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Research mentorship is key for developing future researchers

Shahin S, MMed, FCPATH-ECSA

Although traditionally defined as a relationship between a more knowledgeable individual and one less knowledgeable and experienced, mentorship is a two way mutually beneficial association that spans a continuum from loosely defined, informal relationships to structured, formal agreements between an expert (mentor) and a novice (mentee)¹.

Mentorship dates back from ancient Greece during the Trojan War, when Mentor, a friend of Odysseus, king of Ithaca, was entrusted with the task of teaching and overseeing the social spiritual and personal values of Telemachus, the son of Odysseus².

The goal of any mentoring program is to encourage professional and personal growth for the mentee with each mentor mentee relationship having pre-defined roles and responsibilities in order for the process to succeed.

Mentorship in research promotes the passing on of knowledge from one generation of scientists to the next hence the importance of preparing both the mentor and mentee to navigate this relationship.

Various models and settings of mentoring have been described over the past decade but the fundamental values have largely unchanged; a mentor is described as an individual with more experience who is willing to communicate that experience to a mentee in an effective manner². The mentee on the other hand must demonstrate initiative and actively seek out a mentor depending on their area of research interest. Some of the responsibilities of a mentee include the commitment to put in extra hours with enthusiasm and humility. The mentee should learn to accept constructive criticism and continue to remain engaged in the relationship over time.

A mentor is a teacher, role model, advisor, friend and an advocate who is able to guide a mentee to become a successful

researcher. Examples of research mentors include the senior leader in a research group or a more experienced researcher where the mentee might be junior faculty or research fellow. A supervisor on a research project may not necessarily be a mentor but this relationship has the potential to evolve into mentorship over time².

Personal commitment, trustworthiness, availability and setting aside protected time for mentee are some of the attributes of a good mentor. The mentor through example should provide guidance to the mentee on research methods, critical thinking, preparation and submission of scientific papers, presentation at meetings, selection of appropriate grants to apply for and teaching the ethical and responsible conduct of research. Creating opportunities for networking within the scientific community and providing career advice are some of the other roles a mentor should assume^{2,3}.

A good relationship between a mentor and mentee is one which is active and based on mutual respect and management of mentor-mentee expectations. A pilot mentorship program of junior female academics who were paired with more senior academics at Kings College London found that the pre-mentorship expectations of the mentees was unrealistically higher while mentor expectations of the relationship were exceeded at the end of the program. The mentees expressed highest satisfaction with career progression (39; 89%), and increased confidence (38; 87%) whereas mentors' highest expectations were of satisfaction in seeing mentees progress (26; 69%) among other outcomes⁴.

The most important skill required for a successful mentor- mentee relationship is the ability for effective communication. Providing feedback, holding regular scheduled meetings and

discussions, and setting boundaries are some of the communication strategies that are useful for a relationship to flourish. Support through setting up of research interest groups may also be a strategy for continuous engagement between the mentor and mentee. This provides a forum for the mentee to engage in critical thinking, finding solutions together and eventually owning the process².

Mentoring has its own unique challenges. As an example mentoring busy clinicians in research skills requires an understanding of their work environment. In this regard realistic expectations and goals must be set and the mentor-mentee should learn to work within the confines of this space. Mentorship also allows time for personal reflection and recognition of individual strengths that can be developed over time with the support from the mentor (5).

But is mentoring all that is thought out to be? Does it stifle growth and creativity? Does it cause dependency? Is mentorship a lifelong process? All these are important questions as a poor mentor-mentee match can lead to frustration, resentment and be counterproductive especially if the relationship is forced upon the individuals by departmental rules and regulations. As cited by Sheona⁶, mentors need to understand their role as guides rather than problem solvers otherwise this can lead to dependency on the part of the mentee.

Mentorship is lifelong learning process important for both personal and professional development and essential for the development of researchers who will add to the scientific

literature and further transmit their knowledge and experience to the next generation of the scientific community⁶.

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Prevalence of diabetes mellitus and associated factors in patients with tuberculosis at Kenyatta National Hospital and Mbagathi Level 5 Hospital, Nairobi

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ABSTRACT

Background: Tuberculosis and diabetes mellitus are both global health burdens. Prevalence of both diseases continues to rise in developing countries. Some studies have shown that diabetes mellitus increases the risk for tuberculosis. The relationship between the two diseases has not been fully investigated in Kenya.

Objectives: To measure glycated haemoglobin (HbA1c) levels among patients with tuberculosis attending both Kenyatta National Hospital (KNH) and Mbagathi Level 5 Hospital, and determine the prevalence of diabetes mellitus in these patients.

Materials and methods: This was a cross-sectional descriptive study involving adult patients diagnosed with tuberculosis attending outpatient clinics in the two hospitals. Informed consent was obtained from all participants. Demographic information was then recorded from each participant after which HbA1c was estimated in venous blood using the CLOVER A1c™ analyzer system. The main outcome variables were age, sex, HbA1c value, type of tuberculosis and HIV status.

Results: A total of 124 subjects with tuberculosis were enrolled. Most study participants were male (55.6%), aged 18 to 30 years (43.5%), had pulmonary tuberculosis (69.4%) and were HIV negative (83.1%). Thirteen study subjects (10.5%) had diabetes mellitus and 32 (25.8%) had impaired glucose tolerance. Majority of the patients with diabetes mellitus had pulmonary tuberculosis (8.1%). The mean HbA1c in patients with pulmonary tuberculosis was significantly higher than in those with extra pulmonary tuberculosis (8.3% vs 6.8%; p-value = 0.036). There was no significant difference in mean HbA1c for patients with both tuberculosis and HIV co-infection versus those patients with tuberculosis alone (5.7 versus 5.8; p-value = 0.847).

Conclusion: The overall prevalence of diabetes mellitus was 10.5% and that of impaired glucose tolerance was 25.8%. This is higher than the national estimated diabetes mellitus prevalence of 3.3% and is suggestive of an association between diabetes mellitus and tuberculosis. Routine screening of patients with tuberculosis for diabetes mellitus is recommended to enable early detection and management.

Key words: Diabetes mellitus, Tuberculosis, Glycated haemoglobin (HbA1c), HIV/AIDS

INTRODUCTION

Tuberculosis and diabetes mellitus are major causes of morbidity and mortality worldwide. Global statistics estimate that 9 million new tuberculosis cases were reported in 2013. A quarter of the new cases reported in 2013 were from sub-Saharan Africa, which had the highest rates of cases and deaths relative to population. Kenya is one of the 22 high burden TB countries that together account for more than 80% of the world's TB cases and reported 120,000 new tuberculosis cases in that year¹.

Diabetes mellitus also has a high morbidity and mortality globally and

prevalence is projected to increase markedly by 2030, with most new cases coming from developing countries². It is estimated that obesity is on the rise in Kenya with 25% of all persons in the country being obese or overweight and prevalence being highest in women in their mid to late forties in urban areas. This is anticipated to fuel an increase in the incidence of diabetes mellitus³. This rise has been attributed to a rise in obesity due to poor eating habits and a sedentary lifestyle. In 2012, WHO reports that 1.5 million deaths were secondary to diabetes mellitus and out of this, more than 80% occurred in low

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and middle income countries. In the same year, it was estimated that 1% of deaths in Kenya were attributable to diabetes mellitus although this is likely an underestimate as mortality due to the complications of diabetes mellitus had not been accounted for. The Kenyan Government's National Medium Term Plan (2014-2018) and National Health Strategic Plan (2014-2018) have both identified the prevention and control of non-communicable diseases as priority areas that need to be addressed⁴.

Reactivation tuberculosis has been associated with several immunosuppressive conditions including HIV/AIDS, end-stage kidney disease, diabetes mellitus, lymphoma, prolonged use of corticosteroids, TNF-alpha inhibitors, loss of cell mediated immunity (65 years and above). Several studies have identified a relationship between tuberculosis and diabetes mellitus. Poor glycaemic control, in known diabetics, is a known risk factor for tuberculosis. Diabetes mellitus has also been associated with an increased risk of relapse, treatment failure and 69% increase in mortality in patients being managed for tuberculosis⁵. Whereas the risk associated with well-controlled diabetes mellitus is minimal, poorly controlled diabetes mellitus may increase the risk of developing pulmonary tuberculosis⁶.

In 2008, a study in Hong Kong showed haemoglobin A1c greater than 7% was associated with a three times increased risk of active tuberculosis as compared with those diabetics who were well controlled (haemoglobin A1c less than 7%). Diabetes mellitus was associated with a slight increase in the risk of pulmonary, but not extra pulmonary tuberculosis⁷. In 2009, a South African study showed poor glycaemic control (defined by elevated glycated haemoglobin) increased the risk of active tuberculosis. The study also found the tuberculosis prevalence in children with type 1 diabetes mellitus (using Mantoux tuberculin skin test) at 29.8%. It concluded that poor glycaemic control, as well as contact with a tuberculosis source case, was associated with increased prevalence of tuberculosis⁸.

In Africa, prevalence of diabetes mellitus has also been found to vary. In Tanzania, at Muhimbili Medical Centre, Dar es Salaam, sputum positive pulmonary tuberculosis patients were evaluated for diabetes mellitus using an oral glucose tolerance test. Although 1.8% of patients were already known to have diabetes mellitus, a further 4.9% were diagnosed with diabetes mellitus. This placed the prevalence of diabetes mellitus at 6.7% and that of impaired glucose tolerance at 16.2%⁹.

In Guinea Conakry, capillary blood glycaemia test was done to detect diabetes mellitus. The prevalence of diabetes mellitus in tuberculosis patients was found to be at 3.35%. Of these patients with diabetes mellitus, 31% were undiagnosed before the survey¹⁰.

A study done in Uganda to diagnose diabetes mellitus in smear positive pulmonary tuberculosis patients found a prevalence of 8.5%. Diabetes mellitus was diagnosed using random blood testing combined with the classical symptoms of diabetes mellitus (polyuria, polydipsia and weight loss). The study group was composed of patients in the pulmonology wards who were not yet on anti-tuberculosis drugs. Out of the 8.5% found to have diabetes mellitus, known diabetics were 1.9% and all had poor glycaemic control. Regarding symptoms related to diabetes mellitus, the commonest were polyuria (90.9%), polydipsia (77.3%) and progressive weight loss (68.2%)¹¹.

The prevalence of diabetes mellitus among HIV infected anti-retroviral naïve individuals has been found to be similar to that of the general population¹². However, there has been shown to be a relatively higher level of incidence and prevalence of diabetes mellitus among people living with HIV who are receiving anti-retroviral treatment¹³. The prevalence of diabetes mellitus among patients co-infected with tuberculosis and HIV has not been fully investigated. A case-control study done in Tanzania showed the prevalence of diabetes mellitus was 16.7% in pulmonary tuberculosis patients compared with non-tuberculosis controls for whom the prevalence was 9.4%. The study concluded that diabetes mellitus is a risk factor for TB in HIV uninfected, whereas further studies are needed to examine the association in HIV infected persons¹⁴.

In 2011, WHO and American Diabetes Association (ADA) recommended the following tests to screen for diabetes mellitus: Random plasma glucose (with associated polyuria, polydipsia and weight loss); Fasting plasma glucose; Oral Glucose Tolerance Test (OGTT); Glycated haemoglobin (HbA1c)^{15,16}. HbA1c values were not previously recommended to diagnose diabetes mellitus because of variation in the different assays. Due to standardization of more than 99% of the assays by the National Glyco-haemoglobin Standardization Program (NGSP) to Diabetes Control and Complications Trial (DCCT) standards, there has been improved precision and accuracy of many international assays.

As per the 2011 guidelines, WHO recommended the screening of patients with tuberculosis for

diabetes mellitus¹⁷. The relationship between diabetes mellitus and tuberculosis has not been fully investigated in Kenya. This study sought to determine the prevalence of diabetes mellitus in the population with tuberculosis with the aim of finding out if diabetes mellitus is indeed a significant co-morbidity in the subset of our population with tuberculosis and if screening for diabetes mellitus will be beneficial in such patients. This is currently not recommended in Kenya and is not practiced at the two hospitals we sampled. HbA1c has also not been adopted for diagnosis of diabetes mellitus in the two hospitals. This is the first study to utilize HbA1c for diagnosis of diabetes mellitus in this set-up.

MATERIALS AND METHODS

Broad objective: To determine the prevalence of diabetes mellitus, using HbA1c, in adult patients diagnosed with tuberculosis at Kenyatta National Hospital (KNH) and Mbagathi Level 5 Hospital, Nairobi.

Specific objectives: To determine HbA1c levels in patients with tuberculosis at KNH and Mbagathi Level 5 Hospital, and to determine the prevalence of diabetes mellitus in patients with tuberculosis at KNH and Mbagathi Level 5 Hospital.

Secondary objectives: To determine the proportion of diabetic patients co-infected with tuberculosis and HIV, and to correlate HbA1c values with tuberculosis treatment status.

Study design: The study was a laboratory based descriptive, cross-sectional study.

Study area: Kenyatta National Hospital is a tertiary hospital located on Hospital Road, Nairobi. It is approximately 3.7 km from the Central Business District. It has a bed capacity of 1,455 with 50 wards and 22 out-patient clinics. It serves as a national referral hospital as well as a teaching hospital for the University of Nairobi, College of Health Sciences. The study was carried out in clinic number 16 which is the tuberculosis clinic and serves 10 to 15 patients per day. The clinic is run by highly skilled physicians.

Mbagathi Level 5 Hospital is located on Mbagathi way, Nairobi. It is approximately 7 km from the Central Business District. It has a bed capacity of 200 beds and serves as an extension of the University of Nairobi, College of Health Sciences. The study was carried out in the tuberculosis out-patient clinic which serves 15 to 20 patients per day and is also run by trained physicians.

Case definition: Tuberculosis was defined as a positive sputum smear on Ziehl Nielsen stain for acid-alcohol fast bacilli, a positive Gene X pert/RIF-TB test result, a positive sputum culture for tuberculosis or a histology/cytology diagnosis of tuberculosis. Diabetes mellitus was defined as HbA1c over or equal to 6.5%. Impaired glucose tolerance was defined as HbA1c between 5.7% and 6.4%^{15,16}.

Study population: Study subjects were aged above 18 years, either female or male, who presented to clinic 16 at Kenyatta National Hospital and the tuberculosis clinic at Mbagathi Level 5 Hospital, Nairobi. All study subjects had a diagnosis of tuberculosis, as per the case definition above, which was confirmed from the pre-existing records prior to administration of the questionnaire.

Data collection procedures

Sampling and recruitment of cases: The principal investigator, and research assistant, visited the tuberculosis clinic at KNH every weekday on alternate weeks of the study. The same was done at Mbagathi Level 5 Hospital. Clinic visits lasted a total of ten weeks and a total of 124 subjects were enrolled into the study.

Consent: All patients who met the inclusion criteria, and did not have any of the exclusion criteria, were approached for informed consent. Participants were then recruited until the sample size was achieved. Those who declined to give informed consent were excluded from the study.

Administration of questionnaire: Patient information was obtained by administering a questionnaire. Demographic details of the clients were collected as well as their HIV status, history of diabetes mellitus and details regarding management of tuberculosis. The information was obtained directly from the patient as well as from their medical records.

Laboratory methods

Sample collection and handling: Venous whole blood (2-3mls) was collected aseptically using sterile equipment in an Ethylene Diamine Tetra-acetic Acid (EDTA) vacutainer tube. All tests were run by the principal investigator at the University of Nairobi Clinical Chemistry laboratory.

Test procedure: The specimens were analyzed using the CLOVER A1c™Self kit obtained from Infopia Co., Ltd, using the CLOVER A1c™Self Analyzer. Test method of the CLOVER A1c™

Self system is National Glyco-haemoglobin Standardization Program (NGSP) certified, thus the values of calibration parameters determined to provide an optimal reagent performance, are based on DCCT reference method.

Quality assurance: Quality assurance was maintained according to the laboratory standard operating procedures and all tests were analyzed using the manufacturer's instructions.

Data management: Data was collected in a structured questionnaire and entered into MS Excel computer database, cleaned and verified, then analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 software. All the data that had been collected will continue to be kept in safe custody for five years after completion of the study. The main outcome variables were age, sex, HbA1c value, type of tuberculosis and HIV status.

