

**CYTOMORPHOLOGICAL SPECTRUM OF DIAGNOSES
FROM BONE MARROW PREPARATION AND FINE
NEEDLE ASPIRATION IN COAST AND NYERI LEVEL 5
HOSPITALS, KENYA.**

BY

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DEDICATION

I dedicate my work to my husband Shadrack Shabanji, my children Marcus and Sienna for their unconditional support and understanding during the study period.

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ABBREVIATIONS

AKUH	Aga Khan University Hospital
AML	Acute myeloid leukemia
BMA	Bone marrow aspiration
BME	Bone marrow evaluation
CBC	Complete Blood Count
CM	Centimetre
CML	Chronic myelogenous leukemia
CLL	Chronic lymphocytic leukemia
EDTA	Ethylene diamine tetra- acetic acid
ER	Estrogen receptors
ET	Essential thrombocythemia
FNA	Fine needle aspiration
FNAc	Fine needle aspiration cytology
PUO	Pyrexia of unknown origin
HER 2	Human epidermal growth factor receptors 2
HIV	Human Immunodeficiency Virus
IDA	Iron deficiency anaemia
ITP	Idiopathic thrombocytopenia purpura
KEMRI	Kenya Medical Research Institute
MAI	Mycobacterium avium intracellulare
MDS	Myelodysplastic syndrome
MM	Multiple myeloma
NCI	National Cancer Institute
NIH	National Institutes of Health
PAS	Periodic acid Schiff
PBF	Peripheral Blood Film
PR	Progesterone receptor
PRV	Polycythaemia rubra vera
UON	University of Nairobi
USA	United States of America
TOTs	Trainer of trainees

ABSTRACT

Background: Bone marrow examination and fine needle aspiration biopsy are useful tools for pathological diagnosis of malignancies and other diseases. These procedures are cost-effective, minimally invasive, reliable, fast and well tolerated by patients as they have minimal complications.

These two techniques are under-utilized by medical practitioners universally due to lack of competence in performing these procedures. Lack of consumables also lead to their under-utilization for optimal screening and diagnosis.

This study set out to document the spectra of diagnoses in two level 5 hospitals in Kenya after implementation of a skills-transfer initiative on performing bone marrow evaluation, fine needle aspiration and sample preparation to increase access and to improve diagnosis. The study was part of a larger project working to improve cancer diagnosis by training medical officers and laboratory technologists on procedure performance and technical competence.

Objective: To document the cytomorphological spectrum of diagnoses made using two techniques: - bone marrow examination and fine needle aspiration at 2 level 5 hospitals in Kenya (Coast and Nyeri Provincial General Hospitals) in an operational initiative to improve cancer diagnosis.

Materials and methods: This was a retrospective cross-sectional descriptive study. The study was carried out in Coast and Nyeri level 5 hospitals between January 2013 and December 2016. Bone marrow evaluation (BME) and Fine needle aspiration (FNA) reports from January 2013 to December 2016 were included in the study. A skill transfer operational initiative was implemented in January 2016 to December 2016 of the study. Processed BME and FNA slides were assessed by the principal investigator and three pathologists (supervisors) as part of patient management and results recorded in the data collection book. The reports were examined and findings recorded in a standard proforma including age, sex, relevant investigation and cytomorphological diagnosis made. Data was then analysed to determine the frequencies of different diagnoses made from the two techniques.

Results: The study included 877 FNAs and 75 BMEs. There was a varied age distribution for both the BMA and FNA patients ranging from 2 months to 81 years. The male: female ratio of BMEs reported was 2:1. The 30-39 age bracket contributed to the highest number of BMAs reported, 17.3% (n=75). The mean age for BMEs at Coast level 5 was 32.9 while at Nyeri level

5 it was 41.9. Marrows with reactive changes accounted for most predominant non-malignant haematological diagnosis, Coast 18 (61%) and Nyeri 11 (39%). Acute leukemias were the commonest haematological malignancies reported in both level 5 hospitals 6 (50%) each.

The male: female ratio of FNAs reported was 1:2.5 and the highest number of FNAs reported were from the 20-29 age bracket. Soft tissue lesions were the most frequent diagnoses made in both level 5 hospitals, Nyeri 148 (52%) and Coast 136 (48%) out of 877, followed by breast lesions which were more frequently reported in Nyeri 166(60%) and 112 (40%) at Coast level 5 hospital.

Conclusion

The spectrum of diagnoses was similar in the two regions and reflect the morbidity patterns described for these population by the Kenya Demographic Health Surveys (KDHS).

Marrows showing reactive changes were the commonest non-malignant findings while acute leukemias were the commonest haematological malignancies in the two hospitals.

Lipomas and fibroadenomas were more frequently reported in Nyeri level 5 hospital than Coast level 5 hospital.

There were more malignant conditions picked in Nyeri level 5 hospital compared to Coast level 5 hospital using the two diagnostic techniques.

Recommendations

Similar initiatives can be done in other sites to define disease trends as this will help with documentation of disease types and burdens in the country.

Consecutive reviews to determine the long-term impact of the intervention on cancer diagnosis using BMEs and FNAs in the two hospitals should be done.

1.0 INTRODUCTION

Bone marrow evaluation and fine needle aspiration are simple techniques, valuable and easy to perform and are used to diagnose benign and malignant conditions.

Bone marrow examination (BME) is one of the most valuable tools for evaluation of haematological and non – haematological disorders. Indications for BME include diagnosis, staging and therapeutic monitoring of lymphoproliferative disorders such as CLL, myeloproliferative disorders, myelodysplastic syndrome and multiple myeloma. It can also be used for evaluation of cytopenias, thrombocytosis, leucocytosis and anaemias. Non-haematological conditions can also be investigated for example fever of unknown origin – (FUO) especially in HIV patients. Storage disorders such as Niemann- Pick, Gaucher’s disease and Tay- Sachs can also be diagnosed using BME. Evaluation of the bone marrow can be used in the diagnosis of infectious diseases such as tuberculosis, histoplasmosis, leishmaniasis, mycobacterium avium intracellulare (MAI) and disseminated fungal infections. Analysis of the bone marrow is useful not only for assessment of metastatic diseases but also in the revelation of toxic effect of medications such as alcohol on the bone marrow.

Bone marrow examination consists of bone marrow aspiration (BMA) and bone marrow trephine biopsy; two tests which complement each other (1). BMA yields semi- liquid marrow which can be examined under light microscopy. The aspirate can be used for analysis using flow cytometry, molecular studies, cytogenetics and cultures, this is one of the advantages of BMA over trephine biopsy (2).The other advantages are fast processing and better morphologic assessment of cells. Trephine biopsy yields a narrow cylindrically shaped solid piece of bone marrow which can be examined microscopically sometimes with the aid of immunohistochemistry for cellularity and infiltrative process. Trephine biopsy also has its own advantages such as giving a better bone architecture when it comes to cellular and stromal constitution. Bone marrow trephine biopsies represents all cell types as well as explaining a dry tap. A dry tap is when bone marrow aspiration fails completely and this can indicate aplastic anaemia, idiopathic myelofibrosis, hairy cell leukaemia associated with fibrosis and metastatic cancers. Bone marrow trephine biopsies have the disadvantage of slow processing since they need time for decalcification in EDTA or formic acid (11).

FNA needs very little equipment and is under- utilized as a diagnostic tool. FNA if done correctly, has the potential to improve diagnostic capabilities and provides access to timely and low- cost patient care especially in facilities which have no pathologists. FNA is a first line

diagnostic tool which can be used for diagnosis of superficial lesions in paediatric and adult patients in resource limited settings. It can be performed by both pathologists and non-pathologists in outpatient and in-patient set-ups. It is a feasible diagnostic option in resource limited countries like Kenya.

FNA is a diagnostic tool which was initially applied for inflammatory and infective lesions only. However, cytological investigations expanded its diagnostic application to include confirmation and exclusion of malignancies.

FNA is widely used for evaluation of palpable superficial masses as well as deep seated non-palpable lumps under imaging guidance. FNA is a simple, safe, cost-effective and minimally invasive procedure used to screen, diagnose and follow-up both infectious and neoplastic diseases. FNA has other advantages such as quick diagnosis, less pain compared to tissue biopsy and it does not need anaesthesia (3). Sedation can be used for paediatric and anxious patients. Samples collected from FNA can be used for cytogenetics, flow cytometry, cytological diagnosis and microbiological cultures.

It is a reliable diagnostic tool with few complications and rapid technique for diagnosis of tumour and tumour- like conditions. It is also useful diagnostic tool used for head and neck masses – thyroid, lymphoid and salivary glands. There are no absolute contraindications for FNA though for deep seated lesions in patients with abnormal coagulation profiles and suspected vascular lesions it should be avoided due to risk of haemorrhage. The complications of FNA are minimal, they include pain, bleeding, infection, vasovagal reactions, pneumothorax, seeding along needle tract and perforation of organs (35). Most of the risks are associated with aspiration using small gauge needles (22 G or less) for superficial lesions. When performed by experienced hands the need for tissue biopsy and its risks are averted as a diagnosis can be made from the aspirate.

Fine needle aspiration cytology (FNAC) is applied for diagnosis of malignant and benign lesions, this increases the significance of ancillary studies. The ancillary studies include cellblocks, immunocytochemistry, microbiological cultures and electron microscopy in the final diagnosis of the aspirated material. These tests are done in addition to the cytological evaluation on light microscopy to improve the diagnostic accuracy of FNAC.

FNA is limited by the blind sampling done since some lesions are heterogeneous therefore representative material may not be aspirated. The second limitation is that some diagnoses rely on tissue architecture which can only be assessed by clinical correlation and histology.

Patients are referred from peripheral facilities to county or national referral hospitals for diagnosis and management of malignancies. These patients often have to wait for months before they can access these services and as a result present late when little can be done except palliative care. These hospitals get referrals from within their county and beyond. It is important for these referral hospitals to have updated disease trends within their regions. This will help with organization and planning for diagnostic, preventive, curative and palliative needs of the counties as well as in making projections in the future.

Cancer is an emerging public health problem in Africa. In developing countries like Kenya, there is a double disease burden due to an increase in both non-communicable and communicable diseases which are leading killers. According to the International Agency for Research on Cancer (IARC) there were 715,000 new cancer cases with 542,000 cancer deaths in 2008. Cancer cases are projected to double by 2030 with 1.28 million new cases and 970,000 deaths due to adoption of behaviours and lifestyle associated with economic development such as smoking, unhealthy diet and physical inactivity. Majority of the cancers in Africa are diagnosed at an advanced stage because of lack of screening and early detection services as well as limited awareness of early signs and symptoms of cancer (4).

The cancer burden in Kenya is unknown, mainly because of under-reporting or lack of statistics. Cancer registries rely on good diagnostic techniques which can then be fed into the registries. Cancer ranks third after infectious and cardiovascular diseases as leading causes of morbidity and mortality in Kenya. There are two population-based cancer registries in Kenya, one in Nairobi and the other in Eldoret, the rest of the data is hospital-based therefore the data available is scanty and the true population cancer burden remains unclear. It is estimated that 28,000 cancer cases are diagnosed with 22,000 mortalities annually (5).

