TOPIC

EFFICACY OF FINE NEEDLE ASPIRATE BIOPSY IN DIAGNOSIS OF CLINICALLY SUSPECTED NASOPHARYNGEAL CARCINOMA IN PATIENTS WITH CERVICAL LYMPHADENOPATHY

BY

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DECLARATION

I certify that this study is my original work and has not been presented for a degree in any other university.

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This thesis was supervised and has been submitted for examination with my approval.

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DEDICATIONS

To

My mother the late Charity Wangui who was so much dedicated to my education, my uncle Njogu Njoroge who paid for my secondary school fees and his wife Mary Muthoni for the support she gave me during my education, my late wife Hannah Wangui who encouraged me to pursue post-graduate studies and gave me invaluable support during the initial stages of the program. More dedications to Veronica Wanjugu for encouragement and spiritual support, my children Njogu, Charity, Lincon and Githaga for their jovial moods even during the hard times they underwent as I pursued my post-graduate studies

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TABLE OF CONTENTS

ABBREVIATIONS

ADCC	-	Antibody dependent cellular cytotoxicity
AJCC	-	American Joint Committee on Cancer
EA	-	Epstein Barr Antigen
BTU	-	Blood Transfusion Unit
ENT	-	Ear, Nose and Throat
ENT-HNS	-	Ear, Nose and Throat, Head and Neck Surgery
EUA	-	Examination Under Anaethesia
FNA	-	Fine needle aspirate
FNAB	-	Fine needle aspirate biopsy
FNAC	-	Fine needle aspirate cytology
HNS	-	Head and Neck Surgery
JICC	-	Joint International Commission for Cancer
KNH	-	Kenyatta National Hospital
NA	-	Nuclear antigen
NPC	-	Nasopharyngeal carcinoma
RNE	-	Rigid nasal endoscopy
SCC	-	Squamous cell carcinoma
SIN-2	-	Singapore-2
UICC	-	Union Internationale Contre le Cancer
VCA	-	Viral capsid antigen

ABSTRACT

Nasopharyngeal carcinoma is the most common adult head and neck cancer seen in Kenyan hospitals and a common cause of cervical lymph node malignant diseases in Kenyan adults. The objective of this study was to evaluate the efficacy of fine needle aspiration biopsy (FNAB) in diagnosis of clinically suspected nasopharyngeal carcinoma (NPC) in patients with cervical lymphadenopathy. It was a descriptive cross-sectional study. The study was done in Kenyatta National Hospital (KNH), Department of Ear, Nose and Throat, Head and Neck Surgery (ENT-HNS). A total of 68 patients clinically suspected to be suffering from NPC with metastasis to the cervical lymphnodes were done FNAB of the nodes for cytology and nasopharyngoscopic biopsy for paraffin section histology. The results were compared to find out the degree of correlation of the results from the two procedures. Conclusion was that the efficacy of FNAB of cervical lymphnode in this study was high with sensitivity 93.9%, specificity 100% in diagnosis of clinically suspected NPC with cervical lymphnode metastasis and took a shorter time than histology to obtain the results. Therefore it is an invaluable tool to aid ENT surgeons and other medical service providers in diagnosis and management of clinically suspected NPC with cervical metastasis.

INTRODUCTION

Fine needle aspiration biopsy (FNAB), also known as fine needle aspiration cytology (FNAC) or Aspiration Biopsy (AB), is a screening procedure done using a needle gauge 20 – 25 mounted on 5 – 20 mls syringe. The needle is introduced into the mass under scrutiny and aspirated cells or fluid examined cytologically (1). The aspirate is applied on a slide, fixed with 95% alcohol immediately then stained using papanicolaou technique. The specimen is then examined by a cytologist (2,3). The current gold standard for diagnosis of NPC is tissue biopsy of the primary tumour.

FNAB evaluation of head and neck masses was first reported by Kun in 1847 but did not gain wide acceptance at that time (4). In 1930's, a pathologist in Memorial Sloan Kettering Hospital rediscovered the ability of FNA for evaluation of head and neck masses. Recently Mwangi in his Mmed ENT-HN surgery dissertation did a study on efficacy of FNAB in diagnosis of head and neck masses in Kenyatta National Hospital (KNH) and found that FNAB has an overall sensitivity of 93.6% in the diagnosis of head and neck masses (5). However the focus of the study was head and neck masses and not NPC hence the efficacy on NPC diagnosis could not be ascertained.

Formerly the use of large – bore needles led to frequent complications and occasional seeding of tumour along the biopsy tract (6,7). The frequent morbidity associated with this procedure prevented wide spread acceptance of this technique in America but this was minimized by use of small gauge needles. The procedure was commonly used for cytological examination of mestatic lesions in the neck with good results (8). Its popularity initially due to fear of malignant cells spreading along the needle tract but studies done latter disputed that belief (9) and popularised three decades ago by Scandinavian and European investigators (4). FNAB of solitary neck masses has become a well-accepted, safe and cost – effective tool in the initial assessment diagnosis of head and neck masses.

Complications are rare in FNAB, these include pain, infection and bleeding especially in vascular tumour and in haemorrhagic disorders. The use of large – bore needles used to lead to occasional seeding of tumour along the biopsy tract (6,7) changing the stage and prognosis but this was minimised by use of smaller gauge (20-25) needles.

The procedure is now an established first line diagnostic test for pre-treatment or follow up for Head and neck tumours (10,11,12). Its role in breast (13), thyroid, skin, head and neck lesions has been studied, but no conclusive study of its value in the diagnosis of clinically suspected NPC with cervical lymphadenopathy in KNH has been done. FNAB is relatively a painless, ambulatory procedure. It provides simple, rapid, cost effective and accurate head and neck tumour diagnosis (14, 15).

BACKGROUND

NASOPHARYNGEAL CARCINOMA

APPLIED ANATOMY OF NASOPHARYNX

Nasopharynx is also called epipharynx. It is the hidden, uppermost and poorly accessible part of the pharynx and hence it is difficult to inspect and get biopsies from this site without using endoscopes or patient put under general anaesthesia. It lies behind the nasal cavities and extends from base of skull to the soft palate or the level of the horizontal plane at the level of first cervical vertebra. It is 4cm high, 4cm wide and 3cm long. The roof is formed by the basisphenoid and basiocciput, and therefore NPC can erode it and spread intracranially. The posterior wall is formed by the arch of atlas covered by the prevertebral muscles and fascia. Both the posterior wall and the roof imperceptibly merge with each other. The adenoid lymphoid tissues are at the junction between the roof and the posterior wall. The anterior wall is formed by the posterior nasal apertures or choanae, separated from each other by the posterior border of the nasal septum, this makes nasopharynx to be accessible through the nose. Posterior ends of the nasal turbinates and meati are seen in this wall. NPC can spread through this wall into nasal cavities and give sinonasal symptoms. Lateral walls, each presents the pharyngeal opening of Eustachian tube, situated 1.25cm behind the posterior ends of the nasal turbinates and blockage or invasion of these tubes by NPC gives the aural symptoms. It is bounded above and behind by torus tubaris raised by the cartilage of the tube. Above and behind the tubal elevation is the pharyngeal recess or fossa of Rosenmuller, which is the commonest site for origin of Nasopharyngeal carcinoma. Posterior pharyngeal wall mucosa overlying the pharyngobasilar fascia and the retrophyngeal space containing the lateral retrophyngeal lymph node of Rouviere which are the first nodes NPC spreads into but not palpable and inaccessible for FNAB

The superior constrictor does not reach the base of skull, therefore creating a lateral gap (Sinus of Morgagni) through which NPC tumours can easily infiltrate and spread into the parapharyngeal space and cervical region, hence may be palpable and accessible for FNAB. Parapharyngeal extension of the tumour along the parapharyngeal space involves the superior cervical ganglion resulting in Horner's syndrome, a sign of advanced NPC. The tumour can extend from parapharyngeal space through superior orbital fissure into the orbital cavity causing proptosis. The floor is formed by the soft palate (anterior 2/3) and pharyngeal isthmus (posterior 1/3). The pharyngeal isthmus is the space through which nasopharynx communicates with oropharynx and spread of NPC through this gets into oropharynx.

The nasopharynx is an anatomically difficult area to expose surgically. This area is in close

proximity to several foramina and associated vital neurovascular structures. These include the foramen ovale, foramen spinosum, foramen lacerum, carotid canal, and jugular foramen leading to cranial nerve involvement as well as CNS symptoms if NPC involves these structures or has intra cranial spread. The sub-epithelial connective tissue is rich in lymphoid tissue (16,17,18).

Lymphatic drainage

The nasopharynx has a very rich lymphatic drainage. Efferent flow from the nasopharynx is bilateral and is directed to the lateral retropharyngeal nodes of Rouviere, these are the first station lymphatic filter, not palpable and inaccessible for FNAB, then jugulodigastric and the spinal accessory nodes (16,17,18). The jugulodigastric and the apical node under the sternocleidomastod are the second station nodes and are the first palpable nodes to be involved in NPC and accessible for FNAB (16,17,18). Most of other lymph nodes involved after these in cervical region can be done FNAB. In KNH 97.70% of NPC patients present with cervical lymphadenopathy (19) and therefore FNAB is very important tool in screening and diagnosis of NPC.

NASOPHARYNGEAL CARCINOMA

EPIDEMIOLOGY

The highest incidence occurs in southern China and Hong Kong. Moderately elevated incidence is found in South-East Asia countries, Asian races and North Africans and in Kenyans. Low incidence is found in Europe, America and the rest of the world. It has been found that when and where the Southern Chinese migrate, they retain their high risk for NPC (17).

Data from Nairobi initially indicated an intermediate risk, as with the Malays. However, when the age corrected incidence rate was recalculated by Wen, he found it to be much lower at 0.48 per 100,000 population, which is a typically low rate for data published in 1970. However, almost 25years later, and this time for an American Society for Therapeutic Radiology and Oncology (ASTRO) refresher course on NPC, Kenya was still highlighted as an area of high incidence of the disease. The tumour is quoted as representing 25% of all new head and cancer cases, as compared with 18%-20% for China, Hong Kong and Taiwan and 0.2% for the United States. These instances illustrate the problem of being too categorical about incidence rates for NPC in intermediate-risk populations, that is those between the very high incidence in Guangdong and Guangxi provinces, and the low incidence in the non-Chinese population of the United States, Canada and Europe (20).

NPC was reported as having an incidence of 5-10 per 100,000 population per year and is 6th commonest malignant tumour recorded by Kenya cancer registry. Clifford in 1965 followed up

patients admitted in head and neck surgery unit of KNH with malignant disease and found that 30% had NPC (18).

Race

NPC has more predilection for Mongolian races. In Kenya the Kikuyu and Meru tribes has shown a higher incidence than other tribes (18) (this was a hospital based study and could be biased). Other studies showed that majority (38.4%) of patients resided in Mt Kenya region and most of patients lived in highland regions of the country, irrespective of region of residence (2).

Sex

NPC is more common in males with age standardized M:F ratio of between 2-3:1. Many studies have shown a male preponderance (17,18,21).

Age

NPC has shown a bimodal age distribution of 9-15 (10%) and 25-45 years. In Chinese 1% occurs before age of 25years while majority is seen between 40 and 60 years. Mean age in a study done in Saudi Arabia was seen to be 37.5 years, while in Ghana it was 24.5 years (18).

AETIOLOGY

Aetiology of NPC is still obscure (16,17,18).

In general NPC is thought to be the result of both genetic susceptibility and environmental factors such as carcinogens and infection with Epstein-Bar virus (EBV). Evidence in support of genetic factors is the association of NPC with genotypes HLA-A2 and HLA-Bsin2, which are prevalent in individuals from southern China but rare in Caucasians. Furthermore, abnormalities of multiple chromosomes, including 1,2,3,4,5,6,8,9,11,13,14,15,16,17,22 and X, have been identified (22).

Environmental Factors

Possible environmental or cultural factors that may be associated with NPC include the ingestion of Cantonese-style salted fish and preserved foods containing carcinogenic nitrosamines, especially during childhood. Evidence of Epstein Barr virus-DNA (EBV-DNA) in almost all WHO type 3 NPC cells that were studied supports the association of NPC with EBV (22).

