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Preparedness of clinical laboratories to handle pandemics and emergencies: Lessons learned from COVID-19

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Once in a while health systems are faced with disease outbreaks or such other emergencies that outstretch the capacity of clinical laboratories to meet testing demands. Both public and private clinical laboratories, in delivering their mandates, rely on projections from past workload to plan their day to day operations. With several thousands of infected patients, with part of them needing diagnostic testing and/or hospitalization, the daily activity of clinical laboratories for both routine and urgency testing may be rapidly saturated or even overwhelmed and disrupted[1].

The Clinical and Laboratory Standards Institute (CLSI) have a document, GP36-A, an approved guideline for planning laboratory operations during a disaster. It is an essential document for laboratory emergency preparedness planning during the COVID-19 pandemic. Chapter 10, which deals with planning for pandemic influenza, easily can be adapted to the current COVID-19 situation[2]. The Joint Commission (TJC), a hospital-regulating agency, requires that the laboratory must have an emergency management plan, preferably its own, unless the hospital's plan includes sufficient detail for the lab's response, and so does the College of American Pathologists (CAP)[3].

When SARS-COV-2 emerged at the tail end of 2019, as a novel virus, no clinical laboratory was prepared. The first lesson policymakers and hospital administrators learnt from COVID-19 was that inadequate human and economic resources in the laboratory presented challenges when faced with amplified volumes of fresh samples to test. This was particularly noticeable with molecular diagnostics. Laboratories were asked to enhance their usual throughput and contextually reduce their turnaround time, and this involved recruiting extra staff or shifting staff from other sections to perform molecular testing. Some tests, such as inflammatory markers had a huge surge that depleted existing reagent stocks. There is need therefore to review the test menu and consider what testing might be most useful in a pandemic and plan adequately for the surge in volumes. Disruptions in the supplies chain and dispatching tests outsourced abroad should also be taken into account and communicated with the clients promptly. In the longterm we need to enhance local capacity to perform complex testing and motivate our industries in partneship with the universities to venture into the biotechnology field.

One challenge has been keeping staff safe and healthy while working on essential services. Some laboratory staff have contracted the infection and in some instances has resulted in fluctuating workforce availability, necessitating reorganization of work processes to ensure critical testing continues.

The second lesson is the need to have a network of well equiped and manned regional laboratories. Most of the testing for COVID-19 is happening in the capital city resulting in delayed testing for cases outside the city. Regional laboratories not directly challenged by the oubreak can absorb some of the workload from overwhelmed areas. Thirdly, setting up mobile laboratories including Point of Care Tests (PoCT) which can be rapidly deployed to the site of emergency to support testing needs. This would improve access and turn around time.

Fourthly, is the need to develop evidence based testing and procedural guidelines, and to effectively communicate the clinical laboratories role in handling pandemics or emergencies to the general public.

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Seroprevalence of human T-cell lymphotropic virus 1 and 2 in blood donors at two blood donor centres in Nairobi, Kenya

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ABSTRACT

Background: Human T-cell Lymphotrophic Virus is known to cause Adult T-cell leukemia/lymphoma and HTLV-associated myelopathy/tropical spastic para-paresis. Transfusion of contaminated blood is the major mode of HTLV-1/2 transmission. Many countries have documented the prevalence of HTLV-1/2 in blood donors.

Objectives: To determine the Human T-cell Lymphotrophic Virus -1/2 seroprevalence among eligible blood donors and to correlate HTLV-1/2 seroprevalence with other routinely tested transfusion transmissible infections in Kenyatta National Hospital Blood Transfusion Unit and the Nairobi Regional Blood Transfusion Centre.

Design: Cross-sectional descriptive study.

Methods: One hundred and thirty-eight blood donors who met the national guidelines for blood donation were consecutively recruited into the study. A questionnaire was administered and socio-demographic data recorded. Blood samples were drawn for routine tests and HTLV-1/2 serology which was carried out using HTLV-1/2 immunoglobulin G antibody Enzyme Linked Immunosorbent Assay (ELISA) technique. The results of routinely screened transfusion transmitted infections (Human Immunodeficiency Virus, hepatitis B virus, hepatitis C virus and syphilis) were obtained from the donor registers at the Blood Transfusion Unit at Kenyatta National Hospital and the Regional Blood Transfusion Center.

Results: Ninety eight (71%) of the study participants were male and 40 (29%) females. The age of the participants ranged between 18-59 years, 51% of participants' age ranged between 21 and 30 years while those above 51 years and less than 20 years were the least. None of the study participants tested positive for HTLV-1/2 yielding a seroprevalence of 0% in this population. The prevalence rates of the routinely screened transfusion transmitted infections: HIV, hepatitis B virus, hepatitis C virus and syphilis were 5.7%, 3.6%, 0.7%, 0% respectively. A correlation could not therefore be made between routinely screened infections and HTLV-1/2 infection as the seroprevalence of HTLV was very low.

Key words: Adult T-cell leukemia/lymphoma, HTLV-associated myelopathy/tropical spastic paraparesis, Blood Transfusion Unit, Transfusion transmitted infections

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INTRODUCTION

Blood transfusion is a vital therapeutic procedure used as a lifesaving intervention in all clinical disciplines. Blood transfusion, like all medical interventions, is generally safe but carries the risk of transfusion-transmissible infections including hepatitis B virus, hepatitis C virus, Human Immunodeficiency Virus, syphilis, malaria, Human T cell lymphotrophic virus and Cytomegalovirus. A global study done by Gessain *et al*[1] in 2005 showed regional disparity in prevalence of HTLV-1/2 in Sub-Saharan Africa with a seroprevalence range of 0.2-5.5%, the highest in Nigeria at 5.5% and the same study showed the seroprevalence in Rwanda to be low at 0.2%. In contrast, a study done in Kenya on cervical smears showed a high prevalence of HTLV/HIV co-infection at 19.5%[2], however no studies have been done among blood donors. Many low and middle income countries including Kenya, have a higher prevalence of some TTIs and this poses a greater risk of infection[3]. People with HTLV and co-infected with HIV and hepatitis C have a high risk of developing peripheral neuropathy[4] and liver disease[5] respectively. HBV and HTLV-I viruses are transmitted in same mode and this increases the risk of acquiring either of the infection. HTLV-1/2 can modify the course of syphilis infection and cause severe infection[6]. Blood is normally screened for HIV, HCV, HBV, syphilis but screening of HTLV is not universal. A study done in India revealed that with each unit of blood transfused, there is 1% risk of transmitting a TTIs[7]. This implies that there exists a need to expand the screening of donor blood in these countries, and Kenya in particular, beyond the routinely screened infections to include other TTI like HTLV-1/2.

HTLV-1/2 transmission through blood is only preventable by screening of donated blood. HTLV is a retrovirus of which four types have been documented: HTLV 1-4. Epidemics are caused by type one and two which are found globally.

Modes of HTLV-1/2 transmission include: mother to child transmission that accounts for 20% of the infections[8] and transfusion of contaminated blood accounts for 15-60% of the infections[1] and sexual transmission which occurs in people with genital sores, ulcers or through unprotected sex with an infected partner[9]. Intravenous exposure of contaminated blood is the most efficient way of HTLV-1/2 infection[8]. People transfused with packed red cells are at a high risk of being infected with the virus because transmission of the virus is dependent on cell to cell contact and not cell free virion[8].

HTLV-1/2 affects primarily lymphoid cells. HTLV-2 primarily infects CD8+ cells while HTLV-1 infects CD4+ cells leading to their increased proliferation[10]. With regards to disease burden, HTLV-1 is more significant because it is the aetiologic agent of multiple disorders. However, of the 20 million people affected with HTLV, only 3-5% develop adult T-cell leukemia[11]. HTLV-2 causes mild central nervous system disorders and lung infections. HTLV-3 and HTLV-4 are not associated with any illnesses.

Enzyme immunoassays, particularly Enzyme-Linked Immunosorbent Assay are the most commonly employed method used in diagnosis of HTLV-1/2 virus. The Center for Disease Control and Prevention recommended in 1988 the screening of HTLV-1/2 antibodies in various high income countries including USA, Canada and Caribbean[12].

The aim of this study was to determine the seroprevalence of HTLV 1/2 in healthy donors and further correlate HTLV-1/2 seroprevalence with other routinely tested TTIs.

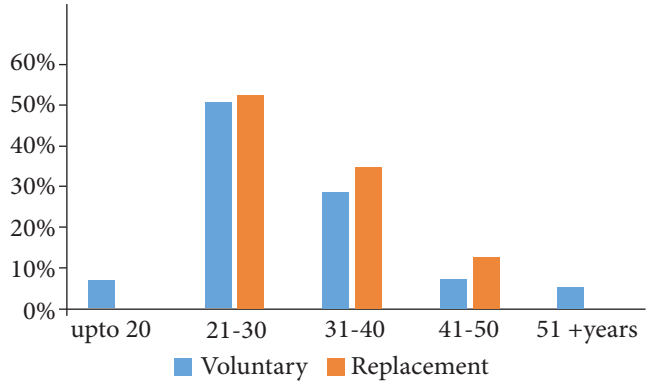
MATERIALS AND METHODS

The study was conducted at Kenyatta National Hospital Blood Transfusion Unit (KNH BTU) and the Nairobi Regional Blood Transfusion Centre (Nairobi RBTC) and UNITID laboratory. This was a cross sectional descriptive study carried out from February 2018 to April 2019. The study was done on samples from blood donors who passed the preset criteria for transfusion screening. HTLV-1/2 serology which was carried out using Wantai Bio –Pharm HTLV-1/2 immunoglobulin G antibody Enzyme Linked Immunosorbent Assay (ELISA) kits. Testing for routinely screened transfusion transmitted infections that include antibodies against HCV, HIV-1/2, HBsAg and syphilis (*Treponema pallidum*) was performed at the NBTS and Kenyatta National Hospital Immunology laboratories. The corresponding results were obtained from the donor registers at KNH BTU and the Nairobi RBTC.

RESULTS

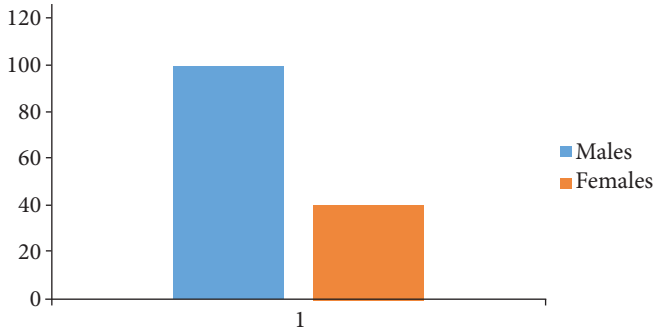
One hundred and thirty eight participants were recruited into the study. Sixty nine study participants were recruited from KNH BTU and the rest from Nairobi RBTC. The sample size was calculated using the Fisher's formula[13]. The age of the participants ranged between 18-59 years. Those aged 18 to 20 years accounted for only 7% and all of them were voluntary donors. Fifty one percent of the voluntary blood donors were between 21 and 30 years while 52% were replacement donors. The study participants whose age ranged between 31-40 years constituted 29% among voluntary donors and 35% in replacement donors. Seven percent of the voluntary donors and 13% of the replacement donors age ranged between 41-50 years. Those above 51 years were voluntary donors being the least at 6%. Figure 1 shows the study participants characteristics.

Figure 1: Age distribution of the study participants (n=138)



The male participants were predominant as they accounted for 98 (71%) compared to 40 (29%) females as illustrated in Figure 2.

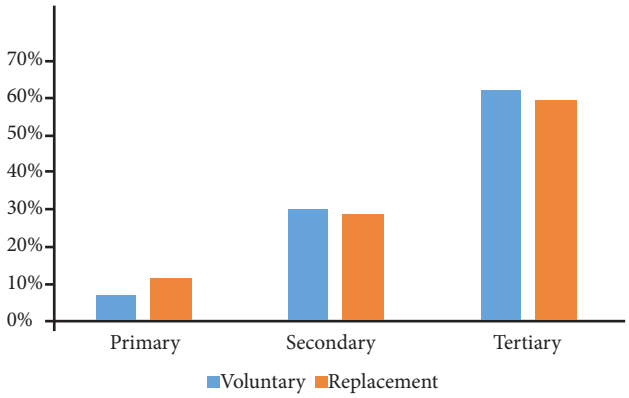
Figure 2: Gender distribution of the study participants (n=138)



Seven percent of the study participants were voluntary donors and had attained primary education. Twelve percent of the study participants were replacement donors and had attained primary education. Thirty percent of the voluntary donors and 29% of the replacement donors had attained secondary education. Those who had attained tertiary

education constituted the majority, accounting for 63% voluntary donors and 59% replacement donors as illustrated in Figure 3.

Figure 3: Education level of the study participants (n=138)



Forty six percent of the study participants earned between Kshs. 10000 to 50000 KShs (US$100 – US$50) and only 32% of the study participants earned less than 10000 KShs. (US$100).

Table 1: Socio-economic status of the study participants (n=*138)*

Social economic status (KShs*)	Frequency No.	(%)
<10000	42	30
10000-50000	63	46
50000-100000	11	8
>100000	7	5
Student	15	11

*KShs 100 = US$1

The marital status of the study participant's revealed that 71 (51%) were single, while 67 (49%) were married as indicated by Figure 4. None of the study participants indicated that they were widows or divorced.

Figure 4: Marital status of the study participants (n=138)

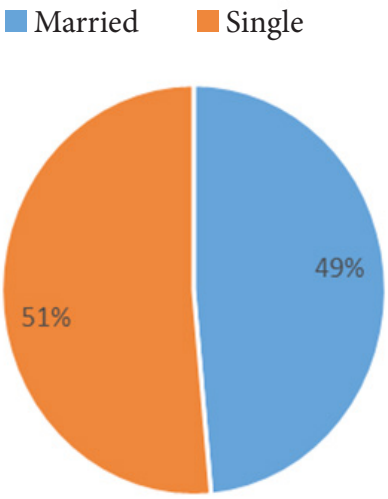
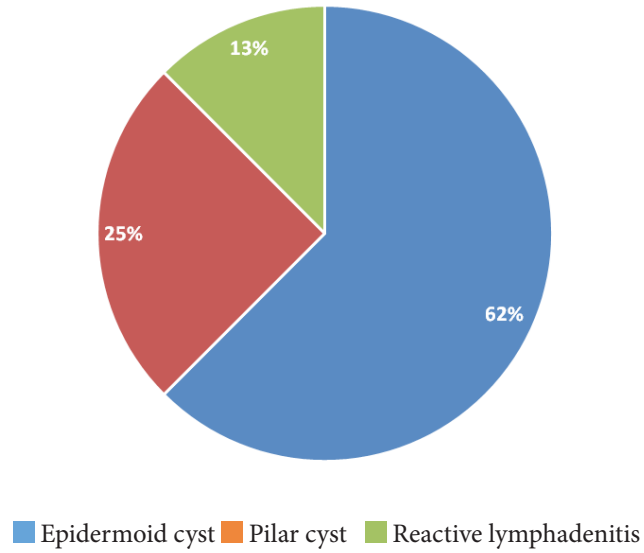


Figure 5 shows 5 (3.6%) of the study participants were seropositive for hepatitis B, 1 (0.7%) was positive for hepatitis C and 8 (5.7%) were positive for HIV antibodies. None of the study participants was seropositive for HTLV-1/2. Therefore, the prevalence of HTLV-1/2 among the blood donors in this study was 0 (0%). A correlation could not therefore be made between routinely screened infections (hepatitis B and C virus, HIV and syphilis) and HTLV-1/2 infection.

Figure 5: Prevalence of the transfusion transmissible infections (n=138)



DISCUSSION

Transfusion of blood contaminated with HTLV-1/2 accounts for 15-60% of infections in areas of high endemicity and screening of blood is the most effective strategy in prevention of transmission[1]. In Kenya, only one study has been done on HTLV-1/2 co-infection with HIV and no study has been done in blood donors. This study found HTLV-1/2 seroprevalence of 0% and this is lower than 19.5% prevalence obtained from a study done in cervical smears of women[2]. Unlike prior studies, this study was the first to be done in blood donors who are certified fit to donate blood. Screening of blood is the most effective strategy in prevention of transmission[12]. In Kenya, only one study has been done on the prevalence of HTLV-1/2 coinfection with HIV and little is known about the seroprevalence among blood donors. The seroprevalence of HTLV-1/2 infection among blood donors in this study was 0%.

The 0% prevalence obtained is similar to the HTLV-1/2 seroprevalence in Rwanda of 0.2%[1] and Uganda with a prevalence of 0.5%[14]. This findings agree with prior studies that indicated the seroprevalence of HTLV-1/2 in East Africa to be low or totally absent in some regions[1]. The findings in this study are also similar to the seroprevalence obtained in Enugu, Nigeria 0%[15]. Europe is not endemic for HTLV virus and the seroprevalence is very low.

For instance, in France the seroprevalence is 0.004% and the seroprevalence is 0.0% in Spain[16]. The 0% seroprevalence obtained is comparable to European rates.

Prior studies have shown certain socio-demographic factors such as advanced age, gender, marital status, low income, geographical region influence HTLV-1/2 seroprevalence[16]. According to Kenyan national blood transfusion service guidelines, donors should be aged between 18 to 65 years. In this study 51% of the participants were aged between 21 to 30 years as compared to those aged 40 years and above. This also reflects the donor population of the whole country in general. The age of the study participants in this study are similar to those in Enugu Nigeria where 53% of the study participants were aged 21 to 30 years[15]. HTLV-1/2 prevalence increases with advanced age with a prevalence of 1.6% in those aged between 18-30 years and 2.9% in those aged more than 50 years old[15]. HTLV-1/2 prevalence increases with increase in age with a prevalence of 1.6% in those aged between 18 to 30 years and 2.9% in those aged above 50 years[17]. A possible explanation for the association is the greater length of exposure to events such as sexual exposure that may result in acquiring HTLV-1/2. Once infected with the virus the infection is carried forward as there is no cure for HTLV-1/2.

Majority of the study participants were male at 71% and this is a reflection of the Kenya donor population. This is because women tend to have lower haemoglobin level and are disqualified as potential blood donors[18]. In a study done in Enugu 96% of the study participants were male while only 4% were female and these findings are similar to this study. These findings not only represent the blood donor population in Africa but also in Europe. A study done in Europe showed that 58% of blood donors in Germany were male and 53% were male in Switzerland[19].

The 0% seroprevalence observed could be attributed to gender disparity. A study done in Nigeria by Onoh *et al*[15] indicated that the prevalence of HTLV-1/2 is higher in females as opposed to males. This is because of the efficient mode of transmission from males to females during sexual intercourse. In both Africa and Europe males donate blood more than females and for this HTLV-1/2 positive cases may be missed.