Cervical and breast cancers are the leading cancers in women while in men oesophageal, head and neck and prostatic cancers are leading. In the paediatric age group, leukemias and lymphomas are the commonest cancers. However, cancers of the gastrointestinal system are also on the rise in the Kenyan population probably due to lifestyle modifications (4).

Regional cancer Registry at KEMRI reported that 80% of cancers are diagnosed when little can be done in terms of curative treatment. This is due to inadequate screening services, poorly structured referral systems and inadequate diagnostic facilities. BME and FNAC are diagnostic tools which are minimally invasive, readily available, accessible and reliable when it comes to diagnosis of haematological and non- haematological conditions. These procedures can be done in out-patient departments and in institutions without facilities for histopathological diagnosis and the specimen sent for evaluation by a pathologist hence making a diagnosis at the earliest opportunity.

A Cancer stakeholders' workshop was held in Naivasha, May 2014. It was hosted jointly by Ministry of Health, Kenya and Centre for Global Health (CGH) at National Cancer Institute (NCI)/National Institute of Health (NIH)- USA. The stakeholders came up with four tracks – 1. Research capacity building, 2. Pathology and cancer registries, 3. Cancer awareness and education and 4. Health system strengthening. Pathology and cancer registries' objective was to strengthen cancer control and care through Pathology and cancer registries by 2017. This would be achieved through strengthening pathology training and service capacity. Diagnostic services- laboratory and radiological – are available in Nairobi and other large cities and towns with variable capacity. In many hospitals and diagnostic centres where cancer diagnosis can be made there is limited human resource (pathologists and laboratory technologists) to help with sample collection and processing to ensure timely and quality cancer diagnosis (5). A situation analysis showed that most of the FNAs in level 5 hospitals are done by pathologists. Most level 5 facilities have only one on-site pathologist who runs both the clinical and anatomic pathology units within the hospitals hence the need to train non- pathologists in diagnostic procedures such as FNA and BME. This will help with early diagnosis and management of most haematological and non- haematological lesions. FNA needs very little equipment and is under-utilized, if done correctly FNA has the potential to improve diagnostic capabilities and provides access to timely and low- cost patient care especially in facilities which have no pathologists. FNA is a first line diagnostic tool which can be used for diagnosis of superficial lesions in paediatric and adult patients in resource limited settings. It can be performed by both pathologists and non- pathologists in outpatient and in-patient set-ups. It is a feasible diagnostic option in resource limited settings like Kenya.

The pathology track resolved that there was need to increase the number of skilled health care providers and facilities with the capacity to perform FNAs, BMA and bone marrow trephine biopsies.

The resident driven programme was one of the action plans for the Pathology track. The University of Nairobi (UoN) and Aga Khan University Hospital (AKUH) pathology residents and laboratory technologists were trained as trainers in proper BMA/ trephine biopsy and FNA sample collection and processing. The trained residents and technologists went to four selected level 5 facilities – Nyeri, Kisumu, Embu and Coast to train clinical officers, medical officers, surgeons, paediatricians and physicians on how to perform these procedures. (A level 5 hospital is a referral hospital for the district/level 4 hospitals and provides specialized care including intensive care, life support and specialist consultation). The trainings were done under the supervision of consultant pathologists from the universities and on-site pathologists, who were also Trainer of Trainees (TOTs) in the programme and assisted with co-ordination of activities in the selected facilities. The rationale for the resident driven project was: To train non-pathologists in FNAC, BMA and trephine techniques since untrained personnel perform these procedures and process the specimen leading to unsatisfactory specimen that cannot be diagnosed accurately. This leads to delay in diagnosis, inaccurate diagnoses and loss of confidence in BME and FNAC procedures as diagnostic tools.

1.1 Literature Review

Bone marrow

1.1.1 Historical aspect of bone marrow examination

Bone marrow examination is one of the oldest procedures known to man. Its use for diagnosis of haematological conditions became relevant in the twentieth century and is increasingly being used with advances in instrumentation, anaesthesia and laboratory processing. Trepanning or trephine biopsy dates as far back as 8000-10000 years back. Skulls with evidence of medical intervention have been found in Europe, Northern Africa, Asia, New Guinea, Tahiti and New Zealand. Most of the information on trepanning history comes from Peru where it is reported that the procedure was carried out to relieve headaches, mental illness and to relieve intracranial pressure. Pianese in 1903 punctured part of the femoral epiphysis to describe a case of anaemia caused by bone marrow infiltration by *Leishmania Infantum*. However, in 1922 advances were made by Monus and Falciner who did tibial bone marrow biopsies using a drill-like instrument to produce a marrow specimen similar to that obtained today. These procedures were carried out without anaesthesia though gowns and gloves were recommended. Seyfar did sternal biopsies in 1922, where he obtained satisfactory specimens on which he did touch preparations,

wet preparations and blocks for sectioning. It was not until 1950 when Rubenstein suggested the use of posterior superior iliac spine as the preferred site for bone marrow analysis. It was during the same period that bone marrow started gaining popularity as a diagnostic tool for haematological conditions (6).

1.1.2 Indications for bone marrow examination

Bone marrow examination (BME) involves both bone marrow aspiration and trephine biopsy. These are two separate procedures which can be done at the same time on a patient. Bone marrow examination is usually preceded by evaluation of medical history, clinical findings, complete blood count, peripheral blood film as well as relevant laboratory tests and radiological investigations (7). BME is an invasive procedure which requires clinical judgement and the application of an inclusion criteria. It is used to diagnose and stage haematological and non-haematological neoplasia, to determine the cause of cytopenias and to confirm or exclude metabolic and infectious conditions suspected on the basis of clinical symptoms and peripheral blood films findings (8).

Aljadayen et.al did a comparative study at the King Hussein Medical Centre, to evaluate the morphological differences between bone marrow aspiration and bone marrow biopsy. They concluded that bone marrow aspiration is complementary to bone marrow biopsy in diseases affecting the bone marrow such as AML, ITP, CML, ALL, MDS, PRV, ET, MM, CLL, nutritional and megaloblastic anaemia. BMA was found to have limited predictive value in infiltrative bone marrow diseases such as lymphomas, myelofibrosis, aplastic anaemia, solid tumour metastases, unexplained leucoerythroblastic blood film where biopsy is mandatory (9). Bone marrow aspiration and trephine biopsies should be performed on all suspected and proven solid tumours and Hodgkin's lymphoma (10). A trephine biopsy is not necessary in cases where an aspirate can give a definitive diagnosis such as ALL. However, it can be done as a baseline for comparison with other biopsies during follow-up. Trephine biopsies are important in the diagnosis and follow-up of lymphomas, adult tumours and paediatric tumours such as neuroblastomas, Ewing's sarcoma, rhabdomyosarcoma and primitive neuroectodermal tumours (11).

In a retrospective study by Okinda NA and Riyat MS to determine the indications of BME at Aga Khan University Hospital. Acute myeloid leukemia was the most common haematological malignancy followed by lymphoproliferative disorders. The most common indication was anaemia followed by bone marrow cultures for diagnostic work up for fever of unknown origin. (12). A similar study done in Benin City, Nigeria where 88 patients underwent BMA most had combined substrate deficiency, acute leukemias, megaloblastic anaemia and malignant plasmacytosis. (13).

BMA and core biopsy are necessary for complete evaluation in the staging of carcinomas since some are missed on aspiration alone. Marrow involvement in lymphomas is infrequent but the inclusion of bone marrow biopsy significantly impacts treatment and outcome of patients where a higher disease stage has not been diagnosed or has been missed entirely (13).

Bone marrow examination can be used for diagnosis of infectious diseases for example Pamnani et.al described four case reports where BMA cytology was used for diagnosis of disseminated histoplasmosis. (14). Trepine biopsies are also indicated for granulomatous diseases like sarcoidosis, Tuberculosis and cryptococcosis (11). A similar study done by Munir et.al in Pakistan showed that 5 % of the 157 patients evaluated were diagnosed with myelofibrosis, malaria , visceral leishmaniasis and paroxysmal nocturnal hemoglobinuria (15).

1.1.4 Sites of bone marrow examination

BMA is commonly carried out on the sternum or ilium. Aspiration from the iliac crest is done either from the posterior or anterior iliac spine. The posterior iliac crest is the preferred site for both adults and paediatric patients. Anterior iliac spine aspiration has the same advantages as the posterior though the cortical bone is thicker but it is preferred in patients who can only lie supine. Sternum is only 1cm thicker therefore cannot be used for biopsy. Sternal aspirations are carried out on the body of the sternum below the angle of Lewis at the level of the second intercostal space. It is the preferred site of aspiration in obese patients. A study carried out on obese 100 patients concluded that sternal aspiration was easier technically and produced more suitable diagnostic specimen, however, patients reported that it was more painful both during the both penetration and marrow aspiration. The medial tibia can be used for bone marrow aspiration in children less than 18 months. It was shown to have no advantage over iliac crest in older children above 18 months. Spinous processes of the vertebral bodies and ribs can also be used for aspiration though are rarely used except when the site is suspicious for lesion

discovered on a radiograph. Bone marrow trephine biopsy is mostly carried out on the iliac crest either anterior or posterior though the posterior is generally preferred (8).

All haemopoietic sites exhibit uniform cellularity and cell lineage proportions therefore evaluation of the bone marrow at one site gives a picture of the body's haemopoietic condition. Evaluation of the bone marrow at multiple sites has not been shown to improve diagnostic accuracy of haematological disorders. There are exceptions where the marrow has patchy involvement for example in lymphomas, multiple myeloma and metastatic disease. These disorders require either a large specimen or evaluation of various sites (16).

1.1.5 Imprint smears

Some conditions such as aplastic anaemia and myelofibrosis can result in a dry tap during bone marrow aspiration. Touch preparations of the trephine biopsy are also useful when the aspiration material is considered haemodiluted or technically inadequate for evaluation. (17). Bone marrow biopsies are thick and preclude the fine cytomorphological details needed to determine cellularity and cellular details. Touch imprints of the biopsies can be done in such cases. Bone marrow imprints help in initiating prompt treatment of patients while they are awaiting histopathological report of the core biopsy. A study done at Varanasi, showed that imprint smears are better than BMAs in assessing cytological details and cellularity therefore increasing its diagnostic accuracy. Bone marrow imprint smears give a better differential count as the cell spreading is better than BMAs (18). Imprint smears permit a differential count similar to that performed on aspirate films according to another study done by Bain BJ (11). Baskota et. al did a hospital based cross-sectional study to determine the use of touch imprints as adjuncts to BMA and they concluded that touch imprints combine features of both BMA and trephine smears. Imprint smears are simple, reliable and rapid techniques with excellent cytomorphological detail (19).