The significance of environmental factors is supportably by the following: -

1. Epidemiological data on geographical clustering in southern China and Chinese emigrant populations.
2. Age-incidence rate curve in high population.

3. Time trend- the high risk for the disease among the Chinese have remained virtually unchanged for many years. This can partly be attributed to the fact that the Chinese have retrained their micro-environment preserving the oriental life-style especially with regard to food custom.

The following various environmental factors are implicated in aetiology of NPC:-

I	Epstein Barr virus		
II	Tobacco use	-	smoking, chewing or sniffing
III	Drugs	-	Herbal medicine
IV	Diet	-	salted fish (contain nitrosamines that induce squamous cell carcinoma and Adenocarcinoma
		-	Nitrosamines
		-	Lack of vitamin C
V	Cooking habits	-	House smoke and fumes
VI	Religion practices	-	Incense and fossil smoke
VII	Occupation	-	Industrial fumes/chemical
VIII	Others	-	Socio-economic status
		-	Previous otolaryngological ailments, for example CSOM.
		-	Metal (arsenic, chromium, nickel)
		-	Weaning habits (16,17).

Age, use of tobacco or ethanol and a number of presenting symptoms have been associated with decreased survival rate in WHO Type I (Squamous cell) tumours. Male gender and total number of presenting symptoms are associated with decreased survival rate for WHO Types 2 and 3 (non keratinizing or undifferentiated) tumour (23).

In Kenya, NPC patients were found to come from highland areas of Central, Eastern, Rift valley and Western Provinces (21).

Familial and genetic factor

Familial NPC has been documented. NPC is thought to result from interaction of genetic and environmental factors including EBV (18,20,24). A review of familial NPC in the white population revealed that in contrast to sporadic NPC, which is usually of the well-differentiated type, familial NPC is usually poorly differentiated (25).

IMMUNOLOGY OF NPC

Cell medicated immunity in NPC

Impaired T-cell functions are found in more than half of the newly diagnosed and untreated patients with NPC. Similar impairments is also observed in treated patients who are in remission. Impaired viral specific T-cell immunity and increased suppressor T-cell activity in patients with NPC suggest immunosuppression (16,17,18).

Epstein-Barr virus and NPC

EBV is a herpes virus. It's lymphotropic action is restricted to B lymphocytes which are found in abundance in the lymph epithelium. EBV primary infection takes place in childhood and is always accompanied by seroconversion and harboring of the virus in a dormant state of life. These viruses may be reactivated resulting in raised serological titres in immunosuppressive states. Association of NPC with EBV is supported by the following -

1. Presence of a humoral immune response (in patients with NPC) against EBV determined antigen including structural antigens for example, viral capsid antigen (VCA), early Epstein Barr antigen (EA) and EBV nuclear antigen (EBNA)
2. Presence of EBV markers. DNA and nuclear antigens in NPC tumour cells (Wolf, Hausen and Becker, 1973).

More than 90% of patients with NPC have elevated antibody titles to EBV determined antigens and only the undifferentiated and poorly differentiated forms consistently express EBNA (16,17)

Important EBV related antibodies in NPC are as follows:-

a. IgA and IgG to VCA
b. IgA and IgG to EA
c. Antibody to NA
d. Antibody dependent cellular cytotoxicity antibodies (ADCC).

These are of clinical importance in evaluating NPC patients for stage of diseases, response to therapy and clinical course and survival (especially the fist three) (16,17). ADCC, a process known to be effective in destruction of virally infected cells appear to act on EBV induced membrane antigens and is capable of destroying infected cells. Serological diagnostic immunoglobulin, IgA/VCA, IgG/VCA, IgG/EA are useful diagnostic markers of NPC. Their titters are related to tumour load (Ho et al, 1981)(16,17).

Serological treatment follow-up.

Titres of IgA/VCA and IgA/EA are useful indices for follow up of NPC after treatment. They decline on successful treatment (Henle et al, 1977). An upsurge of viral capsid antigen, early antigen and nuclear antigen antibodies would indicate clinical recurrence and /or metastasis. Antibody dependent cellular cytotoxicity (ADCC) is of value as a biological titre determining the survival of patients (Chan et al 1979). The higher the titre, the better the survival (17).

NPC genetics

HLA systems shows strong association with NPC. An oriental B antigen was found in high frequency among Singapore Chinese patients with NPC in a study done in 1974. It was designated Singapore-2 antigen (sin 2) (Simons et al, 1976). Another independent study in USA found HLA - antigen (designated HS) occurring among Cantonese Chinese patients with NPC in California. It has now been shown sin-2 and HS are identical and it has been designated BW46 by WHO committee on leukocyte nomenclature. There are three well established associations between HLA with haplotype A2-BW46 and AW 19-B17 (Chan and Simons, 1977; Chan et al 1981) (16).

An important tumour suppressor gene p53 plays a role in the regulation of cell progression and prevention of carcinogenesis. Mutated p53 is related to cell progression and malignancy. An association has been seen to exist between NPC and p53 codon 72 polymorphism. The p53 proline (pro) homozygote are at a higher risk of developing NPC. The arg heterozygotes or homozygote are related to a lower risk of NPC development (16,26,27).

HISTOPATHOLOGY

NPC arises from the crypts of the squamous or respiratory epithelium lining the wall of the nasopharynx. It is a malignant epithelial tumour (WHO classification). It may be preceded by squamous metaplasia. According to World Health Organization classification, three histological types are recognized by light microscopy,

Type 1 squamous cell carcinoma, which has differentiation as follows: -

a Well differentiated

b Moderately differentiated

c Poorly differentiated

Type 2 Non-keratinizing carcinoma

Type 3 undifferentiated carcinoma (commonest sub type seen in high risk countries)

However all show ultrastructural and immunohistochemical evidence of squamous differentiation hence the nonkeratinizing and undifferentiated types (that show no evidence of squamous

differentiation on light microscopy) could be considered variants of the squamous cell carcinoma (16,17,18)

WHO Type 1 cells are large with eosinophilic cytoplasm, obvious keratin production and intercellular bridges. They are morphologically similar to other squamous cell carcinomas of the aero digestive tract. WHO type 2 has cells that range from mature to immature and have little or no keratin (transitional). WHO type 3 forms a diverse group that include anaplastic, clear cell and spindle cell variants. The cells are moderately large and have basophilic cytoplasm, indistinct borders and characteristic large single nuclei (Gustafson 1981)(28). While the majority of NPCs are homogenous, Shanmugaratnam found that 26% of NPCs had features of more than one histologic types. Fee encountered similar findings in 3% of recurrent NPC. These heterologous tumors are classified according to the predominant histologic type (18,29).

Many studies have shown a preponderance of the anaplastic type (18), (80% in UK). In Kenya undifferentiated/anaplastic type was seen to comprise 91.6%. Squamous cell carcinoma comprised 5.2% and transitional cell carcinoma was 3.2% (43). Another study done by Oburra at KNH (1988-1992) showed that SCC was 5.9% and undifferentiated to be 94.1% (n=34) (19).

LOCAL SPREAD OF NPC

NPC has no characteristic macroscopic features. The lesion may appear ulcerative, infiltrative or proliferative (exuberant polypoidal tumour). Early preclinical and infiltrative carcinoma retains a relatively normal mucosal appearance and the diagnosis is based on histopathology. The primary tumour distribution is in the following order of frequency: -

1. Lateral wall (especially around the fossa of Rosen Muller and around the Eustachian cushions).
2. Superior (roof) to posterior wall.
3. More than one wall.
4. Anterior wall and floor.

More than 80% of tumours are unilateral right and left sides are equally affected (17). FNAB cannot detect such spread because these walls are inaccessible.

Due to hidden inaccessible nature of nasopharynx and it's voluminous nature, symptoms do not occur until there is Eustachian tube blockage causing aural symptoms, nasal blockage causing unilateral nasal obstruction, orbital cavity involvement causing proptosis or until base of skull involvement causing cranial nerves paralysis. However the main symptoms that bring the patient for the medical attention is swelling of the cervical nodes. Since cervical nodes are a second level

lymphatic group for nasopharynx, the patients are bound to present late. The accessibility of cervical lymph nodes makes it an ideal candidate for initial FNAB assessment.

Symptoms depend on locoregional and distant spread and organs involved. NPC has marked invasive and metastatic powers. From the primary site the tumour can spread in following directions: -

1. Anteriorly to the nasal cavity and paranasal sinuses, pterygopalatine fossa and apex of orbit causing nasal obstruction, proptosis and Horners syndromes.
2. Posteriorly to retropharyngeal space and node of Rouviere to cause dysphagia and throat discomfort.
3. Laterally to the paraphyngeal space as follows: -
   a. Prestyloid compartment with involvement of mandibular nerve, pterygoid muscles and the deep lobe of the parotid gland causing trismus and neck swelling.
   b. Poststyloid compartment with vascular compression of the carotid sheath and vessels and invasion of the last four cranial nerves IX, X, XI, XII to cause loss of taste, odyno/dysphagia, and compromised laryngeal movements.
4. Superiorly through the sphenoid body and sinus involving parasellar structures, optic nerve, petrous apex and foramen lacerum, spreading along the carotid canal into the cavernous sinus involving cranial nerves III, IV, V and VI. This causes headaches, opthalmoplegia and signs of raised intracranial pressure. Inferiorly to oropharynx and oral cavity causing mass effect and throat discomfort (16,17,18). All the above routes of local spread although easily detected on imaging are inaccessible for FNAB except lateral pharyngeal wall spread.

Distant spread

Using amoeboid motility, cancer cells travel through tissues and penetrate walls of capillaries and veins and are swept by blood to distant sites. Therefore local tissues invasion is a prerequisite to blood borne metastasis (26). When blood borne metastasis occur again the tumour has reach a potentisly incurable stage. It is this distant metastasis that early FNAB strives to forestall before onset of treatment. However such distant sites are also a valuable and suitable for FNAB assessment.

The incidence rates of distant metastasis are about 30% of which skeletal metastasis account for more than one half. The order of frequency is as follows: -

1. Thoracolumber spine
2. Lung
3. Liver

In Kenya a study done at K N H by Gacani et al showed the order frequency of distant metastasis to be as follows: -

1. Lumber vertebra
2. Liver
3. Ribs
4. Pelvis
5. Femur
6. Lungs (26)

Distant metastasis indicate grave prognosis with a median survival of three months. Ninety percent of the patients die within 1 year of diagnosis of the metastasis. Some patients probably have occult metastasis at the time of initial diagnosis (16,17,18,22). Any malignancy has the propensity to metastasis to skin. It is rare in NPC, but has been seen, where patients had a clinical picture of facial, periorbital and lip swelling associated with stridor and dysphasia. This picture of presentation is relatively under diagnosed and should heighten the awareness of oncologists to this form of occurrence of NPC (30). Endocrine changes such as Cushing's syndrome, dermatomyositis and psedomyassthenia gravis (Eaton - Lambert syndrome) have been reported in advanced NPC (17)

Differential diagnosis

When doing FNAB, on a suspected NPC adenopathy one has to consider the differential diagnosis. Conditions that many mimic NPC include the following among others: -

a Angiofibroma
b Burkitts lymphoma
c Mucormycosis
d Wegeners granulomatosis
e Antrochoanal polyps
f Tuberculosis

All these except angiofibroma and antrochoanal polyps (inaccessible by FNAB) can be diagnosed by FNAB. Carotid body tumour that bulges into the oropharynx may also simulate NPC (18). In

angiofibroma and carotid body tumours FNAB should be avoided because of bleeding and are easily assessed by CT scan with contrast. Avascularity, displaced hilar lymph nodes and low intranodal vascular resistance are clues that may suggest the tuberculosis nature of neck nodes. However there is overlap of FNAB appearance between tuberculosis nodes, benign reactive nodes and metastatic nodes and thus histological analysis is often required for a definitive diagnosis (17, 31).

