MAGNITUDE OFLIVER DISEASES DISCOVERED DURING MEDICO-LEGAL AUTOPSIES AT MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA.

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MMed (Anatomical Pathology) Dissertation

Muhimbili University of Health and Allied Sciences

October, 2019

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

MUHAS

SCHOOL OF MEDICINE

DEPARTMENT OF PATHOLOGY

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By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Masters of Medicine (Anatomical Pathology) of the Muhimbili University of Health and Allied Sciences

October, 2019

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of dissertation entitled **‘’Magnitude of Liver diseases discovered during medico-legal autopsies at Muhimbili National Hospital, Dar-es-Salaam, Tanzania”** in partial fulfillment of the requirements for the degree of Master of Medicine (Anatomical Pathology) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Salvatory Makweta Mlaga,** declare that this dissertation is my own original work and that has not been presented and will not be presented to any other university for similar or any other degree award.

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Signature ---------- Date----------

ACKNOWLEDGEMENT

I would like to thank the Lord God and exalt his mighty name for the abundance of merciful protection and guidance during the entire period of this very dissertation work.

I am grateful to thank the Muhimbili University of Health and Allied Sciences (MUHAS) and the Muhimbili National Hospital (MNH) for the knowledge, skills and competence that i have acquired during the memorable three years of my postgraduate training within their premises.

I also direct my appreciation to the Hubert Kairuki Memorial University for their financial support during the entire period of my training.

I also wish to express my special gratitude to my supervisors Prof. A.R. Mwakigonja and Dr. Henry Mwakyoma for their invaluable guidance and encouragement throughout this valuable work period.

With much love I thank my family especially my lovely wife, Angel John Kabanza and my Children since they cordially chose to endure the pain of missing my presence and direct patronage during my study years.

I thank my dear parents Rustina-Mwanahamisi Sanga and Mr. John Mlaga Mwamaluli for their constant support, encouragement and prayers during the whole period of my training.

I extendmy sincere gratitude to the staff of the department of Pathology, MUHAS, especially the head of department of Pathology Prof. A.R. Mwakigonja, Dr. Henry Mwakyoma, Dr. Edda Vuhahula, Dr. PM Ng'walali and Dr. Kabyemela for their mentorship and inspiration to me.

I would like to express my innermost gratitude to all pathologists and laboratory technicians and scientists at MNH especially Laboratory manager Dr. Innocent Mosha and Mrs. Theresia Wakesho, the acting Head of the Histopathology Unit at MNH, for their constant support at various moments of my study.

My sincere appreciation goes to my outstanding classmates; Doctors: Tupokigwe Edna Brown Mwakilima to whom I extend my special appreciations for tireless support, and to others; Henry Stephen, Gilbert Nkya, Asteria Kimambo, Atuganile Mallango and Leonard Mlemwa for their cordial cooperation that made my three years of MMed program enjoyable.

My sincere gratitude goes to the supporting staff at the department of pathology MUHAS, namely Rose Chiduo and Samuel Kasangaya for their cordial assistance at various stages of my study. I also thank Mr. Eric Magorossa, Robert Buchanga and Bernard Nyanza of MNH for their memorable inputs in laboratory works.

My sincere appreciation to Mr. Wilbard Kamara of MNH for his contribution in handling of tissue samples in the meagre spaced archive.

Similarly, thanks to Mr. Rajab Hassan on behalf of the MNH mortuary team for their invaluable assistance in tissue sample collection.

Lastly, to all my enemies both bodily and spiritually, known and unknown since their negative animosity awakened me to work harder as well as enriched my path towards this ultimate abundance of success with certitude by grace in Jesus, the Name above all names.

DEDICATION

To my wife

To my beloved Children

To my parents

To all my teachers, and

To all my friends and colleagues, especially those aspiring to work in Liver transplantation program.

I dedicate this work.

ABSTRACT

Background

The liver is susceptible to various injuries including metabolic, circulatory, environmental and socioeconomic leading to acute or chronic liver diseases. The impact of resultant chronic diseases in many low- and middle-income countries is steadily growing (Naghavi M., et al), but scarcely documented. Lack of awareness about frequencies and patterns of these diseases limits benefiting from treatment and preventive measures (WHO. 2008, Vol 8, number 1). Unlike other chronic diseases which can be detected during routine check-ups with diagnostic tools such as sphygmomanometer and glucometer, most liver diseases including fatty liver requires histological work up, with special stains to describe their patterns (Orah N., et al).

To date no data has been documented on the prevalence of liver diseases discovered during autopsy in our country and thus, occurrence and frequency of these diseases in our settings is not known. Autopsy based studies including those determining the histopathological patterns of the liver diseases are very useful in elucidating occurrence and magnitude of such diseases in our community and this is the aim of the current study.

Objective: To determine the magnitude of liver diseases discovered during medico-legal autopsies at Muhimbili National Hospital, Dar-es-Salaam, Tanzania.

Methodology: We conducted a prospective cross-sectional study, which was autopsy based at Muhimbili National Hospital Histopathology Laboratory premises in which Medical-legal autopsies received at MNH mortuary facility (part of laboratory unit) with causes of death other than liver diseases were enrolled for the study. Demographic information and circumstance of death were established from respective Police Form number 99 (PF 99) and cross-checked with the information from the identifying witnesses of deceased person. Gross appearance of the liver was recorded prior to sampling of the tissue.

Specimen sections were stained by routine H&E protocol, then additional sections were prepared from tissue blocks for selected special stains as directed by H&E sections results, including Reticulin, Per-iodic acid Schiff and Pearl's Prussian Blue special stains for

evaluation of degree of fibrosis, hepatocytes glycogen and iron deposition respectively when necessary.

Results: This study recruited 253 forensic autopsy cases. Majority were males 218(86%) and the mean apparent age in years at death was 36 +/- 12.98(SD). The age range was from 16 to 78 years (M:F ratio of 6:1). The peak age group of the study participants was 26-35years 77(30.4%). The mean ages in years for males and females were 36.74+/-13.23(SD) and 38.23+/-13.38(SD) respectively, and a median of 36years. Bulk of cases had fatty change 83(32.8%), followed by steatohepatitis 37(14.6%), and combined granulomatous lesions 27(10.7%).All six (100%) of liver cirrhosis cases had livers with decreased weight. Regardless of specific disease types majority of cases were crowded in the younger age groups while steatosis cut across all age groups.

Conclusion: There are fatal and prevalent liver diseases afflicting younger forensic decedents in our setting. Steatosis was found to be the most common liver disease entity, reflecting the possible picture in the general population in this demographic region, these were followed by steatohepatitis and combined granulomatous lesions of the liver.

Recommendations: Basing on disclosure of histopathological patterns from this study, modalities for liver biopsy in patients, for evidence of hepatopathy should be scaled up and made widely available in our public health systems.

Pathologists performing forensic autopsies should consider liver histology as routine practice to scale up diagnosis of clinically unnoticed liver diseases.

More studies to underpin the evidence for viral hepatitis and determinants of steatosis among study cases are crucial to elucidate their cause specific magnitude alongside their biochemical and molecular profiles.

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LIST OF ABBREVIATIONS

AFLD	Alcoholic Fat Liver Disease
ALD	Alcoholic Liver Disease
AU	African Union
CRN	Clinical Research Network
DNA PCR	Deoxyribose Nucleic Acid Polymerase Chain Reaction
DPX	Dibutyl Phthalate Xylene
FFPE	Formalin Fixed Paraffin Embedded
H&E	Hematoxylin and Eosin
HCC	Hepatocellular carcinoma
HCL	Hydrochloric acid
HMS	Hyper-reactive malarious splenomegaly
H_2O_2	Hydrogen peroxide
HS	Hepatic steatosis
MNH	Muhimbili National Hospital
MoHCDEC	Ministry of Health, Community Development, Gender, Elderly and Children
MS	Metabolic Syndrome
MUHAS	Muhimbili University of Health and Allied Sciences
NAFLD	Non Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non Alcoholic Steatohepatitis
NCDs	Non communicable diseases

PBC	Primary Biliary Cirrhosis
PF99	Police form number ninety nine
PM	Postmortem
RTA	Road traffic accident
SDGs	Sustainable Development Goals
SOP	Standard Operation Procedures
SPSS	Statistical Package for Social Science
SSA	Sub Saharan Africa
WHO	World Health Organization

DEFINITION OF TERMS

Autopsy: Also, post-mortem examination, obduction, necropsy, or autopsia cadaverum is a highly specialized surgical procedure that consists of a thorough examination of a corpse by dissection to determine the cause and manner of death or to evaluate any disease or injury that may be present for research or educational purposes.

Medico-legal Autopsy: also known as Forensic or coroner's autopsies seek to find the cause and manner of death and to identify the decedent. They are generally performed, as prescribed by applicable law, in cases of violent, suspicious or sudden deaths, deaths without medical assistance or during surgical procedures.

Metabolic Syndrome: Is a clustering of risk factors that greatly increases an individual's probability for developing atherosclerotic cardiovascular disease (ASCVD), type 2 diabetes and chronic kidney disease, and is associated with deranged metabolism of particular nutritional substrate like fat or carbohydrate.

Non Alcoholic Fatty Liver Disease: A chronic induced liver injury encompassing a wide spectrum of liver damage, ranging from simple steatosis to fibrosis, and cirrhosis, with lack of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long term use of a steatogenic medication, or monogenic hereditary disorders.

NAFL is defined as the presence of 5% hepatic steatosis (HS) or more without evidence of hepatocellular injury in the form of hepatocyte ballooning or fibrosis, with no alcohol use.

NASH is defined as the presence of 5% HS or more and inflammation with hepatocyte injury (e.g., ballooning), with or without any fibrosis in the absence of alcohol use.

PF99: Is a special legal document presented as a form, which orders for the performance of a postmortem examination as per inquest ordinance (CAP.24, S.12).

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Liver diseases are not infrequent. Unlike high blood pressure or diabetes, which can be detected with blood pressure machine and glucometer during a routine check-up, liver diseases like Nonalcoholic fatty liver disease (NAFLD) and Alcoholic fatty liver disease (AFLD) are difficult to diagnose due to their silent nature(1). Most people with liver fibrosis from various causes don't present clinical features, and right now there's no straightforward biomarker that a routine blood panel would pick up and for these patients histological work up especially with special stains yields good results(2). The only reliable measure of fibrosis is a liver biopsy. Moreover, the disease progresses slowly-over years or even decades-and researchers have yet to figure out when someone with a fatty liver is at risk for developing the inflammation and fibrosis that define Non Alcoholic Steatohepatitis (NASH) or Alcoholic Steatohepatitis (ASH)(3).Most of the chronic liver diseases, even in advanced stages, may cause no evident clinical signs or symptoms. They either go undiagnosed or are found incidentally during general health check-ups, investigations for other diseases, surgery, or autopsy(4,5).The underlying causes of chronic liver diseases vary in different geographic areas and are based on various factors such as socioeconomic status, life style, diet, local or regional infections, and other endemic diseases.

Liver diseases are a major cause of morbidity and mortality in the population due to its quiet progression to end stage liver disease without important symptoms, and the fact that it affects all population age groups. These have to be detected at an earlier stage to reduce the morbidity(4).

There is a long list of medical conditions known to contribute to the syndrome of silent liver diseases. Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease with a prevalence estimated to be 20-30% in general population of Western countries(5,6).

NAFLD occurs as a histological spectrum of disease and includes the subtypes of simple steatosis and nonalcoholic steatohepatitis (NASH). It was thought to be a benign condition but is now widely recognized as the main cause of liver-related morbidity and mortality. Studies

put to light that NAFLD may progress to cirrhosis, liver failure, and hepatocellular carcinoma(7,8).

Better understanding of treatable and/or preventable diseases is essential for general public interest both economically and socially. Recently, emerging experimental and clinical evidence is starting to show that even liver cirrhosis, one of the end stage in most liver pathologies, can in its early stages be potentially reversible(63).To this end understanding its magnitude and frequency of diseases leading to its occurrence is significant. The fast growing medical science has made it possible for reversing earlier stages prior to liver failure by fibro genetic methods and/or perform Liver Transplantation when necessary(63), all requiring awareness on the frequencies and characteristics of specific disease entities leading to end stage liver diseases, which this work has strived to contribute significant answers.

Tanzania is not an isolated island, is an active member state to AU, and beneficiary of WHO public health improvement strategies, thus she cannot tolerate the lack of knowledge on occurrence of silent liver diseases. This study focuses to examine the hidden possibility of an increased burden of NCDs and communicable diseases at another angle (liver diseases). The work tries to answer such questions like; are we free from the global up surging silent liver diseases, if no, to what extent does the problem affect our community? Who gets affected the most? Answers to all these questions will provide the direction for future plans.

1.2. Literature Review

1.2.1 Overview

This literature review aimed at establishing a common understanding regarding the magnitude and importance of liver diseases as a hidden contributor in the large constellation of NCDs and partly to some infectious liver diseases. This is a public health problem worldwide, such that SSA through WHO umbrella has set specific goals to uncover this burden and work to lower its potential dangers (9). It is about twenty eight years now since 1990, when the planned target of combating health problems through priorities set primarily by epidemics of infectious diseases in sub-Saharan Africa (SSA). For future global health efforts, it is important to understand the growing burden of non-communicable diseases (NCDs) through

research(9,10). These efforts will foster the accomplishment of Sustainable Development Goals (SDGs). The total burden of NCDs in terms of disability-adjusted life-years (DALYs) shows a growing burden of NCDs(10). Mental and behavioral disorders, musculoskeletal diseases, and diabetes and endocrine diseases increased by 3%, 2%, and 1% in 2010, respectively (from 12%, 9%, and 11% in 1990). In southern SSA, truly the proportion of DALYs due to infectious diseases increased by 17% between 1990 and 2010, but an increasing burden of NCDs in Africa shows a growing health iceberg hidden under epidemics of infectious diseases.(8,9,11)

About 20% of chronic disease deaths occur in high-income countries, while 80% occur in low- and middle-income countries where most of the world's populations live as described in detail in the WHO publication "Preventing chronic diseases: a vital investment," the impact of chronic diseases in many low- and middle-income countries is steadily growing(9). In SSA countries, around 28 million people died in 2005 from a chronic disease.

Non-communicable diseases, the silent killers, have insidious onset, provide debilitating complications and result in painful deaths. The estimated number of chronic disease-related deaths in the WHO African Region in 2005 was 2 446 000(9,10).

The rate of increase of deaths from chronic diseases will outstrip that from infectious diseases, maternal and perinatal conditions and nutritional deficiencies more than four-fold in the next 10 years. Populations in the region are not benefiting from primary prevention and cure, which may be due to lack of awareness about occurrences and frequencies of these diseases to both professionals and the community at large(8)

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver diseases, ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis. Nonalcoholic steatohepatitis (NASH) represents a stage in the spectrum of NAFLD, Characterized by presence of inflammation leading to gradual fibrosis of the liver mass. Histology is the unique method for diagnosis of silent liver diseases(1). Fatty liver is a common 'liver disease' often free of symptoms or complaints but might even lead to severe stages. It is characterized by lipid deposition in hepatocytes both, for alcoholic as well as and non-alcoholic fatty liver. An additional inflammatory reaction, liver cell injury and fibrosis results in - alcoholic (ASH) or

non-alcoholic (NASH) - steatohepatitis. Steatohepatitis is characterized by both, inflammatory infiltrates of mixed cells in the small liver lobules as well as liver cell injury in terms of ballooning and resultant fibrosis(12).

