Morbid anatomy of patients with mycobacterial infections, a cross sectional autopsy study at a tertiary Hospital in a high disease burden setting

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Abstract

Background Tuberculosis is a major cause of morbidity and mortality globally. Infections caused by different mycobacteria can be clinically indistinguishable but the differences in their pathological features and mortality are unclear.

Methods To evaluate the contribution of different mycobacterial pathogens to lethal mycobacterial disease and assess subsequent pathological features, we performed autopsies of 49 patients with suspected TB. Autopsy specimens were examined histologically and cultured for mycobacteria. We identified Mycobacterium tuberculosis (Mtb) isolates and non-tuberculous mycobacteria (NTM), and further genotyped the Mtb isolates.

Results Mtb isolates were found in 37 patients and NTM in 12 patients. All patients had signs of caseous pneumonia and 42 patients (86%) had disease involving more than half of the lungs. Two-thirds of the patients with TB or NTM had extrapulmonary engagement in addition to pulmonary pathology. Gross pathology and histopathology were similar in TB patients and patients with NTM, except that patients with NTM had a significantly reduced tendency to cause pleural effusions. Of the Mtb isolates 55% were of the Uganda genotype, which is the predominant Mtb genotype in Uganda. This genotype was significantly more frequent in the younger patients.

Conclusion In this autopsy study of patients with presumptive TB a majority of culture positive cases were caused by Mtb. In 25% of patients NTM were identified, and histopathology was similar in TB and NTM patients, although NTM patients had significantly less pleural effusions. These results suggest that NTM contribute to mortality, and that their identification is important because of the special clinical and therapeutic implications associated with NTM infections.

Introduction

Tuberculosis (TB) is the leading infectious cause of mortality globally, especially in high disease burden countries. According to WHO estimates, there were 10 million new cases of TB in 2016, with 1.7 million deaths (World Health Organization. Global tuberculosis report 2017. Geneva: World Health Organization; 2017), 95% of which were in low and middle-income countries. In sub-Saharan Africa TB is a major cause of death, but many cases are unidentified [1], 3,6 million cases of TB are missed every year according to WHO estimates.

The available WHO data on TB mortality are based on death registers, national TB reports and verbal autopsies. These data are based on microbiology and pathology services which are inadequate in most low income countries [2]. Autopsy is the universal gold standard for establishing the cause of death. It provides accurate morbidity information and identifies undiagnosed disease [3]. Yet autopsy rates are low in sub-Saharan African countries due to poor infrastructure, suboptimal pathology services and cultural beliefs [4, 5].

Nontuberculous mycobacteria (NTM) are an important cause of morbidity and mortality [6]. NTM are ubiquitous environmental opportunistic bacilli with significant regional variation [7]. NTM disease has increased over the past years in many geographical regions following improvement in mycobacterial detection and speciation techniques [8]. In many of these regions, NTM has caused more disease burden than TB [8].

Differentiating NTM infections from TB is important because the treatment requirements of NTM infections differ from those of TB [9]. Diagnosis of TB in resource-poor settings is mainly based on light microscopy of Ziehl Neelsen (ZN) stained smears. Identification and reporting of NTM is not routinely done. This leaves a knowledge gap on NTM prevalence and leads to unnecessary or inappropriate treatment of patients with positive smears caused by NTM but inadvertently interpreted as *Mycobacterium tuberculosis* (Mtb).

To study the pathology and the mycobacteria associated with lethal mycobacterial disease complete whole autopsies were performed on inpatients with suspected TB that died at Mulago National Referral Hospital (MNRH). Pathological examination was done in two phases. Gross pathology was registered, and tissues were taken from all organs for histopathology analysis after appropriate staining. Samples were also taken for culture and molecular analysis to identify the different types of mycobacteria isolated at autopsy.

Materials And Methods

Study setting:

MNRH, located in Kampala, Uganda, is a tertiary care and University teaching hospital receiving patients from across the country, and in particular from the national referral network. Patients presenting to MNRH with medical ailments (communicable and non-communicable disease) are admitted to one of the medical adult inpatient wards in the Department of Medicine. Patients suspected of suffering from TB are admitted and treated in the isolation wings of these wards. Voluntary counseling and testing for Human Immunodeficiency Virus (HIV) is routine for admitted patients at MNRH using WHO-approved kits. MNRH is the largest TB treatment center in Uganda.

