summarizes the study findings and makes recommendations Conclusion

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

FNAC is a safe, cheap and cost-effective diagnostic procedure employed in the diagnosis of most Parotid tumours with minimum risk to the patient and yet it provides a preoperative diagnosis[4]. FNAC involves aspirating tumour cells using a needle and syringe and subjecting aspirated cells to staining then a cytopathologist examines these cells for morphological cellular changes indicating presence of neoplasia or inflammation[1, 4, 9]. While there are complications of seeding of malignant cells in the case of malignant neoplasia, and salivary fistulae formation when the Parotid gland is subjected to incisional biopsy and core needle biopsy, no such complications have been reported for FNAC[4, 6, 9].

FNAC has been widely used to diagnose superficial head and neck tumours; hence it has gained popularity among head and neck surgeons[4]. However, in the case of a Parotid gland tumour, its use has been controversial[1, 4, 9]. The following are some of the controversies in application of FNAC in management of patients with Parotid gland tumours:

1. FNAC has low sensitivity to diagnose malignant tumours[1,4,9].
2. FNAC obviates surgery in 33% of cases, however, it is helpful in surgical planning, most surgeons are opposed to its use as they believe that presence of a Parotid lump is an indication for surgery[18].
3. FNAC is only cost effective in limited circumstances[4,11]. There is high rate of repetition of FNAC collections in the long run increases the cost of managing participants[4,11]. This is because of contamination of FNAC samples due to poor collection technique among those who are not competent in proper collection of samples, is high[4,11].
4. Most surgeons believe that FNAC is not accurate enough to influence decision making, preferring frozen sections which are not readily available even in advanced centres[12].

2.2 Role of FNAC in Management of Parotid Tumours

FNAC has established its role in the management of Parotid gland tumours[4]. It can give a preoperative tissue diagnosis which is crucial in surgical planning. It has acquired a cutting edge

over incisional biopsy and frozen sections[16,17]. FNAC is cost effective, easy to collect and can be done with basic equipment present even in resource limited centre[16,17].

The management problems of Parotid gland tumours has led to more research being done about the application of FNAC in obtaining a preoperative tissue diagnosis[4]. Those who support FNAC use in the preoperative diagnosis argue that FNAC can distinguish neoplastic from inflammatory lesions, and in the case of neoplasia benign from malignant tumours; and incidence of complications with FNAC is rare[4, 6, 9, 10, 11, 12].

Those who argue against the use of FNAC in the diagnosis of Parotid gland tumours feel that FNAC has low sensitivity in the diagnosis of Parotid gland malignant tumours, and that it only changes the management of lymphomas of the Parotid gland[4, 18]. They argue that FNAC obscures diagnosis, there is increased incidence of inadequate sampling therefore it requires multiple sampling, prolongs the period of waiting for operative management and therefore increases the cost of hospital stay[18].

The value of FNAC in the investigation of salivary gland disease has also been widely discussed by both Clinicians and Cytopathologists[4, 9, 10, 11]. Most Clinicians argue that FNAC is not accurate enough to diagnose Parotid gland tumours and influence decision making in terms of whether to operate or not to operate[4, 12].

The Parotid gland has complex anatomy and pathological processes with overlapping morphological features that make diagnosis of Parotid gland tumours difficult[4, 9]. The course of the facial nerve through the gland makes the formulation of a preoperative surgical plan difficulty without a tissue diagnosis[1, 3]. This has bearing on the choice of surgery whether conservative surgery (organ sparing surgery or partial Parotidectomy) or total Parotidectomy. Such decisions are difficult without a tissue diagnosis[4]. The use of incisional and core needle biopsies has complicated in formation of salivary fistulae and seeding of malignant cells in the case of malignant neoplasia[4, 6, 9, 10]. The use of frozen sections only provides an intraoperative diagnosis and is not available in resource limited countries like Zambia[9]. This too does not provide the

surgeon with information to preoperatively counsel the patient for the surgical procedure to be undertaken.[9]

Schmidt et al[18] note that the diagnostic usefulness of FNAC in Parotid gland lesions is controversial. FNAC obviates surgery in 33% of participants and provides useful information for surgical planning, however the clinical information is questioned because of low sensitivity, variation in reported results and the belief that most Parotid gland tumours require surgery. They note that though FNAC is a common procedure, Batsaki et al[19] suggests that FNAC is only cost-effective in limited circumstances. Schmidt et al note that FNAC provides two important key decisions. First is differentiating between neoplastic and inflammatory lesions. Neoplastic lesions require surgery and inflammatory lesions are managed conservatively.