1.2.2. Magnitude of liver diseases

The prevalence of NAFLD in patients undergoing liver biopsy for any reason ranges between 15% and 48% globally. This wide range is naturally related to the differences in the populations studied. In these patient based studies mostly carried in western countries, the prevalence of NASH ranged between 2.1% and 4.8%. Comparable to these results are the studies done in south Africa where the prevalence of NAFLD reached 48%(13)though carried in patients. A study in far east that involved 896 autopsies as study population (not in patients)revealed that 34.9 % of the cases undergoing autopsy for forensic reasons in Tehran forensic facility had evidence of un noticed NAFLD(5), While the same study established a prevalence of (19 cases) 2.1% of Steatohepatitis as the smallest proportion. Majority of these cases in Tehran that is 82.9% had grade I or II fatty change in the hepatocytes. The reason for such a high rate of NAFLD in their study was postulated to be the effect of life style and the changes in the dietary habits in Tehran(5). Although alcohol selling is illegal but not nil as few black markets are allowed to do the business in Tehran, there is risk of alcohol fat liver as well. Considering even the most common emerging sub groups of silent killers affecting the liver, the true incidence and prevalence of NAFLD and NASH are not well known in different populations Tanzania being amongst them. To date, there is no published data on the occurrence and prevalence of this group of diseases in Tanzania. This is partly because liver histology is required as the gold standard for precise diagnosis of this condition, a strong reason in our setting where the complications associated with liver biopsy are most feared, coupled with the obscurity of symptoms in these patients. On top of that, the relatively invasive procedure of liver biopsy is still not considered essential for management of NAFLD by many physicians(14), while the fact is histology remains to be essential towards definitive diagnosis as well as proper staging of most pathologies.

It has been shown that NAFLD is strongly associated with the features of metabolic syndrome. Insulin resistance is a key pathogenic factor in both NAFLD and metabolic

syndrome. Available data from clinical, experimental and epidemiological studies indicate that NAFLD may be the hepatic manifestation of metabolic syndrome (MS) (14).

Contradicting results between MS and NAFLD by different researchers have been observed for example, in a prospective observational study of 4401 apparently healthy individuals Hamagushi et al found that the MS is a strong risk factor for nonalcoholic fatty liver disease. Although these results consolidates on the silence behavior of liver diseases, but it differs with other authors who have seen that NAFLD, in its whole spectrum ranging from pure fatty liver to non-alcoholic steatohepatitis (NASH), might represent another feature of MS(7). What is agreed by most studies is the shared pathophysiologic mechanism between MS and NAFLD basing on clinical associations, and laboratory investigations support that have shown insulin resistance and hyper-insulinaemia to have a central role in pathogenesis of both MS and non-alcoholic fatty liver(7,14).

Furthermore ninety percent of individuals with NAFLD have at least one risk factor of MS, and 33% have all the features of MS. Study have shown that liver fat content is significantly increased in subjects with the MS as compared with those without the syndrome(14,15), independently of age, gender, and body mass index(6,16). NAFLD is associated with significant number of other disease conditions in the group of silently killing NCDs, such diseases includes; Steatosis, Chronic hepatitis, Steatohepatitis, Cirrhosis, Granulomatous hepatitis, Focal nodular Hyperplasia, Hemangioma Hepatocellular Carcinoma as evidenced by Rasoul Sotoudehmanesh et al, in their work on silent liver diseases in autopsies from forensic medicine of Tehran, in Iran(5). Other NCDs like diabetes mellitus may co-exist with silent liver diseases(15), and may affect a broader age group from young ones to elderly patients where it progresses asymptomatically(16–18).

1.2.3. AFLD vis-à-vis NAFLD

Liver is most commonly affected by alcohol abuse followed by lungs(19). Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are the major liver diseases proceeding covertly and have similar pathological spectra, from simple hepatic steatosis to steatohepatitis and liver cirrhosis, although the epidemiological and clinical characteristics of

these two diseases differ (20). Any one or all the three spectra can occur at the same time, in the same patient(21). Alcoholic fatty liver disease occurs after acute alcohol ingestion and is generally reversible with abstinence. Fatty liver is not believed to predispose a patient to any chronic form of liver disease if abstinence or moderation is maintained. Alcoholic hepatitis is an acute form of alcohol-induced liver injury that occurs with the consumption of a large quantity of alcohol over a prolonged period. Alcoholic hepatitis can range in severity from asymptomatic derangement of biochemistries to liver failure and death. Cirrhosis involves replacement of the normal hepatic parenchyma with extensive thick bands of fibrous tissue and regenerative nodules, which results in the clinical manifestations of portal hypertension and liver failure. The prevalence of alcoholic liver disease (ALD) is difficult to define because it is influenced by many factors including genetic (e.g., predilection to alcohol abuse, gender) and environmental (e.g., availability of alcohol, social acceptability of alcohol use, concomitant hepatotoxic insults) factors. The use of alcohol varies widely throughout the world with the highest use in the U.S. and Europe. Men are more likely to develop ALD than women because men consume more alcohol(22). However, women are more susceptible to alcohol hepatotoxicity and have twice the relative risk of ALD and cirrhosis compared with men(23). Testino G. et al., explained that, elevated body mass index is also a risk factor in ALD as well as nonalcoholic fatty liver(24).

In patient studies use of Alcohol Use Disorders Identification Test Consumption (AUDIT-C) questionnaire may help to determine use and concentration of alcohol(25,26), This is not always accurately possible with autopsy based studies due to inability to use the score directly to witnesses without modifications. Attempts to obtain alcohol drinking behavior from next of kin is challenging and most of the time not obtainable, also is associated with lack of clarity(19). Jana et al in their cross sectional study that aimed at establishing the prevalence of alcohol related pathologies in 554 autopsy cases, they obtained drinking history only in subset of 26% (144) of the cases approximating to $1/5^{th}$of sample size(19). In a study of Comparison of Next-of-Kin with Self-Respondents Regarding Questions on Cigarette, Coffee, and Alcohol Consumption it was shown that next of kin particularly spouses provided information as reliable as that of self-informant for cigarette smoking and coffee drinking, but a higher

discrepancy for alcohol drinking (19), thus next of kin appear to be good source of information with limitation to type of information especially alcohol. With exception of vitreous humor Postmortem decomposition spuriously increases blood ethyl alcohol (EA) owing to endogenous production by overgrowth of normal, fermentative flora in the gut with substantial (−0.20%) artifactual elevations. Vitreous humor, typically sterile, is a reliable comparison medium to differentiate ante mortem consumption from post-mortem production(19,27), however there are high cost implications associated with toxicological alcohol detection especially where the service is centralized and scarce as the case is in our setting. Alternatively, characteristic gross and histological pathology in various organ systems may be diagnostic of chronic alcoholism even without established history as evidenced by Donna M. Hunsaker, which its type, amount and duration of use is difficult to establish from witnesses alone. Further to that, they depicted that progressive toxic effects are commonly expressed in the liver, heart, pancreas, and the central nervous system. The diagnostic procedures in patients with suspected fatty liver disease-with or without known alcohol consumption-should be standardized and generally accepted(28). Liver biopsy represents the "golden standard" for confirming diagnosis and determining inflammatory activity and potential fibrosis of fatty liver disease both alcoholic and nonalcoholic related. To some extent, the differential diagnosis of ASH vs. NASH cannot be made on the basis of histological criteria alone. Steatosis, inflammatory changes and hepatocytes injury can be semi-quantified using a "Brunt Score" or "NAS" (NAFLD activity score), providing the basis on which to decide whether or not steatohepatitis is present. However, Brunt scores applies more of histological findings(28,29). The work of Singh D.K et al, delineates Older age, male sex, larger derangement of serum biochemistry, high serum bilirubin, AST/ALT > 1, more ballooning degeneration, portal inflammation, Mallory's hyaline, hepatocytic and ductular cholestasis, ductular proliferation and higher stage of fibrosis to favor a diagnosis of ASH, whereas, younger age, high ALT, AST/ALT < 1, higher grade of steatosis and absence of extensive neutrophilic portal inflammation favors a diagnosis of NASH, this shows the importance of combined clinico-biochemical and histological pointers to deciding between ASH and NASH(30). Both validated non- alcoholic fatty liver disease activity score (NAS)

plus fibrosis, as well as the steatosis, activity, fibrosis (SAF) by pathologists of the American NASH CRN and the European Fatty Liver Inhibition of Progression (FLIP) centers respectively, have shown success in clinical trials and clinical use (Blunt, 2017) in her review paper of ongoing role of liver biopsy evaluation(31). The histological features applied can be used in other studies applying biopsy(30) with good results. Furthermore, Sayantan Ray et al, in their study that correlated clinical biochemical and histologic findings in alcoholic liver diseases they found that, severity of liver damage – clinically and histologically was directly related to the quantity and duration of alcohol consumption. Thus, Histological picture of alcohol liver damage can predict alcohol use in terms of quantity and duration as to acute or chronic effect(32). The histological features of alcohol induced hepatic injury vary, depending on the extent and stage of injury. These may include steatosis, lobular inflammation, periportal fibrosis, nuclear vacuolation, bile duct proliferation, fibrosis or cirrhosis. While steatosis has potential for complete revert to normal liver architecture, steatohepatitis form the rate limiting stage as though seldom possible to reverse with stoppage of alcohol, it is from this stage further damage will be permanent(24). In their study, Testino G. et al,. had shown that, In the subset of patients with AH, a liver biopsy may demonstrate specific histological features, including confluent parenchyma necrosis, steatosis (where AFLD dominated by macro vesicular type more in zone three), deposition of intra sinusoidal and pericentral collagen, ballooning degeneration, and lobular inflammation affecting the perivenular regions in the earliest stages(24). The liver may be infiltrated by polymorphnuclear cells (not found in NASH), typically clustered around cytoplasmic structures known as Mallory bodies, which represent aggregated cytokeratin intermediate filaments and other proteins. According to Testino G. et al., the severity of inflammation (i.e., degree of polymorphonuclear leukocyte infiltration) and cholestatic changes correlate with poor prognosis, whereas the presence of mega mitochondria may be associated with a milder form of alcoholic hepatitis (AH)(24). Further to that, routine histology by H&E supplemented by special stains gives better conclusions in liver studies(2,33,34).

1.2.4. Obscurity of liver diseases

Most liver diseases are commonly discovered incidentally during routine laboratory examination in patients receiving treatment for hypolipidemias and during abdominal ultrasound examination for suspected gallstone and other differentials of the epigastrium and right hypochondrium(1,7). When present, features are not specific and unreliable in evaluating severity of disease(5,16). The commonest signs and symptoms are malaise and right upper quadrant pain or discomfort. Few patients may present with delayed features of ascites, jaundice and liver encephalopathy indicating far more serious hepatic disease. The unnoticeable damages a silent process, resembling high blood pressure or diabetes. But unlike high blood pressure or diabetes, which can be detected with blood pressure machine and glucometer during a routine check-up, liver diseases like NAFLD and NASH are difficult to diagnose(1). Most people with liver fibrosis don't present clinical features, and right now there's no straightforward biomarker that a routine blood panel would pick up and for these patients histological work up especially with special stains yields good results(2). The only reliable measure of fibrosis is a liver biopsy. Moreover, the disease progresses slowly-over years or even decades-and researchers have yet to figure out when someone with a fatty liver is at risk for developing the inflammation and fibrosis that define NASH or ASH for example(3).HIV/AIDS Colluding emerging NCDs has been observed as a new challenge where the giant enemies of public health work synergistically to enhance each other's effect on victim's health. This observation calls for combined and continued effort to combat both infectious and NCDs at the same time(1).

1.2.5. Diagnostic approaches to liver diseases

Many diagnostic approaches are applicable to liver diseases with varying significance in describing disease severity and staging(2). Histology remains superior diagnostic method for liver diseases. Timely diagnosis of liver diseases is strongly hindered by lack of early clinical symptoms for which patients may seek medical care, and possibility of specific infections due to hepatitis virus other parasites causing chronic liver disease and other metabolic diseases further complicating to silent liver diseases requires to be addressed(2,35,36)(33).

Traditionally in clinical routine, diagnostic liver biopsy is only performed for highly selected patients. This is even worse in low income setting were practice of interventional radiology is at its infancy. Therefore, the reported rates which are based on liver biopsies from patients only cannot reflect the true prevalence of liver diseases in the general population(2).

Postmortem examinations, conducted for those who have passed away for reasons other than liver diseases, are certainly better sources for determination of a more reliable prevalence for NAFLD and NASH, as well as other communicable and non-communicable liver diseases. Limited contribution of radiological studies like ultrasound imaging in terms of diagnostic accuracy and providing no indications as to the disease stage, makes this technique less reliable for most of liver diseases(37), despite being capable to capture fatty change. Failure to rely on most biochemical clinical investigations formed the basis for our autopsy study in our settings where open liver biopsies are not performed routinely and FNAB are rarely prioritized to few clinically selected patients and provides limited analytical results.

1.2.6. Tissue biopsy and H&E stains

It is established by previous studies that tissue biopsy is superior to other diagnostic modalities for liver diseases. But tissue biopsy for which H&E alone are evaluated still has limitations for addressing some changes in liver biopsy the gap which can be filled well by use of special stain and clinical biochemical studies(2).

1.2.7. Role of Special stains

Evaluation of liver tissue for diagnosis purposes is mainly based on a thorough examination of sections stained with Hematoxylin and eosin (H&E). Additional special histochemical stains may be used to highlight or identify features that are not easily seen on an H&E stained section. The choice of stain or panel of stains depends on the findings observed on assessment by H&E section, the relevant clinical context, and the preference of the investigator most of the time pathologist(2), and the usefulness of this method is well disclosed by similar studies conducted elsewhere(5,14).

1.2.7.1. Trichrome Stain

Masson's trichrome stain is among the most common special stains applied to liver specimens. The stain imparts a blue color to collagen against a red background of hepatocytes and other structures. It stains type 1 collagen that is normally present in the portal tracts and vessel walls, but also highlights the presence and distribution of reactive fibrosis as a result of liver injury. It is used for staging of chronic liver diseases, and helps to delineate patterns of injury, such as the perisinusoidal fibrosis associated with steatohepatitis and periductal fibrosis in primary sclerosing cholangitis.

1.2.7.2. Reticulin

Stain Reticulin stain uses silver impregnation to detect reticulin fibers, which are made of type 3 collagen. The fibers appear black against a gray to light pink background. In the liver, such fibers are present as part of the extracellular matrix in the space of Disse (33). By highlighting these fibers, the stain helps in the assessment of the architecture of the hepatic plates, such as expansion in regenerative and neoplastic conditions, compression of plates in nodular regenerative hyperplasia, and collapse of the reticulin framework in necrosis.

1.2.7.3. Iron Stain

The Perl's (iron) Prussian blue reaction is a reliable and routinely used stain for detecting iron deposited in tissue or cells. Basically iron is stored in the hepatocytes as ferritin (soluble form) and hemosiderin (an insoluble form) when in excess amounts. By using H&E stain, the latter is seen as cytoplasmic coarsely granular brown refractive granules, while ferritin is not observed. Pearl's stain highlights hemosiderin as coarse blue granules, while ferritin is seen as a faint blue cytoplasmic blush. In diseases like hemochromatosis, iron accumulates primarily in the cytoplasm and initially in the periportal hepatocytes. In secondary iron overload, the accumulation is mainly in the Kupffer cells. When a large quantity of iron is present or if there is concurrent active hepatocellular injury, the distribution of iron may become mixed, both in hepatocytes and Kupffer cells. The staining technique allows unstained lipochrome and bile pigments to be seen against the pale counterstain for comparison and differentiation of the two. In the appropriate clinicopathological setting iron stain may be supplemented as

well with tissue iron quantitation. This can be performed on routinely processed formalin fixed paraffin-embedded tissue biopsies [FFPE](2,38).

Periodic Acid-Schiff Stain: The periodic acid-Schiff (PAS) stain is important for highlighting glycogen, but removing glycogen with diastase digestion enhances detection of un digested material in the same tissue, including the basement membrane, debris within macrophages, alpha-1-antitrypsin globules, and organisms especially fungus. Using PAS with and without digestion confirms the presence of glycogen deposition, such as in the various glycogen storage diseases(33,37).

Orcein staining: This stain is useful for demonstration of hepatitis B antigen in paraffin sections of liver. Modified orcein tissue staining method described by Shikata et al in 1974 may help to detect the Hepatitis B antigen in liver tissue. To date the mechanism of staining is postulated to be related to the presence of disulphide bonds in HBsAg biopsies(33,36) The technique is simple and of use both in fresh and stored material. The deposits were seen in the cytoplasm of liver cells and occasionally in Kupffer cells, but never in nuclei. There observed inverse relationship between staining and parenchymal necrosis. Biopsies from asymptomatic HBsAg carriers were often strongly positive, as were 'ground-glass' hepatocytes in carriers and patients with chronic liver disease (2,33,36,37).