In Kenya the free primary education has improved the level of literacy in the country and this has been further strengthened by easy access to student loans thus enabling Kenyans to further their education. In this study, 61% of the study subjects had acquired tertiary education, 30% had attained secondary education with those only with primary education being the least at 9%. These findings are not similar in the neighboring country, Uganda where 99.7% of the participants had attained secondary education, 0.3% primary education and none had attained tertiary education. This can be explained by the fact that the education system of a country is determined mainly by the governing policies of a country. A study done in Brazil showed that majority of HIV/HTLV-1/2-coinfected cases were either illiterate or had only secondary education[19]. The donors from this study had tertiary education (90%), this can explain the low seroprevalence and also adults with tertiary level of education are less likely to engage in risky sexual activities.

Higher income together with high social status are associated with better health. The same study done in Brazil[19] indicated HTLV/HIV co-infection cases belonged to social classes that earn less than US$250 (Kshs. 25000). From this study majority of the blood donors had an income between Kshs. 5000-10000 (US$50-100) and this could explain the 0% seroprevalence obtained.

Fifty one percent of the study subjects were single with only 49% being married. This is similar to findings from Uganda and Nigeria where majority of the participants were single. In Uganda 98.9% of the study participants were single while 1.2% were married[4] which is similar to Enugu, Nigeria where 76.7% of the participants were single and 22.3% married[15]. The seroprevalence of HTLV virus is much higher in married individuals as opposed to single people and this is explained by the higher frequency of exposure in marriage[20].

Human Immunodeficiency Virus prevalence was 5.8% which is similar to the Kenya HIV estimate report 2018 prevalence of 6.1% in Nairobi County[21]. The prevalence of hepatitis B virus was 3.6% compared to 5.2% from the Kenya national estimates for the periods 2011–2012[22]. This decline can be attributed to strategies to curb transmission including behavioral changes, education among at-risk populations and vaccination. HCV seroprevalence is 0.79–0.99% according to national estimates for the period 2007–2010[22] and this is comparable to the seroprevalence obtained of 0.7%. The national estimates indicate the prevalence of syphilis to be 0.15–0.28%[22] which is similar to findings obtained in this study of 0%. A correlation cannot be done between routinely screened infections (hepatitis B and C, HIV and syphilis) and HTLV-1/2 infection because of the 0% seroprevalence of HTLV-1/2.

Lastly, a low seroprevalence of HTLV-1/2 infection was observed in this study but multicenter studies are required to establish the seroprevalence of HTLV-1/2 in different regions in Kenya.

LIMITATIONS

The prevalence of HTLV-1/2 in this study cannot be generalized as it varies between regions and positive cases may be missed, however this study is the first of its kind and it forms the basis for comparison for future studies.

ELISA method, which has been validated for widespread donor screening in China, was used. Nuclei acid testing has been used to screen and confirm positive HTLV virus antibodies but it's expensive and has not been validated for wide spread use.

The blood donor population age ranges from 18 to 65 years but majority of donors in this study were aged between 21 to 30 years and this is a limitation as prevalence of HTLV virus is high in older age groups.

The sample size used in this study was 138 and 368 sample size was used in a study done in Uganda but despite the large sample size only 2 cases were positive.

CONCLUSION

The seroprevalence of HTLV-1/2 infection among the blood donors who participated in this study was 0%. The low prevalence obtained cannot justify the routine screening of the virus in blood donors in Nairobi County but the prevalence of HTLV varies in different regions.

RECOMMENDATIONS

HTLV-1/2 prevalence varies in different regions and multicenter studies should be encouraged in order to establish the seroprevalence of the virus. The low seroprevalence obtained does not justify routine screening of HTLV-1/2 in Nairobi County.

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Fine needle aspiration cytological findings in HIV positive patients presenting with head and neck masses a Kenyatta National Referral Hospital

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ABSTRACT

Background: Lymphadenopathy is a common sign in Human Immunodeficiency Virus (HIV) infection, and Acquired Immunodeficiency Syndrome (AIDS). However, other masses including malignancies can also affect the thyroid, salivary glands and lymph nodes as well as soft tissues of the head and neck region. Information on the lesions, their cytological characteristics, and their distribution across age-groups and gender, could guide on what to look out for when screening head and neck masses among HIV positive patients at the Kenyatta National Hospital (KNH).
Objective: To describe the fine needle aspiration cytological findings of head and neck masses in HIV positive patients attending selected outpatient clinics at KNH.
Design: A cross- sectional descriptive study.
Subjects: HIV positive patients with head and neck masses, attending Ear, Nose and Throat (ENT) clinic, Fine Needle Aspiration (FNA) clinic and Comprehensive Care Centre (CCC) clinic at KNH.
Methods: Demographic data was collected from consenting patients using a structured questionnaire. Samples (tissue or aspirate) of the masses were collected aseptically, following standard procedure. A 10ml syringe with 23 or 25 gauge needle was used to collect aspirate or tissue sample. Four thin smear slides were prepared per patient; two were fixed in 95% alcohol and stained using Papanicolaou and Haemaoxylin and Eosin stains and two were air dried and stained using Romanowsky and Ziehl- Nielsen (ZN) and Giemsa stains. Slides stained with Papanicolaou and Haematoxylin and Eosin were examined for general cytological characteristics, while those stained with Ziehl Nielsen (ZN) and Giemsa stains were examined for potential TB or lymphoproliferative disorders. Univariate and bivariate analyses were used to describe and compare distribution of cytological findings across age-groups and gender.
Results: A total of 84 HIV positive patients were enrolled, of these 51 (61%) were female. Median age of the enrolled patients was 27 years old [Interquatile range (IQR 22-43 years)]. Common anatomical sites for the masses were lymph nodes (46 of 84, 55%) followed by thyroid gland (22 of 84, 26%). Granulomatous inflammation was the most common identified lesion (27 of 84, 32%) followed by colloid goiter (15 of 84, 18%). Anaplastic carcinoma, pleomorphic adenoma and round blue cell tumour were the least observed lesions at (1 of 84, ~1%) each. Of the patients with granulomatous inflammation (7 of 27, 26%) were positive for tuberculosis. When stratified by age and gender, enlarged lymph nodes were a common finding among adult female patients (≥ 18 years old) (24 of 69, 35%) and in male children (7 of 15, 47%). Granulomatous inflammation was common in adult female and male (16 of 51, 31% and 9 of 33, 27% respectively). The risk of reactive lymphadenitis was significantly lower in adults when compared to children OR 0.10 (0.02-0.43)
Conclusions and recommendations: Our findings highlight patterns in distribution of identified lesions and cytological findings across age-groups and gender, amid diverse head and neck lesions in HIV positive patients seeking out-patient care at the KNH. Benign lesions were common, with tuberculous lymphadenitis and colloid goiter commonly identified among adult females and reactive lymphadenitis among children. Although rarely identified, common malignant masses included squamous cell carcinoma in adults and round blue cell tumour in children. While identification of benign masses may be anticipated in head and neck masses among HIV positive patients, detection of neoplastic tumours highlights a need to look out for malignant masses as well. Since common pathologic processes and swellings in head and neck region can be readily diagnosed based on cytomorphology, FNA can offer a rapid, affordable and effective way of screening for lesions in patients with HIV and should be considered in informing timely management of patients

Keys words: Head and neck masses, HIV positive patients, Fine needle aspiration cytology

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INTRODUCTION

Head and neck region has anatomically complicated structures due to its richness in diverse structures and all these may become involved in disease, often resulting in a clinically apparent mass which may be inflammatory, infective or neoplastic[1]. The masses involving salivary glands, thyroid and scalp are commonly encountered visible lesions and easily accessible for FNA.

Human Immunodeficiency Virus (HIV) infects and destroys CD4+ lymphocytes among other cells and during the process of progression of the disease there is deterioration of the immune system of the individual. The infection of lymphocytes result in enlargement of Lymph Nodes (LNs), which is one of the most common early and consistent signs/symptoms of HIV infection. Lymphadenopathy can present in every stage of HIV infection, but also occurs in HIV negative individuals due to other disease conditions[2].

The earliest data related to the Human Immunodeficiency Virus (HIV) describing development of pneumocystis pneumonia and Kaposi's sarcoma in male homosexuals were published in 1981[3]. In 2002 new HIV infections decreased from 3.3 million to 2.3 million by 2012. In 1990 there were an estimated 300,000 deaths from AIDS. Global AIDS-related deaths peaked at 2.3 million in 2005, and decreased to 1.6 million by 2012. In 2010, the 1.5 million estimated deaths from AIDS represented 2.8% of the 52.8 million deaths and AIDS was the sixth leading cause of Years of useful Life Lost (YLL) worldwide[4,5]. According to estimates by WHO and UNAIDS, 36.7million people were living with HIV globally at the end of 2016. That same year, some 1.8 million people become newly infected, and one million died of HIV- related causes.

There are two genetic types of HIV: HIV-1 which is present in Europe, Asia, America and Central Africa and HIV-2 which is only found in the Western Africa[6,7]. The HIV is an enveloped RNA virus and is transmitted in single stranded, ssRNA form. After transmission the reverse transcriptase, which is also present in the virus, transforms the single-stranded RNA to a double-stranded DNA and then the virus DNA is integrated and transcribed with help from the cellular system of the host. After that there are two possibilities: either the virus becomes latent and the infected cells continue their function without any disturbance to the host cells or the virus becomes active and starts to replicate itself. In the latent stage the HIV can be 'sleeping' for several years. When a huge number of viruses are formed a cell lysis 2 occurs resulting in the spread of the virus to other cells and the extracellular compartment[8, 9]. The process of the selection of target cells that are infected by HIV is based on the recognition of CD4 receptors on the host cells' surface by the virus. The cells that have such CD4 receptors on their cell surface are T-helper lymphocytes, monocytes, dendritic cells and microglia. T-lymphocytes have one of a crucial function of the immune system in the recognition of infectious agents; they are called CD4 'T-helper' cells. The activation of CD4 'T-helper' cells takes place as a response of the immune system to various microbial agents. When these cells are in the naïve state no replication of HIV takes place, only when the T-lymphocytes are activated then the HIV proceed with replication[10]. The main function of the subsets of the CD4+ lymphocyte population is to determine the host response to infection, the subset known as TH1 and TH2[11]. An abnormal activation of the immune system has been shown to be a major factor in disease progression. A pool of activated CD4 T-cells is formed that can be targeted by HIV and this may lead to the exhaustion of the immune system[8].

The spread of HIV in Kenya has been uneven. Although much of Kenya has a low rate of infection, certain places have been more affected than others. In particular, new HIV infections increased by more than 50% from 2013 to 2015. Epidemics are more severe in Coastal and Nyanza regions and in urban centers. As a result, HIV prevalence ranges from 0.1% in Wajir County in North Eastern Kenya, to 25.4% in Homa Bay County in Nyanza.

The earliest manifestations for opportunistic infections in HIV infected patients are lymphadenopathy and haematological alterations[12]. Studies have reported various cytological findings in head and neck lesions in HIV positive patients. In a study done in India on 32 HIV- positive cases presenting with lymphadenopathy the commonest cytological diagnosis, was tuberculous (TB) lymphadenitis in 15 cases followed by reactive lymphadenitis in 10 cases. The others were acute suppurative lymphadenitis, 5 cases, and suspected malignancy, 2 cases[13].

It is estimated that head and neck cancers constitute about (5-8%) of all malignancies worldwide, the trend appears to be increasing[14,15]. Head and neck cancers are relatively uncommon in the West, constituting about 4% of all malignancies, while in the Asian continent and Indian subcontinent, they form 40 to 50% of all malignancies[16]. A study done by Gonzalez *et al*[17], at a hospital in Spain showed a prevalence of 40.3% whereas a study done by Tatomirovic *et al*[18], at the Institute of Pathology in Serbia demonstrated a prevalence of 36.1%. In another study done in Rwanda, Fine Needle Aspiration

(FNA) was found to be a useful tool in the diagnosis of Tuberculous Lymphadenitis (TL). FNA can reduce the number of surgical excisions and provide definite guidelines about further management. In the Rwandan study, a total number of 138 specimens from suspected TL patients were analyzed, of which 14 (10.1%) were ZN positive while cytology revealed 25 (18.1%) cases of tuberculous lymphadenitis[19].

A study done in India by Rajesh *et al*[20] in 2008 on 62 HIV-positive individuals demonstrated that the most common lesion was TB 27 (42.19%) cases, followed by reactive lymphadenitis with 18 (28.12%) cases, acute suppurative lymphadenitis 7 (10.94%), fungal infection 5 cases, and malignancy 3 cases. A study done in paediatric patients by Lucumay *et al*[21] in Western Tanzania showed 43.9% had inflammatory lesions, 38.5% had congenital lesions, while 14.9% had neoplastic.

Based on the paucity of reliable cytological information on head and neck masses in KNH, this study was important to describe the FNA cytological findings of accessible head and neck masses in HIV patients attending ENT and FNA clinics.

The objectives of the study were as follows;

Broad objective: To identify the fine needle aspiration cytological features of accessible head and neck masses in HIV positive patient's attending ENT, Comprehensive Care Centre and FNA clinics in KNH.

Specific objectives: To determine the cytomorphological patterns of the head and neck masses in HIV positive patients attending ENT, Comprehensive Care Centre clinic and FNA clinics in KNH and to determine the proportion of various pathological conditions detected by FNA in HIV positive patients presenting with head and neck masses.

Study design: A cross- sectional descriptive study.

*Study population***:** HIV positive patients attending ENT clinic, FNA clinic and CCC clinic at KNH presenting with head and neck masses.

MATERIALS AND METHODS

The study included HIV patients undergoing fine needle aspiration cytology for various head and neck swellings. A total of 84 patients with lesions in the head and neck regions from the Ear, Nose and Throat (ENT) clinic, Comprehensive Care Centre clinic (CCC) and at the Fine Needle Aspiration (FNA) clinic were processed at the Cytology Laboratory at Kenyatta National Hospital. Using an aseptic technique, aspiration was done using gauge needle 23 or 25 with 10ml syringe in the usual manner. Pressure was applied on completion aspiration to prevent formation of a haematoma.

The material obtained was placed on the microscopic slide using pick; smear technique. Four thin slide smears, were made and 2 fixed in 95% alcohol immediately. The other 2 smears were air dried for staining using Romanowsky and Ziehl- Nielsen (ZN) stains. The 2 alcohol fixed smears were stained using Papanicolaou and Haematoxylin and Eosin stains. The air dried smears were stained with Ziehl Nielsen (ZN) and Giemsa stains for analysis of cases suspicious of TB or lymphoproliferative disorders. Confirmatory interpretation for thyroid lesions was performed using standard system and Bethesda system.

Univariate analyses were used to describe distribution in demographics and cytological findings. Bivariate analyses were used to compare distribution between variables; used Fisher's exact for bivariate analyses: used for categorical variable (presence/absence data); and on data with <5 observations per variable / category.

RESULTS

A total of 84 HIV positive patients were recruited of these 51 (61%) were female.. Patients age range was 0-80 years with a median age of 27 years old and (IQR* 22-43 years). Masses were commonly identified on the lymph nodes (46 of 84, 55%) followed by thyroid gland (22 of 84, 26%) (Table 1). Granulomatous inflammation was the most identified lesion 27 (32%) followed by colloid goiter 15 (18%) (Table 1). Anaplastic carcinoma, pleomorphic adenoma and round blue cell tumour were the least observed lesions at 1(1.2%) each. Seven of the 27 granulomas were positive for TB. Comparing adults with children the risk of reactive lymphadenitis significantly differed between the two groups. Reactive lymphadenitis was 90% (1-0.1*100) less likely to occur in adults compared to children. Out of 84 patients only 8 (6.72%) had HIV clinical staging 76 did not have. Enlarged lymph nodes were a common finding among adult female patients (n=69, 34.8%) and in male children (n=15, 46.7%). Thyroid lesions were more common in female adults (n=69, 20.3%) and less so in children (n=25, 13.3%).

Figure A: Microphotograph showing inflamed epidermoid cyst: (Leica) (X40, H&E stain)
Figure B: Microphotograph showing suppurative thyroiditis: (Leica) (X40, H&E stain)

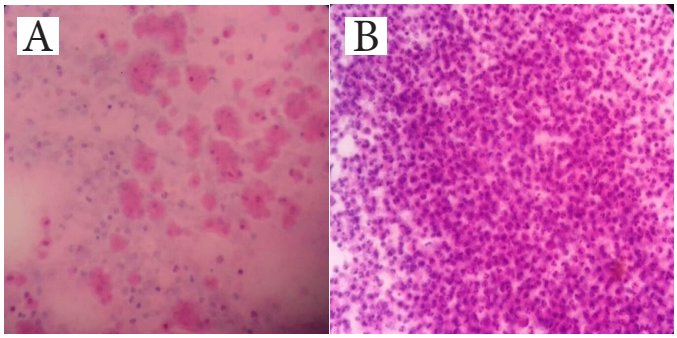


Figure C: Microphotograph showing chronic granulomatous lymphadenitis
Figure D: Microphotograph showing tuberculosis: Acid fast bacilli stained red (Leica) (X100, ZN stain)

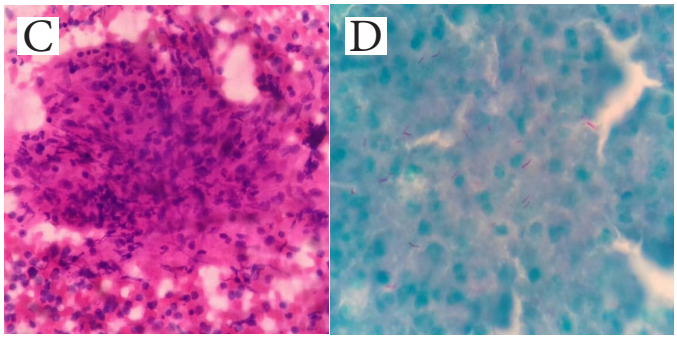


Figure E: Microphotograph showing Hurthle cell neoplasms (Leica) (X100, H&E stain)
Figure F: Microphotograph showing cytomorphological features of reactive lymphadenitis. (Leica) (X40, H&E)

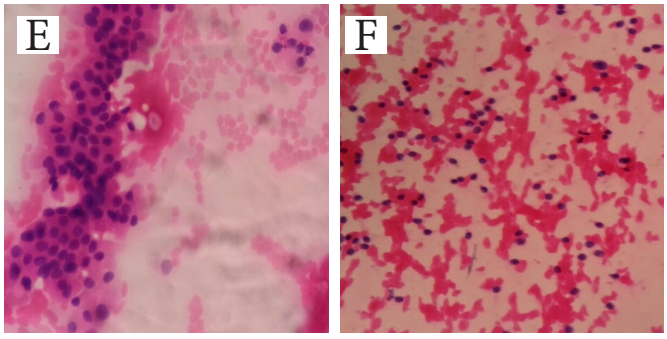


Figure G: Microphotograph showing suspicious for lymphoma (Leica) (X40, H&E).
Figure H: Microphotograph showing positive for malignancy. DDX: Nasopharyngeal carcinoma (Leica) (x40)

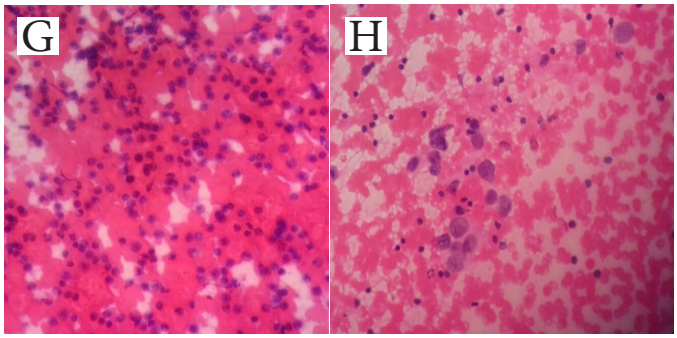


Figure I: Microphotograph showing positive for malignancy round blue cell DDX: Neuroblastoma (Leica) (X40, H&E).
Figure J: Microphotograph showing positive for Squamous cells carcinoma of the thyroid. DDX: Anaplastic carcinoma of Thy (Immunos and histology were recommended). (Lexica) (X40, H&E stain).

