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13th Biennial Kenya Association of Clinical Pathologists conference

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The 13th biennial Kenya Association of Clinical Pathologists conference was held on 16th – 18th October 2019 at the Southern Palms Beach Resort, Diani, Kwale County, Kenya. The theme for the conference was **"BRIDGING THE GAP: PATHOLOGY IN UNIVERSAL HEALTH CARE"** which brought to the fore the important role of pathology and laboratory medicine in making Universal Health Coverage (UHC) a reality in Kenya.

The well attended conference brought together pathologists, scientists and technologists from various hospitals and institutions in East Africa and beyond. Presentations and deliberations covered laboratory management, ethics, haematology, microbiology, histopathology, molecular pathology, immunology, forensic pathology and chemistry. Indeed, the growth of pathology as a profession in Kenya was clear for all to see with several pathologists sharing their unique experiences. Dr Grace Kiraka shared her experience with therapeutic apheresis which she has been carrying out successfully at MP Shah Hospital. Most hospitals with apheretic machines in Kenya only use it for blood component donation. It was therefore quite refreshing to know that we have local expertise when it comes to therapeutic applications. Dr Alice Kanyua from Nairobi Hospital shared her experience on optimizing detection and antibiotic susceptibility testing for *Candida auris* which has recently emerged as a major hospital acquired infection globally as well as in Kenyan hospitals[1]. She highlighted the specific challenges in performing antibiotic susceptibility for this yeast and how she has been able to overcome them in order to guide clinicians on appropriate treatment. These examples highlight the important role that pathologists play both in diagnosis and patient management which is essential in making UHC a reality in Kenya.

The practice of pathology and laboratory medicine is essential for disease detection, surveillance, control, and management. In developed countries, it is estimated that almost 70% of diagnosis made is dependent on a laboratory test result[2]. In contrast, access to quality-assured laboratory diagnosis has been a challenge in Low and Middle Income countries (LMICs) resulting in delayed or inaccurate diagnosis and ineffective treatment[3]. UHC cannot be a reality in LMICs if clinicians do not have access to good quality laboratory services as correct treatment starts with a correct diagnosis. It is therefore important for stakeholders in laboratory medicine to come together and lobby for increased investment in laboratory diagnostic services.

The Kenya Association of Clinical Pathologists (KACP) shall continue to play its role in providing a forum where stakeholders in laboratory medicine can share knowledge and experiences that enrich the practice. In this regard, we shall be hosting the Association of Pathologists in East Central and Southern Africa (APECSA) biennial conference this year and we look forward to a most informative conference. See you at APECSA 2020 in October!

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Histopathology reporting and biomarker testing of invasive breast cancer at Kenyatta National Hospital: An audit of the synoptic reports

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ABSTRACT

Background: Breast cancer is a leading cause of cancer morbidity and mortality in Kenya. Histopathology reports form the basis of management of patients. To improve reporting standards Kenyatta National Hospital introduced synoptic reporting to replace standard text reports.

Objective: To audit the synoptic reporting of invasive breast cancer histopathology at Kenyatta National Hospital.

Methodology: This was a laboratory based retrospective study. Invasive breast cancer synoptic reports from January 2016 to December 2018 were reviewed.

Results: A total of 123 reports were evaluated. Surgical procedure done was documented in 96.7% of the reports, laterality in 96%, specimen integrity in 89.4%, tumour size in 100%, specimen gross description in 76.4%, lymph node sampling in 100%, tumour site in 89.4% and tumour focality in 91.9%. For microscopic details histologic type and grade was indicated in 100%, presence or absence of ductal carcinoma *in-situ* in 97.6%, extensive intraductal component in 45.8%, architecture in 69.4%, nuclear grade in 55.6% and necrosis 47.2%. Presence or absence of lobular carcinoma *in-situ* was indicated in 47.2%. Margin closest to tumour and distance was indicated in 69.4% of the reports and where the margin was involved the margin was indicated in 100% of the reports. Presence or absence of Paget's disease was reported in 91.9%, lymphovascular invasion in 96.7%, skin involvement in 81.3%, skeletal muscle involvement in 80.5%, treatment effect in 47.5%, staging in 83.7% and microcalcifications in 88.6%. The number of lymph node involved was indicated in 100% of the reports. Micrometastasis was indicated in 26% while extranodal extension was indicated in 73.1%.

Conclusions: Introduction of synoptic reporting has led to an increased average level completeness of 82%. Reporting of ductal carcinoma *in-situ*, microscopic margins, treatment effect and lobular carcinoma *in-situ* is still sub-optimal.

Recommendations: There is need for interdepartmental consensus on comprehensive laboratory request forms. Sensitization of anatomic pathologists/registrars on clinical utility and need to report Ductal carcinoma *in-situ*, lobular carcinoma *in situ*, microscopic margins and treatment effect. Based on observed incompleteness, periodic audits should be carried out with a target of achieving complete reports.

Key words: Breast cancer, Synoptic, Narrative reporting, Audit, Completeness

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INTRODUCTION

Breast cancer is the leading cause of cancer morbidity and mortality among women. Globally it's the second most common cancer in women and the leading cause of cancer deaths among women[1]. Due to its increasing incidence a lot of effort has been made to improve screening, laboratory diagnosis and treatment.

Histopathology reports form the basis on which management of breast cancer is based. It is therefore paramount to ensure that all the reports contain all the relevant information important to the clinician[2]. Several organizations including the College of American Pathologists and the Royal College of Pathologists have set guidelines for the handling and reporting of breast cancer specimens.

Previously pathology reports were narrated or dictated. This led to incomplete reports that lack important information needed for management of cancer. This has led to the introduction of synoptic reporting which aims to improve pathology reports. Synoptic reports are standardized as they use a pre-defined checklist. This aims to improve accuracy, timeliness, completeness and proper information transfer[3].

Auditing is a useful tool in a laboratory quality management system. It seeks

to improve patient care and outcome. It evaluates laboratory processes against set standards and it serves to ensure compliance to set standards, identify problem areas and offer corrective measures[4]. Auditing in the histopathology laboratory is part of the internal quality assessment system. It serves to assess, monitor and evaluate histopathology services. Retrospective analysis of performance of new methods is often employed[5]. Assessing adequacy of pathology reports involves examining both gross and microscopic descriptions. This helps to determine the completeness of reports.

Objective: To audit the synoptic reporting of invasive breast cancer histopathology at Kenyatta National Hospital.

MATERIALS AND METHODS

This was a laboratory based retrospective study. Invasive breast cancer synoptic reports from January 2016 to December 2018 were retrieved from the University of Nairobi and Kenyatta National Hospital records department. A total of 123 reports were identified. A data collection tool was developed based on the College of American Pathologists guidelines. The parameters of interest in evaluation of completeness were; procedure done, specimen laterality, integrity, size and lymph node sampling, tumour site and focality, margins involved and uninvolved by invasive carcinoma histologic type and grade, ductal carcinoma *in-situ* and architectural pattern, lobular carcinoma *in-situ*, Paget's disease, lymph-vascular invasion, lymph node, skeletal muscle and skin involvement, micro-calcification and stage. The presence or absence of each parameter in the histology report was assessed individually against the data collection tool and the results were recorded.

RESULTS

Surgical procedures: The type of surgical procedure conducted was documented in 119 (96.7%) and omitted in 4 (3.3%) reports. The type of procedure indicated was Modified Radical Mastectomy (MRM) in all the reports.

Level of completion of macroscopic details: Eight parameters were considered for assessment of completion i.e. specimen laterality, specimen integrity, tumour size, specimen gross description, gross margins, lymph node sampling, tumour site, and tumour focality. Of the 123 reports examined, 94 (76.4%) had 100% completion of the eight parameters. Gross margins and lymph node sampling were documented in all the reports. Specimen laterality was documented in 118 (96%) reports, tumour size in 121 (98.4%) reports, specimen integrity in 110 (89.4%), specimen gross description in 94 (76.4%) reports, tumour focality in 113 (91.9%) and tumour site in 110 (89.4%) of reports as shown in Table 1.

Table 1: Level of completion of macroscopic details (n=123)

	Frequency	
	No.	(%)
Specimen laterality		
Right	60	48.8
Left	58	47.2
Not specified	5	4
Specimen integrity specified	110	89.4
Tumour size specified	121	98.4
Specimen gross description	94	76.4
Gross margins	123	100
Lymph node sampling done	123	100
Tumour site reported	110	89.4
Tumour focality indicated	113	91.9

Level of completion of microscopic parameters: Histologic type, nuclear pleomorphism, mitotic activity, tubular formation and overall histologic grade was indicated in all the reports as shown in Table 2.

Table 2: Reporting of the histologic grade and type (n=123)

Microscopic findings	Whether microscopic findings are reported or not	Frequency	
		No.	(%)
Histological type reported	Yes	123	100
	No	0	0
Nuclear pleomorphism indicated	Yes	123	100
	No	0	0
Mitotic activity indicated	Yes	123	100
	No	0	0
Degree of tubular formation indicated	Yes	123	100
	No	0	0
Grade reported	Yes	123	100
	No	0	0

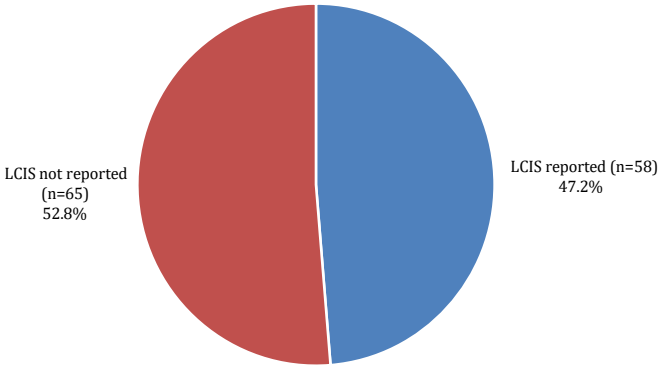
Ductal carcinoma in-situ: The presence or absence of DCIS was documented in 120 (97.6%) reports and omitted in 3 reports. Of these 72 (60%) reports had DCIS. Extensive intraductal component was reported in 45.8%, architectural pattern in 69.4%, nuclear grade in 55.6% and necrosis in 47.2% of the reports as shown in Table 3.

Table 3: Reporting of DCIS (n=120)

Reporting of DCIS	Whether DCIS features are reported or not	Frequency No.	(%)
Presence or absence of DCIS reported(n=123)	Yes	120	97.6
	No	3	2.4
DCIS present (n=120)	Yes	72	60
	No	48	40
Extensive intraductal component reported (n=72)	Yes	33	45.8
	No	39	54.2
Architectural pattern reported(n=72)	Yes	50	69.4
	No	22	30.6
Nuclear grade reported (n=72)	Yes	40	55.6
	No	32	44.4
Necrosis reported (n=72)	Yes	34	47.2
	No	38	52.8

Lobular carcinoma in-situ: The presence or absence of LCIS was documented in 58 (47.2%) reports as illustrated in Figure 1.

Figure 1: Reporting of presence or absence of lobular carcinoma *in-situ* (n=123)



Microscopic margin status: Margin status was reported in all the 123 reports. Margins were indicated as not involved in 98 (79.7%) reports and involved in 25 (20.3%) reports. Where margins were involved by tumour all reports had the specific margin indicated. In uninvolved margins only 68(69.4%) reports had the margin and the distance closest to margin indicated as shown in Table 4.

Table 4: Reporting of microscopic margins

Reporting of margin involvement	Whether features of marginal involvement are reported on or not	Frequency (n)	(%)
Are margins involved (n=123)	Yes	25	20.3
	No	98	79.7
Is margin indicated if there is no marginal involvement (n=98)	Yes	68	69.4
	No	30	30.6
Distance from closest margin indicated (n=98)	Yes	68	69.4
	No	30	30.6
Margin involved by invasive OR DCIS indicated (n=25)	Yes	25	100
	No	0	0

Lymph node status: The number of lymph nodes involved out of all the lymph nodes sampled was indicated in all the reports. The presence or absence micrometastasis was only documented in 32 (26%) of the reports. The presence or absence of extranodal involvement was indicated in 90 (73.1%). This is illustrated in Table 5.

Table 5: Reporting of lymph node status

Reporting of lymph node involvement	Presence or absence reported	Frequency (n)/123	Frequency (%)
Lymph node number involved stated	Yes	123	100
	No	0	0
Micrometastasis	Yes	32	26
	No	91	74
Extranodal extension	Yes	90	73.1
	No	33	26.9

Other parameters examined: The presence or absence of Paget's disease was reported in 113 (91.9%) reports, lymphovascular invasion in 96.7%, skin involvement in 81.3%, micro-calcification in 88.6%, skeletal muscle involvement in 80.5%, treatment effect in 47.5% and staging 84.6% as shown in Table 6.

Table 6: Reporting of Paget's disease, lymphovascular invasion, skin and skeletal muscle involvement, microcalcifications, treatment effects and staging

Type of involvement	Whether features are reported or not	Frequency (n)/123	Frequency (%)
Presence of Paget's disease indicated	Yes	113	91.9
	No	10	8.1
Presence of Lympho-vascular invasion reported	Yes	119	96.7
	No	4	3.3
Skin involvement reported	Yes	100	81.3
	No	23	18.7
Presence or absence of micro-calcification reported	Yes	109	88.6
	No	14	11.4
Presence or absence of skeletal muscle involvement reported	Yes	99	80.5
	No	24	19.5
Treatment effect indicated	Yes	58	47.5
	No	65	52.5
Stage indicated	Yes	104	84.6
	No	19	15.6

Overall completeness: The overall completeness of the synoptic reports was based on documentation of 25 items in the reports. Documentation ranged from 16 to 25 items per report. This corresponds to a completion rate of 64% to 100%. The mean completion for the synoptic report was 82%. Only 6 reports had completion of the 25 items. Majority of the reports had 23 items reported. This is illustrated in Table 7.

Table 7: Parameters completed per report

Items reported/25	n/123	(%)	% level of completion
25	6	4.9	100
24	10	8.1	96
23	30	24.4	92
22	27	22	88
21	23	18.7	84
20	13	10.6	80
19	9	7.3	76
18	2	1.6	72
17	2	1.6	68
16	1	0.8	64

In summary the reporting of tumour size, gross margins, lymph node sampling, histologic type and grade, number of lymph node involved by tumour and margins involved by tumour was at 100%.

The reporting of extensive intraductal component, DCIS pattern, necrosis and nuclear grade, presence or absence of LCIS, margins uninvolved by tumour, micro metastasis and treatment effect showed low levels of completion.

DISCUSSION

Breast cancer is a growing concern worldwide and in Kenya it's the commonest cancer in women. Due to its significant morbidity and mortality a lot of research has been done with an aim of improving outcomes.

Histological features provide the basis upon which management of breast cancer is determined especially now in the era of targeted therapy. It is therefore critical that pathologists relay relevant histological information to clinicians. This is done by ensuring that pathology reports contain all the details on breast cancer characteristics that influence management. Several organizations e.g. the College of American Pathologists and the Royal College of Pathologists have developed checklists for the management of breast specimens. These checklists provide guidance on the information required on a laboratory request form, handling of breast specimens, trimming, processing, the characteristics to report and assessment of receptors[6,7]. The case for standardized reporting has been made by several studies that have demonstrated inadequacies of narrative reporting. Atanda *et al*[8] in Nigeria showed the reporting of tumour size was at 50%, histologic type at 92%, histologic grading at 40%, lymphovascular invasion at 12% and distance from resection margin at 62% when narrative reporting was used. Yesufe *et al*[9] in Ethiopia compared narrative reports with the breast health global initiative guidelines and demonstrated incompleteness in this reports. At KNH, a review and audit of mastectomy reports was done by Macharia[0] and it showed varying levels of completeness of various breast cancer characteristics highlighting a need for a more standardized approach in reporting of breast cancer.

Surgical procedure done was documented in 96.7% of the reports, laterality in 96% and lymph node sampling in 100%. This information is usually derived from the laboratory request form. When not present in the synoptic report it implies that either the surgeons omitted the information, or it was not transcribed in the report. The reporting of these parameters was very good and was only missing in a few reports. To ensure 100% completeness of this information there is need for a specific laboratory request form for breast cancer that guides the surgeon on the information required by the pathologist[11].

The specimen gross description was only documented in 76.4% of the reports. The presence of skin ulceration, edema (peau d' orange), and skin nodules are important in the staging of breast cancer[12]. If these characteristics are not documented and were present then there is likelihood that there was under staging of the cancer. Presence of lymph node sampling was documented in all the reports and was all axillary. Lymph node sampling enables the assessment of lymph node metastasis which is an important component in tumour staging. Tumour focality was indicated in 91.9% of the reports. Focality has prognostic implications. Multifocal tumours have been found to have a high rate of recurrence and have a poor prognosis[12]. Tumour location documentation was at 89.4%. Location has been shown to impact breast cancer survival where upper outer quadrant tumours have a favorable outcome[13]. Majority of the tumours (38.2%) in this study were located in the upper outer quadrant. Specimen laterality was documented in 96%, specimen integrity in 89.4% gross margins in 100% and tumour size in 100%. Only 94 (76.4%) of the 123 reports had all the macroscopic details documented.

Completeness of microscopic details ranged from 100% for histologic type to 45.8% for the reporting of extensive intraductal component for tumours with DCIS.

Lymph node status is very important in the evaluation of breast cancer and has prognostic implications. The number of lymph nodes positive for tumour determines the stage in the TNM classification. From this study reporting of lymph node status was at 100%.