The use of special stain has been suggested to be a routine tests as far as their ability of demonstrating rarely revealed diagnostic features in tissue biopsy(2). And their use in cell block should be investigated further. Whenever possible reporting of liver biopsy must include special stains results. The most distinctive histological feature for identifying HBV aetiology is the ‘ground-glass hepatocyte’. It has a finely granular, and faintly eosinophilia cytoplasm due to proliferation of the smooth endoplasmic reticulum containing accumulated hepatitis B surface antigen. Ground glass cells are highlighted by special stains, including Shikata’s orcein or aldehyde fuchsin and Victoria blue(39), all being largely replaced by Immunohistochemical methods in most centers in recent years.

1.2.8 Use of Immunohistochemistry

Immunohistochemical stains are much less commonly used in the diagnostic evaluation of nonneoplastic liver diseases compared with the histochemical stains described above. The most commonly used stains are those for diagnosing or confirming viral infections involving the liver, including hepatitis B virus, cytomegalovirus virus and herpes simplex virus, although the latter can also be detected via in situ hybridization. Cytokeratin immunohistochemistry is useful in enhancing detection of Mallory Denk bodies (MDB) associated with non-specific hepatic injuries observed in ALD, NAFLD and malignant processes.

1.2.9 The need for accurate prevalence data

Histology remains superior diagnostic method for liver diseases. Timely diagnosis of liver diseases is strongly hindered by lack of early clinical symptoms for which patients may seek medical care. Liver biopsies conducted under clinical bases, and radiological investigations have less contribution towards proper data for magnitude of the silent liver diseases. To this end autopsies, performed for those who have passed away from non-liver diseases, are certainly better sources for determination of a more reliable prevalence for ALD, NAFLD and steatohepatitis(2). Orah and fellows established a trend signifying the missing information, that, there exists a set of commonly diagnosed chronic liver diseases through biopsy namely; Cirrhosis, Chronic hepatitis, Hepatocellular carcinoma and metastatic carcinoma while NAFLD and ALD were not diagnosed especially when no special stains were applied(2). Studies have also shown that, for better post-mortem evaluation of tissue, samples should be collected from dead bodies died within three to five days. Also, the best results are within 72hrs post death especially when early formalinized and / or refrigerated(40) facts which were well established by Mageriu et al on correlations between the autolytic changes and post-mortem interval in refrigerated cadavers. New insights on treatment of some Chronic liver diseases are major concern in the world of medical science. Studies are underway at various phases of trial towards the accomplishment of this goal. The targets include suppression of fibrotic change which is associated with majority of this group of diseases, as well as to alleviate the culprit primary causes(14,17). For quite a long time public health frontiers have

advocated for life style change for treatment and prevention of most NCDs. These changes could not reverse. Early detection of liver cirrhosis is amenable for reversal. Liver fibro genesis is characterized by activation of hepatic stellate cells and other extracellular matrix producing cells. Liver fibrosis may regress following specific therapeutic interventions other than removing agents causing chronic liver damage, but no ant fibrotic drug is currently available in clinical practice, however promising clinical trials are still in different phases in pipeline.

1.2.10. Granulomatous Liver diseases

Granulomas are aggregates of modified macrophages (epithelioid cells) and other inflammatory cells that accumulate after chronic exposure to antigens(41). Manifestations are variable depending on whether the underlying cause is a systemic disease or a primary hepatic granulomatous reaction. Clinical studies have shown that, the prevalence of granulomas ranges between 2.4% and 15% of all liver biopsy specimens(41). Conn and others reported that 66% of their cases of granulomatous reaction were secondary to a systemic disease, 28% to primary liver disorders, and 6% were idiopathic(41). The causes of granulomatous liver disease are diverse. The frequency with which various specific aetiologies are discovered depends on the geographic area and endemicity. For instance, schistosomiasis is a frequent cause of granulomas in areas where this parasitic infection is endemic(41,42). Infections with *Schistosoma mansoni* and *S. japonicum* are frequent in the developing world where waterborne parasitic diseases are prevalent. Chronic schistosomiasis causing granulomas and related fibrosis is the most common cause of portal hypertension in the world, presenting with splenomegaly and bleeding. Tuberculosis and schistosomiasis together makes the most common infectious group of hepatic granulomatous inflammation worldwide primarily in non-Western countries(41). Other infestations like *E. vermiculris* and *F. Hepatica* may occur. Primary liver diseases rarely cause hepatic granulomatous disease except for primary biliary cirrhosis, and hepatitis C(41,42). It is helpful to classify the causes of granulomatous hepatitis into several broad categories such as autoimmune disorders, systemic infections, medications, malignancy, idiopathic causes and, rare causes, which encompass a broad category of systemic granulomatous diseases, connective tissue diseases causing a systemic inflammatory disorder,

and digestive disorders to assist with treatment prediction(41). Histoplasmosis is the most commonly recognized fungal disease associated with hepatic granulomatous infection endemic in the western countries while hepatic candidiasis characterized by granulomas with suppurative central areas containing variable necrosis and giant cells are common in other parts of the world especially Africa(41,43). Aspergillosis characterized by a marked neutrophilic infiltrate or granulomatous inflammation, which is similar to that in mucormycosis and zygomycetosis, also do occur in eastern world. Both hepatic candidiasis and Aspergillosis makes the common fungal infections in onco-hematologic and non oncohematologic patients, and they are leading cause of fungal infections in liver transplants(43). Other fungal infection do occur rarely such as Cryptococcus, Pneumocystis carinii and Blastomycosis dermatitidis and they cause necrotizing inflammatory reaction with granulomatous features in immunocompromised adults to purely granulomatous reactions(41). Hepatitis C is more associated with granulomatous hepatitis than B virus(41). Many drugs have been implicated in the causation of granulomas in the liver or granulomatous hepatitis. The overall incidence of drug-induced hepatic granulomas is thought to be 10%(41). Hodgkin lymphoma, non-Hodgkin lymphoma, and renal cell carcinoma have all been associated with hepatic granulomas. These types of granulomas are non-necrotic and are considered to be distinct from malignant cells. The relationship between malignant lesions and the development of hepatic granulomas is unclear(41). A substantial proportion of patients about 3% to 37% with granulomatous liver disease will never have a cause identified despite extensive work-up. In these cases treatment is targeted towards autoimmune diseases or empirically to *M. Tuberculosis* especially in anergic patients or in those with a positive tuberculin skin test. Sarcoidosis which is a granulomatous disease of unknown cause that can affect several organs, including the liver and primary biliary Cirrhosis (PBC) constitute the common autoimmune diseases, whereas Sarcoidosis accounts for 12% to 30% of granulomatous liver diseases, 25% to 50% of PBC Liver biopsies have granulomas. Understanding of the histomorphological features of granulomatous diseases of the liver is indicated for an appropriate antimicrobial therapy and an overall optimal management including prevention of this entity(41).

1.2.11. Malaria hepatopathy

Malaria is a preventable and certainly treatable parasitic disease, centrally to this, an estimated 225 million cases occur each year across 106 countries Worldwide(44). According to the recent malaria landscape publication, the malaria burden is highest in sub-Saharan Africa, where one in five childhood deaths is caused by malaria (44). It is most prevalent in tropical countries including Tanzania than other parts of the world. From a hepatic viewpoint, it is important to appreciate that whereas *P. falciparum* infection (the severe form of the disease and the only one to cause acute mortality) and *P. malariae* directly utilize the hepatocyte during their life-cycle within Homo sapiens, *P. vivax* and *P. ovale* also undergo a dormant phase (the hypnozoite stage) in these cells, which allows recrudescence(s) to occur(45)." Tanzania is known to harbor all species of malaria parasites with P. falciparum leading in complicating to severe forms. A perplexing question regarding malaria contributing to chronic hepatitis has been explained partly since early 1898 by Dr. (later Sir) Patrick Manson who suggested that 'Under the influence of a succession of acute attacks, hepatic congestion may acquire a more or less permanent character(45)'. This was supported by Lucius Nicholls who in 1913, wrote about association of Cirrhosis of the liver with repeated attacks of malaria leading to being common condition of many tropical countries(45). Hepatocyte changes consist of lipofuscin and occasionally malaria pigment deposition well demonstrated using a bi-refringent staining technique, fat-droplet formation, mitochondrial swelling, and microvillus loss(45). In a small percentage of individuals living in an area of high transmission, hyper-reactive malarious splenomegaly (HMS) - an aberrant immunological response to the four human Plasmodium spp (45). In this syndrome, massive splenomegaly, often accompanied by significant hepatomegaly, is usual; sinusoidal lymphocytosis (mostly T-lymphocytes) (which may be intense), raised serum IgM (polyclonal) and an elevated malarial antibody titre in peripheral blood are co-existent features. Malaria pigment is now known to be haemozoin, an iron protein porphilin complex derived from haemolysis of red blood cells and anaerobic respiration of parasites, and can be detected by Pearl’s Prusian Blue stain(45–47).

1.2.12. Features of chronic viral hepatitis

The most distinctive histological feature for identifying HBV aetiology is the 'ground-glass hepatocyte'. It has a finely granular, and faintly eosinophilic cytoplasm due to proliferation of the smooth endoplasmic reticulum containing accumulated hepatitis B surface antigen(39). Ground-glass cells are highlighted by special stains, including Shikata's orcein or aldehyde fuchsin, and Victoria blue(37,39,48). These infections may be picked by cytopathic changes usually present on the H&E stained sections, such as ground-glass cytoplasm in hepatitis B infection, nuclear and/or cytoplasmic inclusion bodies in cytomegalovirus infection, and nuclear inclusion in herpes infection (37).However study on the frequency of characteristic features for chronic hepatitis B, C, autoimmune and cryptogenic hepatitis concluded that the respective histological patterns have low sensitivity, but high specificity and predictability(39).

1.2.13. Liver Transplantation and liver disease among the donors

Studies have shown that most of liver diseases progresses unnoticed within individuals(5,29,49,50). Tendency of chronic liver diseases to end up in cirrhosis and eventually hepatocellular carcinoma culminating to liver failure led to the resolution of liver transplantation (LT). The choice of good donor for liver transplantation relies on the health state of the potential liver to be donated(51)(52). Some of the diseases progressing covertly may hinder the suitability of the donor's liver. Understanding of liver diseases profiles in a community is essential for preparedness in any country aspiring for LT, as it caters for successful transplantation and recipients' safety at large. Some diseases that may exist silently, yet carry potential for LT failure includes fungal infections due to Candida, Aspergillus and / or Cryptococcus species, A group of hepatitis B and C viruses, Cytomegalovirus infections(43,51,53). Gastrointestinal flora are another potential group causing liver transplant infections(43). These may occur both in immunocompromised and immunocompetent individual, though at a greater prevalence in the former(53).

1.2.14. Cystic and congenital liver diseases

Cystic lesions may be classified as developmental, neoplastic, inflammatory or miscellaneous(54) lesions. Adult polycystic liver disease, of autosomal dominant inheritance with high penetrance, may present at any age with renal manifestations, but although hepatic involvement is common, clinically significant liver disease is rare (<15%), meaning it develops unnoticed and does not usually affect the natural history. Hepatic cysts are rarely seen before puberty but become more frequent with increasing age (<20% in those under 30, and >75% in those over 70); they are more common in women, especially those with children. When liver disease occurs it usually accompanied with renal disease(55). Liver biopsy shows portal tract fibrosis and numerous cystically dilated channels; the hepatic parenchyma is otherwise normal but it is not unusual to find Von Meyenburg (biliary harmatomas) complexes in non-cystic parts of the liver(55). Congenital dilatation of the intrahepatic bile ducts without obstruction recognized by Caroli in 1958. Pure Caroli's syndrome remains an unusual diagnosis, with fewer than 150 cases in the world literature(55). The saccular cystic dilatations (choledochal cysts) more commonly affect the left lobe, and may be associated with biliary stasis, cholangitis, stone formation (in about25%)(55). According to Nambirajan L, (2000) histology proven diagnoses of 22 choledochal cysts, liver biopsies showed varying degrees of bile duct proliferation, cholestasis, parenchymal damage, inflammatory cell infiltration and pericentral fibrosis as clue to diagnosis(55). These features are non-specific and require observation of cysts is important. Simple cysts of the liver do occur, Benign non-parasitic cysts of the liver, once considered rare because they are generally small and asymptomatic, are more commonly shown by modem imaging (prevalence between 0.1 and 2.5%), more so in women (male: female ratio 1:5), more often in the right lobe, and probably of congenital origin. The cysts are lined by biliary columnar epithelium(55), or specifically, Simple non-parasitic hepatic cysts are congenital and are supposedly triggered by chromosome 16. They are lined by cuboidal epithelium and arise as an aberration of bile duct development in utero (56)but it is relatively unusual for the fluid contents to be bile(55). Astonishingly do not communicate with the biliary tree(54). Resembling to simple cysts are Peribiliary cysts but these are typically found in patients with long standing cirrhosis(54), But

Fateh et al., in their review paper described their occurrence in non-cirrhotic cases(57). They develop around the intrahepatic portal venous branches. These lesions may have variable size and morphology(54). Another rare cystic condition of the liver is Peliosis hepatis, histologically there are blood filled cavernous cysts in continuity with the sinusoids. Cysts vary in size but may reach 5mm in diameter. Of note, Sinusoidal ectasia appears to be a milder form of peliosis and may represent its earliest manifestations. Biloma do exist and are encapsulated collection of bile outside the biliary tree. It can form spontaneously, secondary to trauma(54). According to WHO work on echinococcosis, human infection with *E. granulosus* leads to the development of one or more hydatid cysts located most often in the liver and lungs, and less frequently in the bones, kidneys, spleen, muscles, central nervous system and eyes. The asymptomatic incubation period of the disease can last many years until hydatid cysts grow to an extent that triggers clinical signs, however approximately half of all patients that receive medical treatment for infection do so within a few years of their initial infection with the parasite. Depending on the presence of an epithelial lining, livercysts are classified as true or false. True liver cysts include congenital cysts (simple cysts and polycystic liver disease), parasitic cysts (caused by *Echinococcus granulosus* or *Echinococcus multilocularis*), neoplastic cysts (cystadenoma, cystadenocarcinoma, cystic sarcoma, squamous cell carcinoma and metastatic cancers from ovaries, colon, kidneys and pancreas) and biliary duct-related cysts (Caroli disease, bile duct duplication and Peribiliary cysts). False liver cysts may be caused by spontaneous intrahepatic hemorrhage, post traumatic hematoma, or intrahepatic biloma (55,58). multiple simple liver cysts has been classified as follows: Type I, few large cysts (> 7 cm to 10cm); Type II, multiple medium cysts (5 cm to 7 cm);and Type III, diffuse small to medium cysts (< 5 cm)(55).

1.2.15. Statement of the Problem

An increase in NCD is currently a global public health threat that draws attention of health professionals(8–10). Studies conducted in United States and other developed countries have established an abrupt increase of NCDs especially those related to un-noticeable liver diseases. Evidence laid down by studies on the burden of these diseases given the name ‘ silent killers’ have revealed that, there is an increase in the morbidity and mortalities due to

NCDs at an alarmingly high speed, that they are estimated to surpass the magnitude of infectious diseases in the near future.

Liver disease is a major cause of morbidity and mortality in the population due to its silent progression to end stage liver disease without significant symptoms affecting both the economically productive young population and the elderly together. Despite WHO global goal of reaching an additional 2% reduction in chronic disease death rates annually over the next 10 years, an effort that requires contributions from all countries through STEPWISE approach(11), Tanzania like most African countries have not established the necessary scientific evidence like base line data on prevalence and factors associated to the emerging NCDs contributed by silent liver diseases like metabolic syndrome and non-alcoholic fatty liver. The experience from clinical practice, diagnostic liver biopsy is only performed for highly selected patients. Therefore, the reported rates which are based on liver biopsies from patients cannot reflect the true prevalence of liver diseases in the general population. Autopsies, performed for those who have passed away for reasons other than liver diseases, are certainly better sources for determination of a more reliable prevalence for liver diseases especially ASH and NASH(1,7).