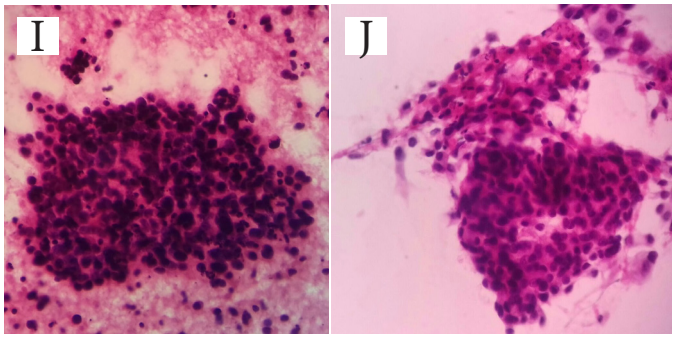


Table 1: Demographics, cytological sites

Characteristics	Cases (n=84)	(%)
Age groups (years)		
Under 5	5	6.0
5-11	6	7.1
12-24	22	26.2
25-34	21	25.0
35-49	14	16.7
50 plus	16	19.1
Median age	27 years (IQR* 22-43 years)	
Gender		
Females	51	60.7
Anatomical site of the lesion		
Lymph nodes	46	54.8
Thyroid gland	22	26.2
Salivary gland	4	4.8
Submandibular	4	4.8
Others	8	9.0
Cytological features		
Granuloma	27	32.1
Colloid goiter	15	17.9
Epidermoid cyst	12	14.3
Reactive lymphadenitis	12	14.3
Lymphoma	5	6.0
Nasopharyngeal carcinoma	3	3.6
Squamous cell carcinoma	3	3.6
Hurthle cell neoplasm	2	2.4
Pillar cyst	2	2.4
Anaplastic carcinoma	1	1.2
Pleomorphic adenoma	1	1.2
Round blue cell tumour	1	1.2

Table 2: Distribution of lesion sites by age and gender

	Adults (n=69)				Children (n=15)			
Site	Female	(%)	Male	(%)	Female	(%)	Male	(%)
Lymph nodes	24	34.8	12	17.4	3	20.0	7	46.7
Thyroid	14	20.3	6	8.7	2	13.3	0	0.0
Others	4	5.8	3	4.3	0	0.0	1	6.7
Salivary gland	3	4.3	1	1.4	0	0.0	0	0.0
Submandibular	1	1.4	1	1.4	0	0.0	2	13.3

Table 3: Distribution of cytological findings by gender and age

	Female (n=51)				Male (n=33)			
Cytological features	Adult	(%)	Child	(%)	Adult	(%)	Child	(%)
Granuloma	16	31.4	1	2.0	9	27.3	1	3.0
Colloid goiter	13	25.5	1	2.0	1	3.0	0	0.0
Epidermoid cyst	5	9.8	2	3.9	3	9.1	2	6.1
Lymphoma	3	5.9	0	0.0	2	6.1	0	0.0
Reactive lymphadenitis	3	5.9	1	2.0	2	6.1	6	18.2
Pilar cyst	2	3.9	0	0.0	0	0.0	0	0.0
Squamous cell carcinoma	2	3.9	0	0.0	1	3.0	0	0.0
Anaplastic carcinoma	1	2.0	0	0.0	0	0.0	0	0.0
Nasopharyngeal carcinoma	1	2.0	0	0.0	2	6.1	0	0.0
Hurthle cell neoplasm	0	0.0	0	0.0	2	6.1	0	0.0
Pleomorphic adenoma	0	0.0	0	0.0	1	3.0	0	0.0
Round blue cell tumour	0	0.0	0	0.0	0	0.0	1	3.0

Table 4: Comparison of cytological finding between children < 18 years old and adults 18 > years old

Final diagnosis	Adult (>=18 years) (n=69)	(%)	Child (<18 years) (n=15)	(%)	Fisher's exact P-value	Odds ratio	
Anaplastic carcinoma	1	1.5	0	0.0			
Colloid goiter	14	20.3	1	6.7	0.2871		
Epidermoid cyst	8	11.6	4	26.7	1.0000		
Granuloma	25	36.2	2	13.3	0.2137		
Hurthle cell neoplasm	2	2.9	0	0.0			
Lymphoma	5	7.3	0	0.0			
Nasopharyngeal carcinoma	3	4.4	0	0.0			
Pillar cyst	2	2.9	0	0.0			
Pleomorphic adenoma	1	1.5	0	0.0			
Reactive lymphadenitis	5	7.3	7	46.7	0.0007	0.10 (0.02-0.43)	
Round blue cell tumour	0	0.0	1	6.7			
Squamous cell carcinoma	3	4.4	0	0.0			

Table 5: Comparison of cytomorphological features between males and female

Final diagnosis	Males (n=33)	(%)	Females (n=51)	(%)	Fisher's exact P-value	Odds ratio
Anaplastic carcinoma	0	0.0	1	2.0		
Colloid goiter	1	3.0	14	27.5	0.004	0.08(0.01-0.61)
Epidermoid cyst	5	15.2	7	13.7	1.000	
Granuloma	10	30.3	17	33.3	0.815	
Hurthle cell neoplasm	2	6.1	0	0.0		
Lymphoma	2	6.1	3	5.9	1.000	
Nasopharyngeal carcinoma	2	6.1	1	2.0	0.558	
Pilar cyst	0	0.0	2	3.9		
Pleomorphic adenoma	1	3.0	0	0.0		
Reactive lymphadenitis	8	24.2	4	7.8	0.054	3.76 (0.89-18.48)
Round blue cell tumor	1	3.0	0	0.0		
Squamous cell carcinoma	1	3.0	2	3.9	1.000	

Figure 1: Proportions of lesion identified on lymph nodes (n=46)

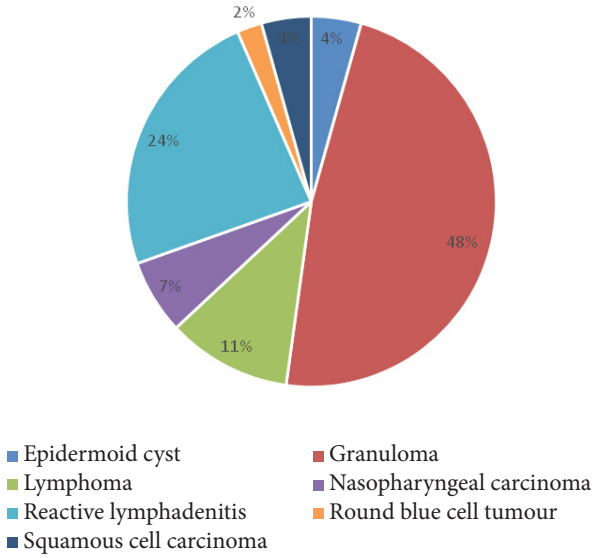


Figure 2: Proportion of lesion on thyroid glands (n=22)

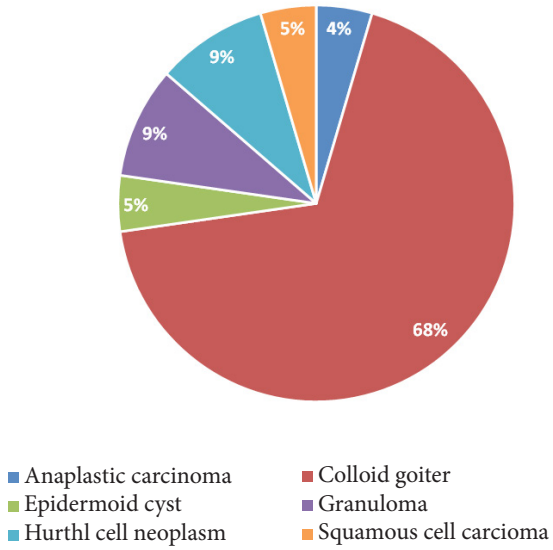


Figure 3: Proportions of lesions identified on salivary gland (n=4)

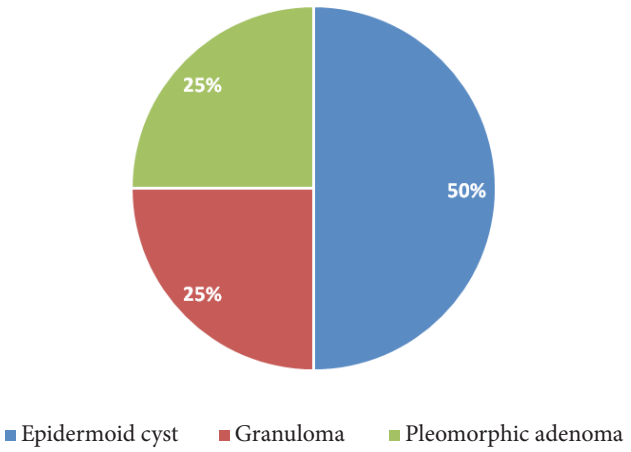
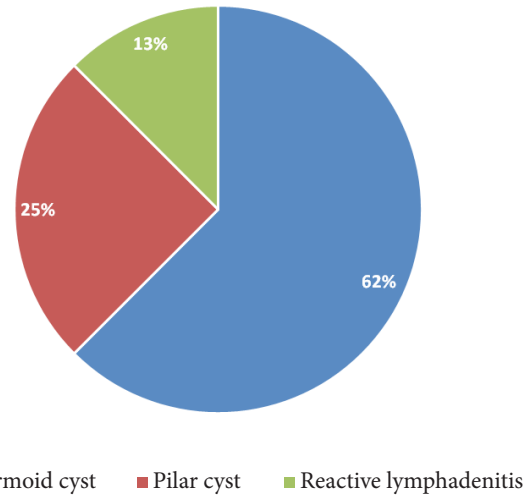


Figure 4: Proportions of lesion identified on submandibular (n=4)



DISCUSSION

Between December 2018 and April 2019, a total of 84 HIV positive patients with head and neck mass were enrolled in the study. Adults aged 20 to 29 years were the majority followed by those aged 40 to 49 years in which maximum numbers of cases are in sexually active age group. Out of 84 participants 51(60.7%) were female, 33(39.3%) were male, children were 15 (17. 86%). We have more females who are HIV infected in the population and has more HIV related conditions[22]. This is in total contrast to a study done by El Hag *et al*[22] in Saudi Arabia which showed an even gender distribution of 50/50.

FNAC has proven to be an easy, quick, reliable, and cost-effective tool for head and neck lesions. It is suitable for an initial rapid diagnosis in HIV positive patients with lymphadenopathy and others masses, among the peripheral lymph nodes involved, cervical lymphadenopathy was common. The majority of cases were found in the age group 20- 29 years, followed by 40-49 years. In a study done by Shenoy *et al*[23] the age group commonly affected was between 25 - 30 years with cervical group of nodes being the most common site.

In Kenya the most common opportunistic infection in AIDS patients is tuberculosis. In our study, majority of the lesions occurred in lymph nodes 46 (55%); granulomas was the most common finding identified, 27 (32.14%) and seven of this were positive for tuberculosis (25.92%). They were: (i) Caseating granuloma showing classical epithelioid granuloma, giant cells, and caseation in a milieu of lymphoid cells; (ii) Granulomatous lymphadenitis showing only granulomas with or without giant cells; (iii) Necrotising lymphadenitis which showed degenerating epithelioid cells in a necrotic background and (iv) Suppurative lymphadenitis where smears showed degenerating and viable neutrophils. In various studies elsewhere different distributions of granulomatous lesions in HIV positive patients with head and masses

have been described. In Mangalore, Shenoy *at al*[23] found 46 (50%), in Malaysia Jayaram *et al*[24] found 53.84%. Other studies in India have shown 57.67% and 58.3% distribution of the granulomas in HIV positive with head and neck masses[25, 26.]

The FNA material obtained from lymph nodes with tuberculous lymphadenitis showed caseation necrosis or epithelioid granulomas identical in appearance with those seen in HIV negative patients. A definite cytologic diagnosis of tuberculous lymphadenitis can be offered in smears with caseating granulomas with or without giant cells, while the necrotizing suppurative smears would be dismissed as acute suppurative lymphadenitis in the absence of Ziehl-Nielsen stain. Thus Ziehl-Nielsen staining should be performed on all aspirates from cases of suspected tuberculosis. Tuberculous lymphadenitis may be more common in HIV patients with superficial lymphadenopathy than is generally believed. Greater use of lymph node aspiration or biopsy may improve the diagnosis of suspected tuberculosis.

Reactive lymphadenitis was the fourth common finding in the present study 12(14.29%). Similar observations were also made by Vanisri *et al*[27] (36.1%). Reactive lymphadenitis was more common in children than in adults 7/12(58.33%,), 5/12(41.66%). In comparing adults to children the risk of reactive lymphadenitis significantly differed between the two groups where the risk of reactive lymphadenitis was 90% less likely to occur in adults compared to children. Lymph node FNAC is avaluable investigation in HIV patients where most opportunistic diseases (bacterial and malignancy) can be correctly identified and high-grade lymphoma can be diagnosed[25].

The second most common site of aspiration was from the thyroid, where thyroid lesions were common in both adults (20.3%) and children (13.3%) female patients. Benign colloid goiter was the most common lesion diagnosed in thyroid in this study (17.86%). Two malignant lesions which are anaplastic carcinoma and squamous cell carcinoma 4.55% each, the study reported two Hurthle cell neoplasm 9.09% which is again similar to those reported in other studies by Caruso *et al*[26]. Twenty percent granulomatous thyroiditis 9.09% and epidermoid cyst 4.55%. Management guidelines recommend follow up in benign conditions, treatment for inflammatory conditions, and lobotomy in neoplastic lesions and thyroidectomy in malignant conditions.

Three types of malignancies were found accounting for 3.6% of the total and included nasopharyngeal carcinoma representing 3.57%, squamous cells carcinoma where two of these were metastases and one was primary in the thyroid, one anaplastic carcinoma, and one round blue cell tumour. Similar observations were made by Rajesh *et al*[20] in 2007 of a low rate of malignancies, which included one metastatic adenocarcinoma, the other two being lymphomas.

Among the cases suspicious for malignancy, five were suspicious for lymphomas, 2.4% were hurtle cell neoplasms same observations made by Nayak *et al*[13] in 2003. The role of FNA in the cyto-diagnosis of lymphoma is controversial. In our study where five cases were suspicious for lymphoma, a surgical biopsy was recommended for a definitive diagnosis. Thus, the role of FNA was limited to the identification of cases for referral for further management as promptly as possible, making it a screening test. The limitation of cytomorphological diagnosis and classification of lymphoma can be overcome by the use of various ancillary techniques such as immunocytochemistry which can be done on cell blocks and aspirates respectively. Based on management guidelines, surgery can be avoided in inflammatory and metastatic lesions.

Salivary gland aspirates (n=5) consisted of two epidermoid cysts, one granulomatous inflammation and one pleomorphic adenoma. The procedure was valuable and reliable in distinguishing inflammatory and simple cystic lesions from neoplastic ones, which are treated differently. Our findings confirm that FNA is helpful for the diagnosis and treatment planning of salivary gland lesions in HIV patients, for instance, a pleomorphic adenoma requires surgery with wide margins to avoid recurrence. Based on management guidelines, surgery can be avoided in inflammatory conditions while benign cystic lesions require surgical excision.

FNA of head and neck in HIV/AIDS patients with clinical correlation can provide most useful information to physicians to determine the further mode of management. However, to obtain maximum benefit from the procedure, co-operation between patients, trained cytopathologist and an experienced clinician is essential. With today's increasing cost of medical practice, any technique which speeds up the process of diagnosis is of tremendous value.

CONCLUSIONS

(i) Commonest benign findings were TB adenitis and colloid goiter in adult females while in children the most common lesion was reactive lymphadenitis.

(ii) Commonest neoplastic lesion in adults was lymphoma followed by squamous cell carcinoma while in children it was only one round blue cell tumour.

(iii) Head and neck swellings in HIV positive can readily be screened for malignancy using FNA.

(iv) In HIV positive patients with head and neck swellings the pathological processes can readily be diagnosed by cytomorphology.

Limitations: The study could not establish clinical stage of HIV disease because the enrolled patients were referrals and did not have clinical information on the stage of the disease. Out of 84 patients only 8 (6.72%) had HIV clinical staging 76 did not have.

RECOMMENDATIONS

(i) FNA should be performed on HIV positive patients with head and neck masses to classify the lesions for better management of the lesions and prevention of unnecessary surgical interventions.

(ii) We recommend that Fine Needle Aspiration Cytology should be the first diagnostic procedure in clinics for the diagnosis of the cause of lymphadenopathy in HIV/AIDS patients.

(iii) Lesions diagnosed as granulomas should have special stains such as Ziehl Neelsen done even as PCR is done.

(v) Further studies on FNA of head and neck for pediatric age groups should be undertaken to determine utility in diagnoses and management of these age group.

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Placental villous changes among hypertensive and normotensive pregnant women at Kenyatta National Hospital

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ABSTRACT

Background: Hypertensive pregnancy disease is a major cause of maternal morbidity and mortality in Africa and is one of the major causes of maternal deaths in Kenyan public health facilities. Previous studies have not demonstrated chorionic villous histological changes specifically observed in chronic hypertension in pregnancy and gestational hypertension. No studies have been done in Kenya to demonstrate variations in chorionic villous histomorphology in normotensive and hypertensive pregnant women. Determining the difference between villous histomorphological findings among the hypertensive pregnancy disease clinical groups (pre-eclampsia eclampsia, chronic hypertensive disease and gestational hypertension) may provide insight onto whether the different groups have a common pathological end point; an altered villous histomorphology. Established associations between placental villous histopathology and clinical variables such as maternal blood pressure status will also help sensitize clinicians on the need for histology examination of placentae.

Objective: To determine placental villous changes among hypertensive and normotensive pregnant women who delivered at Kenyatta National Hospital.

Design: Laboratory based retrospective cross-sectional analytical study.