CLINICAL FEATURES

Because of hidden anatomical characteristic, clinical presentation is late and makes treatment outcomes poor. Most patients seen at KNH between 1973-1983 had duration of symptoms of 6-28 months (32). Most patients have multiple insidious symptoms as follows: -

1. Cervical lymphadenopathy

NPC has a tendency for early lymphatic spread. The lateral retropharyngeal node (of Rouviere) is the first lymphatic filter and is not palpable and therefore not accessible for FNAB. The abundant supply of regional lymphatic vessels in the nasopharynx contributes to the high incidence of cervical metastasis. Approximately 44-57% of patients initially seek medical attention because of a metastatic lymph node that presents as a neck mass. At the time of the diagnosis, 60-85% of patients already have cervical metastasis, which can easily be subjected to FNAB (22). A study by Oburra in KNH between1988-1992 showed 97.7% of NPC patients presented with cervical node/mass (19). The common first palpable node is jugulodigastric and the apical node under the sternocleidomastoid muscle. The parotid gland and node can be involved if the parapharyngeal space is breached. Node involvement can be bilateral. Eventually all the levels and triangles of the neck are involved. Although the T stage does not correlate with the N stage, the less differentiated the primary the more extensive the lymphatic spread.

The Occult Primary

The nasopharynx is the most frequent site of an occult primary tumour in the head and neck with cervical metastasis. In such cases EBV serology serves as adjunct to pathological identification. Should serology be negative and other head and neck regions are clear, an enlarged lymph node should be excised in total with the capsule intact (22). Indiscriminate excision or incisional biopsy of lymph nodes should be discouraged as they offer little in the clinical management, if the primary tumour is still untreated. It also compromises prognosis of NPC and increases the morbidity to radiation should the wound break down as a result of tumour seeding. A small primary NPC is known to give rise to overt tumour deposits in the parotid gland (16,17,22). Therefore FNAB is of

much use in management of such a tumours. FNAB specimen can also be subjected to immunohistochemistry to detect the presence of EBV latent membrane proteins. In the event of positive immunohistochemistry findings the site of acute primary is definitely located in the nasopharynx and treatment can be primarily based on this evidence.

2.Epistaxis

This is more commonly seen in advanced NPC with or without skull base erosion. Blood stained nasal mucus and saliva are more frequently seen. Such findings are also encountered in primary maxillary cancer, especially if there is erosion to the maxillary antrum which could mimic sinusitis. Epistaxis is not a good indicator of NPC as there are many other causes and it also typically appear late.

3. Tinnitus and aural symptoms

Serous otitis media with tinnitus is not uncommon. All unresolving otitis media should be viewed with suspicion especially if the otology symptoms are unilateral. The combination of conductive deafness, elevation and immobility of the homolateral soft palate together with pain in the side of the head, due to trigeminal nerve involvement represent symptoms of local invasion and is called the Trotters triad (13).

4. Neurological palsies

All cranial nerves can be involved either singly or in groups through tumour invasion or compression. Most involved are cranial nerves V, VI, IX, and X (50% of all the palsies). Cranial nerves IX and X are invariably involved together and are the most common group to be affected. Isolated nerve palsies are common with CN V and VI (19,22).

5. Pain and Headache

Pain is an ominous symptom in NPC. Severe pain with headache is hallmark of terminal disease and signifies tumour erosion of base of skull and surrounding structures. Trismus if present signifies tumour involving pterygopalatine fossa and / or pterygoid muscles. A typical facial pain or unexplained headache in absence of obvious clinical findings in the nasopharynx may be a presenting symptom of NPC.

This may be due to involvement of trigeminal nerve especially the 2nd division (18).

6. Other symptoms are:

Diplopia, facial numbness, hypoaesthesia, trismus, ptosis and hoarseness of voice (16,17). A study

done at KNH of NPC patients seen between 1973-1983 revealed the following order of symptomatology; Cervical node enlargement -74%, Nasal symptoms -56%, Aural symptoms -33%, Parapharyngeal/laryngeal symptoms -27.3%, while signs on examination were noted as; cervical enlargement -84.3%, pharyngeal/laryngeal -29%, aural -23%, neurological -19.8%, nasal -8.1%, and orbital -7.3% (31). Another study of patients seen at KNH between 1988-1992 showed the following presentations:- cervical node/mass -97.7%, nasal obstruction -67.6%, hearing loss -64.7%, headache and facial pains -44.1%, and epistaxis -41. 2% (31). Another study done by Muchiri (2003) in KNH showed the presentation as below (21).

Table 1: Signs and Symptoms

Symptom	Frequency	Percentage (%)	Symptom	Frequency	Percentage (%)
Neck Swelling	100	80.0	Dysphonia	7	5.6
Nasal Blockage	89	71.2	Dysphagia	18	14.4
Epistaxis	56	44.8	Trismus	26	20.8
Nasal Discharge	1	0.8	Proptosis	13	10.4
Nasal Growth	13	10.4	Diplopia	8	6.4
Ear Blockage	25	20	Failing vision	8	6.4
Otalgia/Ear Pain	11	8.8	Facial numbness	14	11.2
Tinnitus	17	13.6	Others	24	19.2
Hearing Loss	47	37.6	Total	125	100

It should however be noted that the symptoms above are not as dramatic as cervical mass and are unlikely to drive the patient to sick medical redress. Patient presenting with this symptom should therefore lead to meticulous examination of the neck for nodes or masses which are accessible to initial FNAB assessment.

EXAMINATION OF THE PATIENT

Routine general examination is mandatory. A detailed head and neck examination should then be done before performing a complete and detailed otolaryngology examination (15,16,17,18,26,31)

Nasopharyngeal examination

Examination of the nasopharynx has many draw backs, for example the 'functional' channel for examination is about 2cm only, posterior rhinoscopy is restricted by pharyngeal reflex and patient co-operation and inability to open the mouth and the mirror only gives an 'edge view' of the fossa of Rosenmuller. Biopsy can be taken using mirror examination under general anaesthesia with mouth opened by a Boyles-Davies mouth gag and soft palate retracted by nasal-oral catheter. Transoral nasopharyngoscope can also be used. A tiny growth can be identified and biopsy taken by transnasal rigid endoscopy under local anaesthesia (17). Endoscopic alone has low sensitivity in predicting persistent disease and multiple sites biopsy specimens are therefore indicated. Repeat biopsies are indicated for those with endoscopic findings of 'residual tumour' or positive histologic findings in first session of biopsies to improve detection of persistent disease (30). Positron emission tomography has a higher potential of detecting neck node metastasis of NPC than CT scan (33). An occult NPC may be diagnosed by subjecting tissue obtained from FNA of a neck node to polymerase chain reaction to detect presence of the viral genome of the EBV (34).

BIOPSY METHODS

In KNH, nasopharyngeal biopsy routinely follows preliminary FNAB of any accessible cervical mass. Biopsy from nasopharynx can be taken transnasally by blind biopsy using transnasal Hildyard biopsy forceps, posterior mirror rhinoscopy, or by rigid or fibreoptic endoscopy. It can also be taken transorally by use of Yankauer speculum, rigid endoscopy or by blind curettage using adenoids currette if no obvious tumour is in nasopharynx (no obvious primary).

Flexible fibreoptic or rigid nasopharyngoscopy

The nose is first anaesthetized with local anaesthesia e.g. 5% cocaine or 10% xylocaine or lignocaine. The flexible endoscopy gives a good view of the nasal floor, walls of nasopharynx and fossa of Rosenmuller. A biopsy can then be taken using a Hildyard forceps inserted through the contralateral nostril. The advantages of flexible fibreoptic nasopharyngoscopy are as follows: -

1. Small tumours in any quadrant including fossa of Rosenmuller can be biopsied accurately.
2. Problems of post biopsy bleeding obscuring the vision are avoided (scopes is far away from biopsy site).
3. It can be used to detect and biopsy post irradiation tumour recurrence beneath a necrotic scab. Contact endoscopic biopsies (after staining 1% Methylene blue) is an office procedure that is accurate and allows in vivo diagnosis of NPC (35).

This method of biopsy typically follows the use of FNAB to sample a suspicious cervical node. Economically endowed countries with high NPC preference it may be used for screening purposes.

Value of CT Scan and NPC.

The nasophyrynx can appear deceptively normal when the carcinoma has spread extensively outwards by sub mucosal infiltration. A CT scan then becomes very helpful in delineating the outlying tumour extension and skull base erosion. CT scan of the nasopharynx provides a wealth of information that is extremely useful in differentiating malignant from benign disease as well as evaluating the extent and aggressiveness of any inflammatory disease (28).

In a study done to evaluate the value of CT scan in NPC by the department of Radiology UCLA medical centre, Los Angeles (1982) all the patients with primary malignant neoplasm of the nasopharynx demonstrated obliteration of one or more parapharyngeal fascial planes on CT scanning. A number of squamous cell carcinoma that had spread to the nasopharynx from other sites also demonstrated invasion of the fascial planes. Primary lesions of the nasopharynx tend to invade deeply and extend fairly extensively into the deep structures early while secondary disease has more superficial spread until late in the disease hence CT scan can also be of prognostic value (36) Intravenous contrast given during CT scanning discretely outlines extent of vascular tumours e g angiofibromas or glomus jugulare (28).

Contrast enhanced CT scans also helps in determining deep node involvement. Patients who had aggressive inflammatory conditions e g. mucormycosis, Wegeners granulomatotis or fungal disease in immuno-suppressed patients were observed to have obliteration of parapharyngeal fascial planes. Less aggressive inflammatory conditions such as adenoiditis demonstrated no such invasion on CT scanning (36). MRI which is gadolinium enhanced easily reveals the intracranial extension. Unfortunately, MRI technique are not easily available in our setup.

X-ray findings of bone erosion at the base of skull especially middle cranial fossa usually in the region of the foramina lacerum would give evidence of superior spread of tumor and widening of foramens (31) X-ray of nasopharynx lateral view show as a soft mass in the pharynx. Chest and thoracolumbar X-rays and abdominal ultra sound give information on distant metastasis.

Serological tests are those for IgA and IgG to VCA, IgA and IgG to EA, Antibody to NA and Antibody dependent cellular cytoxicity antibodies (ADCC). Other tests include blood biochemistry, full blood count, ESR, liver function test and Audiometry.

TREATMENT

Treatment is based on histological verification of the disease and not in isolated FNAB findings. Chemoradiation is the mainstay of treatment. Concurrent cisplatin, 5-fluorouracil, and radiotherapy have been shown to improve survival. Other studies have employed neoadjuvant chemotherapy followed by radiation therapy with improvement in local control or progression-free survival rates. However patients in Kenya are usually diagnosed late and treatment is usually palliative. Preliminary results suggest that most patients are dead within three years of diagnosis, hence the importance of screening, early diagnosis and treatment.

Other forms of treatment include the following:-

1. **Locally recurrent disease** - re-irradiation, branchy therapy or stereotactic radiotherapy.
2. **Surgery** - obtaining of biopsy, radio-resistant nodes may be removed by neck dissection, nasopharyngectomy, base of skull surgery, cryosurgery.
3. **Immunotherapy**
4. **Palliative** – symptomatic treatment, feeding and psychological.

Mwangi (2003) did a study on efficacy of FNAB in diagnosis of head and neck masses in KNH. A total of 141 patients with head and neck masses were evaluated using FNAB and open biopsy. The results of the two procedures were compared. He concluded that the efficacy of FNAB is very high with an overall sensitivity of 93.6% in diagnosis of head and neck masses (5). In this study there were only 11 (7.8%) patients who had NPC with metastasis to cervical lymph nodes and FNAB from the nodes had sensitivity of 100%. This figure is too small to make a conclusion of such sensitivity. The focus of the study was head and neck masses and not NPC hence the efficacy on NPC diagnosis could not be ascertained. One of his recommendations is that Radio-oncologist should consider accepting patients with FNAB results especially NPC and institute treatment as the efficacy is very high. Such a recommendation requires a detailed study on this condition with a bigger sample size. Another setback is histological difficulties encountered in differentiating cytological and histological sections of undifferentiated carcinomas from those of lymphomas. Their treatment regimes are entirely different. In centres where facilities are variable, the cytology specimens can be sent for identification of latent membrane proteins (EB virus protein). He also recommended that a detailed study for each condition, in his study, should be carried out to show the efficacy of FNAB and therefore the need for this study.