1.1.6 Complications of bone marrow examination

Bone marrow examination is a potentially hazardous procedure. Some of the complications involved include lacerations of neighbouring structures like a branch of the gluteal artery and soft tissues. Infection and haemorrhage are also common complications. Haemorrhage can occur if the patient has thrombocytopenia or where there is concurrent use of anticoagulants. The sternum is not a recommended site of bone marrow biopsy: Patients with multiple myeloma or osteoporosis are at risk of getting fractures during the procedure (20). The lateral cutaneous nerve of the thigh maybe damaged during bone marrow aspiration or biopsy, a rare

complication, suggestive of poor technique (7). Gluteal compartment syndrome and sciatica have been reported in some patients after undergoing bone marrow biopsy on the iliac crest, though rare it can occur especially in unconscious patients (21). Tumour seeding from the bone marrow into the needle tract for example into the muscle, skin and subcutaneous tissue has been reported in patients with small cell carcinoma of the lung (22).

1.1.3 Contraindications of bone marrow examination

There are no absolute contraindications for bone marrow examination. However, there are relative contraindications related to the general condition of the patient or the risk of anaesthesia or deep sedation. Active infection at the site proposed site of aspiration such as posterior superior iliac spine, would preclude use of the site. Thrombocytopenia and other coagulopathies are not contraindicated if it is executed by a skilled clinician. It is contraindicated in a haemolytic anaemias, aplastic crises, haemophilia and related congenital haemorrhagic disorders (12).

1.1.7 Special techniques in bone marrow diagnosis

Bone marrow aspirates are stained routinely with Romanowsky stains like May-Grunwald-Giemsa stain or Wright- Giemsa stain. Other diagnostic procedures that may be of use include cytochemistry, immunophenotyping which includes immunocytochemistry or flow cytometry. Culture for micro-organisms, cytogenetic and molecular genetic analysis can also be done.

Trephine biopsies histological sections are routinely stained using Haematoxylin and Eosin stains. Reticulin is used for assessment of reticulin fibres while Mason Trichrome is used for collagen fibres (1). Perl's stain or Prussian blue stain is used to demonstrate hemosiderin in bone marrow macrophages and on erythroblasts. Assessment of iron storage is done using Perls's stain. Periodic acid Schiff (PAS) is used for identification of plasma cells, megakaryocytes and tumour cells. Neutrophils are also positive for PAS. Mycobacteria are identified using Ziehl- Neelsen stain (7).

1.1.8 Comparison between the crush and wedge techniques

The wedge and crush techniques are used simultaneously for preparation of BMA smears. The crush technique is valuable for better assessment of megakaryopoiesis because of lack of blood

dilution effect. The wedge spread technique is more beneficial for assessment of total cellularity (23).

1.1.9 Limitations in interpreting bone marrow trephine biopsy

No other specimen type in the histopathology lab represents only half of the sample for interpretation with the half – the BMA being handled in another lab. The BMA is often reported earlier than a trephine biopsy which takes longer to process and can be reported in the histopathology department. Sources of error in interpretation include – inadequate clinical, hematological (blood and aspirate findings) and radiological information. The trephine biopsy specimen is at times too small, too crushed or distorted, poorly decalcified or processed hence inadequate for reporting. The slides are at times poorly stained due to technical inadequacies and the range of stains may be too limited (24).

1.2 Fine needle aspiration

1.2.1 Fine needle aspiration history

FNA is the study of cells obtained by puncturing of organs and tissues in the human body with small gauge needles. This procedure has been used for over eleven centuries though most of the improvements in modern FNA were done in the twentieth century. Albucasis, an Arab physician in the tenth century, was the first to describe modern FNA of the thyroid in the book of Arab Medieval Medicine. In the 19th and 20th centuries FNA was used for the diagnosis of and treatment various diseases as described by the German haematologist, Hans Hirschfeld, who was the first to report a case of cutaneous lymphoma diagnosed by FNA. Leonard Dudgeon was the first pathologist to scientifically establish the need to make fast and secure diagnosis of histological preparations by mounting material from surgical biopsies to glass slides by using touch smears or imprints. Manheim was the first one to suggest the use of the small gauge needle 22 for fine needle aspiration (25).

1.2.2 Indications of FNA

Guthrie while working at John Hopkins Hospital, Baltimore in 1921 was able to successfully diagnose syphilis, tuberculosis, malignant lymphoma, leukemia and metastatic cancers using FNA (25).

FNA is the most reliable and cost-effective method for distinguishing between malignant and benign thyroid nodules. Nodular goitre is the most common FNA cytological diagnosis

followed by non- diagnostic sampling, atypia, thyroglossal cysts, thyroiditis and papillary carcinomas (26).

Breast which includes FNA, nipple discharge and touch preparations identify benign, atypical and malignant pathological changes in the breast specimens. Fibroadenoma was the most common benign lesion diagnosed in premenopausal women, while the most frequently diagnosed lesion in men was gynaecomastia in a study done by Nkonge et.al. (27). Fine needle aspiration is a reliable and acceptable pre-operative procedure. It forms part of the triple test in developing countries (28).

Fine needle aspiration is a reliable diagnostic tool for neoplastic and non-neoplastic lesions of the lymph nodes. In a two- year study done on 130 patients, majority had reactive hyperplasia, tuberculous lymphadenitis and metastatic carcinoma. A small percentage had lymphomas and suppurative lymphadenitis (29).

Granulomatous inflammation can also be diagnosed using FNA. In a study done at Belfast City hospital, 8 out of 22 patients were diagnosed with granulomatous inflammation. Five (5) of the patients had Tuberculosis, 2 Toxoplasmosis, 1 sarcoidosis and the rest had the granulomas as a result of neoplasm (30).

1.2.3 Contraindications of FNA

Most contraindications are relative rather than absolute. Haemorrhagic diathesis and use of anticoagulants are contraindications. Hydatid disease is a contraindication to fine needle aspiration due to the possibility of fatal anaphylaxis upon rupture. Patients with suspected vascular lesion like arteriovenous malformations and angiosarcoma are contraindicated because of the risk of haemorrhage (31).

1.2.4 Complications of FNA

Complications of FNA are rare, though, as a general rule they rise exponentially with increasing diameter of the needle. Superficial lumps and bumps have minor and infrequent complications. Bleeding may occur but this can be prevented by application of local pressure even in patients with bleeding disorders. Local infection can occur at the aspiration site but this is extremely rare if appropriate antiseptic technique is used. Vasovagal reactions can occur though this is rare. Transient nerve paresis especially the recurrent laryngeal nerve post- thyroid FNA has been reported. Tracheal puncture has been reported after aspiration from isthmus lesions of the thyroid nodules and resolves spontaneously. Pneumothorax also occurs when

FNA is performed in the chest vicinity including axilla, breast and supraclavicular regions. Fatalities are almost non-existent though it was reported that a patient died following aspiration of an advanced carotid body tumour which resulted in carotid body thrombosis (31).

1.2.5 Limitations of FNA

Sampling is scanty and the histological architecture is lost thereby making diagnosis difficult. The material collected from breast FNA cannot be used for determination of ER, PR and HER2 receptors therefore there is need to use cell blocks and core biopsies (32).

Core biopsies have taken a lead in the diagnosis of breast tumours though FNAs still have a role. FNA is limited in defining invasion and type of cancer. Its sensitivity is increased when core biopsies are used as adjuncts (33).

One of the pitfalls of FNA as a diagnostic tool is that it has to be used together with histology for the diagnosis of lymphomas, Warthin's tumour and other salivary gland lesions. Ancillary tests such as flow cytometry and in situ hybridization can be useful for the definitive diagnosis but they are not readily available in resource limited settings (34).

Soft tissue tumours are difficult to diagnose with FNA because obtaining sufficient sample material is technically difficult especially when the lesions are small, deep seated and necrotic. The insufficient sample material cannot be used for ancillary studies which are useful for definitive diagnosis of these lesions (35).

1.2.6 Cancer Registries

Kenya has two population- based cancer registries in - Eldoret and Nairobi. The Eldoret cancer registry is based in Moi Teaching and Referral Hospital while the one in Nairobi situated at the Centre for Clinical Research at KEMRI. The two registries have played an important role on the provision of local data to the IARC, which is the cancer research arm of World Health Organisation (WHO) (36).

FNAs and BMEs processed and reported on-site at the level 5 hospitals would not only improve patient outcome but can be registered, documented and fed into hospital-based cancer registries. Data from these hospitals can be used to generate county and national registries. These data can help with hospital / county planning for cancer management.

Cancer registries are opportunities for identification of novel risk factors in Kenya that would help in advancement of prevention measures with respect to the diverse Kenyan culture, dietary

and environmental factors. In a study of cancer registry literature update from all over the world, only 1% of the literature emanated from Africa compared to 34% and 42% from Europe and Asia respectively. The reasons for under-reporting are inadequate diagnostic facilities, limited access to care, inadequately trained manpower and infrastructure as well as poor quality of cancer data systems all contributing to inadequate data on cancer burden (4).

1.2.7 University Curriculum

In the University of Nairobi undergraduate curriculum, students are not expected to perform BMA and trephine biopsy procedures. The students therefore lack competency to perform these procedures upon graduation. These doctors are not confident when performing BMEs and lack skills in getting good material from their aspirates. This delays patient management since the specimen are invaluable and the patients have to either wait for the pathologist to do the procedure or be referred to another hospital.

The number of trephine biopsy specimens received in the Haematology department for reporting are very few compared to those of bone marrow aspirates. The numbers therefore do not provide opportunity for skills acquisition even for the keen student.

2.0 STUDY JUSTIFICATION

Bone marrow aspiration, trephine biopsies and fine needle aspiration are important diagnostic techniques which are used infrequently in our set-up due to perceived technical and procedural difficulties in performing them. Resources for performing the procedures such as the trephine biopsy needles and the reagents for sample processing are often not available. Training non-pathologist health care providers to perform the procedures and giving technologists technical competence will increase their use. This will provide early diagnosis for cancer and other disease conditions resulting in better patient care. In addition, increased cancer diagnosis will provide valuable data for hospital and national population-based cancer registries. This study will assess the clinical indications for FNA/BME and collate the diagnostic findings in the selected level 5 hospitals. The results of this study will also demonstrate the importance of these two procedures in early cancer diagnosis ultimately leading to better clinical outcomes for cancer patients at Level 5 hospital, this part of the objectives of the bigger project.

This is a pilot study in two selected facilities – Nyeri and Coast level 5 hospitals and can be replicated in other county hospitals as efforts continue to set up a national cancer registry.

2.1 Study Question

What is the spectrum of diagnoses made at Coast Level 5 Hospital and Nyeri Level 5 Hospital using Bone marrow examination and FNAC?

2.2 Study Objectives

2.2.1 Broad objective

To determine the spectrum of diagnoses made using bone marrow examination and fine needle aspiration in two level 5 hospitals in the period January 2013 -December 2016.