Study design:

This is a cross-section observational study in which inpatients with suspected TB who died in MNRH between February 2012 and March 2013 were consecutively recruited. Informed written consent was obtained from the patients' next of kin. Information about the research and postmortem procedure was given in English and Luganda, the predominant local language, prior to seeking consent to perform postmortem and further analyses. Next of kin were informed that the study objective was to establish the cause of death and generate useful knowledge for improving community healthcare delivery. Recruitment and grief counseling was done by the principal investigator.

Recruitment:

Subjects were recruited with clinical and radiological features of mycobacterial disease with or without acid-fast bacilli (AFB) smear positive sputum. Included in the study were patients who died with clinical-radiological and laboratory data suggestive of TB including: i) persistent unexplained evening fevers for more than two weeks, ii) cough for more than two weeks, iii) loss of weight or appetite, iv) radiological features suggestive of TB. Patients who died without next of kin or on anti-TB treatment were excluded from the study.

Post mortem procedure:

A complete body postmortem examination was performed on each of the subjects to study the pathological features of the inpatients suspected to have died of TB. The examination was performed within 24hrs after obtaining consent to avoid autolysis and burial delay. All relevant information was reviewed before performing the postmortem examination including written clinical history, laboratory results and radiology. After external examination, careful attention was paid to ensure aseptic technique. Culture samples were collected in situ immediately after entering the body using sterile scalpel and forceps. Different sets of instruments were used for each culture. En masse (Letulle) evisceration was done followed by organ dissection and weighing as described [10]. Organs and regional tissue especially matted caseating lymph nodes were inspected for disease, and representative tissue samples taken. Lungs were examined fresh for extent of disease by cutting sequentially along the arteries, airways and veins following the McCulloch and Rutty method [11]. Culture samples were put in a sterile container containing 5ml of sterilized water and transported on ice to the mycobacteriology laboratory at Makerere University School of Biomedical science.

Tissue analysis:

Samples for histopathology were taken from the lung, the spleen, the liver, lymph nodes, kidney, adrenals, brain and pancreas. All histopathology tissues were fixed with 10% formal saline, sectioned, dehydrated with graded alcohol, and cleared with xylene. The tissues were then embedded with paraffin wax to produce tissue paraffin blocks from which 4µm tissue sections were cut. The tissue sections were stained with Haematoxylin and eosin (HE) for morphological analysis. Tissue sections were screened for AFB after staining with flourochrome (Auramine) and ZN stains. Diagnosis of mycobacterial disease in the tissue sections was based on HE histological findings including chronic granulomatous inflammation, caseous necrosis and AFB positivity on ZN and auramine-rhodamine tissue staining. Tissue was cultured for mycobacteria, isolates of which were subjected to molecular DNA fingerprinting for mycobacterial identification. Diagnostic criteria for NTM diagnosis in tissue samples was based on recognition of mycobacterial histopathologic features and positive NTM growth on tissue culture [9].

Mycobacteria identification

In the mycobacteriology laboratory samples were homogenized and disinfected with sodium hydroxide containing N-acetylcysteine prior to inoculation into Mycobacteria Growth Indicator Tube (MGIT 960) and on solid Loewenstein Jensen media for culture. DNA was harvested from growth following standard protocols *[12]* (Reagents from Sigma life Science, USA).

Isolates were identified by performing polymerase chain reaction (PCR) using 16s reverse and 16s forward primers (Integrated DNA Technologies) targeting the *16s rRNA* region with a conserved sequence typical for the genus Mycobacteria [13–15]. The Capilia TB assay (TAUN, Numazu, Japan) was used to distinguish Mtb complex (MTC) isolates from NTM [16], and MTC isolates were additionally identified by amplification of the insertion sequence IS*6110* using an in-house PCR with aid of reverse and forward IS*6110* primers (Integrated DNA Technologies). Gel bands of approximately 500 bp signified positive results [17].

Region of difference (RD) analysis:

MTC isolates were typed using PCR based typing method [13] which depends on chromosomal region of difference (RD) deletion loci. The patterns of amplification products are visualized by agarose gel electrophoresis. RD 9 confirmed that the cases were *Mtb* and ruled out other species, RD4 and RD 14 ruled out *M. bovis,* the RD724 deletion is characteristic of Uganda genotype.