A study done by Macharia[10] that audited standard text reports showed reporting of histologic type at 100%, axillary node status at 89.3%, tumour margins at 75%, histologic grade at 66.3%, tubular formation at 5.7%, Paget's disease at 36.5%, DCIS 13.8% and vascular invasion at 25%. From this study there was marked improvement in reporting of these parameters with increased level of completeness. Histologic type reporting was maintained at 100%, axillary node status reporting improved from 89.3% to 100%. Histologic type of the tumour was reported in 100% of the reports as compared to 66.3% seen in standard text reports; tubular formation was also reported in 100% of the reports as compared to 5.7%. Presence or absence of DCIS reporting improved to 97.6% as compared to 13.8% in standard text reports. Vascular invasion also improved from 25% to 96.7%. Reporting of Paget's disease also improved from 36.5% to 91.9%. Standard text report showed an average level of completeness of 49.75% compared to 82% in synoptic reports. This study therefore showed a marked improvement in reporting invasive breast cancer histology using synoptic reporting as compared to standard text reports.

A study done by Wilkinson *et al*[2] to examine conformity to the College of American Pathologists reporting guidelines showed reporting of histologic type at 100% and compared to 100% in this study, reporting of tumour grade was at 90% and compared to 100% in this study. Microscopic margin status was reported in 94% compared to 100% in this study. Distance to closest margin reporting was similar in both studies at 69% compared to 69.4% in this study. These findings show that despite standardized

reporting and presence of guidelines, compliance is not achieved fully. This was also seen in a study by Mathers *et al*[14] that assessed the use of a standard proforma compared to narrative reports based on the National Health Service (NHS) guidelines and a study by Idowu *et al*[15] that analyzed lung, breast, colorectal and prostate reports from 86 institutions which demonstrated that although there was marked improvement in reporting 100% completeness had not been achieved. These studies demonstrate that the use of standardized reporting improves reporting standards but does not guarantee the completeness of pathology reports. This emphasizes on the need for regular audits to assess compliance to set guidelines.

To achieve completeness of pathology reports there is need to include input from all the stakeholders of the lab. KNH introduced synoptic reporting based on standards from an external organization but the completeness of each report is based on the discretion of the pathologist. As thorough as the CAP guidelines are, we are in an era of evidence based medicine therefore all the parameters to be included in each report must have evidence of prognostic or predictive implication. As a result, individual pathologists may omit parameters which they deem as not important. The field of breast cancer is growing and as more studies are done more information about the disease is brought to light. It is paramount that all pathologists and trainee pathologists are kept abreast with all the new information. This will make full implementation of reporting guidelines easier.

This study highlights the importance of audits in the histopathology lab. Despite introduction of a standardized reporting format the completeness of reports has not yet reached 100%. Appleton *et al*[16] emphasized the need for continuous audits as guidelines and recommendations tend to be filed away and not implemented especially where there is a high turnover of junior staff. KNH is a teaching hospital and thus experiences a high turnover of junior staff and would therefore benefit from regular audits to ensure that reporting standards are maintained. Because knowledge on breast cancer keeps changing and improved upon there is need for continuous trainings for all pathologists to ensure that reporting standards reflect the current body of knowledge.

CONCLUSION

Introduction of synoptic reporting has led to an increased average level completeness of 82%. Reporting of ductal carcinoma *in-situ*, microscopic margins, treatment effect and lobular carcinoma *in-situ* is still sub-optimal.

RECOMMENDATIONS

There is need for interdepartmental consensus on comprehensive laboratory request forms. Sensitization of anatomic pathologists/registrars on clinical utility and need to report ductal carcinoma *in-situ*, lobular carcinoma *in-situ*, microscopic margins and treatment effect. Based on observed incompleteness, periodic audits should be carried out with a target of achieving complete reports.

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Molecular epidemiology of multi-drug resistant *Klebsiella pneumoniae* in Aga Khan University Hospital Nairobi, Kenya

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ABSTRACT

Background: Multi-Drug Resistant (MDR) *Klebsiella pneumoniae* is rapidly becoming a life threatening nosocomial infection globally. *Klebsiella pneumoniae* causes healthcare associated infections such as Urinary Tract Infection (UTI), pneumonia, blood stream infections, wound or surgical site infections, commonly affecting patients in high risk hospital areas such as Intensive Care Unit (ICU), Neonatal Intensive Care Unit (NICU), and the immune-compromised. *Klebsiella pneumoniae* causes nosocomial outbreaks in the wards which is spread to patients seen at the Outpatinet Department (OPD) by patients who already harbor the bacterial infection. Those patients with MDR infections have difficulties in treatment resulting in high morbidity and mortality.

Objective: The study aimed at determining molecular clonal relatedness of *Klebsiella pneumoniae* isolates from high risk areas of the hospital.

Design: Convenience study design from the Outpatient Department, Intensive Care Unit and Neonatal Intensive Care Unit departments across various age groups and genders.

Methods: A total of 63 MDR *Klebsiella pneumoniae* from clinical isolates were analyzed by Enterobacterial Repetitive Intergenic Consensus (ERIC –PCR). Nineteen percent of the total clinical samples were from NICU, 40% from Intensive Care Unit and 41% from OPD. Clonal relatedness by finger printing between strains from high risk areas of the hospital. The band patterns were determined by dendrogram using computer software.

Results: Neonatal Intensive Care Unit had 2 clusters with an insignificant genetic similarity of <80%. The second cluster had a sub-cluster with a 100% genetic similarity. ICU and OPD unveiled 3 major clusters 3 with a significant genetic similarity of 100%.

Conclusion: This study concludes that there is spread of *Klebsiella pneumoniae* from OPD to NICU and ICU.

Recommendation: This study can be used by the prevention and infection control practitioner in the hospital to control the spread of infection. The study also recommends that a further such study be done targeting health care workers, visitors who come to see their patients and equipment used in the hospital.

Key words: Eric PCR, *Klebsiella pneumoniae*, Multi-drug resistance, Clonality

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INTRODUCTION

Klebsiella pneumoniae, an opportunistic pathogen is responsible for nosocomial infections especially in immune compromised individuals[1]. *Klebsiella pneumoniae* colonizes the mucosal surfaces in humans and causes severe diseases such as septicemia, pneumonia, urinary tract infections, and soft tissue infections. The infections are mainly caused by Extended-Spectrum β-Lactamases (ESBLs) producers that hydrolyzes cephalosporins (cefotaxime, CAZ ceftazidime, ceftriaxone, cefepime). Infections are spread through patients especially those using ventilators (breathing machines), catheters during surgery or those patients taking long courses of antibiotics. *Klebsiella pneumoniae* bacteria cause about 15% of gram –negative infections in the Intensive Care Unit (ICU) wards[2]. The young and the aged are also at risk of the infection. The resistance of carbapenem drugs results in morbidity and mortality of about 30-70% among patients with bacteremia and pneumonia[3]. Critically ill hospitalized patients with MDR *Klebsiella pneumoniae* is increasing due to limited antibiotic options for the treatment resulting in high mortality rates. To prevent spread of *Klebsiella* infections between patients, healthcare personnel must follow specific infection control precautions of adhering to hand hygiene, wearing gowns and gloves when they enter rooms where patients with MDR

Klebsiella related infections are housed. Healthcare facilities must also follow strict cleaning procedures to prevent the spread of *Klebsiella pneumoniae*.

The genus *Klebsiella* are gram negative, non-motile, capsulated rods belonging to the tribe *Klebsiella,* family of *Enterobacteriaceae* and was named after the German bacteriologist Edwin Klebs (1834-1913). *Klebsiella pneumoniae* has a prominent polysaccharide capsule that encases the entire cell surface accounting for the large appearance of the organism on gram strain.

Species of *Klebsiella* that are associated with illness in humans are *Klebsiella pneumoniae, Klebsiella oxytoca* and *Klebsiella granulomatous. Klebsiella rhinoscleromatis* and *Klebsiella ozaene* are rarer causes of disease and are uncommon in developed countries. *Klebsiella rhinoscleromatis* remains endemic in tropic and subtropical areas (North and Central Africa) and is the aetiologic agent of rhinoscleroma, a chronic infection which predominantly affects nasal cavity[4]. *Klebsiella ozaene* causes nasal discharge and also associated with a rare cause of cerebral abscess and meningitis. *Klebsiella pneumoniae is* the most common strain affecting humans. These strains ferment lactose, produces highly mucoid colonies on plates because of the production of a luxuriant polysaccharide capsule[5]. *Klebsiella pneumonia*e have become important pathogens in nosocomial infections[5] which may colonize the skin, pharynx or gastrointestinal tract, sterile wounds and urine.

Klebsiella may be regarded as normal flora in many parts of the colon and intestinal tract. Health-care-Associated Infection (HAI) is a major global concern for both patients and health-care professionals. Infections occur in a patient during the process of care in a hospital or other health-care facility that was not manifested or incubating at the time of admission. These infections are often caused by multi-resistant pathogens resulting in prolonged hospital stay, potential disability, excess costs and sometimes death[6]. The mortality among patients infected with organisms positive for *Klebsiella pneumoniae* carbapenemases is high, perhaps as a result of the limited antibiotic options remaining (often colistin, tigecycline, or aminoglycosides). Triple drug combinations using colistin, tigecycline, and imipenem have recently been associated with improved survival among patients with bacteremia. Bloodstream infections caused by KPC-producing bacteria from New York City hospitals revealed mortality rates of 47% according to Bratu *et al*[7]. The experience outside the US has been similar, as per the studies carried out by Nadkarni *et al*[8].

Outbreaks of KPC production are associated with greater than two-fold increased risk of death. KPC producers also have been reported in many European countries and in South America and so a worldwide geographical distribution[9].

Klebsiella pneumoniae bacteria causes about 15% of gram –negative infections in the ICU wards, globally[2]. Drug resistant *Klebsiella pneumoniae* infections, especially those that produce Extended Spectrum Lactamases (ESBLs) and Multidrug Resistance (MDR), are more difficult and expensive to treat with worse treatment outcome [10].

Antimicrobial resistance: Antimicrobial resistance is the ability of microbes, such as bacteria, viruses, parasites, or fungi, to grow in the presence of a drug that would normally kill it or limit. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. This results in the treatment being ineffective and infection persists, increasing the risk of spread of the microorganisms to other individuals. According to World Health Organization reports hospital settings give rise to antibiotic resistance[11]. This poses a challenge for the health care sector due to limited choices for treatment of the infections[11].

Antimicrobial resistance is a serious global health threat that is undermining the ability to effectively detect, treat and prevent infections. Although antimicrobial resistance is a natural phenomenon, it is exacerbated by the misuse of antimicrobial medicines, poor or non-existent IPC programs, poor-quality medicines, weak laboratory capacity, inadequate surveillance and poor regulation or enforcement of regulations to assure access to high-quality antimicrobial medicines and their appropriate use. The most frequent resistances include resistance to: aminoglycosides, tetracycline, chloramphenicol trimethoprim/sulfamethoxazole, fluoroquinolones and broad-spectrum beta lactams[12]. Some strains of *Klebsiella pneumoniae* are resistant to carbapenem drugs worldwide causing morbidity, mortality among hospital –acquired and long term care associated infections. Mortality is about 30-70% among patients with bacteremia infections and pulmonary pneumonia[3].

Klebsiella pneumoniae can be controlled by hand washing, treating the sick, sterilizing the equipment used especially those used on ward patients and maintaining cleanliness of medical staff handling the patients.

ERIC PCR: Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR is a molecular technique used to assess the clonal variability of bacterial isolates[13]. Eric PCR technique utilizes short repetitive sequences that are dispersed throughout the various bacterial genomes.

They can also be used to resolve the genotype of the large numbers of O antigen serotypes, thereby enabling identification of an appropriate immunogenic strain[14]. Two primers are used in this method which after amplification of DNA, the amplicons are subjected to 1.5% agarose gel electrophoresis and visualized using ethidium bromide staining and a UV gel imager. ERIC method is rapid, sensitive, reliable and allows for

the DNA determination of their evolution which then leads to molecular classification and identification. This method helps to determine the source of the outbreaks by tracing the source of the infection[14].

MATERIALS AND METHODS

Study site: This study was based at the Aga Khan University Hospital (AKUH), Nairobi and Kenya Medical Research Institute (KEMRI). AKUH, Nairobi is a private, none profit institution that provides tertiary and secondary level care services. It is located in 3rd Parklands Avenue. It was established in 1983 with 13 teaching sites in 8 countries. It is a non-denominational institution open to all, irrespective of religion, ethnicity, gender or nationality.

The Kenya Medical Research Institute (KEMRI) is a state corporation established through the Science and Technology (Amendment) Act of 1979, as the national body responsible for carrying out health research in Kenya.

Study design: This was a cross sectional laboratory based study. Samples were from high risk areas (ICU, NICU) and OPD across various age groups and genders. Other wards in the hospital were not included due to low volumes of MDR cases

Study sample: Samples for this study were the MDR *Klebsiella pneumoniae* isolates from clinical specimens routinely isolated in Aga Khan Hospital Microbiology Laboratory.

Inclusion criteria: MDR *Klebsiella pneumoniae* isolates resistant to CAZ ceftazidime (which is used as a marker for ESBL production) and one sample per patient per year were included in the study[15].

Exclusion criteria: Repeat (same type of specimen requested more than once) and those not resistant to CAZ ceftazidime drug were excluded.

Sample size estimation: Convenience sampling technique was adopted since an outbreak is not under human design or control. Ceftazidime resistant MDR isolates of *Klebsiella pneumoniae* were from ICU, NICU and OPD in categories of the hospital between the period of January 2013 and June 2015. Available isolates from each category of departments were used. Clinical isolates were isolated from various specimens.

Microbiological methods: *Klebsiella pneumoniae* was isolated from clinical specimens of blood, urine, sputum, tracheal aspirate and wound swab. Standard procedures were used to culture the clinical specimens on blood agar, chocolate and MacConkey and sabourauds media. The plates were incubated at 35.5-37°C for up to 48 hours. Blood was incubated for a maximum of 5 days in BacTec. Positive flagged blood samples were sub-cultured on blood agar, MacConkey, chocolate and sabourauds media. The grown colonies were identified morphologically by use of bench biochemical tests and gram stain. Gram stain was done from the clinical specimen except from urine samples. The grown colonies were identified using the standard operating procedures according to Clinical Laboratory Standards Institute. The identification of the organism and drug susceptibility was done by the use of the automated bacteriology Vitek 2 compact equipment. MDR *Klebsiella pneumoniae* isolates were stored in glycerol at -80°C before they were sub-cultured for viability.

Morphology identification: The appearance of colonies on the plates was identified and biochemical tests performed where necessary. The *Klebsiella pneumoniae* colonies are gram negative, non-motile, encapsulated lactose fermenting and facultative anaerobic rods.

The gram stain was done to differentiate between gram negative and gram positive cocci or bacilli. Smear from the grown colonies was prepared. The smears were air dried and crystal violet flooded for 30-60 seconds. Excess stain was washed off with water. Grams iodine was applied for 30-60 seconds. Iodine was washed off with water. Decolourizer (Acetone-Alcohol) was rapidly applied and washed off immediately with water. The slides were covered with neutral red for 1 minute then washed off with water. They were air dried and examined under the microscope. The expected results were gram negative bacillus.

Microbial identification using the biomérieux

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system accommodates the colorimetric reagent cards that are incubated and interpreted automatically.

The reagent cards used have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate mixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. The reagent cards available for the identification of different organism are:

(i) GN - Gram-negative fermenting and non-fermenting bacilli

(ii) GP - Gram-positive cocci and non-spore-forming bacilli
(iii) YST - yeasts and yeast-like organisms
(iv) BCL - Gram-positive spore-forming bacilli

A sample suspension is prepared with sufficient number of colonies of a pure culture of the microorganism in 3.0 ml of sterile saline. The turbidity was adjusted and measured using a turbidity meter called the DensiChek™.

Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was placed either manually (VITEK 2 compact) into a vacuum chamber station.

All card types are incubated on-line at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time.

Data is collected, interpreted using different wavelengths in the visible spectrum. Turbidity or colored products of substrate metabolism are measured. Special algorithms eliminate false readings due to small bubbles that may be present.

Calculated raw data was compared to thresholds to determine reactions for each test on VITEK which appear as "+"," – ", "(–)" or "(+)". Reactions that appear in parentheses are indicative of weak reactions and on the VITEK 2, they appear as "?"

The databases of the VITEK 2 identification products is constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and culture collections.

ERIC PCR Method

The techniques used for *Klebsiella pneumoniae* clonal relatedness was ERIC PCR. Bacterial genomes contain repeat sequences such as Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence[13].

These methods were used as molecular biological tools to assess the clonal variability of many bacterial isolates. ERIC-PCR is a genomic fingerprinting technique that generates specific strain patterns obtained by the amplification of repetitive DNA elements present along the bacterial genome[13]. Bacterial DNA was extracted using boiling method. The bacterial colonies were from an overnight subculture of the bacteria. The ERIC sequences have been located in intergenic regions as palindromes of 127 bps[13].

The primers used for the ERIC-PCR reaction was: ERIC lR, 5'ATGTAAGCTCCTGGGGATTCAC3' and EgRIC2 5'AAGTAAGTGACTGGGGTGAGCG3'[13]. The ERIC-PCR fragments obtained were examined by electrophoresis in 2% agarose gel. The gels were stained with ethidium bromide.

The band patterns were interpreted and a difference of more than 2 bands were considered a given major type. Computer software was used for fingerprint analysis. ERIC-PCR fingerprints of amplified DNA fragments obtained by agarose gel electrophoresis were recorded.

*Prevention and control***:** The results of the study will be useful to the infectious prevention control team in monitoring the spread of the MDR *Klebsiella pneumoniae* infections.

