TITLE: AN AUDIT OF FINE NEEDLE ASPIRATION (FNA)
          BIOPSY OF PALPABLE BREAST LESIONS AT
          KENYATTA NATIONAL HOSPITAL (KNH).

A Dissertation presented in partial fulfilment for the degree of master
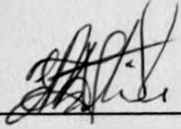of Science in Clinical Cytology in the University of Nairobi.
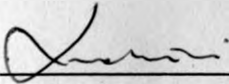
By

E.A. Bulinda

DECLARATION:

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

E.A. Bulinda [B.Sc. (Hons) Biochemistry & Zoology Nbi, P.G.D.E. Moi]

This dissertation has been submitted for examination with my approval as a university supervisor.

Dr. L. Muchiri (M.B.Ch.B., M. Med Path. Nbi, Fellow. Cytopathology]

Date: _____ 20/11/2004 _____

DEDICATION

This work is dedicated to my parents and my wife Petty and children Derrick and Linah for their tolerance of my absence during this study.

ACKNOWLEDGEMENT

LIST OF CONTENTS

ABBREVIATIONS

BRCA1:   Breast Cancer Gene 1

BRCA 2:   Breast Cancer Gene 2

DNA: Deoxyribonucleic Acid

FNA biopsy: Fine Needle Aspiration Biopsy

IP/No: Inpatient Number

KNH: Kenyatta National Hospital

OP/No: Outpatient Number

P53:   A tumour suppresser gene

ABSTRACT

Objective: To audit the FNA biopsy in the diagnosis of palpable breast lesions and assess the influence of the technique on patient management.

Methods: This was a retrospective study of 215 FNA biopsy results of palpable breast lesions in correlation with histology results of 202 patients screened between 1st January 1998 to 31st December 1999 by the Human Pathology Department at Kenyatta National Hospital. The method of analysis used in this study is adopted from the one commonly used for auditing statistical data and quality assurance of FNA biopsy by the National Breast Screening Programme in the United Kingdom.

Results: The results show absolute sensitivity of 76.9%, complete sensitivity of 84.6%, specificity (biopsied cases only) of 83.7%, specificity (full) of 82.2%, positive predictive value of 100%, negative predictive value of 98.2%, false positive rate 0%, false negative rate of 2.6%, inadequate rate of 14.0%, inadequacy rate for cancer of 12.8% and suspicious rate of 5.6%, (confidence interval of 90%). All the accuracy parameters fall within the set standards with exception of inadequacy rate for cancer which is higher than the expected value (<10%). The total number of patients diagnosed with breast carcinoma were 72 among which 71 were female and 1 male. All the carcinomas were ductal carcinoma with the exception of one papillary adenocarcinoma and one intraductal carcinoma (non-invasive carcinoma). Preoperation therapeutic decision was made in 63 (87.5%) patients with breast carcinoma after considering cytology results while 9 (12.5%) of patients with breast carcinoma required confirmation of malignancy. Seventy seven patients with benign breast lesions underwent lumpectomy/excision/incision biopsy after considering cytology results while 34 patients had no histological confirmation of the benign breast lesion.

Conclusion: This study demonstrates that FNA biopsy is an accurate, non-invasive diagnostic procedure for assessment of palpable breast lesions. However, due to the higher inadequacy rate for cancer than the set standard there is need to improve the technical accuracy of FNA biopsy at KNH. This could be done by microscopic assessment of specimen adequacy during sampling when required. The clinician may plan for appropriate therapy for each individual patient based on clinical history and

unequivocal cytology report (without the need of excision/incision biopsy in the majority of the cases).

1.0 INTRODUCTION AND LITERATURE REVIEW

Palpable breast lesions are much more common in women and rare in men (1,2). Palpable breast lesions in women below 35 years are usually benign while in older women carcinoma is more common (1). Breast cancer is the most common cancer in women worldwide (3). Lung cancer approaches or exceeds breast cancer in regions with high prevalence of female smokers in the developed countries (3). In Kenya cervical cancer is the most common cancer among women followed by breast cancer when skin cancer is excluded (4). There is an apparent increase in incidence of breast cancer. In admissions at KNH, in 1998, 111 cases were reported and 28 died while in 1999, 142 cases were reported and 41 died (medical records – KNH). However, this increase may be due to increased awareness of breast cancer cytodiagnosis and referrals from public and private hospitals.

Palpable breast lesions are usually detected by the patients themselves or by the clinician during clinical examination(s). Further investigations of the lesions is required to differentiate between benign and malignant lesions. Studies have shown that the majority of the referrals to a Specialist breast clinic are usually benign breast diseases (2). Therefore delayed investigations prolong patients anxiety and increases outpatient waiting lists. Early stage detection of breast cancer is essential for favourable clinical outcome (3, 5). Two-thirds of palpable malignant breast lesions approximately 4cm in diameter have already metastasized when first discovered while intraductal cancers are rarely palpable lesions and may be diagnosed by examining nipple discharge or by mammography(3).

FNA biopsy has been adopted in most clinics and hospitals in developed countries as a routine clinical procedure replacing a preoperative tissue biopsy (2). In some clinics and hospitals the technique has been developed further into the "triple test" (physical examination, mammography and FNA biopsy) over the last decade (6,7,8). FNA biopsy has been shown to be simple, accurate and cost-effective (10,11). The technique is used to diagnose cystic lesions (2) and to differentiate between benign lesions and malignant lesions (2,5,12,13). Excision biopsy of benign breast lesions detected by mammography usually leaves a scar making subsequent mammography difficult (12). FNA biopsy is a non-traumatic approach and it is a method of choice for suspicious lesions (2).

Studies have shown that mass screening programmes using FNA biopsy technique in developed countries have made several contributions, such as: -

i. The age curve has been modified and the date of tumour diagnosis brought forward;

ii. Diagnosis of less aggressive tumours have increased; and

iii. Increased incidence and survival (3)

The programme has contributed to early detection of breast cancer and hence appropriate and prompt therapeutic decisions undertaken.

Studies by Howell & Goodnight (5) led to the conclusion that FNA biopsy is "useful in diagnosis of palpable breast cancer" for various reasons:

i. It allows preoperative therapeutic decisions to be made;

ii. Breast conservation is achieved without open surgical biopsy and

iii. Mastectomy is done after consideration of clinical examination and FNA biopsy evidence for malignancy.

Martin and Ellis at Memorial Hospital for cancer and Allied diseases, New York, aspirated palpable breast lesions and enlarged lymph nodes using a 16/18 gauge needle and published their findings in 1930 (14). In 1933, Steward emphasized the significance of clinical history and the knowledge of exact site of aspiration for interpretation of the smear (15). Thick air-dried smears were prepared and were stained with haematoxylin & Eosin, no fixation or special stains were required. Small 'clots' were processed as small biopsies and provided histological diagnosis. The aspiration technique was simple and required no special clinical skill. However the smear was often poorly prepared, thick and required massive evidence for diagnosis even in experienced hands and 16/18 gauge needle was not suitable for aspiration of deep seated lesions (6). The aspiration was adopted and modified by the Europeans by the introduction of the thin needle aspiration (fine needle aspiration) using 21/23 gauge needle. This was based on haematologic techniques, using thin, air-dried smears and haematologic stains based on earlier experience of German investigators Huschfield, 1919 and Mannheim, 1931. The current interest in fine needle aspiration biopsy is mainly due to the efforts of the Dutch investigator Soderstrom of Lund and Franzen S. & Zajcek J. et al. Frazen devised a syringe holder to facilitate single handed aspiration (6). The publication from Karolinska

2

Hospital, Sweden, in 1950's observed that little pain and no post operative hemorrhage occurred when a narrow/thin 21/23 gauge needle was used compared to 14 gauge tru-cut needle biopsy (1). The review of 3479 biopsies of palpable breast lesions in late 1960s by Frazen et al and the correlation of FNA biopsy with clinical history and follow-up data emphasized the significance of FNA biopsy and clinicians showed confidence in the technique (16). The technique provided a safe, rapid and cost-effective means of sampling palpable lesions from various body organs and tissues. With the development of imaging technique, aspiration of every body organ is now feasible. The application of radiologists and FNA biopsy marked the return of aspiration to N. America where it was first advocated for by Martin and Ellis (6).