Spoligotyping:

Spoligotyping was performed for all MTC strains following standard protocols [18] and manufacturer's instructions (reagents from Ocimum Biosolution, custom Master Mix from ABgene). Spacers were visualized on film as black squares after incubation with streptavidin-peroxidase and ECL chemiluminescence detection reagents (RPN 2105 Amersham, GE Healthcare Bio-sciences). The spacer hybridization patterns were converted into binary and octal format as previously described [19]. The 43-digit binary code was converted to 15-digit octal code (base 8, having the digits 0–7) [19].

The binary codes of the isolates were entered into the SITVIT2 database of the Pasteur Institute of Guadeloupe and assigned specific shared international spoligotype signatures (SIT) according to the SITVIT2 database [20].

Identification of Uganda genotype

The Uganda genotype, a sub lineage of the T2 lineage, was identified by deletion of RD 724 on RD analysis [21], and absence of spacers 33–36 and spacer 40 and/or 43 by spoligotyping [22, 23].

Ethical consideration:

The study was approved by the Internal Review Board (IRB) of the Makerere University School of Medicine. Final approval was granted by the Uganda National Council of Science and Technology. (HS 1364).

Statistical analysis:

Data was entered and analyzed in SPSS version 21.Univariate and multivariate data analysis was performed and logistic regression models were used to adjust for confounders like age, sex and HIV status. Chi square and Fisher's exact test were used. The independent-samples t test was used to analyze quantitative data for a two sample case to compare means and determine the probability (p) that the means were statistically different from each other; if p 0.05, results were considered statistically significant.

Results

Between February 2012 and March 2013 104 patients with suspected TB were included in the study. Autopsies were performed on 72 patients. In 49 patients mycobacterial culture was positive (Table 1), MTC were identified in 37 patients, all of which were Mtb. Twelve patients had NTM.

Patient characteristics

The 49 patients with positive mycobacterial culture had a median age of 35 years (range 4 years to 75 years).There were 32 males, mean age 38 years with standard deviation of 14, and 17 females, mean age 33 years with standard deviation of 15. p value = 0.372 (Independent-samples T test). Thirty-eight (76%) of the patients that were diagnosed with TB on autopsy had not been diagnosed before death. Thirty-three (67%) of the 49 patients were HIV positive, 14 (29%) were HIV negative and two (4%) were of unknown status. Two patients with TB had co-morbidity with malignant lymphoma.

The average age of TB patients was 36 years and of patients with NTM 36 years, p = 0.459 (Table 1). Sixteen patients had pulmonary disease only, while 33 had pulmonary and extrapulmonary disease. None had extrapulmonary disease only. 38 (76%) of the patients that were diagnosed with TB by autopsy had not been diagnosed during their life.

Twelve (32%) TB patients and four (33%) patients with NTM had pulmonary disease only, while 25 (68%) TB patients and 8 (67%) with NTM had extrapulmonary disease. Of the patients with extrapulmonary engagement 23 (62%) with TB and 4 (33%) with NTM had lymphadenopathy (Table 2). In all patients the location of the involved lymph nodes was in the abdomen, though one person had associated superficial lymphadenopathy and three had additional hilar lymphadenopathy.

Extrapulmonary TB was significantly more prevalent among patients with HIV than among those without (p = 0.000, OR 26 95% CI 5.1–138.4). Cavitating TB lung disease was significantly more prevalent among patients without HIV than those with (p = 0.002, OR 0.1 95% CI 0.–0.4).

Among the 33 HIV infected patients, 26 (79%) had TB and 7 (21%) patients had NTM, while of the 14 patients uninfected with HIV, 10 (71%) were TB patients and 4 (29%) patients had NTM.

Histopathology

All patients had caseous pneumonia. Forty-two patients (86%) had severe disease involving more than half of the lungs. Bronchocentric granulomas were seen in three patients with NTM lung infection and all were HIV negative. Lesions seen in the other organs including the liver, kidney, spleen and lymph nodes (Figure 2) were characterized by chronic granulomatous inflammation (Figure 3&4). The granulomas were characterized by caseous necrosis and variable cellular periphery comprising mature lymphocytes, histiocytes and occasional Langhans giant cells. There was no significant difference in the granuloma morphology and composition between the lesions related to MTC and NTM.