Viguer et al (2005) did a study of Fine-needle aspirate cytology (FNAC) of metastatic nasopharyngeal carcinomas. Cytological features of nasopharyngeal carcinoma (NPC) were reviewed in an attempt to select cytological criteria that permit a specific recognition of metastases. For this purpose, 54 fine-needle aspiration (FNA) procedures from 43 patients with NPC were analysed. Thirty-two (59.3%) procedures were performed before the histological diagnosis. In 25 (46.3%) procedures, smears showed many neoplastic single cells, clusters, and abundant lymphoid cells (mixed pattern). A dissociated (single cell) pattern consisting of individual neoplastic and lymphoid cells was seen in 18 (33.3%) cases. Finally, 11 (20.4%) cases showed cohesive epithelial clusters (cohesive pattern) without relevant cellular dissociation or lymphoid cells. Squamous-cell differentiation was seen in three of these cases. Most single neoplastic cells presented as large, pleomorphic naked nuclei. Other interesting findings were granulomas (n = 3), prominent eosinophilic infiltrates (n = 4), and suppurative changes (n = 5). In most smears with mixed and dissociated patterns, a nasopharyngeal origin could be suggested. On the contrary, those smears with a cohesive pattern were indistinguishable from other head and neck carcinomas. The presence (on cervical lymph nodes) of a dissociated or mixed (single cells and groups) architectural pattern of large, anaplastic cells and naked nuclei accompanied by an abundant lymphoid component is highly suggestive of undifferentiated NPC. He concluded that cytology offers a rapid diagnosis,

establishes the necessity of a complete cavum examination and helps in avoiding unnecessary and harmful cervical node biopsies (37). In this study the efficacy of FNAB of metastatic NPC was 79.6% while in 20.4% could not distinguish from other head and neck carcinomas. This differs with Mwangi (2003) in KNH (5). From this study FNAB can only be a screening but not a diagnostic method

Nadjib et al (1993) did a study of fine needle aspiration biopsy of the nasopharynx. Histological correlation was possible in 121 cases in which cytology was reported as malignant. Malignancy was confirmed in 92 cases. In the remaining 29 cases histological examination revealed only benign or atypical changes. However, all these patients had clinical evidence of NPC, and 21 of them had, in addition, enlarged cervical nodes. Fine needle aspirates from these nodes showed malignant cells similar to those of the primary site. He concluded that fine needle aspiration is more useful and reliable than conventional surgical biopsy in the diagnosis of nasopharyngeal carcinoma and that it improves the diagnostic yield of endoscopic examination (38). This study FNAB from cervical lymph nodes had efficacy of 100% and it showed malignant cells, similar to primary site, which had been missed by histological examination. This concludes that FNAB is a diagnostic not screening method in NPC metastatic disease to cervical lymph nodes. The number of patients with cervical lymph node enlargement in this study was small to make this conclusion.

Mohanty et al (2002) did a study with the aim to describe the cytological features of metastatic nasopharyngeal carcinomas (NPC) to lymph node, which would help in arriving at a definite diagnosis, particularly in cases of occult primary with predominant finding of cervical lymphadenopathy. It was a retrospective study over a period of 2 year and included 15 cases of NPC metastasising to cervical lymph nodes. All cases had histopathologic diagnosis of primary NPC. Two independent observers studied detailed cytomorphology of these cases. The FNAC smears were cellular and except for three cases revealed predominantly discohesive malignant cells admixed with lymphocytes. Cellular degeneration and naked tumour cell nuclear were seen in all cases. The tumour cells showed mild to moderate nuclear pleomorphism, vesicular chromatin, and scanty cytoplasm with ill-defined cytoplasmic borders. All 15 cases were typed according to the World Health Organization (WHO) classification as types I II, and III NPCs. There were 2 (13.3%) cases of type I NPCs, 5 (33.3%) cases of type II, and 8 (53.4%) cases of type III pattern. He concluded that FNAC of metastatic NPC in lymph node is a valuable diagnostic modality in suggesting the site of origin of the tumour and it is also helpful to exclude residual disease and recurrence in a treated patient with lymphadenopathy (39). The FNAB in this study had efficacy of 100% in diagnosis of NPC from lymph nodes and even classification of the tumour. The sample

size is small for these conclusions. The study was retrospective and could be biased in selecting only for positive cases.

Jayaram et al (1999) did a study on cytological appearance of metastatic NPC in 17 cases. Histological correlation was available in all the patients in the form of nasopharyngeal biopsies, and they were classified as per the World Health Organization classification into types I, II and III NPC. Smears from type II NPC showed good cellularity with mainly clustered and occasionally dissociated cells, with focal columnar appearance, vesicular nuclei, prominent nucleoli, and variable amounts of cytoplasm. Clusters of malignant cell closely associated with lymphoid cells and dissociation of malignant cells were more characteristic of type III NPC. He concluded that, FNA cytology is now applied extensively to the diagnosis of head and neck tumours and knowledge of the cytomorphology of NPC would greatly aid in pinpointing the primary of this tumour, which is notorious for presenting with early nodal metastasis (40). The sample size is small, does not indicate the figures in each type and does not mention the appearance of cells in type I. The conclusion mentions other head and neck tumours, which were not included in the study.

In summary, most of these studies had small sample sizes for good results and conclusions made, variable efficacy of FNAB in diagnosis of metastatic NPC, variable findings, conclusions and differing recommendations were observed. Therefore, there is need to do a detailed study on efficacy of FNAB in diagnosis of clinically suspected NPC with metastasis to the cervical lymph nodes in KNH.

JUSTIFICATION OF THE STUDY

Nasopharyngeal carcinoma is a common cause of cervical malignant metastasis in Kenyan adults (5,19,21,41) and up to 97.7% of NPC patients present with cervical lymphadenopathy at first in KNH (19). Although FNAB has been shown as an invaluable screening and diagnostic tool in head and neck masses in many centres worldwide, its detailed study in clinically suspected NPC has not been done in KNH. FNAB is cheap, less invasive and easy to perform for screening of cervical malignancies. Trained technicians can even perform it. This study is one of the recommendations from the only study done on efficiency of FNAB in diagnoses of head and neck masses in KNH by Mwangi in 2003 (5). The focus of his study was head and neck masses and not NPC hence the efficacy on NPC diagnosis could not be ascertained. Although it is currently used in KNH by ENT, dental and general surgeons, its efficacy in evaluation of secondary NPC in cervical nodes has not been documented in a well designed study in KNH set up taking into consideration the skills of cytologist and logistical problems. If its sensitivity and specificity are within acceptable range then it will be justifiable to accept its value as it is evidenced elsewhere, where it is used in the following circumstances: -

1. Help the ENT surgeons/medical service providers in poorly equipped centres in evaluation and early referral of NPC patients to health facilities equipped with Rigid Nasopharyngoscopic and radiological equipments for early diagnosis and treatment.
2. As a reliable diagnostic tool for clinically and/or radiologically suspected NPC in patients who are not suitable or refuse to consent for endoscopic biopsy.

Thus there was need to carry out a study in KNH department of ENT-HNS where NPC is common and FNAB routinely done to help in diagnosis before doing conventional biopsies for histopathology.

THE STUDY HYPOTHESIS

NULL HYPOTHESIS

FNAB is not as effective as tissue biopsy in diagnosis of NPC.

ATERNATIVE HYPOTHESIS

FNAB is as effective as tissue biopsy in diagnosis of NPC.

MAIN OBJECTIVE OF THE STUDY

To evaluate the efficacy of FNAB in diagnosis of clinically suspected NPC in patients with cervical lymphadenopathy in KNH.

SPECIFIC OBJECTIVES

1. To do FNAB in all patients clinically suspected to suffer from nasopharyngeal carcinoma with cervical lymph node metastasis and compare the results with those of rigid nasopharyngoscopic punch biopsy of primary.
2. To determine the time taken to obtain both results of FNAB and nasopharyngoscopic biopsy in KNH

STUDY METHODOLOGY

SETTING (STUDY AREA)

The study site was at the ENT-HNS department of KNH.

STUDY DESIGN

This was a descriptive cross-sectional study.

STUDY POPULATION

All patients who were clinically suspected to suffer from NPC with metastasis to the cervical lymph nodes seen at KNH in ENT-HNS department and were for nasopharyngoscopic biopsy.

INCLUSION CRITERIA

All patients who were clinically suspected to suffer from NPC with metastasis to the cervical lymph nodes seen at KNH in ENT department and were for nasopharyngoscopic biopsy. Such patients may have had painless mobile or fixed cervical lymphadenopathy with or without aural, nasal, oral, ocular, CNS, cranial nerves symptoms and headaches.

EXCLUSION CRITERIA

1. Patients who were suspected to have vascular disorder or tumours with bleeding tendencies e.g. aneurysm, haemangiomas, carotid body tumour or angiofibromas.
2. Patients who were suspected to suffer from NPC and did not have accessible suspicious cervical mass or declined nasopharyngoscopic biopsy.
3. Patients who were clinically suspected to suffer from NPC who were on any form of anti-cancer treatment for NPC or other tumours.
4. Patients who came with FNAB and histology results from elsewhere.
5. Patients who came with ulcerative or fungating mass of the neck.
6. Those patients who declined to be inducted into the study.

SAMPLE SIZE AND DURATION OF STUDY

The sample size was determined by using Fisher formula

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where n = Minimum sample size acceptable to the study

z = Standard errors from mean corresponding to 95%

Confidence level

z =1.64

p = Efficacy of FNA on head and neck masses in KNH ENT-HNS department by Mwangi in 2003 (93.6%)

d = Absolute precision (5%)

$$n = \frac{1.64^2 \times 0.936(1-0.936)}{0.05^2} = 64.44712 \quad n = 65$$

In the study the minimum acceptable sample size was 65 patients.

This study took 6 months (from June 2007 to December 2007) to collect enough data for analysis and a total of 68 patients were inducted.

MATERIALS

1. 21 gauge fine needle.
2. Twenty mls syringe.
3. Clean pair of gloves.
4. Slide carrying container.
5. Glass slides.
6. 95% Alcohol.
7. Stains: pap stain, Hematoxylin and Eosin (H & E).
8. Containers for sharps disposal.
9. Spirit swabs.

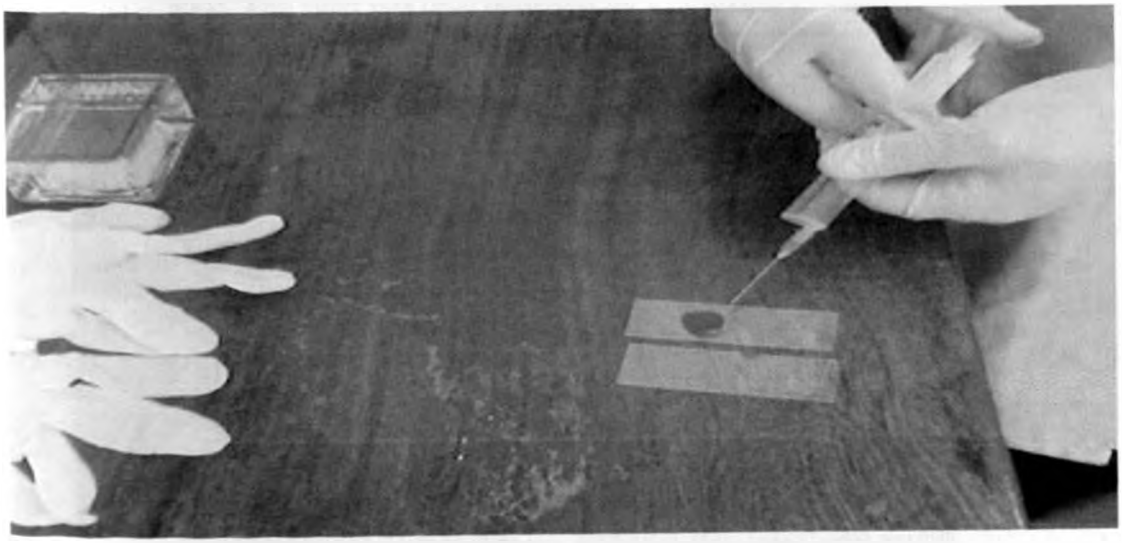
Photograph 1: showing some of the materials used in taking FNAB.