FNA biopsy was not adopted as a routine clinical procedure in clinics and hospitals in United States of America (USA) and United Kingdom (UK) until in early 1970s due to various reasons: -

i. Clinicians were concerned over the accuracy of FNA biopsy (1,17) since open surgical biopsy with frozen sections is considered the gold standard for the diagnosis of palpable breast lesions.

ii. FNA biopsy technique and interpretation require experience and good training (10,17).

iii. Surgeons were not willing to relinquish histological diagnosis of breast lesions (1).

iv. False negative results might delay diagnosis(11).

v. False positive results could lead to mastectomy with clinical and medico-legal implications (1,10,17).

However several studies have established that FNA biopsy is simple, rapid and free of major complications (6,9). Power estimated the complication of FNA biopsy at 0.03% of the total cases studied and made the conclusion that "FNA biopsy is one of the safest invasive diagnostic procedures" (10).

The diagnostic accuracy rates of FNA biopsy of palpable breast lesions determined by various independent researchers are tabulated in table1.

3

Table 1: The accuracy values determined by various researchers independently.

{PRIVATE}Researcher	Diagnostic accuracy	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative Predictive Value (%)	False +ve rate (%)	False –ve Rate (%)
Schofield et al (5)	97.4						
Frable "European Experience" (6)		84.5	96.5				
"American Experience" (6)		70.8	96.7				
Muchiri et al (8)	95	95	75-100	95-100		0-1.8	3.7
Guard & Herman (11)		65-98	34-100				
Kara et al (12)		76.0	96.4				
Nicosia et al (13)	98.87	93.48	100	100	98.65		
Velto et al (18)		96			95		
(18)		100		100			
Sternberg et al (19)		95.5	100				

The data above gives a wide range of values of sensitivity, specificity, positive predictive value, negative predictive value, false positive rate and false negative rate probably due to several reasons:

i. Variable statistical tools used for analysis of their respective data in the evaluation of the diagnostic accuracy and

ii. Differences in study design including factors like sample size, type of institution, education and experience of the pathologist, cytopathologist, and special staining procedure or techniques and availability of image guidance technique.

Studies have shown a high "technical failure rate" to obtain a positive diagnosis in case of carcinoma of 9.8% was found for a single experienced aspirator as compared to 45.5% for a group aspirators. While the more experienced pathologist had a greater degree of accuracy than less experienced ones(9). Diagnostic accuracy and reproducibility depends on adequacy of the sample. Although FNA biopsy technique is a simple technique but it requires skill during sampling, processing and staining to achieve a high level of adequate specimen. Inadequate rate in FNA biopsies is greater than the non-diagnostic rate of histological biopsy (1).

There are two types of reporting system which could be applied to palpable breast lesions the binary system or categorical system. Binary system involves writing the report as either "benign" or "malignant" while categorical reporting consist of five sub-divisions which are inadequate, benign, atypical probably benign, suspicious of malignancy and malignant.

Inadequate smear can be due to hypocellular smear or as a result of aspiration error, processing and staining might render an adequate sample in terms of cellularity unsuitable to interpret. Generally an adequate smear is a representative of the lesion in view of the pathologist (9,17). Benign lesions of the breast consist of a cohesive sheet of cells in a honeycomb appearance. monomorphic nuclei, sharp and distinct cell borders, myoepithelial cells represented by naked bipolar nuclei are usually present, oncocytes, macrophages and leucocytes may be present, and the background is usually clear.

Whereas ductal cells show a cohesive pattern in the wet fixed smear, they tend to dissociate in air dried smears which may lead to false suspicion of malignancy. Benign epithelial cells have a vesicular or "open" chromatin pattern with small nucleoli. Connective tissue stromal fragments may be present in the aspirate, they stain pale blue or pink with Papanicolaou stain and bright pink on the air-dried May Grunwald Giemsa stain with purple nuclei (21). Atypical probably benign cells are usually crowded with enlarged overlapping nuclei and in three dimensional groups or sheets. There is nuclear pleomorphism and loss of cellular cohesion and chromatin clumping is evident. Atypical probably benign cells might be encountered in benign specimen as well as with normal or in highly cellular smear which might be due to epithelial hyperplasia, gynaecomastia, papilloma, hormonal changes, atypical apocrine lesions, columnar cell changes, proliferative lobular lesions and tubular or cribriform carcinoma (10,17). This could be a source of false positive or negative results since "atypical probably benign" and "suspicious of malignancy" is the "grey zone" in the categoric reporting system. "Suspicious of malignancy" category includes highly atypical characteristics in the smear and a firm diagnosis cannot be made due to low cellularity or poorly preserved malignant cells (17). Suspicious of malignancy is associated with lobular, tubular and a cribriform carcinomas, carcinoma-in-situ and epithelial hyperplasia with atypia and low cellularity. "Suspicious of malignancy" requires confirmation by biopsy while "atypia probably benign" requires follow-up. The two sub-classes should be clearly distinguished as they have different clinical implications.

Cytological features which are assessed in making the diagnosis of malignancy include abnormal cells which usually occur in single or loose clusters or both. The abnormal cells have a three-dimensional arrangement with overlapping nuclear, there is high nuclear – cytoplasm ratio and marked pleomorphism. However, low grade invasive carcinoma, in situ lobular carcinoma show cohesive sheets of clusters of cells in three dimensional arrangement rather than the flat sheets of cells observed in benign lesions (17, 28). Lobular carcinoma, some low-grade ductal carcinoma and cribriform carcinoma are composed of very small cell while tubular carcinoma show monomorphic nuclei. Hence other features of malignancy are considered to determine the diagnosis. Carcinoma may show hyperchromatic nuclei with or without uneven chromatin clumping or clearing. Some carcinoma have pale hypochromic nuclei composed

6

predominantly of the active form of chromatin (17,20). The nuclear membrane is usually irregular with indentation. Carcinoma tend to have prominent nucleoli occasionally single staining blue sometimes red with Papanicolaou stain and may be multiple. Abnormal mitosis may be present. Necrosis is a feature of large cell, pleomorphic ductal carcinoma-in-situ, intracystic carcinoma and squamous cell carcinoma of the breast and rare in tubular carcinoma. The well differentiated or low-grade carcinoma are usually difficult to distinguish from benign cells, and may be reported as "suspicious of malignancy" rather than the definitive diagnosis of carcinoma (17, 20).

Studies have shown that inexperienced interpretors of FNA biopsy tend to adopt binary format of reporting with less emphasis on subdivision while the more experienced pathologists adopt the categorical format. Categorical reporting is useful for definite clinical patient management since the report gives the subdivision of benign and malignant categories. Interpretative errors are usually most often responsible for false-positive diagnosis which should be avoided since patient management could be based on cytology results.

Breast cancer usually arises in women as a sporadic event without a family history of the disease. However, risk factors may be present. Studies have shown that the relative risk for a woman who is premenopausal and has first degree relative is 3.0 and if she is postmenopausal the relative risk is 1.8 (21). The molecular pathogenesis of breast cancer involves genetic alteration of breast epithlial cell deoxyribonucleic acid (DNA) resulting progressively into invasive malignant cells. The process is initiated by a variety of carcinogen including chemicals, radiation and possibly retroviruses and may be promoted by psychological and environmental factors such as diet, alcohol, cigarette and radiation.