Methods: The study was carried out at the University of Nairobi (UoN) Histopathology Laboratory. Archived placental tissue blocks (n=143) obtained from hypertensive pregnant women and their normotensive counterparts who delivered at Kenyatta National Hospital between July and December 2015 were processed and analyzed.

Results: Placentae from women with hypertensive pregnancy disease at Kenyatta National Hospital had significantly higher rates of accelerated villous maturity, distal villous hypoplasia, stromal fibrosis, decidual arteriopathy, villous infarction and an increased area (>25%) of intervillous fibrin deposition (p<0.01). The pre-eclampsia-eclampsia clinical group had placentae characterized by lesions associated with placental ischemia supporting evidence that placental hypoperfusion could be characteristic of pre-eclampsia-eclampsia rather than gestational hypertension.

Recommendation: Sensitization of clinicians in Kenyatta National Hospital on clinical utility and need for placental histopathological examination in hypertensive pregnancy disease and related clinical scenarios.

Key words: Placenta, Villi, Hypertension, Pregnancy, Histomorphology

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INTRODUCTION

Hypertension in pregnancy complicates about 10% of pregnancies worldwide; 2-12% and 5-6% pregnancies in the United States and the United Kingdom respectively[1]. It is a major cause of maternal mortality in Africa[2], complicates 2.73% of pregnancies in Kenya[3] and is one of the main causes of maternal deaths in Kenyan public health facilities[3]. Hypertension in pregnancy complicates with premature deliveries, low birth weights, still births, neonatal deaths and long-term neonatal neurological sequelae[4,5]. Maternal complications include risk of recurrence in subsequent pregnancies, an increased cardiovascular disease risk, placenta abruptio[5], renal failure, coagulopathy, cardiac arrest, cerebrovascular accidents, respiratory failure and liver failure[6].

In pregnancy, identification of structural derangement of the placenta through histopathological examination helps establish adequacy of the maternal environment and provides information on expected fetal outcomes. Despite this utility of placental histopathology examination, there are still few requests for placental histopathological evaluation by clinicians in Kenyatta National Hospital and in other hospital settings[6] within the country, probably due to perceived low clinical utility.

Hypertensive disease in pregnancy, particularly pre-eclampsia, has been associated with placental villous

histomorphological changes[7-9]. Few studies have been done to demonstrate these changes and have reported different findings[2,6,8,10–16]. No studies have been done in Kenya to demonstrate variations or changes in chorionic villous histomorphology in normotensive and hypertensive pregnant women. This study hypothesizes that established associations between placental villous histopathology and clinical variables such as maternal blood pressure status will add to the available evidence and help sensitize clinicians on the need for histology examination of placentae in Kenyatta National Hospital and other hospital settings.

Clinical groups of hypertensive disorders in pregnancy include; chronic hypertension, pre-eclampsia-eclampsia, gestational hypertension and pre-eclampsia superimposed on chronic hypertension[17]. None of the previous studies on placental chorionic villi specifically looked at histological changes observed in chronic hypertension in pregnancy and gestational hypertension. It is therefore possible that villous histomorphological changes remain unknown in these clinical groups. Chronic hypertension, pre-eclampsia-eclampsia and gestational hypertension are regarded as distinct entities with respect to their epidemiology including risk factors and recurrence rates, pathogenesis, haemodynamic characteristics and severity of maternal and neonatal adverse outcomes[18]. It however remains unclear whether gestational hypertension and pre-eclampsia are independent conditions with a similar phenotype (hypertension) or if gestational hypertension is an early mild stage of pre-eclampsia[19]. Determining the difference between villous histomorphological findings among the hypertensive pregnancy disease groups (pre-eclampsia eclampsia, chronic hypertensive disease and gestational hypertension) may provide insight onto whether the different groups have a common pathological end point; an altered villous histomorphology. This could possibly explain progression from a mild altered villous histomorphology (chronic hypertensive disease and gestational hypertension) to a more severe alteration (pre-eclampsia).

Objective: To determine placental villous changes among hypertensive and normotensive pregnant women who delivered at Kenyatta National Hospital.

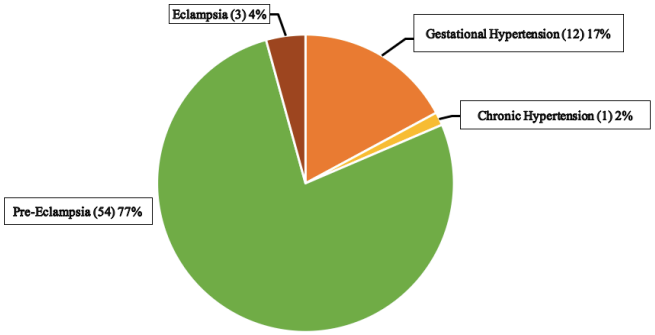
MATERIALS AND METHODS

This was a retrospective cross-sectional analytical study carried out at the University of Nairobi (UoN) Histopathology Laboratory located at Kenyatta National Hospital, Kenya. The study compared villous histomorphology in archived placenta specimens obtained from pregnant women with hypertensive pregnancy disease and those of their normotensive counterparts who delivered at Kenyatta National Hospital between July and December 2015. These were single full thickness placental tissue sections which were retrieved, processed and stained with haematoxylin and eosin to enable chorionic villous histomorphological assessment. A total of 143 cases were examined under light microscopy and histomorphology findings reported. The variables of interest in this study were chorionic villous maturity, stromal changes, presence of inflammation, fibrin deposition and vascular changes. Chi square test was used to determine the association between villous histomorphology findings and hypertensive pregnancy disease. Association between histomorphology findings and the different clinical categories of hypertension in pregnancy was determined using Fisher's exact test. All statistical tests were performed at 5% level of significance.

RESULTS

A total of 73 (51%) placentaes were from normotensive pregnant women and the rest, 70 (49%), from their hypertensive counterparts. Distribution per clinical category for the hypertensive group consisted of: 12 (17%) gestational hypertension, 1(2%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia as shown in Figure 1.

Figure 1: Distribution per clinical category for the hypertensive group



The pregnant women were 26 (SD=5.5) years old on average with a mean (SD) gestation of 37.7(3.2) weeks. The two groups (hypertensive and normotensive) were age-matched in cohorts and therefore no significant age difference was observed between the two groups (Table 1).

Table 1: Age of participants

Variable	Frequency n (%)	Hypertensive n (%)	Normotensive N (%)	P-value
Mean age (SD)	26.2 (5.5)	26.2 (5.3)	26.1 (5.6)	
15-19	10 (7.6)	4 (6.9)	6 (8.2)	
20-24	49 (37.4)	26 (36.2)	28 (38.1)	0.888
25-29	35 (26.7)	19 (27.6)	19 (26.0)	0.955
30-34	25 (19.1)	15 (20.7)	13 (17.8)	
35-39	11 (8.4)	6 (8.6)	6 (8.2)	
40-44	1 (0.8)	0	1 (1.4)	

Villous histomorphology: When compared to their normotensive counterparts, the hypertensive group had significantly higher rates of accelerated villous maturity and distal villous hypoplasia as shown in Table 2. Stromal changes; villous edema and fibrosis were observed in both groups.

Table 2: Placental villous histomorphology findings among participants

Variable	Hypertensive n (%)	Normotensive n (%)	OR (95% CI)	P-value
Delayed villous maturity				
Present	6 (15.4)	17 (25.0)	0.6 (0.2-1.5)	0.244
Absent	33 (84.6)	51 (75.0)	1.0	
Accelerated villous maturity				
Present	26 (44.8)	2 (2.7)	28.8 (6.5-129.0)	<0.001
Absent	32 (55.2)	71 (97.3)	1.0	
Distal villous hypoplasia				
Yes	27 (46.6)	3 (4.1)	20.3 (5.7-72.0)	<0.001
No	31 (53.4)	70 (95.9)	1.0	
Villous edema				
Yes	6 (10.3)	3 (4.1)	2.7 (0.6-11.3)	0.183
No	52 (89.7)	70 (95.9)	1.0	
Villous infarction				
Yes	7 (12.1)	2 (2.7)	4.9 (1.0-24.4)	0.036
No	51 (87.9)	71 (97.3)	1.0	
Villous stromal fibrosis				
Present	13 (22.4)	4 (5.5)	5.0 (1.5-16.3)	0.004
Absent	45 (77.6)	69 (94.5)	1.0	
Presence of villitis				
Present	2 (3.4)	1 (1.4)	2.6 (0.2-29.1)	0.584
Absent	56 (96.6)	72 (98.6)	1.0	
Presence of intervillositis				
Present	3 (5.2)	1 (1.4)	3.9 (0.4-38.8)	0.321
Absent	55 (94.8)	72 (98.6)	1.0	
Percentage of fibrin deposition				
<25%	39 (67.2)	63 (86.3)	1.0	
≥25%	19 (32.8)	10 (13.7)	3.1 (1.3-7.3)	0.009
Villous vascularity				
Increased	8 (13.8)	9 (12.3)	1.1 (0.4-3.2)	0.804
Not increased	50 (86.2)	64 (87.7)	1.0	
Decidual arteriopathy				
Present	8 (20.0)	2 (3.9)	6.3 (1.2-31.3)	0.014
Absent	32 (80.0)	50 (96.1)	1.0	

The hypertensive group had higher rates for both villous edema and stromal fibrosis. When compared to their normotensive counterparts, the hypertensive group had higher rates of villous infarction and decidual arteriopathy.

As regards to placental inflammation, 3.4% and 5.2% of the hypertensive cases had chronic villitis and intervillositis respectively. Fibrin deposition was seen in all the cases. However, the hypertensive group had significantly higher rates of more than 25% microscopic field of fibrin deposition.

Villous histomorphological findings associated with hypertensive pregnancy disease: Proportions of villous histomorphological findings were compared between hypertensive and normotensive groups. A χ^2 test of association with CI = 95% and p = 0.05 as criterion for significance was used to determine association between the villous histomorphological findings and hypertensive pregnancy disease.

Table 3: Villous histomorphology findings associated with hypertensive pregnancy disease

Variable	OR (95% CI)	P-value
Delayed villous maturity	0.6 (0.2-1.5)	0.244
Accelerated villous maturity	28.8 (6.5-129.0)	<0.001
Presence of distal villous hypoplasia	20.3 (5.7-72.0)	<0.001
Presence of villous edema	2.7 (0.6-11.3)	0.183
Presence of villous stromal fibrosis	5.0 (1.5-16.3)	0.004
Presence of villitis	2.6 (0.2-29.1)	0.584
Presence of intervillositis	3.9 (0.4-38.8)	0.321
Intervillous fibrin deposition area; >25%	3.1(1.3-7.3)	0.009
Presence of villous infarction	4.9 (1.0-24.4)	0.036
Chorangiosis	1.1 (0.4-3.2)	0.804
Decidual arteriopathy	6.3 (1.2-31.3)	0.014

Placentae from women with hypertensive pregnancy disease had significantly higher rates of accelerated villous maturity (p<0.01), distal villous hypoplasia (p<0.01), villous infarction (*p*=0.036), stromal fibrosis (p<0.01), decidual arteriopathy (p=0.014 and increased area (>25%) of intervillous fibrin deposition (*p*=0.014). (Table 3).

Difference in villous histomorphological findings between hypertensive pregnancy disease groups: The hypertensive group consisted of: 12 (17%) gestational hypertension, 1(2%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia. Comparisons were made to determine the difference in incidence of villous histomorphology lesions between the two main clinical groups; pre-eclampsia-eclampsia and gestational hypertension. There were significantly higher rates of accelerated maturity and villous hypoplasia among the pre-eclampsia-eclampsia group. The pre-eclampsia-eclampsia group was also characterized by a trend towards higher rates of villous infarction and decidual arteriopathy (Table 4).

Table 4: Villous histomorphological findings among hypertensive pregnancy disease groups

Variable		Pre-Ecl/Ecl. n(%)	Gest HTN n (%)	P-value
Accelerated maturity	Yes	25 (47.2)	0	0.002
	No	28 (52.8)	12 (100.0)	
Villous hypoplasia	Yes	26 (49.1)	0	0.001
	No	27 (50.9)	12 (100.0)	
Villous edema	Yes	6 (11.3)	0	0.621
	No	47 (88.7)	12 (100.0)	
Stroma fibrosis	Yes	13 (24.5)	2 (16.7)	0.781
	No	40 (75.5)	10 (83.3)	
Villitis	Yes	2 (3.8)	0	1.000
	No	51 (96.2)	12 (100.0)	
Intervillositis	Yes	3 (5.7)	0	1.000
	No	50 (94.3)	12 (100.0)	
Fibrin deposition (<25% or >25%)	<25%	36 (67.9)	10 (83.3)	0.632
	>25%	17 (32.1)	2 (16.7)	
Chorangiosis	Yes	7 (13.2)	3 (25.0)	0.471
	No	46 (86.8)	9 (75.0)	
Villous infarction	Yes	6 (11.3)	0	0.621
	No	47 (88.7)	12 (100.0)	
Decidual arteriopathy	Yes	8 (22.2)	0	0.308
	No	28 (77.8)	11 (100.0)	

DISCUSSION

Villous histomorphology in hypertensive pregnancy disease

Evidence suggests that there is impaired physiological conversion in hypertensive pregnancy disease[13,20] where extravillous trophoblast invasion is limited to the decidual portion of uterine spiral arteries. This results in reduced vascular diameters than in normal pregnancies[13,20]. Some utero-placental arteries undergo decidual arteriopathy; fatty infiltration and lipid-laden myogenic foam cells (atherosis), decidual vascular thrombosis and fibrinoid necrosis of the media[20,21]. Similar to other studies[13,20,21] significantly higher rates of decidual arteriopathy was observed in placentae from hypertensive pregnant women.

Decidual arteriopathy, with attendant reduced vascular diameters[20,21] and indirect constriction of fetal stem arteries[22] are thought to cause uteroplacental hypoperfusion and hypoxia in hypertensive pregnancy disease[20]. This can result in proliferation of villous capillaries as an adaptation to chronic oxygen deficiency[23,24]. Increased villous vascularity (chorangiosis), has been associated with hypertensive pregnancy related uteroplacental hypoperfusion[12,23,24] which is contrary to findings in this study. The finding of chorangiosis in normotensive placentae could be explained by other probable causes like pre uterine hypoxia from hypoxemic states and fetal normoblastemia[12]. In other literature, the pathogenesis and clinical significance of chorangiosis remains uncertain although it should be noted in pathology reports[23].

Uteroplacental hypoxia associated with pregnancy-induced hypertension results in different patterns of altered villous development mainly, accelerated villous maturity and Distal Villous Hypoplasia (DVH)[6,11]. These features are seen as a placental reaction to decreased materno-placental perfusion that is associated with pregnancy induced hypertension[25]. The ensuing placental ischemia results in increased villus branching and formation of large and numerous syncytial knots which is characteristic of accelerated villous maturation.

Inadequacy of compensatory mechanisms to uteroplacental hypoperfusion results in villous infarction. In this study, significantly higher rates of villous infarction observed in the hypertensive group could be as a result of deficient intervillous (maternal) circulation due to inadequacy of compensatory mechanisms to uteroplacental hypoperfusion. Significantly higher incidence of villous stromal fibrosis was also observed in the hypertensive group similar to other studies[15,26]. This could possibly be due to placental hypoxia in pregnancy induced hypertension that up-regulates expression of fibrosis related factors including Smooth Muscle Actin (SMA), Collagen Fiber (COL), Fibronectin (FN) and Connective Tissue Growth Factor (CTGF)[15].

Villous edema in pre-eclamptic placentas has been attributed to functional insufficiency of the fetal circulation[6]. This probably explains why the hypertensive group in this study had a higher incidence of villous edema. Perivillous fibrin or fibrinoid deposition is thought to develop following damage to syncytiotrophoblast with subsequent clotting in the intervillous space and closure of the trophoblastic defect by a fibrinoid plug. This study involved third trimester placentae where findings of fibrin deposition is not uncommon[6]. Fibrin deposition was seen in all the cases in this study. However, more than 25% microscopic field of fibrin deposition was observed significantly among the hypertensive group. According to other studies[11,27], a diffuse increase in fibrin deposition possibly reflects chronic intervillous perfusion defects seen in pre-eclampsia.

Villous histomorphology among different hypertensive pregnancy disease groups

In agreement with recent epidemiological findings in sub-Saharan Africa[4,28], this study found chronic hypertension and gestational hypertension to be less common than pre-eclampsia. The hypertensive group consisted of: 12 (17%) gestational hypertension, 1(2%) chronic hypertension, 54 (77%) pre-eclampsia and 3 (4%) with eclampsia. Comparisons were made to determine the difference in incidence of villous histomorphology lesions between the two main clinical groups; pre-eclampsia-eclampsia and gestational hypertension. Women with pre-eclampsia-eclampsia had placentae characterized by significantly higher rates of accelerated maturity and villous hypoplasia. Similar to Maloney *et al*[29], they were also characterized by a trend towards higher rates of villous infarction and decidual arteriopathy. Given that these lesions; accelerated maturity, villous hypoplasia, villous infarction and decidual arteriopathy are associated with placental ischemia[8], the findings are consistent with placental ischemia being characteristic to pre-eclampsia-eclampsia. This supports available evidence that pre-eclampsia-eclampsia and gestational hypertension are different disease entities given their distinct epidemiologic, pathogenesis and haemodynamic characteristics[18].

Correa *et al*[6] found a significant difference in quantity of fibrin deposits between the pre-eclampsia-eclampsia and gestational hypertensive groups which is contrary to findings from this study. Fibrinoid deposition is thought to represent clotting in the intervillous space following damage to syncytiotrophoblast with subsequent clotting in the intervillous space. It is a non-specific finding with variable findings in pregnancy state according to some studies. Maloney *et al*[29] found no statistically significant difference in pathologic lesions present between the different clinical types of hypertensive pregnancy disease. However, placentae from women

with pre-eclampsia were characterized by a higher incidence of decidual vasculopathy (47% vs. 33%; $p = 0.08$) and villous infarction (50% vs. 38%; $p = 0.1$), in keeping with placental ischemia as a key feature in pre-eclampsia.

CONCLUSIONS

Placentae from women with hypertensive pregnancy disease at Kenyatta National Hospital had significantly higher rates of accelerated villous maturity, distal villous hypoplasia, stromal fibrosis, decidual arteriopathy, villous infarction and an increased area (>25%) of intervillous fibrin deposition. Pre-eclampsia-eclampsia group had placentae characterized by lesions associated with placental ischemia; significantly higher rates of accelerated maturity, villous hypoplasia and a trend towards higher rates of villous infarction and decidual arteriopathy. This supports available evidence of placental hypoperfusion as characteristic of pre-eclampsia-eclampsia rather than gestational hypertension.