Both *Mtb* and NTM were found in a variety of organs (Table 2) with similar frequency. However, even after adjusting for confounding factors like age, sex and HIV status serous effusions were significantly less prevalent in patients with NTM than in patients with TB (Table 2).

Uganda genotype

All 29 genotyped MTC isolates were *Mtb* of which 16 (55 %) were Uganda genotype. TB due to *Mtb* Uganda genotype was not significantly associated with gender (p = 0.130) and HIV status (p = 0.688). However there was a significant difference in mean age between patients infected with *Mtb* non-Uganda genotypes (x = 40.77) and patients infected with Mtb Uganda genotype (x = 31.06), p = 0.031, with 95% CI −18.9 to 0.050, the patients with Uganda genotype being significantly younger that those with non-Uganda genotype. There was no significant difference in tendency to disseminate or preference for any organ between the predominant Uganda genotype compared to the non-Uganda Mtb strains (Table 3).

Discussion

Postmortem examination is the gold standard for establishing the specific cause of death and allows for quantification of disease burden. In this study, 38 (76%) of the patients that were diagnosed with TB by autopsy had not been diagnosed during their life.

Very few autopsy studies have been performed on patients with TB in Africa. Those performed have indicated TB as the most common autopsy finding especially in HIV patients [24, 25] and that in many cases, the diagnosis is either delayed or missed. This implies that TB remains a leading infectious

disease associated with mortality and that diagnosing TB remains a challenge, especially in the case of extra pulmonary TB.

All TB patients in this study had signs of caseous pneumonia, which is the last stage in the development of post primary TB [26], and most had severe disease involving more than half of the lungs. Extra pulmonary TB was prevalent in more than two-thirds of the cases, and was significantly associated with HIV infection.

To our knowledge few studies of the genotype of *Mtb* isolates have been performed in autopsy cases [27]. In a Russian study, the predominant Beijing genotype was more frequently identified in autopsy than patient samples, while the Ural genotype was significantly less frequently identified in autopsy samples [27].

In Uganda, the Uganda genotype of *Mtb* is the predominant cause of pulmonary TB, and accounts for up to 70% of isolates [28, 29]. In a study of tuberculous lymphadenitis patients, we found that the Uganda genotype was a predominant cause of extra pulmonary TB, but at a lower frequency (46%) [29]. Patients infected with this genotype had a significantly lower frequency of abdominal lymphadenopathy compared to patients with isolates with non-Uganda genotype [29]. In this study, 55% of the TB cases were caused by the Uganda genotype, including those with extrapulmonary TB. The fact that the patients with the Uganda genotype were younger than those with non-Uganda genotype may be an indication that the Uganda genotype is still on the rise in Uganda.

In a quarter of the 49 cases with mycobacteria NTM were isolated. In general NTM should be identified to the species level [9], since there is large variation in virulence between different NTM species. Although they were not speciated, this is an interesting finding since in 8 of the 12 cases with NTM the isolates came from sterile sites such as liver, kidney or lymph nodes, indicating that they were of clinical relevance. One source of environmental contamination could be the sterile water in which the specimens were transported. However controls in terms of culture of the water did not reveal any contamination with NTM (data not shown).

The clinical features of NTM are indistinguishable from those of TB in immunocompetent people [30]. We isolated NTM in both HIV co-infected patients and patients not infected with HIV. The gross pathology and histopathology in these cases were similar to the findings in TB patients. However, pleural effusions were significantly less prevalent in patients with NTM, both before and after adjusting for confounders like sex, HIV status, and age. This agrees with earlier reports that pleural effusions are rare in disease with NTM [31]. Pleural effusion is one of the most common findings in extrapulmonary TB, and the cause of the effusion is regarded as a delayed hypersensitivity response to mycobacterial antigens in the pleural space. The hypersensitivity reaction generating a pleural effusion depends on pathogen virulence and the host immune status [32]. The lower prevalence of pleural effusions in NTM patients in this study may reflect differences in antigens exposed by the NTM or the immune suppression associated with underlying diseases.