Data management and analysis: Isolates in the study were identified with their coded numbers. Data was entered into spreadsheet where the data was then stored and protected using password known only to the principle investigator. The data collected was entered into database created in Microsoft excel and was subjected to computer software and dendrograms were obtained. The clonality of distribution of clonal relatedness was determined.

*Ethical considerations***:** The study was approved by Ethical Review Committee (ERC) of approval of Kenyatta University (KU), Nairobi, Kenya.

RESULTS

The study had a total of 63 clinical isolates, 12 (19%) clinical samples from Neonatal Intensive Care Unit (NICU). Out of these 7(11%) were blood samples. The ages of these 7 neonates were between the ages of 2 days to 3 months. From Intensive Care Unit (ICU) there were a total of 26 (41.3%) clinical isolates of which 14 were tracheal aspirates. The ages of these patients ranged between 3 years to 90 years of age. The third group of isolates were from outpatient department (OPD), they were 25(39.6%) and were all urines. The ages of the outpatient isolates were ranging between 3 and 81 years.

NICU cluster analysis results: The method defined two major clusters (C-1 and C-2) based on banding patterns. The two clusters had an insignificant genetic similarity of < 80%. However, the first cluster had a sub-cluster (S.c-1) with two organisms which had a significant homogenous genetic similarity of

100%. Each of the two blood culture isolates were obtained from different patients aged 2 months and 2 days.

The second cluster had a sub-cluster (S.c-2) with a 100% genetic similarity from a patient aged 2 days and another who was 2 months old. The two isolates in this sub-cluster were obtained from different blood cultures.

ICU and OPD cluster analysis: Cluster analysis of isolates obtained from ICU and OPD unveiled three major clusters (C-1, C-2 and C-3) based on banding patterns. The three main clusters had a genetic similarity of <80% and these three were further divided into sub-clusters.

The first cluster was divided into five sub-clusters (S.c-1, 2, 3, 4 and 5). The sub-clusters were further divided into minor clusters (M.c-1,2,3,4,5,6,7,8,9 and 10) based on branching patterns percentage similarity. Minor cluster 1 (M.c- 1) unveiled two organisms obtained from OPD) with a significant genetic relatedness of 80%. The second minor (M.c-2) cluster had three organisms with a significant genetic similarity of 100%. Two of these isolates were obtained from patients admitted in ICU ward while one was from OPD. The second sub-cluster had three minor clusters (M.c 3, 4, and 5) obtained from OPD patients from urine samples, all with a homogenous significant genetic similarity of 100%. The 3rd, 4th, and 5th sub-cluster had an insignificant genetic similarity of < 80%.

However, the 6th and 7th sub-cluster uncovered two minor-clusters (M.c-6 and 7) both with a 100% genetic similarity. The two minor clusters comprised of organisms obtained in patients in OPD and ICU ward respectively. The 8th, 9th and 10th minor cluster also uncovered a significant genetic similarity of > 80%.

DISCUSSION

A total of 63 clinical isolates were collected from critical care wards of NICU, ICU and OPD from the year 2013 to 2015. The isolates were identified using Vitek 2 compact. All the isolates that were resistant to CAZ at a MIC concentration of ≥64 were subjected to the study. A total of 19% isolates were from NICU ward, 40% from ICU and 41% from OPD. The youngest age of the patients was 2 days old.

This study reports three major cluster and sub clusters .The finding regarding *Klebsiella pneumonia* clonal relatedness reflects a previous study done in Burujerd hospital[16].

CONCLUSION

The findings of the study show that there is a spread of *Klebsiella pneumoniae* from health care providers to the patients. This can be shown clearly by 2 day old neonate in NICU being infected. The study also shows that there is a spread of the *Klebsiella pneumoniae* from outpatient to the ICU and NICU by either the visitor's, health care provider or medical equipment's used on patients not being properly sterilized. The study shows that there is a significant genetic relatedness which is a clear indication of cross-infection within and between wards in this hospital facility.

RECOMMENDATIONS

This report can be used by the prevention and infection control practitioner in the hospital to control the spread of infection.

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Challenges in the investigation and prosecution of sexual offences in relation to forensic medical evidence in Kiambu County, Kenya

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ABSRACT

Background: Kenya police annual reports show that sexual offences are escalating in Kenya. This study was done to find out the challenges the police officers within Kiambu County faced in the course of seeking justice for the sexual violence victims in relation to collecting forensic evidence. Forensic medical evidence is crucial for conviction or exoneration of a suspect.

Objective: The purpose of this study was to study the challenges of investigation and prosecution of sexual offences in Kiambu County as a representative of the national situation.

Design: This was a descriptive cross sectional study.

Setting: Gender Departments at Kikuyu and Tigoni police stations in Kiambu County.

Materials and methods: The research involved conducting of interviews and administration of Likert scale questionnaire for police officers trained on handling sexual offenses as the key informants, and use of data abstraction tool to collect data from the police record files in the year 2016. Sixteen police officers (key informants) participated in this research and 22 sexual offences files were availed for purposes of this research. The analytical software MAXQDA 2017 was used to compile all typescript segments according to their established codes. The percentage and frequencies were determined using IBM statistics.

Results: The analysis of P3 forms indicated 50% of reported victims had hymen broken, 40.9% had genital lacerations and 9.1% had hymen intact. It was established common laboratory tests ordered in rape cases are: high vaginal swab (77.3%), HIV (95.5%), pregnancy (77.3%) and DNA analysis (13.6%). The great amount of evidence (77%) collected in rape investigation is not sent to the forensic laboratories for analysis. The other setbacks include lack of collaboration between the police gender department and other government agencies like health facilities handling cases of sexual offences, and inadequate support for the gender offices to effectively handle cases of sexual offences. Only a third of reported sexual offence cases reach full trial. Inadequacies regarding filling of the P3 and Post Rape Care (PRC) forms noted were: omissions, poorly captured and documented forensic medical evidence. There was insufficient or lack of chain of custody for exhibits or forensic evidence.

Conclusion and Recommendations: There are challenges in investigation and prosecution of sexual offences and this is adversely affecting justice for the victims. We recommend continuous specialised training to clinicians on forensic medical evidence, infrastructural upgrade, equipping police gender departments with modern tools and a multidisciplinary approach in handling sexual offences.

Key words: Chain of custody, Forensic medical evidence, Sexual violence

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INTRODUCTION

Sexual violence crimes happen throughout the world with varying incidence in different countries, areas like in the Latin America the study shows that among the sexually assaulted adults less than 5% report to law enforcing institutions. Sexual violence by partners reported by women, between the ages of 15-49 years was 59% in Ethiopia which was the highest in the country included in the survey, in majority of the countries the rate falls between 10% and 50%[1].

Kenya Demographic Health Survey (KDHS)[2] findings was that one in every five or 21% of Kenyan women aged between 15 and 49 years were exposed to various forms of sexual violence. Moreover, KDHS found that 12% of women reported that their first encounter of sexual intercourse was against their consent. Further 40% and 44% of women and men respectively aged between 5 and 49 years have experienced physical violence from the age of 15 years, of these 20% and 12% of women and men respectively have been physically abused within one year before the survey[2].

According to the National Police Service (NPS) 2015 annual report, Kiambu County ranked third in crimes against morality at 238 cases after Nakuru and Bungoma Counties. There was 19% increase in offences against morality in 2015

compared to 2014 in Kiambu County. The report cited an increase of the same offences by 8% in the year 2014. Tigoni Police Station recorded 9 cases in 2015 but there was a sharp increase in 2016 recording 19 cases[3].

Sexual assault offences are defined, outlined and stipulated in the Kenya Sexual Offences Act 2006[4]. In Kenya, only 25% of sexual assault cases presented before a court of law leads to successful conviction. This poor outcome is as a result of lack of adequately skilled health workers and law enforcement agents to gather evidence from victims promptly and in effective way[5].

Examination of victims of suspected sexual assault requires a combination of skills in clinical history taking, thorough physical examination, collection and preservation of substantial physical evidence for forensic assessment. Clinicians' capacity in handling of sexual offences and through collection, preservation of physical evidence, analysis, reporting and conclusion are key for sexual offences cases.

In the year 2006, Kenya successfully enacted policing laws to help curb Sexual and Gender Based Violence (SGBV)[4], nevertheless these offences continue to be endemic in the country. Clinicians' capacity in handling of sexual offences by collection and preservation of physical evidence, analysis and reporting, and conclusion were among key aspects for conducting this research. Forensic medical evidence is vital in prosecution of sexual offences because of its utility in aiding determination of the cases in a court of law[6].

There is need for a study to establish challenges in the investigation and prosecution of sexual offences in relation to forensic medical evidence in Kiambu County**.**

MATERIALS AND METHODS

The research involved interviews using a guide with specified questions that begins with *how* or *what* and Likert scale questionnaire for police officers trained on investigation and prosecution of sexual offenses. The police in Tigoni, Kikuyu and Thika police stations were the key informants. Information on challenges and experience in the probation and prosecution of sexual offence was obtained.

Triangulation was used during data collection as a data management tool. All the police files on sexual offences for the year 2016 were examined. The files sample frame comprised of the files in the year 2016, involving sexual offences.

Research design: This was a descriptive cross sectional study, to assess challenges in the probation and prosecution of sexual offences in relation to forensic medical evidence in Kiambu County.

Study area: Research was conducted within Kiambu County in Tigoni, Kikuyu and Thika police gender department. These police stations were selected for this study because they are considered to apply best practices in handling sexual offences in Kiambu County, their population density and they are situated in main sub counties hence the outcomes of the study are representative.

Study population: Four police officers trained in handling sexual offences and two senior most police officers were recruited from each of the three police stations. An interview was conducted on them and then Likert scale questionnaire was administered. Two senior police officers were not available due to other official engagements. One hundred and ninety four files (NPS, 2016) on reported cases against morality in Kiambu County were perused. The reported cases included rape, defilement, sodomy, incest, indecent act, bestiality among others. In this the accessible population to the study was in Tigoni, Kikuyu and Thika police gender department. Each of the gender department reports an average of 20 cases in a year, comprising of 60 cases of accessible population.

Data collection methods and procedures: The researchers conducted interviews to 16 key informants who were police officers trained and experienced in handling sexual offences. Likert scale questionnaire was administered to the same officers including their supervisors. The interviews were face to face and involved audio recording and taking notes lasting for 30-40 minutes followed by Likert scale questionnaires lasting for 8-12 minutes. The researchers accessed the police files involving sexual offenses for year 2016 and required information captured in the data capture tool. Each file perusal took 10-15 minutes. The data was captured using a structured questionnaire, Likert scale and audio. The data was cleaned through frequency checks, physical count and double entry. Analysis was done using IBM statistics.

Ethical considerations: Permission to conduct this study was obtained from Mount Kenya University (MKU) Research Ethics Committee and National Commission for Science, Technology and Innovation (NACOSTI). Permission from Tigoni, Thika and Kikuyu police gender departments was sought before commencement of data taking. Information obtained during the study was confidential and anonymity was observed as no names or any other personal identification was captured.

RESULTS

(i) Qualitative results

The study sought to establish the challenges that are encountered in obtaining forensic medical evidence for sexual offences during investigations. The use of descriptive study design was to find out facts without manipulation of data, seek opinions, analyse and interpret findings. Triangulation of information was used to aid in validation of the results, and helped to collaborate the themes identified from the manuscripts. The most noticeable themes were;

(i) Loss of vital evidence before reporting as most victims of sexual assault not being aware how to preserve evidence before reporting to police or visiting a health facility
(ii) Clinicians lacking full insight on how to handle victims of sexual offences, collect and preserve forensic medical evidence
(iii) Lack of collaboration between the police gender department and health facilities in handling cases of sexual offences
(iv) Inadequate support to the police gender department to effectively handle cases of sexual offences

The study found out that the lack of awareness by the victim on the right thing to do after such incidences have taken place led to the interfering with evidence by the victims through taking showers and having a change of clothes. The delay of the victims in reporting of such cases leads to the degradation of evidence with time. In addition, the study found out that the lack of adequate training of the medical personnel handling the victims was equally a challenge in obtaining the required forensic medical evidence. Thus, these challenges encountered had an effect on the prosecution of the sexual offences which in some cases led to the acquittal of the offenders and therefore justice is denied to the victims.

The study established the poor collaboration between the medical personnel and the police officers, the police stations and the government hospitals affected the processing and presentation of forensic medical evidence during prosecution. The processing and presentation of this evidence is further impeded by the lack of proper storage facilities for the exhibits. The analysis of DNA samples took unnecessarily long duration but in majority of cases the DNA sample was not collected in the first place which would have served to place the offender at the scene of crime. Therefore, these challenges in processing and presenting of the forensic medical evidence affected the strength of prosecution of the sexual offences.

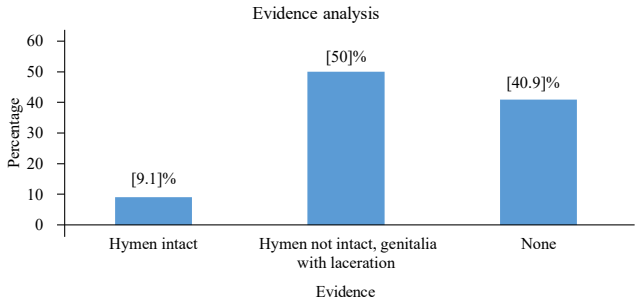
Triangulation used to assess the accuracy of the key informant interview confirmed most of the information gathered. There existed several inadequacies in regard to the police documents that are used in the gathering of evidence. Most of the documents did not capture all the necessary information, and were not accompanied by the evidence and specimens collected. In cases where exhibits were available, there was a generally poor preservation of the same. This therefore served to weaken the prosecution of offenders involved in sexual offences. There was glaring discrepancies between the P3 and PRC forms of the same victims.

In regard to the challenges in the chain of custody of forensic medical evidence, the study found that the chain of custody was not well established in most cases that were studied. Thus the lack of a well-established chain of custody can affect the overall prosecution of sexual offences.

(ii) Quantitative results

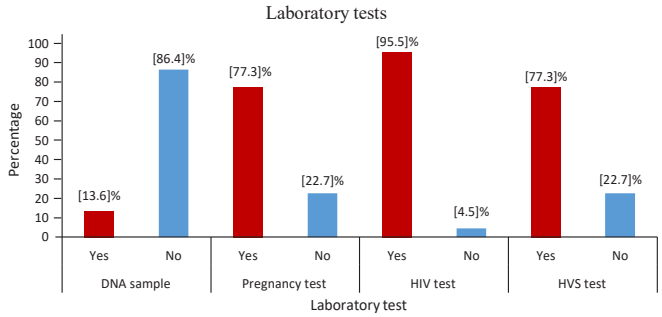
Evidence analysis in the P3 Form: From the findings, it was established that 11 (50%) cases indicated that based on the evidence analysis carried out, the hymen was not intact, with the genitals having lacerations, 9 (40.9%) indicated there was no evidence analysis that was carried out, 2 (9.1%) reported that the evidence analysis revealed that the hymen was intact. The findings therefore indicate that evidence analysis revealed that there was actual offence committed as evidenced by the broken hymen and the lacerations. However, the findings also show that there was no evidence analysis in a number of cases which serves to compromise the weight of evidence being presented during the prosecution of the cases (Figure 1).

Figure 1: Clinical assessment of sexual offences victims



Laboratory test and results: Types of laboratory tests ordered on the victims as part of sexual offences investigation include: DNA, Pregnancy, HIV and HVS. It appears the forensic DNA which is deemed most crucial in evidential preparation was least requested by the investigators. The HIV test may have been requested for patient management but utility is limited in proving sexual offences (Figure 2).

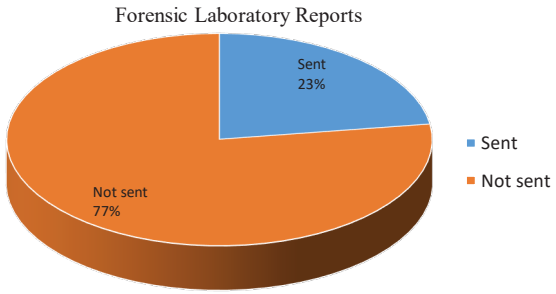
Figure 2: Laboratory test on the victims



Forensic laboratory results: The study sought to find out whether specimens obtained from the victims and perpetrators was sent to forensic laboratories for analysis.

From the findings, majority of the specimens, 77% collected from the victims and perpetrators were not sent to forensic laboratories for analysis while 23% of the specimens were sent to forensic laboratories for analysis. However, even for the specimens that were sent for analysis, no results were received back. The findings therefore indicate that most of the collected evidence was not sent for further forensic analysis which otherwise would have provided more evidence in the prosecution of the cases (Figure 3).

Figure 3: Frequency of utility forensic laboratory in processing specimen from sexual offences



Likert scale questionnaire: The Likert questionnaires were administered to the key informants with an expectation to respond how they agreed or disagreed with the statements. A three point Likert scale was used to rate the responses namely: (1) Agree, (2) Neutral and (3) Disagree.

More than 81% of the respondents observed that sexual offences are not reported to the law enforcing authorities. There was parity (50%) on victims of sexual violence reporting to police before bathing or changing clothes. The 93.75% of the respondents observed that sexual violence victims who are below 18 years of age need company of a parent or a guardian (Table 1).