Newer treatment modalities are being devised including the anti-fibrotic change in the liver to supplement the lifestyle focused treatment and prevention measures. The applicability and will to priorities for the use of these strategies depends on being aware about the frequencies of the disease at hand. To date no data has been published on the prevalence of liver diseases in our locality and thus, occurrence and distribution of these diseases in our setting is not known. Determining the prevalence and factors associated with the occurrence of these diseases and the histopathological features ascribed to specific disease entities is of paramount significance to understand the contribution of this group of NCDs in the general group of NCDs known to exist in our community. To what extent these diseases contribute to deaths mistakenly ascribed to other simultaneous disease conditions other than the true liver disease is wealthy being answered. And the proper methodology towards explanation for this occurrence is through autopsy based studies especially the ones which determine broadly histologic features on liver biopsies.

1.2.15.1. Conceptual Framework

This is the plan of conceptualized model of variables which will be operationalized to accomplish the set objectives of this study. Really it is a diagrammatic representation of the theory. The theory is presented as a model where research variables and the relationship between them will be translated into a visual picture to illustrate the interconnections between the independent, and dependent variables.

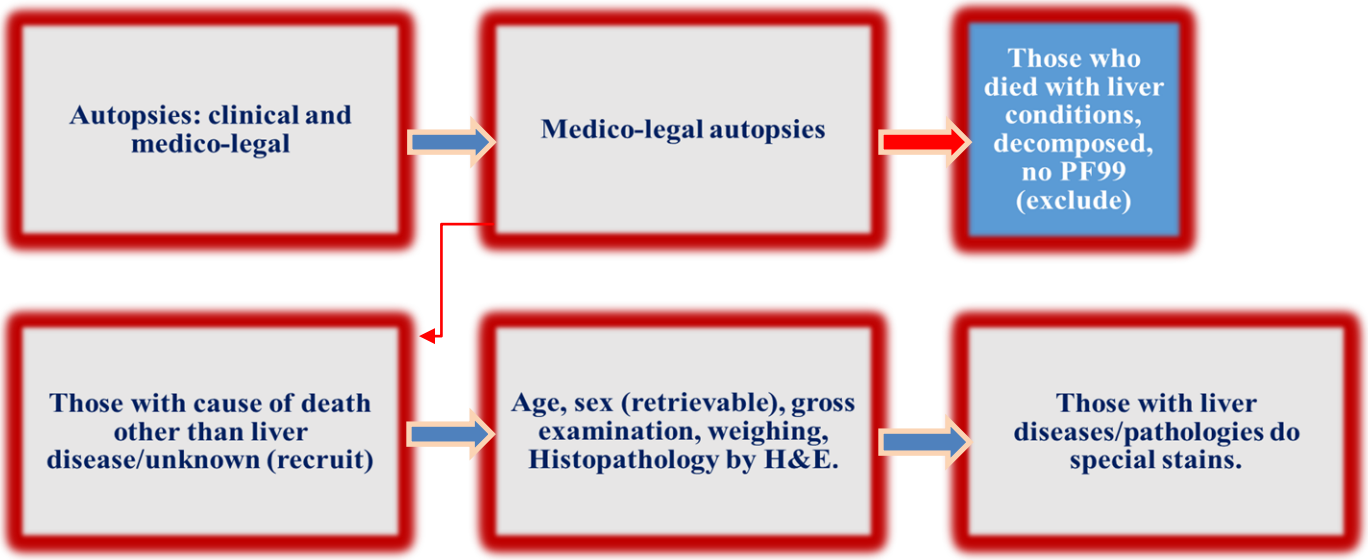

Figure 1: Conceptual framework

1.2.15.2. Rationale of the study

This study will provide a true picture of the prevalence and occurrence of the deadly liver diseases in our community. To this end therefore, the research results will assist both to update the registry of NCDs and some communicable diseases as well as to provide policy makers with grounds for informed policy formulation as far as the alarmingly major public health threat of NCDs is concerned, furthermore it will enable appropriate planning towards implementing evidence-based interventions. The results of this work will stand as the baseline data for future research around the subject matter.

Hypothesis
There are liver diseases prevalent among forensic decedents at MNH.

1.2.15.3. Research Questions

1. What is the prevalence of liver diseases among medico-legal autopsy liver specimens at MNH?
2. What are the histopathological patterns in liver biopsies among medico-legal autopsies?
3. What are the proportions of histopathological patterns among medico-legal autopsies with regard to age and sex at MNH?

1.2.15.4. Objectives

1.2.15.4.1. Broad Objective

To determine the magnitude **of** liver diseases discovered during medico-legal autopsies at Muhimbili National Hospital, Dar-es-Salaam, Tanzania.

1.2.15.4.2. Specific Objectives

1. To determine the prevalence of liver diseases discovered amongst medico-legal autopsy cases at MNH.
2. To determine histopathological patterns of liver tissue biopsies among medico-legal autopsy cases.
3. To determine the proportions of specific liver histopathological patterns among medico-legal autopsies with regard to age and sex.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study design

This was a prospective, cross-sectional study design, in which medical legal autopsies received and scheduled for post-mortem examination at MNH mortuary facility were enrolled.

2.2. Study duration

The study was conducted for a period of seven (7) months from June to December 2018.

2.3. Study area

The study was conducted at Muhimbili National Hospital specifically in the Mortuary facility, whereby both dead bodies emanating from inpatients and outpatients department within MNH premises, as well as from outside the hospital who were officially received for medico-legal autopsies were recruited. Muhimbili National Hospital is the national referral and teaching hospital with Mortuary facility equipped with five maximum autopsy beds for carrying postmortems at a goal. Muhimbili National Hospital has a histopathology department with integrated forensic activities performs both clinical and medico-legal postmortem services. On average about 1200 autopsies are carried annually basing on2017 and 2018 autopsy records at MNH mortuary.

2.4. Study population

All dead bodies formally received in mortuary facility for medical-legal autopsy from June to December 2018 at MNH.

2.5 Inclusion criteria

All dead bodies scheduled for autopsies at MNH from children to elderly, who died from diseases other than liver conditions and whose necessary information is retrievable from PF99.

2.6. Exclusion criteria

i. The received dead bodies for whom PF99 forms are nowhere to be found.
ii. The registered postmortem bodies with liver disease ascribed to circumstances of death.
iii. All decomposed dead bodies.

iv. Cases that showed bad quality sections and features of autolysis under H&E were considered inadequate and excluded from the study.

2.7. Sample size estimation

The sample size was calculated from Fischer's formula:

n = [DEFF*Np (1-p)]/ [($d^2/Z^2_{1-\alpha/2}$*(N-1)+p*(1-p)] where:

n= Minimum required sample size

N=Population size (896)

D=Confidence interval limits as % of 100 (absolute +/- %):5%

DEFF= Design effect: $1Z^2_{1-\alpha/2}$ = 1.96 at 95% Confidence Interval which will be assumed for the study

P = Proportion silent liver disease with the characteristic of interest of which it's estimated proportion of patients with NAFLD in liver autopsy biopsies was 34.9% (Ali Ali-Asgari et al, 2006)

d^2 = Margin of error which is conventionally taken as the sampling error at 1.96 and is thus taken as 5% in this study.

The minimum estimated sample size (n) is thus taken to be 252 dead bodies.

2.8. Sampling procedure

Convenient sampling method was applied whereby all dead bodies received from within or outside the hospital for formal medico-legal postmortem examination, from June to December 2018 at MNH, got included in the study population.

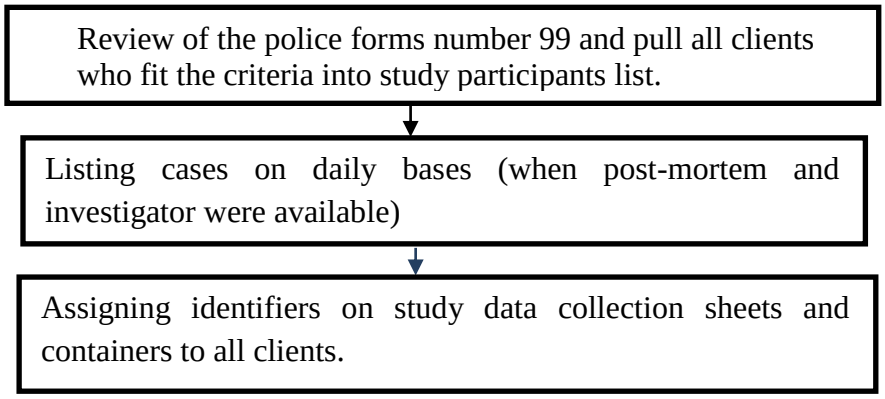

Figure 2: Sampling procedure

2.9. Autopsy and laboratory methods

Medical legal autopsies scheduled at MNH with causes of death other than liver diseases were enrolled for the study. Demographic information were obtained from PF99 forms and inquired from identifying witnesses of deceased. The routine Postmortem examination was thoroughly conducted and the respective reports produced as per normal procedure, but special attention was paid to the Liver which was removed for further examination as per the need of the study. The weight of each liver was taken using a special scale BCD/20450, By W&T AVERY (currently Avery Weigh-Tronix), available in MNH autopsy facility. Gross appearance of the liver was described prior to sampling the tissue. Sampling was done by principle investigator and when necessary involving one senior Pathologist.

Three biopsies measuring at least (2 × 2 × 2 cm) each, from the right and left lobes and one biopsy of the same size from deeper areas of the liver parenchyma were obtained in each case. Any grossly abnormal areas were sampled as an additional fourth biopsy. Thereafter, containers of specimen were labeled with respective registration number (PM no.) and additional identifier for the specific site were assigned specific names **R, L** and **D** for right lobe, left lobe and deeper area respectively. When an obvious lesion was present its slide was given an additional label of **'A'**, for abnormality on the side of cassette. All the liver specimens were then immediately fixed in 10% neutral buffered formalin. The specimens were processed, sectioned, and stained with Hematoxylin and eosin following MNH standard

operating procedures, later the initial microscopy was done by principle investigator followed by review by a senior pathologist. Then additional sections were prepared depending on the need for special stain established by H&E sections, for example Reticulin, Masson's trichrome, and Pearl's Prussian blue special staining evaluation of degree of fibrosis, liver architecture and iron deposition respectively were conducted when necessary. Orcein special stain as well as alternative stains for demonstration of Ground glass hepatocytes could not be conducted though indications were present from H&E sections due to hindrances by logistical affairs. If we could manage to get them they could help to demonstrate histologic features of hepatitis B viral infection in affected hepatocytes.

In case of any diagnostic discrepancy, between the principle investigator and senior Pathologist, the result had to be reported according to the consensus of a joint slide review. Histologic findings were recorded in a standard form prepared.

2.10. Microtome and Sectioning

The rotary microtome (SAKURA model SRM 200 CW) was used to cut the thin sections of three micrometers for H&E staining and four micrometers for special staining whereby three or four slide sections were analyzed per case, and up to six slides per case could be analyzed when special stains were applied. The thin section were then allowed to float on water bath containing distilled water at 45^0C and got mounted on standard frosted glass slides. Thereafter, slides labeling with respective registration number (PM no.) was done on cassettes of the autopsy biopsies and additional identifier for the site were assigned specific names **R, L** and **D** for right lobe, left lobe and deeper area respectively. When an obvious lesion was present its slide was given an additional label of **'A'**, for abnormality. Then the slides were then allowed to drain before dewaxing them on the hot plate at 60^0C for 50 minutes.

2.11. H&E staining procedure

H&E staining was conducted manually, and the following procedure was followed for all sections as per SOP attached in appendices.

2.12. Histopathological bases for interpretation and diagnosis of common liver lesions

Steatosis: In her conclusion remarks, Brunt E, stated, liver biopsy evaluation has provided and will continue to provide pertinent information for clinical decision making and care as well as for investigation into pathogenesis, progression, and disease correlates and effectiveness of therapeutic interventions in clinical trials in NAFLD (Brunt 2017)(28,31). Here we adopted the criteria as described below.

The criteria for diagnosis of Fat liver disease at various stages has been decribed by Brunt et al, and widely accepted as shown in appendix VIa, VIb and VIc (Brunt et al, 1999, Andrew)(28). The NAS (NAFLD activity score) is a refinement of the Brunt score, derived by separate semi quantification of each of the three components – steatosis, hepatocyte ballooning and lobular inflammation – and addition to form a total score were also used to decide whether or not steatohepatitis was present.

Granulomatous liver diseases: (Appendix VI, a-c).Criteria for diagnosis and describing granulomatous liver diseases adopted from Coash. M et al, appendix V, was used as guidance. Specific for Tuberculosis; Ziehil Neelsen special stain was used to confirm diagnosis after initial H&E impression.

Fungal infections: PAS special stain was used to confirm fungal infections.

Malaria heapatopathy:These were diagnosed basing on H&E features like presence of reticuloendothelial cells proliferation and sinusoidal dilatation, alongside malarial haemozoin pigments on H&E as well assubjectto Pearl's Prussian Blue staining to illuminate the haemozoin pigment. Also visualization of malaria parasites on H&E and post GIEMSA stain.

Cirrhosis: presence of regenerative nodules and fibrosis (with or without remnants of steatosis)

2.13, Description of study variables

The first objective is to determine the prevalence of liver diseases discovered during medico-legal autopsy at MNH from June to December 2018.

In this case the morphological features of liver tissue biopsies prepared were determined using H&E staining method to find out the frequency and the pattern of the liver conditions in the specimens obtained. These, then were further related for age and sex to cater for the third objective.

Dependent variables: These were frequencies of specific liver diseases in liver autopsy specimens from dead bodies scheduled for postmortem examination at MNH.

Independent variables: This comprised of the number of occurrences of a specific disease features.

The second objective was to determine histopathological patterns of liver tissue biopsies among medico-legal autopsy cases

In this case the morphological features of liver tissue biopsies prepared in autopsy unit were determined to find out the gross and microscopic histomorphology and the pattern of liver conditions in the specimens obtained. It was determined by gathering information from gross features, H&E and selected special staining method.

Dependent variables: These were histomorphological patterns of hepatic autopsy specimens from clients scheduled for and in whom forensic postmortem examination were conducted at MNH.

Independent variables: These comprised of the gross features, H&E and special staining behavior on each autopsy specimen of liver biopsy.

The third objective was to determine the proportions of specific liver histopathological patterns among medico-legal autopsies with regard to age and sex.

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In this regard, all histopathological patterns found were cross tabulated with pre-determined variables of age groups and sex among medico-legal autopsies

Dependent variable: This comprised of histopathological patterns of hepatic autopsy biopsy specimens from H&E findings.

Independent variable: This was determined by age and sex

2.14. Data collection techniques

The preformed data collection sheet was used. It comprised of demographic information, specimen identification and study results summary as detailed in the attached appendix I.

2.15. Data analysis

The raw data was captured in the SPSS computer software version 20 and the dataset was managed electronically in the computerized software program for analysis.

Variables were summarized as mean, median and percentages. A two-tailed P-value <0.05 was considered significant. SPSS computer software version 20 was used for data cleaning, analysis and drawing tables and graphs.

2.16. Validity and Reliability of the study

2.16.1. Specimen collection**:** autopsy biopsy specimens for the study was obtained after recording gross morphologic features through prospective procedure from all dead bodies died from causes other than liver diseases whose identification were obtained via PF99 as they formally get registered for ordered medical-legal autopsies. Liver tissue specimens were immediately fixed in 10% neutral buffered formalin, and processed as per SOP at MNH for both H&E and special stains protocols

2.16.2. Examination and reporting**:** All processed slides were assessed by the principal investigator and a senior pathologist. Three to four sections for each case were stained by H&E. Afterwards the principle investigator established the histological diagnosis according to morphology. Similar procedure were observed for special stains according to their SOPs, then first got read by principle investigator and were reviewed by senior pathologist as well.

The findings were recorded on the same collection data sheet as demographic and H&E data.

Cases were considered positive for each H&E stained section and where necessary special stain according to adopted SOPs attached in this proposal.

The H&E sections were analyzed to diagnose various lesions according to the criteria as follows:

Steatosis: presence of fatty change; graded as 0+ to 3+ depending on the percentage of cells containing fat. The easiest method follows the acinar architecture of the liver parenchyma and describes the involvement by steatosis in thirds — <33% (or 0–5%, 5–33%), 33–66% and >66% (NAS-NAFLD activity score, a refinement of the Brunt score) — the alternative criteria as mild, moderate or severe, was not used in our study. Grade '0' steatosis were not included in the final results of steatosis due to their occurrence in normal individuals subjected to trivial transient insults, instead we only considered grade 1+ to 3+.