2.2.2 Specific objectives

- 1) To document the diagnoses made using FNA and BME over a four- year period.
- 2) To compare the different disease spectra between the two sites
- 3) To determine the frequency of malignant conditions detected using FNA and BME in the two level 5 hospitals.

2.2.3 Secondary Objective

- 1) To identify the short term impact of the intervention on cancer diagnosis in the two sites.

3.0 MATERIALS AND METHODS

3.1 Study Design

This was a cross-sectional, descriptive study. It was a retrospective study with pre-intervention and intervention arms. The study period was January 2013-December 2016.

3.2 Study Setting

The study was carried out at the Nyeri and Coast Level 5 hospitals. Nyeri level 5 hospital is located in Nyeri county, 160 km northeast of Nairobi. It is a level 5 hospital with a bed capacity of 350 beds and an average of 700-800 patients are seen at the outpatient department per day. Nyeri level 5 hospital serves neighbouring counties such as Laikipia, Muranga, Nyandarua and Kirinyaga.

Coast level 5 hospital is a 700-bed capacity hospital located on the coast of the Indian Ocean in the city of Mombasa, in south-eastern, Kenya. The hospital serves Kwale, Kilifi, Taita Taveta and Lamu counties. At least 800-1000 patients are seen at the outpatient department daily. Out of these between 50 – 80 patients qualify for FNAs in a month.

3.3 Study Population

The population encompasses all diagnostic slides from FNAC and bone marrow evaluated at the two level 5 facilities.

3.3.1 Inclusion criteria

Medical records of patients who have had BME

Medical records of patients who have had FNA.

Diagnostic BME and FNA slides processed during the intervention period.

3.3.2 Exclusion criteria

Inconclusive biodata

Ambiguous and missing diagnosis

3.4 Sample size determination

The sample size for bone marrow examination and fine needle aspiration was determined using the formula for finite population (Daniel, 1999). This formulae was used with the assumption

that 50% of all the specimen reported would yield malignant diagnoses in line with objective 3 of the study. The calculation was as follows:

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where

n' = sample size with finite population correction,

N = size of the target population = 94

Z = Z statistic for 95% level of confidence = 1.96

P = Estimated proportion of malignancies/diseases amongst all specimens from bone marrow = 0.5

d = desired precision = 0.05

A sample size of **76** was obtained for the study. This was distributed proportionally to Coast hospital as 40 and Nyeri hospital as 36.

The sample size for FNA was determined using the formula (Daniel, 199);

$$n = \frac{Z^2P(1-P)}{d^2}$$

Where;

n = sample size

Z = Z statistic for 95% level of confidence = 1.96

P = Estimated proportion of malignancies/diseases amongst all specimens from FNA = 0.5

d = desired precision = 0.05

The sample size was **385**. This was distributed proportionally to Coast PGH as 133 and Nyeri PGH as 252.

3.5 Sampling method

A sampling frame for all specimens from BME and FNA was obtained from the two hospitals. All BMEs and FNAs which met the inclusion criteria were included in the study.

3.6 Archived Slides Retrieval and Assessment

Upon ethical approval, permission was sought from relevant authorities at Nyeri level 5 hospital and Coast level 5 hospital to retrieve and review medical records and slides. All FNA and BME reports from January 2013 to December 2016 in both hospitals were retrieved and those that met the inclusion criteria were used as part of study data.

3.7 Examination and Reporting

All slides processed during the intervention period were assessed by the principal investigator and three pathologists (supervisors). The reports were generated as part of patient care and were recorded and used as part of study data. Discrepant findings were discussed by the pathologists and consensus arrived at. Reporting proformas are in the Appendix.

3.8 Data collection tools

Clinical and histological data were collected using a designed questionnaire and reporting proforma respectively.

3.9 Variables

The variables that were analysed include BME and FNAC findings and were categorised using age, sex, and pathological diagnosis.

3.10 Data analysis

Data obtained from the BME and FNA reports was entered and stored using Microsoft excel 2016. Data was imported using STATA, coded, cleaned and analyzed.

Data was analysed using socio-demographic variables and indications for BME or FNAC.

Results were analysed and presented in proportions and percentages in the form of tables, pie charts, graphs and narrative.

Pearson chi square was applied to test if there was a relationship between the frequency of disease diagnosis in the 2 level 5 hospitals. A p-value of ≤ 0.05 was considered statistically significant.

3.11 Quality Assurance

Standard operating Procedures (SOPs) were used and adhered to during FNA and BME specimen processing and reporting in the implementation period.

Materials and reagents used in the project were obtained from certified distributors. The reagents were stored at the recommended temperatures and were changed regularly according to SOPs.

A records officer well versed with medical record keeping was hired as a research assistant to help with retrieval of medical records using ICD-10 coding system and manually. The retrieved slides were given unique identification numbers and were clearly labelled. Data was carefully entered into respective data collection forms to avoid mix-ups and transcription errors.

All the diagnostic FNA and BME slides were analysed by the PI and reported by the haematopathologists and cytopathologists as part of improving patient care. Discrepant findings and all positive findings were seen by an independent pathologist.

3.12 Ethical consideration

Permission was sought from the KNH Ethical and Research committee (KNH-ERC) and the study undertaken after formal approval.

Approval was also sought at the study sites – Nyeri and Coast Provisional General Hospitals.

The slides and requisition forms were assigned unique numbers.

Confidentiality of the study results data was maintained with only the principle investigator, supervisors and statistician allowed to view the data

4.0 RESULTS

4.1 BME RESULTS

4.1.1 Gender distribution for BME in Coast and Nyeri level 5 hospitals

A total of 75 BMEs were reported in both Coast and Nyeri level 5 hospitals. The males were (66.7%) n= 50 while the females were (33.3%) n=25. The male to female ratio was 2:1.

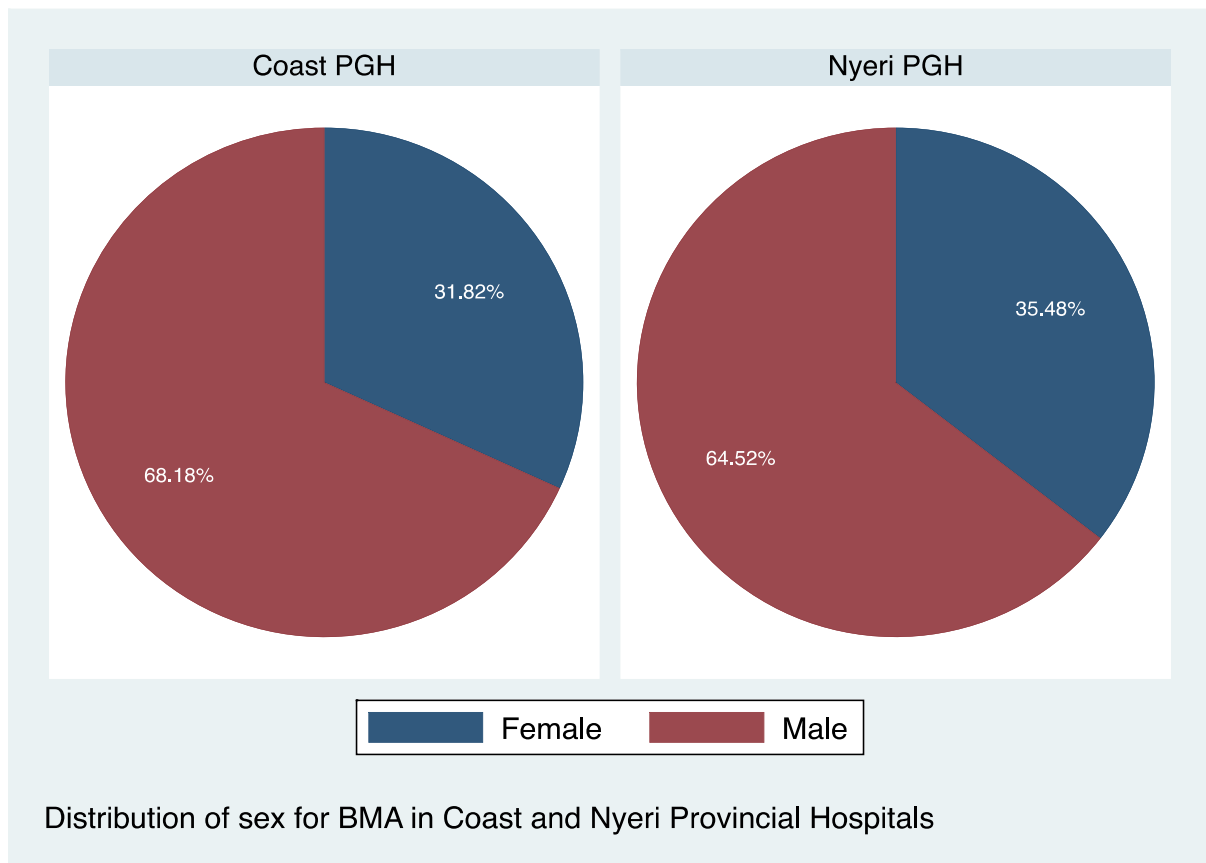


Figure 1: Gender distribution in Coast and Nyeri level 5 hospitals

4.1.2 Association of gender with diagnosis using BME

Table 1 shows there was a higher proportion of males with reactive marrow changes 17 (22.7%) and malignant diseases 25 (33.3%) compared to the females from the 75 BMAs done. This distribution of disease between the two gender was not statistically significant with a Fisher's exact = 0.503.

Table 1: Association of gender with diagnosis using BME

Gender	Normal	Benign	Malignant	Non-Diagnostic	Reactive	Total
Female	1	1	11	1	11	25
Male	3	3	25	2	17	50
Total	4	4	36	3	28	75

4.1.3 Age distribution of BMEs in Coast and Nyeri level 5 hospitals

The age distribution for BMEs was varied and ranged from 1 year to 88 years, the 30-39-year age bracket accounted for the highest number of BMA patients, 17.3% (n= 75) of the total BMAs while the lowest age bracket was >80 years which accounted for 2.7% of the BMAs. The mean age for BMEs at Coast Level 5 was 32.9 while at Nyeri level 5 hospital it was 41.9.