Table 1: The manner of reporting sexual offence

Statements	Agree (%)	Neutral (%)	Disagree (%)	No.
All sexual offences are usually reported to police station for action	12.5	6.25	81.25	16
Minors of less than 18 years need a parent/guardian while reporting sexual offence against them	93.75	0	6.25	16
Most victims of sexual offences report incidences to police stations before going to seek medical attention	87.5	0	12.5	16
Most victims of sexual offences seek medical attention first before reporting incidence to police stations	18.75	6.25	75	16
Most victims of sexual offences report offence to police station before taking a bath or change of clothing	50	0	50	16

Table 3 indicates that there is poor collaboration between the police and public health facilities in collecting forensic evidence (87.5%). This affects the quality forensic evidence which is critical in the investigation and prosecution of the sexual violence offences (93.75%).

Table 2 show that victims of sexual offences report to the police long after the incidence (81.5%). It was reported that the medical facilities receiving the sexual offence victims have limited capacity to collect forensic medical evidence (62.5%) but highly admitted (100%) that the forensic medical evidence is very important in corroborating sexual offences.

Table 2: Handling of forensic medical evidence after sexual crime incidence

Statements	Agree (%)	Neutral (%)	Disagree (%)	No.
Most victims of sexual offences report offence to police station after taking a bath or changing of clothing	50	0	50	16
Most victims of sexual offences report offences when it's too late to collect forensic medical evidence	81.5	0	18.5	16
Most victim of sexual offence report the offence early enough that aids gathering forensic medical evidence	25	0	75	16
Forensic medical evidence is very vital to corroborate victims of sexual offence allegation	100	0		16
The clinicians attending the victims of sexual offences are conversant with forensic medical evidence	25	0	75	16
The hospitals where the victims of sexual offences are first attended to are conversant on how to collect and preserve forensic medical evidence	37.5	0	62.5	16
The local facilities are well facilitated to collect and preserve forensic medical evidence	62.5	0	37.5	16

Table 3: Relationship between agencies dealing with sexual crimes

Statements	Agree (%)	Neutral (%)	Disagree (%)	No.
There is good collaboration of the police and government health facilities in handling the victims of sexual offences	62.5	0	37.5	16
There is poor collaboration between the police and government hospitals in collecting and preservation of forensic medical evidences in relation to sexual assault	87.5	0	12.5	16
Forensic medical evidence is usually the main evidence the investigating officer is usually interested in sexual offences	56.25	0	43.75	16
The police investigating officer usually encounter challenges in building a strong case due to poor collaborative forensic medical evidence	93.75	0	6.25	16

The respondents observed that clinicians attending do not need chain of custody for forensic medical evidence (75%) and that it is not observed (56.25%). However, the respondents consider it necessary chain of custody is important for forensic medical evidence (100%) (Table 4).

Table 4: Chain of custody for the specimen for sexual offences

Statements	Agree (%)	Neutral (%)	Disagree (%)	No.
Chain of custody is important in handling forensic medical evidence	31.25	12.5	56.25	16
All the cases of sexual offences there is strict adherence to the chain of custody in handling forensic medical evidence	25	0	75	16
In your own opinion the clinician handling forensic medical evidence are conversant with the chain of custody in handling this type of evidence	37.5	0	62.5	16
There is need to support the existing structure on chain of custody in handling forensic medical evidence	100	0	0	16

DISCUSSION

The study identified the challenges the gender departments in Kiambu County faced in their investigation and prosecution of sexual offences in relation to forensic medical evidence, and in pursuit for justice to the victims of sexual violence.

Based on the findings from key informants, most are proud working in the gender department since it assists in enhancing the knowledge regarding gender based issues. It also provides an opportunity for those working there to learn about what happens in the communities they live in. Working in this particular department provides one with an opportunity to educate the public on issues around gender based violence. Professionalism and integrity are virtues for those working there. There should be a full informed consent of the victim when medico-legal evidence needs to be collected and there after stored and analysed[7].

According to those interviewed, there are several problems that are encountered when dealing with sexual offences. Among these problems include: under-reporting of majority of cases at the station, late reporting of the offences, up to 72 hours or more when most of the evidence is lost or degraded, with some assault victims taking baths/showers and changing their clothing before attending a health facility to report the offences. Most victims are not conversant with the procedures of reporting such incidences. This may also explain as per the police report why the victims often feel unsupported when they come forward, and premature prosecution of cases before investigations are complete which can result in wrongful acquittals[8].

There is poor documentation and capture of evidence in both PRC and P3 forms, poor storage facilities for specimens that will be used in analysis, failure of the victims to provide full information on the incidence(s) and there is a general fear of being exposed alongside intimidation from the offenders together with their next of kin. With regard to the challenges that are encountered when gathering forensic medical evidence, the key informants pointed out that some clinicians were not conversant with what type of specimen to take for the forensic analysis. Evidence, when collected promptly, is adequately recovered such as microscopic evidence of spermatozoa which can be lost in late examination[9].

It was also pointed out that there are several factors that prove to be a challenge in gathering of forensic medical evidence such as tampering of the crime scene, victimization of the victims because of tribal or cultural norms and poor facilities and infrastructure at the police stations where confidentiality is needed in such cases. According to Amnesty International Kenya, only 25% of sexual assault cases presented before a court of law leads to successful convictions. This poor outcome is as a result of inadequacy of skills of health workers and law enforcement agents to gather evidence from victims promptly and appropriately[5].

Regarding how the challenges which are usually encountered in gathering of forensic evidence eventually affect the prosecution of these offences, the key informants pointed out that the forensic medical evidence provide more weight to the prosecution, but when this type of evidence is not collected and there is no clinician to act as medical expert, successful prosecution of such a case becomes a challenge. They suggested that in the P3 form, the clinician should state the degree of injury as required in column 5 of the P3 form, clearly indicating whether its harm, grievous harm or maimed. The P3 form should also have a section for DNA match.

With regard to how other working environments affect the investigations and prosecution of sexual offences, it was noted that storage of the forensic medical evidence was very poor at the police stations. This finding is contrary to other countries like Australia, Canada, United Kingdom and United States

where the evidence is secured in a sexual violence kit and may be frozen or stored while the victim decides whether they will pursue recourse in the legal system[7]. Some stations lack private rooms for interrogation of the victims and where they can give their evidence freely, lack of transport and facilitation to the Government Chemist and the inability of some officers to attend victims of sexual offences due to lack of sensitization on how to handle such cases.

The officers interviewed seemed not to be so conversant with the chain of custody and how to go about it. According to WHO the creation of a secure chain of custody is key to effective processing of medico-legal evidence to avoid compromise before analysis and possible court use. From specimen collection, sealing in separate containers to avoid cross-contamination, labelling, signing by the person who gathered them[10].

CONCLUSIONS

There still exists challenges in investigation and prosecution of sexual offences and this is adversely affecting justice to the victims. The immediate course of action should be to enhance collaboration between the police, the health workers and other stakeholders to form multidisciplinary teams to investigate and prosecute sexual offences. There should be community awareness of how to report and preserve evidence after the ordeal. Regular special training to the police and clinicians in line with their roles in handling victims of sexual offences. More research needs to be done on clinician's knowledge in forensic medical evidence and their roles as medical forensic experts during prosecution.

RECOMMEDATIONS

The study recommends that necessary steps be taken to improve the collaboration of the police gender departments, health facilities and other stakeholders who handle victims of sexual assault through joint special training, workshops and sensitization programmes. This will enhance preservation of the evidence and seamless flow of investigation and to secure more convictions of sexual offenders. There is need for national forensic DNA database.

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Declaration of conflict: The authors declare no conflict of interest

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An audit and review of histopathological reporting of prostate cancer on prostatic tissue specimens in Kenyatta National Hospital

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ABSTRACT

Background: Cancer of the prostate is the commonest cancer in males in Kenya. Gold standard of diagnosis is histopathology. A complete report is required for holistic patient management. Auditing of histopathology reporting is a key element of a quality assurance programme. Studies have established that there have been significant changes in patient grading using the new modification of Gleason system and that there is observer variability in grading using this system.

Objectives: To evaluate the completeness of information provided in request forms, completeness of prostate cancer reporting using the College of American Pathologists protocol, identify changes in grading with the 2014 Gleason modifications and to assess the level of inter-observer variability in grading at Kenyatta National Hospital.

Design: Retrospective descriptive study.

Materials and Methods: Consecutive request forms, reports and paraffin blocks for 137 cases previously reported as prostate cancer were retrieved. Information from request form and reports was entered into data collection tool. Histological slides were prepared. Diagnosis and grading were reviewed, using the International Society of Urological Pathologists 2014 Gleason system, by the principal investigator and two consultant pathologists. The initial Gleason grades and grade groups were compared with review findings. Kappa coefficient was used to obtain level of inter-observer variability.

Results: The patient name and hospital number were indicated in all cases. Other request form details were incomplete. Macroscopic features were inconsistently provided. Histological type in all cases was prostate adenocarcinoma not otherwise specified. Other microscopic features were inconsistently reported. Gleason scores were upgraded in 51.8% of cases whereas grade groups were upgraded in 43.1% of cases. The level of agreement was fair for the primary pattern (*k* 0.25), poor for the secondary pattern (*k* -0.31) and slight for the Gleason score sum (*k* 0.20).

Conclusions: Request forms are not adequately filled. Presence and use of an inclusive and comprehensive reporting protocol ensures complete reporting. There was an upward shift in grading with the modified system. Strength of agreement between the initial and review Gleason grades and scores ranged from poor to fair.

Recommendations: Sensitization of the clinicians on the importance of providing adequate information through continuous medical education. Use of the CAP cancer reporting protocol to enable the generation of a complete report. Strengthen measures aimed at reducing observer variability including consensus grading of difficult cases, use of common 'reference images' with architectural patterns and continuous training on any new changes in the system.

Key words: Prostate cancer, Audit, Gleason grading system, Inter-observer variability, completeness, Standard reporting protocol

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INTRODUCTION

Audits compare and examine current practice against a set standard procedure and guidelines. In the laboratory, audits are done to provide evidence on the quality of services provided and that quality requirement processes are being met[1,2].

The significance and effectiveness of auditing cancer reporting has been demonstrated in a number of studies. Idowu *et al*[3] showed a greater degree of completeness of reporting of breast cancer with an overall improvement from 0.9% to 20.5% between their first and second audits. Imperato *et al*[4] demonstrated an improvement in reporting of breast and prostate cancer that ranged from 12.6% to 19.9% and 1.4% to 23.9% respectively.

Gleason grading system was developed in 1966 by Donald. F. Gleason[5]. It has been refined and modified first by Gleason *et al* in 1974 and 1977 then by the ISUP in 2005 and 2014[5-8]. It is the most recommended system by WHO since 1993[9]. Gleason grading plays a major role in determining

treatment modality, risk stratification and patient prognostication[10]. A new prognostic grade grouping system with five grade groups is the latest highlight in grading of prostate cancer[11]. This new system has been found to be advantageous by providing a more simple and accurate stratification. It also provides the potential for reducing overtreatment of indolent cancer given that the lowest grade is 1[12]. It was approved for use by World Health Organization in 2016[13].

Studies have been done to demonstrate the effect of modification of Gleason system on the overall patient grades and also the impact it has on patient stratification during prognostication[14-16]. Studies that have made a comparison between the old traditional Gleason system versus the ISUP 2005 modified system have shown a shift towards a higher grade and a decrease in the number of lower grades. Chen *et al*[15] documented an increase of scores 7-10 from 59% to 72% and a decrease in scores 2-5 from 27% to 0% in core biopsies. Studies comparing the 2005 modification and the 2014 modification have also demonstrated similar findings[17]. Shah *et al*[17] documented a marked decrease (80%) in Gleason score 6, a 28.57% decrease in Gleason score 8 and 60% increase in Gleason score 9 attributed to the new criteria for pattern 4.

Studies have been done to assess the level of observer variation in grading using the Gleason system. Many have shown a fairly acceptable level of observer variation. Delahunt *et al*[11] in 1982 demonstrated an inter-observer exact agreement rate of between 47-60% (k 0.605-0.836) whereas Rousselat in 1986 and de las Morenas in 1988 found inter-observer agreement rates of 65% and 66% respectively. McLean *et al*[18] in 1997 found the extent of inter-observer variation (weighted kappa) for the raw Gleason scores (2-10) as 0.16-0.29 (poor to fair) with a total agreement rate of 9.9% and total disagreement rate of 43.7%. In 2001 Allsbrook *et al*[19] found an overall kappa (k) coefficient for inter-observer agreement as 0.435 (moderate agreement) with a k range from 0.00 to 0.88. Abdollahi *et al*[20] in 2010 demonstrated a fair inter-observer agreement of k 0.29 and an almost perfect intra-observer reproducibility rate of 85.2%. The observer variability is attributed to differences in training, experience, practice and familiarity with the system[19]. These discrepancies in grading can potentially affect patient management[21,22].

The completeness of a histopathology report requires that the information provided should include the diagnosis and pathological features of prognostic and predictive significance[23]. From time to time standard reporting protocol and checklists have been in use. Cancer reporting protocols provide an avenue for generation of an adequate report. A multi-institutional study by Idowu *et al*[3] demonstrated that centres which regularly utilized checklists reported all the necessary elements as opposed to those that did not use (88% versus 34%). CAP cancer protocols are used by many pathologists[24]. They provide complete and uniform reporting of malignant tumours.

Adequate clinical information has been shown to affect the accuracy and completeness of pathology reports. Clinicians need to give adequate demographic and clinical information that will have an impact on the diagnostic process or affect its interpretation. The microscopic details in a prostate cancer specimen report should include histologic type, Gleason grade, tumour volume, tumour location, extra prostatic extension, seminal vesicle invasion, perineural invasion, lymphovascular invasion, resection margins, lymph node status, intraductal carcinoma, any additional findings and pathologic stage[25].

Objectives: The aims of this study were to determine the completeness of information provided in the request forms, the completeness of documentation of macroscopic and microscopic features using the CAP reporting protocol, to determine Gleason scores, grade groups and their changes from the initial report using the ISUP 2014 modified Gleason system and the inter-observer variability in Gleason scores between the initial and review findings in the years 2016 and 2017.

MATERIALS AND METHODS

This was a retrospective descriptive study conducted at the Kenyatta National Hospital/University of Nairobi histopathology laboratory on prostatic tissue specimen including core biopsies, transurethral resection prostatic chips and whole prostate glands previously reported as prostate cancer starting from September 2013 to January 2017. Cases in which the request forms, reports and paraffin blocks were missing were excluded. A total of 137 cases were selected. Consecutive reports and paraffin blocks for prostatic tissue specimen were retrieved. Clinical, demographic, macroscopy and microscopy information was obtained from the request forms and reports and then entered into the data collection tool that incorporated the College of American Pathologists protocol. Corresponding specimen paraffin wax embedded blocks were retrieved from the histopathology department for sectioning and hematoxylin & eosin staining. All the prepared slides were reviewed and reported by the principal investigator and thereafter confirmed by the two supervisors (consultant pathologists). The final Gleason scores and grade groups for the review were then derived using the ISUP 2014 Gleason system. The supervisors were blinded on the initial report microscopy findings. The initial and review report histological grade and grade group were then compared to assess the changes. Data was analysed using SPSS version 20. The level of agreement in Gleason grading was obtained using Cohen kappa (k) statistic.

RESULTS

There were 106 core biopsies, 18 transurethral resection prostatic chips, 9 simple and 4 radical prostatectomy specimens. Age was indicated in 116 (84.7%) cases. Mean age was 59 years with a range of 25-90 years. Peak age was 71-80 years. The patient name and hospital number were included in all the request forms. Other request form details are as shown in Table 1.

Table 1: Request form details

	Indicated	Not indicated	Total
	No. (%)	No. (%)	No. (%)
Hospital number	137 (100)	-	137 (100)
Patient name	137 (100)	-	137 (100)
Date of procedure	80 (58.4)	57 (41.6)	137 (100)
Date specimen received	127 (92.7)	10 (7.3)	137 (100)
Clinical history	62 (45.3)	75 (54.7)	137 (100)
Clinical diagnosis	100 (73)	37 (27)	137 (100)
Type of procedure	107 (78.1)	30 (21.9)	137 (100)
PSA level	27 (19.7)	110 (80.3)	137 (100)
Clinician details	128 (93.4)	9 (6.6)	137 (100)

The number and length of cores was provided in 100 (94.3%) cases and 98 (92.3%) cases respectively. Out of the 18 TURP cases reported, 11 (61.1%) cases had the number of chips provided, 10 (55.6%) cases were weighed and 12 (66.7%) cases were measured. The weight of prostatectomy specimens was provided in 12 (92.3%) cases whereas the dimensions was provided in 11 (84.6%) cases.

All the cases were diagnosed as prostate adenocarcinoma not otherwise specified. One hundred and thirty three (97.0%) cases were graded completely using the Gleason system. Two (1.5%) cases could not be assessed. These were small and fragmented cores. Two (1.5%) cases were incompletely graded in that there was a final score but no mention of the primary and secondary patterns. The predominant primary pattern was 3 accounting for 38.7% (53 cases) while pattern 2 was least diagnosed (9 cases, 6.6%). Pattern 4 was the predominant secondary pattern accounting for 58 (42.3%) cases while pattern 2 was the least diagnosed (7 cases, 5.1%). The predominant Gleason score was 7 accounting for 43 (31.4%) cases. Least predominant scores were 4 and 5 each with 4 (2.9%) cases. Scores 6, 8, 9 and 10 accounted for 18 (13.1%) cases, 18 (13.1%) cases, 38 (27.7%) cases and 9 (6.6%) cases respectively. Tumour volume was quantified in 69 (50.4%) cases. It could not be quantified in 2 (1.5%) cases.