The disease outcome following infection with mycobacteria depends on environmental, host and pathogen factors [33]. NTM infection can occur throughout the body, though pulmonary infections are the most common form of NTM infection [33]. Most NTM infections generate a chronic granulomatous inflammation and are clinically indistinguishable from TB. Various species of NTM have been identified as potential pathogens in a large proportion of pulmonary disease both in immunocompetent and immunocompromised hosts [34–36], and are associated with pulmonary structural changes like bronchiectasis, chronic obstructive pulmonary disease and cystic fibrosis [9]. Although clinical infections with NTM are rare, they are increasingly being recognized, and in some regions occur more frequently than TB, even in patients without predisposing factors [37] but especially in immunocompromised patients [38]. Disseminated infection has mainly been reported in patients with immunosuppression[39]. NTM was reported in HIV patients with less than 50 CD4 cell/μl[40], and in patients with abnormalities of Interferon gamma (IFN-γ) or Interleukin–12 (IL–12) [41, 42]. NTM are most prevalent in HIV subjects and most of the NTM were found to be clinically significant [43]. In a study involving HIV patients in Miami, USA, NTM were disseminated in the majority of cases and mortality was 10% for disseminated disease [44]. However other studies have reported cases of disseminated NTM in patients without any predisposing conditions [45, 46].

Our study has a number of limitations. The sample size was restricted due to low consent and recruitment levels attributed to customary and religious beliefs. The small sample size may not be representative of the entire study population. Our study designed to establish the mycobacteria causing fatal TB disease in a tertiary referral hospital does not represent cause of death in the entire health care strata. Most of the patients diagnosed with TB in Uganda are treated as outpatients and are difficult to track. Because of the small sample size, binary logistic regression odds ratio are exaggerated, which should be considered in interpreting results of small sample size studies.

Significance/Implication: The results of this study may be valuable in policy formulation with regard to diagnosis and management of mycobacterial diseases. Better protocols for prevention, early identification and management of NTM disease should be developed. NTM disease morbidity and mortality may be reduced by developing appropriate screening tools, early diagnostic strategies and good reporting systems.

Conclusion: In patients with presumptive TB, a majority of culture positive cases were caused by *Mtb,* and histopathology was similar in TB and NTM patients, although NTM patients had significantly less pleural effusions. These results suggest that NTM contribute to mortality, and that their identification is important because of the special clinical and therapeutic implications associated with NTM infections.

Abbreviations

TB, Tuberculosis; HIV, Human immunodeficiency virus; ZN, Ziehl-Neelsen; *Mtb, Mycobacterium tuberculosis;* NTM, Nontuberculous mycobacteria; FNA, Fine needle aspiration; MGIT, Mycobacterial

growth indicator tube; CAS, Central Asian Strain; EPTB, Extrapulmonary TB; DR, direct repeat locus; PCR Polymerase chain reaction; SIT, spoligotype international type.

Declarations

Ethics approval and consent to participate:

The study was approved by the Internal Review Board (IRB) of the Makerere University School of Medicine. Final approval was granted by the Uganda National Council of Science and Technology. (HS 1364).. Informed written consent was obtained from the patients' next of kin. Information about the research and postmortem procedure was given in English and Luganda, the predominant local language, prior to seeking consent to perform postmortem and further analyses.

Consent for publication:

Consent for publication was obtained from all the patients' next of kin.

Availability of data and materials:

All data generated or analysed during this study are included in this article. The datasets used and/or analysed during the study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

DW: Participated in the research conceptual development, drafting the manuscript, research implementation, data acquisition and interpretation and intellectual content. MJ: Participated in the research conceptual development and design and contributed to the intellectual content of the manuscript. GK: Participated in conceptual development, drafting and critically revising the manuscript. Contributed substantially to the intellectual content and gave the final approval of the manuscript.

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Tables

Table 1. HIV status and demographic parameters of the patients infected with MTC as compared to those infected with NTM

HIV Status	MTC n=37 (75.5%)	NTM n=12 (24.5%)	P-Value
HIV +	26 (72%)	7(64%)	0.710
HIV -	10 (28%)	4 (36%)	
Age			
mean	36 (SD 13)	36 (SD 18)	0.459
Sex:			
Female	13 (35%)	4 (33%)	1.000
Male	24 (65 %)	8(67%)	

Table 2 Visceral involvement in patients with TB and NTM: Pathological and microbiological findings.