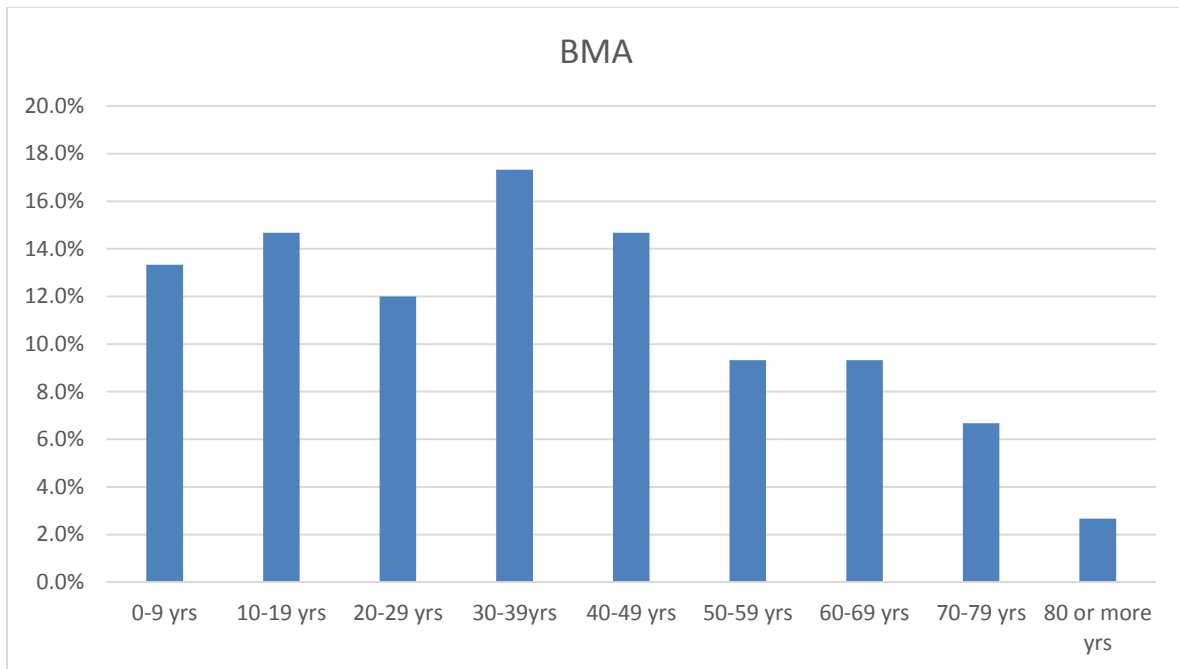


Figure 2: Age distribution of BMEs in Coast and Nyeri level 5 hospitals

4.1. 4 Disease classification using BME at Coast and Nyeri PGH

Coast and Nyeri level 5 hospitals had high proportions of malignant diseases accounting for 21 (48.4%) and 15 (47.7%) respectively of the total BMEs followed by marrows with reactive changes which accounted for 18 (40.9%) and 10 (32.3%) at Coast and Nyeri hospitals respectively. This is demonstrated in **Figure 3**.

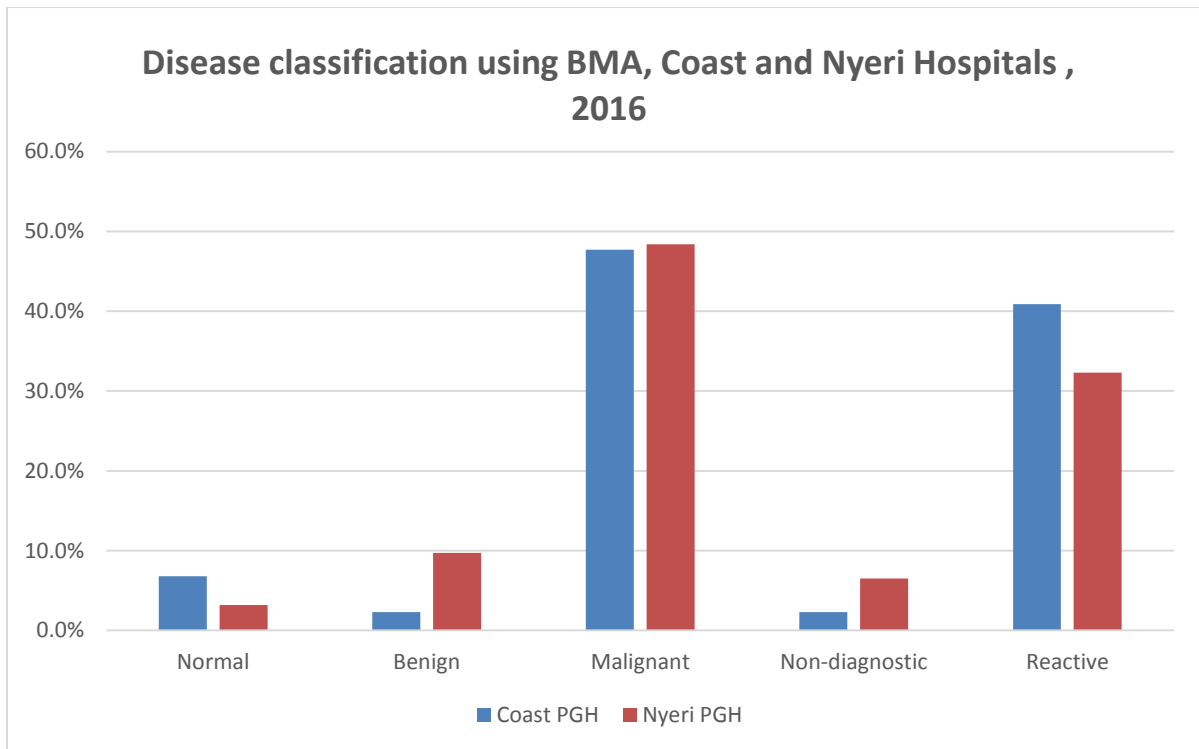


Figure 3: Disease classification using BME at Coast and Nyeri level 5 hospitals

4.1.5 Diagnoses made using BME in Coast and Nyeri level 5 hospitals

Figure 4 shows that marrows with reactive changes was the most predominant diagnoses made at Coast level 5 hospital 17 (61%) and 11 (39%) Nyeri level 5 hospital. Acute leukemia contributed to 6 (50%) each in both facilities while chronic leukemia was 6 (75%) and 2 (25%) in Coast and Nyeri PGH respectively.

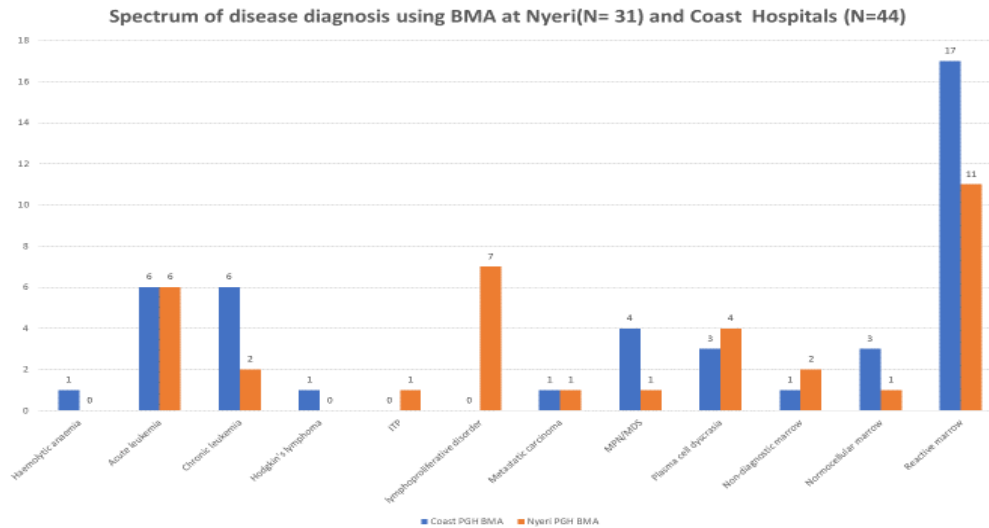


Figure 4: Diagnoses made using BMA at Coast and Nyeri hospitals

4.2 FNA RESULTS

Table 2 shows the number of FNAs done per year in each hospital during the study period.

Table 2 : Number of FNAs reported at Coast and Nyeri level 5 hospitals

Hospital	2013	2014	2015	2016
Coast level 5	74	70	79	149
Nyeri level 5	93	100	119	193

4.2.1 Trends of FNAs done at Coast PGH and Nyeri level 5 hospitals

There was a gradual rise in FNAs reported at Nyeri PGH between 2013 and 2016 with a rise of up to 38.22% whereas Coast PGH showed a decline in FNAs reported from 19.9% to 18.8% in 2014 with a rise to up to 40.0% in 2016 in the same duration as shown in **Figure 5**.

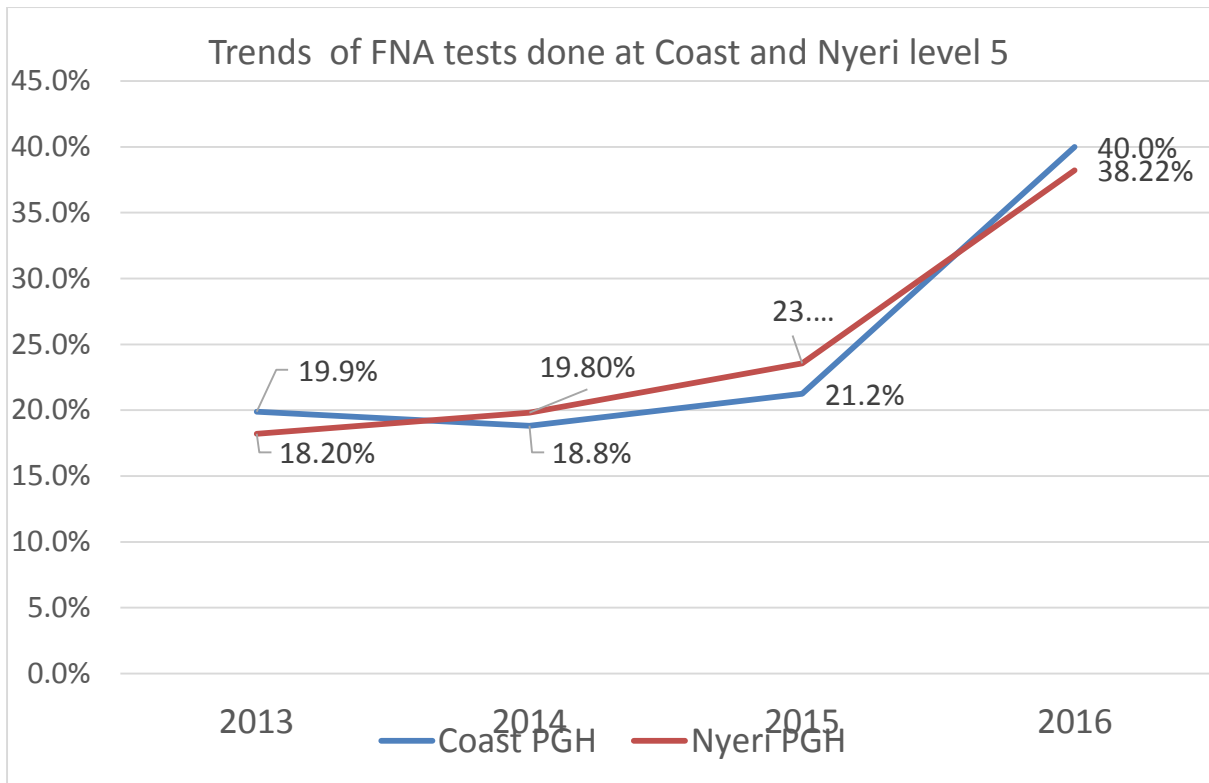


Figure 5: Trends of FNAs done at Nyeri and Coast level 5 hospitals

4.2.2 Distribution of gender for FNA in Nyeri and Coast level 5 hospitals

A total of 877 FNAs were reported in both Coast and Nyeri level 5 hospitals. The gender distribution was similar in both hospitals with a male to female ratio of 1:2.5.