Lymphovascular invasion was present in 15 (10.7%) cases, not identified in 23 (16.4%) cases and not recorded in 99 (70.7%) cases. Perineural invasion was identified in 26 (18.6%) cases, not identified in 23 (16.4%) cases and not reported in 88 (62.9%) cases. Extra prostatic extension was identified in 6 (4.4%) cases and not reported in 123 (89.8%) cases. There was no seminal vesicle invasion identified in 3 (75%) radical prostatectomy specimens. Three radical prostatectomy specimens had positive margins. Three (75%) cases were staged all of which were pT2NxMx (stage I).

One hundred and twenty nine (94.2%) cases were graded in the review using the ISUP 2014 modified Gleason system. Predominant primary pattern was grade 4 (74 cases, 54%) and the least was grade 3 (23 cases, 16.8%). Predominant secondary pattern was 5 (63 cases, 46%) and the least was grade 3 (26 cases, 19%). Predominant Gleason score was 9 with 60 cases (43.8%). Least score was 6 with 12 (8.8%) cases. Scores 7, 8, and 10 comprised of 14.6% (20 cases), 15.3% (21 cases) and 11.7% (16 cases) respectively. In the review the predominant prognostic grade group was 5 with 77 (56.2%) cases. The least was group 2 with 8 (5.8%) cases. Prognostic grade groups 1 (12 cases), 3 (12 cases) and 4 (20 cases) accounted for 8.8%, 8.8% and 14.6% respectively.

Table 2: Changes in Gleason scores between initial and review findings

	Frequency	(%)
Unchanged	44	32.1
Upgraded	71	51.8
Downgraded	14	10.2
Changed diagnosis	8	5.8
Total	137	100

Figure 1: Gleason scores in the initial and review findings

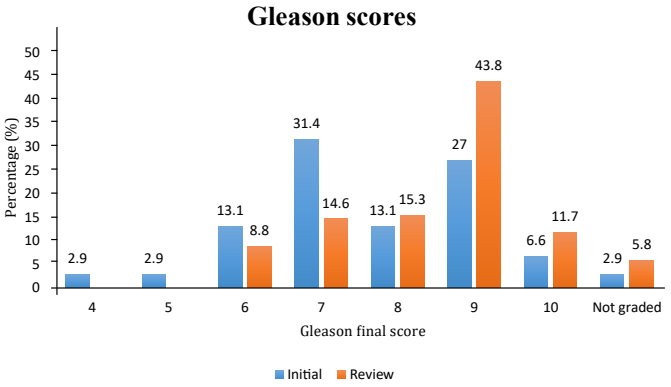

Table 3: Changes in the grade groups between the initial and review findings

	Frequency	(%)
Unchanged	60	43.8
Upgraded	59	43.1
Downgraded	10	7.3
Changed diagnosis	8	5.8
Total	137	100

Figure 2: Grade groups in the initial and review findings

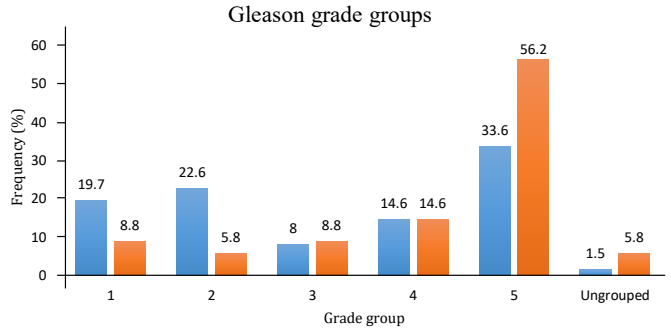

Table 4: Level of agreement between initial and review Gleason grade for 2016/2017

	Percent agreement (%)	Kappa	Strength of agreement	P value
Primary pattern	46.7	0.25	Fair	0.043
Secondary pattern	20.0	-0.13	Poor (less than chance agreement)	0.806
Gleason score	33.3	0.20	Slight	0.026

DISCUSSION

Demographic parameters help in patient identification and permit correlation with previous reports. Hospital number and the patient name were the only consistent parameters included in all forms. This correlates with a study done by Alagoa *et al*[26] in Nigeria where all forms included the patients name. Patient's age was missing in 15.3% of the cases. This is similar to other studies however slightly higher than in studies by Alagoa *et al*[26] and Nutt *et al*[27] who found age missing in 11.5% and 3.7% of cases respectively. The date for the surgical procedure wasn't indicated in 41.6% of cases. This is higher compared to the study by Nutt *et al*[27] which had (3.3%). This date helps in assessing turnaround time. Type of procedure performed and the specimen helps in proper grossing and avoidance of identification errors. This was missing in 21.9% of cases, similar to Alagoa *et al*[27] who however had lower number of cases (11.0%).

The histologic type in all cases was prostate adenocarcinoma not otherwise specified. This concurs with findings in a study by Idowu *et al*[3] in which there was inclusion of histologic type in prostate cancer specimens. The type helps in determining the grade and prognosis. Gleason grade guides management and risk stratification. One hundred and thirty three (97.0%) cases were graded completely. In a study on 101 core biopsies by Siddiqui[28], there was indication of the grade in all cases.

Other inconsistently reported features included tumour quantity, lymphovascular invasion, perineural invasion, extra prostatic extension, margins and stage. In the study by Idowu *et al*[3] missing elements included extent of invasion, tumour volume, and lymphovascular invasion. In another study by Aumann *et al*[29] the inconsistently reported features in descriptive reports included margin status, extraprostatic extension, seminal vesicle invasion, perineural invasion, lymphovascular invasion and stage. In the study by Siddiqui[28] in which a standard reporting protocol was used, all features were reported. In our study the reports were descriptive in nature hence the possibility of missing out some features.

Predominant primary pattern in the initial and review reports were 3 and 4 respectively. Least pattern in the initial and review were 4 and 3 respectively. This may be explained by the change in criteria for the patterns. All cribriform glands were re-classified as pattern 4. Branching and irregular glands with clear lumina and stroma in between them were regarded as pattern 3. In the review and initial report the predominant secondary patterns were 5 and 4 respectively. The change may be explained by the fact that the second pattern in the review was assigned to the worst pattern present as opposed to the old system of awarding the second predominant pattern.

There were no scores 4 and 5 in the review because no case was assigned patterns 1 and 2 as per the 2014 guidelines. In Shah's study there were also no scores 4 and 5. The predominant final score in the review was 9 (43.9%), comparable to Shah *et al*[17] who found a predominance of score 9 (45.5%). They classified their cases using the ISUP 2014 criteria.

In our study the least common score was 6 (8.8%) which also compares with Shah's study where score 6 was the lowest (3%). The final Gleason score in 51.8% were upgraded whereas only 10.2% of them were downgraded. There was an increase scores 8, 9, 10 and a decline in scores 6 and 7. Differences are attributed to change in pattern and reporting criteria.

Commonest prognostic grade group was 5 in both the initial and review reports, correlates with the findings of Shah *et al*[17] who reported group 5 as predominant (48.5%). However, in a study by Gupta *et al*[6] the predominant grade group was 3 accounting for 41.7%. The grade group in 43.8% remained unchanged. Cases upgraded (43.1%) were more than those that were downgraded (7.3%). There was a 22.6% increase in cases of group 5. Groups 1 and 2 were decreased. Changes were as a result of increased reporting of higher scores and decreased reporting of lower scores in the review.

The primary pattern had a fair agreement (*k* 0.25) comparable to Ozkan *et al*[30] (*k* 0.34). The secondary pattern had poor agreement (*k* -0.13). Ozkan *et al*[30] had a fair agreement (*k* 0.37). Level of agreement in Gleason score sum was slight (*k* 0.20), comparable to a study by Mc Lean *et al*[18] (*k* 0.15-0.29). Documented problematic factors contributing to poor-fair agreement include difficulties in differentiating benign glandular structures that mimic pattern 3, small amount of tissue especially in core biopsies, patterns that fall in the interphase between classic patterns and inherent subjectivity[19,30]. Subjectivity is because Gleason grading is a qualitative parameter therefore inherently bound to have different interpretations that may not always be interpreted as an error[20]. Specimen quality is also another factor. Core biopsies that are fragmented or compressed during processing have been associated with difficulties in diagnosis and grading[20].

CONCLUSIONS

The histopathologic request forms for prostatic specimens were not adequately filled. There was incomplete assessment of various macroscopic and microscopic features. This study was able to reveal deficient areas in reporting that require attention. We highlighted changes in grading that come with the use of the modified system and the level of agreement in grading previously unknown in KNH.

RECOMMENDATIONS

(i) Clinicians need to be sensitized on the importance of providing adequate information through continuous medical education.

(ii) Use of the CAP cancer reporting protocol to enable the generation of a complete report.

(iii) Strengthen measures aimed at reducing observer variability in grading including consensus grading of difficult cases, use of common 'reference images' with the architectural patterns and continuous training on any new changes in the system.

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Diagnostic utility of modified cell blocks in fine needle aspirates of thyroid nodules at Kenyatta National Hospital, Kenya

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ABSTRACT

Background: Fine Needle Aspiration Cytology (FNAC) is considered to be a standard screening test for the diagnosis of thyroid nodules. It is a safe, fast and cost-effective way of evaluating thyroid nodules. However, this technique has some limitations such as false negative or positive results, high rate of unsatisfactory results and inability to classify border line lesions which result into indeterminate results. Use of cell block as an adjunct method of diagnosis has been demonstrated to increase the diagnostic efficacy of diagnosing organ specific lesions.

Objective: To establish the utility of modified cell blocks as an adjunct test to FNAC in the diagnosis of thyroid lesions.

Design: This was a descriptive cross-sectional study.

Subjects: A total of 52 cases suspected of clinically having thyroid lesions at Kenyatta National Hospital (KNH) FNA clinic were evaluated.

Setting: This study was done at Kenyatta National Hospital FNA clinic.

Methodology: Fine Needle Aspiration (FNA) materials for both conventional smears (CS) and modified Cell Blocks (CB) were collected simultaneously at FNA Clinic. Ethical clearance was obtained from KNH/UoN Ethics and Research Committee (ERC) before carrying out the study. Written informed consent was sought from all participating patients. Cellularity, morphological and architectural preservation, as well as cytologic diagnosis on CS was compared with CB sections. Data was entered on Microsoft excel and analyzed using SPSS software version 20. McNemar's Chi-square statistical test was performed at 95% confidence level.

Results: Out of 52 participants, majority (88%) were women while 12% were men with a female to male ratio of 1:7.7. Age ranged from 21 to 73 years with mean age of 41 years. Most (75%) of thyroid FNA were reported as benign with a high unsatisfactory rate of 25% on conventional smears. The benign lesions included; colloid goiter (69.2%), thyroiditis (3.8%), and ACUS (1.9%). Modified cell block preparations had high cellularity, minimal obscuring background material with excellent architecture compared to conventional smears. Modified cellblock provided additional information for diagnosis of thyroid lesions in 15.4% of the total cases. Unsatisfactory rate of thyroid FNA cytology was reduced from 25% to 13.4% when both methods were used thereby increasing the diagnostic efficacy to 86.6%. The diagnosis of suspicious/follicular neoplasm which was missed on conventional smear was picked up by modified cell block preparation. Comparing the diagnosis on McNemar's Chi square test, there was no statistically significant difference in the two methods (p-value >0.05).

Conclusion: In this study, modified cell block preparation provided additional information which was helpful in establishing new diagnosis and also decreased the unsatisfactory rate of thyroid FNA cytology from 25% to 13 % when both methods are combined.

Recommendations: Modified cellblock should be considered as an adjunct test to the conventional smear on thyroid FNA cytology. Proper training and monitoring of clinician performing FNA procedure should be provided in order to reduce unsatisfactory rates of the thyroid aspirates.

Key words: FNAC, Cell blocks, Conventional smears and thyroid lesions

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INTRODUCTION

Thyroid nodule is a common clinical presentation in fine needle aspirate cytology clinic at Kenyatta National Hospital. The prevalence of thyroid nodules ranges from 4% to10% in the general adult population[1]. Majority of these nodules are benign, and only 5% to 30% are malignant which require histological evaluation[2]. The main goal of evaluating these nodules is to distinguish benign nodules from malignant

ones. Diagnostic tests like ultrasound, thyroid nuclear scan, clinical chemistry, and Fine Needle Aspiration Cytology (FNAC) are used for evaluation of thyroid nodules.

Fine needle aspiration cytology is considered the gold standard screening test in the evaluation of thyroid nodules[1,3]. The technique is simple, safe, fast, easily repeated, and cost effective procedure, with excellent patient compliance. The main aim of FNAC is to identify nodules that require surgery and those benign nodules that can be observed clinically and decrease the overall thyroidectomy rate in patients with benign disease. However, FNAC has limitations[4]. Some of the limitations include false negative or positive results. In addition, FNAC has inability to define malignant follicular lesions in the absence of nuclear features of papillary carcinoma. These limitations are as a result of compromised specimens which are poorly preserved, and can be due to sparsely cellular or excessive clotting. Furthermore, follicular adenoma and follicular carcinoma have overlapping cytomorphologic features and cannot be accurately distinguished by FNAC. Like other conventional FNA smears, thyroid FNAC also offers limited material for ancillary tests.

Attempts to improve the diagnosis on FNAC have been made for most organ specific lesions including the thyroid gland. These efforts include preparation of cellular material as cell blocks which mimic histological tissue biopsy. Cell blocks are micro-biopsies embedded in paraffin wax, suitable for sectioning, staining, and microscopic study. This technique has been in use for over a century. There are different methods of cell block preparations. These include: bacterial agar method, plasma method, thrombin clot method, Tissue Coagulum Clot (TCC) method, and automated CB preparation system. Cell Block (CB) preparations have been implied to increase cellular yield and improving the diagnostic accuracy of lesions. They also offer extra material for other ancillary tests[5].

Just like paraffin tissue sections in histology, cell block section yields histologic tissue architecture which are useful when making diagnosis. Architectural pattern may not be present on conventional smear. However, a few drawbacks are known for CB techniques and include increased cost and lengthened turnaround time (TAT). To mitigate for increased cost, a modified cell block preparation technique has been developed. This technique offers excellent cytomorphologic features and ensures optimal preservation of histochemical and immunocytochemical properties. It is simple and reproducible and uses routine safe laboratory chemicals.

At KNH, cell block preparation is not a common practice for thyroid lesions and other lesions. To the best of our knowledge, no study has been done to demonstrate the utility of cell block preparations in evaluation of thyroid lesions in Kenya, moreover, using the modified technique. The aim of this study was to establish whether the diagnosis of thyroid lesions could be improved by using combination of modified cell-block and FNAC (conventional smears).

MATERIALS AND METHODS

This was a laboratory based, descriptive cross sectional study which was conducted at KNH surgical clinic from February to April, 2016. Ethical approval was obtained from KNH-UoN ERC; study number P767/12/2015. This study included patients aged 18 years and above referred to KNH FNA clinic with palpable thyroid lesions. All the participants in this study gave informed consent. Patients below 18 years old, those with clinical history of thyroidectomy, and those with impalpable lesions were excluded from this study. A total of 52 patients presenting with thyroid nodule lesions participated in this study and convenient sampling method was used until the required sample size was achieved.

Specimen collection

Thyroid FNA procedure was done by consultant pathologists/pathology registrars on patients with palpable thyroid masses who presented to the surgical clinic during the study period. One-inch 23-gauge needles and 5 cc syringes were used to collect the FNA material. Three to five passes were made on the thyroid masses. Rapid onsite evaluation of the smears was not done. Two clean labeled slides were used to prepare conventional smears (An average of 2 slides per case was prepared). The slides were fixed immediately in 95% alcohol for a minimum of 15 minutes. Needle rinses of residual material in the needle and syringes were made (for cell block preparation) using acetic Acid Alcohol Formalin (AAF) fixative (95% ethyl alcohol 34 ml + formalin 4 ml + Glacial acetic acid 2ml) from each patient after preparing conventional smears.

Laboratory procedures

The suspension (from needle rinses) was centrifuged for 10 minutes at 2000 rpm to obtain cell buttons. The cell buttons were re-suspended in AAF fixative and centrifuged for a further 10 minutes at 3000 rpm. The tubes containing the cell buttons were set aside for 4 to 6 hours. The cell buttons were then removed and wrapped in lens papers and were processed

as histology specimens using an automatic tissue processor. Briefly, the cell buttons were dehydrated using ascending grades of alcohols; 50%-70%-100%, cleared in 3 changes of Xylene, infiltrated and embedded using paraffin wax. From each block, two cell block sections between 3-5µm were prepared. These were stained using Papanicolaou staining and Hematoxylin & Eosin staining procedures respectively. Conventional smears were also stained using the same staining procedures.

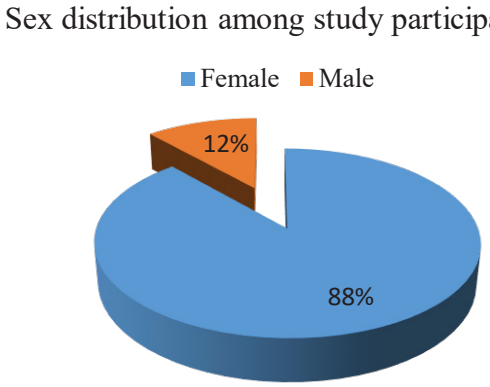
Both the conventional smears and modified cell blocks were processed at KNH cytology and UoN histology laboratories. The quality and suitability of both conventional smears and modified cell block sections were assessed using Modified Mair *et al* Scoring System which assesses background obscuring material, degree of cellular degradation, cellularity, and finally architectural and cellular arrangement[6]. A cumulative score between 0 and 8 was obtained from each specimen and categorized as diagnostically inadequate, Category 1 (score 0), or diagnostically adequate, Category 2 (score 1-8). Both the cell blocks and conventional smears were reported using the Bethesda System of Reporting Thyroid Cytopathology (BSRTC)[7].