Viscera involved	TB/NTM n (%)	Crude		Adjusted*	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Lung: Pleural effusion	19 (51%)/1 (8%)	0.1(0.0-0.7)	0.025	0.1(0.0-0.9)	0.038
Lung: Cavitation	7(19%)/2(17%)	0.9(0.2-4.8)	0.861	2.0(0.2-21.4)	0.554
Liver	13(35%)/1(8%)	6(0.7-51.4)	0.105	0.2(0.0-1.8)	0.142
Spleen	20(57%)/4(33%)	02.7(0.7-10.5)	0.162	3.9(0.6-26.6)	0.163
Brain: Meningitis	14(38%)/4(33%)	1.2(0.3-4.8)	0.7791	1(0.2-4.9)	0.903
Pancreas	12(33%)/3(25%)	2(0.4-8.6)	0.420	4.9(0.7-33.3)	0.105
Lymph nodes	23(62%)/4(33%)	3.3(0.8-12.9)	0.089	3.5(0.6 -19)	0.140
Kidney	17(46%)/2(17%)	4.3(0.8-22.1)	0.086	4.6(0.7 -29)	0.114
Adrenal glands	3(8%)/ 0(0%)		0.999		0.999
Extrapulmonary (any kind)	25(68%)/8(68%)	1(0.2-3.8)	0.954	2.5(0.3-21.2)	0.396
Adjusted* = Adjusted for Age, Sex and HIV status; OR = Odds ratio, Cl= Confidence Interval Logistic regression.					

Table 3. Visceral tuberculosis: Pathological and microbiological findings.

Viscera involved	Uganda/Non-Uganda n (%)	Crude		Adjusted*	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Lung: Cavitation	5(71%)/11(50%)	2.5(0.4-15.7)	0.321	0(0)	0.998
Lung: Pleural effusions	7 (44%)/8 (62%)	0.5(0.1-2.2)	0.343	0.2(0.-1.8)	0.166
Spleen	9(60%)/8(67%)	0.8(0.2-3.7)	0.722	0(0-)	0.998
Liver	6(38%)/6(46%)	0.7 (0.2-3.1)	0.638	0.1 (0.0-2.2)	0.133
CNS: meningitis	7(44%)/4(31%)	1.8(0.4-8.1)	0.476	1.1(0.2-8.5)	0.917
Pancreas	6(38%)/4(31%)	1.3(0.3-6.4)	0.705	0.6(0.1-5.7)	0.682
Lymph Nodes	10(63%)/9(69%)	0.7(0.2-3.5)	0.705	0.3(0 -3.5)	0.319
Kidney	8(50%)/7(54%)	0.9(0.2-3.7)	0.837	0.1(0 -3.0)	0.192
Extrapulmonary TB (any kind)	11(69%)/9(69%)	1(0.2-5)	0.978	2(0.1-29.3)	0.623
Adjusted* = Adjusted for Age, Sex and HIV status; OR = Odds ratio, Cl= Confidence Interval Logistic regression.					