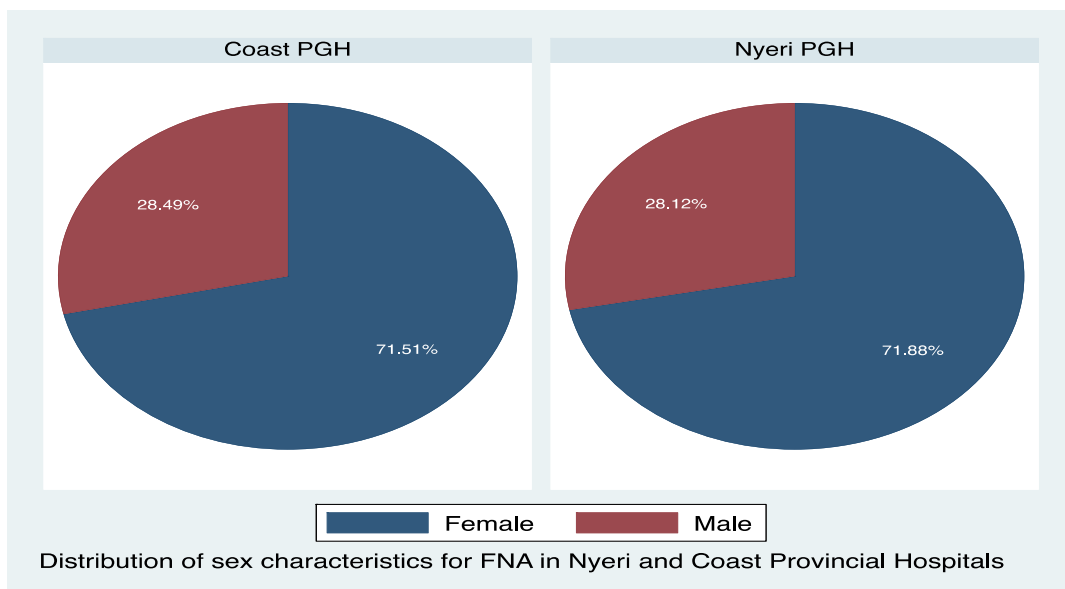


Figure 6: Distribution of gender for FNA in Nyeri and Coast level 5 hospitals

4.2.3 Association of gender with diagnosis using FNA

Out of the 877 FNAs, 629 (71.7%) were from females while 248 (28.3%) were from males. This distribution between the two gender shows that there was a statistically significant difference in sex and diagnoses with a P value of <0.0001, a higher proportion of females had benign and malignant diagnoses than males. This is demonstrated in **table 2**.

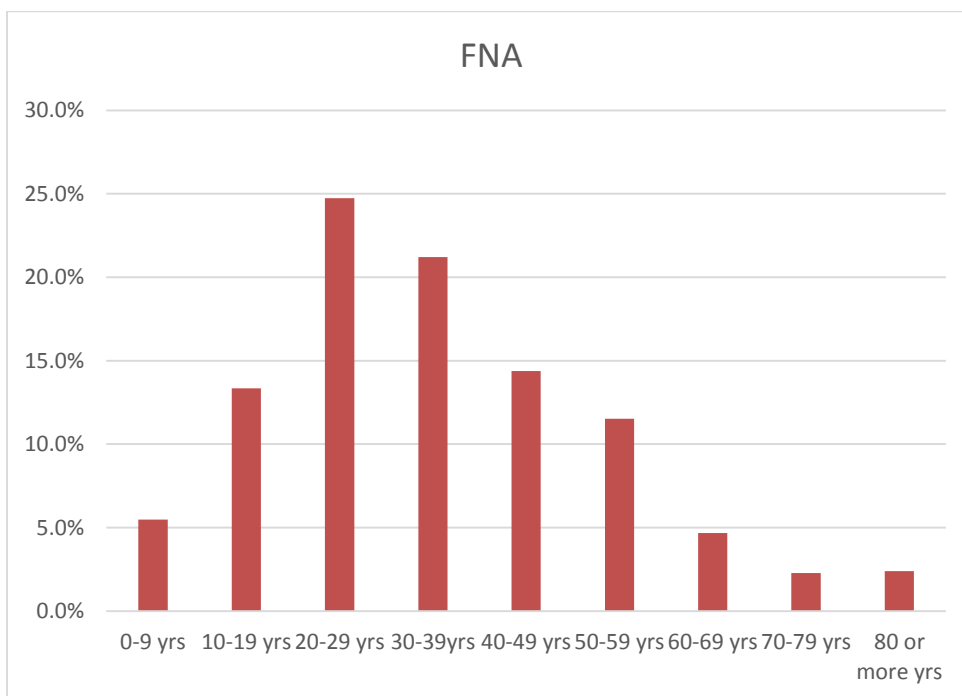
Table 3: Number of FNAs reported at Coast and Nyeri level 5 hospitals

Gender	Normal	Benign	Infective	Malignant	Non-Diagnostic	Total
Female	6	453	95	70	5	629
Male	1	147	75	22	3	248
Total	7	600	170	92	8	877

4.2.4: Age distribution of FNA in Coast and Nyeri level 5 hospitals

FNAs were performed in patients ranging from 3 months to 78 years. The highest number of FNA patients were in the 20-29-year age bracket accounting for 24.7% (n=877) of the total FNAs while the lowest number was in the 70-79-year age bracket accounting for 2.3% of the FNAs.

Figure 7: Age distribution of FNA in Coast and Nyeri level 5 hospitals



4.2.5 Disease classification using FNA at Coast and Nyeri level 5 hospitals

A total of 877 FNAs were included in the study, these were then classified into 47 disease categories. The diseases were further sub-divided into 6 categories according to site of aspiration. Out of the 505 FNAs done at Nyeri level 5 hospital, benign conditions accounted for majority of the diagnoses 344 (68.1%) followed by infective conditions which were 94 (18.6%) whereas at Coast level 5 benign and infective conditions accounted for 256 (68.8%) and 76 (20.4%) out of the 372 FNAs done there respectively.

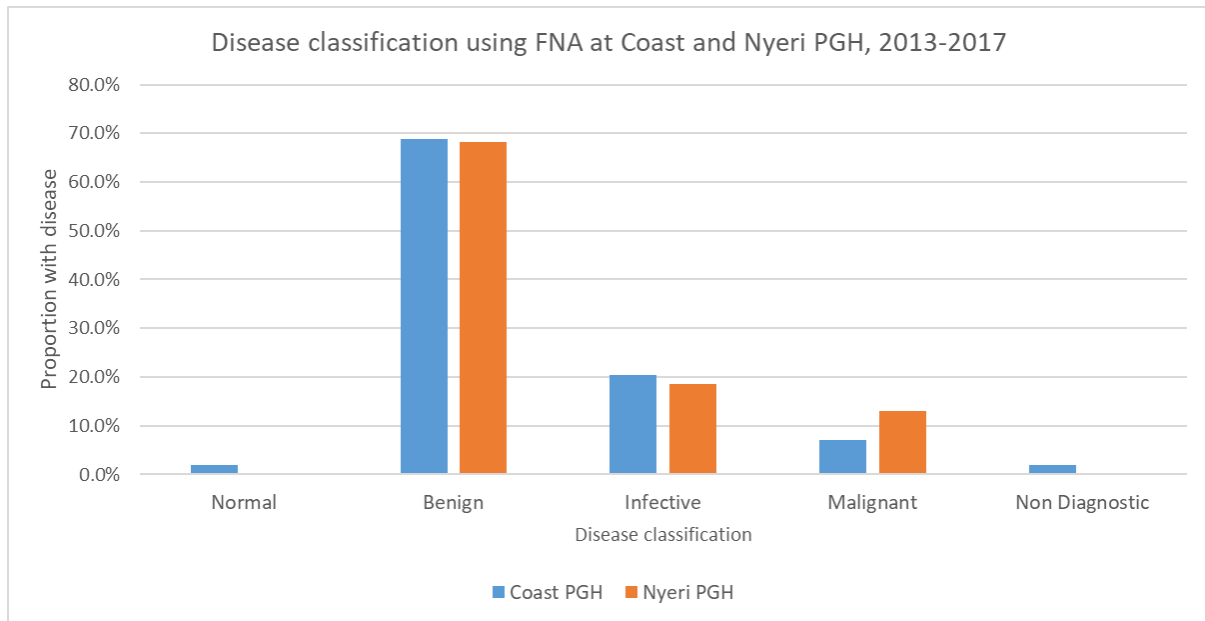


Figure 8: Disease classification using FNAs at Coast and Nyeri level 5 hospitals

4.2.6 Classification of diseases according to site of FNA

Breast lesions were the commonest diagnosis made at Nyeri level 5 hospital contributing to 18.9% (n=166) followed by soft tissue lesions which accounted for 16.8% (n= 148) out of the 505 FNAs reported in the hospital. At Coast level 5 hospital soft tissue lesions were the most common followed by breast lesions accounting for 15.5% and 12.2% respectively out of the 372 FNAs reported there.