Second, FNAC determines whether a neoplastic lesion is malignant or benign, and this information is vital as it determines the extent of surgery to be done and it also determines whether the facial nerve is to be saved or not. Schmidt et al[18] has raised two important research questions on the clinical usefulness of FNAC for Parotid gland lesions. Firstly, is FNAC sufficiently sensitive to exclude neoplasia and avoid surgery? Secondly, is FNAC sufficiently sensitive to diagnose malignancy to allow facial nerve sparing surgery? The resolutions to these research questions require an accurate assessment of diagnostic performance of FNAC and an understanding of the causes of variations in the studies done on the Parotid gland. More research needs to be done on the diagnostic accuracy of FNAC, eliminating bias by blinding, and correlating it to histopathology which is the most accurate way of diagnosing any tumour. If a correlation exists, then FNAC is useful for diagnosis of Parotid gland tumours and it is sufficiently sensitive and can avoid surgery and allow facial nerve sparing surgery. Hence, in order to practice evidence-based medicine, this study was undertaken in Zambia, with our local cytopathologists with the need to determine whether our pathology laboratory services could accurately diagnose Parotid tumours by FNAC and whether the clinicians/surgeons could use the diagnostic information to make decisions in the management of patients with Parotid gland tumour.

Choudhury et al[4] note that FNAC has established its role in diagnosis of Parotid gland tumours and that there is no evidence that FNAC when performed results in formation of salivary fistulae

and implantation of malignant cells in the case of malignancy[6]. They note that though FNAC has gained popularity among Head and Neck surgeons in the assessment of thyroid and neck masses, its usefulness in the evaluation of Parotid gland tumours has not attained similar enthusiasm, main reason being the belief that the presence of a Parotid lump is an indication for its removal[11], and the reported sensitivity of 57-95% and specificity of 86-100% is believed not to be accurate enough to influence the decision making process[12]. Choudhury et al[4] reported a diagnosis of benign tumour sensitivity of 95.2% and specificity of 80%; and for malignant tumours sensitivity of 75% and specificity of 92%. They also found FNAC to be a safe, cost effective, quick and easy diagnostic procedure that caused little discomfort to the patient.

Kotwal et al[9] noted that FNAC of suspected salivary gland tumours has established a role, it provides a preoperative diagnosis and influences management of patients. It has acquired an edge over incisional biopsy and frozen sections[16,17]. They note that diagnostic criteria of FNAC depend on cytological features, architectural organization and synthetic cellular product. But the diverse morphological patterns and overlapping features are the character of salivary glands. They note that no two pleomorphic adenomas look alike thus it becomes a challenge at times to give a diagnosis. Their study exposed the pitfalls in which FNAC and histopathology did not correlate. They emphasized that sampling and genuine problems occur in typing of salivary gland tumours for instance pleomorphic adenoma and mucoepidermoid carcinoma were difficulty to accurately distinguish on FNAC and squamous cell metaplasia was difficult to distinguish from squamous cell carcinoma. They note that high grade of accuracy can be achieved if established diagnostic criteria are present and are strictly observed. They note that some cases will be problematic. Uncertainty by the cytopathologist becomes the acceptable limitation of FNAC. When such happens, it must be conveyed to the surgeons/clinicians who in the first instance must be aware that this is a limitation of FNAC.

CHAPTER THREE: METHODOLOGY

3.1. Study Design

This was a hospital based cross-sectional study.

3.2 Study Duration

This study was conducted over a period of 2 years and 6 months from April 2014 to October 2016. Data was collected from April 2014 to June 2016. Data analysis was done from July to October 2016.

3.3 Study Variables

3.3.1 Dependent Variable (Outcome)

1. FNA Cytopathology reported diagnosis
2. Histopathology reported diagnosis

3.3.2 Independent Variables (Exposure)

These included:

1. Age
2. Gender
3. presenting complaint
4. Procedure done whether facial nerve sparing or not

3.3.3 **Categorical variables** included: Gender (male/female), presenting complaint, FNAC diagnosis, histopathological diagnosis and procedure done.

3.3.4 **Continuous Variables** included: age, duration of presenting complaint.

3.4 Study Site

This study was conducted in the departments of surgery at University Teaching Hospital in Lusaka, Ndola Central Hospital in Ndola, Chipata General Hospital in Chipata, and Livingstone General Hospital in Livingstone

3.5 Target Population

All patients with Parotid gland tumours scheduled for operation were included.

3.6 Study Population

This included patients with Parotid gland tumours satisfying the inclusion criteria.

3.7 Study Sample Size

Using the prevalence formula and using the hypothesis, sample size was calculated as:

$$N = \frac{Z^2 \text{ X } P\ (1 - P)}{D^2}$$

Where N = sample required

Z = Z statistic (usually 1.96)

P = the expected prevalence (in this case we shall use 6%)

D = accepted accuracy range (+/- 10%)

$$N = \frac{1.96^2 \text{ X } 0.06\ (1 - 0.06)}{0.1^2}$$

$$N = 21.67$$

$$N = \mathbf{22}$$

3.8 Procedure for Sample Collection

This was a prospective study done in the Departments of Surgery at the following hospitals: University Teaching Hospital (Lusaka), Ndola Teaching Hospital (Ndola), Chipata Central Hospital (Chipata) and Livingstone Central Hospital (Livingstone), in Zambia; from April 2014 to October 2016. A total of 25 participants with Parotid tumours were recruited in this study and all were later operated. All the 25 participants were subjected to thorough history and physical examination with appropriate imaging (Parotid ultrasound). A preoperative FNAC was done either in the theatre before operation under General anesthesia. The postoperative excision samples were coded and submitted for histopathological diagnosis.