The most important and best established genes involved in initiation and early promotion of breast cancer are: breast cancer gene 1 (BRCA1), breast cancer gene 2 (BRCA2) and p53 gene. BRCA1 is located on chromosome 17q21. It is an autosomal dominant gene that codes for a tumour suppresser protein. Mutation of this gene has been identified in patients with familiar breast and ovarian carcinomas. Inheritance of breast cancer gene 1

7

confers a 65% risk of ovarian cancer and 87% risk of breast cancer by age 70. BRCA2 is located on chromosome 13q12-13. It is involved in familiar and early onset of breast cancer. The risk of breast cancer in women with BRCA2 is approximately 87% by age 80. P53 is a tumour suppresser gene with putative role in DNA replication, transcription and cell cycle control. It inhibits cell transformation by *myc* and *ras* oncogenes. Mutation of p53 leads to abnormal accumulation of p53 in the cytoplasm. This prevents p53 from entering the nucleus and regulating transcription (22).

Generally family history of breast cancer is an important factor to be considered by the clinician as well as pathologist for appropriate diagnosis and therapeutic management of patients with palpable lesions. It is considered that at least 3 cases of breast carcinoma will occur in close relatives following an autosomal dominant trait (22).

Hormonal regulation of the breast is important in the development of breast cancer. Women with first pregnancy before 20 years have a reduced risk of developing cancer compared with women who are 35 years and above at the time of first pregnancy where the relative risk is 1.5-4.0. Studies have shown those women who have had oophorectomy before 35 years have a relative risk of 0.4 while those with extensive anovulating cycle have relative risk of 2.0-4.0. Women with early menopausal before 45 years have relative risk of 0.5-0.7 and those after 55 years the relative risk is estimated at 1.5. Non-proliferative fibrocystic disease, duct ectasia have no significant increased risk. Proliferative breast disease without atypia (hyperplasia, papilloma) have a relative risk of 1.5-2.0. While proliferative breast disease with atypia especially atypical hyperplasia have a relative risk of 4-5. Women with carcinoma-in-situ have significantly increased risk of 8-10 of developing invasive breast carcinoma. Therefore the clinician/surgeon require a definitive diagnosis for him/her to plan for an appropriate therapeutic procedure (21).

Further classification of the tumor allows the surgeon to plan management even more carefully on an individual basis. The aspiration of breast lesion and axillary lymph node will assess the stage of the disease. For non-invasive breast carcinoma conservative therapeutic options exist which includes lumpectomy, with or without breast irradiation excluding axillary dissection. Lobular carcinoma-in-situ requires local excision or

mastectomy with careful follow-up (21). For invasive carcinoma tamoxifen, mastectomy and radiotherapy is usually advocated (23). Tamoxifen is a non-steroid antiestrogen that competes with estradiol at the estrogens receptor level.

For benign breast lesion further classification is essential for appropriate therapeutic decisions for example, in cases of gramulomatous mastitis which is caused by either systemic granulomatous disease (Wegener granulomatosis, sarcoidosis) which involve the breast and the breast is the presenting site, or mycobacterial or fungal infection. Detailed FNA biopsy result will guide the clinician to appropriate therapy or further investigation rather than a simple diagnosis of "benign breast lesion", no malignant cells seen. Mastitis is usually associated with lactation. Most common cause of mastitis is *Straphylococcus aureas* hence appropriate antimicrobial treatment is necessary if unresponsive incision biopsy is considered with appropriate anaesthesia and irrigation of abscess cavity with hydrogen peroxide and saline solution. Part of the aspirate is taken to microbiology laboratory for culture and sensitivity (17, 21).

Therapeutic modalities of fibrocystic disease are varied. Patients may be counselled to put on comfortable supporting bra, use mild diuretics eg. hydrochlorothiazide for 2-3 days prior to menses. Other therapeutic procedures include use of vitamin A and E, change in diet, steroid therapy (oral contraceptives) for women who do not respond to conservative treatment. Other therapeutic options recommended are sex steroid inhibiting substance (damazol) a synthetic androgen and surgical excision biopsy. Fibroadenoma found in women over 25 years should be excised (21)

Factors contributing to false result are determined by interpretative limitation which include:-
Sampling problems leading to false negative diagnosis which might be due to incorrect localization of the lesion and faulty aspiration technique due to patient factors: small superficial lesions, vascular lesions, necrotic lesions and fibrous lesions (some lobular carcinoma, scirrhous carcinoma) make aspiration painful and the sample hypocellular (17).

    i. Sampling problems leading to false positive diagnosis include artefact, poor fixation, aspiration of recently needled area and overstraining.

ii. Interpretive problems leading to false negative diagnosis include the small size of malignant cells (lobular and tubular carcinoma) and moderate to hypocellular smear.

iii. Interpretive problems leading to false positive diagnosis is mainly due to interpretative errors (14) which might be due to inexperience, overzealous and making a diagnosis on inadequate smear (9,17).

This study was carried out to determine the technical accuracy as well as the diagnostic accuracy of F.N.A biopsy technique. Evaluation of the clinicians or surgeons therapeutic decisions made based on FNA biopsy is essential as it reflects the confidence they have in the technique for patient clinical management.

2.0 JUSTIFICATION OF THE STUDY.

Routine F.N.A biopsy technique of various body sites (thyroid lesions, lymph node lesions, head and neck nodules, palpable breast lesions etc) has been adopted as a diagnostic technique at K.N.H. Previous prospective studies have reported a high accuracy rate, a factor which supports the adoption and the usefulness of the technique. However, considerable training and experience are required for reliable results. The study attempts to audit F.N.A biopsy technique in the diagnosis of palpable breast lesions by determining the inadequacy rate, sensitivity, specificity, positive predictive value, negative predictive value, false negative rate and false positive rate and inadequacy rate for cancer. The results are analysed in comparison with the general sensitivity and specifity range of 60 to 85% rather than the 90 to 100% for corresponding figures found in the literature where inadequate results and suspicious result are excluded. This study incorporates inadequate and suspicious results since excluding them biases the figures which should be reflected in the results as the pathologist/clinician should ensure correct sampling, processing and staining is done to provide adequate specimen for diagnosis. Whereas inadequacy rate depends on the characteristic of the lesion aspirated and care, experience and training of the aspirator; the inadequacy rate for cancer is not dependent on the characteristics of the lesion with exception of the rare desmoplastic carcinomas, lobular and tubular carcinomas, therefore a higher rate (>10%) suggests the need for improvement of the diagnosis of breast lesions. In addition high suspicious rates and inadequacy rates limit the usefulness of the technique. Over the last decade the diagnosis

of breast lesions has been modified into a more accurate test known as the "triple test" (physical examination, image guidance (mammography) and F.NA. biopsy) in some institutions but it is yet to be adopted at K.N.H. The current principle of multi-disciplinary assessment is important and should not be compromised. The clinical history as well as imaging features are significant in cytologic diagnosis of palpable breast lesions. Systemic therapy using either chemotherapy or hormonal therapy has higher survival rates primarily in premenopausal patients and has some effect in postmenopausal patients, thus adjuvant systemic therapy is advocated far more often than previously. Therefore the clinicians or surgeons need reliable cytology results for clinical patient management compared to a tru-cut or core biopsy since F.N.A biopsy is less traumatic, simple and accurate.

Therapeutic decision made based on the FNA biopsy results with or without histology results is determined. This indicates the confidence or lack thereof the surgeons have in the technique. For example, mastectomy done after considering F.N.A. biopsy results and clinical impression without incision or excision biopsy provides an indication of confidence the surgeons have in the F.N.A. biopsy technique.