RECOMMENDATIONS

There are demonstrated placental histopathology findings in pregnancies complicated by hypertensive pregnancy disease. Clinicians therefore need to be sensitized on these findings that can enhance the need to submit placental histopathology examination requests for placentae related clinical scenarios. These include intrapartum management of hypertensive pregnant women, postmortem evaluation of unexplained perinatal mortality with unclear antenatal history especially where hypertensive pregnancy needs to be ruled out and evaluation of poor neonatal outcomes for possible aetiology.

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Autopsy findings of the thymus in cases of fatal paediatric severe acute respiratory conditions at the Kenyatta National Hospital Farewell Home

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ABSTRACT

Background: The thymus is responsible for T-helper and T-cytotoxic cell maturation together with their immune regulatory functions. It then gradually reduces in size and function from teenage with complete atrophy in adulthood. Thymus size and function is influenced by various factors i.e. genetics, nutritional status and infections. In thymic pathology, paediatric health is adversely affected as the thymus plays a critical role in immune homeostasis from birth to adolescents; particularly involving infectious agents.
Objectives: To describe the thymic autopsy findings and clinical correlations among fatal paediatric severe acute respiratory infections at the Kenyatta National Hospital Farewell Home from the year 2014-2016.
Design: Cross-sectional descriptive study.
Setting: The University of Nairobi's Anatomic Pathology Core Histopathology laboratory.
Methods: The formalin fixed thymic and splenic tissue blocks were sectioned and stained using routine haematoxylin/eosin stain. Cellular populations were assessed by staining for the distribution and quantity of T lymphocytes, B lymphocyte and NK cells using CD3, CD5, CD20, CD79a, PAX5 and CD56 immunohistochemical stains. Specific clinical data i.e. age, weight, I.P number, mid-upper arm circumference and lymphocyte differential counts were obtained.
Results: From this study, 96% of the thymi were atrophic and there was significant association between nutritional status and CD3, CD20 lymphocyte expression in both thymi and spleen.
Recommendation: Clinicians need to be advised to assess thymus size ultrasonographically, as a marker of morbidity/mortality in children who present with chronic infection plus malnutrition.

Key words: Autopsy, Thymus, Paediatric, Acute respiratory conditions, Farewell home

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INTRODUCTION

The thymus, being a major lymphoid organ in children plays an important role in primary lymphopoiesis, in conjunction with the bone marrow. It's a restrosternal organ that closely borders the thyroid and parathyroid glands. Grossly, it is composed of two lobes while ultrastucturally it's divided into lobules within which is a cortex and medulla. From the 10th to 14th weeks of embryogenesis, it receives T-lymphoid precursor cells from the bone marrow[1].

In the under five paediatric age group, it is the largest lymphoid organ and is responsible for T-helper and T-cytotoxic cell maturation together with their immune regulatory functions. Cells of the B lineage and macrophages also play an important role in thymic physiology, as well as infectious disease surveillance[2].

The high rates of infectious disease in Sub-Saharan Africa has previously been attributed to overcrowding, poverty, poor health systems and malnutrition[3]. A recent paediatric respiratory aetiology autopsy study at the Kenyatta National Hospital in Kenya, demonstrated a high proportion of thymic hypoplasia among fatal paediatric severe acute respiratory infections. Severe thymic hypoplasia was observed in children with multiple infections including *Pneumocystis Jirovecii* pneumonia and *Klebsiella*, reminiscent of HIV associated pathology[4].

Africa has the largest infectious disease burden and accounts for 50% of global under five mortality[3]. Kenyan demographics document under five mortality rate of 52/1000 with the leading cause of death in this age group being pneumonia at 15%[5]. Paediatric patients who suffer from chronic respiratory tract, Ear, Nose and Throat (ENT) and skin infections; or have poor response to antibiotic therapy for severe respiratory tract infections, could be having an underlying immune deficiency[6]. Autopsy studies in African children dying from

respiratory diseases have shown that most children who die from respiratory diseases have preventable and treatable aetiological agents such as acute pyogenic pneumonia, PJP, TB and CMV[4]. It is therefore likely that thymic hypoplasia representing primary or secondary immune deficiency could be a common cause of infectious disease related deaths in Africa. Compounded with the paucity in documented cases, this is often overlooked both ante- and post-mortem.

Study justification: While thymus size and functions is determined by numerous intrinsic and extrinsic factors, thymic dysfunction can thus be classified as being of either primary or secondary aetiology. Thymic involution, whether primary or secondary, has been associated with progressive deterioration in thymopoiesis with resultant ineffective immune homeostasis and increased morbidity from infectious diseases[5].

Secondary thymic atrophy with lymphocyte depletion has commonly been associated with infectious diseases such as HIV, measles, and malnutrition[2]. However, there is paucity of information on effects of common infectious organisms such as *Streptococcus pneumonia* and *Haemophilus influenza* on the thymus, yet these are known to cause significant morbidity and mortality in the paediatric age groups. In Sub-Saharan Africa, malnutrition is a frequent contributor to early childhood mortality and thus infectious disease states with co-morbid malnutrition invariably have adverse outcomes[6].

At autopsy, gross evaluation, conventional histology and immunohistochemistry may be useful for the diagnosis of immunodeficiency, which could be useful in establishing the pathogenesis of thymic hypoplasia and its contribution to SARI risk.

Broad objective: To describe the thymic autopsy findings and clinical correlations among fatal paediatric severe acute respiratory infections at the Kenyatta National Hospital farewell home.

Specific objectives:

(i) To describe the morphological changes on gross pathology and histology of thymi derived from fatal paediatric severe acute respiratory infections.
(ii) To determine the populations of T lymphocytes, B lymphocytes and Natural Killer Cells in the thymic tissue and respective splenic tissue using immunohistochemistry.
(iii) To describe the association of above findings with nutrition status and lymphocyte count on complete blood count report.

MATERIALS AND METHODS

Study design: This was a cross-sectional descriptive study using autopsy samples obtained from a previous paediatric respiratory aetiology surveillance study that was carried out at The Kenyatta National Hospital from the year 2014 to 2016.

Study area: The University of Nairobi's Anatomic Pathology Core Histopathology Laboratory located at the Kenyatta National Hospital histopathology department.

Study population

Sample size calculation: The sample size was determined using a sample size formula for cross sectional studies with finite population correction[1].

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

Where:
n' = Sample size
N = Finite population (estimated as 6 patients per month for 1 year = 72)
Z = Z value (1.96 at 5 % type 1 error [P < 0.05] at 95% confidence level)
P = Expected proportion of children under the age of 5 years with thymic pathology.
d = Margin of error = 5 %
Substitution into the formula gives the sample size as 61.

A total of 64 autopsy specimens obtained from fatal paediatric severe acute respiratory tract infections who received care at Kenyatta National Hospital, got enrolled in the PRESS study but unfortunately died during treatment. Autopsies were subsequently conducted on these decedents with the autopsy specimens being archived at the University of Nairobi's Anatomic Pathology Unit's Core Histopathology Laboratory.

Inclusion criteria: Well preserved tissue blocks containing adequate tissue with no evidence of autolysis and thymic and splenic specimens that were retrieved from decedents aged 01-59 months of age who met the SARI case definition criteria during the PRESS study period i.e. history of reported fever >38°C and cough that required hospitalization during the PRESS study period.

Exclusion criteria: Where specimens were unavailable for analysis. Autolyzed samples on gross inspection.

Data collection procedures: Permission was sought from the relevant University of Nairobi, Pathology Departmental authority for retrieval of 64 thymic and splenic formalin fixed paraffin embedded tissue specimens. Tissue blocks retrieved from the repository were assigned a unique study number sequentially.

All tissue blocks were sectioned, mounted on slides and stained with haematoxylin and eosin. Standard operating procedures applied.

Histological examinations assessed for the following features:

Thymic atrophy: Using a pre-established criteria in malnutrition and infection[2]; hyper cellular medulla with hypo cellular cortex; reduced number of cortical lymphocytes; increased number of tingible body macrophages with apoptotic bodies; - Shrinkage of thymic lobules; increased prominence of interlobular septae; blurring of normal cortico-medullary demarcation; epithelial cell proliferation with development of glandular structures containing eosinophilic material. Degree of thymic involution was thereafter graded.

Immunohistochemical quantification of T, B and N.K cells: Subsequent immunohistochemical staining of thymic and respective splenic tissues for CD3, CD5, CD20, CD79, Pax5 and CD56 quantitative and distributive assessment was done. Retrieval of previous data i.e MUAC measurements, age of child, weight of child and the lymphocyte count on complete blood count was subsequently done.

Quality assurance

Pre-analytical: The retrieved FFPE tissue blocks were clearly assigned a unique research number and matched for respective block number. A trained technologist on histologic and immunohistochemical tissue processing was engaged in sample sectioning and staining. The retrieved blocks were processed adhering to both histologic and immunohistochemical S.O.P. for immunohistochemical staining, H&E slides were scrutinized and only best preserved blocks containing adequate tissue selected for immunohistochemical assays.

Analytical: The principal investigator and a pathologist (supervisor) reviewed the histological and immunohistochemical features. In cases of lack of consensus, the slides were reviewed by a second experienced blinded pathologist as the tie-breaker. Immunohistochemical stains were acquired only from a reputable distributor. Each immunohistochemical stain was run in tandem with the manufacturers' recommended positive control samples. Every tenth study slide was reviewed by the second blinded pathologist.

Post analytical: Data was entered immediately into respective data capture sheets to avoid any post-analytical errors.

Ethical considerations

Permission to conduct this study was sought from KNH/UON-ERC. Study specimens had been obtained only from decedents whose parents/guardians had consented to the postmortem procedures during the previous 2014-2015 PRESS study. Permission to conduct the PRESS study was sought from the Institutional Review Board at KNH.

Data management and analysis

The clinical characteristics of the study population were summarized using measures of central tendency and presented in tables and charts. Morphological changes on gross pathology and histology of thymi and populations of T lymphocytes, B-lymphocytes and Natural Killer Cells in respective splenic tissue using immunohistochemistry was described using descriptive statistics and presented as proportions in a graphical format.

Nutrition status was classified as Severe Acute Malnutrition (SAM), Moderate Acute Malnutrition (MAM), at risk of AM and well-nourished using the previously collected MUAC and weight measurements.

Correlation between independent variables like nutritional status, lymphocyte counts and the thymic histologic findings was determined using fisher's exact test. Similarly, the relationship between these variables on the cell populations (T lymphocytes, B-lymphocytes and Natural Killer Cells) was determined using bivariate and multivariate analysis.

RESULTS

Demographic characteristics of the decedents: The age distribution of the decedents was right skewed, with a median (IQR) age of 8 months. The youngest was 1 month old, and the oldest was 48 months. The females were 29 (48.3%) and males were 31 (51.7%). The male to female ratio was 1.07:1.

Table 1: Demographic characteristics of the decedents

Variable	Frequency (%)
Sex	
Male	31 (51.7%)
Female	29 (48.3%)
Nutritional status	
SAM (MUAC<110mm)	20 (33.3%)
MAM (MUAC110-125mm)	17 (28.3%)
At risk of AM (MUAC 125- 135mm)	6 (10%)
Well nourished (MUAC>135mm)	17 (28.3%)

N= 60; Mean= 11.48; SD= 10.99; Median= 8; IQR=10

Figure 1 : Density plot showing age distribution of the decedents

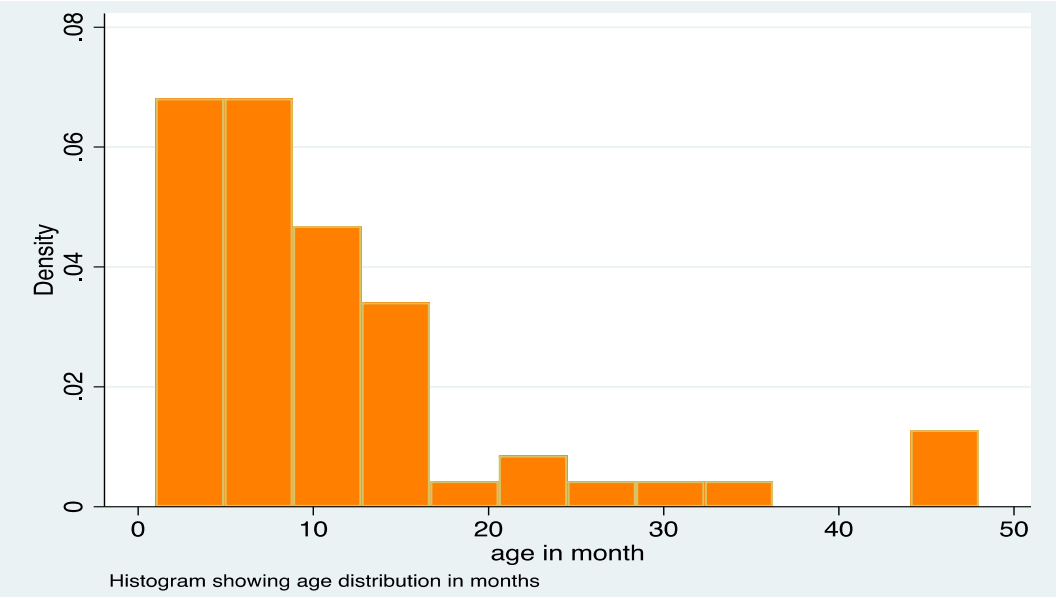
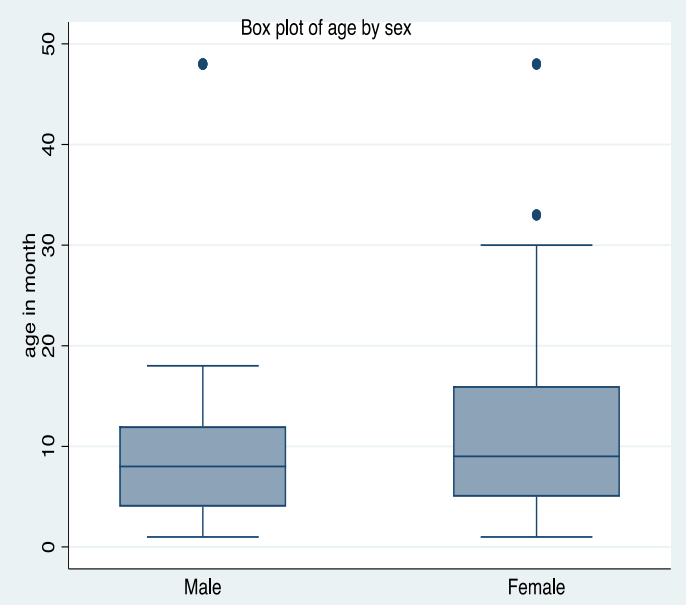


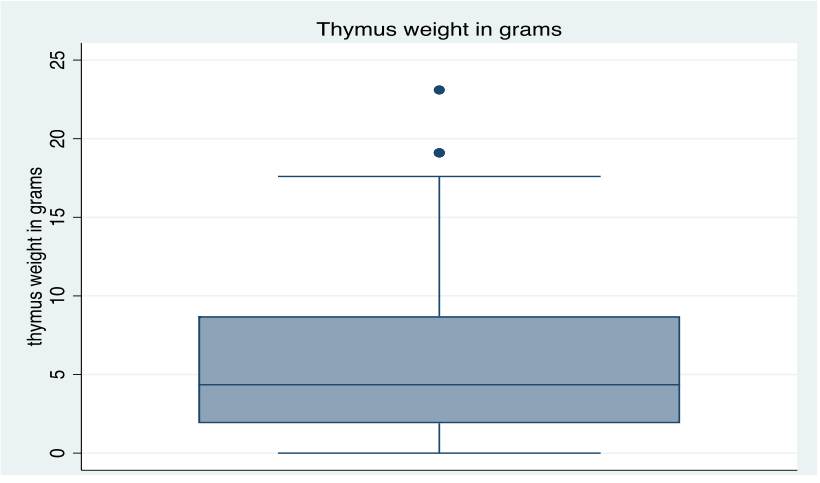
Figure 2: Box plot of age by sex



Variables	Male	Female
Frequency	31	29
25th Percentile (Q1)	4	5
Median (Q2)	8	9
75th Percentile (Q3)	12	16

Thymus morphology: As shown in Figure 3, the mean thymic weight from the 60 decedents was 6.06 grams with a median of 4.35 grams. 78.3% of the thymi weighed less than 10 grams while 21.67% had normal thymic weight of more than 10 grams.

Figure 3: Thymic weights



N = 60
Mean= 6.02gm
SD= 5.5
Median = 4.35gm
IQR= 6.8

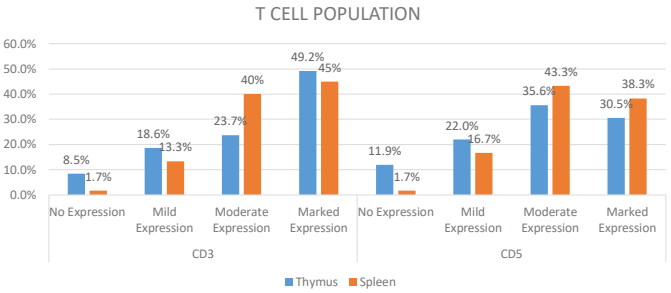
As shown in Table 2, only 2 (3.3%) of the decedents had normal (grade 1) thymi on histology. Sixteen (26.7%) had features of fibrosis (grade 4), 24 (40.0%) were grade 3 and 18 (30.0%) had grade 2.

Table 2: Distribution of microscopic findings of 60 specimens

Microscopic findings	No. (%)
Grade 1	2 (3.3%)
Grade 2	18 (30%)
Grade 3	24 (40%)
Grade 4	16 (26.7%)

Majority of the thymic tissues had marked (49.2%) and moderate expression (23.7%) of CD3. This is comparable to respective splenic specimens where 45% had marked expression while 40% of the splenic tissues had moderate expression. CD5 was almost similar, where 30.5% of the thymic tissues showed marked expression while 35.6% exhibited moderate expression of the marker. In the splenic tissues majority had marked and moderate CD5 expressions of 38.3% and 43.3% respectively.

Figure 4: Comparison of T-lymphocytes in thymus and spleen



Majority of the thymi had mild (39.0%) and moderate (35.6%) CD20 expression; mild (52.5%) and moderate (28.8%) CD79a expression. Contrary to this, majority of the splenic tissues showed moderate (40%) and marked (33.3%) CD20 expression; moderate (28.8%) and marked (40%) CD79a expression.