FNAC was done by using a 10cc syringe and a 20-22G needle in a patient who had given informed consent. The smears were done on 6 glass slides, 3 fixed in alcohol and 3 air dried, then stained with Papanicolaou stains, subsequently reported. The post-operative excision samples were fixed in 10% formalin and then gross and microscopic examinations performed after staining with H&E stains.

The FNAC and histopathology reports were then retrospectively reviewed, compared and analyzed. A comparison of preoperative FNAC and post-operative histopathology diagnosis was done and the data analysis was performed by calculating the sensitivity and specificity, and positive and negative predictive values of FNAC in the diagnosis of Parotid tumours. All samples were coded to eliminate bias. The blinding method eliminated bias. All Cytopathological examinations were done by a one pathologist and this pathologist did not examine any histopathology specimen of the Parotid samples.

The FNAC and histopathology report were reported to units in-charge of participants and results were also communicated to the participants.

3.9 Technique

FNAC samples were aspirated using an appropriate size of needle (20-22 G) and syringe (10 cc). After aspiration, samples were smeared on a glass slide, and then stained appropriately using Papanicolaou staining, to highlight the nuclear and cytoplasmic details. The slides were then read

by a Cytopathologist who examined the cytological features, architectural organization of the cells and presence of synthetic cellular products.

All medical staff involved in this study to collect FNAC samples were trained to ensure standardization in the method of collection of samples.

The appropriateness of the needle size and syringe size used was to avoid injury to blood vessels which could have distorted the quality of slide; therefore, a dilution of 100cells/0.1 ml of blood was satisfactory.

The basic aspiration procedure was undertaken using the following steps:

1. The skin was wiped with an alcohol pad.
2. The Parotid Tumour was located, palpated, and stabilized.
3. The needle passed through the skin and advanced into the tumour.
4. Suctioned was applied by raising the plunger.
5. The needle was moved rapidly back and forth, sampling different areas of the tumour.
6. Suction pressure was released, then needle was removed from the patient.
7. The needle was detached from the syringe.
8. The syringe was filled with air.
9. Reattached the needle with the sample onto the glass slide.
10. The needle tip touched a glass slide with bevel side down.
11. The specimen was expressed onto the glass slide.
12. Oval smears were made.
13. The slides were fixed in alcohol then stained in Papanicolaou stain and transported for reading by a cytopathologist.

3.10 Inclusion and Exclusion Criteria

3.10.1 Inclusion criteria

1. All participants with Parotid gland tumours booked for operation and operated upon.
2. Consenting to be enrolled in the study.

3.10.2 Exclusion criteria

1. Participants with Parotid gland tumours not scheduled for operation or without an indication for operation.
2. Refusing to consent to inclusion in the study.

3.11 Data Collection Procedures

This was done with the aid of data collection sheets (as attached in appendix III) and histopathology and FNA cytopathology reports.

3.12 Data Management and Analysis

Patient recruitment was done on admission days for all surgical units. Data was entered on an excel spread sheet ready for analysis. Data was analyzed both manually and by use of Microsoft excel. The primary outcome was the correlation of FNA cytopathology and histopathology reports. The predictive values, likelihood ratio, specificity and sensitivity of FNAC were also calculated.

3.13 Ethical Consideration

On recruitment, the purpose of the study was explained to participants. It was explained that participation in the study was on voluntary basis, participates had the right to choose to be part of the study or not, and refusal to be part of the study would not affect the treatment outcome in any way. For those who chose to participate in the study, it was explained that this study has no risks to the participants. Participants did not benefit in this study. Participants were assured of anonymity and confidentiality. All this was explained as attached in the information sheet in Appendix I, after which each participant gave a written consent and for those below age of consent; guardians/parents consented on the behalf of the minors as attached in appendix II.

Having addressed ethical issues, ethical approval was sought from University of Zambia Biomedical Research and Ethics Committee (UNZABREC). Permission to conduct the study was obtained from University Teaching Hospital, Chipata General Hospital, Livingstone General Hospital and Ndola Central Hospital managements.

3.14 Limitation

The only Zambian government hospital that was able to read cytology at the time of this study was UTH therefore, all FNAC samples were sent to UTH and only read by one Cytopathologist. This cytopathologist was blinded from Parotid tumour histopathology samples and the results of their histopathology. The Cytopathologist failed to give a malignant diagnosis on some FNAC specimen due to overlapping and diverse morphological features of Parotid gland neoplasia. FNAC was unable to characterize the malignant tumours. Few clients were recruitment from two hospitals, Ndola Teaching and Livingstone Central Hospital but we still recommend FNAC as a preoperative assessment even in these Hospitals.

CHAPTER FOUR: RESULTS

4.1 Results

The study involved a total of 25 participants recruited from four hospitals as shown in figure 1, who had preoperative sample collected for FNAC and a final histological post-operative diagnosis. In this study 14 were females and 11 were males as shown in Figure 2.