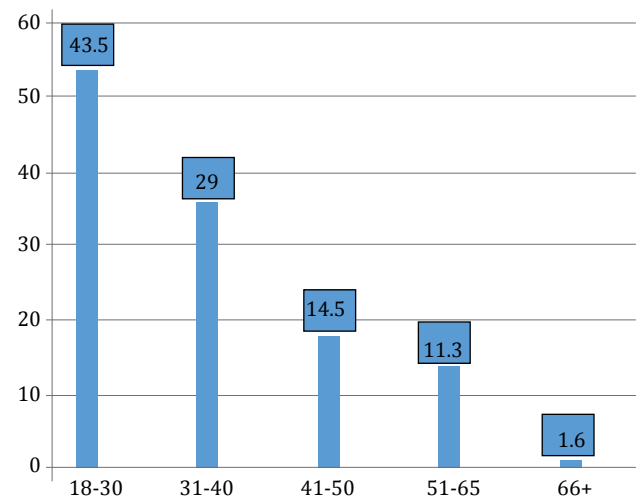
RESULTS

General characteristics of study subjects: A total of 124 subjects were recruited into the study over a period of ten weeks. Majority were enrolled from Mbagathi Level 5 Hospital (51%). Males comprised 55.6% of the study subjects. Regarding age, the range was 18-72 years with a median age of 32 years and mean of 34 years. Most of the subjects were in the 18-30 year age bracket (43.5%). Only two subjects were more than 65 years old and both were females. Results of HIV serology were available for all the study subjects and majority were HIV negative. Only 21 subjects (16.9%) were HIV positive. These findings are shown in Table 1 and Figure 1.

Table 1: Demographic characteristics of study subjects (n =124)

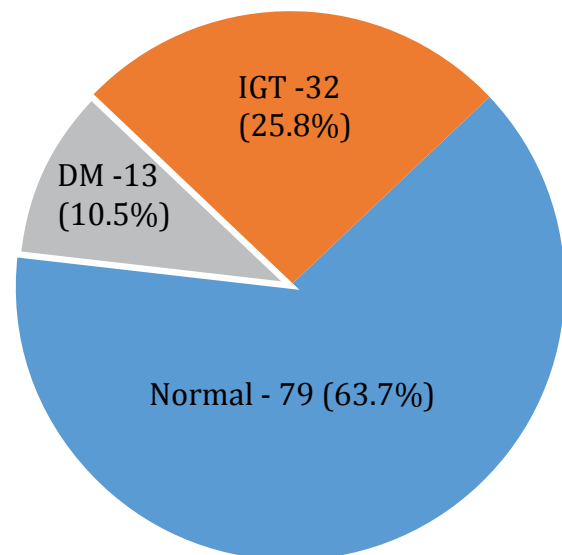
| Variable | No. (%) |
|---------------------------|------------|
| Recruitment site | |
| Mbagathi Level 5 Hospital | 63 (51) |
| Kenya National Hospital | 61 (49) |
| Sex | |
| Female | 55(44.4) |
| Male | 69 (55.6) |
| HIV status | |
| Negative | 103 (83.1) |
| Positive | 21 (16.9) |

Figure 1: Age distribution of study subjects (n=124)



HbA1c values in study subjects with tuberculosis: Most of the study subjects (63.7%) had normal HbA1c values. A total of 13 subjects (10.5%) had elevated HbA1C values diagnostic of diabetes mellitus giving a prevalence of 10.5%. The rest (25.8%) had HbA1C values indicative of impaired glucose tolerance. This is represented in Figure 2.

Figure 2: Distribution of HbA1c values in subjects with tuberculosis (n=124)



Mean HbA1c values in study subjects with tuberculosis: The mean HbA1c of the total population was 5.7 (SD 1.1), median was 5.5 (IQR 5.2-6.1) and mode 5.6. The mean HbA1c of those diagnosed with diabetes mellitus was 7.9 and those with impaired glucose tolerance was 6.1 (Table 2).

Table 2: Mean HbA1c values in study subjects with tuberculosis (n=124)

| Diagnosis | Frequency (%) | Mean HbA1c (SD) | 95% CI |
|----------------------------|---------------|-----------------|---------|
| Normal | 79 (63.7) | 5.3 (0.5) | 5.1-5.4 |
| Impaired glucose tolerance | 32 (25.8) | 6.1 (0.2) | 6.0-6.1 |
| Diabetes mellitus | 13 (10.5) | 7.9 (1.7) | 6.9-9.0 |
| Total | 124 | 5.7 | 5.6-6.0 |

Study subjects previously tested for diabetes mellitus: Out of the 124 subjects sampled, only 22 (17.7%) had been tested for diabetes mellitus prior to recruitment into the study. Out of these 22 subjects, most (81.8%) had undergone testing less than 1 year before enrolling in the study. Only 5 (22.7%) of them had been diagnosed with diabetes mellitus while 3 (13.6%) were found to have impaired glucose tolerance (Table 3).

Table 3: Results of previous tests for diabetes mellitus (n=22)

| Results | Total (%) | HbA1c Ranges | Mean HbA1c (SD) |
|----------------------------|-----------|--------------|-----------------|
| Normal | 14 (63.6) | 5.1 – 5.6 | 5.7 (0.5) |
| Impaired glucose tolerance | 3 (13.6) | 5.9 – 6.4 | 6.2 (0.3) |
| Diabetes mellitus | 5 (22.7) | 6.5 – 11.7 | 8.5 (2.1) |

Mean HbA1c in pulmonary and extra pulmonary tuberculosis: The HbA1c was normally distributed in the study and population. The mean HbA1c of the total population was 5.7 (SD 1.1), median was 5.5 (IQR 5.2-6.1) and mode 5.6. This allows for use of mean as an appropriate summary for statistical analysis of the results.

The mean HbA1c in the study subjects with pulmonary and extra pulmonary tuberculosis were 5.76 and 5.7 respectively. The mean HbA1c in the study subjects with diabetes mellitus among those with pulmonary tuberculosis was however higher than in subjects with extra pulmonary tuberculosis (8.3 versus 6.8; $p = 0.036$). There was no significant difference in the mean HbA1c values for normal and impaired glucose tolerance subjects with pulmonary and extra pulmonary tuberculosis as shown in Table 4.

Table 4: Mean HbA1c in pulmonary and extra-pulmonary tuberculosis (n=124)

| Diagnosis | Pulmonary TB (n=86) | Extra-pulmonary TB (n=38) | P value |
|--|---------------------|---------------------------|---------|
| Normal Mean HbA1c (SD) | 5.2 (0.6) | 5.4 (0.3) | 0.324 |
| Impaired glucose tolerance Mean HbA1c (SD) | 6.1 (0.2) | 6.0 (0.1) | 0.086 |
| Diabetes mellitus Mean HbA1c (SD) | 8.3 (1.9) | 6.8 (0.3) | 0.036 |
| Total Mean HbA1c (SD) | 5.76 (1.2) | 5.7 (0.5) | 0.170 |

Diagnosis in relation to length of treatment for tuberculosis: Out of the subjects with diabetes mellitus 11 (10.9%) had been on treatment for less than a month whereas 2 (8.7%) had been on treatment for over a month. For the subjects with impaired glucose tolerance, 30 (29.7%) had been on treatment for less than a month and 2 (8.7%) had been on treatment for over a month (Table 5).

Table 5: Diagnosis in relation to length of treatment for tuberculosis (n=124)

| Length of treatment | Less than 1 month (n=101) | Over 1 month (n=23) |
|--------------------------------|---------------------------|---------------------|
| Normal (%) | 60 (59.4) | 19 (82.6) |
| Impaired glucose tolerance (%) | 30 (29.7) | 2 (8.7) |
| Diabetes mellitus (%) | 11 (10.9) | 2 (8.7) |

Mean HbA1c in tuberculosis/HIV co-infection and tuberculosis alone: There was no difference in mean HbA1c values between patients with TB/HIV co-infection and those with TB alone. The mean HbA1c in subjects with TB/HIV co-infection was 5.7% whereas the mean HbA1c in patients with TB alone which was 5.8% (p -value = 0.847) (Table 6).

Table 6: Mean HbA1c in tuberculosis/HIV co-infection and tuberculosis alone (n=124)

| Diagnosis | Frequency | Mean | P value |
|-----------|------------|-----------|---------|
| TB/HIV | 21 (16.9) | 5.7 (1.1) | 0.847 |
| TB alone | 103 (83.1) | 5.8 (0.8) | |

DISCUSSION

The main aim of the study was to determine whether there is a relationship between tuberculosis and diabetes mellitus in Kenya. Diabetes mellitus has been recognized as a major comorbidity in patients with tuberculosis in Kenya³. The specific objective of this study was to determine the prevalence of diabetes mellitus in individuals diagnosed with tuberculosis at KNH and Mbagathi Level 5 Hospital, Nairobi. A total of 124 patients were recruited and most study subjects were males aged 18 to 30 years. This is in keeping with national statistics which indicate tuberculosis is most prevalent in males. National statistics also indicate that incidence of tuberculosis can be up to 30% greater in males aged 35 to 44 years compared to females in the same age bracket. In this study, the number of males with tuberculosis aged 35 to 44 years was also slightly higher than females in the same age bracket (19.3% versus 13.7%)³.

The prevalence of diabetes mellitus in this study was 10.5% which is significantly higher than the national prevalence of 3.3%. Although it is worth noting that 3.3% is most likely an underestimate due to minimal data in our population⁴. Out of 10.5% diagnosed with diabetes mellitus in the study, 6.5% were newly diagnosed and 4.0% were previously known diabetics. Comparison of our findings to those from other reports around the world revealed that the prevalence of diabetes mellitus among tuberculosis patients varied from 3.3% to 34%. This wide variation could be due to the diagnostic test used, sample size, the study location and the prevalence of known diabetics who were recruited in each study⁹⁻¹¹. Although all the studies were hospital based, each study used a different test and some even combined several tests to increase accuracy. This may explain the wide variation in results. Despite this however, most of the reports showed a higher prevalence of newly diagnosed diabetics versus known diabetics in the populations studied. This indicates that a significant number of unknown diabetics may present initially with tuberculosis. This finding validates the WHO recommendation of screening of patients with tuberculosis for diabetes mellitus¹⁷.

In this study, mean HbA1c was significantly higher in diabetic patients with pulmonary tuberculosis compared with those who had extra pulmonary tuberculosis (8.3 versus 6.8; p-value = 0.036). The mean HbA1c for the known diabetics was 8.5% which is indicative of poor glycaemic control¹⁶. This shows that poor glycaemic control is associated more with pulmonary tuberculosis⁷.

Another significant finding from our study was that a total of 25.8% of the total study population was diagnosed with impaired glucose tolerance, and out of this only 7.3% were already known to have impaired glucose tolerance prior to enrolling in the study. Mugusi *et al*⁹, in their study in Tanzania, reported an impaired glucose tolerance prevalence of 16.2%. Impaired glucose tolerance remains a significant risk factor for developing diabetes mellitus and associated cardiovascular disease. This risk has been shown to persist despite successful treatment for tuberculosis¹⁸.

Most of the study subjects with both diabetes mellitus and impaired glucose tolerance had been on treatment for less than a month. The duration of treatment may affect the HbA1c value as impaired glucose tolerance has been recognized in patients on medication for tuberculosis and has been described as a form of transient hyperglycaemia which usually normalizes following cessation of the anti-tuberculous therapy and sputum conversion (in smear positive patients)¹⁹. Rifampicin was expected to act as a confounder in this study as it has been shown to cause transient hyperglycaemia in patients on management for tuberculosis. According to Waterhouse *et al*²⁰, impaired glucose tolerance and insulin-requiring diabetes mellitus were reported in patients on rifampicin therapy which resolved following cessation of this drug. HbA1c measures long term glycaemia (two to three months) relatively unaffected by recent acute fluctuations in glucose levels. Thus, the findings can rule out Rifampicin as a confounder since most of the study subjects were in the intensive phase of treatment and had been on treatment for less than a month. We can therefore conclude the glycaemic changes we documented had been occurring prior to initiation of rifampicin.

All the study subjects had been tested for HIV and HIV co-infection was found in 16.9% of the total study population. The national prevalence of TB/HIV co-infection is 37%³. This disparity could be attributed to the fact that one of our exclusion criteria was a patient on anti-retroviral therapy. There was no significant difference in mean HbA1c for patients with both tuberculosis and

HIV co-infection versus those patients with TB alone (5.7 vs 5.8; p-value = 0.847).

CONCLUSION

Prevalence of diabetes mellitus in patients with tuberculosis was found to be high (10.5%) when compared to that of the general population (3.3%) and is suggestive of an association between diabetes mellitus and tuberculosis.

There was a significant difference in the mean HbA1c for patients with pulmonary tuberculosis versus those with extra pulmonary tuberculosis (8.3 versus 6.8; p-value = 0.036).

There was no significant difference in mean HbA1c for patients with both tuberculosis and HIV co-infection versus those patients with tuberculosis alone (5.7 versus 5.8; p-value = 0.847).

RECOMMENDATIONS

The study recommends routine screening of patients with tuberculosis for diabetes mellitus as per the WHO guidelines¹⁷. Further studies are needed to describe the relationship between impaired glucose tolerance and tuberculosis.

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Molecular characterization of Human Papilloma Virus (HPV) in urine among HIV infected men attending a high volume hospital, Nairobi-Kenya

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ABSTRACT

Background: Infection with Human Immunodeficiency Virus (HIV) and the resulting immunosuppression are associated with an increased risk for Human Papilloma Virus (HPV) persistence and related malignancies. Currently, the epidemiology of HPV infection in HIV infected men in Kenya is largely uncharacterized.

Objectives: This study was aimed at detecting and characterizing HPV in urine from HIV infected men in a Kenyan population.

Design: This was a descriptive cross-sectional study.

Setting: A high volume hospital in Nairobi Kenya.

Study population: Eighty-three HIV positive men on Antiretroviral Drugs (ARVs).

Methodology: The study was approved by Kenyatta National Hospital/University of Nairobi/Ethics Research Committee and informed consent given by patients. Data was analyzed using STATA version 13 (Stata Corp, College Station, TX, USA) at the significance level of $P \leq 0.05$.

Results: Twenty five point three percent of all samples were positive for HPV DNA, while 74.7% tested negative for HPV DNA test. Twelve different HPV subtypes were detected; HPV-18 (10.8%), HPV-35 (6.1%), HPV-39 (4.8%), HPV-52 (4.8%), HPV-66 (3.6%), HPV 56 (2.4%) and HPV-16 (2.4%), HPV-31 (1.2%), HPV-51 (1.2%), HPV-58 (1.2%), HPV-59 (1.2%) and HPV-68 (1.2%).

Conclusion: From the results of this study, it was demonstrated that HIV infected men have a high prevalence of HPV infection with high risk HPV (HR HPV) genotypes and HPV 18 was the most common in this population. These results are of particular importance in our country where HPV screening in men is not practiced.

Recommendation: Screening HIV infected men for HPV infection should be mainstreamed into comprehensive care programs for men. Gardasil 9 can be considered in our setup as it protects against a wide range of genotypes (6, 11, 16, 18, 31, 33, 45, 52 and 58) - as detected in our study. However, a larger study should be conducted to validate our findings.

Key words: HPV, HIV, ARVS, DNA, LR HPV types, HR HPV types

INTRODUCTION

Human Papilloma Virus (HPV) is an epitheliotropic species-specific virus containing a small circular double-stranded DNA (approx. 8 Kb) within an icosahedral coat or capsid comprised by 72 pentameric capsomers. Its footprint is small (55 nm) resembling a golf ball under the electronic microscope. HPV comprises a family by itself (Papillomaviridae) and its types are classified in 16 genera. The genera are classified in many species and within species so far¹. HPV is a major cause of cervical carcinoma and intraepithelial neoplasms worldwide. There are more than 100 HPV genotypes classified into

HPV Low Risk (LR) and High Risk types (HR). LR HPV types mainly cause genital warts and condylomas. LR HPV includes; types 6, 11, 40, 42, 44, 54, 61, 70, 72 and 81. HR HPV types have been associated with precancerous and cancerous lesions. They include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82^{2,3}.

The International Agency for Research on Cancer has classified both Human Papillomavirus (HPV) and human immunodeficiency virus type 1 (HIV-1) as carcinogens. HPV is a direct carcinogen and HIV-1 is an indirect carcinogen through immune suppression⁴. Not all HPV-infected people will develop cancer,

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but it is important to note that persistent HPV infection, with specific HPV types, and high viral load are important cancer risk predictors⁵. Also, multiple HPV infections are regarded as an independent risk factor associated with a higher chance of developing high-grade lesions in men and women⁶.

Epidemiology has shown that persistent infection with oncogenic HPV types is the cause of squamous cell cancers of the cervix, penis, vagina, vulva and anus with an increased number in the HIV infected men and women⁷. The male population represents an important reservoir for the virus and may play a role in the transmission and perpetuation of the infection in the general population⁸. Emerging evidence points to a significant role for HIV infection in promoting HPV prevalence, incidence and persistence⁹. While much is known about the natural history of HPV infection in cervical cancer in women, less is known about the development of HPV-associated diseases in men¹⁰. This study sought to detect and identify HPV types in urine from HIV positive men on ARVs in one of the high volume hospitals in Kenya.