Figure 5: B lymphocytes in thymus and spleen

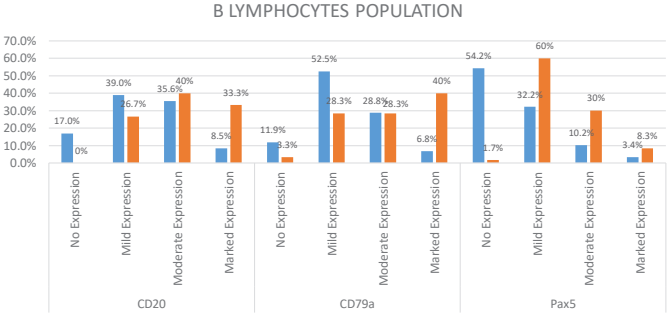
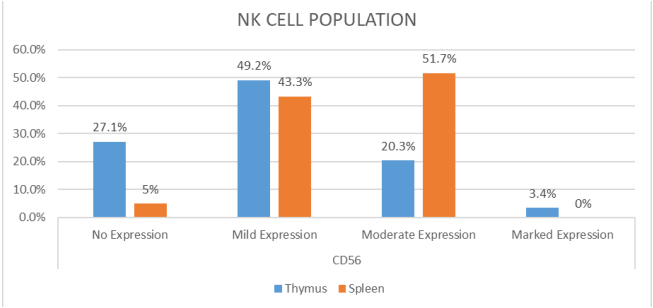


Figure 6: NK cells in thymus and spleen



Lymphocyte count on complete blood count

Figure 7: Lymphocyte count in *10^9/L on CBC

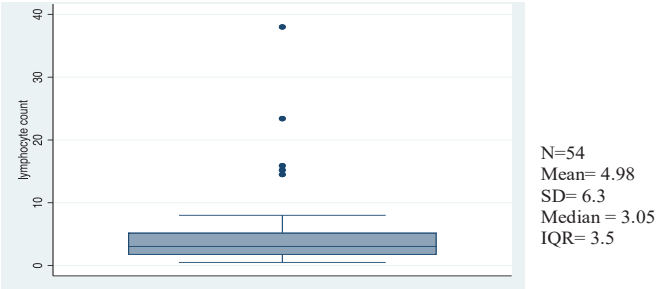
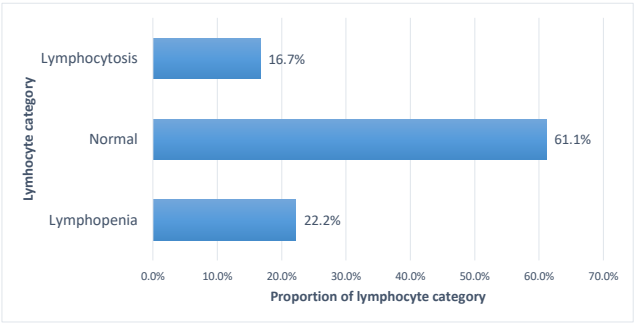


Figure 8: Distribution of lymphocyte categories based on lymphocyte count on 54 specimens



Fifty four decedents had complete blood count results documented from previous PRESS study. The mean lymphocyte count was 4.98*10^9 with a median of 3.05. Age specific reference range for lymphocyte count (1.5-7*10^9/L) was used. 61.1% of the decedents had normal lymphocyte counts, 22.2% had lymphopenia and 16.7% had lymphocytosis.

Table 3: Association between nutrition status and microscopic findings

Nutrition status	Thymic morphology				Total	Fishers exact test
	Grade 1	Grade 2	Grade 3	Grade 4		
SAM	1	2	10	7	20	0.1
MAM	0	6	6	5	17	
At risk of AM	0	2	1	3	6	
Well nourished	1	8	7	1	17	
Total	2	18	24	16	60	

Fisher's exact test was performed to examine the association between nutrition status and microscopic findings. There was no significant association between the nutrition status and microscopic findings (P>0.05) (Table 3).

Table 4: Association between lymphocyte count and microscopic findings

	Microscopic findings					
Lymphocyte count	Grade 1	Grade 2	Grade 3	Grade 4	Total	Fishers exact test
Lymphopenia	0	1	5	6	12	0.219
Normal	0	11	14	8	33	
Lymphocytosis	1	3	3	2	9	
Total	1	15	22	16	54	

A Fishers exact test was performed to examine the relationship between lymphocyte count and microscopic findings. There was no significant association between the lymphocyte count and microscopic findings (P>0.05) (Table 7).

Table 5: Association between nutrition status and CD3 (Spleen)

	Thymus CD3					
Nutrition status	No Expression	Mild expression	Moderate expression	Marked expression	Total	Fishers exact test
SAM	2	8	3	7	20	
MAM	1	1	5	10	17	
At risk of AM	1	2	1	1	5	
Well nourished	1	0	5	11	17	
Total	5	11	14	29	59	0.03

A Fishers exact test was performed to examine the association between nutrition status and CD3 (Splenic T-lymphocytes). There was a significant association between the nutrition status and CD3 (Spleen) (P<0.05) (Table 5).

Table 6: Association between nutrition status and CD20 (Spleen)

	Thymus CD20					
Nutrition status	No Expression	Mild expression	Moderate expression	Marked expression	Total	Fishers exact test
SAM	6	6	8	0	20	
MAM	0	9	7	1	17	
At risk of AM	1	3	1	0	5	
Well nourished	3	5	5	4	17	
Total	10	23	21	5	59	0.009

A Fishers exact test was performed to examine the association between nutrition status and CD20 (Splenic B lymphocytes). There was significant relationship between the nutrition status and CD20 (Spleen) (P<0.05) (Table 6).

Table 7: Association between lymphocyte count and CD3 (Thymus)

	CD3 IHC staining in thymus					
Lymphocyte count	No Expression	Mild expression	Moderate expression	Marked expression	Total	Fisher's exact
Lymphopenia	2	5	3	1	11	0.016
Normal	2	5	8	18	33	
Lymphocytosis	1	1	0	7	9	
Total	5	11	11	26	53	

A Fishers exact test was performed to examine the association between lymphocyte count and CD3 (Thymus). There was significant association between the lymphocyte count and CD3 (Thymus) (p<0.05) (Table 7).

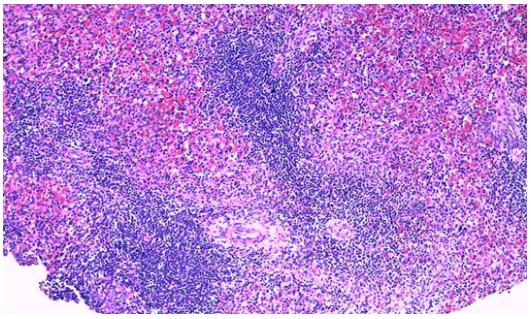
Table 8: Association between lymphocyte count and CD20 (Thymus)

	CD 20 IHC staining in thymus					
Lymphocyte count	No Expression	Mild expression	Moderate expression	Marked expression	Total	Fisher's exact
Lymphopenia	3	5	3	0	11	0.769
Normal	5	14	10	4	33	
Lymphocytosis	2	2	4	1	9	
Total	10	21	17	5	53	

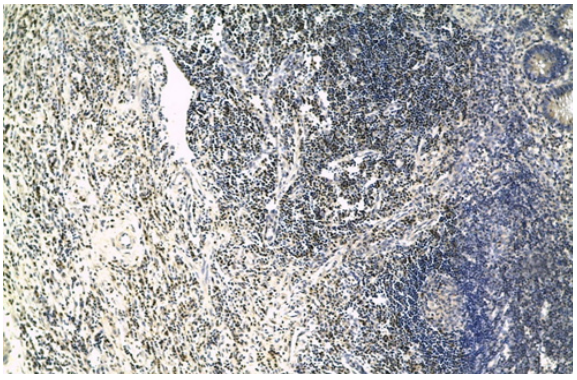
A Fisher's exact test was performed to examine the association between lymphocyte count and CD20 (Thymus). There was no significant association between the lymphocyte count and CD20 (Thymus) (P>0.05) (Table 8).

Figure 9: Histomorphological photomicrographs

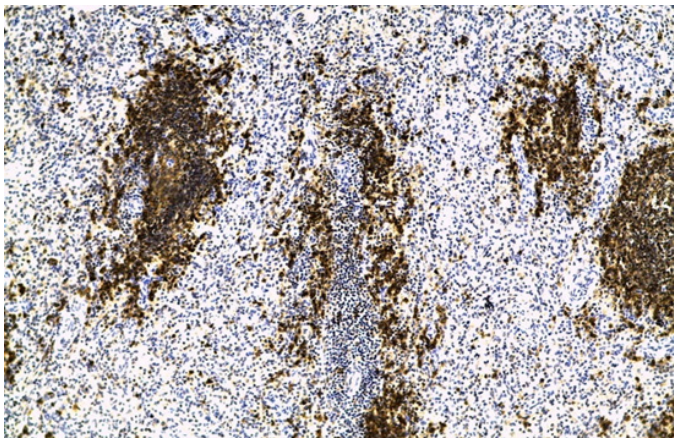
H/E staining of the spleen on TMA showing PALS, white and red pulps



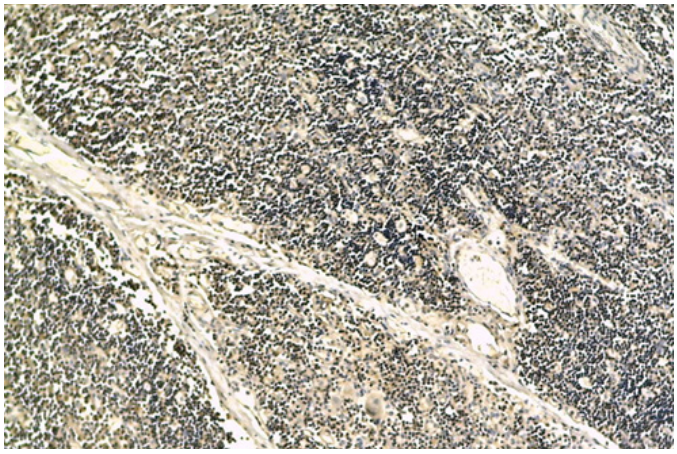
CD3 positive control using appendicular lymphoid tissue



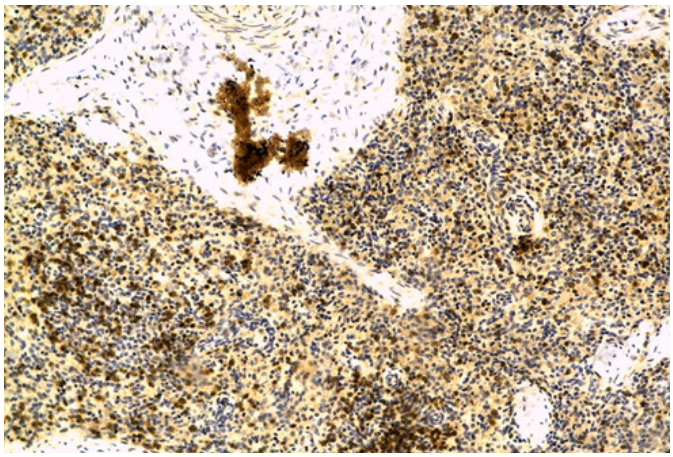
Marked CD3 (>50% cells staining) expression in the spleen. Higher magnification showing cytoplasmic staining



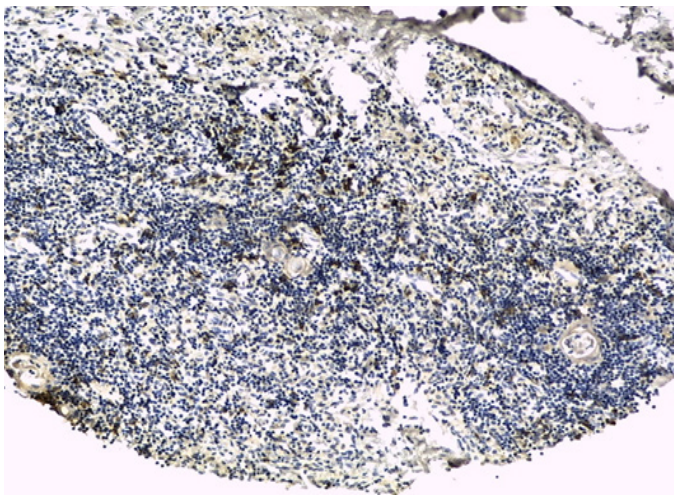
Marked CD5 expression in normal thymic lobules



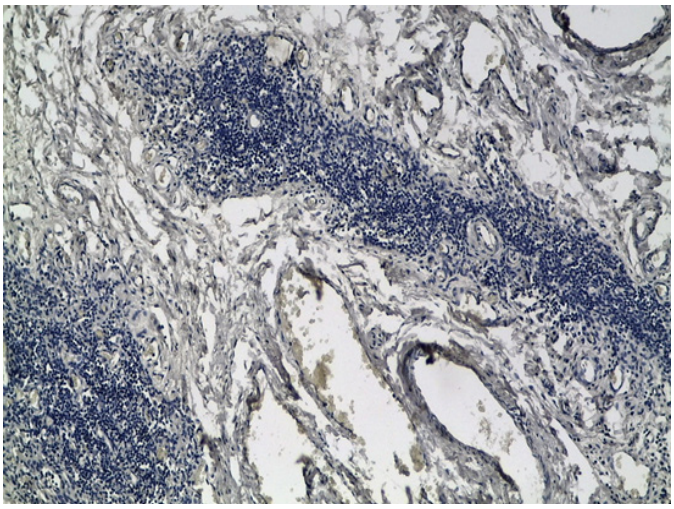
Moderate CD3 (10-50% cells staining) expression in thymic lobules



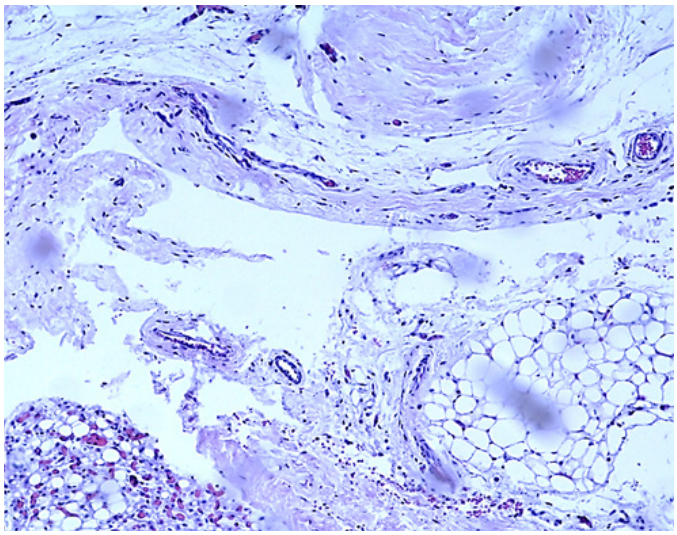
Mild CD5 (<10% cells staining) expression in the thymus- few scattered positively staining cells



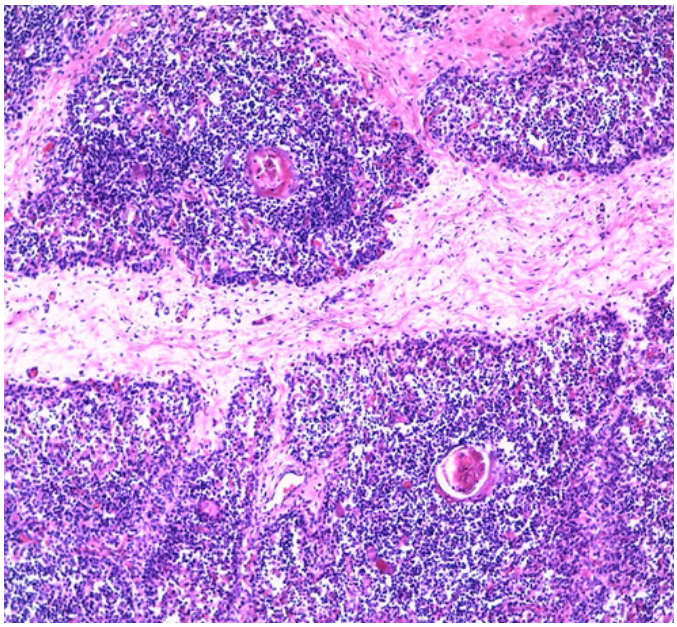
No cells took up CD20 stain on markedly atrophic thymic lobules



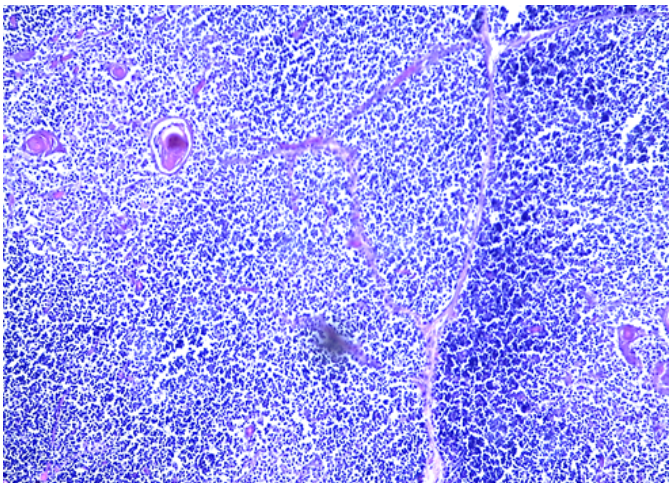
Grade 4 thymic atrophy with predominantly fibrous septae and replacement of lobules by fat tissue



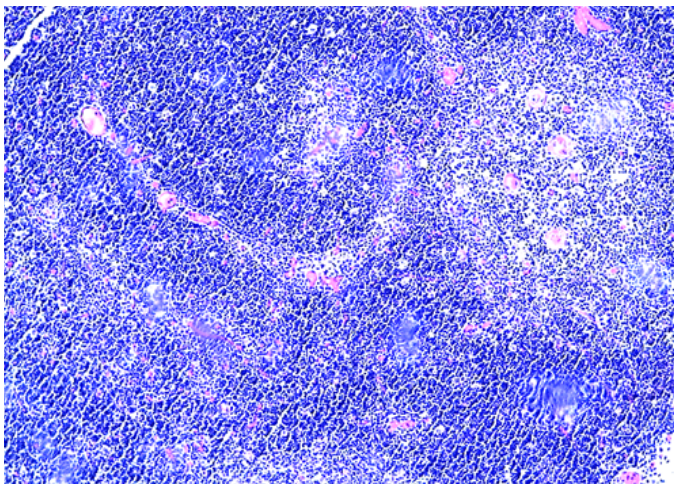
Marked thymic atrophy (grade 3) with C:M ration reversal, shrunken thymic lobules, prominent interlobular septae and prominent thymic epithelial cells



Grade 2 thymic involution showing reversal of cotrico-medullary ratio and moderate increase in thymic epithelial cell



Normal grade 1 thymus- lobular preservation with hyprcellular cortex and a hypocellular medulla, thinned out interlobular septae and few small epithelial cells



DISCUSSION

This study found that 78.3% of the study population had thymus weights below 10 grams, with a mean thymus weight of 6 grams which is considerably lower than the normal documented weight of 10-15 grams. This result was similar to a study done by Abe[7] that found reduced thymic/body weights ratios on autopsy, in infants and neonates who were exposed to various stressors i.e injury, child abuse, severe infection and congenital disease. Ninety six point seven percent of the decedents in this current study had significant features of thymic involution, whereby marked predominance of stromal extracellular matrix was classified as grade 4; scant thymic lobules with increased tangible body macrophages plus epithelial cells and prominent interlobular septae was classified as grade 3; intact lobules but with reversal in cortico-medullary ratio and mild increase in tangible body macrophages was termed as grade 2. 26.7% of thymi

showed grade 4, 40% showed grade 3 and 30% exhibited grade 2 features. Only 3.3% had normal histologic findings (grade 1). The results were similar to a study done by Toti *et al*[8], where grade 2 and grade 3 thymic involution were present in spontaneously aborted fetuses due to chorio-amnionitis. Abe[7] in his study found higher grades of involution i.e. 2,3 and 4; in the thymic tissue from stressor positive infants and neonates as compared to the control group which exhibited normal histology. A study by Garly *et al*[9] concluded that small thymus size at 6 months of age is a strong and independent risk factor for mortality in cases of childhood infections.