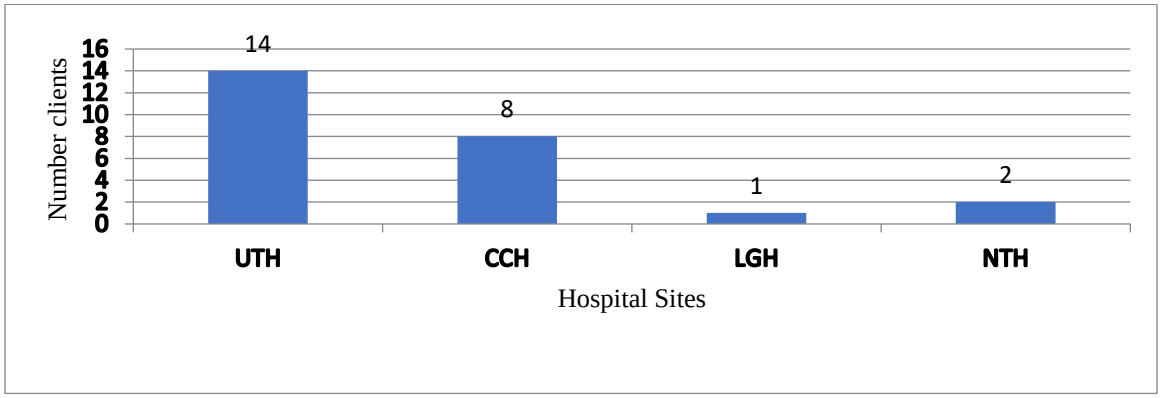

Figure 1. Hospital Distribution of Participants

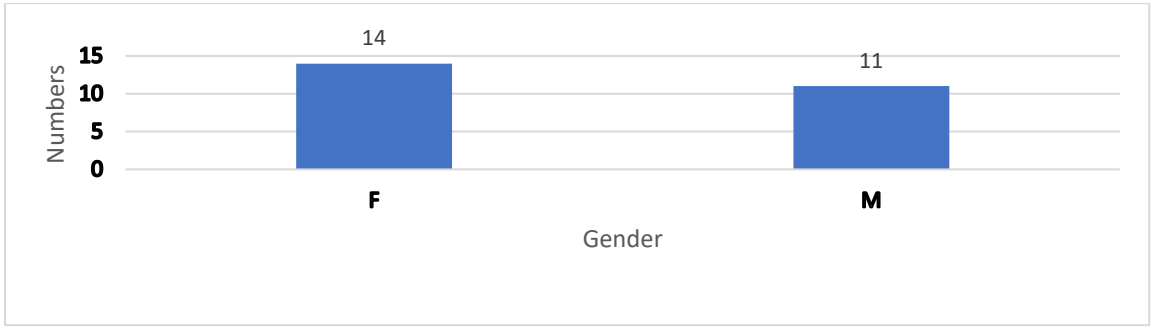

Figure 2: Gender Distribution of Participants in the study

The age range was from 19 to 74 years with a mean of 40.6 years and a median age of 34 years as shown in figure 3 and Table 2.

Table 3 compares FNAC and Histological diagnoses of the study.

Table 2: Age distribution of participants in the study

No	Age Range of Participants (years)	Number of participants
1	0-10	0
2	11-20	4
3	21-30	7
4	31-40	5
5	41-50	2
6	51-60	1
7	61-70	3
8	71-80	2
9	81-90	1

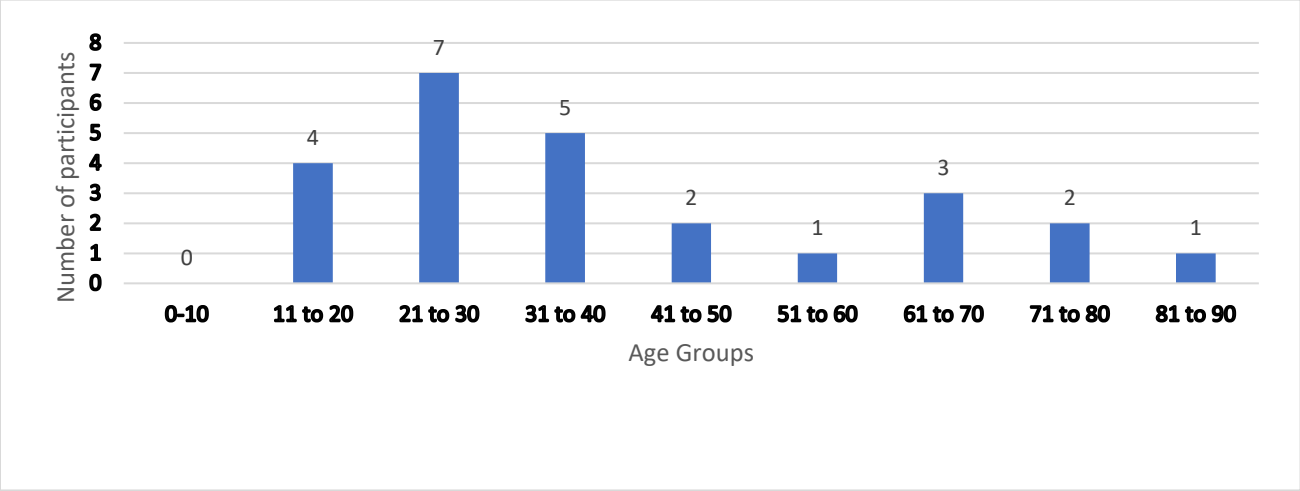

Figure 3: Age Distribution of participants in the study

Table 3 FNAC and histological diagnoses of the participants involved in the study