MATERIALS AND METHODS

Participant recruitment: Men (infected with HIV) seeking ARV treatment in a high volume public hospital and those admitted in the same facility were recruited into the study. Sensitization meetings were held at the reception of the comprehensive Care Clinic (CCC) of the hospital. A one on one talk was given to the HIV infected male patients in the wards by the principal investigator. Ethical approval was obtained from the KNH/UON/ERC.

Samples: A total of eighty-three urine samples were obtained from HIV positive men on ARVS after consent was given.

Collection and processing: Five mls each of first voided morning urine samples were collected from participants and samples transported to Kenya Aids Vaccine Initiative (KAVI - ICR) molecular Laboratory for storage at -70°C within two hours of collection until ready for DNA extraction.

High risk human papilloma virus detection and amplification

The HR HPV detection was carried out using the PCR test according to the manufacturer's instructions as described previously in the kit -HPV Genotypes 14 Real-TM Quant.¹¹ The PCR test

detects 14 different HR HPV types 16/18/31/33/35/39/45/51/52/56/58/59/66/68. DNA was extracted from 4 ml of the preserved urine sample solution using the Gen find™ DNA extraction kit as per the manufacturer's instructions. All reagents were equilibrated to room temperature prior to use and all prepared reaction mixtures were not stored for later use. 10 mM Tris, pH 7.5, solution from a 2M Tris stock solution was prepared. The Lysis Buffer and Proteinase K (96 g/mL) in an appropriate-sized conical tube was combined as per number of samples and mixed by pipetting up and down. The extracted DNA was stored at -20°C. HPV genotyping was done using multiplex. A 10µl volume of extracted DNA was added to an equal volume of reaction mixture containing 2µl primer set 1, 0.5µl primer set 2, 5µl PCR gold buffer, 7µl of 25Mm MgCL₂, 7µl DNTP mix (10mM each) and 0.2µl DNA polymerase (5 U/ µl). PCR grade water was added to 40 µl per sample. The PCR tubes were then placed in a thermocycler with the following protocol: 94°C for 15 minutes, (94°C for 20 seconds, 38°C for 40 seconds, 71°C for 80 seconds) 40, and 71°C for 4 minutes.

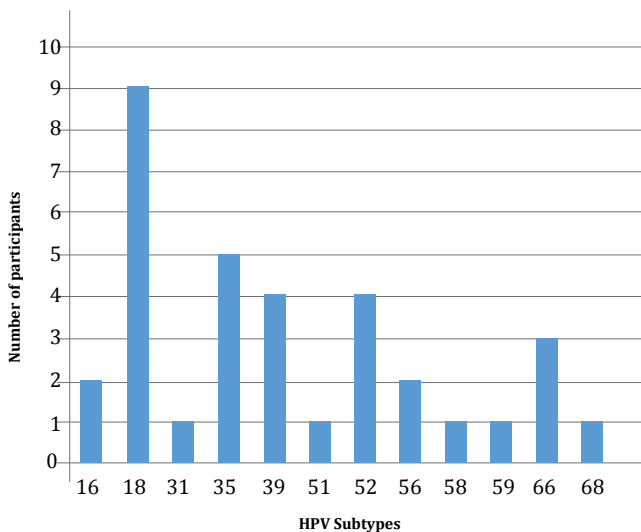
Human Papilloma Virus genotyping

Genotyping was performed using a Multiplex HPV genotyping kit (Multimetrix, Progen, Germany) as per the manufacturer's instructions. Each 200 ng DNA was subjected to PCR using HPV consensus primers for amplification of the HPV L1 gene. Amplified DNA was genotyped by applying a bead based hybridization with HPV type-specific probes using the Luminex technology, allowing for typing of the high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, and 82. PCR primers and Luminex reagents were both used from the Multimetrix HPV genotyping kit (Progen, Heidelberg). Two sets of primers are provided by the manufacturer. Primer set 1 contained all HPV primers: 9 forward and 3 biotinylated reverse primers necessary to amplify the HPV types under investigation. Primer set 2 (DNA quality control primers) contained primers for the amplification of a -globin gene fragment which were included in each run to ensure sufficient DNA integrity. After resuspending the beads, the read-out in the Luminex analyzer was performed. HPV types are discerned according to the unique bead signature, whereas the presence of PCR products is determined by phycoerythrin fluorescence. An analytical sensitivity cut-off was calculated based on the negative controls as per the manufacturer's instructions.

RESULTS

Urine samples from 83 HIV positive men on ARVs were tested for the presence of HPV. Out of this, 25.3% tested positive while 74.7% tested negative for HPV DNA. Oncogenic HPV genotypes identified in this study were 12: (HPV-18 (10.8%), HPV-35 (6.1%), HPV-39 (4.8%), HPV-52 (4.8%), HPV-66 (3.6%), HPV 56(2.4%) and HPV-16 (2.4%), HPV-31 (1.2%), HPV-51 (1.2%), HPV-58 (1.2%), HPV-59 (1.2%) and HPV68 (1.2%) as shown in Figure 1.

Figure 1: Distribution of HPV subtypes among HIV infected men



Multiple infections were detected in 28.6% of the patients with positive HPV DNA. Those infected with one type of HPV accounted for 71.4%, infected with 2-3 genotypes were 9.5%, those with 4 genotypes were 19% and those with 5 genotypes were 32.8% respectively. The age group most infected was 41-50 years (46%) and the least infected was 21-30 years (2%). Infection rate was high among the married (81.9%) followed by 10.8% among the divorced/widowed and 7.2% among the single category. Among the study participants 62.7% had attained secondary education, 19.3% primary education and 18.1% tertiary education.

DISCUSSION

Few studies have been conducted on the prevalence and genotyping of HPV in men in sub-Saharan Africa, including Kenya. Most of the data that are available are from HPV studies in women as data on infections in men are very limited. Most research has focused on HPV infection in women because of the association of HPV infection and cervical cancer using cervical smears but in men

HPV is mostly asymptomatic. Therefore, to the best of our knowledge this is the first study in Kenya to report HPV infection and genotypes in the urine of HIV infected men.

Among 83 male participants in our study, 25.3% HIV infected tested positive for HPV DNA. Comparable results have been reported in a study done in Thailand¹². However, in a study carried out in the Netherlands reports a proportion of 39.4%¹³ which is slightly higher than that from our findings. This could be due to the less samples analyzed (45 urine samples versus 83 in our study). In yet another study done in Argentina on men whose wives suffered from cancer of the cervix, out of 185 cases, a proportion of 75% tested positive for HPV genotypes¹⁴. This high proportion could be due to the high risk of their wives being infected with high risk HPV and also more than twice the sample size used. In our study, we had no history of cervical cancer in the spouses of our participants but rather they were a high risk group due to their HIV status. The sampling procedures could also attribute to differences in proportions because of the methods used for urine collection. In our study we used First Voided Urine (FVU) whereas the other studies used random urine samples¹³.

The age group most infected was 41-50 years (46%) and the least infected was 21-30 years (2%). Infection rate was high among the married (81.9%) followed by 10.8% among the divorced/widowed and 7.2% among the single category. Among the study participants 62.7% had attained secondary education, 19.3% primary education and 18.1% tertiary education. These different percentages of infection in the various age groups are mainly dependent on individual life styles.

In one study done in Thailand using urine samples from HIV infected men, HPV genotypes 16 (26.6%), HPV 31 (6.3%) and HPV 58 (6.3%) were identified¹⁵. Whereas from our study, we were able to identify the following genotypes; HPV-18 (10.8%), HPV-35 (6.1%), HPV-39 (4.8%), HPV-52 (4.8%), HPV-66 (3.6%), HPV 56 (2.4%) and HPV-16 (2.4%), HPV-31 (1.2%), HPV-51 (1.2%), HPV-58 (1.2%), HPV-59 (1.2%) and HPV-68 (1.2%). HPV 16 (2.4%), 31 (1.2%) and 58 (1.2%) occurred in much lower frequencies in our setup. There is substantial geographical variation in the relative frequency of different HR HPV types¹⁶. These findings have led to the development of two vaccines against HR HPV. A quadrivalent prophylactic vaccine against HPV types 6, 11, 16 and 18 was licensed in the USA¹⁷ and more recent a bivalent vaccine against HPV 16

and 18. While commercial vaccines against HPV 16 and HPV 18 are now available, global variations in HPV type specific prevalence could affect their regional effectiveness¹². Despite the widespread efforts to ascertain the burden of HPV infections in male populations across diverse regions, little systematic data are available on the prevalence of HPV infections in Kenyan male population¹⁸. However in a study done at Argentina, the frequencies of HPV 16 (21.5%), 18 (6%) and 31 (4.4%)¹⁴ were comparable. Yet in another study at Japan, High-risk HPV genotypes identified were as follows; HPV 16 (20%), 58 (13.3%), 18 (6.7%), 33 (6.7%), 31 (6.7%), 39 (3.3%), 52 (3.3%), 59 (3.3%), and 68 (3.3%)⁵. There is no much variation from our findings in the frequency of HPV 16 and 58. The same reasons given above attribute to this scenario as well.

A study by Shigehara *et al*⁵ in Japan detected 9 HR HPV genotypes in urine of male participants suffering from urethritis. These findings are relatively comparable to ours in which 12 HR HPV genotypes were detected. However, contrary reports also exist in which only 3 HR HPV genotypes were detected in studies done in Thailand and Argentina⁵. The difference in number of HR HPV genotypes detected in our study could be due to the application of a high risk cohort compared to the former and later studies¹⁹. The other reason for disparities could be due to the method of sample collection used. In this study we sampled the First Voided Urine (FVU), which contains many exfoliated cells compared to random samples which contain few exfoliated cells. The methods used to process the samples in the various studies were also different.

Findings from a study carried out in Argentina indicates that 11.1% of the study participants had double infection¹⁴, whereas in our study 28.6% of the study participants had multiple infections with up to five different HR genotypes in one participant and four in another participant. This could be due to the type of kit used and also method of sampling specimens from the participants. Our study utilized the PCR testing kit which removes inhibitors such as nitrites in urine known to easily inhibit amplification²⁰.

Results from this study indicate a high proportion of HR HPV genotypes and multiple infection rates among HIV positive men in our setting. High risk HPV type 18 is the most common detected. It is also evident that urine can be a valuable specimen for screening for HPV infection. Since this study demonstrates 12 different genotypes (18, 35, 39, 52, 66, 56, 16, 31,

51, 58, 59 and 68), vaccination of HIV infected men with Gardasil 9 can be considered in our setup as it protects against a wide range of genotypes (6, 11, 16, 18, 31, 33, 45, 52 and 58)^{18,21,22}. However, a larger study should be done to validate the findings from this study.

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DISCLAIMER

The principal investigator did not receive financial support from the companies which supplied the kits.

CONFLICT OF INTEREST

The authors have no conflict of interest in this paper whatsoever.

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Serum vitamin D levels in male blood donors and male patients co-infected with human immunodeficiency virus and tuberculosis: a comparison study

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ABSTRACT

Background: Vitamin D plays a role in the immune function and its deficiency is associated with higher incidence of immune system disorders and faster progression of some infectious diseases. Tuberculosis (TB) is a major cause of death among people living with Human Immunodeficiency Virus (HIV) Acquired Immunodeficiency Syndrome (AIDS). Evidence that vitamin D protects against tuberculosis has been supported by some preliminary clinical studies.

Objective: To compare vitamin D levels in healthy males and HIV and tuberculosis co-infected males.

Materials and methods: This was a cross-sectional study carried out in Mbagathi District Hospital Tuberculosis Clinic and blood donor collection sites. Co-infected males and male blood donors were recruited to the study. Vitamin D was run on the Abbott Cobas platform. Pearson correlation tests were used to evaluate the linear relationship between variables. Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations.

Results: The distribution of serum vitamin D was significantly different between blood donors and HIV/TB patients. Among blood donors, serum vitamin D level ranged from 42.6nmol/L to 106.7 nmol/L. Median 68.7nmol/L. For HIV/TB patients' vitamin D levels ranged between 33.9-89.8 median 44.0 nmol/L. Out of 121 patients, 91(75.2%) were on the intensive phase of treatment tuberculosis and 28 (23.1%) in the continuation phase. Among the patients vitamin D deficiency was more frequent (68.5%) among patients in the intensive tuberculosis compared to those in the continuation phase. There was a significant association between tuberculosis phase and vitamin D status of the patient ($p<0.001$).

Conclusion: HIV/TB co-infected patients have a lower serum vitamin D level as compared to blood donors. Co-infected patients in the intensive phase have lower vitamin D than in those in the continuation phase.

Recommendations: Patients with human immunodeficiency virus co-infected with tuberculosis should have their serum vitamin D measured.

INTRODUCTION

Vitamin D is a hormone of great physiological importance; it is an essential micronutrient for bone mineralization. In the immune system vitamin D has been shown to activate macrophages and restrict intracellular *Mycobacterium tuberculosis* growth by up regulating cathelicidins¹. Vitamin D has a potential effect on Human Immunodeficiency Virus (HIV) for it influences the immune response to TB, a common cause of morbidity and mortality in this group of patients. Vitamin D Deficiency (VDD) has been associated with higher incidence of TB and faster progression of disease.

Increased tuberculosis risk in vitamin D deficient individuals has been noted. High rates of TB infection and VDD have been reported in African immigrants in Australia, the United States, and Europe. The association between hypovitaminosis D and TB has been described in several case-control studies². Investing in supplementation in poor settings such as sub-Saharan Africa where HIV/TB co-infection rates are high could be a cost-effective approach towards the eradication of TB morbidity and mortality. Vitamin D testing has increased in the last few years worldwide and associations have been made with HIV, TB and HIV/

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TB co-infection and several non-communicable diseases.

Several publications have been made in the West documenting deficiency in apparently healthy populations and in special groups. There is no consensus on what optimal vitamin D status is. There is need to establish a reference interval in the local population due to difference in skin colour, geographical latitude, age, gender and season. Currently controversy remains on recommended levels of vitamin D. Most laboratories agree that 75nmol/L (30ng/mL) and above is considered to be sufficient while individuals with lower concentrations are considered to be vitamin D insufficient or deficient³.

Dark skin interferes with the cutaneous synthesis of vitamin D. Holick⁴ demonstrated non-Hispanic black subjects require 6 times the amount of UV radiation necessary to produce a serum vitamin D concentration similar to that found in non-Hispanic white subjects. This is because melanin absorbs ultraviolet radiation thus the increased radiation needed. Decreased vitamin D production by dark-pigmented skin explains the higher prevalence of vitamin D insufficiency among darker-skinned adults. Dawson-Hughes and colleagues⁵ demonstrated that in Boston, 73% of elderly black subjects were vitamin D insufficient, compared with 35% of elderly non-Hispanic whites.

Vitamin D has been shown to improve macrophage phagocytic capacity, cell-mediated immunity, and increase natural killer cell number suggesting an important role in response to infections⁵. In the presence of adequate VitD, Vitamin D Receptor (VDR) up regulation leads to cathelicidin induction, an antimicrobial peptide with direct action against intracellular pathogens such as MTB. Increased resistance to tuberculosis could potentially prolong survival in patients with HIV and slow HIV disease progression and reduce associated mortality⁷.

Liu *et al*⁸ demonstrated MTB sensing by the Toll-like receptor 2/1 (TLR2/1) complex increases expression of VDRs in macrophages. Synthesis of active Vit D promotes VDR-mediated transactivation of cathelicidin and killing of intracellular TB. Cathelicidins have direct antimicrobial function in addition to anti-bacterial effects such as cationic membrane disruption. *In vitro* studies show macrophages are most efficient in producing cathelicidin (LL-37) after infection with TB, suggesting this antimicrobial peptide is important during mounting of the primary immune response towards TB⁸. A further study

by Liu *et al*⁸ demonstrated that transcriptional regulation of cathelicidin is mediated by the active form of VitD. Stimulation of TLR receptors by microbial products results in increased production from the inactive form of the hormone to the active form⁸. Liu *et al*⁸ noted serum from donors with hypovitaminosis D had low levels of LL-37 in macrophages compared to donors with normal vitamin D levels. Similar conclusions were made by Adams *et al*⁹.

Martineau *et al*¹⁰ observed that VDD was highly prevalent among black African adults living in Cape Town and was found to be associated with susceptibility to active TB in both the absence and the presence of HIV infection. The association was noted to be stronger in HIV-infected people. The study recommended testing for this group and supplementation for deficient individuals.