RESULTS

Demographic characteristics of the study participants

A total of 52 patients with thyroid gland lesions were enrolled in this study. The patients age ranged from 21 to 73 years with age mean of 41 years. The majority of patients were females accounting for 88% (46/52) of total participants (Figure 1).

Figure 1: Distribution of study participants by gender



When considering participants in various age groups, out of the 52 participants, 8 were between 20 to 29 years representing 15.4% of the total. Majority of the participants 36.5% (19/52) were in their third decade of life followed by 23.1% (12/52) of participants in their fourth decade. Thirteen (25%) participants were aged 50 years and above.

Duration and location of the thyroid lesions

Out of the 52 participants, nine participants (17.3%) had the thyroid nodule for duration of less than one month. The majority of patients (40.4%) had thyroid gland enlargement for the period of one to twelve months. Fourteen participants (26.9%) had thyroid nodules for duration of more than 12 months while 15.4% of the participants were not able to recall its duration. In this study a majority of patients (64%) had centrally placed thyroid enlargement while the left and right sided lesions accounted for 21% and 15% respectively.

Background and cellular patterns/characteristics of the conventional and modified cell block methods

Using Mair *et al*., scoring system, 34.6% of cases had minimal background obscuring material on modified cell block method as opposed to 15.4% on conventional smears. Conventional smears had marked background obscuring in more than half of the cases (53.8%) while on modified cell block method, the cases with marked obscuring was 29%. Both conventional smears and modified cell block were comparable on moderate background obscuring which was 30.8% and 36.5% respectively. On cellularity, modified cell block cases were rated to have marked cellularity in 44.2% as compared to 21.1% in conventional smears. Moderate cellularity was scored in 40.4% of conventional smear. Minimal cellularity was seen in 38.5% and 28.9% in conventional and cell block cases respectively. Degree of cellular degeneration was comparable across all categories on both modified cell block and conventional smears. Forty percent of modified cell block cases were categorized to have marked architectural and cellular arrangement as compared to 19% on conventional smears. Conventional smears had minimal architectural arrangement in 48.1% of the cases as compared to 34.6% cases on modified cell block. On the other hand, moderate architecture and cellular arrangement were 32.7% and 25% on conventional smears and modified cellblock respectively. Excellent architecture was 19.2% on CS while on modified cell block, it was 40.4% (Table 1).

Table 1: Cytomorphologic features of conventional smears and modified cell blocks based on Mair *et al* scoring system

Criteria	Conventional smears (%)	Modified cell block (%)
Background		
Marked	53.8	28.8
Moderate	30.8	36.5
Minimal	15.4	34.6
Cellular degradation		
Marked	25	28.8
Moderate	7.7	5.8
Minimal	67.3	65.4
Cellularity		
Minimal	38.5	28.9
Moderate	40.4	26.9
Marked	21.1	44.2
Architecture arrangement		
Minimal	48.1	34.6
Moderate	32.7	25
Excellent	19.2	40.4

Cytodiagnosis of thyroid lesions on both conventional smears and modified cell block

Using the Bethesda System of Reporting Thyroid cytopathology, unsatisfactory results were reported in 25% cases of conventional smear and slightly higher (28.8%) in modified cellblock section. Among the 13 samples that were unsatisfactory on conventional smears, six samples were satisfactory on modified cell block method preparation. Out of two cases of lymphocytic thyroiditis that were reported on conventional smears, modified cellblock preparations reported one case as Hashimoto's thyroiditis and another one was unsatisfactory.

Colloid goiters were reported in 69.2% and 67.3% in conventional smears and modified cell block cases respectively. One case that was reported as atypical cell of undetermined significance on conventional smear was unsatisfactory on modified cellblock. One case of colloid goiter on conventional smear was reported as Hürthle cell neoplasm on modified cellblock. There was no case of malignant/suspicious for malignant on both conventional and modified cell blocks.

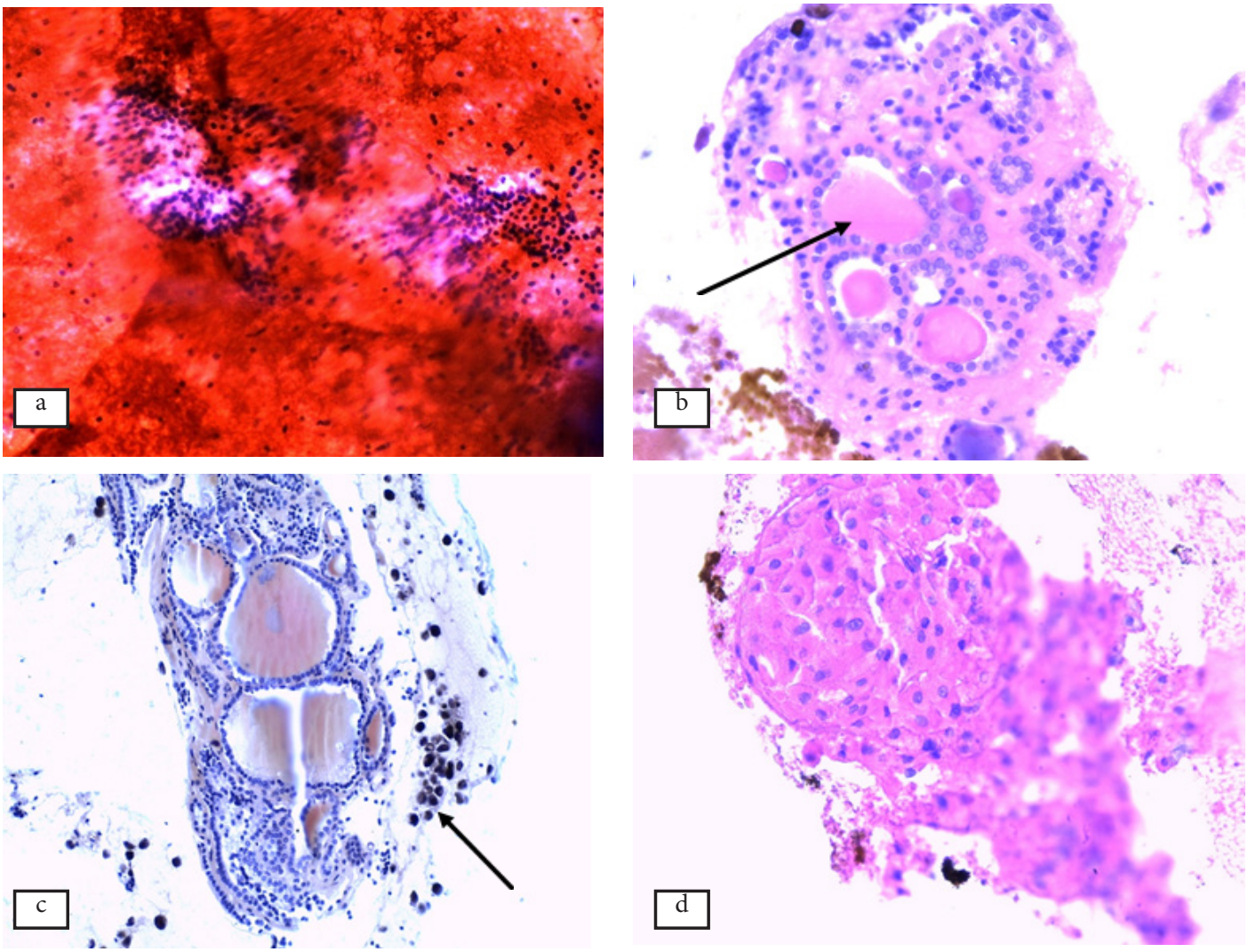
Table 2: Comparison between the diagnosis of thyroid lesions on conventional smears and modified cell blocks

Results	Conventional Smear No. (%)	Modified cell block No. (%)
Unsatisfactory	13 (25)	15 (28.8)
Thyroiditis	2 (3.8)	1 (1.9)
Colloid goiter	36 (69.2)	35 (67.3)
Hürthle cell neoplasm	0 (0.0)	1 (1.9)
ACUS	1 (1.9)	0 (0.0)
SFN	0 (0.0)	0 (0.0)
Suspicious for Malignant	0 (0.0)	0 (0.0)
Malignant	0 (0.0)	0 (0.0)
Total	52 (100)	52 (100)

Comparing the two methods on McNemar's Chi Square test, there was no statistically significant difference in the two methods at *p-value* of 0.791.

Figure 2: Photomicrographs showing microscopic results on conventional smears and modified cell blocks

Photomicrographs of conventional smears and modified cell block sections



(a) Conventional smear showing benign follicular cells obscured by blood cellular elements x10, H/E; (b) Modified cellblock section showing benign follicular cell forming macrofollicles with colloid (arrow) x40, H/E; (c) Modified cell block section showing benign follicular cell forming macrofollicles with colloid and hemosiderin laden macrophages (arrow) x10 PAP and (d) Modified cellblock section showing Hürthle cells x20, H/E.

DISCUSSION

In this study a total of 52 patients were recruited at Kenyatta National Hospital FNA clinic. The age of patients ranged from 21 to 73 years with mean of 41 years. This age range and mean is slightly higher compared to the study done by Gupta *et al*[1] who found that the age range was from 22 to 58 years with mean age of 38.7 years. However, in another study done by Raafat *et al*.[8] age range was from 15 to 65 years. This shows that thyroid nodules are found within a large age range. In this study we found that majority of patients (37%) were in their third decade of life followed by 23% of patients in their fourth decade. These findings are in agreement to those documented by Gupta *et al*.[1] and Zarika *et al*.[9] who found out that the majority of patients were in their third decade of life. The majority (46/52) of participants in this study were female accounting for 88% of total participants with a male to female ratio of 1:7.7. This finding agrees with Zarika *et al*[9]. who in a similar study, found that 86.67% of their study participants were females.

More than half (53.8%) of conventional smears samples had marked obscuring background on Mair *et al* scoring system while only 28.8% were reported to have marked obscuring background on modified cell block sections. The main background obscuring material was blood cellular elements because thyroid gland is a highly vascular organ. However, 34.6% of modified cell block cases had a clear background (minimal obscuring background) as compared to 15.6% of conventional cases. This difference can be accounted for since modified cell block fixative fluid used had acetic acid which lyses red blood cells. Nithyananda *et al*[5]. also found that cell block sections showed clearly recognizable normal and abnormal cells with minimal shrinkage after using AAF fixativ.

Marked cellularity was scored in 44% (23/52) and 21% (11/52) cellularity in modified cell block cases and conventional smear cases respectively. Marked cellularity in cell block could be due to the clots in the needle hub which trap cell and these clots were removed for cellblock preparation. This agrees with Basnet *et al*[10] who showed that cell block method allows the recovery and processing of minute amounts of cells hence high cellularity.

In this current study, the degree of degradation was comparable on both techniques with minimal degradation rate of 67.3% and 65.4% on conventional and modified cell block cases respectively. This minimal cellular degradation which was more than half of all cases in both methods was achieved because samples were fixed immediately after collection. This result agrees with Nithyananda *et al*[5]. However, Khan *et al*.[11] showed a contrary result of marked cellular degradation on cell block than conventional smears. In their study material for cell block was aspirated after 3 to 4 times of the aspirations for the conventional FNA and this may have contributed to a more traumatized and poorly preserved specimen.

Modified cell block showed 40.4% marked architecture and cellular arrangement compared to 19.2% in conventional smears. Forty eight percent (25/52) had a minimal architectural arrangement on conventional smears as compared to 34.6% in modified cell block. This agrees with Brown *et al.*[12] and Kulkarni *et al*.[13] who concluded that cell block preserve architecture pattern with excellent nuclear and cytoplasmic details.

Twenty five percent of conventional smears cases were reported as unsatisfactory. This was a high unsatisfactory rate. The majority of studies have shown that unsatisfactory rate ranges from 2% to 20%[14]. The high unsatisfactory rate in this study could be because almost all FNA procedures were done by pathology residents who had variable experience. However, six cases out of 13 unsatisfactory cases on conventional smears were satisfactory on modified cell block reducing the unsatisfactory rate of thyroid FNA cytology from 25% to 13%. In a similar study done by Vance *et al*[15], they found out that cell block preparation reduced unsatisfactory rate from 16.4% to 9.3%. During the FNA procedure, aspirated material could clot in the needle hub which was difficult to flush onto the slide for conventional smears. This clotted material in the hub was removed and used for cell block preparation. This could explain why six samples which were unsatisfactory on conventional were satisfactory on modified cell block.

The majority (73.1%) of the cases were reported as benign. This is high as compared to Vance *et al*.[15] who reported 55.7% of cases in their study as benign. However our current study compares very well with Cibas *et al*.[14] who documented that 70% of all thyroid FNA are reported as benign. Overall, modified cell block aided in diagnosis of 8 cases (15.4%) which is in concordance with Brown *et al.*[12] and Thapar *et al*[16] who showed in their studies that cell block aided in diagnosis of 14% and 13% of cases respectively.

CONCLUSION

This study has not demonstrated statistically significant differences in the diagnostic methods. However, modified cell block preparation provided additional information which was helpful in establishing new diagnosis and also decreased the unsatisfactory rate of thyroid FNA cytology from 25% to 13%.

RECOMMENDATION

Modified cellblock should be considered as an adjunct test to the conventional smear on thyroid (FNA cytology) especially in suspected thyroiditis and neoplasms. In resource constrained settings, the cost implications should guide in the selection of the method to use. Proper training and monitoring of the clinicians performing FNA procedure should be provided in order to reduce the unsatisfactory results in thyroid aspirates.

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Solid pseudo-papillary neoplasm of the pancreas: a case report of a rare tumour

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ABSTRACT

We present a case report of a 28-year-old lady who presented with a tumour in the pancreas. Physical examination revealed an abdominal swelling which had cystic and solid components on imaging. The tumour was resected at surgery and histopathological examination together with immunohistochemistry revealed features of Solid Pseudopapillary Neoplasm (SPN) of the pancreas. These tumours are rare, of low malignant potential and it is important to diagnose them accurately, distinguishing from other pancreatic tumours due to relatively good prognosis.

CASE PRESENTATION

A 28 year old lady presented with a history of abdominal pain, nausea, occasional vomiting and fever. She had also reported weight loss over the preceding year. A physical examination was conducted and it revealed an abdominal swelling.

Subsequently, an abdominal Computerized Tomography scan (CT scan) was conducted and it revealed a mass which was arising from the pancreas. The mass had varying solid and cystic components in addition to areas of haemorrhage. Administration of intravenous contrast showed enhancing solid peripheral areas and central cystic areas. Peripheral calcifications of the solid tumour areas were also noted.

Surgery was performed and the pancreatic mass was wholly resected, after which it was submitted for histopathological examination.

On gross examination the pancreatic mass was well circumscribed and was separated from the adjacent pancreatic tissue by a fibrous pseudocapsule. Its cut surface had a variable appearance which included solid, cystic, haemorrhagic and necrotic areas.

Histopathological examination showed a tumour with a combination of solid components consisting of pseudopapillae which had fibrovascular cores and cystic areas which had haemorrhage. The pseudopapillae were lined by small cells with amphophilic cytoplasm and rounded to ovoid nuclei. The nuclei showed grooving. Mitoses were rare. There were Periodic Acid Schiff (PAS) positive intracytoplasmic and extracytoplasmic hyaline globules. The surrounding stroma showed foamy marcophages and cholesterol clefts. Ancillary tests done revealed a wide range of immunophenotypes. The tumour cells were positive for Vimentin, CD99 (paranuclear dot-staining), CD10, E-cadherin, CD56 and cyclin-D1.

Based on the histological and immunohistochemical profile, a diagnosis of Solid Pseudopapillary Neoplasm (SPN) of the pancreas was established. While the immunohistochemical profile done was limited by cost, we feel the panel used was sufficient in correlation with morphology to arrive at the diagnosis.