No	gender	age	FNAC diagnosis	Histological diagnosis	Positivity status	Negativity Status
1	F	35	Pleomorphic adenoma	Pleomorphic adenoma	TP	
2	M	29	Cystic lesion	lymphoepithelial Cyst	TP	
3	F	19	Cystic lesion	lymphoepithelial Cyst	TP	
4	M	44	Acellular/unsatisfactory	lymphoepithelial Cyst		FN
5	F	68	Pleomorphic adenoma	Pleomorphic adenoma	TP	
6	F	29	Carcinoma	Epithelial-myoepithelial carcinoma	TP	
7	F	34	Acellular/unsatisfactory	lymphoepithelial Cyst		FN
8	F	45	Pleomorphic adenoma	Pleomorphic adenoma	TP	
9	F	35	Pleomorphic adenoma	Pleomorphic adenoma	TP	
10	F	15	Pleomorphic adenoma	Pleomorphic adenoma	TP	
11	F	84	Pleomorphic adenoma	Pleomorphic adenoma	TP	
12	F	22	Pleomorphic adenoma	Pleomorphic adenoma	TP	
13	M	28	Pleomorphic adenoma	Pleomorphic adenoma	TP	

14	M	30	Pleomorphic adenoma	Pleomorphic adenoma	TP	
15	M	68	Carcinoma	Invasive SCC	TP	
16	M	28	Cystic lesion	lymphoepithelial Cyst	TP	
17	M	56	Carcinoma	Invasive SSC Metastatic	TP	
18	F	40	Cystic lesion	lymphoepithelial Cyst	TP	
19	M	71	Carcinoma	Invasive SCC	TP	
20	F	74	Carcinoma	Invasive SCC	TP	
21	F	31	Pleomorphic adenoma	Pleomorphic adenoma	TP	
22	M	23	Pleomorphic adenoma	Pleomorphic adenoma	TP	
23	M	19	Pleomorphic adenoma	Pleomorphic adenoma	TP	
24	F	19	Pleomorphic adenoma	Pleomorphic adenoma	TP	
25	M	69	Chronic Sialadenitis	metastatic carcinoma		FN

where:

TP is true positive

FP is false Positive

FN is False Negative

Table 4 shows the analysis of non-neoplastic and neoplastic tumours in the study. Commonest tumour was pleomorphic adenoma.

Table 4: Analysis of non-neoplastic and neoplastic tumours in the study

Histology and Cytological diagnosis analysis					
	Histological Diagnosis	Cytological Diagnosis			
Benign Lesions					
Pleomorphic adenoma	13	13			
lymphoepithelial Cyst	6	4	(2 samples where acellular)		
	0.76	0.68			
Malignant lesions					
Carcinoma	6	5			
	0.24	0.2			
Non- Neoplastic					
chronic sialadenitis	0	1	(wrong diagnosis)		
		0.04			

Figure 4 shows diagnoses by FNAC. Pleomorphic adenoma was the commonest tumour.

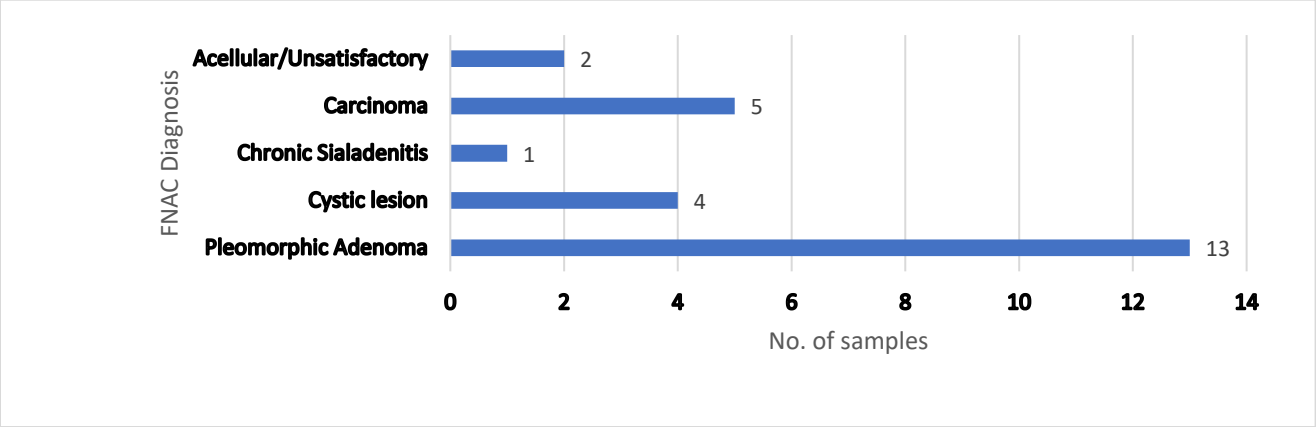

Figure 4: FNAC Diagnosis

Figure 5 shows diagnosis by histopathology. Pleomorphic adenoma was commonest tumour.