In a meta analytical study published by Nnoaham *et al*¹¹ the association between low serum vitamin D and risk of tuberculosis was assessed. Findings from the meta-analysis were that the probability of 70% that a healthy individual would have higher serum vitamin D level than an individual with TB if both were chosen at random from any population. Lower levels were associated with a higher risk of active TB. The study concluded that the potential role of vitamin D supplementation in people with tuberculosis and VDD associated conditions like chronic kidney disease should be evaluated.

MATERIALS AND METHODS

A cross-sectional study was conducted with the aim of comparing the distribution of serum vitamin D among male blood donors and male HIV patients co-infected with tuberculosis. Participants from Mbagathi District Hospital and blood donors were recruited consecutively until the sample size was achieved.

Selection criteria for blood donors were: age over 18 years and males who consented. An exclusion criterion for blood donors was transfusion transmissible illness positive. Inclusion criteria for HIV positive patients with TB: Consent, confirmed HIV + and TB +, age above 18 years, males newly diagnosed TB or on treatment for TB. An exclusion criterion were patients on VitD supplementation. Sample size was 240, 120 for male blood donors and 120 for patients co-infected with HIV/TB.

Data was entered and stored using Microsoft excel 2013. Data was imported using STATA 13, coded, cleaned and analyzed. Numeric

(continuous/categorical) data were summarized using measures of central tendency and dispersion; summaries were presented in tables. Histograms were plotted to show distributions. Serum vitamin D level in patients and donors were compared to the WHO and Holick cut off levels: Normal ($>75\text{nmol/L}$), insufficient ($50\text{--}74\text{nmol/L}$) and deficient ($<49\text{nmol/L}$).

Pearson correlation tests were used to evaluate the linear relationship between variables. Pearson correlation statistic and corresponding p-values was reported. Two independent sample t-tests were used to compare the means of serum vitamin D between the two populations. The t statistics with corresponding p-values were reported.

Approval for study protocol was obtained from Kenyatta National Hospital/ University of Nairobi –Ethical and Research Committee and Mbagathi Level 5 Hospital Ethical Review Committee.

RESULTS

Serum vitamin D level was normally distributed among blood donors and right skewed among patients. The vitamin D range among donors was $38.9\text{--}106.7\text{nmol/L}$ with a median of 68.7nmol/L retrospectively. Among HIV/TB co-infected patients the vitamin D level range was $33.8\text{--}89.8\text{nmol/L}$ and median of 48.9nmol/L . The medians between the 2 groups were statistically different with blood donors having higher levels. The distribution of serum vitamin D was significantly different (K-sample test for equality of medians: $\text{chi-sq}=86.38$; $\text{p-value}<0.001$) between blood donors and HIV/TB patients (Figure 1).

Figure 1: Distribution of vitamin D in HIV/TB patients and blood donors

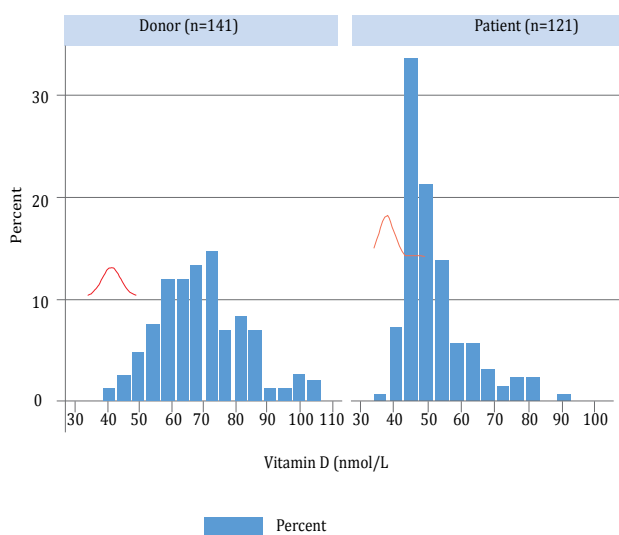


Table 1: Association between tuberculosis phase and vitamin D level

| TB Phase/ Vitamin D level | Deficiency | Insufficiency | Normal | Chi-sq (df) | P-value |
|---------------------------------|------------|---------------|--------|----------------|---------|
| Continuation | 6 | 16 | 6 | 27.67 (2) | <0.001 |
| Intensive | 63 | 28 | 1 | | |

Vitamin D deficiency was more frequent (68.5%) among patients in the intensive phase of treatment compared to those in the continuation phase. Majority of patients in the continuation phase had insufficient Vitamin D levels. There was a significant association between tuberculosis treatment phase and vitamin D status of the patient ($\text{chi-sq}=27.67$; $\text{p}<0.001$) (Table 1).

DISCUSSION

There is no universal agreement on the optimal concentration of VitD D. The question on what is an optimal vitamin D status remains a great topic of discussion. Some studies suggest that risk assessment on indicators of health such as bone and muscle function should be used to define optimal levels of this vitamin. Serum PTH levels and bone density should be assessed to truly assess for deficiency after low serum vitamin D level is realized. Many specialists consider the commonly used population based reference values too low and health based reference values are recommended. Requiring patients to have levels greater than 80nmol/L implies that greater than 80% of the European population is VDD¹². The World Health Organization¹³ and Holick⁴ defined vitamin D insufficiency as serum levels below 50nmol/L , a level that avoids skeletal and muscular problems.

The level of serum vitamin D was significantly different between blood donors and HIV/TB patients. This is similar to findings from a meta-analysis where the medians of TB patients were compared to matched healthy controls. A significant difference in medians between the healthy controls and TB patients was found. This meta-analytical study concluded that the probability of a healthy individual having higher serum vitamin D levels than an individual with tuberculosis if both were chosen at random from a population was 70%⁴.

This study confirms previous studies that patients presenting with active TB have significantly lower mean concentrations of serum VitD where majority had vitamin D deficiency

57.0% (69/121) and insufficiency 37.2% (45/121) using WHO cut off values mentioned previously.

In a case control study done in Guinea Bissau¹⁴ vitamin D levels in TB patients were compared to levels with healthy controls. Deficiency was found to be 46% (167/362) of the TB patients and 39% (193/494) of the healthy controls. Hypovitaminosis was defined as VitD<50nmol/ L. The study made the conclusion that hypovitaminosis D was highly prevalent among TB patients and healthy controls. Hypovitaminosis D was more frequent in this study at 57% as compared to the Bissau study at 46%. Hypovitaminosis D in the blood donors was lower at 8% vs. 39% in the healthy control subjects in Bissau study, this could be because the controls used in the Bissau study were contacts of the patients thus if it was a nutritional deficit the contacts would also be deficient.

In a study done by Kibirige *et al*¹⁵ where HIV/TB co-infected patients had VDD, VitD insufficiency, severe VDD and very severe VDD at 44.2%, 23.5%, 13.5% and 4.2% respectively. This is in concordance with findings in this study where most co-infected patients were deficient 57% (69/121) and insufficient 37.2% (45/121). No patients in this study were found to have severe and very severe deficiency. A reason contributing to this is the patients who were recruited to the Mulago study who were all in-patients thus more ill corresponding to lower serum vitamin D levels due to perhaps the acute phase response to infection.

In a multicenter study done in 22 study sites in Canada and United States of America, a markedly high prevalence of vitamin D insufficiency was seen in the cohort of patients with active pulmonary tuberculosis, where 86% of the study subjects had measured concentrations of serum VitD<75 nmol/L. These findings echo the results in this study where among the patients' the majority had vitamin D deficiency (57.0%) and insufficiency (37.2%).

Vitamin D deficiency was more frequent 68.5% (68/92) among patients in the intensive phase of treatment for tuberculosis as compared to those in the continuation phase where 57.1% (16/28) had insufficient levels. These findings correspond to a study done in Malawi¹⁶ where patients serum vitamin D levels were followed up from diagnosis of tuberculosis to treatment. Trends in serum 25 (OH) D concentrations over time were assessed for 133 patients who reached a final outcome. Median serum VitD D rose to 62 nmol/L by week

8 of treatment and 64 nmol/L by end of treatment. This occurred despite daily administration of RMP and INH to all patients and increased use of ART by HIV-infected participants.

The significance of an association between vitamin D deficiency and tuberculosis is 2-fold. First, already low vitamin D levels in tuberculosis patients may fall further on commencement of treatment. Secondly, treatment Isoniazid (INH) inhibits both hydroxylation steps of Vitamin D cutaneous synthesized into VitD and 1,25OHD. rifampicin induces alternative enzyme activity to degrade VitD into a waste product. Combined rifampicin and isoniazid treatment may reduce serum concentrations of useful vitamin D metabolites by 23–34%¹⁷.

CONCLUSION

HIV/TB co-infected patients have a lower serum vitamin D levels as compared to blood donors HIV/TB co-infected patients in the intensive phase have lower vitamin D than in continuation (68.5% vs. 21.4%).

RECOMMENDATIONS

Patients with human immunodeficiency virus co-infected with tuberculosis should have their serum vitamin D measured. A further study to establish serum vitamin D levels in females is recommended. Vitamin D supplementation is recommended based on clinical correlation and laboratory investigation.

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Evaluation of quality of packed red blood cells prepared at Regional Blood Transfusion Center Nairobi

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ABSTRACT

Background: Preparation of packed red cell components is the one most well-known functions of the blood transfusion service in Kenya. Therefore its of great importance to assess the quality of the prepared packed red cells blood components.

Objective: To assess quality of Packed Red Blood Cells (PRBCs) prepared at Regional Blood Transfusion Center Nairobi (RBTC).

Design: Descriptive cross-sectional study.

Setting: The study was conducted at Nairobi Regional Blood Transfusion Center.

Sample size: Eighty packed red cells were selected for the study as the size was convenient and cost effective

Results: Out of 80 packed red blood cells assessed, 86% met the criteria for acceptable red cell count, 76.3% for acceptable platelet count, 98.7% for acceptable volume, 45% for white blood cell count and 82.5% for haematocrit level.

Conclusion and recommendation: Among the prepared packed red blood cells, not all met the quality criteria. Based on these findings, there is need to enhance the quality assurance protocol and focus on the processes used to prepare packed red blood cells namely centrifugation, separation and storage to attain quality standards set by the Kenya National Blood Transfusion Services (KNBTS), European Directorate for the Quality of Medicine (EDQM), American Association of Blood Banks (AABB).

Key words: Quality, Packed Red Cells Components, Regional Blood Transfusion Center Nairobi

INTRODUCTION

Blood transfusion services are required to provide blood and components which are safe¹, affordable and accessible for transfusion into patients who require the blood products². Therefore, one donated unit can benefit more than one recipient after components preparation³. The current transfusion therapy depends on the availability of blood components⁴. These components, used separately or in various combinations, can adequately meet most patient transfusion needs while keeping the risks of transfusion to a minimum. Component transfusion therapy has the added benefit of using a limited natural resource more effectively by providing the needed therapeutic component to several patients from a single donation. In addition there is reduced burden on blood banks because of reduced transfusion related events and overall wastage when whole blood is transfused⁵.

Packed red blood cells are prepared by removing approximately 80% of plasma from a unit of whole blood. Standards require that the final haematocrit of a red blood cell unit not exceed 80%⁶. Multicomponent aphaeresis for deriving combinations of Red Blood Cells (RBCs), Platelets (PLT) and plasma units⁷ and collection of double RBC units are increasingly used to alleviate blood shortages, especially in the U.S⁸. Average haematocrit is between 65% and 80%.

Packed Red Blood Cells Units (PRBCS) contains the same RBC mass and therefore the same oxygen -carrying capacity as whole blood, but with approximately one-half the volume. Other advantages of using PRBCs rather than whole blood are: significant reduction in the level of plasma thus facilitating transfusion of group O cells to non O recipient, reduction of potassium and reduction of adverse events associated with WBC, platelet and plasma volume⁹.

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With the development of appropriate processing techniques, blood component preparation has become a routine procedure worldwide¹⁰. Packed red blood cells are the component most commonly prepared and used in Kenyan health facilities. Each unit of whole blood has a volume of about 450mls of which approximately 200mls consists of red blood cells. It is expected that one unit of packed red blood cells will usually raise haematocrit by approximately 4%. International requirements (European, AABB) for haematocrit (50%-70%), European Directorate for Quality of Medicine & Healthcare (EDQM) recommends that red cells in additive solutions contains greater part of whole blood WBC (about 2.5 to 3.0×10^9) and variable platelets depending on the method of centrifugation and the mean platelet count $< 200 \times 10^9$ per unit.

Guide to the preparation, use and quality assurance of blood components

| Parameter to be checked | Requirements | Frequency of control |
|-------------------------|-----------------------|-------------------------|
| Volume | 280 ± 50 mL | 1 per cent of all units |
| Haematocrit | 0.65-0.75 | 4 units per month |
| Haemoglobin | Minimum 45 g per unit | 4 units per month |

Kenya Regional Blood Transfusion Center Nairobi recommends the volumes of components as follows²

- (i) Packed red blood cells 220-340mls
- (ii) Red cells suspended in SAGM- adult volume 285-340mls
- (iii) Paediatric bag 145-170mls.

Kenya National Blood Transfusion Center has not stated their reference range for other parameters other than volume.

Quality assessment of RBC count in our packed red blood cells units was done using the 10/30rule¹⁶. The rule of 10/30 states that haemoglobin is equal to RBC count multiply by 3 plus or minus 1 and the haematocrit is equal to haemoglobin multiply by 3 plus or minus 3. This was considered in the study because the AABB has not given reference range for RBC count in packed red blood cell.

MATERIALS AND METHODS

This was a descriptive cross-sectional study conducted at the Kenya National Blood Transfusion

Center Nairobi Region located in Nairobi. Nairobi region has a capacity of 4 million people and this center takes care of this population.

Sample size: A convenient sample size was selected and was dependent on resources and time constraints. A total of 80 packed red blood cells collected from Regional Blood Transfusion Centre Nairobi between February and June 2017 were used as the study sample.

Sampling processing: 5 ml of blood was collected aseptically into an EDTA vacutainer tube from the prepared packed cells units. The volume of the packed red blood cells units were measured; these aliquots from the PRBCS units were analyzed for haematocrit, white blood cell count, platelets count and red blood cell count using the XN1000 haematology analyzer. The results were printed and then entered into an excel sheet.

Data analysis: All data was analyzed using STATA version 13. The results were presented in percentages using tables and charts as well as ranges, mean and standard deviation.

Ethical consideration: Approval to conduct the study was sought from Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (PROTOCOL NUMBER: P691/10/2016). A written consent was obtained from the Director of National Blood Transfusion Service Headquarters as well.

RESULTS

Eighty PRBCs were sampled for the study.

Complete blood count and volume measurements of packed red cells

Table 1: The volume, white blood cell count, red blood cell count, platelet count and haematocrit concentration of the packed cells units

| Parameter | PRBCs | Met requirement | Failed | Percent meeting requirement |
|-----------------|-------|-----------------|--------|-----------------------------|
| Volume | 80 | 79 | 1 | 98.7 |
| Haematocrit | 80 | 66 | 14 | 82.5 |
| RBC count | 80 | 69 | 11 | 86.3 |
| WBC count | 80 | 36 | 44 | 45 |
| Platelets count | 80 | 61 | 19 | 76 |

Table 2: Distribution of mean, range and reference ranges quality parameters in packed cells

| Parameter | Mean | Range | Reference ranges |
|-------------|-------|-----------|----------------------|
| Volume | 266.1 | 220-335 | 220-240ml |
| Haematocrit | 55.8 | 32.7-84.9 | 45-75% |
| RBC count | 6.4 | 4.1-10 | 5-8*10 ⁶ |
| WBC count | 5.4 | 1.3-14.1 | <5*10 ⁹ |
| Platelets | 204.5 | 102-484 | <200*10 ⁹ |

DISCUSSION

Blood components are produced using methods that depend on the blood separation equipment manufacturer's established procedures. Differences in production processes exist across different organizations. Standard operating procedures are applied, in part to address these concerns and ensure quality of the components.

At a minimum, the following critical parameters should be checked during the quality control assays: volume, haemoglobin or haematocrit and residual leukocytes, if leukocyte reduction is performed. (© World Health Organization WHO Technical Report Series, No. 961, 2011).

One element that is emerging as an important mediator for packed red blood cells quality is the manufacturing methods that are used to separate the RBCs, platelets, and plasma from whole blood. The method used to separate blood components from whole blood¹²⁻¹⁴ the additive solutions used¹⁵ have all been shown to affect the quality characteristics of transfused products. Whole blood units are collected in an aseptic manner in a ratio of 14mls of anticoagulant-preservative solution per 100mls of whole blood. Apheresis components are collected into anticoagulants as recommended by the manufacturer. Depending upon the collection system used, a single whole blood donation typically contains either 450mls ($\pm 10\%$) or 500mls ($\pm 10\%$) of blood collected from blood donors with a minimum haematocrit of 38%, withdrawn in a sterile container that includes an anticoagulant solution licensed for this component. Occasionally, units of other volumes are collected and those volumes are stated on the label.