Steatohepatitis: presence of pericellular fibrosis, portal and acinar inflammation, ballooning degeneration, hepatocyte necrosis associated with fatty change.

Chronic venous congestion: presence of sinusoidal dilatation, congestion and presence or absence of centrilobular necrosis.

Chronic hepatitis: interface hepatitis, portal inflammation and spotty necrosis +/- clues to viral hepatitis.

Cirrhosis: presence of regenerative nodules and fibrosis (with or without remnants of steatosis).

Liver cell dysplasia: cellular enlargement, nuclear pleomorphism, increased multinucleation with normal nuclear: cytoplasmic ratio.

Brunt protocol for deciding presence or absence of steatohepatitis was applied as delineated in appendix number six. Considerations for diagnosis and describing granulomatous liver diseases into various specific sub types was adopted from Coash. M et al, as attached in appendices.

2.16.3. Data handling**:** Each case was assigned identifier which were its Postmortem number denoted as PM number, plus the additional identifier of tissue site for the study. This enabled cases not to mix up during series of processes and reporting. However, in order to ensure reliability of the data, laboratory specific standard operating procedures were adopted in entire period of the study.

2.17. Quality Assurance

The principle investigator has been trained in handling and processing histologic specimens, histotechnology methods including special staining techniques. The tissue specimens clearly labeled and processed while adhering to standard operating procedure (SOP).

The principle investigator established histological features and diagnosis basing on H&E and special stained sections. Then one supervising pathologist independently reviewed the findings.

Data was then carefully entered into respective data collection forms as soon as they are generated to avoid mix-ups.

2.18. Ethical considerations and Approval

The proposal was presented to the Department of Anatomical Pathology of the Muhimbili University of Health and Allied Sciences were it was approved.

Ethical clearance was sought from the Research and Publication Committee of the School of Medicine and from the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences.

Administrative permission to conduct the study was obtained from Muhimbili National Hospital as per the hospital management protocols. All study postmortem cases were only recruited in the study when duly filled and officially received PF99 forms (which orders and thus allows the Autopsy must be conducted) have been observed directly by the Investigator. The postmortem cases were identified by serial number and PM numbers recorded on the record sheet without names of deceased (anonymously). Confidentiality was observed and unauthorized people had no access to the data collected. The data sheets were immediately entered into the computer software which then got encrypted with password which only the investigator had the access.

2.19. Study Limitations and delimitation strategies.

Weight of total body for description and comparison of our findings with real deceased weight to establish BMI, was impossible owing to lack of this important facility in our mortuary facility. Never the less to help in describing and later diagnosis of some findings of possible

non communicable disease changes we measured weight of the liver for each body. Other biochemical and serological tests for risk factors of liver diseases were not conducted due to logistical challenges and most components were out of the scope of the study direct objectives since no exact list of specific diseases to be found could be figured out prior to data collection and generation of results. Never the less, non-modifiable parameters of age and sex were analyzed accordingly.

Blood tests for markers of Hepatitis virus (especially hepatitis B, prevalent in our setting) or cancer were not done due to financial constraints and neither Orcein stain nor Victorian blue planned to cover as an alternative for enhancing histological features for hepatitis B virus could not be obtained timely from various venders due to logistical hindrances beyond our capacity especially scarce of reagents for the same as they are largely replaced by IHC which was not our initial target. Here meticulous observation of H&E sections assisted by special stains was done by investigator and two more senior pathologist to capture for all viral hepatopathy and conclusions regarding this entity were limited to suggestive findings.

CHAPTER TRHEE

RESULTS

3.1. General findings:

A total of 608 (forensic and clinical) autopsies were carried from June to December 2018, out of which 604(99.3%) were medical legal. Of these 347cases were not recruited due to various reasonsincluding64being decomposed, 7 had restricted autopsy orders (not to open), 3 cases had clinical liver related disorders and 274were not recruited at all as were received after sample size was attained or due to missed opportunities from investigator availability. Of the remaining 257 autopsy cases, 3 cases were further excluded during examination of slides due to autolysis interfering interpretations. Therefore, 253 autopsies were pulled into the final list of study cases. There were various liver diseases discovered in this study that were not known prior to autopsy examination of the deceased. Liver diseases discovered includes fatty change, steatohepatitis, venous congestion, Pseudo-cysts, granulomatous lesions without specifying individual entities, Cirrhosis, Cholangitis, HCC, and poly-cystic liver disease. There is an alarmingly prevalent steatosis (around 33%) among allegedly healthy population of forensic autopsy cases.

The frequent causes of deaths were traumatic brain injuries, polytrauma, both being secondary to RTA and assaults, other causes included respiratory failure due to various underlying causes, asphyxia due to fatal pressure on the neck or drowning, hemorrhagic/septic shock associated with sharp object injuries or burn events respectively, and poisoning suspects.

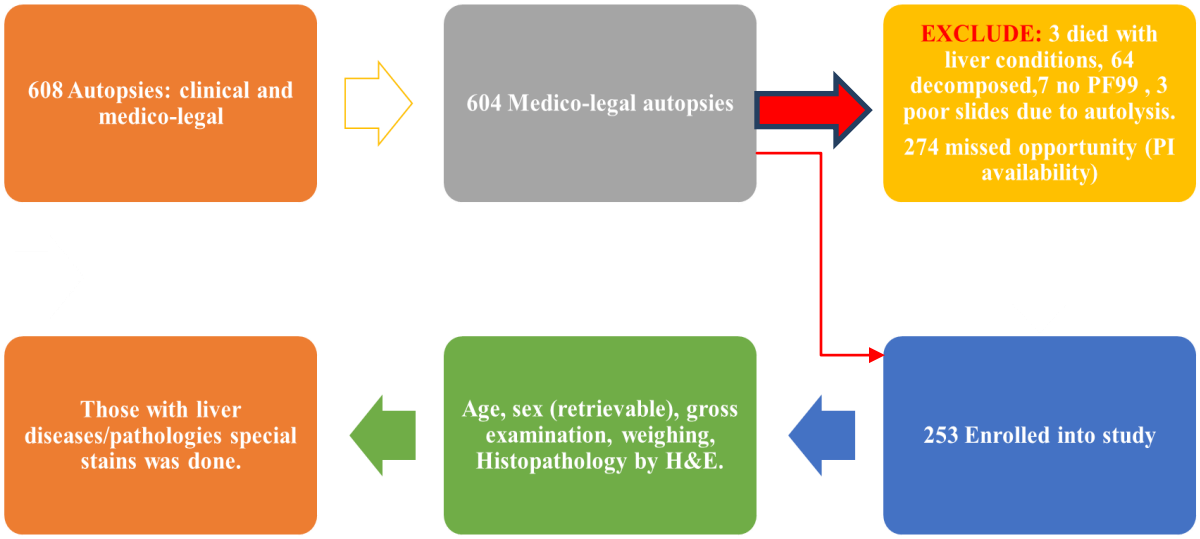
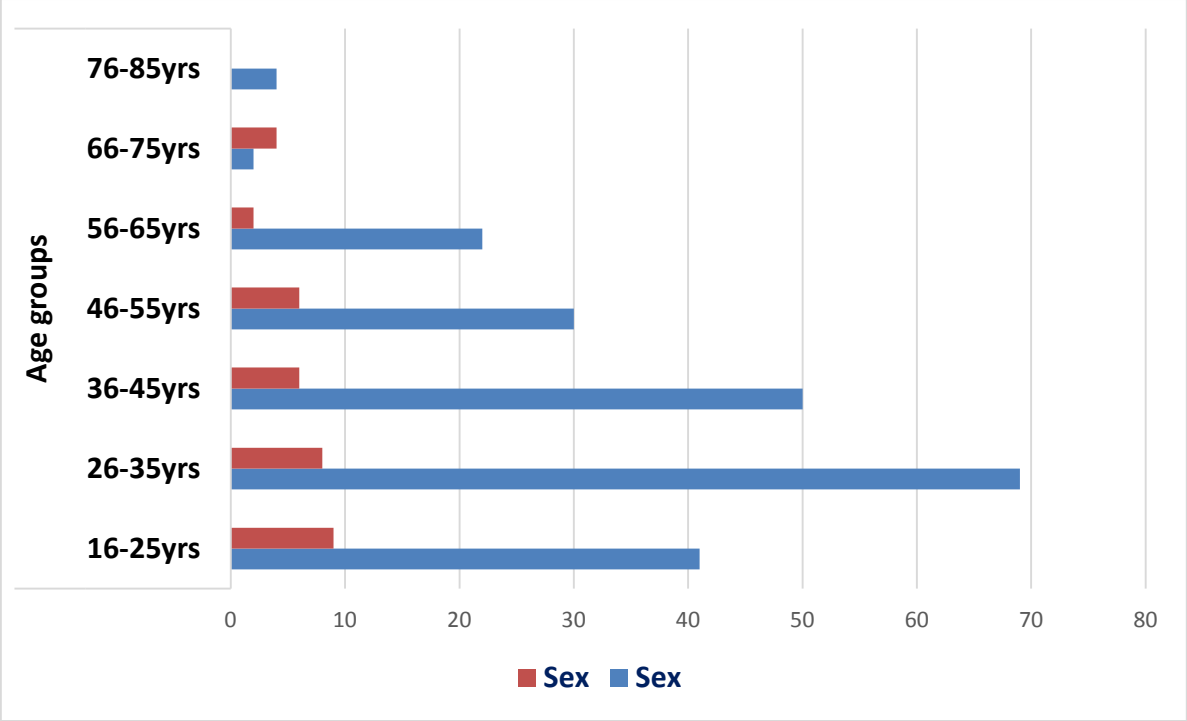


Figure 3: Flow chart of study cases

3.2. Demographic characteristics of study cases

Figure 4: Demographic Characteristics



Male predominance in the study cohort is evident in almost all age groups, except a meagre proportion among 66-75years group (Figure 3).

Table 1: Age and sex distribution

		Sex		Total (%)
		males	females	
Age groups	16-25yrs	41	9	50(19.8)
	26-35yrs	**69**	**8**	**77(30.4)**
	36-45yrs	50	6	56(22.1)
	46-55yrs	30	6	36(14.2)
	56-65yrs	22	2	24(9.5)
	66-75yrs	2	4	6(2.4)
	76-85yrs	4	0	4(1.6)
Total		**218(%)**	**35(%)**	**253(100)**

Among 253 autopsy biopsies recruited and analyzed in our study, Majority were males 218(86%) and the mean apparent age at death was 36 +/- 12.98(SD). Male to female ratio of 6:1 and the age range from 16 to 78 years. The peak age group of the study participants was 26-35yrs 77(30.4%). The mean ages for males and females were 36.74+/-13.23(SD) and 38.23+/-13.38(SD) respectively, and a median of 36years (Table 1).

3.3. Prevalence of liver diseases amongst medico-legal autopsies.

Table 2: Prevalence of liver diseases, N=253

Histopathology	Total cases (%)
Fatty change related lesions	
Steatosis(grade 0 excluded)	**83(32.8)**
Steatohepatitis	37(14.6)
Cirrhosis	6 (2.4)
Granulomatous liver diseases	
Schistosomiasis	8(3.1)
Fungal infections	7(2.7)
Tuberculosis	3 (1.2)
Viral Hepatopathy	7(2.7)
Granulomatous lesions (*NOS)	9 (3.6)
Vascular and other inflammatory lesions	
Liver congestion	9 (3.6)
Cholangitis	3(1.2)
Neoplasms	
HCC	2(0.8)
Cysts	
Pseudo-cysts	10 (4)
Poly-cystic liver disease	1(0.4)
No liver Pathology	68(26.9)
Total	**253(100)**

Note: * NOS = not otherwise specified

Bulk of cases had fatty change 83(32.8%), followed by steatohepatitis 37(14.6%).Combined infective and infestations sum up to 34(13.4%) when suspected viral hepatitis is added to granulomatous lesions. Combined granulomatous lesions 27(10.7%).Of the 27, the majority (18 cases) were specified as hepatic schistosomiasis 8(3.2%), Fungal infections 7(2.7%) and Tuberculosis 3(1.2%). The remaining 9 cases were unspecified granulomatous lesions. Liver cirrhosis accounted for 6(2.4%) whereas 2(0.8%) cases of malignant lesion were of hepatocellular carcinoma.

3.4. Histopathological patterns of liver tissue biopsies among medico-legal autopsy cases

3.4.1 Histopathological patterns with liver weight

Majority of steatosis cases 62.7% had normal liver weight followed by a proportion of 22.9% with increased liver weight. Most cases of steatohepatitis 45.9% had normal liver weight, though followed by potential fraction with decreased liver weight 32.4%.All (100%) of liver cirrhosis cases had livers with decreased weight. Liver weight was decreasing with increase in stage of fibrotic lesion.

Table 3: Histopathological patterns vis-a-vis liver weight

		Steatosis		**steatohepatitis**		**cirrhosis**	
		Yes	No	Yes	No	Yes	No
Liver Weight	Normal	**52(62.7)**	112	17(45.9)	147	0(0)	164
<1400		12(14.5)	23	12(32.4)	23	**6(100)**	29
>1600		19(22.9)	35	8(21.6)	46	0(0)	54
	Total	83(100)	170(100)	37(100)	216(100)	6(100)	247(100)

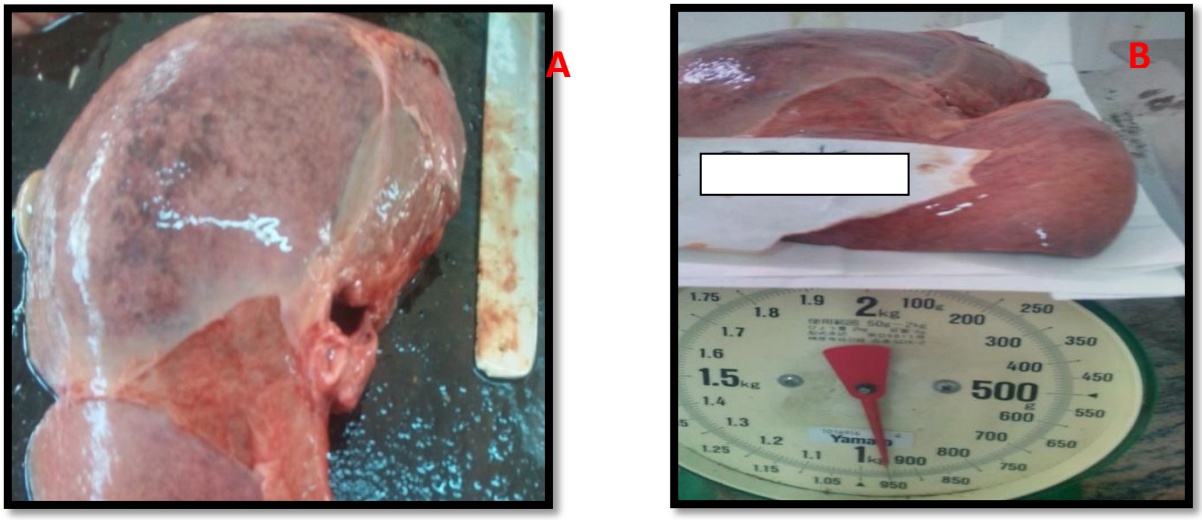


Figure 5: A) Gross: mottled tan-yellow. B) Decreased weight (950gms) of the same case of steatohepatitis.