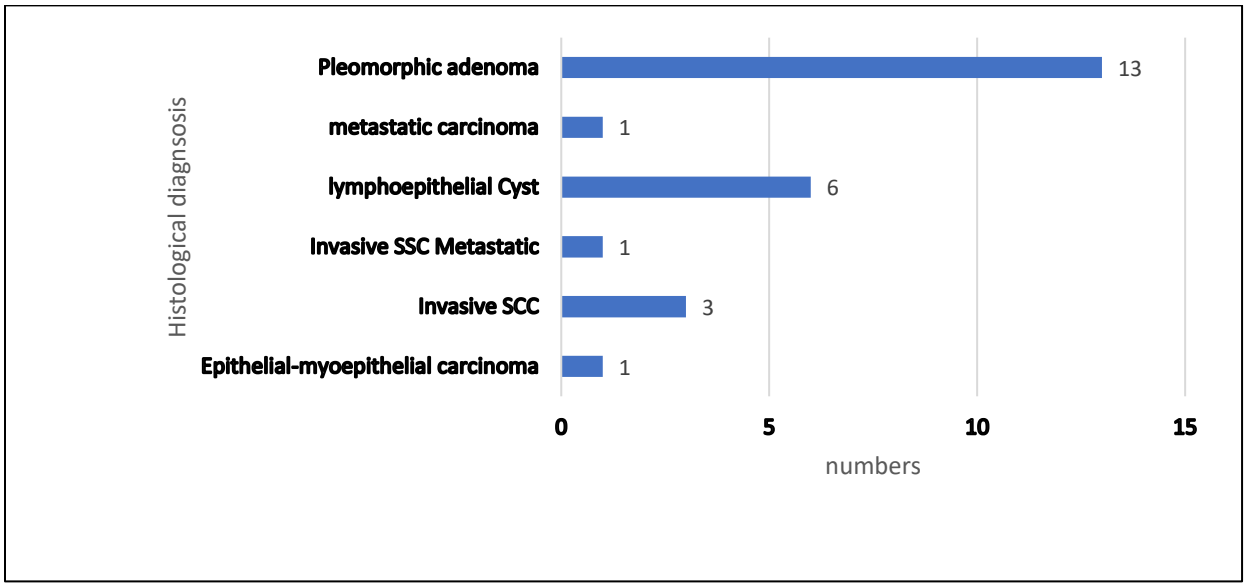

Figure 5: Histopathological diagnosis

FNAC did not correlate with 3 specimens which were diagnosed as 1 chronic sialadenitis (wrong diagnosis, histopathology showed metastatic invasive squamous cell carcinoma); and 2 samples which were acellular.

Based on this data the following calculations were done based on Table 3.

Table 5: Two by two Table Analysis of the Results

	TRUE	FALSE
Positive	22 (a)	1 (b)
Negative	0 ©	2 (d)

Calculations

Sensitivity = a/(a+c)

22/(22+0)

22/22

1.00 (100%)

Specificity= d/(b+d)

2/(1+2)

2/3

0.6667 (66.7%)

Positive predictive value= a/(a+b)

22/(22+1)

22/23

0.9565 (95.7%)

Negative Predicative value= d/(c+d)

2/(0+2)

2/2

1.0 (100%)

Likelihood ratio= sensitivity/ (1-specifity)

1.00/(1-0.667)

1.00/.3333

3.003

3

The study shows that FNAC has a sensitivity of 100% and specificity of 66.7%. the Positive Predictive value was 95.7% and the Negative Predictive value was 100%. The Likelihood ratio was 3.

CHAPTER FIVE: DISCUSSION

5.1 Role of FNAC in Diagnosis of Parotid Tumours

The management of Parotid tumours involved an assessment of whether the Parotid tumour was inflammatory or neoplastic, and If neoplastic then whether benign or malignant. Inflammatory lesions are management conservatively while neoplastic tumours are subjected to surgery.[4]

Benign tumours of the Parotid gland in the superficial lobe are treated with superficial Parotidectomy while those in the deep lobe are treated with total Parotidectomy with facial nerve excision, while in some centres facial nerve sparing surgery is performed. Malignant tumours are treated with total Parotidectomy with facial nerve excision[4]. Some centres perform superficial Parotidectomies for malignant tumours in the superficial lobe with facial nerve sparing.