After plasma is removed, the resulting component is packed red blood cells, which has a haematocrit of 65% to 80% and a usual volume between 225mls and 350mls¹⁵. Additive Solutions (AS) may be mixed with the red cells remaining after removal of nearly all of the plasma.

Contents of Anticoagulant-Preservative Solutions include:

1. Citrate Acid Phosphate Dextrose Adenine with a Shelf Life of 21 days
2. Citrate-Phosphate Dextrose (CPD) with a shelf life of 21 days
3. Citrate-Phosphate-Dextrose-Dextrose (CP2D) with a shelf life of 21 days
4. Citrate-Phosphate-Dextrose-Adenine (CPDA-1) with a shelf life of 35 days.
5. ACD is used for apheresis components.

Nearly all packed red blood cells units in our study met the volumes requirements. Seventy nine out of the 80 packed red cells had volume within the normal range (220mls - 340mls) while one didn't according to "Guidelines for the Blood Transfusion Services in the United Kingdom" 8th Edition, 2013.

In this study, the average packed red cells haematocrit was 55.8% which is close to a study by Upadhyay and Pangtey⁸ whose mean HCT was 54% on quality analysis of packed red blood cells.

Another study done by Hussen *et al*⁹ on clinical and quality evaluation of red blood cell units collected via apheresis versus those obtained manually, 70% of the packed red blood cells had HCT values of 66% to 80%. White blood cell count in the packed red blood cells units in our study were high compared to the recommendation by the European Directorate for Quality of Medicine & Healthcare (EDQM) which suggests a value between 2.5 and 3.0*10⁹. A guideline from Food and Drug Administration Center for Biologics Evaluation and Research August 2013 Updated in 2014 recommends a manufacturing process resulting in a final product containing with white cell count less than 5.0 *10⁹. In our study only 45% of packed red blood cells had white blood count of less than 5.0 *10⁹ and this may be due to the fact that we do not leucodeplete our blood products.

Quality assessment of RBC count in our packed red blood cells units was done using the 10/30 rule. The rule of 10/30 states that haemoglobin is equal to RBC count multiply by 3 and the haematocrit is equal to haemoglobin multiply by 3. The rule of "10/30" for RBC, HB and haematocrit was met in this study.

PRBCs units that met the platelet count quality criteria in this study was 76.3% (61/80). According to AABB platelets amount in the packed cells have no significance since after storage the shelf life is less than 8hrs in the fridge at 2-6°C.

CONCLUSION AND RECOMMENDATION

Among the prepared packed red blood cells many did not meet quality criteria. Based on these findings, there is need to enhance the quality assurance protocols and focus on the processes used to prepare packed red blood cells namely centrifugation, separation and cell suspension to attain quality standard set by Kenya National Blood Transfusion Services and AABB

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Correlation between apolipoprotein B and lipid profile as markers of cardiovascular risk in patients with type 2 diabetes at Kenyatta National Hospital, Nairobi

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ABSTRACT

Background: Diabetes Mellitus (DM) is associated with significant mortality and morbidity which are far worse in the developing countries than elsewhere. Vascular diseases in DM account for the majority of deaths in diabetics. Dyslipidaemia is a major potential modifiable risk factor for the macrovascular complications in diabetics. Diabetic dyslipidaemia consists of a pattern characterized by high plasma triglycerides, High Density Lipoprotein Cholesterol (HDL-C) and increased concentrations of small dense Low Density Lipoprotein Cholesterol (LDL-C) particles which are all atherogenic. A routine lipid profile does not accurately assess the presence of small dense low density cholesterol particles. Apolipoprotein B (apo B) is the principal lipoprotein moiety of Very Low Density Lipoprotein (VLDL), Intermediate Density Lipoprotein (IDL), Lipoprotein a (Lpa) and both large buoyant and small dense LDL and therefore more accurately assess the cardiovascular disease risk. This study aimed at assessing the correlation between apo B and the routine lipid profile as markers of cardiovascular risk in type 2 diabetic patients at outpatient diabetic clinic of Kenyatta National Hospital.

Objectives: The main objective of the study was to assess the correlation between apolipoprotein B and lipid profile as markers of cardiovascular disease risk in type 2 diabetic patients attending an outpatient diabetic clinic.

Materials and methods: This was a descriptive cross-sectional study carried out in the diabetic clinic of Kenyatta National Hospital in Nairobi, Kenya. Ninety six type 2 diabetic patients not on lipid-lowering drugs were studied. Blood samples were collected and analyzed for total cholesterol, LDL-C, HDL-C and triglycerides and apolipoprotein B using Humastar 600® Biochemical analyser.

Data management and analysis: Demographic and laboratory data was collected using a structured questionnaire. The data was analysed using SPSS version 18.0. The 5% level of significance (95% confidence interval) with p-values of <0.05 was considered statistically significant.

Results: Of the 96 patients studied, 60.7% were females. The age range was 33-88 years with mean of 59.5 years and a median of 60 years. Eighty percent of the patients studied had low HDL-C (<1.35mmol/L), 74.5% had high total cholesterol (> 4.38mmol/L), 71.3% had high triglycerides (> 1.05mmol/L), 69.1% had high LDL-C (> 2.49mmol/L), 78.7% had high non-HDL-C (>3.03mmol/L) and 61.7% had high apo B (>0.65g/L). The most frequent lipid disorder was low HDL-C with the least frequent being high apo B. There was a strong positive correlation between apo B and non-HDL-C (p value < 0.001). In addition, 20.7% of patients with normal total cholesterol had high apo B and 22.4% of patients with normal triglycerides had high apo B. Further, 27.6% of patients with normal LDL-C had high apo B.

Conclusion and Recommendations: A significant proportion of the patients studied had dyslipidaemia cutting across all the parameters. Apo B has helped identify additional dyslipidaemia phenotypes in patients with normal total cholesterol and normal LDL-C. Non-HDL-C should routinely be calculated for type 2 diabetic patients to aid in cardiovascular risk assessment since it measures total atherogenic potential that may be missed by LDL-C.

Key words: Diabetes mellitus, Dyslipidaemia, Cardiovascular disease risk, Lipid profile, Apolipoprotein B

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INTRODUCTION

Diabetes Mellitus (DM) is associated with significant morbidity and mortality despite improving standards of health care. This is worse in the developing countries where resources are scarce¹. Macrovascular complications that include Coronary Heart Disease (CHD), Cerebrovascular Accidents (CVA), and Peripheral Vascular Disease (PVD) which are different facets of the same vascular disease account for more than 70% of all deaths in diabetic patients². The incidence of coronary heart disease is about 2-4 fold greater in diabetics than non-diabetics, occurs at a younger age and has a much greater case fatality. In fact, diabetic patients with no history of vascular disease have the same risk of having a myocardial infarction or dying from a vascular disease as non-diabetic patients with prior history of coronary heart disease^{3,4}.

Potential modifiable risk factors for the macrovascular complications in type 2 diabetic patients include persistent hyperglycaemia, hypertension, dyslipidaemia and smoking⁴. Therefore, for successful management of type 2 DM, there needs to be an aggressive integrated approach that aims to control blood glucose while also targeting the associated factors.

In diabetes mellitus, there are abnormalities in lipid and lipoprotein metabolism but dyslipidaemia may also herald future diabetes. A spectrum of diabetic dyslipidaemia can include all phenotypes identified in the general population however; one phenotype is particularly common in DM and is attributed mainly to insulin resistance and insulin deficiency⁵. It has a characteristic pattern consisting of high plasma triglycerides (TG), low HDL cholesterol (HDL-C), and increased concentration of small dense LDL cholesterol (LDL-C) particles that may be more susceptible to oxidation^{5,6}. Chronic hyperglycaemia promotes glycation of LDL-C and this together with the small dense lipoprotein particles increase the atherogenicity of LDL-C⁶.

Typically, in the assessment of cardio-vascular risk in diabetic dyslipidaemia a fasting lipid profile is done. This consists of Total Cholesterol (TC), triglycerides, HDL-C and LDL-C. Some facilities also include TC/HDL-C ratio. Ideally this should be done at the time of diagnosis of diabetes mellitus and then as clinically indicated.

However, Apolipoprotein B (apo B) has been shown to be a better risk marker than LDL-C for cardio-vascular disease in patients with high cardiometabolic risk such as diabetes mellitus and metabolic syndrome⁷. There is one apo B

for each molecule of LDL, VLDL and IDL which are all atherogenic. Therefore, total apo B levels correspond to the total number of atherogenic particles.

Patients at high risk of cardiovascular disease such as in diabetes mellitus and metabolic syndrome require accurate risk assessment and focused treatment. Measurement of apo B as part of routine lipid profile could play a pivotal role in optimal cardiovascular disease risk reduction.

MATERIALS AND METHODS

The study was conducted between November 2012 and February 2013. This was a descriptive comparative study carried out on outpatients at the diabetic clinic of Kenyatta National Hospital. All type 2 diabetic patients not on lipid lowering drugs, more than 18 years and who provided consent were included in the study. Fasting blood were drawn for lipid levels and apo B.

Analysis of the specimens for total cholesterol, triglycerides, LDL-C, HDL-C and Apo B was done using Humastar 600® automated biochemical analyser. Non- HDL-C was calculated as HDL-C subtracted from total cholesterol. Interpretation of results was based on NCEP guidelines. For this study, the cut off levels were based on the 50th percentile values as obtained from a local study and dyslipidaemia was defined as follows, when any of the following parameters was found to be abnormal- low HDL-C <1.35mmol/L, non-HDL-C \geq 3.03mmol/L, total cholesterol \geq 4.38mmol/L, triglyceride \geq 1.05mmol/L, LDL-C \geq 2.49, Apo B \geq 0.65g/L⁸⁻¹⁰.

The study was approved by the Department of Human Pathology (UoN) and the KNH/UoN Scientific and Ethical Research Committee.

The collected data was coded and entered into Microsoft Access Database and SPSS version 18.0 was used to analyse the data. Categorical variables such as gender and medical history data were summarised into proportions while continuous data such as age and physical examination data was presented as means/medians.

Laboratory results namely apo B, total cholesterol, LDL-C, TG and HDL-C were summarised into means/medians then categorised into normal and abnormal levels based on the 50th percentile cut off values. Apo B was correlated with total cholesterol, LDL-C, TG and HDL-C using Pearson correlation coefficient (r). In addition, the categorised data of the parameters were tested for association using Chi-square test of association. Odds ratio was used to

estimate the risk of abnormal levels of apo B in type 2 diabetes. The 5% level of significance (95% confidence interval) with p-values of <0.05 being considered statistically significant.

RESULTS

Ninety six samples were analysed for lipid profile and 94 samples were analysed for Apo B. Table 1 shows the clinical and laboratory features.

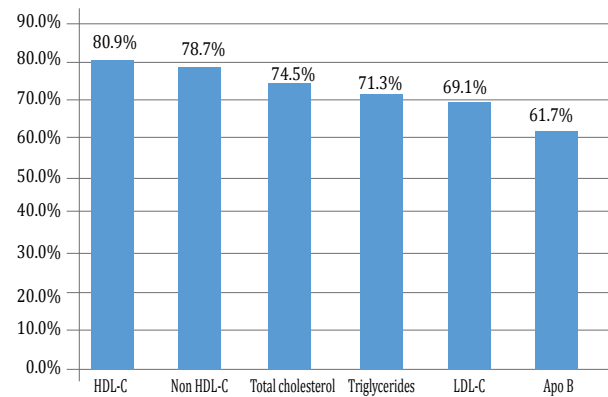
Table 1: Clinical and laboratory features of study subjects

| Patients | Male | Female | Total |
|---------------------------------------|-------------|-------------|-------------|
| Physical examination | | | |
| Gender (Number, percentage) | 38 (39.6) | 58 (60.4) | 96 (100) |
| Age(yr), mean(1SD) | 59 (9.7) | 59.9 (11.5) | 59.5 (10.8) |
| BMI (kg/m ²), mean(1SD) | 27.7 (5.2) | 29.7 (6.4) | 28.8 (5.8) |
| Waist to hip ratio, mean(1SD) | 0.99 (0.09) | 0.91 (0.1) | 0.94 (0.1) |
| Systolic Bp(mmHg), mean(1SD) | 139 (21) | 139 (16) | 139 (18) |
| Diastolic Bp(mmHg), mean(1SD) | 79 (12) | 79.8 (11) | 79 (11) |
| Laboratory results | | | |
| Total cholesterol (mmol/L), mean(1SD) | 4.99 (1.30) | 5.68 (1.68) | 5.4 (1.6) |
| Triglycerides (mmol/L), mean(1SD) | 1.76 (1.06) | 1.9 (1.64) | 1.8 (1.4) |
| LDL-C (mmol/L), mean(1SD) | 3.06 (1.37) | 3.5 (1.42) | 3.3 (1.4) |
| HDL-C (mmol/L), mean(1SD) | 1.02 (0.42) | 1.12 (0.31) | 1.1 (0.4) |
| Apo B (g/L), mean(1SD) | 0.68 (0.22) | 0.71 (0.31) | 0.69 (0.27) |
| Non-HDL-C (mmol/L), mean (1SD) | 3.98(1.18) | 4.55 (1.48) | 4.32 (1.39) |

Majority (82%) of the patients were between 40 and 69 years. 60.4% were females while 39.6% were males. The mean age of both the females and males was 59 years. The mean total cholesterol of females was 5.68mmol/L while in males was 4.99mmol/L with 74.5% having elevated cholesterol. The mean triglycerides level for males was 1.76mmol/L while that of females was higher at 1.9mmol/L with 71.3% of the patients having elevated triglyceride levels. The mean LDL-C for males was 3.06mmol/L while the mean for females was higher at 3.5mmol/L with 69% of the patients having elevated levels. The mean HDL-C for males was 1.02mmol/L while the mean among females was 1.12mmol/L with 80.9% of patients having low HDL-C levels. The mean Apo B levels for males was 0.68g/L while the mean for females was 0.71g/L with 61.7% of patients having elevated Apo B levels. A majority of the patients had dyslipidaemia as depicted in Figure1.

There was a strong positive correlation between apo B and non-HDL-C (p value < 0.001), (Figure 2 and 3). Of importance is that 20.7% of patients with normal cholesterol had high apo B and 22.4% of patients with normal triglycerides had high apo B. 27.6% of patients with normal LDL-C had high apo B while 63.9% of patients with normal apo B had high LDL-C (Table 2).

Figure 1: Summary of frequency of dyslipidaemias in the study population



(Cut-offs for low risk values HDL-C ≥ 1.35 mmol/L, non-HDL-C < 3.03 mmol/L, total cholesterol < 4.38 mmol/L, triglyceride < 1.05 mmol/L, LDL-C < 2.49 , Apo B < 0.65 g/L).

Figure 2: Correlation between apo B and LDL-C in the study population

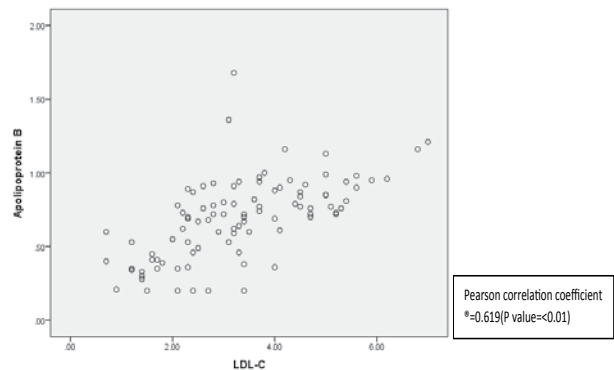


Figure 3: Correlation between apo B and non-HDL-C in the study population

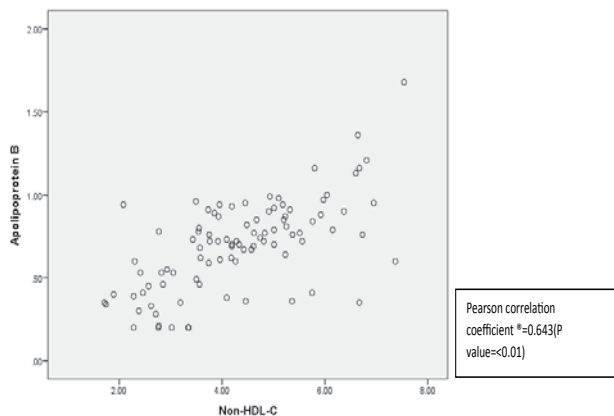


Table 2: Odds ratios between apolipoprotein B and lipid profile (TC, TG, LDL-C, HDL-C, non-HDL-C) in the study population (N=94)

| Variable | Apo B | | OR (95% CI) | P value |
|-------------------|-----------|-----------|----------------|---------|
| | High | Normal | | |
| Total Cholesterol | | | | |
| High | 46 (79.3) | 24 (66.7) | 1.9 (0.7-4.9) | 0.175 |
| Normal | 12 (20.7) | 12 (33.3) | 1.0 | |
| Triglyceride | | | | |
| High | 45 (77.6) | 22 (61.1) | 2.2 (0.9-5.5) | 0.089 |
| Normal | 13 (22.4) | 14 (38.9) | 1.0 | |
| LDL-C | | | | |
| High | 42 (72.4) | 23 (63.9) | 1.5 (0.6-3.6) | 0.384 |
| Normal | 16 (27.6) | 13 (36.1) | 1.0 | |
| HDL-C | | | | |
| Low | 44 (75.9) | 33 (91.7) | 0.3 (0.1-1.1) | 0.053 |
| Normal | 14 (24.1) | 3 (8.3) | 1.0 | |
| Non-HDL-C | | | | |
| High | 56 (96.6) | 18 (50.0) | 28 (5.9-132.5) | <0.001 |
| Normal | 2 (3.4) | 18 (50.0) | 1.0 | |

DISCUSSION

This study gives an insight into the levels and patterns of dyslipidaemias in the local type 2 diabetic population where a total of 96 patients were studied.