3.4.2. Histopathological patterns of medical legal autopsies by H&E staining

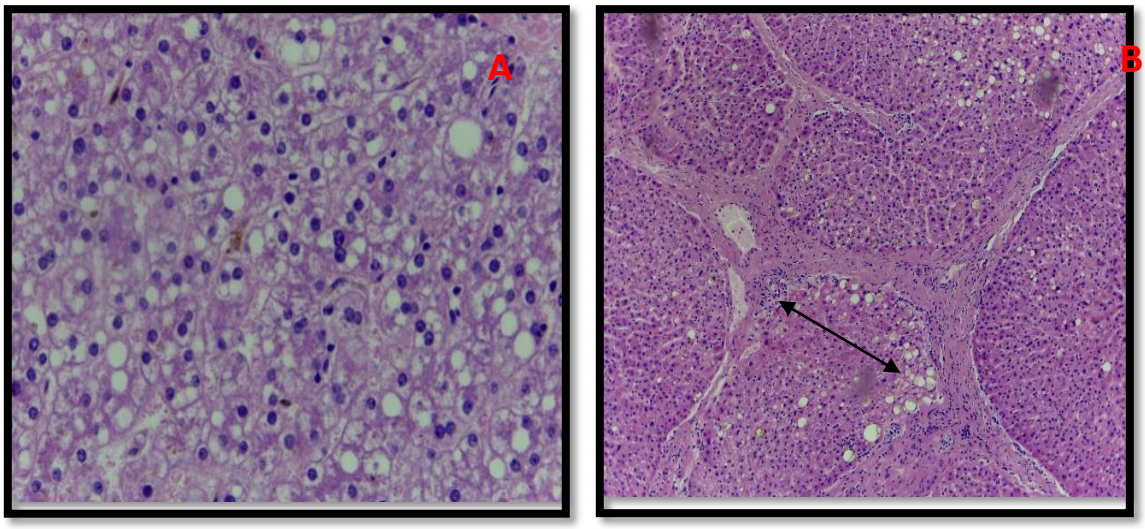


Figure 6: (A) Mixed macro and micro vesicles in a case of Steatosis. (B) Mixed Nodular cirrhosis with focal evidence of remnants of steatohepatitis (arrow ends), H&E X40.

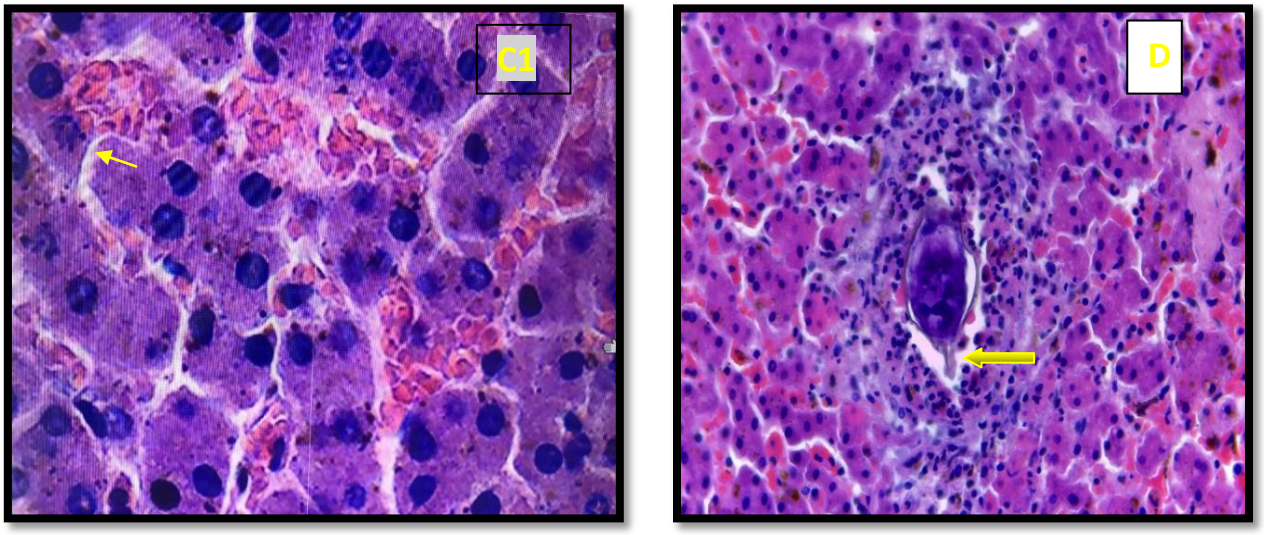


Figure 7: (C1) Hepatic congestion with focal pericellular fibrosis (yellow arrow). (D) Schistosomiasis of liver: Note esinophilic rich granuloma and Schistosoma egg with terminal spine pointing downward in the centre (yellow arrow); H&E, X40

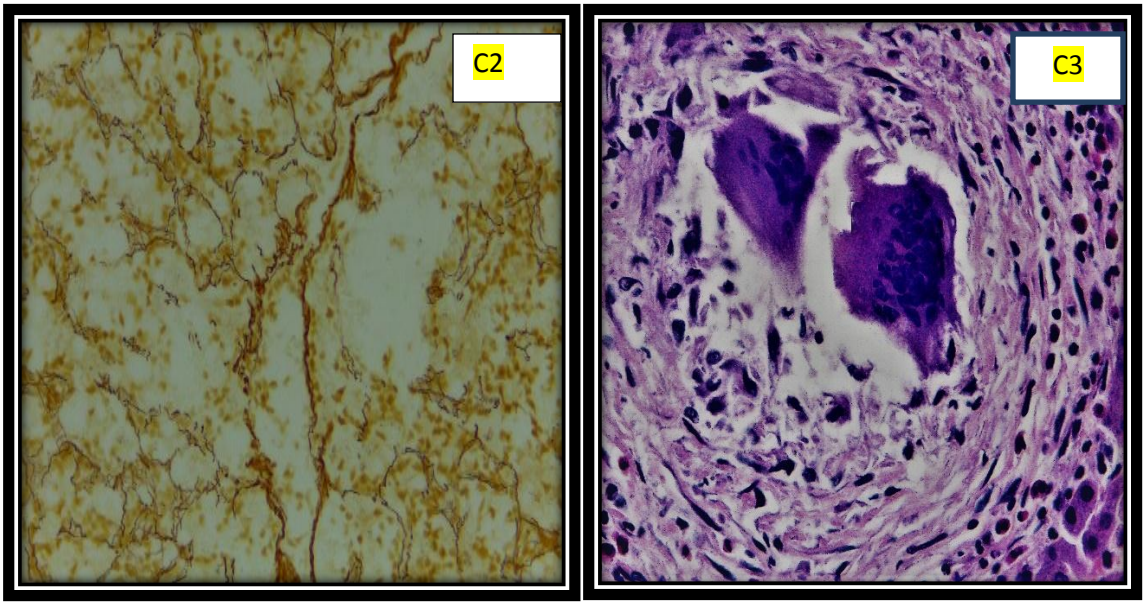


Figure 8: (C2) Reticulin stain enhancing visualization of dilated sinusoids and pericellular fibrosis even in a setting of hepatic necrosis, a case of Steatohepatitis. (C3) Granulomatous Hepatitis in a case of Tuberculosis. H&E, X40.

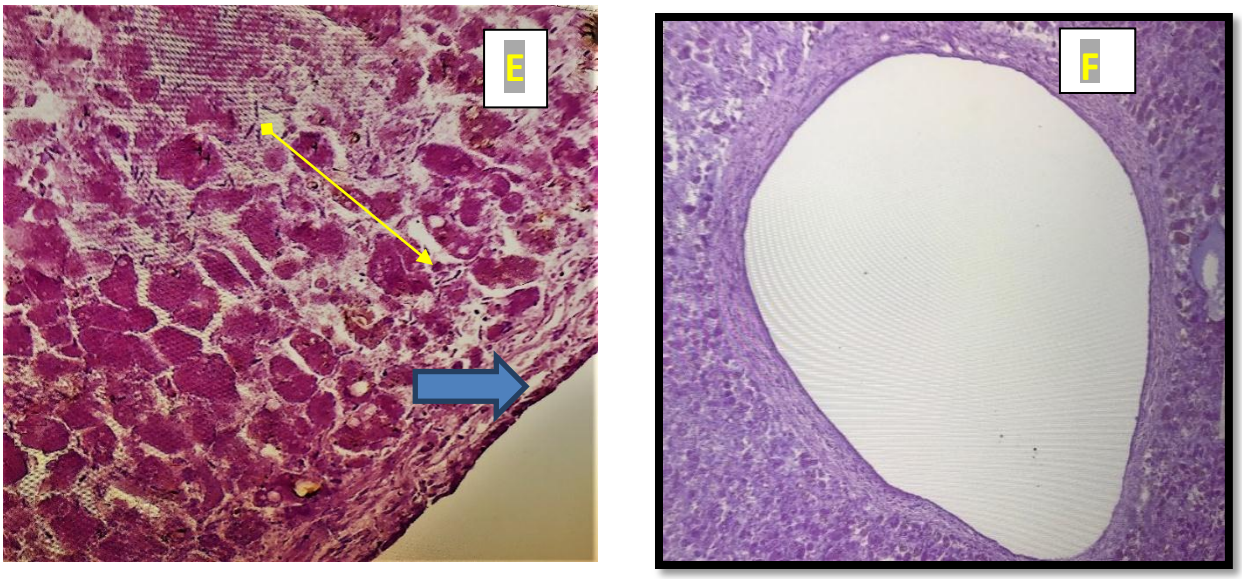


Figure 9: (E) Fungal hyphae (double heads arrow) adjacent to cyst wall (lower right-arrow), (F) Pseudo-cyst, note laminated non epithelial wall, H&E X40.

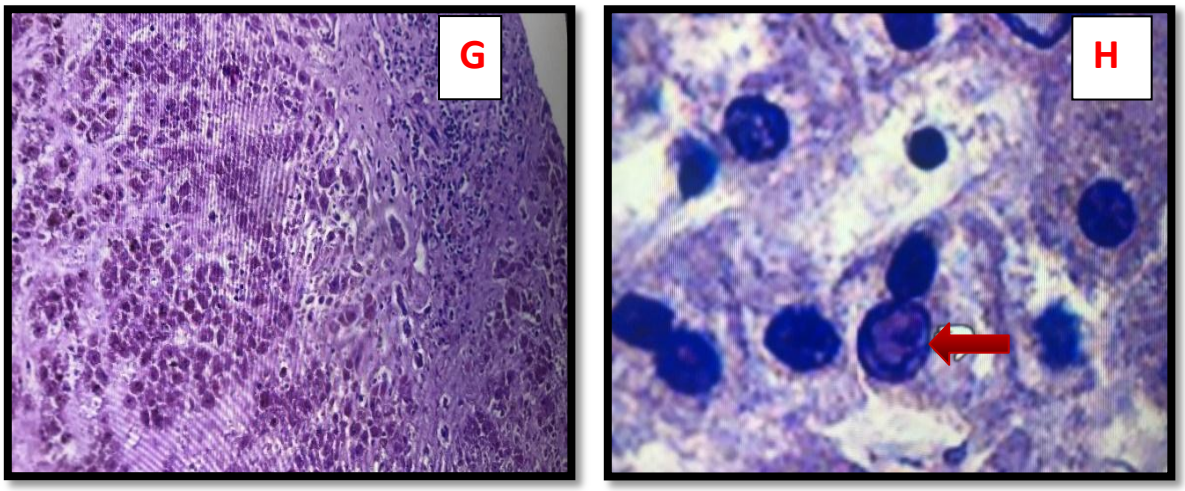


Figure 10: (G) Hepatocellular carcinoma exhibiting trabecular + focal acinar patterns. (H) Powderly intra nuclear inclusion with ground glass hepatocytes suggesting Viral hepatopathy (red arrow), H&E, X40

3.4.3. Grading of fatty change/ steatosis

Majority of steatosis fall under grade I and II (64.4%) (Table 4). Grade '0' fatty change cases were excluded from final histopathological diagnosis of steatosis which considered grades I to III only and constituted 83 (32.8%) cases (Table 2).

Table 4: Grades of steatosis, n=101. (NAS criteria & Brunt et al)

*Sr. No	Grading	Number of cases (%)
1	0	18 (17.8)
2	I	39 (38.6)
3	II	26 (25.7)
4	III	18 (17.8)
Total		**101 (100)**

Note: ***Sr. No**; refers to serial number

3.4.4. Histopathological staining patterns with regard to special histochemical stains.

Special stains were applied to the specimens as directed by H&E findings to enhance diagnosis. PAS conducted for improving visualization of the presence of specific parasitic organisms and hepatocytes glycogen showed positivity in a total of 31 cases in the following distribution; seven cases of fungal infection, three of the 7 cases of suspected viral hepatitis, 9 cases of steatohepatitis and 12 cases with steatosis.

Table 5: Distribution of special stains positivity frequency in various Histopathologies

Histopathology	**Special stains**				
	PAS	**Pearl's**	**Giemsa**	***ZN**	**Reticulin**
Steatosis	12	12	-	-	-
Steatohepatitis	9	14	-	-	7
Liver congestion	-	4	-	-	-
Cirrhosis	-	4	-	-	6
Fungal infection	7	-	7	-	2
Pseudo-cysts	-	-	3	-	1
Suspected viral hepatitis	3	7	-	-	-
Tuberculosis	-	-	-	3	-
Malarial pigments	-	29	29	-	-
Normal histology	-	6	-	-	-
Total	**31**	**76**	**39**	**3**	**16**

Note: *ZN = Ziel-Neelsen stain

Perl's Prusian Blue stain for iron to assess the random prevalence of hepatic iron pigments was conducted in all cases. Distinction between malarial pigment and other iron pigments was based in the histomorphological patterns, such that all cases with reticuloendothelial cells proliferation and sinusoidal dilatation, alongside positive malarial haemozoin pigments both by H&E and Perl's prusian blue stain were considered to be malarial sequel. Basing on H&E stained sections there were 201(79.4%) cases with pigments mimicking both malarial haemozoin and other pigments including iron and hemosiderin pigments. Pearl's Prusian Blue stain showed positivity in 76 cases (30%) in association with various patterns. Of these 29(39.7%) cases were malaria pigment (Table 5) and of 47 remaining cases considered to be probably hemosiderosis were distributed as follows maximum number 14(18.4%) was seen in steatohepatitis, 12 were associated with steatosis and the rest were distributed in suspected viral hepatitis 7 cases, normal histology 6 cases, 4 cases of liver congestion and cirrhosis contributed 4 cases.

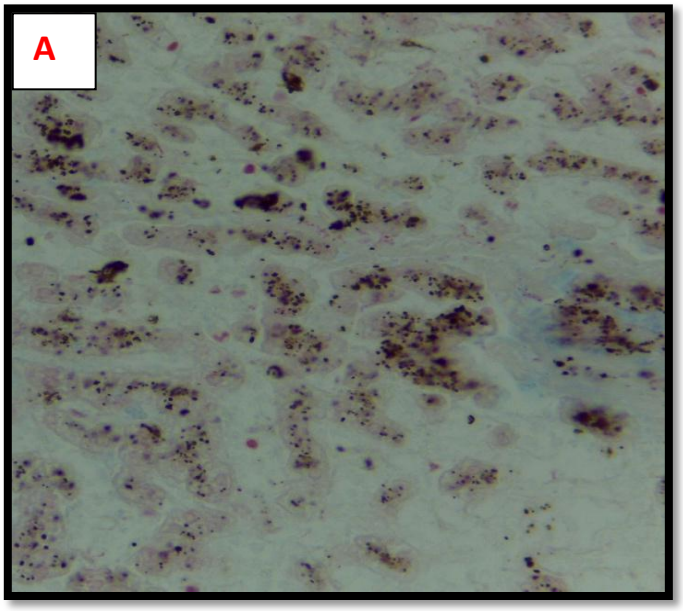


Figure 11: (A) positivity of iron in dilated sinusoids and intra-cytoplasmic both in Von Kupffer cells and hepatocytes, Perl's stain X40.

Reticulin which was applied to enhance visualization of reticulin collapse in liver parenchyma. Sixteen cases were subjected to this special stain, all (100%) were positive, among which the maximum positivity was observed in 7 cases of steatohepatitis, followed by all cases of cirrhosis, others were associated with extensive fungal infection and one case of multiple pseudo-cysts. Giemsa stain was carried to visualize possible bacterial and protozoa related hepatitis and to explore malarial related changes, it was run in 52 cases. Of the 52 cases majority 29 showed features suggestive of malaria sequel predominately reticuloendothelial cells proliferation and sinusoidal dilatation, alongside malarial haemozoin pigments (Figure 9) and these were further subjected to Pearl's Prussian blue stain to illuminate the haemozoin pigment. Giemsa stain showed well enhanced fungal hyphae as well in all fungal infection cases (Figure 12). Ziel-Neelsen stain was done in 27 cases with granulomatous hepatitis. Of these only 3 (11.1%) were positive for Acid Fast Bacilli, all the positive cases were males.