METHODS

The principal investigator identified the patient and informed consent was sought for inclusion in the study. Those who met the criteria were included and their details entered in the study proforma shown in appendix II. A detailed history was taken and physical examination was done. FNAB and nasopharyngoscopy and biopsy were done on all the patients. Both results were entered in the study proforma.

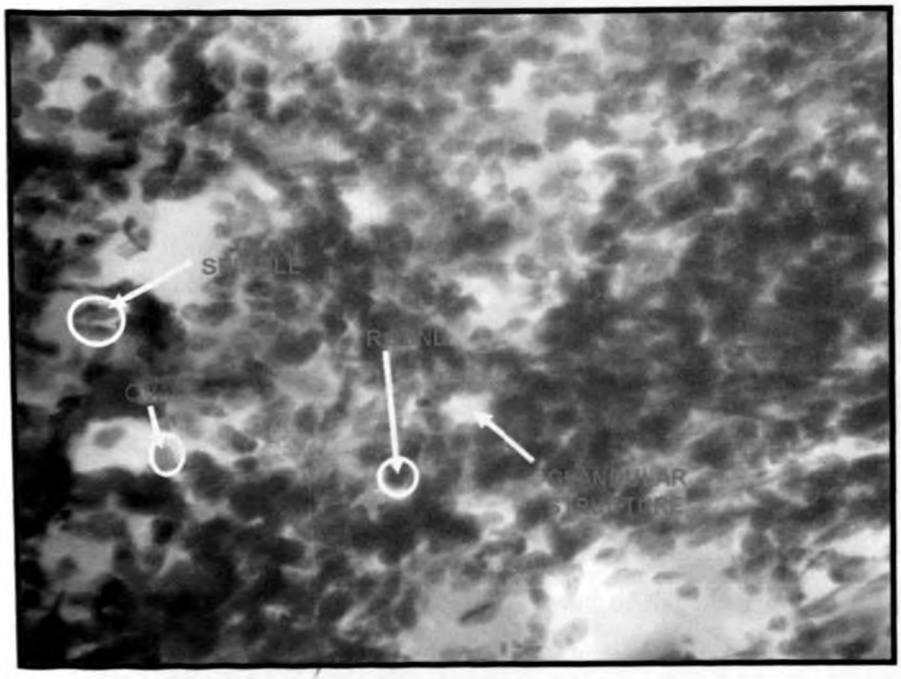
FNA PROCEDURE

FNAB was done to all the patients inducted in the study as described below using the method described by Kun (41). Patient was positioned to allow the best digital palpation of the mass. The skin overlying the mass was prepared with a pre-packaged, sterile, alcohol prepared sponges containing 70% Isopropyl alcohol. The investigator grasped the mass with the left hand and held it in a fixed and stable position. A 20cc disposable syringe with an attached 21-gauge needle was placed just under the surface of the mass and negative suction was applied to the syringe. The best negative pressure was created and maintained with the syringe plunger pulled back to 10cc mark. The mass was entered and multiple (about 6) passes were made without exiting the surface of the mass. The vacuum on the syringe was then released and the mass was exited. From the aspirated fluid a small drop was applied on the glass slide. A smear was made by laying another glass slide on top of the drop of fluid and pulling the slide apart to spread the fluid. Wet smear were placed in 95% ethyl alcohol and treated with the papanicolaou technique and stains.



Photograph 2: showing an aspirate being applied on glass slide. After the 20-cc disposable syringe with an attached 21-gauge needle had been applied into the mass and aspirated, a small drop of aspirated fluid was placed on a glass slide.

Photograph 3: showing the smear technique for plating a sample aspirate. After a small drop of fluid was applied on a glass slide, a second slide was used to smear the aspirate evenly over the surface of the slide. The slide was then prepared for cytological evaluation.



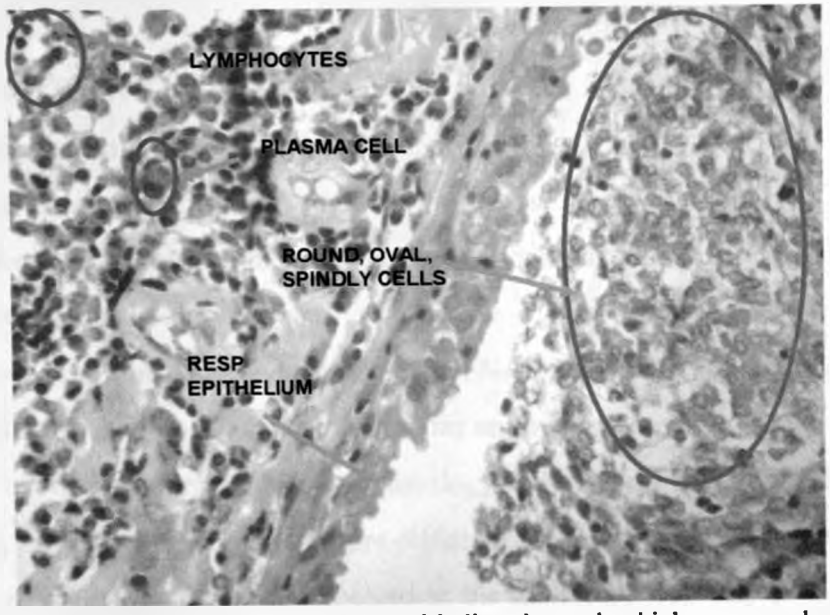
Photograph 4: Cytology slide:- Haphazardly arrange cells exhibiting pleomorphism - round, oval and spindle cells with hyperchromatism. They exhibit coarse chromatin. These are features of anaplastic carcinoma.

PROCEDURE OF PREPARING SECTION FOR HISTOPATHOLOGY

The specimen from Nasopharyngoscopic biopsy were preserved in formalin and taken to the histology laboratory when labeled. In the laboratory, the technologist did put the specimen in three changes of formalin 30 minutes each for proper fixation. Dehydration of the specimen was done using ascending order of alcohol concentration 70% - 90%. For the tissue to mix with paraffin wax it was put in two changes of chloroform, first one hour and second for three hours.

To provide internal support of the tissue it was put in two changes of paraffin wax. Cooling the wax into blocks for easier sections provided external support. The blocks were mounted on microtome machine and using microtome knife thin sections of less than 5 micrometer were made.

The tissue specimens were mounted on the slide and melting of the wax was done in an oven. Dewaxed slide was put in xylene to remove all the wax. The prepared slide was stained routinely with H/E stain. Cover glass was mounted on the stained tissue using Dibutyl phenelyphthaline Xylene (DPX). The prepared slide was examined and reported by the histopathologist.



Photograph 5: Histology slide:- Respiratory epithelium beneath which was anaplastic tumour composed of oval, round and spindle cells. Occasional nucleus and many chronic inflammatory cells- Plasma cells and lymphocytes were seen. Features of anaplastic carcinoma.

QUALITY CONTROL

The questionnaire was pre-tested before commencement of the study and appropriate modifications were made. All FNABs were done by principal investigator to avoid interpersonal variation. All RNEs were done by registrars in the department of ENT-NHS of KNH. All cytology and histology specimens were examined by qualified cytopathologist and histopathologist respectively. Neither the former nor the later had advance knowledge of the cytological or histological findings.

DATA ANALYSIS

All data were analysed for completeness, consistency and accuracy. It was then transferred into a coded sheet for computer analysis (SPSS Package) with the help of a statistician. The results were presented in text, graphs, tables and charts. Conclusion and recommendations were made based on the results.

ETHICAL CONSIDERATIONS

Informed consent was obtained from all patients before induction into the study. The patient or guardian gave consent voluntarily. The study was done after being approved by the ethical committee of KNH. The results of the FNAB and nasopharyngoscopic biopsy were treated as confidential. Both results were communicated to the patients and counseling done. Patients were treated using conventional modalities after FNA and nasopharyngoscopic biopsy. The patients did not incur any extra costs, as FNAB is the first line of investigation of patients with head and neck masses.

CONSTRAINTS IN THE STUDY

The following constraints were encountered:-

1. Financial constraint of patients for RNE and biopsy.
2. Long booking of RNE leading to delay in performing it and constraint to the patients who came from far and very sick ones.
3. Only two days were allocated for FNAB in FNAB room in a week so inconveniencing to the patients especially those who come from far and very sick ones.
4. Delay in processing and reporting of histology and cytology biopsies leading to patients seeking private services and therefore difficulties in following up specimen and results.
5. Filing of the result after being reported in the laboratory was very slow, poor and some times being misplaced forcing use of carbon copies in the laboratory files or repeating the procedures.

RESULTS

A total of sixty eight (n=68) NPC patients were inducted in the study. Cervical lymphnode FNAB for cytology and nasopharyngoscopic biopsy for histology were taken and examined independently. The results were as follows:-

Table 2: Sex Distribution

SEX	FEMALE	MALE	TOTAL
FREQUENCY	26	42	68
PERCENTAGE	38.2%	61.8%	100.0%

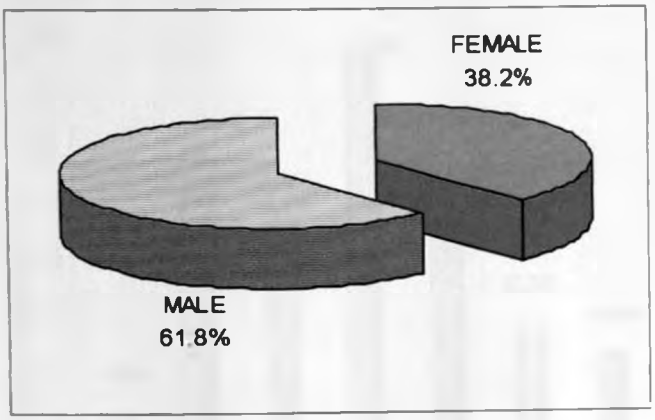


Figure 1: Sex Distribution :-

These included 42 (61.8%) male and 26 (38.2%) Female. The overall ratio of male to female was 1.6 : 1

Table 3: Age Distribution

AGE	Frequency	Percent
<20	4	5.9%
20-29	12	17.6%
30-39	11	16.2%
40-49	17	25.0%
50-59	11	16.2%
60-69	7	10.3%
70+	6	8.8%
Total	68	100.0%

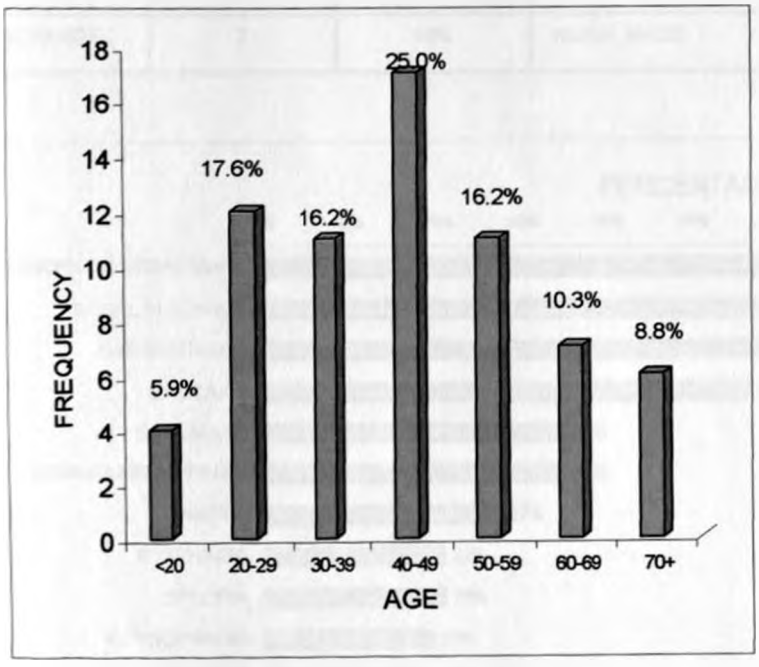


Figure 2: Age distribution:-

Those inducted in the study ranged between 14 and 83 years, with a mean age of 44 and a multiple mode of 25, 46, 48 and 70 years and a median of 46. The most seen age group was between 40 and 49 years.