Figures

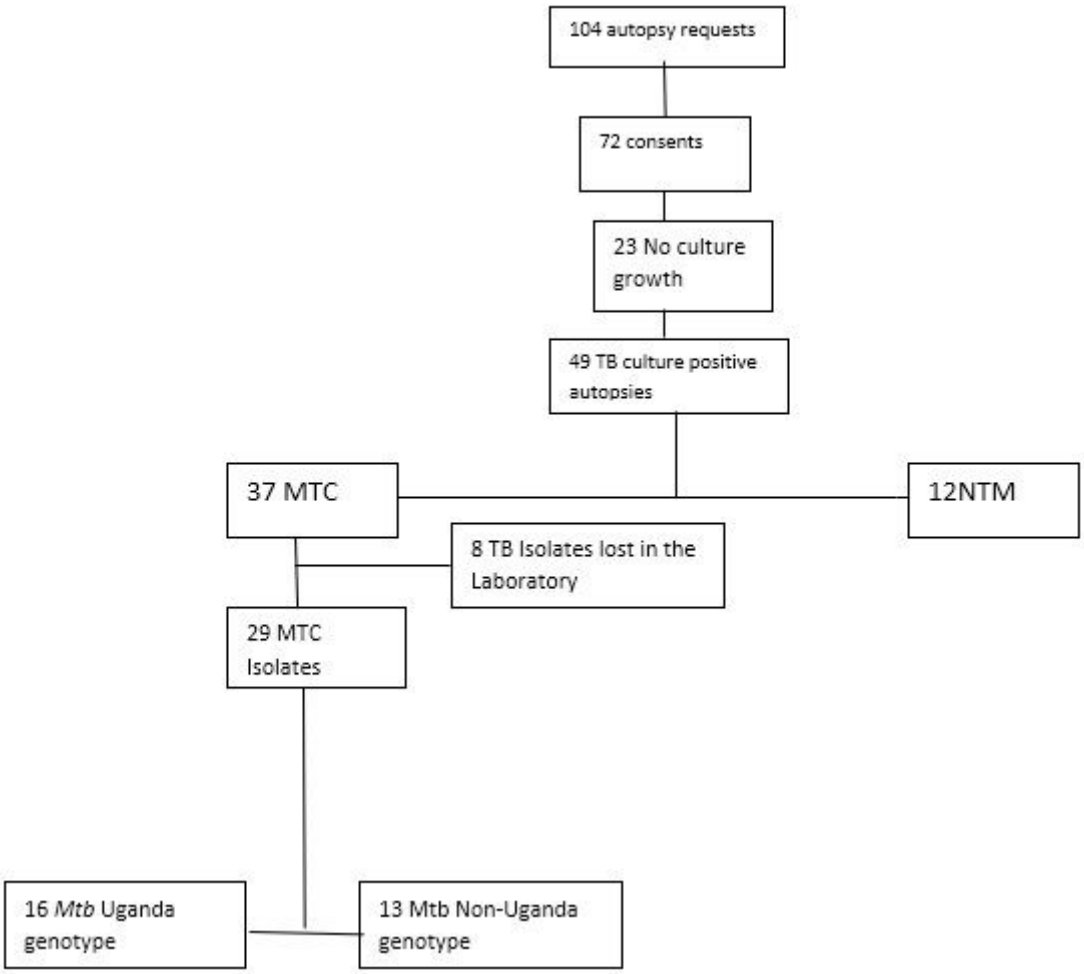

Figure 1

Study flow diagram

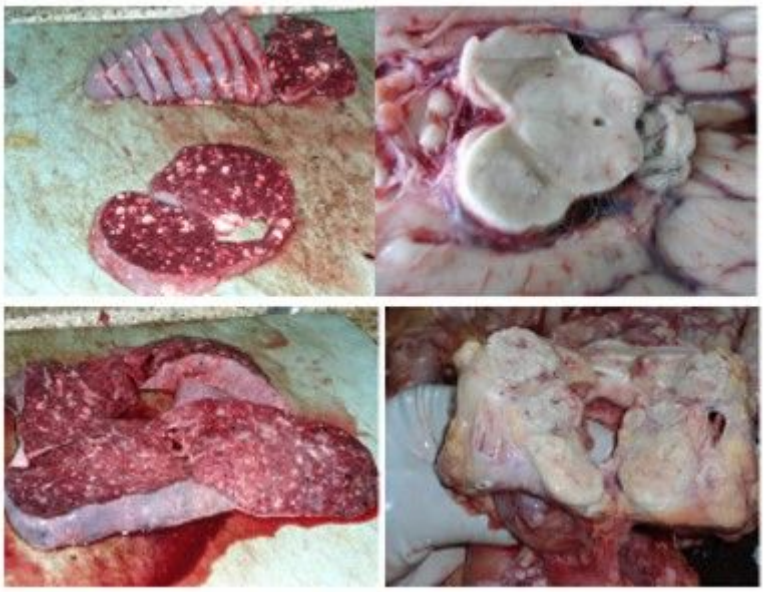

Figure 2

Disseminated TB involving a) tubercles in the spleen parenchyma b) tubercles at the base of brain c) Miliary TB of lung showing numerous 1-2 mm yellow nodules in the lung parenchyma d) large matted caseating mediastinal lymph nodes.