All FNACs in the study were collected preoperatively few minutes before the scheduled operation from participants whose indications for operations were determined clinically.[18] As reported by Schmidt et al[18], most surgeons believe that the presence of a Parotid tumour is an indication for removal. All participants in the study had superficial Parotidectomy with facial nerve sparing based on a preoperative clinical diagnosis that diagnosed all the Parotid tumours recruited in this study as benign Parotid tumours. The patients were managed under different surgical units by different consultants who performed the Parotid surgeries, All FNACs were read by one pathologist based at University Teaching Hospital who was blinded from histopathology specimen results, Histopathology samples were read by other pathologists and these were blinded from the cytopathology results.

In this study, 88% of the samples from Parotid tumours where diagnosed as neoplastic Parotid tumours and 12% where unsatisfactory/acellular (2 out of 25) and wrong diagnosis (1 out of 25) on FNAC; while histology diagnosed 100% as neoplastic Parotid tumours. FNAC diagnosed 68% as benign Parotid tumours and 20% as malignant Parotid tumours. Histopathology diagnosed 76% as benign Parotid tumours and 24% as malignant Parotid tumours. This finding is comparable with what other authors have found and reported, benign tumours at 40%, 61% and 69%; while malignant tumours have been reported at 6%, 13% and 37%[4].

The commonest benign Parotid tumour was pleomorphic adenoma at 52% and commonest malignancy was invasive squamous cell carcinoma on FNAC and histopathology. Other authors have reported pleomorphic adenoma as commonest benign tumor, however, the malignant tumours are varied.[4] Choudhury et al[4] found commonest benign tumour as pleomorphic adenoma while commonest malignant tumour was adenocystic carcinoma.

FNAC was unable to characterize the malignant tumours and therefore reported as carcinoma but histopathology showed Invasive squamous cell carcinoma as commonest malignant tumor of the Parotid. The failure to characterize the phenotype of the malignant tumours has been reported by other authors as a limitation of FNAC and that at times it may even fail to give a diagnosis due to overlapping morphological features.[4,17] The study has shown that FNAC is accurate and can diagnose malignant Parotid tumours which were at 20% compared to 24% diagnosis by histopathology. This has also been established by other authors.[4]

The age range was from 15 to 84 years. This shows that Parotid tumours affects all age groups and has equal distribution between males and females.

Parotid tumours were seen more commonly in females than in males with a female to male ratio of 1.2:1. Other studies have reported a ratio of 1.8:1 (F:M) ratio[4]. Pleomorphic adenoma was more common in females than in males at 69.2% of all pleomorphic adenomas. Carcinomas were more common in males than in females at 66.7% of all carcinomas in the study.

Two samples were acellular/unsatisfactory because of the cystic nature of the Parotid tumours and therefore difficult to harvest any cells on aspiration. This phenomenon has been described by some authors and therefore a known limitation of FNAC[4,6]. Other authors have noted poor collection technique leading to high rate of repeating the procedure, delayed operation and increased cost of management. [4,17] This has been the reason other authors like Batsaki et al[19] who report that FNAC is only cost effective in limited circumstances, the high rate of repeating the procedure increases cost.[19] But this study has shown that FNAC was able to diagnose 88% of neoplastic tumors of the Parotid into benign and malignant tumours. Therefore, it can be relied upon to diagnose Parotid

tumour and influence management in terms the type of surgery to undertake and whether the facial nerve excision should be done or not.

Technique becomes important in ensuring proper sample collection otherwise the results may be unsatisfactory necessitating repeating the procedure or the result maybe wrong altogether[4,11] as was seen in one of the samples that showed chronic sialadenitis on FNAC, but histopathology showed metastatic invasive squamous cell carcinoma. Sample collection on FNAC harvested necrotic tissue and inflammatory cells hence missing the diagnosis. This raises concern that FNAC requires proper technique of sample collection to ensure proper specimen that is representative of the tumour is collected.

In this study, the sensitivity of FNAC was 100% while specificity was 66.7%. Other authors have reported 57-98% sensitivity and specificity of 86-100%[4,6]. The findings in this study are therefore comparable. The positive and negative predictive values were 95.7% and 100% respectively. Other authors have reported positive and negative predictive values of 66%, 94%, 75% and 95%, 71%, 100% respectively.[4] This is very significant. The likelihood ratio was 3.03 which is very significant for the application of FNAC as a diagnostic test at the four centres were the study was conducted. The likelihood ratio of 3 increases the pretest probability that the positive result of FNAC will more likely indicate a correct pathology in the Parotid gland of the patient and can therefore be adopted as an evaluation tool for preoperative assessment of Parotid tumours at the four Hospitals in Zambia.

This study has shown that FNAC correlates with histopathology and therefore can provide a preoperative cytological diagnosis which can influence management of Parotid tumours in terms of whether to operate or not, and If a patient should be operated then what would be the extent of surgery and would the facial nerve be spared or not. The study has shown that Parotid organ conserving surgery is possible as FNAC was able to distinguish benign from malignant tumours hence establishing a role in management of Parotid gland tumours and providing surgical information required for decision making about the extent of surgery.