3.0 AIM OF THE STUDY:

The aim of the study was to audit F.N.A. biopsy technique in the diagnosis of palpable breast lesions and assess the influence of the technique on patient clinical management without open surgical biopsy.

3.1 SPECIFIC OBJECTIVES:-

1. To determine the technical accuracy of FNA biopsy.
2. To determine the diagnostic accuracy of FNA biopsy.
3. To determine how surgeons or clinicians make therapeutic decisions based on FNA biopsy result.

4.0 MATERIALS AND METHODS.

FNA biopsy file was used to retrieve data of outpatients who attended weekly FNA biopsy clinic and were screened for breast cancer by the attending pathologist in the

Department of Human Pathology (KNH) between 1st Jan 1998 and 31st Dec. 1999. Data collected included outpatient /inpatient number (OP/IP No), cytology laboratory number, age, sex and FNA biopsy cytology results. Using OP/IP No, histology or follow-up results are retrieved from the patients' file. The clinician or surgeon therapeutic decisions made based on F.N.A. biopsy results are also included.

Cytology results were then correlated with histology results as shown in appendix I.

FNA biopsy samples, which show discrepancy with histology results, are reviewed using a binocular microscope in order to determine the diagnostic pitfalls and diagnostic limitations. At KNH wet fixation of smear followed by the Papanicolaou staining technique is favoured with or without air dried smears, stained with May Grunwald Giemsa staining technique for additional information.

The diagnostic accuracy rates are calculated using the statistics formulae given in appendix I. This is because the FNA biopsy reporting criteria is not binary but consists of five categories i.e. benign, atypical probably benign, suspicious of malignancy, malignant and unsatisfactory/inadequate (as observed from 1988 - 1999 diagnostic FNA biopsy reports). Hence sensitivity and specificity are not the best statistical tool to measure the accuracy because sensitivity and specificity evaluates the ability to detect the presence or absence of the disease and categories like atypia, suspicious will not fit in a binary scheme. The method of analysis used in this study is adopted from the "National Breast Screening Programme in the United Kingdom" for auditing statistical data and quality assurance of fine needle aspiration cytology (17). It includes inadequate and 7suspicious categories and the accuracy parameters can be compared with the standardized statistics and difficult areas identified and rectified by education and improvement of the technique. The minimum standardized statistics applied are:

> Absolute sensitivity - > 60%,
>
> Complete Sensitivity > 80%
>
> Specificity >60% (include non-biopsied cases)
>
> Positive predictive value for malignancy > 95%
>
> False negative rate <5%
>
> False positive rate <1%
>
> Inadequate rate <25% and

Inadequate rate or malignancy <10%

Suspicious rate < 20% (17)

4.1 Sample Size:

Sample size = 174 (appendix II)

Significance level: = 0.10

Confidence coefficient; 0.90

4.2 Inclusive Criteria: -

i.   Cytology results for patients who had palpable breast lesions screened for breast cancer (between 1st Jan 98 to 31st Dec. 1999) using FNA biopsy technique

ii. Cytology results with clearly labelled laboratory requisition form i.e. IP/OP No. and cytology lab. No.

iii. Excision biopsy histology results and follow-up results of the patients screened for breast cancer in correlation with their IP/OP Nos.

iv. Histology results of mastectomy specimens of patients screened for breast cancer in correlation with their IP/OP Nos.

4.3 Exclusive Criteria: -

i.   Cytology results for non-palpable breast lesions screened for breast cancer using Mammography.

4.4 DEFINITIONS:

Technical Accuracy:

Technical accuracy of the aspiration procedure is to assess the accuracy of F.N.A biopsy in producing diagnostic material; therefore results are either adequate or inadequate.

Diagnostic Accuracy:

Is the measure of how accurately a diagnostic test (FNA biopsy) places patients into the appropriate categories.

Absolute Sensitivity:

Is the percentage of patients with cancer and FNA biopsy results are positive (malignant).

Complete Sensitivity:

Is the percentage of tumours in which abnormal cells (malignant, suspicious and atypical cells) are observed.

Specificity (Biopsied cases only)

Is the percentage of histologically benign biopsied cases in which the cytology results are benign.

Specificity (full)

Is the percentage of benign cases (biobsied or reviewed with no evidence of cancer) in which a benign cytological diagnosis was made.

Positive Predictive Value

Postive predictive value of a malignant lesion is the probability that a patient who gives a positive test (malignant) has cancer.

Negative Predictive Blue

Is the probability that a patient with a negative result (benign) does not have cancer.

False Positive Rate:

Is the percentage of patients who do not have cancer and the test result is positive (malignant).

False Negative Rate:

Is the percentage of patients who have cancer but gives a negative (benign) test results.

Inadequate Rate:

Is the percentage of cases where inadequate material was aspirated to enable a diagnosis to be made.

Suspicious rate:

Is the percentage of result which are felt to be suspicious of malignancy or atypical.

14

4.5 STRENGTH OF THE STUDY DESIGN:

i.   Open surgical biopsy or an excision biopsy of palpable breast lesions is considered as a gold standard for the diagnosis of palpable breast lesions (11).

ii.   It is a cost-effective study design used to evaluate the diagnostic accuracy and make suggestions to improve its performance, and can be used for continuing education purposes.

iii.   It is important to evaluate tests on which such far reaching clinical decisions such as mastectomy and chemotherapy are made.

4.6 CONSTRAINS OF THE STUDY DESIGN:

i.   The study assumed that the gold standard is 100% accurate.

ii.   Some benign or malignant breast lesions did not have histologic results, follow-up results was considered in such cases and in areas where the samples are reported malignant they were assumed to be so and also those reported benign were assumed to be so.

iii.   Data retrieval from patient file was a problem due to lack of sound record keeping.

iv.   Patients also influence therapeutic decisions.

4.7 ETHICS CONSIDERATION:

i.   Patients names were deleted from the data collected and OP/IP Nos. and Lab Nos. were used.

ii.   Results determined and recommendations made were treated as confidential and forwarded to the relevant departments/section.

RESULTS:

The study included 215 FNA biopsy results of 202 patients with palpable breast lesions screened for breast cancer between 1st January 1998 and 31st December 1999 by the human pathology department at KNH. Repeat FNA biopsy results are included in the study.

Correlation of cytology and histology results of patients screened for breast cancer between 1st January 1998 and 31st December 1999 at KNH. Histology results are based on excision/incision biopsy or mastectomy (Table 3).

HISTOLOGY	CYTOLOGY					
	Malignant	Suspicious for malignancy	Atypical probably benign	Benign	Inadequate	Total
Histology malignant	41	3	3	2	10	59
Benign	—	—	1	77	14	92
No histology	19	2	3	34	6	64
Total cytology results	60	5	7	113	30	215

Using the table above and statistical formulae given in appendix II accuracy parameters are determined and tabulated in Table 4 below.

Accuracy Parameters	Standard NBSP (UK) %	Study Percentage (%)
Absolute sensitivity	>60	76.9
Complete sensitivity	>80	84.6
Specificity (biopsied cases only)	>60	83.7
Specificity (full)	-	82.2
Positive predictive value	>95	100
Negative predictive value	-	98.2
False positive rate	<1	0.0
False negative rate	<5	2.6
Inadequate rate	<25	14.0
Inadequacy rate for cancer	<10	12.8
Suspicious rate	<20	5.6

NBSP (UK) – National Breast Screening Programme (United Kingdom).

All the accuracy parameters fall within the set standards with exception of inadequacy rate for cancer which is higher than the set standard value.

Therapeutic decisions made based on cytology results or histology results for patients diagnosed with breast carcinoma (Table 5).