In this study, various levels and patterns of dyslipidaemias were identified. The patterns of dyslipidaemia in the current study consisted of high total cholesterol, high triglycerides, high LDL-C, low HDL-C, high non-HDL-C and high Apo B. Diabetic patients tend to have a characteristic dyslipidaemia consisting of high triglycerides mainly triglyceride-rich VLDL particularly post-prandially (post-prandial lipaemia), low HDL-C and increased concentrations of small dense LDL-C.

Majority of the patients (74%) had hypercholesterolemia which is consistent with a previous study done at Kenyatta National Hospital where about 70% of the patients had high cholesterol¹¹. This shows that most of the diabetic patients in the local population either have undetected or poorly controlled high cholesterol and are therefore at risk of cardiovascular disease.

More than 70% of the patients had hypertriglyceridaemia and this is consistent with previous studies in American and European regions that showed that diabetic patients had higher triglyceride levels than non-diabetic patients^{2,6}. In type 2 diabetic patients there is increased free fatty-acid release from insulin-resistant fat cells, the increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production^{6,12}. However, the high levels of triglycerides differs from a previous study at KNH, that showed only about

30% of the patients had high triglycerides¹¹. One of the reasons for this discrepancy could be the lower cut-offs used in this study which used local data for the reference values.

About 70% of the patients had a high LDL-C while data from a previous study showed about 50% of the patients with high LDL-C¹¹. However, proportion of patients with abnormal LDL-C in both studies is much higher than most published data where LDL-C was normal or only slightly raised in type 2 diabetic patients^{12,13}. Diabetic patients tend to have small dense LDL particles which are not measurable with available assays of the total LDL-C¹². Data from both studies at KNH show a significantly high proportion of the diabetic patients having high LDL-C as opposed to other studies may reflect that this pattern of dyslipidaemia could be different from other regions.

Apolipoprotein B was raised in about 60% of the patients which is a significantly high proportion of patients but the least frequent lipid disorder in the local type 2 diabetic population. Apo B was positively correlated with all the basic lipid parameters. In a study done by Wagner *et al*,¹⁴ apo B was found to be the most frequent lipid disorder in normocholesterolaemic type 2 diabetic patients and was found to identify additional dyslipidaemic phenotypes¹⁴. Further, Wagner *et al*¹⁵ found that apo B identifies dyslipidaemic type 2 diabetic patients in the normotriglyceridaemic group.

LDL-C has been established as an independent risk factor for atherosclerosis. Consequently, NCEP ATP III has published guidelines on lipids and identified LDL-C as the main target of therapy⁸. However, it has been noted that many patients who receive treatment and achieve the recommended LDL-C target still develop atherosclerotic complications¹⁶. One explanation for these discrepancies is the mis-match that has been described in many patients between the LDL-C concentration reported on a basic lipid panel and the number of atherogenic lipid particles, which is often expressed as Low-Density Lipoprotein (LDL) particle number or the number of apo B-containing lipoproteins¹⁷.

In this study, the population had a higher frequency of abnormal LDL-C than that of abnormal apo B. However, in the normocholesterolaemic group 6.2% had high LDL-C while 20.7% had high apo B indicating that apo B identifies more dyslipidaemia in this group. In addition, 27% of patients with normal LDL-C had high apo B, further showing the additive value of apo B.

Among the hypertriglyceridaemic group about the same proportion of the study population (~78%) had high LDL-C and high apo B levels. In the normotriglyceridaemic, there was similar pattern 21.5% had high LDL-C while 20.7% had high apo B. This is in contrast to the study by Wagner *et al*¹⁵ that showed that apo B identified more dyslipidaemic patients than LDL-C.

This study shows that 81% of the patients had low levels of HDL-C. This is consistent with available data where type 2 diabetic patients tend to have low HDL-C due to increased concentrations of VLDL in hypertriglyceridaemia and also inability to upregulate apolipoprotein A-1 production (main HDL-C lipoprotein moiety) owing to insulin resistance^{6,13}.

In addition, 79% of the patients had high levels of non-HDL-C. In the current study, non-HDL-C correlated strongly with apo B and actually there was a higher percentage of patients with high non-HDL-C than with high apo B. Non-HDL-C estimates all the cholesterol in LDL-C, IDL-C and VLDL. NCEP has recognized non-HDL-C as a secondary target of statin therapy in patients with hypertriglyceridaemia⁸. Studies have shown that both apo-B and non-HDL-C are superior to LDL-C in predicting cardiovascular risk since they identify the total atherogenic risk¹⁸⁻²⁰. Some studies (including INTERHEART study) have shown that apo B is superior to non-HDL-C^{15,18,19,21}. However, recent meta-analysis studies have shown that it may not be advisable to select one over the other since each of these reflects different measures of risk²²⁻²⁶. In addition, in a study among statin treated patients non-HDL-C was shown to be a stronger predictor of cardiovascular events than both LDL-C and apo B^{23,26}. The advantage of non-HDL-C is that there is no added cost since it is derived by subtracting HDL-C from total cholesterol and this may be more applicable in our set-up where apo B is not widely available.

In conclusion, we found that the majority of the patients studied had low HDL-C, elevated non-HDL-C, elevated total cholesterol, elevated triglycerides, elevated LDL-C and elevated apo B. Apo B can help identify additional dyslipidaemic phenotypes in patients with normal cholesterol and normal LDL-C and therefore should be considered as part of the lipid panel. In addition, non-HDL-C should routinely be calculated for type 2 diabetic patients to aid in cardiovascular risk assessment since it measures total atherogenic potential that may be missed by LDL-C.

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Bone marrow morphology in paediatric mortalities associated with severe acute respiratory illness at Kenyatta National Hospital, Nairobi, Kenya

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ABSTRACT

Background: In Kenya, Severe Acute Respiratory Tract Infections (SARI) are the leading causes of death among children. These children frequently have haematologic abnormalities such as anaemia, leukocytosis, thrombocytopenia and thrombocytosis. The contribution of the haemophagocytic syndromes and the diagnostic potential of bone marrow biopsies in SARI patients in our paediatric population are unknown. In addition, the contribution of specific infectious disease morbidity to bone marrow pathology is also unclear.

Objectives: The aim of the study was to describe the morphological changes in bone marrow obtained from autopsies of SARI related mortalities in children under 5 years.

Materials and methods: Fifty nine archived Formalin Fixed Paraffin Embedded (FFPE) bone marrow tissue specimens from the Paediatric Respiratory Surveillance Study (PRESS) were retrieved, sectioned and stained with routine and special stains.

Results: Fifty two bone marrow biopsies were satisfactory for evaluation. Erythropoiesis was increased in 16(30.8%) cases and under-represented in 11 (21%) cases. Granulopoiesis was hyperplastic in 31(59.6%) cases and reduced in 7 (13.5%) cases. Megakaryopoiesis was well represented in 40 (76%) cases. Plasma cells were increased in 5 (9.6%) cases and lymphocytes increased in 15(28%) cases. Histiocytic hyperplasia was seen in 14 (26.9%) cases and Histiocytic Hyperplasia with Haemophagocytosis (HHH) was present in 13 (25%) cases. There was no association found between bone marrow morphology and the infectious aetiological agents.

Conclusion: The main changes observed in bone marrow were hyperplasia of the granulocytic (59.6%) and erythroid (30%) lineages and histiocytosis (26.9%). Dysplasias involving the erythroid (44%) and myeloid lineages (14%) were common features. Haemophagocytosis seen in 25% cases, was a significant finding in Kenyan SARI mortalities.

INTRODUCTION

The main haematological complications of acute respiratory infection are anaemia, thrombocytopenia, alterations of white cell count and function, immunocytopenia, and alterations in haemostasis¹. Bone marrow abnormalities associated with bacterial infections include: quantitative changes in haematopoietic tissue, interstitial edema, megakaryocytes with or without dysplastic changes, necrosis, bone marrow hypoplasia, and haemophagocytic syndrome².

Viral infections increase bone marrow lymphocytes, plasma cells, and macrophages with or without haemophagocytosis. The bone marrow is hypercellular with an increased myeloid erythroid ratio. Morphological changes in viral infection include plasmacytoid lymphocytes, intranuclear inclusions,

giant pronormoblasts and bizarre megakaryocytes^{2,3}.

Fungi have no specific haematological manifestations. The bone marrow reaction to fungal infection is formation of granuloma and necrosis. The fungi are found within the histiocytes or admixed within necrotic debris².

The Histiocytic Hyperplastic Haemophagocytic Syndrome (HHPS) is a group of disorders that is found in children with severe acute respiratory illness and they cause proliferation of macrophages and dendritic cells. It is an inflammatory condition that is characterized by fever, cytopenia, hepatosplenomegaly, adenopathy and coagulopathy. The other findings are increased serum transaminases, low albumin, and increased serum ferritin levels⁴.

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In a study of seventy autopsies done in Japan it was found that HHH (41.4% cases) was associated with excessive inflammation especially in sepsis and haematological diseases. The bone marrow in these deceased patients was categorized in terms of activity of haemaphagocytosis through microscopic examination⁵.

Clinical management of haematological comorbidities seen in respiratory tract infections appears neglected yet important. The aim of this study was to provide information to medical personnel on the anticipated haematological complications that would arise from severe acute respiratory illness. Provision of relevant interventions may reduce the mortalities and morbidities that may arise in children with severe acute respiratory illness⁶.

A complete diagnostic autopsy that included microbiologic investigations and histologic examination of bone marrow tissue provided information about qualitative assessment of haematopoietic activity and presence of haemophagocytosis. Bone marrow samples were processed using routine morphological techniques and special stains. Any associations between the respiratory infectious agents and the bone marrow morphology were analysed.

MATERIALS AND METHODS

The main objective was to describe the morphology of bone marrow in children dying due to SARI at the Kenyatta National Hospital (KNH). The specific objectives were to describe the haematopoietic characteristics of bone marrow in children dying due to SARI, describe histiocytic characteristics of bone marrow and to correlate the bone marrow morphological findings with the infectious agent.

The study was conducted at the KNH, a 2,000-bed teaching and referral hospital, between August 2014 and December 2015. A cross-sectional descriptive study design was adopted to investigate the morphology of bone marrow in children dying due to SARI. The sample size was fifty nine archived bone marrow Formalin Fixed Paraffin Embedded (FFPE) specimens obtained from Paediatric Respiratory Surveillance Study (PRESS) SARI autopsies. This study was part of the larger PRESS which was performed on September 2014-February 2016 at KNH. Fifty nine FPE blocks were retrieved, sectioned and stained using with Haematoxylin and Eosin (H/E), May Grunwald Giemsa (MGG) for staining haematopoietic cells, Perl's Prussian for iron stores, Periodic Acid Schiff (PAS) for parasitic

infections and Ziehl Neelson stain for acid fast bacilli. The specimens were examined using light microscopy by the principal investigator, haematopathologist and anatomic pathologist. These were examined using a standardized Royal College of Pathologists of Australia (RCPA) 2014 protocol approach. Variables were bone marrow cellularity, nature of erythropoiesis, nature of myelopoiesis, megakaryocytes, lymphocytes, plasma cells, histiocytes and haemophagocytosis. Ethical approval was sought from Kenyatta National Hospital and University of Nairobi Ethical Research Committee (KNH/UON-ERC) and permission to use data from the PRESS study, from the principal investigator.

The data was collected using a predesigned proforma and was stored in Microsoft Access database. Data was coded, cleaned and analyzed using statistics and data version 13. Numeric variables were summarized using means (standard deviations) or medians (interquartile range) that were reported in histograms and dot plots. Categorical variables were summarized using frequencies and proportions that were reported in tables. Chi-square test of homogeneity was done to compare the distribution of morphological findings by type of infection. The chi-square statistic and corresponding p-values were reported.

Clinical data on total blood count parameters were often missing from the files and were not documented, making it difficult to correlate the antemortem haematological findings to the post mortem bone marrow morphology.

RESULTS

The study was conducted on all fifty nine archived bone marrow specimens. Clinical data on total blood count parameters were often missing from the files and were not documented, making it difficult to correlate the antemortem haematological findings to the post mortem bone marrow morphology.

Fifty two (88.1%) cases were satisfactory for evaluation. Normal marrow architecture was observed in 37 (71.2%) cases. Normal cellularity was observed in 35 (67.3%) cases, hypercellularity in 7 (13.5%) and hypocellularity in 10 (19.2%).

Haematopoietic characteristics: Erythropoiesis was increased in 16 cases (30%); preponderance of immature forms was present in 13 cases (25%) and dysplasia in 23 cases (44%). Granulopoiesis

was increased in 31/52 (59.6%) cases. Dysplastic features were observed in 14/52 (28.6%) cases. Left shifted granulopoiesis was reported in 38/52 (73.1%) cases. Megakaryocytes were well represented in 40(76.9%) cases and normal morphology in 46(92.0%) cases. Increased lymphocytes were observed in 15/52 (28.9%) cases while lymphoid aggregates were present in only 2/52 (3.9%) biopsies. Increased plasma cells were reported in 5/52 (9.6%) biopsies. All the plasma cells had normal morphology (Table 1).

Table 1: Bone marrow haematopoietic characteristics in children with SARI

| Erythropoiesis | Category | Frequency | Proportion (%) |
|---------------------------|---------------------------------|-----------|----------------|
| Cellularity (n=52) | Normal | 25 | 48.0 |
| | Increased | 16 | 30.8 |
| | Reduced | 11 | 21.2 |
| Maturity (n=52) | Normal | 39 | 75.0 |
| | Preponderance to immature forms | 13 | 25.0 |
| Morphology (n=52) | Normal | 29 | 55.8 |
| | Abnormal | 23 | 44.2 |
| Granulopoiesis | | | |
| Cellularity (n=52) | Normal | 14 | 26.9 |
| | Increased | 31 | 59.6 |
| | Reduced | 7 | 13.5 |
| Maturity (n=52) | Normal | 14 | 26.9 |
| | Left shift | 38 | 73.1 |
| Morphology (n=49) | Normal | 35 | 71.4 |
| | Abnormal | 14 | 28.6 |
| Megakaryopoiesis | | | |
| Number of cells (n=52) | Normal | 40 | 76.9 |
| | Increased | 4 | 7.7 |
| | Reduced | 8 | 15.4 |
| Location (n=52) | Interstitial | 51 | 98.1 |
| | Paratrabecular | 1 | 1.9 |
| Morphology (n=52) | Normal | 48 | 92.3 |
| | Abnormal | 4 | 7.7 |

Histiocytic characteristics: Histiocytosis was observed in 14 (26.9%) cases and histiocytic hyperplasia with haemophagocytosis was present in 13 (25%) cases. Evidence of infection seen in the bone marrow were tuberculosis (n=1), malaria pigment (n=2) and parvovirus related changes (n=1). There was no significant association between the type of infection and presence of HHH (P-value=0.274) (Table2).