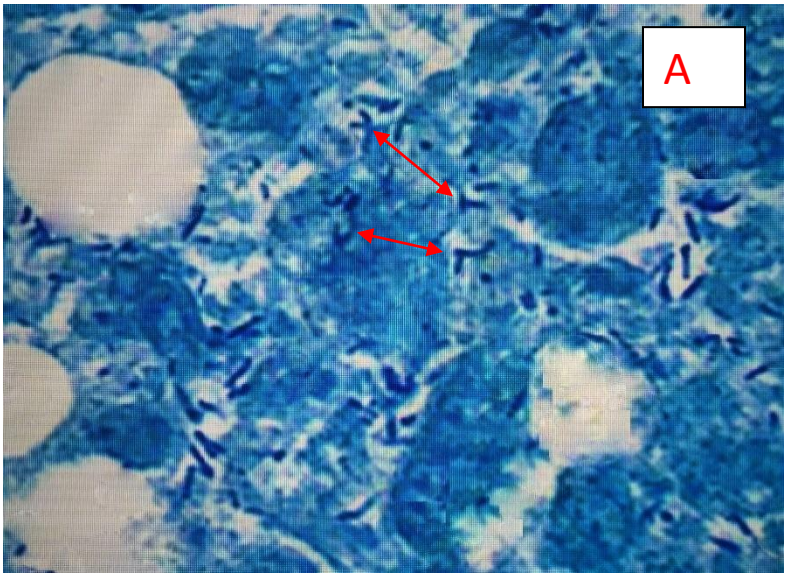
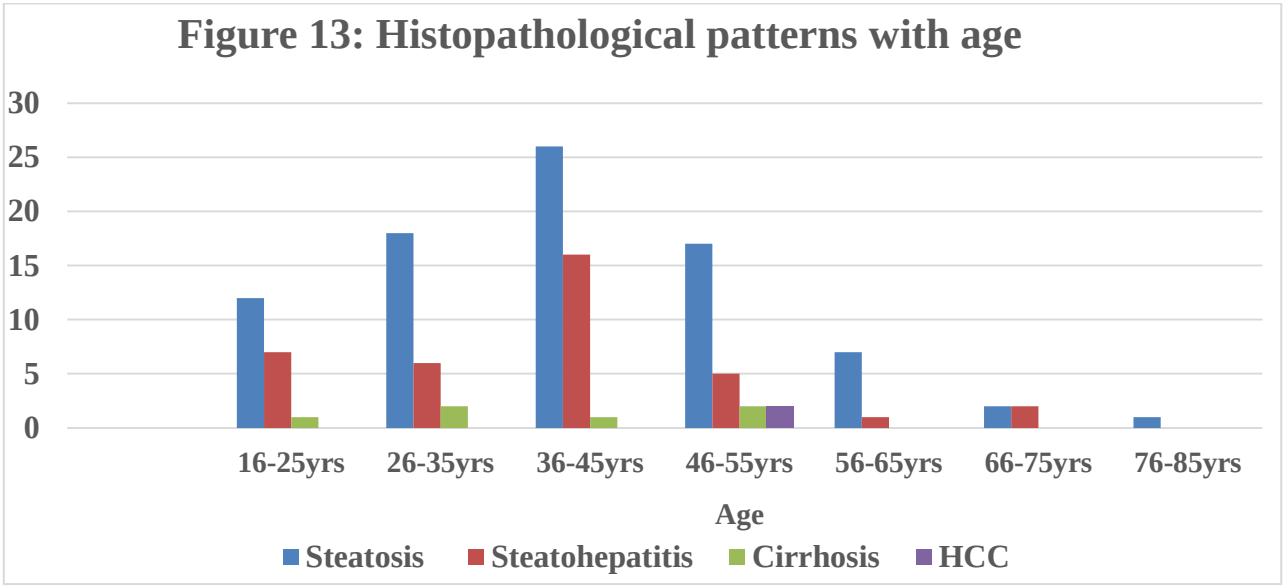


Figure 12: A) Giemsa stain showing branching fungal hyphae in a case of actinomycosis (note angle of some hyphae on red arrows).Associated pseudo cysts are shown. Giemsa x40.

3.5. The proportions of specific liver histopathological patterns among medico-legal autopsies with regard to age and sex.

3.5.1. Specific liver histopathological patterns with regard to age

Of the 32.8% cases with most prevalent liver disease (fatty change), 30 (11.9%) were in the age group 36-45yrs, and steatosis cuts across all age groups. Five (83%) out of six cases of liver cirrhosis were within noteworthy young age groups between 26 and 55 years.



Majority of cases in this study were crowded in the age group 36 - 45years which encountered 56(22.1%) cases across various patterns (see also Table 1). Age-wise distribution of liver diseases was statistically strongly significant (p-value < 0.001)

3.3.1. Specific histopathological patterns with regard to sex

Of the 218 males 184(84%) contracted one or more disease(s), while within 35 females 24(68.5%) were afflicted by liver disease(s).

Table 6: Proportions of liver histopathological patterns by sex

Sr. No	Histopathology	Males	Females	P-value (Fisher's)
1	Fatty change	77	12	0.848
2	Steatohepatitis	35	2	0.127
3	Pseudo-cysts	8	2	0.633
4	Liver congestion	9	0	0.288
5	Granulomatous lesions (NOS)	9	0	0.616
6	Schistosomiasis	6	2	0.306
7	Fungal infections	5	2	0.250
8	Hepatitis	5	2	0.218
9	Cirrhosis	6	0	1.000
10	Tuberculosis	3	0	1.000
11	Cholangitis	2	1	0.166
12	HCC	2	0	1.000
13	Poly-cystic liver disease	1	0	0.340

Majority of males and females had Fatty change (steatosis) accounting for 77(30.4%) and 12(4.7%) respectively in our cohort. Majority of silent liver diseases were clustered among males (p-value >0.05) as in table 6.

CHAPTER FOUR

DISCUSSION

The liver is vulnerable to a wide diversity of metabolic, toxic, Microbial, social and circulatory insults. Disorders of the liver can emanate as primary while in others the hepatic involvement is secondary to alcoholism, extrahepatic infections, and systemic diseases. To the limit of my knowledge, this is the first study on liver diseases among forensic decedents in our setting, as opposed to scarce data conducted in limited size core biopsies from patients (not autopsy) or radio imaging modalities within SSA including Tanzania as well. Thence, this study had the opportunity to elucidate histopathological patterns in larger tissue biopsies. Thus, the results of which have a worthy contribution in explaining the burden of this entity in our setting.

This study was limited to determine Body mass index (BMI), and other risk factors for liver diseases due to logistical challenges like lack of weighing scale for dead bodies, however age and sex associations with various histopathological patterns were described. History of alcohol use, for instance, was not taken from possible next of kin as the majority of them were in the unconducive psycho-social state owing to mourning. The consideration for future follow up for the same history of alcohol was impractical in our case due to time constraints characterizing the dissertation works.

In this study, majority of cases were males 218(86%) with male to female ratio of 6:1, and mean ages in years for males and females were 36.74+/-13.23(SD) and 38.23+/-13.38(SD) respectively. This tendency compares with other studies carried elsewhere which indicates that women make a small contribution in the general cohort of medical-legal cases globally, ranging from 0 to less than 40% (4,5,16,59–61). Young male's predominance was mainly a selection bias in our study due to the tendency of the majority of medico-legal cases to be males in our settings. This can be partly explained by the risky nature of circumstances of death among medical-legal events worldwide. Further to that, meager women proportion appears mostly as victims rather than doers in most circumstances of death. A similar and recent study in India (60) enrolled 409 cases including 349(85%) males and sixty females (15%). On the other hand, a study conducted in India as well (16), found a relatively higher

number (doubling) of female proportion in a cohort of 50 cases, females were 13 (26%), probably due to involvement of clinical autopsy cases in their study (16,60).

The mean apparent age at death in this study was 36 +/- 12.98 (SD) and the majority of the study participants were clustered between 2nd to 4th decades of life accounting for 52%. This trend correlates with many other studies conducted elsewhere. One study in Iran found a mean age of 43.8 ± 19.7 (SD) years (5). Most liver diseases were crowded among males a distribution shown to have occurred by chance (p-value >0.05). This could have probably been influenced by a meager number of females among medical-legal cases seen in this study.

Steatosis is considered to be benign, and remains a hallmark of consequential ASH and NASH. However, the risk of development of cirrhosis in patients with simple fatty liver disease is 0.5% to 1%(62). Furthermore, hepatic steatosis is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage (62). Steatosis is the most prevalent finding in most similar studies and is amenable to complete reversal to normal with the removal of agents initiating liver injury (63). In this study bulk of cases had fatty change 83(32.8%) followed by steatohepatitis 37(14.6%). Of the 83 cases of steatosis/ fatty change majority had grade I and II fatty change (64.4 %), and grade zero were excluded from the final prevalence of this disease entity as they may be associated with normal transient changes post trivial insults and may reverse to normal unnoticed. The prominence of fatty change among incidental liver diseases is a common finding of many previous studies. Studies done in India had shown a similar trend ascribed to alcohol abuse (4, 60-61). Similarly, a common event of alcohol abuse, especially among youths and unmonitored use of over the counter drugs and local medicinal herbs both being common in our geographic region, are known to be associated with advanced stages of liver diseases as seen by previous local studies (64). Liver cirrhosis had 6(2.4%) magnitude in this study. Most likely the known sequential events from steatosis through steatohepatitis to regenerative nodular cirrhosis can explain this trend in our study results. There is a high frequency of fatal liver disorders among younger age groups in our region which have been shown by both the current work and previous loco-regional studies. This could be ascribed to early exposure to relevant risks including indulging in alcohol use among youths in our demographic area both

local and industrial beers, an event which is on its upsurge. Further to that, increased caloric intake and reduced physical activity in recent years have undoubtedly contributed to increased obesity and a parallel increase in the prevalence of the fatty liver disease in the arm of nonalcoholic as well. These events deserve further studies to establish the medical sociological gaps in determining their occurrences to help in planning preventive measures. A significant proportion of steatosis cases (32.8%) had increased liver weight. Contrarily to this observation, all (100%) of liver cirrhosis cases had livers with decreased weight. Generally, Liver weight was decreasing with an increase in the stage of the fibrotic lesion. These findings were expected, and they parallel other previous studies. Alagarsamy J, described a similar trend in India (16) and she went further to show the preponderance of decreased weight of liver among males, but this could be explained partly by the influence of a high number of males (74%) in their study sample, which was comparable to our findings. On the other hand, an increase in the weight of livers among the deceased with steatosis can be perceived as the result of an increase in deposition of fat and its consequential increase in liver parenchyma mass evidenced by hepatomegaly in most instances.

When undetected and not timely cared for, granulomatous lesions can lead to fatal outcomes like portal hypertension and its sequelae like bleeding varicosities. In diagnosing hepatic granulomatous diseases a reasonable initial approach is to search first for the most common causes of granulomatous liver disease in a particular population to avoid unnecessary testing and guide targeted therapy (41). In this study combined granulomatous lesions constituted 10.7%. Of the total 27 cases of granulomatous hepatitis majority of them (66.7%) were assigned specific causes as hepatic schistosomiasis 8(3.2%), Fungal infections 7(2.7%) and Tuberculosis 3(1.2%). The remaining 9 cases were unspecified granulomatous lesions which could account for autoimmune or drugs and chemicals not explored in this study specifically. However this trend was comparable with previous works as seen by Coash M, et al and Fiore M, et al, in their studies of granulomatous and fungal liver diseases correspondingly(41,43).

Infections with Schistosoma mansoni and S japonicum are frequent in the developing world where waterborne parasitic diseases are prevalent. In the current study, hepatic schistosomiasis incidences reached 3.2% with Schistosoma hematobium and mansoni seen at frequencies of 3

(37.5%) and 5(62.5) respectively. This showed similar inclination within these geographic regions, a trend which is opposing to western countries where the disease is not endemic (41). Also, schistosomiasis was found to have a prevalence of 51.5% in one previous local, countrywide study, signifying the endemicity of this disease in our setting (65). Also in the current study fungal infections constituted 7(2.7%) of granulomatous hepatitis. Of these, 4(57.1%) were candidiasis and 3(42.9%) were actinomycosis. Further to that, all candida associated fungal lesions formed ill-defined granulomas which can be explained by the possibility of an immunosuppressive state in some of these deceased. Although our study was limited to the performance of some special stains or immunohistochemistry to confirm evidence for viral hepatitis, highly suggestive features for the same were recorded basing on H&E sections microscopy including ground glass hepatocytes, nuclear vacuolation, and nuclear and cytoplasmic inclusions. Intranuclear inclusions, in this case, were suggestive of cytomegalovirus infection whereas ground glass hepatocytes point to hepatitis B virus infection. Further to that, combined infective lesions in this work may sum up to 13.4% when suspicious viral hepatitis is added to granulomatous lesions, scaling up an infective component in our setting, a trend not common in studies conducted in the western population. In the current work, Tuberculosis constituted 1.2% of all cases. The current magnitude is relatively low as compared to previous local studies that showed pulmonary tuberculosis (at MNH) with a prevalence of 33.8%. Despite both being autopsy studies carried at the same center the previous findings were comparatively higher probably due to the anatomical site in which case the lungs are more affected by tuberculosis as compared to other sites like the liver (65) this being explained by the commonest route of transmission being air droplet infection resulting into pulmonary tuberculosis as an initial disease. However, general reflection studies carried elsewhere especially Northern parts of the world and Arabic countries especially, found granulomatous lesions at lower prevalence ranging from 0 - 1.5 (in autopsy studies). But the same could rise to 2.5 to 15% in clinical studies and even higher among people living with HIV/AIDS (16-75%)(41). This can be due to relative variability in incidences of HIV and HIV/AIDS in these demographic regions, as well as the endemicity of most communicable diseases in our setting (66) vested with limited preventive measures. Generally, with few

exceptions like Tuberculosis and viral related hepatitis, infections and infestations are more frequent and of great public health importance in our region compared to western countries due to their magnitude.

Another important observation in the current study was the finding of ten cases (4%) of variable-sized liver multilocular pseudo-cysts. Strikingly 40% of these showed co-existence with fungal infections in both the surrounding liver parenchyma as well as within the luminal sides of laminated cysts wall which implied a possible causal relationship between fungal infections and hepatic cysts formation. This possibility was highlighted by some previous works (54, 57-58). Fungal species can lead to liver disorders by direct infective process or through aflatoxins generated by some fungus growing on grains stored in a humid environment, and their potential effects may culminate in fatal liver diseases like liver cirrhosis and HCC (64). The consequential effects of secondary fungal infections leading to liver transplant failure is a known fact (62), and it is even fatal in the coexistence of HIV/AIDS. Therefore this should alert health systems aspiring for future liver transplantation services on the existence of invasive fungal infections amongst people in our demographic region.

Occurrence and distribution of diseases in age groups and sex are important epidemiological benchmarks for disease management plans. In this study, it has been shown that liver disease mostly affects individuals between the 3rd and 5th decades of life (p-value 0.016) and that Steatosis cuts across all age groups. This finding parallels earlier studies locally and elsewhere. Also, having seen this reflection in specific disease entities like steatosis, Cirrhosis, HCC, TB and hepatic schistosomiasis especially in loco-regional studies signifies the drift that most fatal liver diseases afflict younger (economically active) generation in our setting (64,65). Five (83%) out of six cases of liver cirrhosis were within noteworthy younger age groups between 26 and 55 years. These can be explained as an outcome probably ascribable to high frequencies of pre-cirrhotic lesions like steatosis observed mainly among youths in this research work, most likely secondary to early exposure to risk factors for liver injury including alcohol abuse, chemical liver injuries by over the counter medical drugs and herbal medicines. Also, obesity and other metabolic disorders upsurge may have a significant contribution to this

growing burden. Extraordinarily, the occurrence of steatosis in a sixteen years old boy may be linked to different causal factors such as malnutrition and hypoxic states including anemia, since other factors plausible in explain the same among young adults and elderly counterparts may not confidently explain the same incidence at this age. A similar inclination of liver cirrhosis about HCC was described earlier in the lake zone of Tanzania (64). In the current study hepatocellular carcinoma was seen in the 5th and early 7th decades accounting for 0.8%. This age distribution of HCC is comparable to most previous studies done elsewhere (67) as well as previous local studies. Jaka et al. found mean age of 45years, but a higher prevalence of 4.6% of all malignancies among patients in Lake Zone of Tanzania, but this was a longer duration (5yrs) study involving specifically a cohort of cancer patients. This occurrence can be thought of partly as a sequel of steatosis culminating into cirrhosis and HCC thereof, ascribed in part to risk factors such as early exposure to risk factors such as abuse of alcohol, ingestion of local medicinal herbs, aflatoxins, and un-prescribed drugs use as well as metabolic disorders from reluctance to physical exercises and improper dietary intake not explored in the current study individually, as well as hepatitis B infection which is known to be more prevalent in the developing world. Of special emphasis is the contribution of earlier incidences of HBV associated HCC in developing countries including Tanzania compared to developed countries as seen by other local and non-local studies. However, this work has been limited by logistical factors in characterizing HBV related hepatopathy by special histochemical stain or immunohistochemistry. A comparatively younger age group being affected by most fatal liver diseases in our findings opposes few previous similar works done elsewhere (5, 17, 32, 59-60). This can be explained by geographical and demographic differences in these study regions. The distribution of liver histopathological patterns by sex was probably by chance (p-value >0.05). This might have been influenced partly by the meager number of females in our cohort of forensic autopsies.