Therapeutic decision	Number of patients
?? Adjuvant tamoxifen and mastectomy with or without axillary lymph node clearance after considering cytology results of malignancy.	41
✍✍Planned for mastectomy after considering cytology results of malignancy.	19
✍✍Adjuvant tamoxifen after considering cytology results of malignancy.	1
✍✍Referred to hospice for palliative care after considering cytology results of malignancy.	1
Mastectomy after considering cytology results and histology results consistent with malignancy.	9
Total	72

?? Cases with positive histology results

✍✍Cases without histological confirmation of malignancy.

The case excluded from the table above (table 5) is that one of an adult male who underwent mastectomy based on a benign breast lesion (gynaecomastia). Among the 72 patients diagnosed with breast carcinoma, 71 were female while 1 was a male. Preoperative therapeutic decisions were made in 63 (87.5%) of patients after considering cytology results of malignancy while 9 (12.5%) patients required histological confirmation of malignancy after considering FNA biopsy results. The clinician/surgeon required histological confirmation of breast carcinoma in one case diagnosed as suspicious of malignancy and another one diagnosed as malignant in an adult male. Histological diagnosis was required in 2 cases reported as inadequate. 2 cases reported as atypical probably benign and 2 cases reported as benign.

Twenty millilitres of blood stained fluid was aspirated from a cystic breast lesion and on cytological evaluation, a diagnosis of intracystic carcinoma was made.

Three cases reported as "atypical probably benign" showed invasive ductal carcinoma, intraductal caarcinoma and lactating adenoma on histologic diagnosis. Two cases with "atypical probably benign" diagnosis showed ductal carcinoma on repeat FNA biopsy and one had histologic confirmation of malignancy. Two cases with "atypical probably benign" diagnosis had no follow-up or histology results.

Five cases of "suspicious of malignancy" were reported, among which two repeat smear showed ductal carcinoma and three had histological confirmation of malignancy. The clinician/surgeon planned for mastectomy in two cases and malignancy was confirmed. The pathologist usually requested for repeat smear or excision biopsy for a definitive diagnosis whenever "atypical probably benign" and "suspicious of malignancy" was reported. The clinician/surgeon on the other hand requested for a repeat smear, excision biopsy or planned for mastectomy based on the cytology report and clinical findings.

Seventy seven patients with benign breast lesions underwent lumpectomy/excision biopsy after considering the cytology results while 34 patients did not have excision biopsy. Nine patients with inadequate cytology results had histological diagnosis of benign breast lesions. While there were 6 patients with inadequate cytology report without histology results or follow-up results.

Pathologists always request for repeat FNA biopsy whenever an inadequate smear was encountered while the clinician/surgeons planned for repeat FNA biopsy or mammography or excision biopsy or mastectomy based on the individual clinical findings. The table below shows the correlation of cytology benign diagnosis and histology benign lesion, false negative results are excluded.

Correlation of cytology benign diagnosis and histology benign lesions. (Table 6).

CYTOLOGY	HISTOLOGY					
	Fibroadenoma	Fibrocystic disease	Mastitis	Others	No histology	Total
Fibroadenoma	38	12	—	6	18	74
Fibrocystic disease	6	8	—	—	8	22
Mastitis	—	2	3	—	6	11
Others	—	—	—	2	2	4
Inadequate	6	6	—	2	6	20
Total	50	28	3	10	40	131

Others include Galactocoele, duct ectasia, tubular adenoma, papilloma and benign phylloides tumor.   Atypical probably benign, suspicious of malignancy and false negative results are excluded. Repeat smears were included. (Table 6).

The distribution of palpable breast lesions in correlation with the patients age groups based on cytology with or without histology results. (Table 7).

Age-group	Malignant	Fibroadenoma	Fibrocystic disease	Mastitis	*Others	Total
15-24	1	45	8	3	3	60 (32.4%)
25-34	10	15	10	4	5	44 (23.8%)
35-44	11	7	6	—	2	26 (14.1%)
45-54	22	1	2	2	2	29 (15.7%)
55-64	9	—	—	—	—	9 (4.9%)
65-74	8	—	2	1	—	11 (5.9%)
75-84	4	—	—	—	1	5 (2.7%)
85-94	—	—	—	—	—	—
95-104	1	—	—	—	—	1 (0.5%)
Total	66	68	28	10	13	185

Others include Galactocoele, duct ectasia, tubular adenoma, papilloma, benign phylloides tumor and atypical probably benign lesion.

The distribution of palpable breast lesions in correlation with the patient age groups based on cytology with or without histology results is well illustrated in figure 1 below.
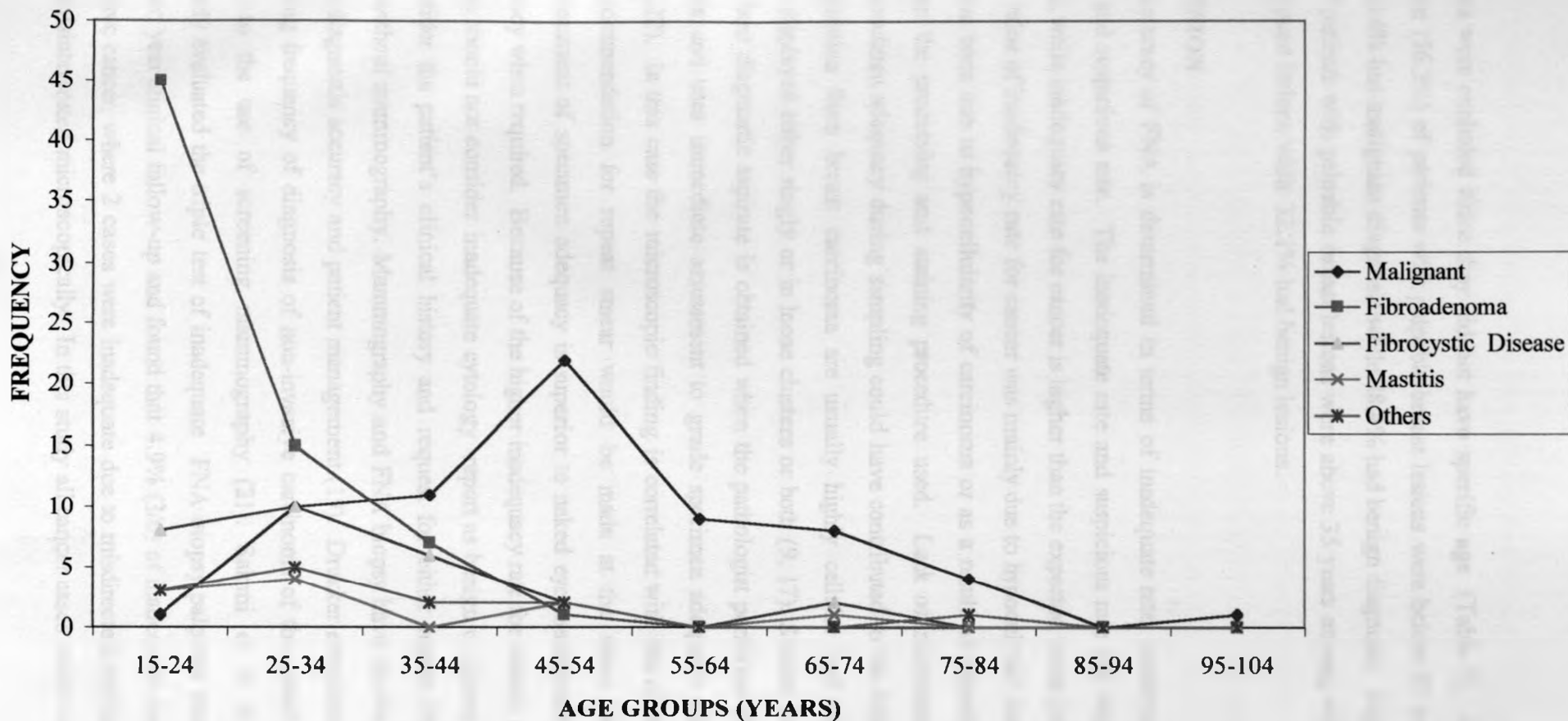
FIGURE 1
THE DISTRIBUTION OF PALPABLE BREAST LESIONS IN CORRELATION WITH THE PATIENTS AGE GROUPS BASED ON CYTOLOGY WITH OR WITHOUT HISTOLOGY RESULTS

Seventeen cases were excluded since they did not have specific age (Table 7). One hundred and four (56.2%) of patients with palpable breast lesions were below 35 years among which 10.6% had malignant diagnosis while 88.4% had benign diagnosis. Eighty one (43.8%) of patients with palpable breast lesions were above 35 years among which 67.9% had malignant lesions while 32.1% had benign lesions.