Figure 1: Light microscopy (20x and 40x) showing histopathological features of SPN

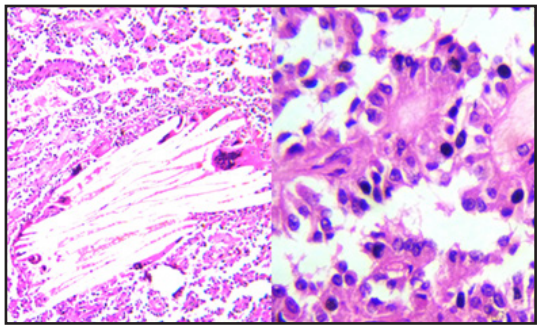

Figure 2: Immunohistochemistry showing nuclear overexpression of cyclin-D1

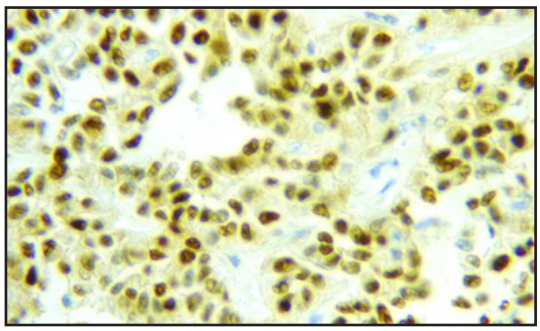

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Figure 3: Tumour cells showing positivity for CD10

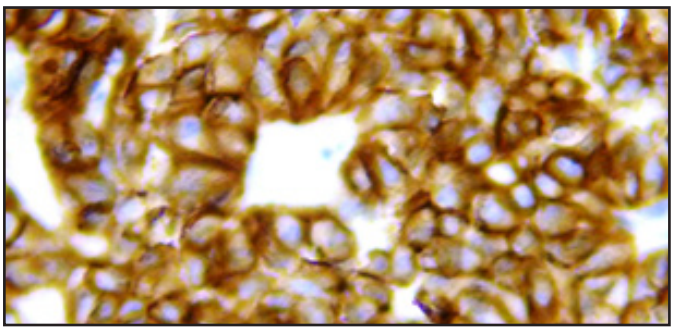


Figure 4: Tumour cells positive for vimentin

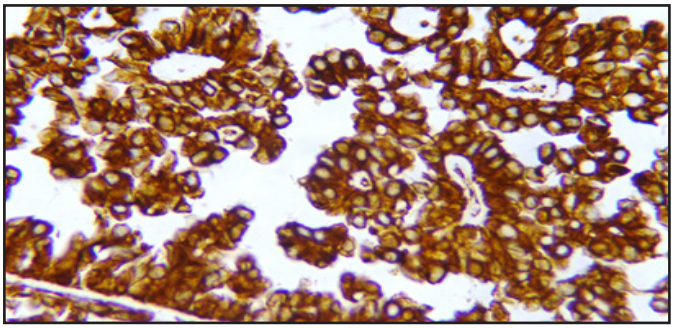


Figure 5: Tumour cells showing paranuclear dot-like positivity for CD99

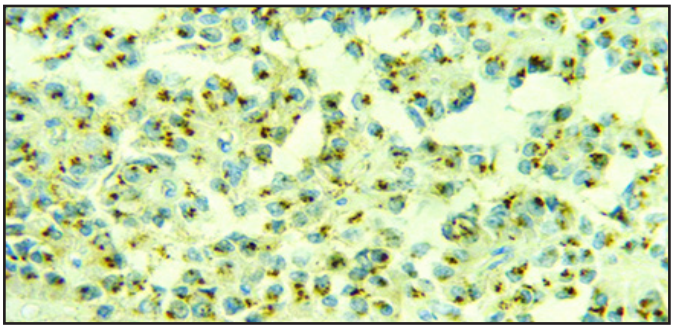
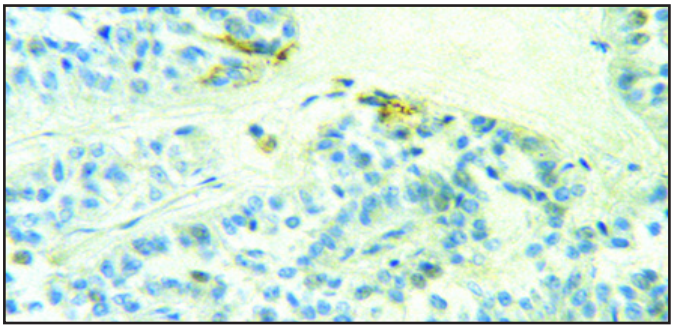


Figure 6: Membrane loss of e-cadherin by tumour cells



DISCUSSION

Solid Pseudopapillary Neoplasm (SPN) of the pancreas is rare, accounting for less than 2% of pancreatic tumours[1]. It is predominantly seen in young women (over 90% of tumours reported in women) with a reported average age of 22 years[2]. The tumour was initially described by Franz in 1959[3] and has been known by other names which include papillary cystic carcinoma, solid and papillary neoplasm, solid and cystic tumour and low grade papillary neoplasm.

The patients who have this tumour present with variable symptoms, most of which are not specific. Some of the tumours are found incidentally[1].

Imaging: These tumours are often detected by imaging, mostly during examination of patients who present with non-specific abdominal symptoms. CT scan and Magnetic Resonance Imaging (MRI) are the preferred modalities, where the tumours are found to be well circumscribed, encapsulated and heterogeneous with areas of haemorrhage and cystic degeneration. The solid tumours and solid parts of solid-cystic tumours are T2 hyperintense and T1 hypointense with progressive enhancement[4].

Pathological findings: Minimally invasive procedures such as CT-guided Fine Needle Aspiration (FNA) or CT guided core biopsies may be useful in diagnosis[5,6]. Cytological smears tend to be cellular with delicate papillary fronds which are lined by bland cells which have moderate cytoplasm and show nuclear grooving[7].

Tumours which have been surgically resected are usually well circumscribed and encased by a fibrous pseudocapsule. They have variable sizes and appearance with solid, cystic and haemorrhagic cut surfaces[2,7]. Microscopically the tumours show solid and cystic components with fibrovascular cores which are lined by cells with amphophilic cytoplasm, ovoid nuclei and have nuclear grooves. Foamy macrophages, cholesterol clefts and haemorrhage are often seen. The tumours show hyaline globules which are PAS positive[7,8].

Immunohistochemistry: The SPNs show overexpression of cyclin-D1, consistent with disruption of the Wnt signaling pathway. They are also positive for CD56, CD10, estrogen receptor, progesterone receptor, vimentin, beta catenin, alpha 1-antichymotrypsin, alpha 1-antitrypsin, SOX 11, androgen receptor, claudin7, claudin5, NSE, synaptophysin, cytokeratin, CD99 and loss of membranous e-cadherin[9].

Molecular pathology: There is evidence of *CTNBB1* gene mutation at exon 3 (beta catenin mutation) in most SPNs. This mutation results in accumulation of cytoplasmic beta catenin, which complexes with Tcf/lef and translocates to the nucleus. This results in activation of oncogenes such as cyclin-D1 and transcription of molecular targets of Wnt. These tumours do not show mutations associated with other pancreatic neoplasms such as SMAD4, Tp53 and KRAS suggesting distinctive histogenesis[9].

Treatment and prognosis: The management of choice in SPNs is complete surgical resection. Reports indicate that even with locally advanced tumours, surgical outcomes are good[10].

The Solid Pseudopapillary Neoplasms (SPN) are classified as tumours of low malignant potential/ low-grade tumours. The probability of metastasis is low in these tumours and overall five and ten year survival rates are over 95%[11].

CONCLUSION

Solid Pseudopapillary Neoplasms (SPN) of the pancreas are rare tumours which are considered to have low malignant potential. Their origin is not known. These tumours have overall good outcomes, however, select cases may develop metastasis and result in fatality. It is therefore important to accurately diagnose and classify these tumours.

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Non-malignant hyperleukocytosis

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ABSTRACT

Hyperleukocytosis is a White Blood Cell count (WBC) of ≥ 100 X 10^9/l in the peripheral blood. This is a haematological emergency, usually associated with various haematological malignancies: leukemia (acute and chronic) and myeloproliferative neoplasms. It is very rare in non-malignant conditions. This is a case report of 11 years old boy who presented to Kenyatta National Hospital (KNH) with a WBC count of 102x10^9/l not associated with a haematological malignancy or a solid tumour. The patient was managed on antibiotics and other supportive therapy with improvement.

This hyperleukocytosis associated with bronchitis and presenting mainly with generalized lymphadenopathy. The condition was effectively treated with 2nd line antibiotics, supportive IV fluids and antipyretics.

Key word: Hyperleukocytosis, Leukostasis

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INTRODUCTION

White Blood Cells (Leukocytes) are produced in the bone marrow from the pluripotent stem cell then go on to differentiate into granulocytes (eosinophils, monocytes, neutrophils and basophils) and lymphocytes (plasma cells, natural killer cells and T-Cells)[1]. Leukocytosis is an elevated leukocyte count on the haemogram above the set reference for age. A marked elevation above 40,000X10^9/l is designated as a leukemoid reaction. A leukocytosis of ≥50,000x10^9/l is mostly due to haematological malignancies such as leukemia acute or chronic as well as a myeloproliferative disease. It can also be as a result of a para-neoplastic process in other non-haematological malignancies such as solid tumours[2]. In rare cases, hyperleukocytosis may be related to non-malignant causes[3]. The few cases that have been reported are related to pertussis infection[4] hemolysis and others[5].

The number of circulating WBC is dependent on the size of the precursor cells, their pool in the bone marrow, the rate at which they are released into the circulation, the size of the marginated pool and the rate of WBCs extravasation from the circulation into the tissues as well as their rate of clearance[1].

The pathophysiology of hyperleukocytosis is related to leukostasis where the increased blood viscosity leads to poor perfusion of tissues resulting in hypoxia and ischemia with end organ damage. In cases of acute myeloid leukemia like the monocytic and monoblastic types, the symptoms related to leukostasis may appear at a lower WBC count of 50,000x10^9/l and this is related to the large size of myeloblasts. The latter induce inflammation through their ability to produce cytokines, promoting cell adhesion molecules and enhancing the leukostasis[6]. These are the Leukocyte Adhesion Molecules (LAM): the Selectins (E, P and L-Selectins), integrins, immunoglobulin family molecules (intra-cellular adhesion molecule, the vascular adhesion molecules) and mucin-like glyco-proteins. These molecules together with cytokines (interleuki-1, tumour necrosis factor and chemokines) play an integral role in the recruitment of leukocytes from the vasculature to the site of inflammation: extravasation[7].

Leukostasis is an emergency and is managed by leukocytapheresis as well as other supportive therapy such as intra-venous fluids administration and cytoreduction[8].

CASE REPORT

An 11 year old male presented with bilateral neck swellings, drenching night sweats, intermittent fevers with abdominal swelling for 2 weeks. Two weeks prior to arrival at the hospital, he had a mild cough, and fever that were treated at a local health facility with oral medications: antibiotic and antipyretic. Both were in tablets forms that he took for 5 days. The fever and cough subsided. Shortly after completion of the treatment, he developed

a skin rash, papular, itchy, generalized rash with bilateral neck swelling associated with fever, sweating and abdominal swelling. These new symptoms appeared within one week. He had no known history of allergies or contact with a case of tuberculosis and had received his vaccinations as per schedule. He had no other admission reported in the past.

On examination, he was in a fair general condition, afebrile, had generalized lymphadenopathy and a healing dry papular rash on the trunk and extremities sparing the mucosal linings. There was hepato-splenomegaly on abdominal examination. Other systemic examination findings were normal.

Other investigations done were an abdominal ultrasound that reported the presence of a hepato-splenomegaly; while a chest radiograph showed bilateral peri-hilar and basal peri-bronchial thickening with increase in lung markings likely due to bronchitis.

An initial haemogram done in the previous facility showed a WBC of 97,650x10^9/l with normal platelets count (300x10^9/l) and haemoglobin level of 11.2 g/dl. The liver and renal function tests were within acceptable limits. He was referred for further investigation to rule out a leukemic process.

A repeat haemogram was done at the same setting as the peripheral blood film and the bone marrow aspirate. The haemogram showed a WBC of 102x10^9/l with neutrophilia (26.7x10^9/l), lymphocytosis (16.8x10^9/l) and monocytosis (59.2x10^9/l) while the eosinophils (0.05x10^9/l) and basophils (0.57x10^9/l) were within acceptable ranges. The peripheral blood film showed normocytic, normochromic Red Blood Cells (RBCs), marked leukocytosis with atypical mononuclear cells of 9% and a satisfactory count of platelets. The bone marrow aspirate cytology showed a hyper cellular marrow with a high myelo-erythroid ratio of 18:1, macronormoblastic erythropoiesis, a left shifted granulopoiesis and adequate megakaryocytes. The plasma cells and lymphocytes were unremarkable. The conclusion was that of reactive settings in the marrow.

Serology tests done were for: Hepatitis A (Abs), Hepatitis B (Surface Ag), Hepatitis C (Abs), VDRL and CMV; all were negative. Serum Lactate Dehydrogenase (LDH) and uric acid levels were within acceptable ranges for age. Blood and urine culture tests were negative for growth of pathogens. The histology from one of the cervical lymph nodes reported: Reactive follicular lymphoid hyperplasia.

He was started on cefuroxime axetil for 5 days. The fever subsided, the skin rash dried up and the tenderness from the lymphnodes reduced but they remained enlarged and the hepatosplenomegaly was persistent. Ten days later, he had a spike in the body temperature (38.4^{0}C) and was treated with piperacillin (5 days), vancomycin (5 days) and azithromycin (3 days). By the 4th day he was afebrile, with a reduction in the lymphnodes size, the liver was barely palpable and the splenomegaly was reduced from 6cm BCM (Below costal margin) to 3cm BCM. The supportive treatment with IV fluids, analgesics and antipyretics was ongoing throughout the treatment period. On the haemogram, the WBC count came down to 58x10^9/l with a marked reduction in neutrophils to 11.3x10^9/l as lymphocytosis (40.3x10^9/l) persisted while monocytes were 6.86x10^9/l. Haemoglobin level and platelets counts remained within the reference range for age. The WBC count kept a downwards trend to 17,800x10^9/l at the time of discharge. The neutrophils and monocytes were within acceptable ranges. The lymphocyte count kept downward trend. The other parameters (Serum LDH and uric acid levels) were within acceptable values with no new symptoms reported (Table 1).

Table 1: Haemogram values recorded during hospitalization

	Baseline	After 2 days	After 6 days	After 36 days
WBC (Total) x10^9/L	102.4	95.1	58	17.8
Neutrophils	26.7	14.9	11.3	4.1
Lymphocytes	16.8	64.8	40.3	13.1
Monocytes	59.2	14.8	6.8	0.4
Eosinophils	0.05	0.04	0.06	0.00
Basophils	0.57	0.5	0.2	0.04
RBCs x10^{12}/l	4.1	4.1	3.9	4.2
Haemoglobin (g/dl)	10.7	10.6	10.2	10.1
Platelets x10^9/l	247	254	274	350

DISCUSSION

Hyperleukocytosis is rarely associated with non-malignant conditions. The documented cases have been mostly associated with pertussis and hemolysis due to haemoglobinopathies or in new-borns[5].

The patient in the presented case had four major findings: generalized lymphadenopathy, hepatosplenomegaly, hyperleukocytosis and bronchitis on the chest X-ray. In infectious settings, leukocytosis appears as a result of a stimulus that up-regulates the factors controlling the proliferation and differentiation of WBC. These are factors such as: growth factors (granulocyte—colony stimulating factor), adhesion molecules (e.g. CD11b/CD18), and cytokines (Il-1, IL-6, IL-8 and TNF)[3].

Leukocytosis may involve one or more portions of the white blood cells. The causes vary depending on the portion of WBC that is elevated. Neutrophilia is commonly associated with sepsis, infection, inflammation, use of some medications and inherited conditions[9]. With the hyperleukocytosis as an overproduction of white blood cells from the bone marrow; this process is likely to involve the other haematopoietic organs such as the lymph nodes, the liver and the spleen resulting in "extra-medullary haematopoiesis". This explains the presence of enlarged lymph-nodes with follicular lymphoid hyperplasia and hepato-splenomegaly[10].

Acute bronchitis in children is due to viral infections in over 90% of cases while bacterial infections and other causative factors such as air pollutants or mixed causes constitute around 10%[11].

Though this patient had an initial exposure to medication he received for the treatment of a respiratory tract infection and developed a dermatological reaction, the lack of an eosinophilia excludes the likelihood of an allergic reaction.

In the face of a leukocytosis, morphological examination of the peripheral blood film and the bone marrow is adequate to exclude a haematological malignancy as it was in this case in addition to the serum LDH and uric acid levels within acceptable limits.

This hyperleukocytosis had a mixed cellular inflammatory response with: neutrophilia, monocytosis and lymphocytosis. The positive findings on the chest X-ray point towards an infective process. Exposure to pathogen(s) activates the immune response with cellular changes. The inflammatory cells responding to the stimulus get mobilized from the peripheral blood to the site of inflammation. Although bronchitis in children is mostly due to viral infections, superimposed bacterial infections are very common and this could explain the mixed pattern of infectious response on haemogram[11].

As a haematological emergency, leukostasis is managed by leukapheresis a facility that is lacking in our institution; this patient received a treatment based on empiric antibiotherapy to control the infection and supportive therapy consisting of fluids and analgesics. The response with reduction in WBC count is supporting our hypothesis of a reactive process as opposed to a malignant one where a response would be unlikely.

Where available, further tests such as cytogenetic studies for CAD1, CD11a/CD 18 to exclude a congenital deficiency of leukocytes adhesion molecules as seen in familiar cold auto-inflammatory syndrome would be valuable[9].

RECOMMENDATIONS

Further tests such as cytogenetic abnormalities to exclude a congenital deficiency of leukocytes adhesion molecules by the lack of CD11b/CD18 as well as the assessment for familiar cold auto-inflammatory syndrome through CAD 1 mutation.

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Abstracts presented during the 13th Biennial Scientific KACP Conference

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Content

A one year retrospective study of the seroprevalence of transfusion transmissible infections among blood donors at the Nairobi Hospital

Kairu G

ABSTRACT

Introduction: Blood transfusion is essential in the management of many clinical diseases and conditions and at the moment there is no substitute product that can completely replace it. However, the potential risk of Transfusion Transmissible Infections (TTIs) remains high. The WHO recommends mandatory screening for the following TTIs, HIV-1 and HIV-2, hepatitis B, hepatitis C and syphilis. Screening for other infections, such as malaria or chagas disease, is based on local epidemiological data. At The Nairobi Hospital we have two types of donors, voluntary and replacement. donors are screened for all the mandatory TTIs and malaria.

Aim: To determine the seroprevalence of TTIs in healthy blood donors at the Nairobi Hospital.

Methods: A retrospective review of blood donor records over a one-year period from June 2018 to June 2019 was done. Using immunoassay methods donors were screened for hepatitis B surface antigen (HBsAg), antibodies to HCV, syphilis, malaria, HIV Type 1 and 2 and P24 antigen.