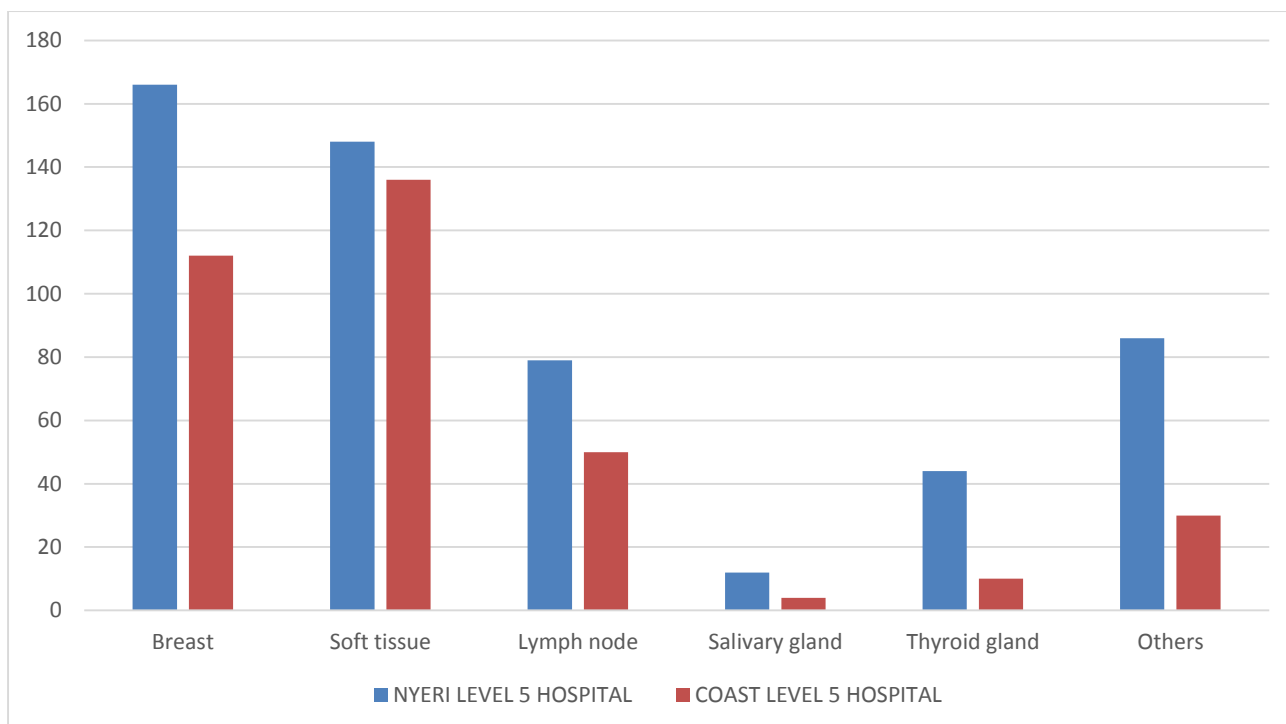


Figure 9: Classification of diseases according to site of FNA

4.2.8 Comparison between the pre- and post- intervention periods

A bivariate analysis was done to compare the frequency of disease diagnosis reported during the pre-intervention period (average of 3 years) and post-intervention period (1 year). The Fisher's exact test was applied to test this and we found a P value of 0.055. This was not statistically significant. This could be explained by the fact that as much as there was an increase in the numbers of FNAs done during the post-intervention period, the disease patterns remained the same.

*A P value of <0.5 was considered statistically significant.

All BMEs were reported post-intervention so no assessment was done to compare pre and post- intervention periods.

Table 4: Bivariate analysis of disease diagnosis using FNA during pre-& post-intervention period

Type of intervention	Normal	Benign	Infective	Malignant	Non-diagnostic	Total
Pre-	0	124	30	21	2	178
Post-	6	227	80	28	1	342
Total	6	351	110	49	3	520

4.2.8 Frequency of malignant diseases between the two hospitals

Table 5 shows that there was a higher frequency in the malignant diseases reported in Nyeri level 5 (13%) hospital compared to Coast level 5 (7%) with a $P=0.004$ which was statistically significant.

There was no statistical difference in malignant disease diagnosis using BME between the two facilities.

Table 5: Bivariate analysis of malignant diagnosis using FNA between the two hospitals

Diagnosis	STUDY SITE		Total
	Coast level 5 hospital	Nyeri level 5 hospital	
Malignant	26 (28.3%)	66(71.7%)	92
Non-Malignant	346 (44%)	439 (66%)	785
Total	372	505	877

5.0 DISCUSSION

Bone marrow evaluation and fine needle aspiration are diagnostic techniques which can be used for screening for infectious, benign and malignant diseases. However, they are under-utilized in government health facilities. This study set out to document the disease spectra in two level 5 hospitals using the two techniques. Comparisons were made between the pre- and post-intervention periods.

A total of 952 cases met the study inclusion criteria; 877 FNAs and 75 BMEs (all BMEs were reported in the post-intervention period). A total of 535 FNAs (mean of 35.4 per year) were reported during the pre-intervention period while 342 FNAs (34.5 per year) and 75 BMEs were done in the post-intervention period. The number of BMEs and FNAs requested in both facilities was low considering the fact that both level 5 hospitals serve a minimum of 700 patients at the OPD daily. These low numbers could be attributed to the fact that there are few on-site pathologists who do both clinical and anatomic pathology work. However, there was an increase in FNAs and BMEs during the post-intervention period. The increase in BMEs and FNAs after the skills transfer training for non-pathologists which was one of the initiatives of the pathology track after the situation analysis presented at the 2014 Cancer Stakeholders' Workshop in Naivasha. One of the objectives of the bigger project was to train non-pathologists in performing diagnostic procedures such as FNA, BMA and trephine biopsy due to the shortage of pathologists in the country.

A total 877 FNAs were included in the study. Out of these, 535 were reported in the pre-intervention while 372 were reported in the post-intervention period. During the post-intervention period 40% and 38.2% of the FNAs were reported at Coast and Nyeri level 5 hospitals respectively. There was an increase in FNAs reported during the post-intervention period considering it was a one-year period compared to the three-year pre-intervention period. The increase in FNAs done in both facilities could be attributed to more non-pathologists performing the procedures. The clinicians also became more aware of FNA as a screening and diagnostic tool and started requesting for it.

Seventy-five (75) BMAs were reported in the post-intervention period. This reflects 100% rise from the 3-year pre-intervention period. This increase can largely be attributed to the skills transfer to non-pathologists and laboratory technologists. Provision of BMA needles, reagents

and consumables which were previously unavailable in both hospitals also contributed to the increase.

Haematological malignancies had a male preponderance across all age groups for myeloid and lymphoid subtypes. Our study findings correlate with those of Smith et.al who also found the male incidence of haematological tumours double that of females (49). Non- haematological conditions due to nutrient deficiency and HIV changes were slightly more in females in a study done by Okinda and Riyat at AKUH, these findings are similar to ours since most females had marrows with reactive changes due to haematinic deficiency (12). In contrast, a study done in North Sudan showed an almost equal male: female ratio of haematological conditions (13).

There was a female predominance in FNAs reported in both level 5 hospitals with male: female ratio of 1:2.5 (71% female and 29% males). Breast, soft tissue and thyroid lesions are easily accessible, palpable and more predominant in the female population; this could have contributed to the increased frequency seen in this study. This finding is similar to a retrospective study done by Florentine et.al. in which females comprised 74% of the patients (46). Majority of the female patients had infective, benign and malignant conditions compared to the males in both hospitals during both the pre and post- intervention periods.

In Coast and Nyeri level 5 hospitals there was a varied age distribution for both FNAs and BMEs. The ages of the patients ranged from 1 month to more than 80 years for both procedures. Most of BMEs reported were in the 30-39-year age group. This could be attributed to marrows with haematinic deficiency. Okinda and Riyat also found nutrient deficient marrows to be more common in the 15-40 year age group (12). Haematinic deficiencies are not uncommon in this age group especially in females due to high physiological demands during growth and reproduction. The highest number of FNAs were reported in the 20-39 age. FNA is a screening tool for breast lesions such as fibroadenomas which are more prevalent in women between 20-30 years.

The spectrum of diagnoses made using BMEs in both hospitals was wide. The 75 BMAs were initially classified into 5 disease groups. Non-malignant haematological conditions were 52% while the malignant haematological conditions reflected 48%. Marrows with reactive changes were the most predominant non-malignant haematological BMEs reported. They were due to haematinic deficiency and HIV related changes. Megaloblastic anaemia was the main cause of haematinic deficiency in the BMEs. Study findings are similar to those of Gundapar Khan et.al. in Abbottabad Pakistan where the commonest non-haematological conditions in the marrows

were megaloblastic anaemia, iron deficiency anaemia and visceral leishmaniasis (38). These results also correlate with a study at AKUH which showed that reactive marrow changes were the most common conditions. (12). In addition, findings are similar to a study done by Asif et al in a tertiary hospital where anaemia was the commonest non-malignant disorder and leukemia was the commonest haematological malignancy (15). Although iron deficiency is the commonest nutritional deficiency according to the National Iron and Folic acid Supplementation Communication Strategy 2013-2017 (39), it is not an indication for bone marrow evaluation. This explains the increased number haematinic deficient marrow due to megaloblastic anaemia.

Acute leukemia peaks in the first decade of life; with a slight male preponderance. In Kenya, cases of acute leukemia are sporadic and true incidence remains unknown. It was the second most frequent diagnosis reported in Coast and Nyeri level 5 hospitals. Furthermore, it was also the commonest haematological malignancy reported. Findings are comparable to Adewoiyin AS et.al. in Nigeria who reported acute leukemia in 18% of the BMAs (40). Results from a study done at Moi Teaching and Referral Hospital differ since Non-Hodgkin's lymphoma was the most frequent diagnosis in the paediatric age group followed by ALL (41). BMA and trephine biopsy were both complementary in the diagnosis of 5 out of 12 acute leukemias reported in our study. The other 7 were reported on BMA. Gupta et al also reported acute leukemia as the commonest haematological malignancy. In their case, however, all 20 acute leukemia diagnoses were reported on both BMA and trephine (42).

Chronic leukemias can occur at any age though they are more prevalent in the 5th and 6th decades. In our study, chronic leukemias accounted for the second commonest haematological malignancy reported with a higher frequency in Coast level 5. They were reported mostly in males in the 40-59 age group. These findings are similar to a study done in Western Kenya that determined burden and pattern of cancers in which lymphomas, acute leukemia and chronic leukemia were the commonest haematological malignancies in descending order (43). These findings are comparable to ours except for the reversal in order of frequency. This could be due to the fact that lymphomas are not usually diagnosed on BME.

Lymphoproliferative disorders and plasma cell dyscrasias were the 4th most diagnosed haematological disorders in this study. However, the Lymphoproliferative disorders were all reported in Nyeri level 5 while plasma cell dyscrasia had an almost equal proportion in the two hospitals. Plasma cell dyscrasias mostly multiple myeloma are common in males in the 50-70

year age bracket though some cases have been reported in younger patients. In this study all the cases were reported in males in that age group. Our study findings on plasma cell dyscrasia differed from a North Sudanese study which reported that 2 (1.8%) out of 112 BMEs had multiple myeloma (13). In our study plasma cell dyscrasia accounted for 7 (9.3%) of the reported marrows. Lymphoproliferative disorders represent a heterogeneous group of clonal expansion in the lymphoid cells and occur across all age groups though mostly in children. We were however, unable to classify and characterize them further due to lack of ancillary tests. Our findings differ from those of a similar Ghanaian study in which Lymphoproliferative disorders were the most common cause of bone marrow infiltration for 47 (58%) of the 80 patients included in that study (44).

FNAs are used for diagnosis of variable palpable masses hence the wide spectrum of diagnoses made in both level 5 hospitals. A total of 877 FNAs were included in the study. Benign diseases were the most predominant in the two hospitals. Soft tissue, breast and thyroid were the most aspirated sites since they are easily accessible and can be sampled on palpation. Soft tissue lesions accounted for the most frequent diagnosis reported. Lipomas are the commonest benign soft tissue tumours in adults and have no gender predilection. They usually occur in accessible sites such as the trunk, back and extremities hence their high frequency in the cases reported in both hospitals. These findings resemble a study done in Los Angeles on benign soft tissue tumours which concluded that lipoma and lipoma variants were the commonest soft tissue tumours (45).