Table 4: Signs and Symptoms

SYMPTOMS	FREQUENCY	PERCENTAGE	SYMPTOMS	FREQUENCY	PERCENTAGE
NASAL BLOCKAGE	49	74%	NASAL FB SENSATION	5	8%
EAR BLOCKAGE	42	63%	EAR GROWTH	1	2%
EPISTAXIS	38	57%	**SIGNS**	**FREQUENCY**	**PERCENTAGE**
TINNITUS	18	27%	NASOPHARYNGEAL MASS	59	87%
DYSPHAGIA	14	21%	ORAL MASS	23	35%
DIPLOPIA	14	21%	CRANIAL NERVE PALSY	23	35%
ODYNOPHAGIA	13	20%	TRISMUS	11	17%
EYE PAIN	13	20%	PROPTOSIS	8	12%
ORAL GROWTH	8	12%	NASAL MASS	3	5%
EAR DISCHARGE	7	10%	AURAL MASS	3	5%

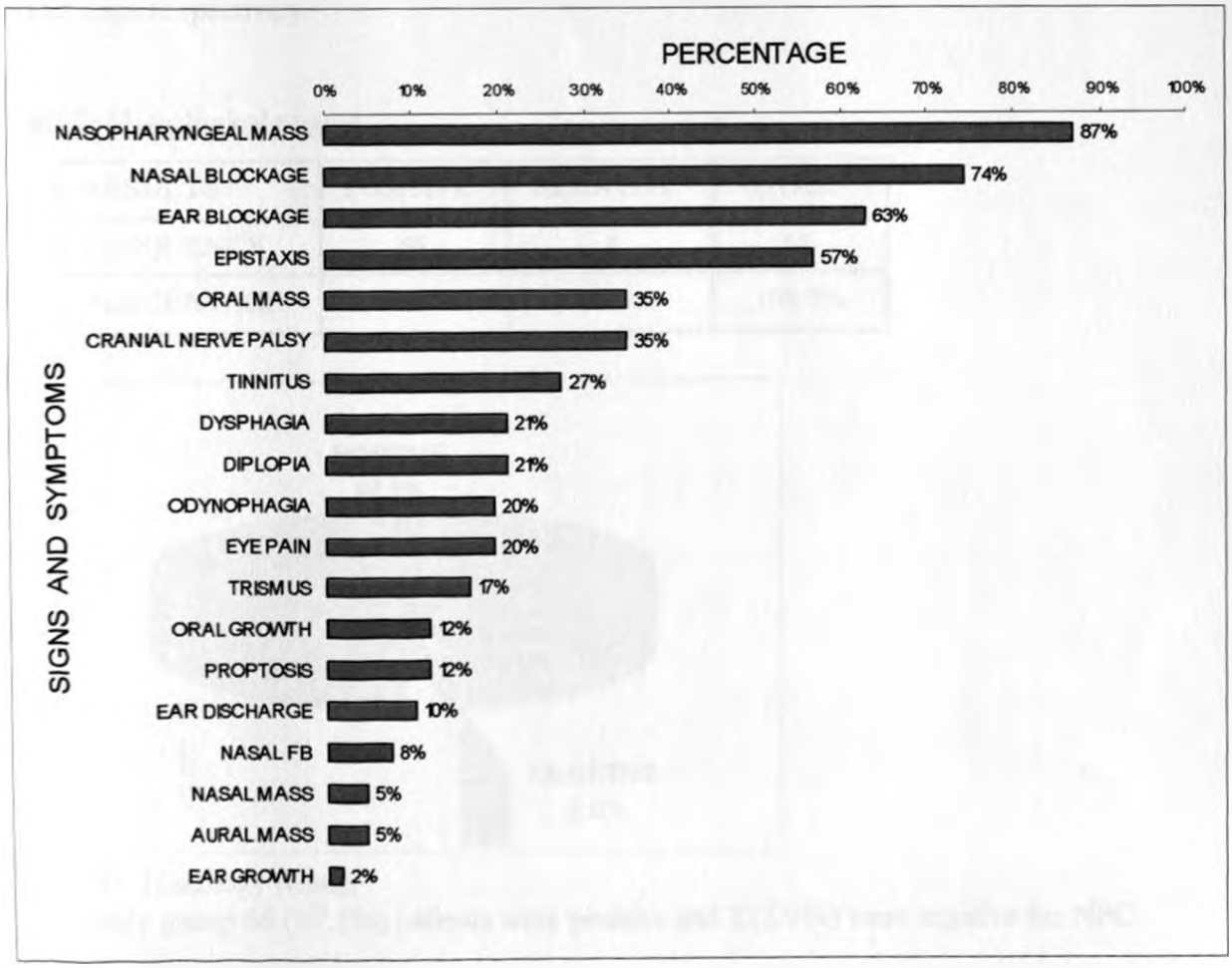


Figure 3: Signs and Symptoms:-

The commonest presentation of NPC in this study group (apart from neck masses which was an inclusion criteria) was nasopharyngeal mass followed by nasal blockage.

Table 5: Tumour Stage Grouping

	STAGE 2	STAGE 3	STAGE 4	TOTAL
FREQUENCY	3	11	54	68
PERCENTAGE (%)	4.4%	16.2%	79.4%	100.0%

79.4% of the patient in this study group had stage IV and there were no any stage I disease observed clinically.

Table 6: Duration taken for the results of cytology and histology.

	No. OF PATIENTS	MEAN	MINIMUM	MAXIMUM
Duration of FNAB(days)	68	10.45	2	39
Duration of Histology(days)	68	19.59	2	62

It took minimum of 2 days for both cytology and histology to obtain results and a maximum of 39 and 62 days respectively

Table 7: Histological results

RESULTS	POSITIVE	NEGATIVE	TOTAL
FREQUENCY	66	2	68
PERCENTAGE	97.1%	2.9%	100.0%

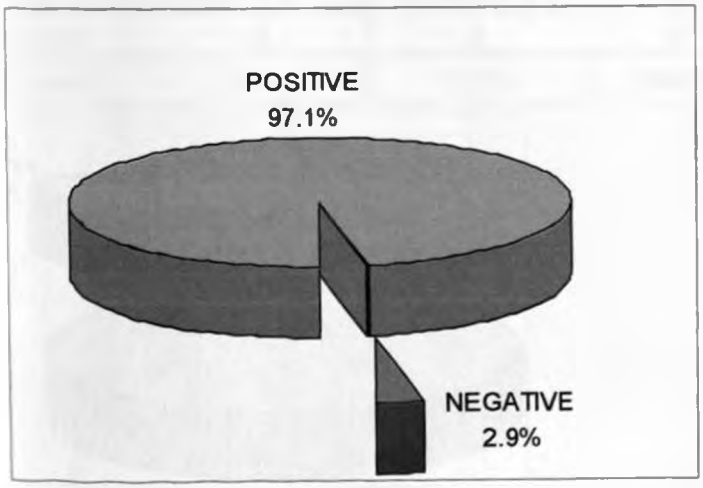


Figure 4: Histology results

In the study group 66 (97.1%) patients were positive and 2 (2.9%) were negative for NPC.

Table 8: Histological WHO Classifications

CLASSIFICATION	TYPE 1	TYPE 3	NEGATIVE	TOTAL
FREQUENCY	16	50	2	68
PERCENTAGE	23.53%	73.53%	2.94%	100.0%

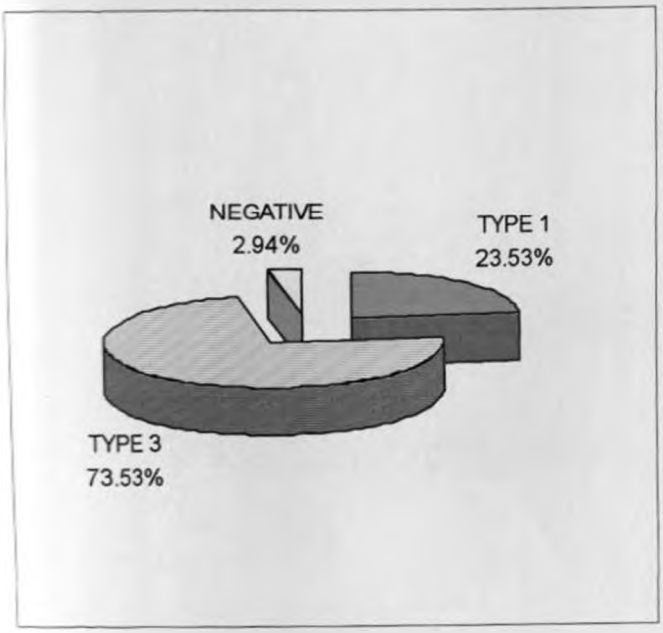


Figure 5: Histological WHO classification

Using the WHO histological classification 73.53% were type 3 and 23.53% type 1.

None had type 2.

Table 9: FNAC Results

RESULTS	POSITIVE	NEGATIVE	TOTAL
FREQUENCY	62	6	68
PERCENTAGE	91.2%	8.8%	100.0%

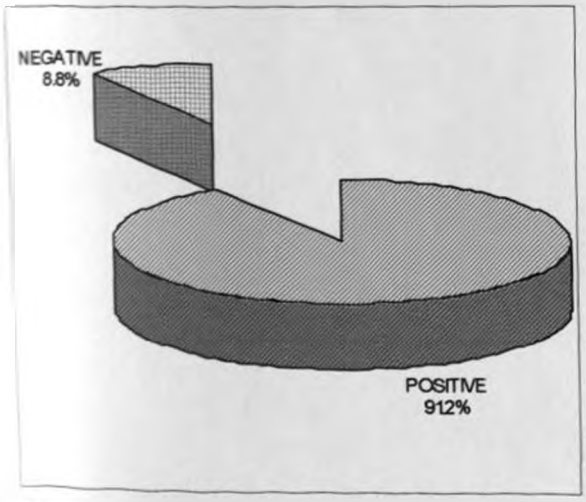


Figure 6: FNAC Results:-

Fine needle aspirate cytology of the cervical lymphnode for the study group was positive in 62 (91.2%) and negative in 6 (8.8%) of the patients.

Table 10: FNAB WHO Classifications

CLASSIFICATION	TYPE 1	TYPE 3	NEGATIVE	TOTAL
FREQUENCY	13	49	6	68
PERCENTAGE	19.1%	72.1%	8.8%	100.0%

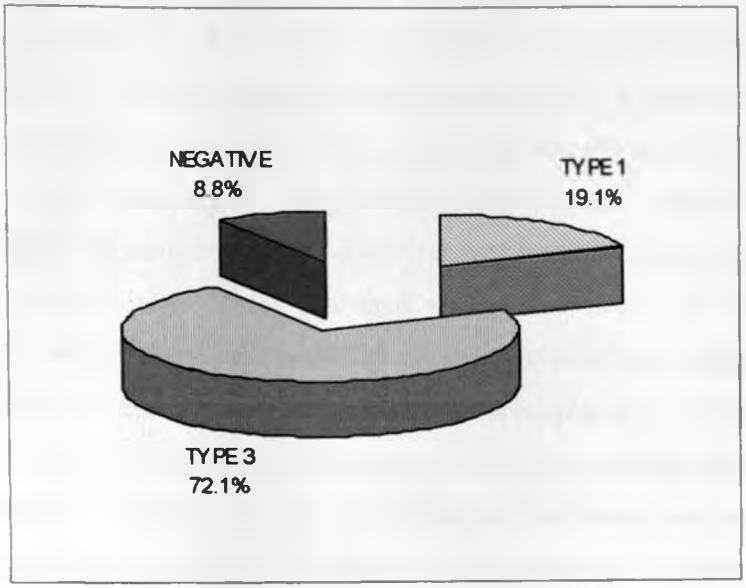


Figure 7: FNAB WHO Classification:-

Cytologically 72.1% were in type 3, 8.8% were negative and there were no type 2 detected.