Results: A total of 6,982 donors were included in the study, 3.41% of all donated blood was positive for at least one TTI. Seroprevalence for HBV, HIV, HCV, VDRL, and malaria was 1.07%, 0.60%, 0.50%, 0.60%, and 0.52% respectively.

Conclusion: The study shows that hepatitis B virus is the most prevalent TTI, which is in keeping with other studies done in Kenya, while the overall seroprevalence of TTIs remains low. Strict donor selection and TTI screening is key in maintaining a safe donor pool.

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A review of physician requested peripheral blood films at the Aga Khan University Hospital Nairobi, Kenya

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ABSTRACT

Background: A Peripheral Blood Film (PBF) is a cytological method that is both simple to prepare and can provide invaluable diagnostic information. The Aga Khan University Hospital receives requests for PBF review from both within and without the hospital. The bulk of PBF examination however is prompted by the laboratory on samples that are flagged by the haematology analyzer.

Methodology: We examined PBFs requested by physicians at the Aga Khan Hospital for the period from 1st June 2018 to 18th August 2019.

Results: Overall, 55% of all requests were external requests. The bulk were from Outreach clinics in Nairobi, followed by Aga Khan Mombasa and Kampala and those clearly indicated as being from other hospitals (28%, 24%, 10% and 12% respectively). Sixty percent of requests provided no history and 11% provided indications that were non-haematological. The most common morphological diagnosis was that of a reactive picture accounting for a quarter of all diagnoses. Twenty percent of all films were of normal morphology while Iron deficiency anaemia accounted for 10% of all morphological diagnoses. Ten percent of the films showed features suggestive of a neoplastic disorder. Fourteen out of two hundred and seventy five films had storage changes and 9/14 of these were deemed non-diagnostic. Nine percent of films had no definitive conclusion from the examiner. Therapeutic advice and a follow-up algorithm were provided for 13% of cases.

Conclusion: PBF examination should be made a reality to remote hospitals either by training of local staff or the strengthening of a referral system. We also recommend the use of a guided reporting system to optimize benefit.

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Application of a pathology-supported genetic testing strategy for return of research results to Kenyan breast cancer patients

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ABSTRACT

Background: Obtaining informed consent from study participants and disseminating the findings responsibly is a key principle required for ethically conducted clinical and genetic research. Reports from African researchers providing feedback on insights gained during return of whole exome sequencing results to study participants in resource-limited settings is lacking.

Objective: The empirical process used to fill this gap in relation to BRCA1/2 mutation screening provided unique insights incorporated into a pathology-supported genetic testing algorithm for return of research results to Kenyan breast cancer patients.

Methods: The informed consent form approved by the Moi Teaching and Referral Hospital was adopted from a translational research study conducted in South Africa. A total of 95 female and two male breast cancer patients were enrolled in the study from 2013 to 2016. Immunohistochemistry (IHC) results of Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal growth factor Receptor 2 (HER2) status were obtained from hospital records. DNA of patients with a family history of cancer was extracted from saliva and sequenced to screen for pathogenic mutations in the BRCA1/2 genes.

Results: Data on IHC used as a proxy for molecular subtype were available in 8 of 13 breast cancer patients (62%) with a family history of cancer. Five BRCA1/2 variants of uncertain clinical significance were detected, as well as a pathogenic BRCA2 mutation (c.5159C>A; S1720*) in a female patient eligible for return of research results.

Conclusion: Detection of a BRCA2 mutation in a patient with familial breast cancer, frequently associated with hormone-positive breast carcinoma as reported in this case, led to a high level of confidence on which to base risk management in future. Implementation of new technologies alongside standard pathology provides a practical approach to the application of genomic medicine in Africa.

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Automated indirect immunofluorescence microscopy in antinuclear antibody testing: Is the time ripe for artificial intelligence to take over?

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ABSTRACT

Background: Indirect immunofluorescence microscopy using Human Epithelial cell line-2 (HEp-2) or acceptable derivatives such as HEp-2010 cells, is regarded as the gold standard for screening for antinuclear antibodies (ANA). Conventional manual microscopy is labour intensive and often lacks proper standardization. As a result, several automated systems have been developed that generate results based on software-driven pattern recognition systems.

Objective: In this study, we evaluated the performance of a fully automated indirect immunofluorescence system (Europattern suite, Euroimmun) in detection of ANA, compared to visual interpretation by a skilled observer as the reference standard.

Methods: A total of 88 patient samples were analyzed by the automated system. Results generated by the software-driven pattern recognition system were compared against visual interpretation made by the skilled observer; the latter regarded as the reference standard.

Results: The software (Europattern) had an overall sensitivity of 88% and a specificity of 66% in discriminating positive and negative results. The software correctly identified antinuclear antibody patterns in 64% of the positive samples. In addition the software was able to correctly estimate the titer in 57% of the positive samples. However, the system incorrectly identified 42% of the positive samples as mixed patterns with the majority of these (51%) classified as having cytoplasmic patterns in addition to nuclear patterns

Conclusion: The results obtained by the software were in good agreement with the visual interpretation by the observer. However, visual interpretation by a skilled observer is critical as the pattern recognition systems are still evolving and require optimization to suit each laboratory's requirements. All in all, automated indirect immunofluorescence evaluation offers the prospects of a less arduous yet effective tool for ANA testing.

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Cancer prevention and management at county level: The Machakos County experience

Nzioka AK

ABSTRACT

Background: Article 43 (1) (a) of the Kenyan constitution states that every person has the right to the highest attainable standard of health which includes the right to health care services[1]. Healthcare services include diagnostic pathological services. Machakos County is one of four Universal Health Coverage (UHC) pilot counties. UHC embodies three objectives; Equity in access, affordability and quality health care services[2]. In Kenya, cancer is the third leading cause of mortality accounting for 7% of overall mortality rate[3]. According to International Agency for Research on Cancer, Globocan 2018[4] 47,887 new cancer cases were diagnosed in 2018. However, authentic local data on the cancer burden at county level is lacking.

Objective: The aim of this abstract is to outline the journey Machakos County has undertaken in its cancer prevention and management programme under UHC.

Setting: Machakos County, a devolved government in Kenya and one of the four UHC pilot counties.

Machakos County cancer prevention and management programme: Using the Hub and Spoke referral model where level 5 and 4 hospitals act as the Hubs while the level 1, 2 and 3 facilities serve as the spokes, the county government of Machakos has rolled out the following 7 key strategies;

A. Human resource for health. Several specialists including pathologists, surgeons, gynecologists, oncologists, oncology nurses, radiologists, cytologists, histotechnologists and cytotechnologists were recruited.
B. Health education and promotion on cancer prevention including;
   - Talk shows - Public barazas, FM stations, TV, social media
   - Sensitization outreaches in the 40 wards of Machakos County using Beyond zero truck
   - Vaccination against known oncogenic viruses (High risk HPV) ongoing
   - Training 2,530 CHVs on basic cancer information and early signs and symptoms
C. Cancer screening at all levels of care. This includes;
   - Self and clinical breast examinations
   - VIA/VILI and Pap smears for cervical cancer
   - Mammography for breast cancer
   - Digital rectal examination and Prostatic Specific Antigen (PSA) test for prostate cancer
   - Fine needle aspiration for solid body masses
   - Fluid cytology
   - FHG and PBF for blood cancers
   - Occult blood for colonic cancer
D. Cancer diagnosis using cytopathology, histopathology, tumour markers, molecular studies and radiology and imaging modalities.
E. Cancer treatment strategies including surgical oncology, chemotherapy, cryotherapy, nutritional support and blood transfusion services.
F. Palliative care encampasing pain management, blood transfusion services, nutritional support and counselling services.
G. Cancer research including setting up of Machakos County cancer registry and collaboration with research institutions and organizations.

Results: In one month, the following results were achieved 301 Pap smears, 69 fine needle aspiration cytologies, 5 BMAs, 95 histopathology specimen reported.

Conclusion: Counties can roll out effective cancer prevention and management programmes.

Key words: Machakos county, Cancer prevention, Cancer management, Universal Health Coverage

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Infectious disease molecular syndromic testing; a Gertrude's Childrens' Hospital experience

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ABSTRACT

Background: Molecular tests, usually targeting one or two organisms per assay, have revolutionized our ability to diagnose infectious diseases. Syndromic testing has come to the forefront and potentially represents the next frontier in diagnostic testing for most infections. At Gertrude's Children's Hospital, molecular testing is done on gastrointestinal, respiratory and the meningitis/encephalitis panels.

Objectives: This study was to describe the profile of molecular testing done on three infectious disease panels for a period of one year and to correlate clinical syndromes/ diagnosis with molecular laboratory tests findings.

Methods: Results and clinical data for molecular testing done on stool, CSF and respiratory samples were analyzed on BioFire® FilmArray®**.** Patients admitted at Gertrude's Children's Hospital aged 0 months to 20 years were included in the study.

Results: A total of 156 CSF samples were included in the study, 19(12.18%) tested positive for a single organism and only 1(0.64%) tested positive for polymicrobial organisms. Respiratory panel, 62 samples were tested, 22(35.48%) tested positive for single organism and 21(33.87%) tested for polymicrobial organism detection. Gastrointestinal panel had 30 stool samples, 7(23.33%) tested positive for single organism and 15(50%) for polymicrobial organisms.

Conclusion: Syndromic infectious disease testing offered a relatively easy way, quicker results to patient diagnosis confirmation. In addition, the panels offered broad coverage, including up to 20 viral and bacterial targets in an all-in-one test. This contributed to management decisions, including infection prevention and control measures and choice of antimicrobials.

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Application of molecular diagnostics to detection of microorganisms in tissues

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ABSTRACT

Background: Molecular testing for microorganisms offers an opportunity for generation of rapid, clinically actionable information. It may be performed in culture isolates, fresh or frozen fluids or tissue, and Formalin-Fixed, Paraffin Embedded tissue (FFPE). The advantage of FFPE is that it can be screened for microorganisms and inflammation suggestive of infection, and the disadvantage is decreased sensitivity due to cross-linking if nucleic acids during fixation. The selection of molecular assay is important and varies from highly targeted (single pathogen) to targeted panel(s) based on syndrome (e.g. pneumonia) rather than pathogen suspicion. Single pathogen testing offers high sensitivity and rapid turnaround but requires a high index of suspicion for a specific microorganism. Multiplex testing has lower sensitivity and specificity and may miss unusual organisms.

Methods: Broad spectrum sequencing interrogating the 16sRNA gene is a powerful tool that provides genus and often species for bacteria. The 16sRNA gene is composed of 1500 base-pairs and contains multiple conserved and hypervariable regions, the latter distinct and unique for different bacteria thus allowing precise identification. A similar approach with 18sRNA or ITS regions may be used for identification of fungi.

Results: The presence of necrotizing granulomas, although highly characteristic of tuberculosis, is nonspecific and may be observed in other infectious and non-infectious entities. Somewhat more useful, but still non-specific, is the identification of Acid-Fast Bacilli (AFB) in tissues by conventional stains (e.g. Ziehl-Neelsen) or immunohistochemistry, which highlight all mycobacteria. Mycobacterial molecular testing is a frequent useful way to identify mycobacteria at the species level, particularly when cultures are negative or not performed. It consists of sequencing of the 16sRNA gene, the hsp65 gene, and the IS6110 gene. Recent studies suggest that a combined approach, with detection of AFB by histochemistry or immunohistochemistry as an initial step, followed by molecular testing, offers the best sensitivity and specificity to detect mycobacterium tuberculosis and identify non-tuberculous mycobacteria. Studies suggest the more AFB visually identified in sections, the higher yield of molecular identification.

Molecular testing is also necessary for microorganisms that can be identified on FFPE to the genus level without problem, but that need speciation for treatment. One example is *Leishmania spp.* The identification of amastigotes in tissue is diagnostic of leishmaniasis. For speciation, culture with isoenzyme analysis and Leishmania PCR are necessary. The CDC (Atlanta, USA) aids with these assays at no cost in the USA.

Conclusions: In summary, targeted assays are useful when a specific pathogen is suspected on clinical or histological grounds. It may be qualitative (e.g. influenza), quantitative (e.g. *Toxoplasma gondii*) or provide specific classification (e.g. mycobacteria, leishmania). Testing should be done in conjunction with clinical decision making (e.g. instituting or modifying antimicrobial therapy). Multiplex/panel testing should be considered if broad differential exists.

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Laboratory accreditation: Bridging the gap/Pathology in Universal Health Coverage

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ABSTRACT

Background: The journey to accreditation has been long and tortuous spanning about 10 years within the practice of laboratory medicine in Kenya.
Methods: Most of the institutions and particularly public sector came from not being trusted to being credible through the acclaimed process of WHO Stepwise Laboratory Improvement Towards Accreditation (SLIPTA). The catalyst for this was demand for credible results in the management of HIV/AIDS in the era of donor funding.
Results: The setting up of the national accreditation body KENAS vide legal notice 55 of CAP 446 set up the ball rolling. Initially set up as a department within KEBS and now fully recognized by other accreditation bodies including ILAC, AFRAC and IAF. KENAS cerebrated 10 years in august 2019 with considerable achievement seen in accreditation of 71 medical laboratories besides others accredited non medical institutions.
Conclusions: The pathologists play an important role, in ensuring and maintaining quality health management systems within public and private laboratories. Pathologists can no longer be spectators in the era of Universal Health Coverage (UHC) we must be active participants.

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Red cell abnormalities in donor blood

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ABSTRACT

Background: During transfusion, components of blood including red blood cells, platelets or plasma are directly administered into the recipient to treat conditions such as anaemia and haemostatic deficiencies among others. Red cell transfusions are indicated for treatment of severe life threatening anaemia. Effective red cell transfusions will positively impact prognosis. The efficacy of a red blood cell unit depends on the amount of blood delivered, the quality of cells and the life span of the unit.
Methods: This descriptive cross-sectional study was done at the Regional Blood Transfusion Centre (Mombasa) and the Technical University of Mombasa, Kenya. Consecutive blood samples were analyzed for selected red cell parameters with the aim of establishing the occurrence of red cell abnormalities in donor blood.
Results: A total of 676 samples were analyzed. A significance level of $p<0.05$ was set for all statistics. The study found that 31.07% of the donor samples had abnormal values. There was a significant variation (t - 0.03, CI 95%) in the total red blood cell count. A significant Pearson's correlation was realized between the osmotic fragility and haemoglobin concentration ($r= .195$, and $p< 0.001$).
Conclusion: The study concludes that a significant proportion of donated red cells had one or more abnormalities.
Recommendation: The study recommends further research on red cell abnormalities and their effect on both the donor and the recipient. It also recommends the development strategies that may be used to enhance the screening out these abnormalities.

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Seroprevalence of human herpesvirus 8 and selected associated factors among blood donors at two blood donor centres in Nairobi, Kenya

Mwangi WM, Rajab J, Githanga JN, *et al*

ABSTRACT

Background: Human Herpesvirus 8 (HHV8) has been isolated as the causative virus for Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman disease. Studies have reported significant risk of HHV8 transmission through blood transfusions in endemic regions that include sub-Saharan Africa. In Kenya the seroprevalence of HHV8 and its associated factors among healthy blood donors has not been documented.
Objectives: To determine the seroprevalence of HHV8 and its associated factors among healthy blood donors in Kenya.
Design: Cross-sectional descriptive study.
Setting: The Regional Blood Transfusion Centre, Nairobi and Blood Transfusion Unit, Kenyatta National Hospital (KNH).
Methods: One hundred and sixty five blood donors who met the preset national guidelines for blood donation were consecutively recruited. A questionnaire was administered for socio-demographic data. Testing was done using HHV8 IgG antibody ELISA technique. Results of routinely screened TTIs (HIV, Hepatitis B and C, syphilis) were obtained from the donor registers.
Results: The HHV8 seroprevalence was found to be 43.6% and there was no statistically significant associations between HHV8 seroprevalence and socio-demographics. Routinely screened TTIs did not reveal any influence on HHV8 status.
Recommendations: Studies are needed to quantify the absolute risk of acquiring HHV8 infection from blood transfusion and determine the need for screening of blood units for HHV8 in endemic areas especially when blood is to be transfused to immunocompromised individuals.

Key words: HHV8, Voluntary, Replacement, Transfusion transmitted infection, Seroprevalence

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Tuberculous aneurysm of the abdominal aorta: Case report

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ABSTRACT

Background: A mycotic aneurysm is a dilation of an artery due to damage of the vessel wall by an infection and account for 2.6% of aortic aneurysms. Mycotic aneurysm secondary to tuberculous infection of the aorta is an even more rare and life-threatening disease. The infrequency with which it is encountered makes it a formidable diagnostic challenge.
Methods: A 29 year old male presented with a history of right loin pain radiating to the back with a concurrent reduction in range of motion of the right hip. On physical examination, he appeared in fairly good general condition, heart rate of 65 beats per minute, respiratory rate of 19 bpm, blood pressure 98/63mmHg and oxygen saturations of 93% on ambient air. Temperature was at 36.6^0C. Pertinent cardiovascular examination findings were peripheral pulses of the right femoral, popliteal, posterior tibial arteries plus the dorsalis pedis artery were not palpable. An abdomino-pelvic CT scan showed features suggestive of a mycotic aortic aneurysm with extension to the right common iliac artery. Surgical intervention ensued during which a mycotic aneurysm was confirmed. Tissue submitted for histology showed several layers of a recent thrombus plus a small reactive lymph node. The overlying wall showed necrosis with palisading histiocytes suggestive of granulomas. Special staining with Ziehl-Neelsen stain was positive for acid fast bacilli.
Conclusion: The case illustrates the importance of early diagnosis as the cornerstone of effective treatment especially in this era of increasing mycobacterial infections.

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