6.0   DISCUSSION

The technical accuracy of FNA is determined in terms of inadequate rate, inadequacy rate for cancer and suspicious rate. The inadequate rate and suspicious rate fall within the set standards while inadequacy rate for cancer is higher than the expected value (table 4). The higher value of inadequacy rate for cancer was mainly due to hypocellular smear which could have been due to hypocellularity of carcinoma or as a result of aspiration error rather than the processing and staining procedure used. Lack of microscopic assessment of specimen adequacy during sampling could have contributed to the higher ratio. Most aspiration from breast carcinoma are usually highly cellular and show malignant cells displayed either singly or in loose clusters or both (9, 17). Studies have shown that the best diagnostic aspirate is obtained when the pathologist performs FNA biopsy technique and uses immediate assessment to grade specimen adequacy when required (17,21,22). In this case the microscopic finding is correlated with the clinical history and recommendation for repeat smear would be made at the same visit. Microscopic assessment of specimen adequacy is superior to naked eye assessment of specimen adequacy when required. Because of the higher inadequacy rate for cancer, the clinician/surgeon should not consider inadequate cytology report as benign or ignore it, but should consider the patient's clinical history and request for either repeat FNA biopsy with or without mammography. Mammography and FNA biopsy have shown to achieve the best diagnostic accuracy and patient management (19). Drukker emphasized that the increasing frequency of diagnosis of non-invasive carcinoma of the breast is directly related to the use of screening mammography (21). Salami et al in a retrospective study evaluated the triple test of inadequate FNA biopsy palpable breast lesions with a two year clinical follow-up and found that 4.9% (3/6) of inadequate cases were found to have cancer, where 2 cases were inadequate due to misdirected aspirates and 1 case was misinterpreted microscopically. In this study all cancer cases underwent

22

preoperative surgical removal of the mass as a result of clinical history and radiological findings (25). Zakhour and Wells argue that hypocellular smear could provide a diagnostic clue when the pathologist is aware of the clinical history and mammographical finding that the lesion in question is suggestive of a lipoma. Therefore, the presence of fatty tissue in hypocellular smear is consistent with the clinical and imaging characteristic of a lipoma (17). This could be the reason behind the clinician/surgeon plan for mammography in some of the cases reported as inadequate. Excision biopsy is recommended where recurring masses occur after two aspiration have failed (21).

The diagnostic accuracy is determined in terms of sensitivity, specificity, positive predictive values, negative predictive value, false positive rate and false negative rate. Result of this study indicate that the positive predictive value was 100% with no false positive result. Zakhour and Wells emphasized that the positive predictive value is the best measure of diagnostic accuracy of FNA biopsy since sensitivity and specificity incorporate suspicious and inadequate result. They advocate that the clinician and surgeon consider the positive predictive value as an essential statistical parameter as it indicates the proportion of positive cytology results that comes from carcinoma (17). There was no false positive diagnosis since conservative approach to the diagnosis of the palpable breast lesion was used. The clinician/surgeon made therapeutic decision based on positive cytology results with exception of one case of an adult male with cytology results of breast carcinoma. The clinician/surgeon wanted to rule out any possibility of false positive diagnosis as breast carcinoma is very rare in male. Similar results of positive predictive value have been observed in various studies (1, 8, 17, 26, 27). The negative predictive value of 98.2% gives the proportion of negative cytology results that comes from a benign lesion. The false negative rate of 2.8% (2 cases) was due to aspiration error or carcinoma which show bland cytological features. Lobular and monomorphic pattern of ductal carcinoma usually show bland cytological features especially in elderly individuals (9,10).

Boerner argues that benign cases require periodic follow-up as benign cytological diagnosis does not entirely exclude the possibility of carcinoma (28). The surgeon/clinician should request for repeat FNA biopsy or excision biopsy whenever the

clinical findings are not consistent with cytology results. For a definitive specific diagnosis of the benign lesion the clinician/surgeon should consider the cytology results in addition to the clinical history and/or excision biopsy results. The subjective nature of cytologic diagnosis and further classification of benign breast lesion is demonstrated in table 7. Studies have shown that further classification of benign diseases cannot always be reliably performed using FNA biopsy alone (8, 17). McKee emphasised that the cytologic features of fibroadenoma and fibrocystic disease may overlap to some extent (20). Therefore the clinician/surgeon require multi disciplinary assessment for benign breast lesions. Boerner et al found that cases of invasive tubular carcinoma were more common in false negative smear with fewer that six epithelial clusters in which no interpretative error was made. He emphasised the need of correlation of clinical history with cytology results to reduce the rate of false-negative cases (28). In this study one case of false negative was diagnosed as fibroadenoma in a 45 year old female. This could have been due to misdirected aspiration. Studies have shown that carcinoma may occur in association with fibroadenoma in very rare occasions (29). The other case was diagnosed as reactive inflammatory process as cytology showed ductal cells singly admixed with chronic inflammatory cells and cellular debris. The epithelial cells showed reactive and degenerative changes as well as apocrine changes. Suen argues that reactive cellular changes i.e enlarged nuclei and prominent nucleoli mimic cancer cells but benign diagnosis is made on the strength of the cells occurring in sheets rather than singly and are admixed with acute inflammatory cells in case of breast abscess (9). Zakhour and Wells recommended peripheral aspiration in case of a necrotic lesion (17).

Absolute and complete sensitivity are within the set standards and similar to other studies (1,8,17, 27). Absolute sensitivity gives an indication of the number of tumors which can potentially be treated by one stage operation (Table 4 & 5). Specificity (full) and specificity (biopsied cases only) are within the set standards and since they include inadequate cases values higher than 90% are not expected. Therapeutic decision could be made in the majority of patients diagnosed with benign breast lesion using FNA cytology without the need of excision biopsy; however, follow-up is advocated by Zakhour and Wells for cases which have benign cytology results and are clinically benign (17).

Among the "atypical probably benign" diagnosis two repeat FNA biopsy had ductal carcinoma diagnosis among which one case had histological confirmation of invasive ductal carcinoma. One case was diagnosed as invasive ductal carcinoma and another as intraductal carcinoma on histology. One case was histological benign and two cases had no follow-up results or histological diagnosis (table 4). Cytological diagnosis of atypical probably benign breast lesion required repeat FNA biopsy and/or excision biopsy for a definitive diagnosis. Trott suggests that atypical features of epithelial cells in an adequate smear may be suggestive of a well-differentiated carcinoma and for diagnosis of malignancy a definitive characteristic features should be observed. A diagnosis of carcinoma may be considered if the smear shows monomorphic epithelial cells with individual pleomorphism and smaller in size with absence of atypical myoepithelial cells (1). Mckee emphasized that well differentiated or low grade carcinoma are usually difficult to differentiate from benign cells hence they may be reported as "suspicious of malignancy" (20). While Silverman argues that underdiagnosis of tubular carcinoma in low-to- moderate smear is due to relatively monomorphic tumor cells showing mild atypia. The diagnosis of tubular carcinoma is confounded by presence of myoepithelial cells in some cases (10). The tumors are therefore diagnosed as "suspicious of malignancy" and excision biopsy is required for definitive diagnosis (10, 20, 26, 28, 30). Studies by Zakhour and Wells highlights that tubular carcinoma; carcinoma-in-situ, epithelial hyperplasia with atypia and poorly cellular aspirates from breast carcinoma are associated with "suspicious of malignancy" category (17). All the "suspicious of malignancy" results had histological or follow-up diagnosis of breast carcinoma in this study. Since the study has demonstrated to have a high position predictive value, the clinician/surgeon should request for either repeat FNA biopsy or excision biopsy for definitive diagnosis for cases reported as "atypical probably benign" and "suspicious of malignancy". The conservative approach is also advocated by Suen if the cytology diagnosis is the basis for radical mastectomy (9).