Table 11: FNAB/histology results- Cross tabulation count.

RESULTS		HISTOLOGY		
		POSITIVE	NEGATIVE	TOTAL
CYTOLOGY	POSITIVE	62	0	62
	NEGATIVE	4	2	6
	TOTAL	66	2	68

$$\text{SENSTIVITY} = \frac{62}{(62 + 4)} \times 100 = 93.9\%$$

$$\text{SPECIFICITY} = \frac{2}{(0 + 2)} \times 100 = 100.0\%$$

From the above results the sensitivity of FNAB was 93.9% and specificity of 100%.

DISCUSSION

NPC is the most common head and neck cancer seen at KNH. It is also a common cause of cervical lymphnode malignant metastatic disease in Kenyan adults (5,19,21) and up to 97.7% of NPC patients present with cervical lymphadenopathy (19). It has been noted in this study and previous studies that many patients with NPC present late (21, 44). In view of this it was important to explore diagnostic methods applicable to local population where we have high incidences (21). This was a prospective study carried out for six month from June 2007 to December 2007 at KNH, ENT-NHS department. A total of sixty eight patients who certified inclusion criteria were inducted into the study. Among these 42 (61.8%) were male and 26 (38.2%) were female. This gave a ratio of male to female 1.6:1. The age ranged between 14 and 83 years with a mean age 44 years, median of 46years and a multiple mode of 25, 46,48 and 70 years.
Clinically all patients must have had cervical lymphadenopathy which was a mandatory inclusion criteria. The patients in this study presented with multiple signs and symptoms which included cervical, nasal, otological, oropharyngeal, neurological, and ophthalmologic features in order of decreasing frequencies. It is important also to note that on rigid nasopharyngoscopy 59 (87%) of the patients had nasopharyngeal mass and 9 (13%) had no frank mass on nasopharyngoscopy which is common with early pre-clinical and infiltrative NPC (16, 17, 41). For each patient, the disease was staged using the AJCC TNM classification and tumour grouping shown in appendix III. The patients in stage IV were 54 (79.4%) indicating that many patients presented in the late stage. In this study there were no patients in stage I because of the inclusion criteria of cervical lymphadenopathy. CT scan findings were not included in the study which could have added more value to the clinical findings.

The duration taken from the date that each sample was received in the laboratory and the date of reporting were noted to determine the length of time it took to obtain the results of both FNAB and RNE biopsy. The FNAB took a minimum of 2 days and a maximum of 39 days with a mean of 10.45days. The RNE biopsy took minimum of 2 days and a maximum of 62days with a mean of 19.5 days. In view of these, FNAB took a shorter time than RNE biopsies to obtain the results. Mwangi et al (5) found that the mean for FNAB was 7.29 days which was shorter than in this study, may be due to increase in work load with time, and tissue biopsy 19.91 days which is almost the same as in this study. The two studies indicated that to obtain results of FNAB takes a shorter time than those of RNE biopsy. The possible reason of such a wide range in obtaining the result was the processing of slides for histology is much involving and takes longer time than cytology.

For all the patients inducted into the study, FNAB of cervical lymphnode for cytology and nasopharyngoscopic directed tissue biopsy for histology were taken and examined independently then results compared. For all the patients inducted in this study the histology, which is the gold standard for diagnosis of NPC, was positive in 66 (97.1%) and negative in 2 (2.9%). Kimani et al (44) had 96.6% positive which

corresponds with the findings in this study. At this point it should be noted that even the 9 (13%) patients mentioned earlier who had no frank mass on nasopharyngoscopy, the histology was positive in keeping with infiltrative NPC (16,17,41). For each patient the disease was classified according to WHO histological classification using both histology and FNAB results. By histology 16 (23.53%) patients were in type 1, 50 (73.53%) in type 3, 2 (2.94%) negative. By cytology 13 (19.1%) patients were in type 1, 49 (72.1%) in type 3 and 6 (8.8%) were negative. It was noted that out of the 6 patients FNAB was negative 3 were in type 1, 1 in type 3 by histology and 2 were negative. There were none in type 2 in both histology and cytology. Therefore the study showed that majority of the tumour were in type 3 both histological and cytologically. Oburra HO et al (19) showed squamous cell carcinoma were 2 (5.9%) and undifferentiated 32 (94.1%) (n=34). Mwangi JC et al (32) showed squamous carcinoma 5.2%, transitional cell carcinoma 3.2% and anaplastic carcinoma 91.6%. Mohanty et al (39) using cytology showed type 1 -13.3%, type 2 -33.3% and type 3 -54.4% (n=15). Jayaram et al (40) used cytology but did not give figures in each type but gave features to differentiate them. Other studies done internationally have shown type 3 being most common (18, 27,39). Therefore in this study both histology and cytology correspond with other studies done locally and internationally which have shown preponderance of undifferentiated histological spectrum.

Fine needle aspirate Biopsy was done in all the 68 patients. FNAB was positive in 62 (91.2%) of the patients and negative in 6 (8.8%). Two of the above cases which were negative FNAB were reported as chronic inflammatory condition suggestive of TB adenitis and tissue biopsy were negative. The other four FNAB results were reported as inflammatory and reactive lymphnode while histology tissue biopsy was positive. It should be noted that even the 9 (13%) patients mentioned earlier who had no frank mass on nasopharyngoscopy and histology was positive FNAB was also positive in all of them. From the results FNAB had a sensitivity of 93.9% and specificity of 100% in this study. Mwangi et al (n=11), Nadjib et al (n=21) and Mohanty et al (n=15) had a sensitivity of 100% (5, 38, 39) but their sample sizes were small for such sensitivity. Mwangi et al (n=141) on FNAB on neck masses had an overall sensitivity of 93.6% which is almost equal to the sensitivity in this study (5). Viguer et al (n=54) FNAB of cervical lymphnode in NPC patients had a sensitivity of 79.6% (37) which was lower compared with this study, may be due to difference in sample sizes. Other factors that might have contributed to these differences could be due to varying skills and liability of the cytologist and the investigators, mode of transport to the laboratories, time taken before preparation of the cytological studies and examination of the slides.

Other malignant tumours of aerodigestive tract, breast, skin, lymphoid and other systems can present with cervical metastatic diseases. The FNAB cannot predict the exact primary of the metastatic diseases. Squamous cell carcinoma of upper aerodigestive tract microscopically look the same and therefore it is difficult to predict the primary by both histology and cytology unless it has clinically and/or radiologically been suspected. Likewise metastatic tumours of undifferentiated

carcinomas may be both cytologicaly and histologicaly difficult to differentiate from lymphomas and immunohistochemistry may be required (45,46). Because of these factors FNAB compliments clinical presentation and radiological findings in making diagnosis of clinically suspected NPC. Tissue biopsy of nasopharynx for histology remains the gold standard in diagnosis of NPC.

CONCLUSION

From this study it is evident that the efficacy of FNAB of cervical lymphnode is very high with sensitivity of 93.9% and a specificity of 100% in diagnosis of clinically suspected nasopharyngeal carcinoma in patient with cervical lymphadenopathy. The duration of obtaining results of FNAB was shorter time than RNE biopsies. Other primary malignancies can present with metastatic malignant diseases of the neck region. FNAB of cervical lymphnode then remains a powerful method in diagnosis and management of clinically suspected NPC with lymphadenopathy. Tissue biopsy for histology of the primary remains as the gold standard for diagnosis of NPC. Therefore FNB of cervical lymphnode is an invaluable tool to aid ENT surgeons and other medical service providers in diagnosis and management of clinically suspected NPC with cervical metastasis.

RECOMMENDATIONS

From this study the following recommendations were made:-

1. FNAB of cervical lymphnode should routinely be done on all patients clinically suspected to suffer from nasopharyngeal carcinoma with cervical lymphadenopathy.
2. FNAB can be used as reliable diagnostic tool for clinically suspected NPC with cervical lymphadenopathy in patients and radiological investigations clearly indicate NPC and all other possible primary sites have been ruled out but endoscopic biopsy results have repeatedly been negative, patients who are not suitable or refuse to consent for endoscopic biopsy. Therefore the radio-oncologist should consider accepting such patients and institute treatment.
3. FNAB should be used to assist in assessment of NPC patients being followed up after treatment with persistent or recurrent cervical masses to exclude residual diseases or recurrence disease.
4. Another study should be done to clinically suspected NPC to show correlation between radiological (example CT scan), clinical, histology and FNAB findings.

APPENDIX I

GENERAL PATIENT INFORMATION

My name is Dr David W Njoroge from ENT department KNH. We are doing a study on NPC. This study involves only patients who agree to take part. Please take your time to make your decision. We would like to find out how effective it is to make a diagnosis of NPC by taking material from the lymph node on your neck as which may be enlarged due to the illness you are suffering from. Although it has been done elsewhere its accuracy has not been confirmed in circumstances prevailing at the KNH. If the study proves to be an effective method of making the diagnosis, it will confirm the usefulness of FNAB as an early diagnostic method or screening method for NPC and help patients in the early referral and treatment.

If you are recruited in the study, a history about your illness will be taken and physical examination as well as thorough examination of your head and neck will be done. The results will be recorded in a form. We will take a FNAB from your mass or lymph node on your neck and the biopsy taken for cytology. This is a procedure done to all patients who have a mass or lymph node on the neck. The procedure entails introducing a needle mounted on a syringe and using the syringe the biopsy is aspirated. The material aspirated is taken to the laboratory for examination. There are no added risks peculiar to the procedure since it is just like any other injection.

If you do not participate in the study there will be no any penalties at all and those who choose not to participate in the study will be given the same treatment as those who chose to participate.

If you choose to participate in this study there may or may not be a direct benefit to you. We hope the information learned from this study will benefit other patients with NPC.

Records of your history, physical Examination and Cytology results in the study will be kept in confidential form.

Taking part in this study will not lead to any added cost on top of what you pay the hospital for normal investigations.

Taking part in this study is voluntary; you may choose not to take part or may leave the study any time. Leaving the study will not result in any penalty or loss of benefits that you are entitled to.

The information that we get, may not be of very immediate benefit to you but will help us to know how reliable it is to use this method in diagnosing NPC and like any scientific information, we will seek to share our findings with other people undertaking the similar studies. Therefore we may publish our findings in scientific journals or present them at meetings.

If you require to discuss this matter with your family, friend or associates you are free to do so and we will be ready to answer any question.

If you are satisfied with our explanation and you are willing to participate then please fill and sign the consent below.

CONSENT FOR STUDY

I.....ID No.....

Study No.....of.....

Do hereby consent to be included in the study on diagnosis of nasopharyngeal carcinoma using FNAB at K.N.H. The nature of the study has been explained to me by Dr.....

And I have not been promised any material gain to be included in this study.

Signed.....(self/parent/guardian)

APPENDIX II

PROFORMA FOR THE STUDY

Patient's Name: .....

Study No:.....

Sex:..... Age (in years).....

IP.NO. ..... Date.....

Staging of tumour----------

	SYMPTOMS	YES	NO	DURATION
Nasal	Nasal blockage	( )	( )	----------
	Epistaxis	( )	( )	----------
	Growth	( )	( )	----------
	FB sensation	( )	( )	----------
	Others ----------			

		YES	NO	
Aural	Feeling of blockage	( )	( )	----------
	Hearing loss	( )	( )	----------
	Tinnitus	( )	( )	----------
	Ear discharge	( )	( )	----------
	Growth	( )	( )	----------
	Others----------			

		YES	NO	
Oral	Growth (mass)	( )	( )	----------
	Trismus	( )	( )	----------
	Difficulty in swallowing	( )	( )	----------
	Pain on swallowing	( )	( )	----------
	Others ----------			

		YES	NO	
Neck	Growth/Swelling	( )	( )	----------
	Difficulty in breathing	( )	( )	----------
	Others----------			

		YES	NO	
Eyes	Protrusion	( )	( )	----------
	Pain	( )	( )	----------
	Double vision	( )	( )	----------

Other symptoms: ----------

SIGNS AT PRESENTATION

ENT-HN EXAMINATION.