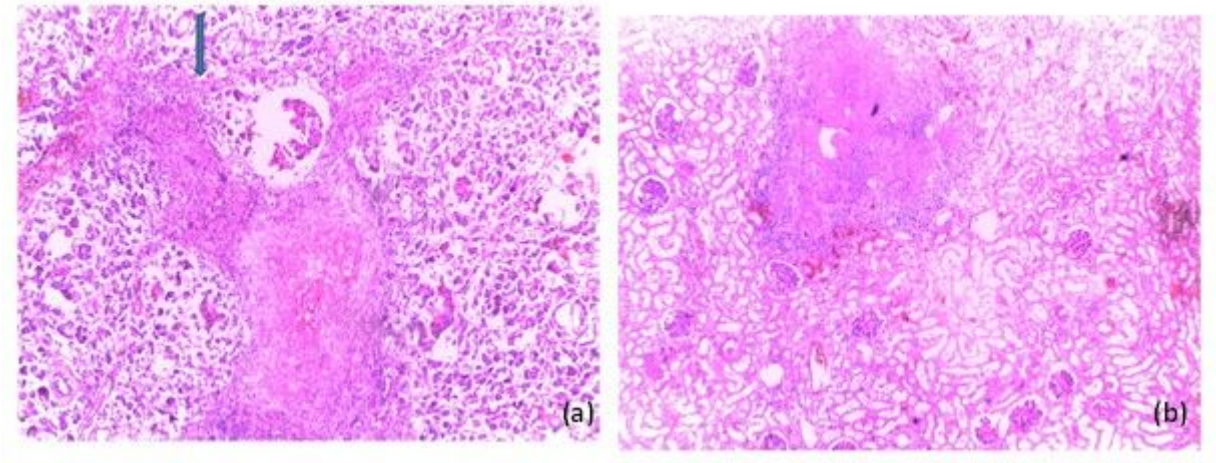

Figure 3

(a) Tuberculous pancreatitis: Chronic caseous granulomatous inflammation involving the interlobular fibrous connective tissue septa and destroying adjacent acini and interlobular ducts. The inflammation spreads along the interlobular septa. (b) Tuberculous nephritis: Chronic caseous granulomatous inflammation involving and destroying cortical labyrinth.

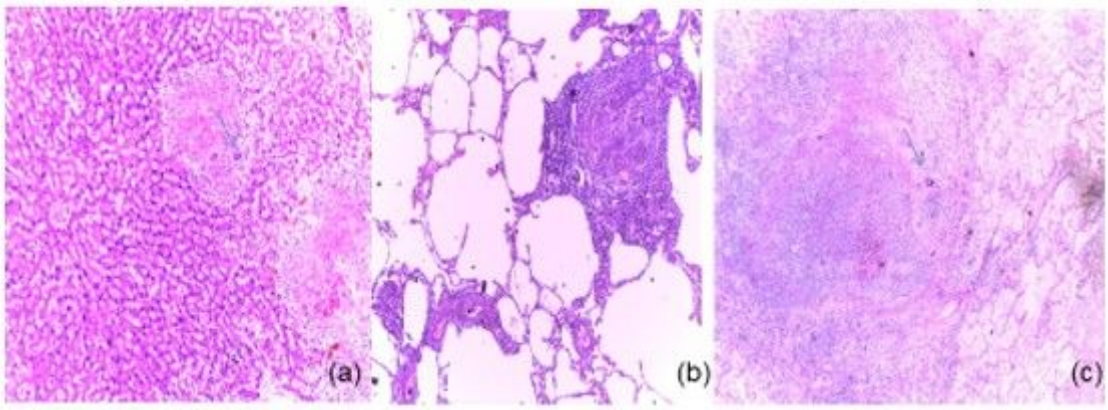

Figure 4

(a) Hepatic TB. Multiple granulomas destroying hepatocytes. Central caseous necrosis and Langhans giant cells (Arrow). HIV positive with disseminated TB (b) Bronchial centric and bronchiolar centric granulomas. HIV Negative with NTM. c) Caseous pneumonia characterized by central caseous necrosis, granuloma and Langhans giant cells (Arrow). Narrowing of the bronchioles is accompanied by destruction of interalveolar septa resulting in emphysema (E).