Table 2: Bone marrow histiocytic characteristics in children with SARI

| Variable | Category | Frequency | Proportion (%) |
|---------------------------|-----------|-----------|----------------|
| Number of cells (n=52) | Normal | 38 | 73.1 |
| | Increased | 14 | 26.9 |
| Grading (n=52) | Absent | 39 | 75.0 |
| | Mild | 6 | 11.5 |
| | Moderate | 2 | 3.9 |
| | Severe | 2 | 3.9 |
| | Hypo | 3 | 5.8 |
| Infection (n=48) | Absent | 44 | 91.7 |
| | Present | 4 | 8.3 |

Correlation of bone marrow features to respiratory infections: There was no significant (p-values>0.05) difference in the distribution of haematopoietic and histiocytic abnormalities among biopsies with history of bacterial and viral infections.

Table 3: Distribution of haematopoietic and histiocytic characteristics by type of infection

| Variable | Category | Bacterial (n=13) | Viral (21) | Chi-square statistic | P-value |
|------------------|----------|---------------------|---------------|-------------------------|---------|
| Erythropoiesis | Abnormal | 10 | 15 | 0.125 | 0.724 |
| | Normal | 3 | 6 | | |
| Granulopoiesis | Abnormal | 13 | 20 | 0.638 | 0.425 |
| | Normal | 0 | 1 | | |
| Megakaryopoiesis | Abnormal | 2 | 6 | 0.776 | 0.378 |
| | Normal | 11 | 15 | | |
| Lymphocytes | Abnormal | 6 | 7 | 0.559 | 0.455 |
| | Normal | 7 | 14 | | |
| Plasma cells | Abnormal | 1 | 4 | 0.825 | 0.364 |
| | Normal | 12 | 17 | | |
| Histiocytes | Abnormal | 9 | 16 | 0.200 | 0.655 |
| | Normal | 4 | 5 | | |

Figure 1: Photomicrographs of bone marrow morphology

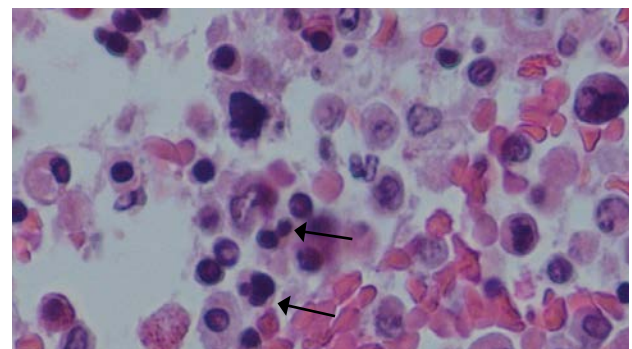


Figure 1a: BMB section arrow shows binucleate erythroid forms, and nuclear budding. Paraffin-embedded H&E X40

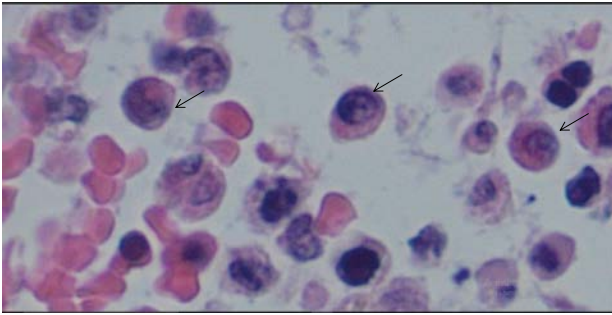


Figure 1b: BMB section arrows shows left shifted granulocytic cells. Paraffin-embedded H&E X100

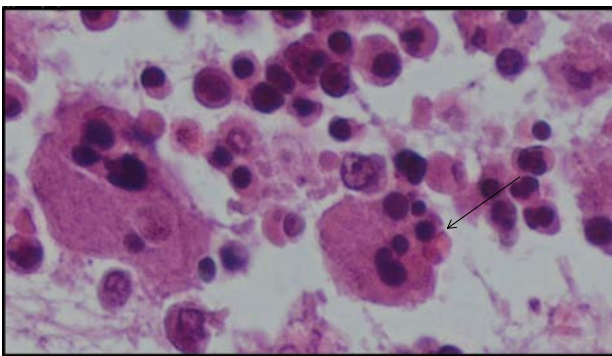


Figure 1c: BMB section, arrow showing haemophagocytic histiocyte. Paraffin-embedded H&E X100

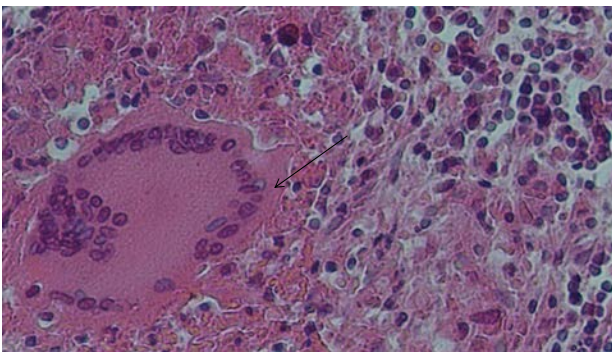
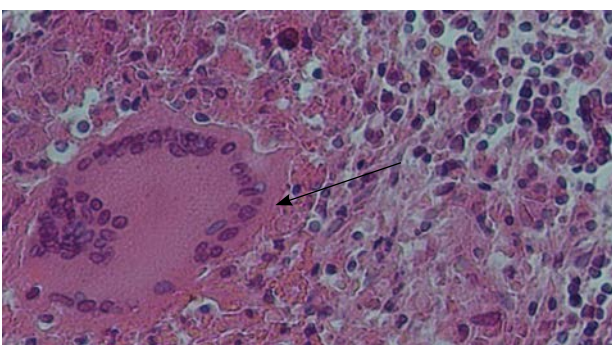


Figure 1d: BMB section, arrow showing Langerhans giant cell. Paraffin-embedded H&E X100



DISCUSSION

Erythropoiesis was increased in 16 (31%) cases which was similar to a study by Costa *et al*⁷ that showed 22/77 (30%) neonatal patients with acute respiratory illness had erythroid hyperplasia. In this study erythroid dysplasia was seen in 23 (44%) cases. Karen *et al*⁸ reported 46/77 (59%) cases with erythroid dysplasia due to fetal hypoxia and degenerative postmortem changes. The study showed that erythroid dysplasia was due to postmortem changes which was similar to this study. Studies by Wang *et al*⁹ showed dyserythropoiesis in bone biopsies stored at room temperature for up to 72 hours. Similarly, in this study storage temperature for up to 6hrs may have been a contributing factor to dyserythropoiesis observed in the marrow. The bodies remained at ambient room temperatures prior to collection from the wards, during transportation to the morgue and while awaiting storage.

There was granulocytic hyperplasia in 31 (59%) cases with a predominant left shift granulopoiesis which was similar to the Schid *et al*¹⁰ study which showed hyperplastic left-shifted granulopoiesis in 34% cases. The morphology of the granulocytic lineage was predominantly normal except in 14 cases where there were dysplastic forms seen in adenovirus, HIV and strep pneumonia infections. Megakaryopoiesis was suppressed in four (15%) cases which was due to infection similar to studies by Edward *et al*¹¹, where 4/14 (17%) cases demonstrated reduced megakaryopoiesis in neonatal autopsies with infection. In this study, 13 (25%) cases had HHH. Studies performed at two different hospitals in India identified HHH in 35 of 107 deceased patients (33%) and 102 of 230 autopsies (44%) respectively^{12,13}. This difference may be due to bacterial sepsis and recent blood transfusion which contributed significantly in developing HHH in these two hospitals. However, from this study there was no significant association between infection type and HHH. In this study 25% of cases had HHH which was an important finding that has often been ignored or even unnoticed by haematopathologists. Even though bone marrow examination is not indicated in SARI cases, severe pancytopenia is a clinical feature of HHH. Other clinical features include fever; lymphadenopathy and hepatosplenomegaly which if diagnosed and treated early may prevent bone marrow failure, multiple organ dysfunction and death.

Infections associated with HHH in this study were malaria (n=2) cases and tuberculosis (n=2) which was somewhat similar to that of Chandra

*et al*¹² where the infections associated with HHH were viral, tuberculosis and malaria. Although two patients had tuberculosis, the evidence of TB was seen in the bone marrow only in one case while the other was seen in the lung. Evidence of other infections seen in the bone marrow were parvovirus related changes and malaria pigment.

Bain *et al*² demonstrated that viral infections cause reduced megakaryopoiesis, left shift granulopoiesis, lymphocytosis and a hypercellular marrow. In addition, bacterial infections cause granulocytic hyperplasia, increased megakaryopoiesis, reduced erythropoiesis and a hyper cellular bone marrow. In this study there was no correlation of bone marrow morphological findings to the aetiological infectious agent.

CONCLUSIONS

The main changes observed in bone marrow were granulocytic hyperplasia (60%), erythroid hyperplasia (30%) and histiocytosis (27%). Dysplasias involving the erythroid (44%) and myeloid lineages (14%) were common features. Histiocytic hyperplastic haemophagocytosis (25%) was a significant finding in Kenyan SARI mortalities. However, there was no correlation of the bone marrow morphological findings to the infectious disease aetiology.

RECOMMENDATIONS

Histiocytic hyperplastic haemophagocytosis is a significant finding in acute respiratory illness and its clinical significance to infectious agent should be analyzed using immunohistochemical methods for detection of infectious agents. Microorganisms that are difficult to identify using routine or special stains can be identified using monoclonal antibodies.

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Hypereosinophilia presenting as paraneoplastic syndrome: Case report

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ABSTRACT

Eosinophilia is described as an increase of above $0.5 \times 10^9/l$ of eosinophils in peripheral blood count. There are three levels of severity of eosinophilia. Hypereosinophilia refers to severity levels above $1.5 \times 10^9/l$. It encompasses a broad group of disorders of mainly primary and secondary haematological disorders with potential tissue and end-organ damage. Clinical evaluation begins with excluding secondary causes of eosinophilias followed by morphological assessment of blood and bone marrow, immunophenotyping, standard cytogenetic, fluorescent *in situ* hybridization and other molecular techniques for clonality and risk stratification especially for primary clonal haematological disorders. This is a case presentation of a child who presented with right limb pain, limping gait and hypereosinophilia as a paraneoplastic syndrome. It highlights the importance of a systematic clinical approach and excluding neoplastic disorders when evaluating persistent hypereosinophilia.

Key words: Hypereosinophilia, Eosinopoietic cytokines

INTRODUCTION

Eosinophilia is an increase in the eosinophils above $0.5 \times 10^9/l$ in peripheral blood count. The normal range of eosinophils in the peripheral blood is $0.35-0.5 \times 10^9/l$. Based on the levels of severity, three levels are recognized; mild $0.5-1.5 \times 10^9/l$, moderate $1.5-5 \times 10^9/l$ and more than $5 \times 10^9/l$. Hypereosinophilia refers to severity levels above $1.5 \times 10^9/l$ with tissue infiltration and possible end organ damage¹. This was proposed by the Working Conference on Eosinophil Disorders and Syndromes in 2010². The World Health Organization update for 2015 classification is focused on diagnosis, risk stratification and management³. This is arrived at on the basis of clinical presentation, morphological evaluation of blood and bone marrow, tissue biopsy, molecular techniques mainly cytogenetics, immunophenotyping using flow immunocytometry, Fluorescent In Situ-Hybridization (FISH), Polymerase Chain Reaction (PCR) and T-cell clonality assessment. Therefore, there are three main broad categorization of hypereosinophilia mainly: secondary (reactive), clonal (primary) and idiopathic⁴.

The clinical presentation differs variably between patients with eosinophilia. The features can be due to the primary disease causing the

reactive eosinophilia. In developing countries mainly parasitic infections and the developed allergic disorders with their associated systemic effects⁵. The eosinophils are predominantly tissue cells after release from circulation. Hypereosinophilia is accompanied by tissue eosinophilia and may itself be responsible for the symptoms causing end organ damage. The vulnerable tissues to damage include the heart 60%, others skin, nervous system, respiratory and gastrointestinal causing morbidity and mortality⁶.

Eosinophils are derived from CD34+ haematopoietic stem cell and myelopoietic neoplasms in the marrow. Primary eosinophilias are typically bone marrow primary myeloid disorders which can be acute or chronic. They include chronic myeloid leukemia, chronic eosinophilic leukemia, other myeloproliferative neoplasms, some acute leukemias, advanced mast cell disorders and rare forms of myelodysplastic syndromes. The hallmark for clonal hypereosinophilia is bone marrow morphological features and associated molecular features. They are diagnosed after exclusion of secondary causes using, a combination of morphologic review of peripheral blood and bone marrow, standard cytogenetics, FISH, flow cytometry and T-Cell clonality assessment to detect evidence of

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lymphoproliferative, acute or chronic myeloid disorders. The use of molecular techniques especially genetic analysis has increased the number of clonal specific haematopoietic entities⁷.

In order to get better treatment outcomes for clonal eosinophilias, in 2008 the World Health Organization (WHO) adapted molecular techniques for risk stratification and therapy. A new major entity; myeloid and lymphoid neoplasms with eosinophilia and abnormalities of Platelet-Derived Growth Factor Alpha (PDGFA), Platelet-Derived Growth Factor Beta (PDGFB) or Fibroblast Growth Factor Receptor 1 (FGFR1) has been introduced. Chronic eosinophilic leukaemia-not otherwise specified (CEL-NOS) is also in this category.

Secondary or reactive hypereosinophilia entails conditions that cause extrinsic increase in eosinophils like drugs, infections, allergic disorders, systemic diseases, malignancies, food and any other medical condition. The malignancies include the non-myeloid or lymphoid malignancies where eosinophilia is usually triggered by eosinopoietic cytokines such as interleukin 3 (IL-3), IL-5, and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) which promote eosinophil differentiation and survival. These immunoregulatory factors are released by T-cells in the marrow after appropriate stimulation. These cytokines may be elaborated from CD4+, CD8+ cells in the peripheral blood, inflamed cells as well as malignant cells in Langerhans cell histiocytosis, solid tumours, Hodgkin's disease, Non-Hodgkins lymphoma, T-cell lymphomas, and acute lymphoblastic leukemias⁸.

Idiopathic hypereosinophilic syndrome is a diagnosis of exclusion after evaluation for primary and secondary causes as well as lymphocyte variant hypereosinophilia. The cause of symptomatic eosinophilia is unclear despite all the investigations. Traditional diagnostic criteria for idiopathic hypereosinophilic syndrome was end-organ damage, persistent eosinophilia above $1.5 \times 10^9/l$ for six months⁹.

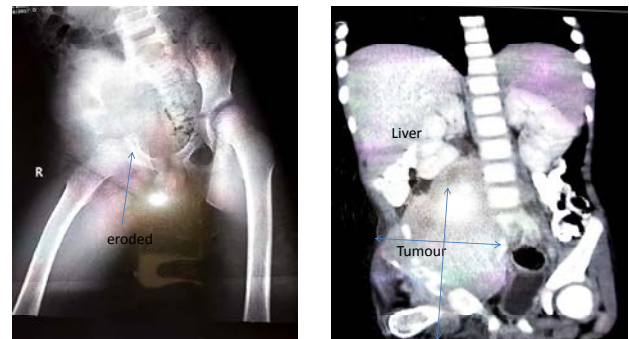
CASE REPORT

This was a 5 year old, 4 months old child who was referred to Kenyatta National Hospital. The mother noticed a painful swelling developing on the right thigh extending to the hip and also involving the right iliac fossa. Five months prior to this presentation, there was alleged trauma to the right leg which was stabilized by application of external fixation using plaster of Paris that was

removed a month later. The child complained of persistent pain on the same limb, was unable to walk and continued with physiotherapy for 3 months. A month later, there was still no improvement; the limb progressed to swell, increasing in pain prompting the mother to seek further medical attention in Kenyatta National Referral Hospital in Nairobi.

Past medical report of full haemogram showed an increase of the white cell count. Examination revealed mild pallor, hepatosplenomegaly, right iliac fossa mass poorly defined extending to the right hip and causing tenderness to right hip which was immobile. The complete blood count showed elevated white blood cell count $211 \times 10^9/L$, neutrophils $175 \times 10^9/L$ (83%), lymphocytes $12 \times 10^9/L$ (6%), eosinophils $15 \times 10^9/L$ (7.2%), haemoglobin 7g/dl, MCV 98 and platelets $250 \times 10^9/L$. CT-scan was done which revealed bony erosion of the right iliac bone, pubis and partial sparing of the ischium, a pelvic mass, ill-defined para aortic and right inguinal nodes. Other positive findings included hepatosplenomegaly. Differential diagnosis included Ewing sarcoma and osteogenic sarcoma.