Table 7 and figure 1 illustrates the distribution of palpable breast lesion in correlation with the patients age group based on cytology and/or histology diagnosis. Majority of the patients below 35 years had benign diagnosis. Fibroadenoma was the most common benign lesion followed by fibrocystic disease. Fibrocystic disease was more common in patients above 35 years as compared to fibroadenoma. Inflammatory breast lesion,

benign phylloides tumor, atypical probably benign lesions were rare across the age-groups. Breast carcinoma is more common in patients over 35 years with peak incidence between 35-64 years. These findings are consistent with Trott's observation that palpable breast lesions in patients below 35 years are usually benign while in older patients carcinoma is more common (1).

Majority of patients with benign breast lesion in this study underwent lumpectomy/excision biopsy after considering benign cytology results (table 4 & 7). Bruce points out that fibrocystic disease has a varied therapeutic decision which include dietary change, vitamin A and E use, analgesic and hormonal manipulation as well as excision biopsy. Complex fibrocystic disease (sclerosing adenosis, epithelial calcification or papillary apocrine changes) have a 2-to-3 fold increased risk of developing breast carcinoma. Individual conservation is advocated for women under 25 years of age on condition that the fibroadenoma is not increasing in size or causing psychological stress (21). Duport and Page et al determined a long term relative risk of 2.17 for development of invasive breast carcinoma in patients with a history of fibroadenoma and a relative risk of 3.88 for patient with complex fibrocystic disease (23). Follow-up studies have established that sclerosing adenosis with or without atypical hyperplasia has an increased risk of developing into invasive breast carcinoma (17). The clinician should individualize therapeutic decisions made based on clinical findings and cytology report keeping in mind the risk of each benign breast lesion developing into breast carcinoma.

Appropriate antibiotics was administered to patients with inflammatory breast lesion. Bruce et al advocate that treatment with antibiotics is necessary and incision biopsy and drainage done with appropriate anaesthesia should be considered if the disease persist after 36-48 hours (21). FNA biopsy serve as a therapeutic and diagnostic procedure whenever a cyst is encountered; 20ml of blood stained fluid was aspirated from a cystic breast lesion and on cytological evaluation a diagnosis of intracystic carcinoma was made (Table 6). Rosemond et al points out that cystic lesions should not be ignored since intracystic carcinoma is a rare lesion (9). Adjuvant tamoxifen is administered in the majority of pre-and-post menopausal women after cytologic diagnosis of malignancy as they await mastectomy with or without axillary lymph node clearance. Radiotherapy

was also planned after mastectomy (table 5, 7). The "world view" of breast carcinoma management advocates for adjuvant tamoxifen, mastectomy and radiotherapy (31). Studies have established that survival benefits attributed to adjuvant tamoxifen and chemotherapy increased with longer follow-up. Premenopausal women with oestrogen receptor positive tumors significantly benefited from tamoxifen (31). Mulmistrom et al observed a 3 times higher local recurrence in the surgery only group compared with surgery and radiotherapy (31). Patients who opted for palliative care without mastectomy were allowed to do so after considering cytology results of malignancy (table 5). Therefore, FNA allowed pre-operative therapeutic decision to be made without the patient's need of excision/incision biopsy in the majority of the patients.

7.0   CONCLUSION AND RECOMMENDATION

This study demonstrates that FNA biopsy is an accurate, non-invasive diagnostic procedure for assessment of palpable breast lesions in experienced clinical team. The clinician or surgeon made preoperative therapeutic decision(s) based on unequivocal cytology result and clinical history in the majority of patients with breast carcinoma. Equivocal cytology results such as "atypical probably benign" and "suspicious of malignancy" were very few and required follow-up or histology results for a definitive therapeutic decision to be made. Majority of the cases reported as "atypical probably benign" and all cases reported as "suspicious of malignancy" had follow-up or histology results of malignancy. This reaffirms that it is good clinical practice to obtain either cytological and/or histological proof of breast carcinoma before making a definitive therapeutic decision such as mastectomy or radiotherapy. Conservative approach was adopted in treatment of the few patients with breast carcinoma who opted for palliative care under the hospice while on adjuvant tamoxifen after considering cytology results of malignancy. Multidisciplinary assessment such as physical examination, mammography, cytology and/or histology results is required for further classification of benign breast lesions for appropriate therapeutic decision. The clinical management of patients with benign breast lesions should be individualised and their clinical history as well as the risk associated with the lesion developing into a breast carcinoma assessed.

The higher value of inadequacy rate for cancer is a good indicator for the need of improvement of the technical accuracy of FNA biopsy at KNH. FNA biospy smears

were inadequate due to sampling error which resulted in hypocellular smear or the fibrous characteristic of the scirrhous carcinoma. Sampling error could be minimized to pitfalls arising from the nature of the breast lesion by incorporation of microscopic assessment of sample adequacy using toluidine blue staining technique whenever necessary. This will allow recommendation of a repeat smear if appropriate during the same visit. Multidisciplinary assessment is important when inadequate smears are consistently obtained to avoid delay in diagnosis of the palpable breast lesion. The higher inadequacy rate for cancer could be partly attributed to doctors undergoing training. However, further research should focus on routine multidisciplinary approach to inadequate FNA biopsies of palpable breast lesions at KNH.

Lastly, the need to computerise and harmonise cytology and histology results of the patients with palpable breast lesions is necessary at laboratory level. This will aid in rapid retrieval of data and assessment of technical and diagnostic accuracy. A good quality assurance programme will ensure a high quality practice, enhance the teaching/learning process at department level and improve the services provided to the patients.

Table 2 Correlation of cytology and histology results. (adopted and modified from Zakhour et al (17)

HISTOLOGY	CYTOLOGY					
	Malignant	Suspicious of Malignancy	Atypical but Benign	Benign	Inadequate	Total
Histology Malignant	a	b	c	d	e	f
Benign	g	h	i	j	k	l
No Histology	m	n	o	p	q	r
Total Cytology results	s	t	u	v	w	x

Formulae for calculation of accuracy

Parameter using table 2 - appendix I (14)

(i) Absolute Sensitivity = $\dfrac{a+m \times 100}{f+m}$

Assumption Unbiopsied cases reported as malignant by cytology are considered carcinomas

(ii) Complete Sensitivity = $\dfrac{a + b + c +m \times 100}{f+m}$

(iii) Specificity (biopsied cases only) = $j/l \times 100$

(iv) Specificity (full) = $\dfrac{i + p \times 100}{l + 0 + p + q}$

Assumption: atypia which are not biopsied are considered benign

(v) Positive predictive value = $\dfrac{s - g \times 100}{s}$

29

(vi) Negative Predictive value = $\dfrac{v - d \times 100}{v}$

(vii) False positive rate = $\dfrac{g \times 100}{f + m}$ (this excludes inadequate results)

(viii) False negative rate = $\dfrac{d \times 100}{f \times m}$

(ix) Inadequate rate = $w/x \times 100$

(x) Inadequacy rate for cancer = $\dfrac{e \times 100}{f + m}$

(xi) Suspicious rate = $\dfrac{t + u \times 100}{x}$

APPENDIX II

Calculation of the sample size; (24)

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

Where **n** is sample size,

Z is reliability coefficient

P is the proportion of FNA biopsy result with correlating histology result from the 1998 to 1999 pilot study.

d is the proportion in which the accuracy of n is determined.