Lymphadenopathy

Neck Present: ----------

Absent: ----------

If present, state site i.e. above supraclavicular or supraclavicular:----------

Size. Less than 6cm: ----------

More than 6cm: ----------

Multiplicity single ----------

Multiple ----------

Nose:

Nasal endoscopy. Rigid: ----------

Flexible: ----------

Nasopharynx

Lateral walls:

Right----------

Left ----------

Roof ----------

Posterior wall --------

Other signs

Ears auditory meatus ----------

Tympanic membrane ----------

Tuning fork tests

Rinnes: ---------- Weber: ----------

Throat: Oral mucosa ----------

Tongue ----------

Oropharynx ----------

Soft palate ----------

Cranial nerve palsy

Present----------

Absent----------

If present, state the nerve/s involved ----------

Respiratory system ----------

	+Ve	-Ve		+Ve	-Ve
Histology results	( )	( )	Cytology results	( )	( )

If positive, state the type:

WHO-type 1 ---------- 1 ----------

2 ---------- 2 ----------

3 ---------- 3 ----------

Date received in lab ---------- Date reported ----------

APPENDIX III

STAGING OF NASOPHARYNGEAL CARCINOMA

Sites

Postero-superior wall extend from the level hard palate to the base of skull

Lateral wall- including the fossa of Rosenmuller

Inferior wall- consists of the superior surface of the soft palate.

Note: The margins of the choanal orifice including the posterior margins of the nasal septum are included with the nasal fossa.

Primary tumour

T1 - Tumour confined to the nasopharynx

T2 - Tumour extends to soft tissues of oropharynx and/or nasal fossa

T2a -Without parapharyngeal extension

T2b -With parapharyngeal extension

T3 - Tumour invades bony structures and/or paranasal sinuses

T4 - Tumour with intracranial extension and/or involvement of cranial nerves, infratemporal fossa, hypopharynx, or orbit (33).

Regional Lymph nodes

NX - Regional lymph nodes cannot be assessed.

NO - No region al lymph node metastasis

N1 - Unilateral metastasis in lymph node(s), 6cm or less in greatest dimension above the supraclavicular fossa

N2 - Bilateral metastasis in lymph node(s), 6cm or less in greatest dimensions, above the supraclavicular fossa

N3 - Metasis in a lymph node(s)

N3a -Greater than 6cm in dimension

N3b- Extension to the supraclavicular fossa (33).

G - Histopathological grading

GX - Grade of differentiation cannot be assessed

G1 - Well differentiated tumour

G2 - Moderately differentiated

G4 - Poorly differentiated

Distant metastasis

Mx – Distant metastasis cannot be assessed

M0- No distant metastasis

M1- Distant metastasis

Stages

Stage I - T1, N0, M0

Stage IIA-T2a, N0, M0

Stage IIB-T1/T2, N1, M0; T2b, N0, M0

Stage III-T1/T2/T3, N2, M0; T3, N0/N1/N2, M0

Stage IVA-T4, N0/N1/N2, M0

Stage IVB-Any T, N3, M0

Stage IVC-Any T, any N, M1 (44)

Majority of patients seen at KNH present in stage IV (19,21,32,43)

REFERENCES

1. Weymiller EA, Jr Kiviat, NB Duckert. Aspiration cytology, an efficient and cost – effective modality. Laryngoscope 1983; 93 (5): 561 – 4.
2. Amedee, RG, Dhurandhar at al. Fine-Needle Aspiration Biopsy. Laryngoscope September 2001; Vol. 111 (9): 1551 – 1557.
3. Martin H, Ellis EB. Biopsy of needle puncture and aspiration. Ann Surg 1930; 92: 169-81.
4. Lee Z. FNA of Neck masses. E-medicine Jun 2002.
5. Mwangi MJ. Efficacy of FNAB in diagnosis of H & neck masses in KNH. A dissertation in part fulfillment of the degree of M. Med. ENT-HNS 2003; 19-40.
6. Ryd W, Hagmar B, Eriksson O. Local tumour cell seeding by fine-needle aspiration biopsy. A semi-quantitative study. Acta Pathol Microbial Immunol Scand (A) 1983 Jan; 91(1): 17-21.
7. Von ST, Arner O, Skovsted G, et al. Renal Adenocarcinoma. Is there a risk of spreading tumour cells in diagnostic puncture? Scand J Urol Nephrol 1967; 1(3): 270-6.
8. Birchall MA, Stafford ND, et al. Malignant neck lumps. a measured approach. Ann R Coll Surg Engl 1991; 73: 91-5.
9. Audi – Rogena EA. Evaluation of FNA in diagnosis of Accessible Tumours at KNH 1996. Dissertation in part fulfillment for M.Med Pathology 1996; 25-36.
10. Guyot JP, Obradovic D, Krayenbuhl M, et al. Fine-needle aspiration in the diagnosis of head and neck growths. is it necessary? Otolaryngol Head Neck Surg 1990 Nov; 103(5): 697 -701.
11. McGuirt WF, McCabe BF. Significance of node biopsy before definitive treatment of cervical metastatic carcinoma. Laryngoscope 1978 Apr; 88(4): 594 – 7.
12. Shockley Pillsbury. The neck, diagnosis and surgery. 1st ed. Mosby 1994;16-17.
13. Eckert R, Howell LP. Number, size, and composition of cell clusters as related to breast FNA adequacy. Diagn Cytopathol 1999; 21(2): 105-11.
14. Bottles K, Miller TR, Jeffrey RB, et al. Aspiration cytology characterization of inflammatory masses. West J Med 1986 Jun; 144(6): 695-9.
15. Muchiri LW, Penner DW, Adwok J, et al. The role of FNA in the diagnosis of breast lumps at KNH. East Afr Med J1993; 70:31.
16. Chew CT. Nasopharynx. Scotts Brown's Otolaryngology. 5th.ed. Ian S M.Bull T R Butterworth Co.Ltd.1987; 315-330.
17. John W.Mark G, Janet W. Nasophargeal carcinoma. Head and Neck Surgery (Stell and Maran). 4th.ed. Butterworth-Heinmann Ltd.1994; 397-407.
18. Rynd M. Tropical and Geographical medicine. Nasopharyngeal cancer review 1993; 43: 59-65.

19. Oburra HO. Late presentation of Laryngeal and Nasopharyngeal cancer in KNH. East Afr Med J1998; 75: 223 – 226.

20. Willand EF jnr., Joseph B R jnr., Gaffinent. Long-term survival after surgical resection of recurrent NPC after radiotherapy failure. Arch of Otolaryngol Head Neck Surg JNov 1991; 117:1233-36.

21. Muchiri M. Demographic pattern and clinical characteristic of NPC seen in KNH. A dissertation in part fulfillment of the degree of M. Med. ENT-HNS 2003; 34-47.

22. Teo P,leung SF, Yu P, et al. A retrospective comparison between different stage classifications for NPC. Br j Radiol 1991; 64:766:901-8.

23. Henry Gray. The pharynx. Descriptive and Surgical Anatomy. 2nd ed. Paragon.562-3.

24. Chaursias BD. Nasopharynx. Human anatomy. Regional and Applied. 2nd ed. Satish Kumar Jan 1997.

25. William FG. Review of medical Physiology. 19th.ed. Lange 4: 25-26.

26. Gacani W, Oburra HO, Babu MA et al. Distant metastases from nasopharyngeal carcinoma below the clavicle at KNH. East Afri Med J2001; 78: 678-681.

27. August M, Dodson TB, Nastri A, at al. Nasopharyngeal carcinoma. Oral Surg Oral Med Oropath OroRadio and Endod (United States) 2001; 91: 205.

28. Chong VT, Fan YT, Khoo JB. Computed tomography and MRI findings in paranasal sinuses involvement in NPC. Ann Med, Singapore (SINGAPORE) 1998; 27:800-4.

29. Leon SS, Wee J, Tan NG. Recurrent NPC presenting as diffuse dermal lymphatic infiltration in the neck. Head Neck J(United States) 2001; 23: 34-41.

30. Pak NW, To KF, van Hesselt GA. In vivo diagnosis of NPC using contact rhinoscopy. Laryngoscope (United States) 2001; III: 1453-8.

31. Kao CH, Ho YJ. Comparison of Positron emission tomography in detection of cervical lymph node metastasis of NPC. Ann Otol Rhinol Laryngol (united states) 2000; 109:1130-4.

32. Mwangi JC. Nopharyngeal carcinoma seen at KNH. Dissertation in part fulfillment for M.Med, Surgery, 1985; 32-37

33. Chien CR ,Chen SW, Hsieh CW. Retrospective comparison between the AJCC 5th ed. And 4th ed. classifications of NPC. Jpn j clin oncol 2001;31:363-369.

34. Tsai Ming-H, Lin CD, Hsein YY. Prognostic significance of p53 codon 72 polymorphism in NPC. Laryngoscope (united states) 2002; 112:116-9.

35. Negan RKC, Yiu HNY, Cheng HMK, et al. Central nervous system metastasis from NPC. Cancer (unite states) 2002; 4:398-405.

36. Tai THP, Mould RF. Descriptive epidemiology of NPC. Current Oncology 2001; 2:2-68.

37. Viguer JM, Jimenez-heffernan JA, Lopez-ferrer P,et al. Fine-needle aspiration cytology of metastatic nasopharyngeal carcinoma. Diagn Cytopathol 2005 Apr; 32(4): 233-7.

38. Nadjib M, Lubis D. The technical procedure and the value of fine needle aspiration biopsy of the nasopharynx. Pathology 1993 Jan; 25(1): 35-8.

39. Mohanty SK, Dev P, Ghoshal S, Saikia UN. Cytologic features of metastatic nasopharyngeal carcinoma. Diagn Cytopathol. 2002 Dec; 27(6): 340-2.

40. Jayaram G, Swain M, Khanijow V, Jalaludin MA. Fine-needle aspiration cytology of metastatic nasopharyngeal carcinoma. Diagn cytopathol 1999 Sep; 21(3): 230-2.

41. Dhingra PL. Diseases of Ear, Nose and Thorax. 3rd ed; Elsevier. 289-305.

42. Koeller KK. Alamo L, Adair, et al. Congenital Cystic Masses of the Neck: Radiological-Pathologic Correlation. Radiographic 1999; 19(1): 121 – 146 1999.

43. Kun M. A new instrument for the diagnosis of tumours. Month J Med Sci 1847; 7:853 -4.

44. Kimani WK. Evaluation of exfoliative cytological screening methods in patients suspected to have nasopharyngeal carcinoma at Kenyatta National Hospital. A dissertation in part of fulfillment of the degree of M.Med. ENT-NHS 2007; 19-26.

45. Carbone A, Micheau C. Pitfall in microscopic diagnosis of undifferentiated carcinoma of nasopharyngeal type (lymphoepithelioma). Cancer 1982; 50: 1344-1351.

46. Giffler RF, Gillespie JJ, Ajulu AG. et al lymphoepthelioma in cervical lymph nodes of children and young adults. Am J Surg Patho 1977; 1: 293-302.

47. Vinold KJ. Nasopharyngeal carcinoma. ENT news 2002; 11: 84-85.

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15th June 2007

Dr. David W. Njoroge
Dept. of Surgery
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Dear Dr. Njoroge

RESEARCH PROPOSAL: "EFFICACY OF FINE NEEDLE ASPIRATE BIOPSY IN DIAGNOSIS OF NASOPHARYNGEAL CARCINOMA IN PATIENTS WITH CERVICAL LYMPHADENOPATHY" (P20/2/2007)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your revised research proposal for the period 15th June 2007 – 14th June 2008.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

Prof. A.N. Guantai
SECRETARY, KNH-ERC

c.c. The Deputy Director CS, KNH
Prof. K.M. Bhatt, Chairperson, KNH-ERC
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