In infectious states, the thymus is directly affected by localized infectious agents or through systemic mediators such as increased glucocorticoids and pro-inflammatory agents from distant infectious sites[10]. Rice *et al*[11] and Walong *et al*[12] reported that malnutrition is strongly associated with increased risk of mortality from acute lower respiratory tract infections and pneumonia. In another study done by Njuguna *et al*[13] found that 75.9% of the 60 decedents who were included in the current study died within 4 days of hospital admission with majority of the deaths occurring within the first day of admission.

Despite the high prevalence of thymic involution, there was significant expression of T-cell markers where majority of thymic tissue had moderate-marked expression (72.9% CD3 staining) of the T-cell markers. This scenario was replicated in the spleen (85% CD3 staining). Contrary to this, majority of the thymi had mild-moderate expression (74.5% CD20 staining) of the B-cell markers with moderate-marked B-cell expression (73.3% CD 20 staining) in the spleen. This finding is similar to the Toti *et al*[14] study which did not show difference in T, B and NK cellular populations of atrophic thymi from fetuses with chorioamnionitis as compared to the normal group. A study by Nobrega *et al*[15] showed that homing of peripheral T-lymphocytes in an attempt to mediate protection against infection resulted in significant T-cell population within the affected thymi. The increased lymphocyte expression in the thymus can thus be explained by peripheral lymphocyte infiltration.

Malnutrition has been associated with escalated childhood mortality rates due to various factors such as impaired gut barrier functions, reduced plasma immunoglobulin and complement levels and diminished thymus sizes as a result of decreased thymocyte proliferation and increased thymocyte apoptosis[2]. In this study, 81.7% of the decedents were malnourished, with 33.3% having severe acute malnutrition, 28.3% having moderate acute malnutrition and 10% were at risk of malnutrition. Only 28.8% of the decedents were well nourished. Similarly, Chavalier *et al*[16] documented marked thymic involution - on ultrasonography, in children who were followed up and treated for malnutrition. Although there was no significant relationship found between the microscopy of the thymus and nutritional status, we found that the relationship between CD3 T-cell populations and CD20 B-cell populations in the thymus with nutritional status to be significant ($P<0.05$). These findings are similar to a study done by Shushimita *et al*[17] who reported significant reduction in immature and mature B-cells in lymphoid organs of mice that were subjected to dietary restriction and fasting. Similarly, their thymi exhibited T-cell depletion with arrested thymopoiesis.

In this study, we found that 61.1% of the decedents had normal lymphocyte counts while 16.7% had lymphocytosis. Only 22.2% of these decedents had lymphopenia; despite the high prevalence of malnutrition and thymic involution in this study population. This finding compares with a previous systemic review article by Rytter *et al*[18] that reported high lymphocyte counts in malnourished children. We did not find significant relationship between malnutrition and peripheral lymphocyte counts. This is contrary to a study done by Varga *et al*[19] that found a positive correlation between thymus size and peripheral lymphocyte count.

CONCLUSIONS

(i) The common histologic changes in the under five children presenting with acute respiratory infections; include fibrosis, marked reduction in lobular sizes, increase in thymic epithelial cells, fat replacement, increased interlobular extracellular matrix, increase in macrophages.
(ii) Cellular populations of B and T and NK cells in the secondary lymphoid organs were not adversely affected by thymic involution.
(iii) T and B cell populations in the thymus and secondary lymphoid organs were significantly affected by malnutrition states of the decedents.
(iv) Peripheral lymphocyte counts on complete blood count were not affected by the atrophic thymi.

LIMITATIONS

We relied on pre-established thymic weight ranges from western countries as there are no documented normal weights from our local population. This might have affected interpretations of thymic weights.

Not all decedents had their clinical data readily available, this could have led to the negative clinical correlations.

RECOMMENDATIONS

Clinicians could be advised to assess thymus size ultrasonographically, as a marker of morbidity/ mortality in children who present with chronic infection plus malnutrition.

Aggressive clinical management of malnutrition in children is recommended to avoid preventable fatalities from SARI.

Future studies that target a wider study population that incorporate C4/CD8 markers, infectious aetiologic agents, immunoglobulin levels could be done to further analyze thymic function, and the effects of malnutrition and infections on immunologic status of these children.

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ABO and Rh D blood group distribution among blood donors at the Kenyatta National Hospital

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ABSTRACT

Background: Since their discovery, ABO and Rh D blood groups have been shown to be of clinical importance in blood transfusion and organ transplant procedures due to their expression on red cells, endothelial and epithelial membranes. For success of blood transfusion, there should be compatibility between the donor and the recipient. Incompatibility is indicated in cases of haemolytic reactions and HDN during pregnancy. The distribution of these blood groups varies between races and geographical locations.
Objectives: The main aim of this study was to determine the distribution of the ABO blood groups and Rh D antigens among the donor population between January 2017 and January 2018. The donor characteristics of age and gender were also considered.
Methodology: Data used in this study was retrieved from the hospital's donor records and included; donor blood group, age and gender. The sample size was determined from the total number of donors within that period and systematic sampling method used to pick the subjects. A total of 500 donors were included in the study. The data was collected and analysed using Excel to present the distribution of the blood groups, age and gender of the donors. The data was presented in forms of tables and bar graphs.
Results: Out of the 500 donors in this study, 44.8% were blood group O, 27.8% were blood group A, 20% were blood group B and 7.4% were blood group AB. Ninety four point six percent were Rhesus D positive while 5.4% were Rh D negative. Seventy percent were males and 30% were females. Forty point eight percent of the donors were in the 18-27 years age group, 38% were in the 28-37 years age group, 16.6% were in the 38-47 years age group, 4.4% were in the 48-57 years age group and 0.2% were above 58 years.
Conclusion: The most common blood group was O and the Rh D antigens were present in 94% of the participants. Male donors outnumbered their female counterparts and the majority of donors belonged to the 18-37 years age group.
Recommendations: The results from this study provided useful information that would influence policy making in recruitment of donors and proper planning in management of the blood banks inventory to ensure that there is no deficit of any of the blood types. Mobilization of the female gender into blood donation is necessary to boost the voluntary blood donation numbers in the country.

Key words: Haemolytic disease of the new-born, Voluntary, Blood donor, Blood recipient, Blood bank, Transfusion reaction, Service, Unit

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INTRODUCTION

There are several blood group systems on the basis of different blood group antigens but the ABO and Rh D system is the most important in clinical practice[1]. In 1901, Austrian Karl Landsteiner discovered the common blood types A, B and O based on the presence or absence of A and B antigens on the surfaces of red blood cells[2]. A year later in 1902, Adriano Sturli and Alfred von Decastello, working under Landsteiner, discovered type AB[3]. In the Rh D system discovered by Landsteiner and Alexander Weiner in 1940, based on the presence or absence of Rh D antigens on the surfaces of red blood cells, blood groups are Rh D positive or Rh D negative[4].

The expression of ABO antigens is controlled by three genetic loci: *ABO* found on chromosome 9, *H* and *Se* both found on chromosome 19[5]. The genes from each locus are inherited in pairs as Mendelian dominants and each gene codes for a different glycosyltransferase enzyme which attaches specific monosaccharides

onto precursor disaccharide chains[6]. Expression of A and B antigens depend on the *H* and *Se* genes which both produce glycosyltransferases that add L-fructose producing the H antigen[6]. Presence of the A or B gene or both genes lead to the production of further glycosyltransferases which convert the H substance into A and B antigens by the terminal addition of N-acetyl-D-galactosamine and D-galactose respectively[6].

The *RH* locus is on chromosome 1[8]. The clinical importance of the Rh system is that individuals who are D negative, when transfused with D positive blood or if exposed to D positive foetal red cells having crossed the placenta during pregnancy are often stimulated to make anti-D[9].

The compatibility of ABO and Rh D blood group between a donor and recipient is crucial in the success of blood transfusion practice. It is preferable for patients to receive blood of the same blood group and Rh antigen.

To meet the needs of patients in a hospital it is important to collect the right types and quantities of blood for transfusion hence the knowledge of the distribution of the blood groups is important in managing transfusion services. Moreover, gender participation in blood donation has been of interest. It has been shown that males constitute a majority of the blood donors[10].

Study justification: Knowing the donors blood group will enable the research to conclude on the most common blood group within the study period and therefore provide information that will help the hospital in making future decisions such as managing their inventory and starting targeted donor campaigns for those blood groups that are less common.

Demographic information of the blood donors is important in formulating and monitoring recruitment strategies. This study highlights the rate of male and female participation and therefore concludes on the most dominant gender in blood donation within the study period. This information can be used to implement gender targeted motivational donor campaigns and address perceptions that may hinder a particular gender not to participate in blood donation. The study also indicates the age group distribution of blood donors which will also help in donor recruitment.

Broad objective: To determine the ABO and Rh D blood group distribution among blood donors at the KNH.

Specific objectives: To determine the age group distribution among the blood donors and to determine the gender distribution of the blood donors.

MATERIALS AND METHODS

Study design: This was a cross sectional descriptive study carried out at the Kenyatta National Hospital (KNH) - Blood Transfusion Unit (BTU) involving blood donors for a period of one year between the periods of January 2017 to January 2018.

Study area description: The study was carried out at the BTU in KNH and used the donor records kept by the department.

Study population: This study included eligible donors whose blood was collected after the pre-screening donor selection stage.

Sample size determination: The sample size was calculated using the Slovin's formula[11] as follows:

$$n = \frac{N}{1 + Ne^2}$$

Where;
n = Sample size
N = Total donor population within the study period
e = Margin of error

The BTU receives approximately 30 donors per day. Using this number, the donor population in a year is around 10,800 and this was taken as the total donor population (N) in the study period. This study also took a 95% confidence interval and a 0.05 error tolerance level.

Using the above formula, sample size, *n*, was calculated to

$$n = \frac{10800}{1+10800 \times 0.05^2}$$

$$n = 385.71$$

$$n = 386 \text{ subjects}$$

After analysis of the first data collected, there was a huge difference between the number of males and females hence not creating a good correlation. An additional number of females were purposively added to the sample size bringing the final number of participants to 500 donors.

Sampling method and analysis: After the sample size was calculated, the study participants were selected using the systemic sampling from the donor records. Using this method, individuals were selected at regular intervals from the sampling frame. Every 10th donor recorded from the start study date was marked and recorded in a data collection sheet and the cycle was repeated all through the study period until the sample target size was reached.

ABO and Rh status were analyzed by tube method using commercially prepared anti-A, anti-B, anti-

AB and anti-D antisera blood types using specific procedures outlined in the manufacturer's manual. Prepared 5% suspensions of red blood cells in normal saline were used. Four different tubes labelled with donor unit numbers were added with one drop of antisera A, B, AB and D. To every tube with specific antisera one drop of 5% cell suspension was added and each sample was macroscopically observed for agglutination. An indirect antiglobulin test to identify patients with the Weak D phenotype was done on Rh negative donors to ensure they are truly D negative.

Variables: Variables included blood group, age and gender for individual blood donors.

Data collection instruments: The sampled donors' details were collected using a data collection sheet. The details included donor ID, ABO, Rh D blood groups, age and gender. The data was then represented in dummy tables to indicate the frequencies of the ABO blood groups and Rh D antigens, the distribution of Rh antigens per ABO blood group and the age and gender distribution of the participants.

Data management: Statistical analysis of the data included calculating percentages to indicate the distribution of blood groups, age groups and genders. Calculations were done using the Excel Program and data represented in form of pie tables and bar graphs. The data collected was only accessed by the researcher and was properly stored and secured.

Ethical consideration: Ethical approval to conduct this study was sought and received from the KNH/UON/ERC. Since this was a retrospective study, it will essentially form a service audit to improve quality of care in KNH by proper blood inventory planning. Permission to use the blood bank data was also sought from the BTU of the KNH because data was retrieved from donor records in that department. During data collection, donor names were not indicated but were replaced by unique identification numbers.

RESULTS

This study included 500 adult participants and the blood group characteristics of the donors are as shown in Table 1.

Table 1: Distribution of ABO blood group and Rh D antigens among the participants

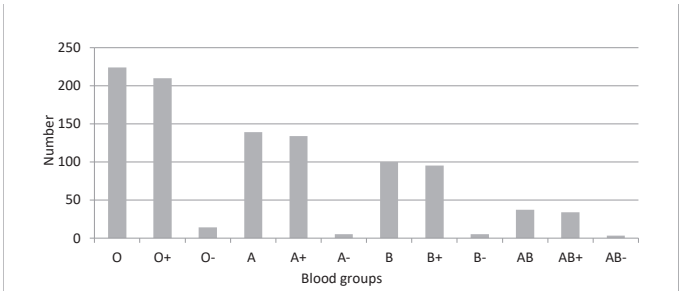
Blood group	No.	(%)
A	139	27.8
B	100	20
O	224	44.8
AB	37	7.4
Rh D positive	473	94.6
Rh D negative	27 (11 females)	5.4

The Rh D antigens per ABO blood group are distributed as shown in Table 2.

Table 2: Distribution of the Rh D antigens per ABO blood group

Blood group	Rh D Negative (%)	Rh D positive (%)
A	5 (1%)	134 (26.8%)
B	5 (1%)	95 (19%)
O	14 (2.8%)	210 (42%)
AB	3 (0.6%)	34 (6.8%)

Figure 1: Blood group distribution



Out of the 500 participants who were included in the study, there were more males (70%) than the females (30%) (Table 3). Majority of the participants belonged to the age group of 18-27 years and the least were 58+ years (Table 4).

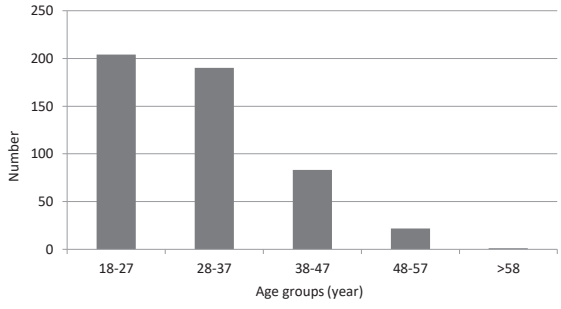
Table 3: Gender distribution

Sex	No.	(%)
Male	350	70
Female	150	30

Table 4: Age distribution

Age group (years)	No.	(%)
18-27	204	40.8
28-37	190	38.0
38-47	83	16.6
48-57	22	4.4
>58	1	0.2

Figure 2: Age distribution



DISCUSION

This study shows that the most common ABO blood group is O and the least is AB among the participants at the KNH in Nairobi. These findings are consistent with other previous studies done within the Nairobi population[12, 13]. They are also consistent with other studies done elsewhere in Africa[14 - 20] and the European countries[21 - 23]. In contrast to this study, blood group B is the most common in countries such as India, Pakistan and Bangladesh[24 - 27] while blood group A is the most frequent in countries like Istanbul, Nepal and France[28-31]. In all of the studies, blood group AB has the least distribution.

The frequency of Rh D negative in this study is 5.4% which is similar to other studies done elsewhere in other African population [13-16] in contrast is statistically lower than in other populations where Rh D negativity is as high as 15-17% [28-32]. Globally we share the same blood group types however there are some geographic, regional, and ethnic differences. Ensuring adequate Rh positive blood supply is important in the context of patient safety especially in pregnant mothers and multiply transfused patients.

This study found that the majority of the donors were males (70%) and this is consistent with other studies done across Africa, Asia and Middle East which also indicate that females constitute a lower number compared to their male counterparts[14-32]. Women may present to give blood, but are not allowed to make a donation, most frequently because of a low level of haemoglobin. The second factor is the higher rate of adverse reactions in women, which is related to the lower weight of women compared to that of men, which increases the probability of adverse reactions such as dizziness and fainting[33-35]. Other factors are cultural taboos, pregnancy and breastfeeding[34,35]. This is unlike other studies done in European countries of Finland, France, Denmark, Netherlands, Belgium, United Kingdom, Spain and Portugal where the female donors are more than 40% of the donor population[29-31].

The most common age group identified in this study is 18-37 years (78.8%) which is consistent with a report released by the WHO in 2011 [36, 37]. This increased ability among younger adults to donate may be related to awareness, better physical health, and greater mobility. Older individuals may suffer from medical conditions such as ischemic heart disease, diabetes mellitus, malignancy and hypertension hence negatively impacting their ability to be well enough to donate blood[35, 36].

STUDY LIMITATIONS

1. This study was conducted at the BTU in KNH; the results should not be generalized to reflect Kenya as a whole.
2. The sample size is only 500 which may not be representative of the Kenyan population.

CONCLUSIONS AND RECOMMENDATIONS

The most common blood group is O and the Rh D antigens are present in 94% of the participants. Male donors outnumber their female counterparts and the majority of donors belong to the 18-37 years age group.

Knowledge of the distribution of blood types in a local setting is critical to the functioning of any national blood service. This study provides information concerning the blood type and demographic information of blood donors in the BTU at KNH and this will help in blood drives to ensure adequate blood stocks.

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Factors associated with sample rejection at Kenyatta National Hospital Haematology Laboratory

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ABSTRACT

Background: During specimen collection, handling and analyzing there are various factors that can interfere with the integrity of the sample resulting to rejection. These factors need to be considered before receiving and analyzing the sample; if the sample does not meet the set criteria, it is then rejected. Rejection of a sample is followed by notifying the authorizing clinician or processing the specimen as it is and indicating on the request form when it is difficult to obtain another sample.
Objective: The aim of this study was to identify factors that lead to sample rejection at the reception area of the laboratory. This factors fall under pre-analytical errors that may occur during specimen analysis.
Design: This was a retrospective study.
Setting: Kenyatta National Hospital (KNH) Haematology Laboratory.
Methods: The study included the entire population of all the samples rejected in the year 2018 at the laboratory. Data was collected from KNH haematology laboratory sample rejection records with the approval of KNH/UON Ethics and Research Committee and the laboratory in-charge. It was then analyzed using Microsoft Excel.
Results: In the year 2018, 644 samples were rejected at the KNH haematology laboratory. Clotted samples (85.25%) being the main reason for sample rejection. Sample less than 1 ml and mismatched details on the request form and sample with 6.21% and 2.64% respectively.
Conclusion: Sample rejection is inevitable in the laboratory but it can be reduced. This is made possible through following the set ISO standards and laboratory SOPs, a designated team of laboratory personnel that deals with phlebotomy where possible and keep up to date with the latest advances in laboratory technology like use of barcode system.