FNAc is an important diagnostic tool for screening breast lesions. In our study breast was the 2nd commonest aspirated site, with Nyeri level 5 accounting for majority of the cases. Most of the cases reported were benign breast lesions like fibroadenoma, unspecified benign breast diseases, duct ectasia, fibrocystic changes and gynaecomastia. Fibroadenomas are the commonest breast lesions in females in the 20-35 age group this explains their predominance in females in the study. These findings are similar to a study done by Nkonge et.al at KNH which revealed that fibroadenomas were the commonest diagnoses from cytomorphological evaluation of breast diseases (27). In contrast, Florentine et.al reported fibrocystic changes of the breast in females of the same group as the most frequent diagnosis which is contrary to our study results (46). Breast cancer and breast lesions suspicious for malignancy were reported in females mostly in the 40-49 age group. This finding is in keeping with a study done in Sub-Saharan Africa which showed that breast cancers occur a decade earlier in females in developing countries with most presenting late with advanced disease (50).

Head and neck FNAs are usually done to investigate clinically suspicious lymphadenopathies, lymph node was the third most sampled site for FNAs in our study. Acute and chronic reactive lymphadenitis were the commonest diagnosis, followed by granulomatous lymphadenitis and lymphoproliferative disorders. These findings are comparable to those of Mohanty and Wilkinson. Histopathological correlation was in keeping with cytology findings (47). Study findings from Iraq differ from ours, in theirs 63.1% of the lymph nodes were malignant mostly metastatic (48).

Malignancies comprised 0.6% of the FNAs reported. They were predominant in Nyeri level 5 hospital compared to Coast level 5 hospital. FNA as a diagnostic modality is limited in defining the invasion and type of malignancy (32). In our study we experienced this limitation regarding the broad spectrum of malignant diseases reported. Some tumours were reported as papillary neoplasm of the breast, phyllodes tumours and follicular neoplasm. They were difficult to determine whether benign or malignant unless supported by histological findings.

Study limitations

Some of the FNA and BME reports had missing biodata – age and sex. Eight (8) of the FNAs from Nyeri level 5 and two (2) BMAs from Coast level 5 were missing biodata. This made it difficult to correlate the cytomorphological findings given that certain conditions affect different gender and age groups. These FNAs and BMEs were excluded from the study. Special techniques such as immunocytochemistry, flow cytometry and immunophenotyping would have been useful in characterizing some of the malignant conditions reported during the study period. This would have been useful especially for the acute and chronic leukemias as well as lymphoproliferative disorders which were not classified due to unavailability of these techniques. Some of the BMA and FNA requisition forms and reports lacked clear indications from the requesting clinicians. This affected our findings especially in the BMEs where it was difficult to determine whether a BME was requested for staging, follow-up or diagnosis of a malignant condition. In spite of the limitations our findings are still valid.

Conclusion

The spectrum of diagnoses was similar in the two regions and reflect the morbidity patterns described for these population by the Kenya Demographic Health Surveys (KDHS). Marrows showing reactive changes were the commonest non-malignant findings while acute leukemias were the commonest haematological malignancies in the two hospitals. Lipomas and fibroadenomas were more frequently reported in Nyeri level 5 hospital than Coast level 5 hospital. There were more malignant conditions picked in Nyeri level 5 hospital compared to Coast level 5 hospital using the two diagnostic techniques.

Recommendations

Similar initiatives can be applied to tertiary hospitals and other levels of health care to determine disease trends and increase cancer diagnosis.

Increase in availability of these two techniques across similar facilities in the country will contribute to defining disease patterns.

Consecutive reviews to determine the long-term impact of the intervention on cancer diagnosis using BMEs and FNAs in the two hospitals should be done.

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APPENDICES

APPENDIX I: Approval letter from KNH/UON ERC



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Dr. Leah Obosy Okuoro
Reg. No. H58/75560/2014
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Dear Dr. Okuoro,

REVISED RESEARCH PROPOSAL –CYTOMORPHOLOGICAL SPECTRUM OF DIAGNOSES FROM BONE MARROW PREPARATIONS AND FINE NEEDLE ASPIRATIONS IN COAST AND NYERI LEVEL 5 HOSPITALS, KENYA (P310/06/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 3rd November 2017 –2nd November 2018.

This approval is subject to compliance with the following requirements:

- Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
- Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
- Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
- Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
- Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

APPENDIX II: Haematoxylin and Eosin Staining Preparation

Reagents

1. Eosin 1% aqueous solution

Eosin 10g distilled water- 1litres

2. Harris-haematoxylin solution

Haematoxylin-5g

Ethyl alcohol-50ml

Ammonium aluminium -100g

Distilled water-1 litre

Mercuric oxide red 2.5g

3. Scotts tap water

Na hydrogen carbonate-3.5g

MgSo₄ -20g

Distilled water-1 litre

Acid alcohol

-0.5%hcl in 70% alcohol

Procedure for staining

1. Dissolve the ammonium aluminium in distilled water heat, stirring frequently.
2. Dissolve the haematoxylin in the alcohol and add to aluminium solution.
3. Bring to the boil while stirring.
4. Mix and allow cooling.

5. Filter into a glass stain bottle and the solution is ready for use.
6. De-wax sections with two changes of xylene.
7. Re-hydrate sections with two changes of absolute alcohol and wash in running tap water.
8. Stain with haematoxylin sol for up to 5 minutes.
9. Wash in running tap water.
10. Differentiate in acid alcohol for approximately 5 minutes.
11. Wash in running tap water.
12. Blue in Scotts tap water for few seconds.
13. Wash in running tap water.
14. Stain with eosin for approximately for 5 minutes.
15. Wash in running tap water.
16. Dehydrate, clear and mount section.

APPENDIX III: May Grunwald Giemsa

Reagents

1. May Grunwald stain
2. Giemsa stain
3. Phosphate buffer ph. 6.8
4. Tap water

Procedure for preparing stain

Prepare working stain solutions by: Mixing equal parts of the 0.3% MGG by dissolving 0.3g of MGG in 100mls of absolute ethanol.

Warm in water bath at 50 degrees Celsius for 30 minutes then 20 degrees Celsius for 30 minutes with equal parts of PH of 6.8

1. Mix one part commercially available Giemsa with nine parts of P.H 6.8 buffered water in the second staining jar.
2. Label the slide.
3. Fix with Methanol for 10 minutes.
4. Transfer it into a jar of MGG working solution for 15 minutes.
5. Transfer into a jar of Giemsa stain working solution for 15 minutes.
6. Wash in three successive buffered water changes for 5 dips.
7. Allow to stand for three minutes.
8. Dry slide and examine under the microscope.

Quality control

- Changing of the working staining solution and the buffer washes every start of a shift.
- Ensure that water isn't mixed with ethanol.

APPENDIX IV: Ziehl Neelsens Stain Preparation

Reagents

Carbolfuschin 1L

Sulphuric acid 1L

Malachite green 1L

Procedure for preparing stain

1. Bring section to water.
2. Cover the section with a piece of filter paper.
3. Flood the section with filtered carbolfuschin for 15minutes.
4. Remove the filter paper and wash well in tap water.
5. Decolorize with 20% sulphuric acid for 3 minutes and rinse in water.
6. See if the sections are pale pink.
7. Repeat step 5 and 6 until the desired picture is obtained.
8. Counter stain with methylene blue or malachite green for 2-3minutes
9. Wash in water.
10. Dehydrate in 3 changes of Ethanol.
11. Clear in 3 changes of xylene.
12. Mount in DPX.

Results:

Nuclei-blue

AAFB- bright red

RBC-pale pink

APPENDIX V: Periodic Acid Schiff's Stain Preparation

Reagents

Periodic acid 1L

Schiff's 1L

Procedure for preparing stain

1. Bring section to water.
2. Oxidize for 10 minutes in 1% periodic acid.
3. Wash in running tap water for 5 minutes.
4. Rinse in distilled water.
5. Place in Schiff's reagent for 15 minutes or until section turns magenta colour.
6. Rinse in three changes of freshly prepared 0.5% sodium metabisulphite.
7. Wash in running tap water for 10 minutes.
8. Stain in alum- haematoxylin for 6-7 minutes.
9. Differentiate in 1% acid alcohol-3 dips.
10. Blue in running tap water for 10 minutes or Scott's tap water for 1 minute.
11. Counter stain in 0.3% tartrazin in cellosolve for 3 minutes.
12. Dehydrate in absolute ethanol, clear in xylene and mount in DPX.

Results:

Nuclei-blue

Cytoplasm-yellow

Positive controls –red or magenta

APPENDIX VI: Iron Stain Preparation

Reagents

Xylene

Absolute alcohol

Neutral red

4% potassium Ferrocyanide

4% HCL

Procedure for preparing stain

1. Bring section to water
2. Flood section with a mixture of equal parts of 4% potassium Ferrocyanide and 4% HCL for 15 minutes
3. Rinse in distilled water
4. Counter stain with 0.5% neutral red for 30seconds
5. Rinse in distilled water
6. Dehydrate in 3 changes of absolute alcohol
7. Clear in 3 changes of xylene and mount in DPX

Results

Nuclei – red

Ferric iron- blue

APPENDIX VII: BMA/ Trephine Biopsy Proforma

Lab. Specimen number:

Age in years:

Sex: Female

Male.....

Hospital:.....

OP/IP no:.....

Ward:.....

Date of procedure:.....

Date

received:.....

Time of collection: am/pm

Clinical diagnosis:.....

Microscopic examination (will be done after evaluation and interpretation of the CBC and PBF)

(1)BMA

- Particulate

- Lymphocytes

- Foreign cells

- Parasites

- Megakaryopoiesis

- Lymphocytes

- Foreign cells

- Parasites

- Iron stain:.....

(2) Trephine biopsy

- Length of core

- Bone architecture

- Cellularity

- Erythropoiesis

cells

- Granulopoiesis

- Megakaryopoiesis

- Lymphocytes

- plasma cells

- marrow macrophages

- Foreign cells/ non- haemopoietic

3) Special stains

-H &E

- Reticulin

-PAS

- Iron stain

-MT

APPENDIX VIII: FNA Proforma

Lab. Specimen number:

Age in years:

Sex: Female

Male.....

Hospital:.....

OP/IP no:.....

Ward:.....

Date of procedure:.....

Date

received:.....

Time of collection: am/pm

Clinical history and examination :-.....

- Site of lesion
- Duration
- Tenderness Tender Non-tender.....
- Consistency

Clinical diagnosis

Specimen reporting

Specimen adequacy - satisfactory.....

Unsatisfactory.....

Negative for malignancy

Suspicious for malignancy

Positive for malignancy

Infections

Cytopathological diagnosis.....

APPENDIX IX: Plagiarism report

Turnitin

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