Some participants in this study had a benign diagnosis as indication for surgery but post-operative histopathological diagnosis was a malignant diagnosis and there at initial operation, the Parotid tumour was not adequately excised and therefore they will have recurrences and increased morbidity of reoperations. This practice could be avoided in future by adopting preoperative cytological diagnosis of Parotid tumours with FNAC. This shows that clinical assessment alone is not enough to evaluate Parotid tumours and decide the extent of surgery.

This study has shown the usefulness of FNAC and its potential to influence management of Parotid tumours. The preoperative cytological diagnosis can guide the extent of surgery to be done and whether the facial nerve should be excised or spared. However, it has been shown that FNAC cannot give definitive diagnoses of some Parotid tumours compared to histopathology or but can determine whether the tumour is neoplastic or inflammatory and if inflammatory whether benign or malignant[4].

CHAPTER SIX: CONCLUSION

6.1 Conclusion

The study found that FNAC was a safe and effective preoperative diagnostic procedure for assessment of Parotid tumours that offered a cytological diagnosis important in influencing management of Parotid tumours and this correlates with other studies[4,6] This study has demonstrated that FNAC correlates with histopathology in the diagnosis of Parotid tumours. The following can be deduced about FNAC:

1. FNAC was able to diagnosis 88% of Parotid tumours with Pleomorphic adenoma being commonest benign tumour.
2. Commonest benign tumour on histopathology was pleomorphic adenoma and commonest malignant tumor was invasive squamous cell carcinoma.
3. FNAC is highly sensitive at 100% and specific at 66.7%.
4. The positive predictive value was 95.7% and negative predictive value was 100%, likelihood ratio was 3.
5. FNAC was found to be useful as a cheap, cost effective, safe procedure in the evaluation of Parotid tumours and that it can be relied upon to influence management of Parotid tumours. FNAC is therefore more reliable than clinical examination in distinguishing between malignant and benign Parotid tumours.

6.2 Recommendation

FNAC should be adopted as a preoperative assessment procedure of evaluating Parotid tumours at the four major hospitals in Zambia but more studies need to be done about the application of FNAC in the diagnosis of Parotid tumours.

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APPENDICES

Appendix I: Information Sheet

My name is Dr. Kazuma Seke M. E. I am in training to become a General Surgeon- a doctor who does operations. I am doing a research to find out the best way to manage Parotid tumors by comparing Cytopathology and histopathology diagnoses of Parotid tumours. This will enable me to evaluate the accuracy of FNAC diagnosis of Parotid tumors. This will enable us (clinicians/surgeons) have a preoperative diagnosis for future participants which will determine whether a patient with a Parotid tumour needs an operation or not and if they need an operation then what type of operation should be done. This is because not all Parotid gland tumour operations require surgical management. Others can be treated medically but this depends on the diagnosis.

Fine needle aspiration cytology means collection of cells from your Parotid lump using a needle and a syringe. It will be done just before your surgery and while you are under anesthesia (sleeping), so there will be no pain.

Fine needle aspiration cytology has no risks to the participants in this study. The risks are those related to the surgical procedure (Parotidectomy, which means removal of the Parotid gland) that you will consent for separately.

The collected aspirated sample (by FNAC) and the tissue that will be excised (by Parotidectomy) during your surgery will be analyzed in the laboratory (by Cytopathology for FNAC samples and Histopathology for excised tissue) and later compared for consistency (the result of Histopathology and that of FNAC will be compared).

Names will not be used in the research to protect the participants from being identified and all the information will be kept confidential.

Participants will not benefit in this study. The findings of the study may benefit future participants with Parotid gland tumours.

Taking part in this study is at free will and will not affect treatment in any way should you choose not to be involved.
If you accept to take part in this study, then you will need to sign the attached consent form.

If you want more information or ask questions, please get in touch with me on:
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Lusaka, Zambia.
Assurance No.

Appendix II: Informed Consent

I_____ do hereby agree at free will to take part in this
(Full Name)
study having been fully informed.

Participant signature or Thumb print: …….
Date: …….

FOR MINORS OR THOSE BELOW AGE OF CONSENT
I…… on behalf of……., who is my
(Name of Parent/Guardian) *(Name of Minor)*
……., do hereby agree at free will to take part in this study having been fully informed.
(Relationship)

Parent/Guardian's signature or Thumb print: …….
Date: …….

Witness
signature: ……Full name: ……
Date: …….

If you want more information or ask questions, please get in touch with me on:

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Assurance No.

Appendix III: Data Collecting Sheet

Demographic Data
Code: .....
Age of Patient: .....
Sex:
a) Female ☐
b) Male ☐
Date of admission: .....
Date of operation: .....

Clinical data
Presenting complaint: ……

Duration of Presenting complaint: ……
Diagnosis: .....
Type of Operation: .....
With facial nerve preservation:
a) Yes.....
b) No .....
Tissue Diagnosis
FNA Cytopathology report including tumour type……
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Histopathology report including tumour type……
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