Figure 1: Radiology of the lesion



CT-scan coronal view of the lesion

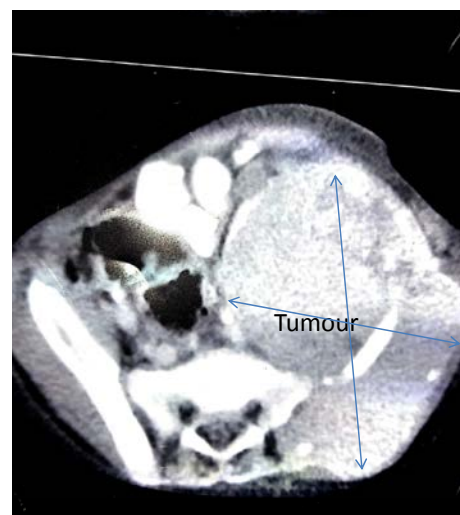


Figure 2: Peripheral blood film, Leishman stain: showed polychromasia, eosinophilia, hypersegmented and hypogranular eosinophils 72%, neutrophils 19%, atypical lymphocytes 6% and monocytes 3%

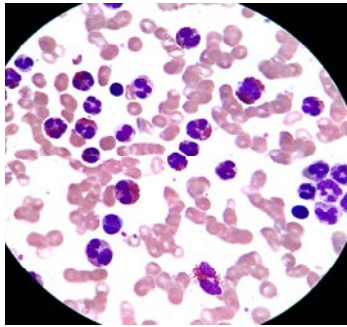
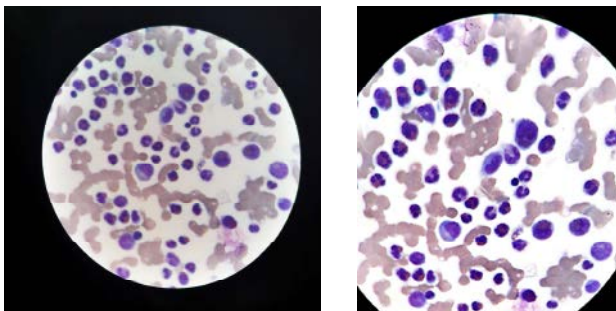


Figure 3: Bone marrow cytology, Leishman stain

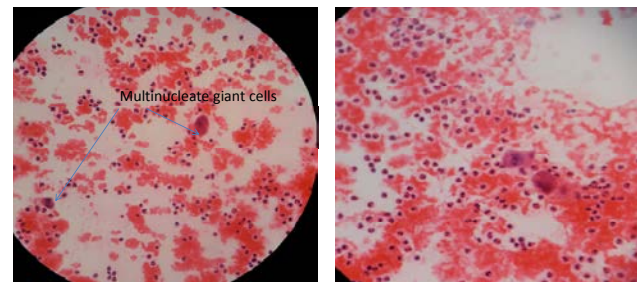


Bone marrow cytology was particulate with increased cellularity. The Myeloid: Erythroid ratio 48:1. Granulopoiesis had increased eosinophilia. Erythropoiesis was reduced and normoblastic with normal morphology of the precursors. Megakaryocytes present but reduced, showing budding activity. The lymphocytes and plasma cells unremarkable. The conclusion was hypereosinophilic syndrome caused by myeloid neoplasm associated with eosinophilia.

The chemistry levels were as follows; urea 5.3 (1.7-8.3)umol/L, uric acid 469(120-350) micromol/L, creatinine 97(20-70) micromol/L, lactate dehydrogenase level 469 (60-225) umol/L. Possible cellulitis was considered and treatment received included amikacin, ceftriaxone, clindamycin, paracetamol, morphine, lactulose.

Fine needle aspirate report of the right thigh mass demonstrated the presence of inflammatory cells mainly eosinophils, neutrophils and giant Langerhans cells. There were also Touton giant cells scattered with large nuclei and abundant cytoplasm. On the basis of these cytological features, a diagnosis of Langerhans cell histiocytosis was considered and child started on steroids. The condition continued worsening and the child succumbed with complications of congestive cardiac failure after 3 days.

Figure 4: Fine needle aspirate from the right thigh mass Haematoxylin Eosin



DISCUSSION

Assessment of a child with hypereosinophilia is a multidisciplinary approach with the aim of classifying the disease, identify the primary cause and the extent of end-organ damage for appropriate targeted therapeutic intervention. Detailed clinical history of parasitic infection, allergy, drug, travel history and physical examination of the skin for allergic disorders, and systemic examination to detect neoplastic disorders and end organ damage is essential in work up¹⁰. Neoplastic disorders are associated with hypereosinophilia. In the paediatric age group, early features of haematological malignancies include bone pain, limping gait and features mimicking arthritis. In assessment of any child with such presentation preceding mild trauma or a possible diagnosis of arthritis; a malignancy of any type must be ruled out.

Physical examination should detect the presence of abnormal masses, clinical signs specific for certain syndromes and other deformities. Basic tests include complete blood count, peripheral blood film, routine chemistries for hepatic, renal function tests and stool examination to exclude possible parasitic infections. The peripheral blood report confirmed more eosinophils than neutrophils and this also served the role of quality validation for complete blood count report. The lactate dehydrogenase and uric acid level were elevated indicating high tumour burden and possible lysis syndrome. The inflammatory markers, autoimmune antibodies, C-reactive proteins, quantitative IgE levels, serology for antineutrophilic cytoplasmic antibodies and parasites as well as stool for parasitic screening were not done.

Radiological evaluation of the abdomen, chest, pelvis and other symptomatic areas is useful for assessment of lymphadenopathy, splenomegaly, hepatomegaly and other occult neoplasms. A child with limping gait should have both knee and hip X-rays with appropriate views to assess

possible lesions. In the initial evaluation in the peripheral facility, only posterior anterior view of the knee joint was done which had no lesions. However, during admission in the referral facility, a repeat standard radiological assessment of both the knee and the hip using X-ray revealed an erosion of the right ischium bone responsible for the pain and inability to walk. Systemic survey for metastasis employs radiological assessment of the abdomen, skeletal and other systems using ultrasound, CT-scan and MRI¹⁰. The ultrasound revealed a right iliac fossa mass 8cm by 7cm. The CT-scan confirmed a pelvic mass in the right iliac fossa associated with para-aortic nodes and hepatosplenomegaly with erosion on the right ischium.

Generally, the whole haematopoietic system is involved in hypereosinophilia. Bone marrow examination techniques aims at classifying as reactive, clonal or idiopathic. The cytological findings vary depending on the cause of eosinophilia and may reflect the primary clonal disorders, disseminated disease involving the marrow, the neoplastic non-myeloid and myeloid causes of reactive eosinophilia. Bone marrow morphological evaluation should rule out acute lymphoblastic leukaemia, lymphomas, histiocytic disorders, metastatic tumours that cause reactive eosinophilia¹¹. Tissue biopsy for histopathology is key in solid tumour diagnosis and is more accurate when combined with immunostaining to demonstrate eosinophil derived basic proteins. Morphological evaluation of the bone marrow core biopsies should include immunophenotyping for CD34, CD117 and CD25¹².

The bone marrow cytomorphology report consensus conclusion was hypereosinophilic syndrome caused by myeloid malignancy. There were no blasts, no features of myelodysplastic syndrome and the eosinophils were more than the neutrophils. Based on the age, splenomegaly, cardiomegaly and bone marrow findings; acute myeloid leukaemia, MDS, other MPNS without eosinophilia the two main differentials considered were chronic eosinophilic leukaemia-not otherwise specified (CEL-NS) and a subcategory of myeloid and lymphoid neoplasms associated with eosinophilia with abnormalities of PDGFRA, PDGFRB or FGFR1.

The only bone marrow evaluation study done was cytology. If trephine biopsy for histology was done it would add value in assessment using special stains for reticulin or mast cells. Features of myeloproliferative neoplasms include splenomegaly, presence of early myeloid

precursors in peripheral blood, elevated serum B12 and tryptase levels. Serum B12 levels which is elevated in myeloproliferative neoplasms and autoimmune lymphoproliferative syndromes was a requirement.

The clinical data highly supported the diagnosis of myeloproliferative hypereosinophilic syndrome which is associated with Fip-1-like 1/ platelet derived growth factor alpha(FIP1L1/PDGFA) fusion gene detected using FISH or real-time PCR for the CHIC2 locus which is lost during the fusion and therefore negative is this condition. T-cell receptor and immunoglobulin (IgH) rearrangement studies using flow cytometry on blood and bone marrow is used to detect aberrant T- or B-cell populations with basic panel CD3, CD4 and CD8 to detect lymphocytic variant of hypereosinophilic syndrome. The facility is limited with molecular diagnostic armamentarium hence these conditions could not be confirmed¹³.

The fine needle aspirate of the thigh mass suggested Langerhans cell histiocytosis, which is a spectrum of three variants of a disorder with similar cytological and histological features but differ in clinical severity. The child was very sick with localized bone lesions, hepatosplenomegaly, para-aortic lymphadenopathy and cardiomegaly. In eosinophilic granuloma, the bone lesions are solitary with less severe disease; in multifocal eosinophilic granulomas, there are several lytic bone lesions and organ involvement, however in Letterer-Siwe disease the disease is multifocal with acute constitutional symptoms, organomegaly, extensive rashes and blood cytopenias. In view of the cross-cutting features, diagnosis remained as Langerhans cell histiocytosis. Histopathology with the use of special stains is key in establishing the diagnosis using S100 and Vimentin to identify Langerhans cells and should only be done when patient is stable.

The standard approach for treatment is to assess the need for urgent intervention, classify the clinical variant for specific therapy and steroid responsiveness. The first line of treatment is systemic high corticosteroid therapy to reduce the symptoms. Recommended dosing is 1mg/kg prednisone to 1gram methylprednisolone depending on the severity and should not be delayed at all even if definitive diagnosis is pending. If the eosinophil count and symptoms do not improve in 2 days, a second agent is added based on clinical suspicion. Imatinib mesylate is most appropriate for myeloproliferative disease. In the steroid refractory patients, hydroxyurea, vincristine and mepolizumab (the humanized

monoclonal anti-IL-5) has been used to rapidly lower the counts. Definitive treatment is based on underlying cause and molecular classification to target eosinophils using mepolizumab (the humanized monoclonal anti-IL-5) and newer agents on trial¹⁴.

Part of clinical evaluation is to investigate for end organ damage. The presenting clinical features were complications end organ damage; mainly cardiomegaly, hepatosplenomegaly, respiratory distress and features of congestive heart failure. These are features of paraneoplastic syndrome associated with hypereosinophilia. Evaluation of heart damage involves serum troponins, electrocardiogram and radiological test for cardiomegaly using echocardiogram, chest X-ray or MRI. These are life threatening and are the most common cause of death.

There are rare syndromes with specific organ pathologies that cause hypereosinophilia that should be distinguished from hypereosinophilic syndromes¹⁵. These five syndromes include Churg-Straus Syndrome(CSS), Ommenn syndrome, Hyper-IgE syndrome, Gleich's syndrome (episodic angioedema and eosinophilia) and eosinophilia myalgia syndrome. The syndromes form a distinct group of hypereosinophilia since the relationship between the observed pathologies is uncertain with the hypereosinophilia¹⁵.

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Peritoneal gliomatosis in a mature ovarian teratoma: Case report

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ABSTRACT

Peritoneal gliomatosis is a rare condition characterized by implantation of mature neural glial tissue within the peritoneal cavity in patients with ovarian teratomas, immature ones in most cases and rarer in mature teratomas. We present a case of peritoneal gliomatosis in a patient with mature ovarian tumour with imaging and pathological features and a review of literature.

INTRODUCTION

Ovarian teratomas are the most occurring germ cell tumours. They are classified into mature, immature and mesodermal teratomas depending on the cell content¹. Gliomatosis Peritonei (GP) is a rare condition associated with ovarian teratomas and is characterized by presence of nodules of mature glial tissue within the peritoneal cavity of these patients. GP is commonly found in immature teratomas and rarely occurs in mature teratomas^{2,3}. World Health Organization grading system for immature teratomas classifies GP as grade 0 teratoma since its presence does not indicate poor prognosis⁴. However, malignant transformation has been documented with GP⁵. We report a rare case of GP in a patient with mature ovarian teratoma.

CASE REPORT

A 15-year-old girl presented to Kenyatta National Hospital with history of progressive abdominal swelling for 1 year. Reported associated flank pains. Computed Tomography (CT) scan showed a mass within the peritoneal cavity measuring 21× 21×13 cm, displacing the small bowel loops laterally. The mass appeared well circumscribed with soft tissue attenuation areas as well as hypo-attenuating areas indicating cystic necrosis.

During laparotomy, a large cystic mass arising from the left ovary was found. The greater omentum had multiple visible firm nodules. Left salpingo-oophorectomy and biopsy of the greater omentum were performed.

On gross inspection, capsulated cystic ovarian mass measured 29 × 17 × 14 cm.

Cut section was variegated with solid and cystic areas. Gritty and necrotic areas were identified. The omentum was received as fibrofatty tissue with multiple nodular deposits.

Microscopically, sections from the ovarian mass showed a mature cystic teratoma elaborating mature tissue derived from the three germ layers and included abundant mature glial tissue along with choroid plexus, skin and adnexa, adipose tissue, hyaline cartilage, bone trabeculae, gut mucosa and respiratory epithelium. Sections from the omentum showed proliferated glial tissue in variably size nodules.

Figure 1: Mature teratoma composed of skin and adipose tissue

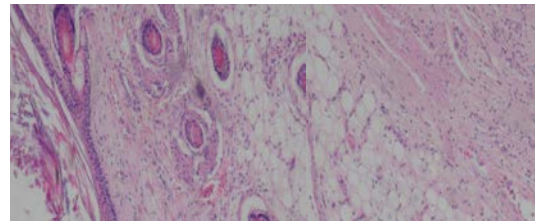


Figure 2: Neural tissue

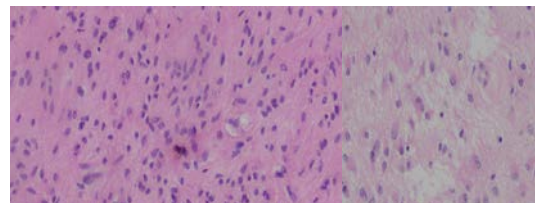
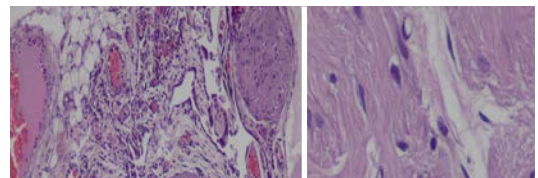


Figure 3: Mature glial tissue implants in the omentum

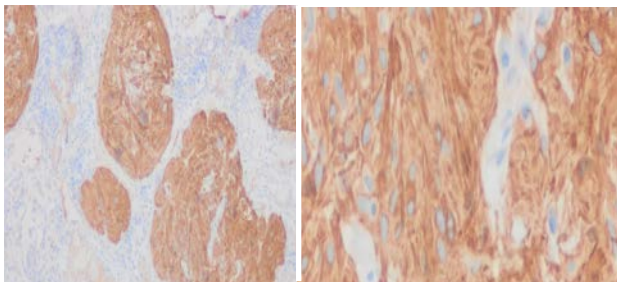


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Figure 4: GFAP positive stain glial tissue in the omentum



DISCUSSION

Peritoneal gliomatosis is defined as presence of mature glial tissue within the peritoneum usually in association with both mature and immature ovarian teratomas. Some reports however indicate its occurrence in association with ventriculo-peritoneal shunts and pregnancy². Regarding the pathophysiology of GP, two theories have been proposed. The first theory suggests direct spread of glial tissue from the primary ovarian teratoma through capsular rupture or angiolymphatic metastasis. The other theory postulates that glial tissue implants derive from Mullerian stem cells within the peritoneum under the stimulation from factors secreted by the ovarian teratomas^{3,5,6}. Majority of the studies support the latter hypothesis. Molecular analysis performed by Mrabti *et al*⁶ and Ferguson *et al*⁷ on glial implants and ovarian teratomas established that these tumours are genetically different indicating that GP arises from stem cells within the peritoneum. Abe *et al*⁸ from their study showed that the glial implants are distinct neoplasms from the associated teratomas and are derived from trans-differentiation of mesenchymal cells within the peritoneum.

The appearance of GP intraoperatively is indistinguishable from other intra- abdominal multifocal diseases like tuberculosis, peritoneal carcinomatosis, endometriosis and leiomyomatosis therefore microscopy and immunohistochemistry are recommended for definitive diagnosis.

GP is classified as Grade 0 teratoma with good prognosis. However extensive sampling is indicated to rule out presence of immature elements associated with adverse outcome. Malignant transformation of mature glial implants has been documented and therefore long term follow up is recommended in patients with GP.

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