Key words: Sample, Rejection, Laboratory, Haematology, Request

INTRODUCTION

The laboratory plays a major role in patient diagnosis and treatment hence the results obtained from the laboratory should be of quality. It is estimated that 70-80% of the clinical decisions that are made mostly rely on laboratory results[1]. However, not all the samples brought forward to the laboratory get analyzed due to various reasons which occur before and during sample analysis. If the mistakes that occur during sample analysis are not corrected, they may affect diagnosis and treatment of the patient through misdiagnosis and increase in the cost of treatment. At the same time, there is loss of crucial resources such as reagents, time and manpowered that could have been used to process another sample[2].

During analysis samples go through a laboratory testing system called Total Testing Process (TTP). This system is further sub-divided into three main categories which are; pre-analytical, analytical and post-analytical[3]. It has been shown that even with the increasing rate of laboratory automation, it focuses more on elimination of errors at the analytical phase yet pre-analytical nearly accounts for 68.2% of laboratory errors which lead to sample rejection[4].

Pre-analytical phase is further divided into pre-analytical and pre-analytical phase or true pre-analytical phase[5]. The pre pre-analytical phase starts by the clinician selecting the test to be done, preparing the patient for the test, withdrawing, handling and transporting the sample. On the other hand, pre-analytical phase starts at the laboratory where the sample is received, sorted and rejected if it has not met the requirements for the test to be done[6]. Once the sample has reached the laboratory it is recorded in the sample logbook with all the details including the test to be done. If rejected, the same sample should be recorded in a sample rejection book clearly stating the reason for rejection.

Analytical phase is the actual sample analysis where after sample sorting the sample is analyzed using the various laboratory equipment and results generated. At this phase a sample is rejected if it is insufficient to carry out all the tests authorized by the clinician or if the remaining amount is not enough to re-run the test. In the haematology

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laboratory, a blood sample might pass the pre-analytical phase but are rejected at the analytical phase. This can happen if it is required to run multiple tests but is not sufficient for all of them. For instance, a specimen might get rejected if it is only enough to do CBC but not ESR. The other errors that occur at this stage are associated with the laboratory equipment, reagents and the analytical techniques used. Post analytical phase is also divided into two; Post-analytical and post post-analytical phase. The former involves recording and reporting of results while the latter involves interpretation and release of results after authorization[7].

Justification of the study: The data obtained from sample rejection should be analyzed after certain periods of time to assess the TTP, if a problem arises this should be addressed. This will improve the TTP by ensuring and promoting quality results, save on resources require to analyze the samples, lowers the rejection rate thus improve TAT. Patients will get their accurate and valid results on time, this means they get prompt medical intervention, which in turn improves the prognosis and rate recovery. Overall, reducing rejection rate improves patients' satisfaction with the medical services they receive at a particular hospital and have confidence in the general healthcare system.

Broad objectives: To establish the factors that lead to sample rejection.

Specific objectives: To identify the leading cause of sample rejection and to identify the rate of sample rejection in the year 2018.

MATERIALS AND METHODS

Study design: This was a retrospective study which reviewed data on samples rejected and recorded at KNH Haematology laboratory during the year 2018.

Study area: The study was conducted at KNH Haematology laboratory where the samples are received at the reception and recorded before any analysis are done. Samples that are rejected are recorded in the rejection book.

Study population: The study included all the available and viable data on the samples rejected at the KNH Haematology laboratory.

Inclusion criteria: The study included records of samples rejected from KNH Haematology laboratory in the year 2018.

Exclusion criteria: The study excluded any entry of a sample that was rejected and the reason behind the rejection not clearly indicated.

Limitations: The study was limited to where the reason for rejection is not clearly indicated in the rejection book. Besides, most laboratories do not record cases where samples that were analyzed while they should have been rejected. This may result in misleading findings that show a lower rate of rejection than it actually is.

Data management: The data was recorded on a laboratory notebook where it was verified and corrected manually. It was then be entered in Microsoft Excel Spreadsheet 2010 for sorting, cleaning and analysis.

Ethical considerations: Ethical approval was sought from the UON/KNH Ethics Review Committee (KNH-ERC). Any information obtained from the records will not be disclosed to unauthorized persons and it will only be used for the intended purpose.

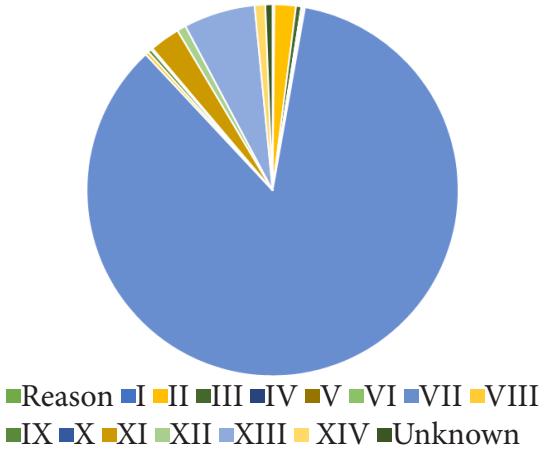
RESULTS

During the year 2018, 644 samples were rejected for various reasons. Table 1 shows the number of samples rejected based on the sample rejection criteria used by the laboratory.

Table 1: Rejection criteria

Serial No.	Reason for rejection	Frequency	(%)
I	Request form not fully filled	1	0.16
II	Unlabeled specimen	12	1.86
III	Wrong specimen container	3	0.47
IV	Leaking specimen container	0	0.00
V	Request with no specimen	1	0.16
VI	Specimen with no request	1	0.16
VII	Clotted sample	549	85.25
VIII	Request test not done in the lab	2	0.31
IX	Outpatient specimen not paid for	2	0.31
X	Contaminated container	1	0.16
XI	Mismatched details on request form and container	17	2.64
XII	Unknown or illegible test	5	0.78
XIII	Blood specimen less than 1ml	40	6.21
XIV	Any other i.e. empty specimen container	6	0.93
XV	Not clearly indicated reason for rejection	4	0.62
Total	Samples rejected	644	100.00

Figure 1: Data representation



Out of the 644 samples rejected in the year 2018, the main reason for rejection was clotted sample which is 85.25% of the total samples. Blood sample less than 1ml together with mismatched details on the request form and sample container follow with them accounting for 6.21% and 2.64% respectively for rejection.

DISCUSSION

Based on the rejection criteria, most of the reasons are associated with the pre-analytical phase of the TTP. Clotted sample has been identified as the main reason for rejection based on the data collected. A study carried out by Shiferaw and colleagues[8] at decentralized phlebotomy centers showed that clotted sample was the main reason for sample rejection with 23.3%. This cause for clotted sample might be associated with not observing the order of draw of blood as recommended in SOPs. Order of draw of blood is observed during a closed system where by multiple samples are been taken from one patient. Clinical & Laboratory Standards Institute (CLSI) [9] recommends the following order for draw of blood: blood culture tube, coagulation tube, serum tube with or without clot activator or serum gel tube with or without gel, heparin tube, EDTA tube, glycolytic inhibitor tube. This aims at minimizing contamination of samples collected and ensure that the results obtained are accurate[9,10]. Clotting of a sample may also be brought about by the phlebotomist not mixing the sample well after collecting. After collection the sample should be inverted 5-10 times slowly to allow proper mixing of the anti-coagulant with the sample[11,12]. Other factors that may lead to clotting of blood include dispensing of the sample into the sample container via the needle, use of butterfly needles and forceful collection of blood[12].

Blood sample collected of less than 1ml is the second reason for sample rejection from the data. Studies carried out by Salvagno *et al*[14] and Plebani *et al*[15] together with their colleagues indicate that inadequate sample volume is among reasons for rejection at 21% and 15% respectively. Sample collected below 1ml is as a result of difficulties encountered during blood collection via the peripheral blood veins. This is mostly associated with neonate paediatrics and oncology patients because locating a suitable vein to draw is a challenge[16,17]. The main reason as to reject samples less than 1 ml is because the sample anticoagulant ratio is not met.

Mismatched details on the request form and on the sample followed by unlabeled specimen were among other notable reasons for sample rejection with 2.64% and 1.86% respectively[15,17]. Mismatched details are information on the request form and sample is not similar to patients with identical names but there are no unique details to distinguish between the two. This may be avoided by providing more than unique identifying features on the sample like laboratory number and patient identification number which are unique to the sample collected and patient.

RECOMMENDATIONS

Sample rejection is avoidable and can be reduced through continuous training and education of the clinicians and laboratory personnel. Even the new recruits joining the field should be informed on proper ways or advances that have been used in sample container collection. For clinicians it is important to provide information on the tests done at the laboratory and to which the sample is collected into. Establishment of a dedicated staff that only deal with phlebotomy. This will reduce the chances of sample rejection because they are dedicated to only phlebotomy. Phlebotomy should only be done if possible by trained personnel to reduce high rates of sample rejection. Introduction of automatized laboratory sample labeling through a barcode system. The barcode is attached to the sample which contains all the patient information and tests to be done. This also has an added advantage that it will cost as the system will tend to turn paperless. A proper system should be put in place to identify this reason for sample rejection through use of quality indicators and continuous assessment.

CONCLUSIONS

The laboratory plays a major role in diagnosis and treatment of patients. It is important to ensure that the results produced are accurate and acceptable. This is made possible by following set standards, guidelines and laboratory protocol. By doing so this improves the patient confidence in the hospital and trust the set health care system structure works hence believes and faith in the stakeholders and the healthcare system.

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Priapism - a rare presentation in chronic myeloid leukemia: case report

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ABSTRACT

Priapism is a rarely seen in leukemia. We report a 17-year-old young adult, recently diagnosed with chronic myeloid leukemia, who presented with persistent painful erection of the penis for over 24 hours at home. Surgical treatment of the priapism was initiated by corporal aspiration and phenylephrine irrigation in theatre. The erection gradually reduced following further aspirations and washes.

Key words: Priapism, Chronic myeloid leukemia, Erection, Painful, ischemia

INTRODUCTION

Priapism is a prolonged erection of the penis that continues hours beyond (more than four hours) and is not associated with sexual stimulation[1]. It is a rare condition that occurs commonly in certain groups such as sickle cell anaemia[2]. Ischemic priapism is marked by rigidity of the corpora cavernosa with little or no cavernous arterial inflow[1, 2]. The patient typically complains of penile pain, and the examination reveals a rigid erection. Causes of veno-occlusive priapism cases in the literature apart from sickle cell disease include urinary retention, insect bites and haematologic dyscrasias[3]. In haematologic dyscrasias priapism may be seen in patients with leukocytosis with incidence of 1%–5%[3, 4]. One of the causes of leukocytosis is Chronic Myeloid Leukemia (CML). CML is characterized by the clonal increase and unregulated growth of myeloid cells in the bone marrow and the accumulation of these cells in the blood and is associated with Philadelphia chromosomal translocation[5]. We report a case of a 17 year old young adult who presented with priapism as a manifestation of chronic myeloid leukemia.

CASE REPORT

A previously healthy 17-year-old male referred from a peripheral hospital for haematological evaluation with high white blood cell count and no anaemia. He reported feeling fairly well, no fever, no night sweats, no history of weight loss, joint pains or cough. He had been treated for non-specific fever for which he was put on antibiotics and analgesics. A total blood count was done revealing that he had increased white cell count. That is when he was advised to seek a haematological review. The only notable finding on examination was a splenomegaly of 4cm below the left costal margin. A total blood count revealed excessive white cell count of over 512,000/mm^3 with a thrombocytosis of 498,000/mm^3 and borderline haemoglobin level of 10 g/dl. Further investigations including a bone marrow aspirate and Philadelphia chromosome studies were done confirming a diagnosis of chronic myeloid leukemia (Stable phase). Since the patient was stable he was put on hydroxyurea awaiting definitive treatment with imatinib. He was also put on allopurinol 300 mg daily to mitigate for potential tumour lysis syndrome. He was advised to have adequate hydration.

Figure 1: Bone marrow aspirate

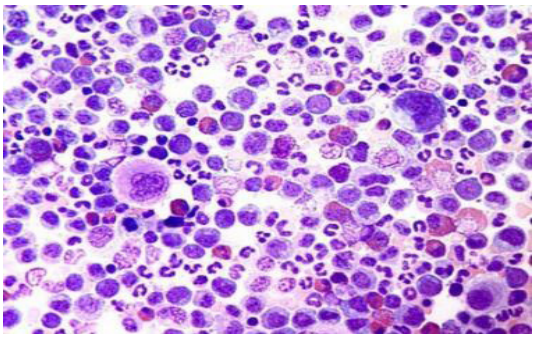

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Figure 2: Chromosomal aberration in CML

A/c Status : P **Ref by** : UNKNOWN **Report Status:** Final

Fluorescence in-situ Hybridization (FISH)

BCR - ABL t(9;22) Translocation Assay *

Specimen : Peripheral Blood

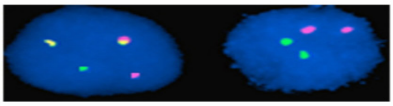
Clinical Indication : To rule out BCR/ABL translocation

Result : nuc ish (ABL,BCR)×3,(ABL con BCR×2)[160/200]

160/200 (80%) Interphase nuclei show 1O 1G 2Y fusion signals for BCR/ABL

Interpretation : Specimen is **Positive** for t(9;22)(q34;q11.2)

Interphase nuclei analyzed	Normal nuclei 2 Orange 2 Green Signals	Abnormal nuclei 1 Orange 1 Green 2 Yellow Signals
200	40	160



Method : FISH analysis performed on 200 Interphase nuclei

Probe : ZytoLight SPEC BCR/ABL1 Dual Color Dual Fusion probe

Note : Absence of an abnormal clone does not rule out the presence of a neoplastic disorder.
: This is a Laboratory developed test (LDT), developed at Dr. Lal PathLabs and has not been cleared or approved for specific uses by FDA.

Comments: Fusion of BCR/ABL1 is observed in all patients with Chronic Myeloid Leukemia (CML); in approximately 25% of adult patients with precursor B cell Acute Lymphoblastic Leukemia (B-ALL) & in 1% of patients with pediatric B-ALL. The chromosome mechanism resulting in BCR/ABL1 fusion is a t(9;22) (q34;q11.2) in approximately 85% cases; a complex 9;22 translocation with one or more additional chromosomes in approximately 15% cases and a chromosomally cryptic or insertional translocation in <1% of patients.

Cut off for the normal individual is 4%.

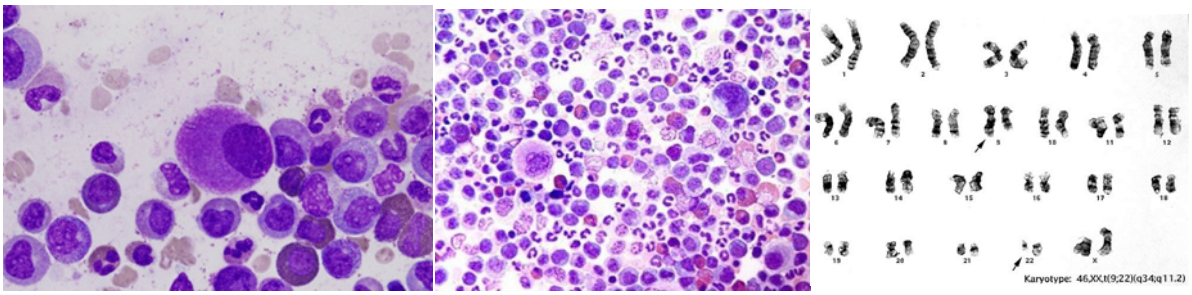
Five days later the patient noticed his penis remained erect and was painful. He had never had any similar episodes previously. This time the conjunctiva appeared pale with no jaundice. The penis was erect, firm and tender with superficial venous engorgement. Full haemogram showed haemoglobin (Hb) 8.9 g/dl, haematocrit 24.3%, White Blood Count (WBC), 349,000/mm^3, and platelet 754,000/mm^3. Liver and kidney function tests were unremarkable. He was admitted as an emergency and put on intravenous cytosar awaiting urological review. Surgical treatment of the priapism was initiated by corporal aspiration and phenylephrine irrigation in theatre. The erection gradually reduced following further aspirations and washes.

DISCUSSION

Priapism was named after the Greek god Priapus. It is believed that a jealous Hera or Aphrodite cast a spell over his mother while pregnant causing Priapus to be born with the affliction priapism[6]. Priapism is a rare condition and about 20% of the cases are seen in haematological conditions including sickle cell diseases and leukemia[4]. About 1 to 5% of male patients with leukemia may present with priapism, with 50% of these patients being diagnosed with chronic myeloid leukemia[7]. However, about only in 1–2% of patients with CML present with priapism[7,8]. While priapism in CML patients has been described in all age groups, it tends to have a bimodal age distribution in males aged 5–10 and 20–50 years[7].

American Urological Association recommends a multifaceted approach to treatment of priapism including systemic treatment with high dose hydroxyurea, TKIs and intra-cavernous treatment which should be administered concurrently[9-11]. Leukapheresis to reduce hyperviscosity is also advised especially in extremely high counts such as in this case[12, 13].

Figure 3: Chromosomal aberration in CML



CONCLUSIONS

Priapism is a rare presenting feature of chronic myeloid leukemia. It is a urological emergency requiring urgent intervention to prevent permanent penile damage. It is associated with hyperleukocytosis and associated leucostasis. A combined management including systemic therapy, therapeutic leukapharesis as well as surgical intervention is advised. Besides the initial relief of priapism, the further workup and management of the underlying disease are more important. In our case, with use of a combined urological therapy and oncological treatment to priapism, the patient rapidly had relief of his clinical problem.

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The total word count for research papers should be no more than 4000 words, case reports should have no more than 2000 words and reviews not more than 5000 words

Manuscript text

- Font size 12 point type
- Double-spaced throughout the text marked and consecutive page numbers including cover page
- Font Times New Roman
- Word document and not pdf

Tables

Number tables and figures submitted in separate files to match authors pdf and each format Microsoft word (.doc)

Illustrations*:* Scanned copies of JPEG photos should be sent. They should not be inserted in the text but the file should be saved or named with the figure numbers, title of paper and authors name. The photographs should be scanned as they should appear in the text. Photographs, graphs and diagrams will be referred to as figures and should be numbered in Arabic numerals and typed on a separate sheet. Each illustration should be accompanied by a legend clearly describing it in the text. The position of illustrations in the text should be clearly indicated.

Tables: The Editor will only accept those tables that are essential. Maximum number of tables allowed is 5. Tables should be numbered consecutively, in text, in Arabic numerals.

References: Only papers clearly related to the author's work should be referred to, exhaustive lists should be avoided. When reference is made to work by more than