The sample size required to estimate the proportion of patients

Within 0.05 with 90% confidence is,

(given that; $z = 1.645$; $p = 0.20$, $d = 0.05$)

therefore $n = \dfrac{1.645^2 \times 0.20\,(1-0.20)}{0.05^2}$

$$= 173.1856$$
$$n = 174$$

The minimum sample size required is 174

REFERENCES

1. Trott PA. Breast In: Young AJ, ed. Fine Needle Aspiration. Cytopathology. London, Blackwell Scientific Publication; 1993: 1-5, 78-96.

2. Lester CS, Cotran SR. The Breast. In: Contra R, Kumar V, Collins T, eds. Robbins Pathologic Basis of Diseases. Philadelphia, WB Saunders Co; 1999: 1095-1119.

3. Colemn M, Estern J, Damiecki P, et al, WHO International Agency for Research on Cancer. Lyon, I.A.R.C Scientific Publication, 1993:121(433).

4. Kungu A. National Cancer Registry. In: Parker DM, ed. WHO Cancer Occurrence in Developing Countries, Lyon, I.A.R.C. Scientific Publication, 1968:47.

5. Schofield K, Segatol - Islami Z, Biingle Y, et al. Cytologic and Histologic Correlation of Fine Needle Aspiration of breast lesions. Acta cytol. 1991; 35(5): 610.

6. Koss LG. Diagnostic Cytology and its Histopathologic Bases.Philadelphia, JP Lippincott Co; 1992: 8-9, 1234-1250, 1293-1315.

7. Cibas ES, Ducatman BS. Cytology Diagnostic principles and clinical Correlation. Philadelphia, WB Saunder Co; 1996: 190-191.

8. Muchiri L, Penner D, Rana FS. The role of FNAB in the Diagnosis of Breast lumps at KNH. East Afi Med J. 1993; 70(4): 31.

9.   Suen CK. Atlas and Text of Aspiration Biopsy Cytology. Baltimore, The William & Wilkins Co; 1990; 105-124.

10. Silverman JF. Breast. In: Bibbo M, ed. Comprehensive Cytopathology 2nd Edition. Philadelphia, WB Saunders Co; 1997: 634, 731-732, 770-771.

11. Raab S S, Bottles K, Cohen BM. Technology Assessment in Anatomic Pathology: An Illustration of Test Evaluation using Fine Needle Aspiration Biopsy. Arch Pathol Lab Med. 1994; 118: 1173-1180.

12. Kara R, McGregor SE. Duggan AM. Breast Fine Needle Aspiration: an Analysis of 1068 cases. Acta cytol. 1991; 35(5): 609.

13.   Nicosia VS, Horoulitz AS, Williams AJ, et al. Fine Needle Aspiration Biopsy of palpable Breast Lesions, Review and Statistical analysis of 1,311 cases. Acta cytol. 1991; 35(5): 609.

14.   Martin H.E, Ellis E.B: Biopsy by needle puncture and aspiration. Ann Surg 1930; 92:169 – 181.

15.   Stewart F. W: The diagnosis of tumours by aspiration. Am J Pathol. 1933; 9:801-812.

16.   Franzen S Zajicek J: Aspiration biopsy in diagnosis of palpable lesion of the breast. Critical review of 3,479 consecutive biopsies – Acta Radiol Ther Phys Biol. 1968; 7:241 – 262.

17.   Zakhour H, Wells W. Houston MJ, ed. Diagnostic Cytopathology of the Breast. London, Churchill Livingstone; 1992: 1-57, 103-132.

18. Velto J, Pommier R, Schimolt W, et al. Use of the "triple test" for palpable breast lesions yields high diagnostic accuracy and cost savings. Am J Surg. 1995; 169(5): 519-22.

19. Steinberg JL, Trucleau M E, Ryder DE, et al. Combined Fine Needle aspiration, Physical examination and mammography in the Diagnosis of palpable breast masses, their relation to outcome for women with primary breast cancer. Can J Surg. 1997; 40 (1): 9.

20. Mckee TG. Cytopathology. Barcelona, Mosby-Wolfe; 1997: 113-184.

21. Bruce HD. Breast disease. A primer on diagnosis and management. Int J Fertil. 1997; 42(5): 278-287.

22. Easton DF, Ford BP, Bishop DT. The breast cancer linkage consortium: Breast and ovarian cancer incidence in MCA1 Mutation Carriers. Am J Hum Genet. 1995; 56: 265-271.

23. Duport ND, Page DL, et al. Long term risk of breast cancer in women with Fibroadenoma. N Engl J Med. 1994; 331: 10-15.

24. Milton SJ. Statistical methods in the Biological and health sciences, 2nd Edition. New York, McGraw-Hill; 1980: 237-238.

25. Salami N, Hirschowitz SL, Nieberg RK. Triple test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. Acta Cytol. 1999; 43(3): 339-343.

26. Brown LA, Loghills SB, Powis SJ. Audit of diagnostic accuracy of fine needle aspiration cytology specimen taken by the histopathologist in symptomatic breast clinic. Cytopathol. 1991; 2: 1-7.

27. Silverman JF, Lannin DR, O'Brienk, et al. The triage role of fine needle aspiration biopsy of palpable breast masses. Acta cytol. 1987; 31: 731-736.

28.   Boerner S, Snerge N. Specific adequacy and false negative diagnosis rate in fine needle aspirates of palpable breast masses. Cancer. 1998; 84(6): 344-348.

29.   Yoshida Y. Takaoka M. Fukumoto M. Carcinoma arising in fribroadenoma. Case report and review of the world literature J surg oncol. 1985: 29: 132.

30.   Bacher International Comparison of Performance in breast FNA cytology. Arch pathol Lab Med. 1991; 115:743-750.

31.   Mokbel K. The 23rd Annual San Antorior Breast Cancer Symposium – 2000. Curr Med Res Opin. 2001; 16(4): 276-284.

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723, Nairobi
Telegrams: "MEDSUP", Nairobi
Email: knh@healthnet.cr.ke

Ref......KNH-ERC/01/952   Date...8..March..2001......

Mr. Eric Bulinda
Dept. of Human Pathology/Clinical Cytology
Faculty of Medicine
University of Nairobi

Dear Mr. Bulinda,

RE: **RESEARCH PROPOSAL** "AN AUDIT OF FINE NEEDLE ASPIRATION BIOPSY
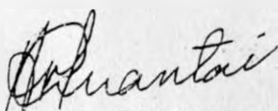OF PALPABLE BREAST LESIONS AT KNH"   (P940/11/2000)

This is to inform you that the Kenyatta National Hospital Ethical
and Research Committee has reviewed and **approved** your above cited
research proposal.

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 data base that will be consulted
in future when processing related research study so as to minimize
chances of study duplication.

Thank you.

Yours faithfully,

PROF. A.N. GUANTAI
SECRETARY, KNH-ERC

c.c.   Prof. K.M. Bhatt,
       Chairman, KNH-ERC,
       Dept. of Medicine, UON.

       Deputy Director (CS),
       Kenyatta N. Hospital.

       Supervisor: Dr. L. Muchiri, Dept. of Human Pathology, UON

       The Chairman, Dept. of Pathology, UON

       The Dean, Faculty of Medicine, UON