Evaluation of liver tissue for diagnosis purposes is mainly based on a thorough examination of sections stained with Hematoxylin and eosin (H&E). Additional special histochemical stains may be used to highlight or identify features that are not easily seen on an H&E stained

section. One of the important findings highlighted during special stains in this study was the prevalent malarial pigment and high frequency of iron pigments. The question as to whether malaria is a predecessor of hepatitis, and later cirrhosis, is one that has puzzled physicians, including hepatologists since long time ago. Studies have shown that Hepatocyte involvement, which is crucial to the life-cycle of Plasmodium spp., does not produce significant clinical disease, also no evidence of long-term sequel (for example, hepatitis/cirrhosis) exists, apart from residual reticuloendothelial changes and the occasional development of hyperactive malarious splenomegaly (HMS). Therefore specific therapy to counteract hepatic involvement consequent on severe (complicated) Plasmodium spp. infection is not indicated (45-46). In this study features of malaria, liver involvement was evidenced mainly by Haemozoin pigments, sinusoidal dilatation and reticuloendothelial hyperplasia observed in 11.5% of study cases distributed among various liver lesions and histologically normal liver specimens. No hypnozoite was seen. Malaria is known to be prevalent in tropical countries like Tanzania. These findings, therefore, were predictable, but the same was uncommon in most previous autopsy based studies probably due to geographical differences in malaria endemicity being higher in our setting. I suppose it could not be mentioned by some studies in Asia and South Africa with low endemicity most likely due to less attention on the same as it is perceived not a disease entity. This probes for further studies to explore the long term effects of this prevalent disease in its endemic regions. We also found a considerable amount of iron pigments distributed among various diagnoses, importantly in normal liver histology which signifies hemosiderosis (Iron deposition without liver injury) that could be ascribed to the known truth concerning the high prevalence of iron overload in Sub Saharan Africa linked partly to the consumption of iron-rich fermented drinks. In this regard, a genetic predisposition may play a role in the same in this region though no gene has been documented yet (68).

The finding of one case of polycystic liver disease in the current research work was peculiar as this condition is rare. This was uncommon even in the previous studies carried elsewhere. Although the initial diagnosis was made on the liver biopsy at autopsy, the finding of concurrent bilateral renal cysts of variable sizes in a middle-aged adult suggests this to be

polycystic kidney disease. Therefore, most likely liver cysts occurred as a secondary effect of the autosomal dominant kidney disease. This remains to be proven by genomic studies.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

There are fatal and prevalent liver diseases afflicting younger forensic decedents in our setting. There is alarmingly prevalent steatosis (around 33%) among the allegedly healthy population of forensic autopsy cases, this might reflect the picture in the general population in this demographic region, and it was followed by steatohepatitis and combined granulomatous lesions of the liver. We have a knowledge gap in the need for further researches to explain the association of specific liver diseases with various risk factors in our region, spanning from clinical, biochemical to histopathological aspects.

5.2. Recommendations

To the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC)

Since we have unveiled severe and prevalent liver diseases through tissue biopsies on autopsies, modalities for liver biopsy in patients, for evidence of hepatopathy should be scaled up and made widely available in our public health systems.

To the Pathologists and Physicians involved in performing autopsies

Pathologists and/or clinicians performing forensic autopsies should consider liver biopsies for histological examination as routine practice to scale up the diagnosis of clinically un-noticed liver diseases.

To all Scientists / Researchers

More studies to underpin the evidence for viral hepatitis and determinants of steatosis among study cases are crucial to elucidate their cause-specific magnitude alongside their biochemical and molecular profiles.

To the future liver transplantation teams

Lastly but not list, Can the plan for liver transplantation (in our setting) be informed on the existence of fatal liver diseases threatening the fate of transplants or excluding donor fitness as in invasive fungal infections and cirrhosis respectively?

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APPENDICES

Appendix I: Checklist Guide (Data sheet/ data record form)

PART A:

1. Serial no:-……
2. PM no:-……
3. Date:-……
4. **Age groups**
    1. 16-25yrs
    2. 26-35yrs
    3. 36-45yrs
    4. 46-55yrs
    5. 56-65yrs
    6. 66-75yrs
    7. 76-85yrs
5. **Sex** 1. Males, 2. Females

PART B: Gross description of the liver specimen

6. **Gross** changes of the liver specimen YES-1 / NO-2
7. **Weight of the liver** – group 1. Normal ; 1400-1600gms
   Group 2. Decreased; < 1400gm
   Group 3. Increased; > 1600gms
8. **Color& C/S;** (MULTIPLE) **1**. Tan 2**.** Tan yellow 3. Pale yellow **4**. Brownish red **5**.whitish –Brown **6**. Greenish **7**. Nutmeg
9. **C/S 1**-Greasy, 2-smooth, 3-cystic , 4-Nutmeg, 5-nodular, 6- heterogeneous
10. Consistency (MULTIPLE) 1. Soft 2. Firm 3. Hard 4. Cystic 5. Mixed
    Others: mention ……..

PART C: H&E based diagnosis:

11. **Steatosis**, **(multiple)YES -- 1**, **NO – 2**

    Sub-categorization of prominent features;

12. Among - Steatosis YES – 1, define vesicular lesion
    1. Macro-vesicular YES 1, NO -- 2.
    2. Micro-vesicular YES 1, NO -- 2.
    3. MIXED(Micro/macro) YES –1, NO --2
13. Important distinguishing features
    1. Lobular/ portal inflammation (circle appropriately)
    2. Monomorphs / polymorph predominant (circle appropriately)
    3. pericellular /Periportal bridging / perivenular fibrosis (circle appropriately)
    4. Ballooning degeneration YES- 1, NO- 2
    5. Zonal or none zonal fatty change
    6. Alcoholic foamy degeneration YES-1, NO-2
    7. Ground glass hepatocytes YES -- 1, NO -- 2.
    8. Mallory-Denk bodies (MDB): YES -- 1, NO -- 2 (H&E+cytokeratin based)
    9. Mega mitochondria: YES-- 1, NO—2, (cylindrical cytoplasmic esinophilic bodies)
    10. Lipogranuloma YES -1, NO- 2
    11. Fibrin ring reaction YES -1, NO-2
14. Above steatosis features favors
    1. AFL; YES – 1, NO -- 2
    2. NAFL: YES -- 1, NO -- 2

15. **Steatohepatitis,** (MULTIPLE) **YES -- 1, NO -- 2**
    1. ASH; **YES –1, NO – 2**
    2. ASH Cirrhosis **YES – 1, NO -- 2**
    3. NASH: **YES -- 1, NO -- 2**
    4. NASH Cirrhosis **YES -- 1, NO -- 2**

16. Cirrhosis Nodularity: (MULTIPLE) **YES -- 1, NO -- 2**

    1. Macronodular. **YES -- 1, NO -- 2**
    2. Micronodular. **YES -- 1 , NO – 2**
    3. Mixed nodularity. **YES 1, NO 2**

17. chronic venous congestion: **YES -- 1, NO -- 2**

18. Infections/ Infestations

    1. Tuberculosis (ZN stain): **YES -- 1, NO -- 2**
    2. **Malaria Hepatopathy** (Haemozoinpigmentintra hepatocytes and in KC with clearing at lobular centers,REC hyperplasia+/- parasites hypnozoites in RBCs, hepatocytes lipofuschin and fat droplets, megamitochondria): **YES -- 1, NO -- 2**
    3. Non-specific granulomatous disease. YES -- 1, NO – 2
    4. Worm infestations (specify; Schistosomiasis YES -1. NO – 2
    5. Fungal infection YES- 1, NO - 2
    6. Granulomatous Hepatitis, YES - 1, NO- 2 (r/o - ddx granulomatoid reaction)
    7. Suggestive of **viral hepatitisYES -- 1, NO – 2**
    8. Clues favors **CMV / HBV/ HCV, undetermined**VIRUSES (Circle appropriately)
    9. Others, specify

       ……. **YES -- 1, NO -- 2**

19. Liver cell dysplasia: **YES -- 1, NO -- 2**

20. HCC **YES -- 1, NO -- 2**

21. Liver metastasis **YES -- 1, NO -- 2**

22. Metabolic disorders, specify ……**YES --1, NO – 2**

23. Congenital anomaly. Specify (gross&/histological) …… **YES -- 1, NO – 2**

24. Peribiliary pseudo-cysts (PBPC)+/- Peliosis **YES -1, NO -2**

25. PBPC with fungal infection **YES -1, NO—2**

PART D: Special stains results

i. PAS: **YES -- 1, NO – 2**
ii. PAS-D **YES – 1, NO -- 2**
iii. ZN Stain: **YES -- 1, NO -- 2**
iv. Reticulin: **YES -- 1, NO -- 2**
v. Pearls Prussian: **YES -- 1, NO -- 2**
vi. Masson trichrome: **YES -- 1, NO – 2**
vii. Giemsa YES -1 / NO -2

26. IHC: CK for MDB; **YES -- 1, NO – 2**
27. Others disease conditions, specify ……**YES 1, NO 2**

Appendix II: Standard operating procedure (SOP) guidelines for H&E stain

1.0 Purpose	To provide instructions during hematoxylin and eosin staining.
2.0 Scope	This procedure is applicable during manual Hematoxylin and eosin staining at Muhimbili National Hospital, Central Pathology Laboratory.
3.0 Responsible staff	The head of Unit Histopathology is responsible for ensuring the effective implementation and maintenance of this procedure.
4.0 Principle	Tissue structures contain groups of cells that are made up of the nucleus and cytoplasm. The nuclei of the tissues which are acidic in nature (due to their nucleic acid content i.e. DNA and RNA) have the affinity for basic dyes. Haematin is the oxidation product of Hematoxylin. When used in conjunction with a mordant (e.g. Potassium alum which is included in Hematoxylin solution) it will provide a stable link called lake which binds to the acid phosphate groups of DNA and RNA and stain the nuclei into blue colour. The cytoplasm on the other end is basic in nature and will have an affinity for the acidic dyes. Eosin which is acidic in nature is the most suitable stain to combine with alum- Hematoxylin for demonstration of cytoplasm architecture by staining it red/shades of pink. It is vital to use the correct concentration of reagents such as 1% HCL acid in 70% ethyl alcohol for differentiating the stains to avoid undesired staining results.
5.0 Reagents and Supplies	Xylene Harris Hematoxylin 1%Acid in 70%ethyl Alcohol Scott's Tap Water Substitute 1% aqueous Eosin Staining Solution Absolute ethyl alcohol DPX. (Mountant). Containers for Reagents and Solutions Gloves, frosted slides and Laboratory coat.

6.0 Reagent preparation	Refer to reagents preparation sop HISTO no 29
7.0 Equipment	Light microscope Timer
8.0 Sample and Container Types	Histological tissue sections
9.0 Special Safety Precautions	All specimens should be considered as potentially infectious. Xylene is carcinogenic; always wear gloves, mask and goggles when handling it.
10.0 Quality control	Positive control - Well processed tissue which should be cut and stained each week and every time when the reagents are changed. Negative control - poor processed tissue which should be cut and stained each week and every time when the reagents are changed.
11.0 Detailed procedures	Dewax sections and bring down to water. Stain in Harris hematoxylin for 10 minutes. Rinse in running tape water. Differentiate in 1% acid alcohol for 5-10 seconds. Rinse in tap water Blue the sections in tap water for 10 minutes. Counter stain in 1% aqueous eosin for 3 minutes. Rinse in tap water. Dehydrate the sections through ascending grades of alcohols. Clear in two changes of xylene (3 minutes in each) Mount with DPX or mounting machine.
13.0 Interferences and Limitations	Poor preparation of reagents. Poor storage of stains. Poor quality of tissue sections
14.0 Results Interpretation	Nuclei - Will stain blue color Cytoplasm and intercellular substances - shades of pink and red Cells with much RNA or acid mucopolysaccharide - Purplish

Appendix III: SOP for staining of iron - Prussian blue reaction - Mallory's method

PURPOSE: To demonstrate ferric iron in tissue sections.

Small amounts of iron are found normally in spleen and bone marrow. Excessive amounts are present in hemochromatosis, with deposits found in the liver and pancreas, hemosiderosis, with deposits in the liver, spleen, and lymph nodes.

PRINCIPLE: The reaction occurs with the treatment of sections in acid solutions of ferrocyanides. Any ferric ion (+3) in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called 'Prussian blue" or ferric ferrocyanide.

CONTROL: A known positive control tissue.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections 4µ.

EQUIPMENT: Microwave oven, acid-cleaned glassware, non-metallic forceps. REAGENTS: 5% Potassium Ferrocyanide: Potassium ferrocyanide 25.0 gm Distilled water 500.0 ml Mix well, pour into an acid-cleaned brown bottle. Stable for 6 months.

CAUTION: Low toxicity if not heated. Nuclear-fast Red: See Retic 5% Hydrochloric Acid: Hydrochloric acid, conc. 25.0 ml Distilled water 475.0 ml Mix well, pour into brown bottle, stable for 6 months. CAUTION: Corrosive, avoid contact and inhalation. Working Solution: 5% potassium Ferrocyanide 25.0 ml 5% hydrochloric acid 25.0 ml Make fresh, discard after use. CAUTION: Avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat.

Avoid contact and inhalation.

Potassium ferrocyanide; Low toxicity as long as it is not heated, it will release cyanide gas. Hydrochloric acid; target organ effects on reproductive system and fetal tissue.

Irritant to skin eyes and respiratory system.

PROCEDURE

1. Deparaffinize and hydrate to distilled water *Working solution, * microwave, 30 seconds.
2. Allow slides to stand in solution for 5 minutes, in the fume hood.
1. Rinse in distilled water.
2. Nuclear-fast red, 5 minutes.
3. Wash in tap water.
4. Dehydrate, clear, and coverslip.
5. *Conventional method: room temperature for 30 minutes.
6. RESULTS:

Iron (hemosiderin) blue, Nucleired, Background pink and RBCs pale yellow.

Appendix IV: SOP for Gordon and Sweets Reticulin method

SOLUTIONS

1 Silver Solution

To 5cm3 of 10% silver nitrate, add concentrated ammonia drop by drop until the precipitate has just dissolved. Add 5cm3 of 3% sodium hydroxide and redissolve the precipitate formed with a few drops of ammonia. Dilute to 50cm3 with distilled water.

2. 1% Acidified Potassium Permanganate

3. 1% Oxalic Acid

4. 2.5% Iron Alum

5. 10% Aqueous Formalin

6. 0.5% Cold Chloride

7. 5% Sodium Thiosulphate ("HYPO")

8. 1% Eosin

METHOD

1. Sections to water.

2. Treat slides with 1% acidified potassium permanganate for 2 minutes.

3. Rinse in distilled water.

4. Bleach in 1% oxalic acid solution.

5. Rinse in distilled water.

6. Treat slides with 2.5% iron alum for 10 minutes.

7. Wash well in several changes of distilled water.

8. Treat slides with silver solution (kept at 4イ) for 20 seconds.

9. Wash well in several changes of distilled water.

10. Reduce in 10% aqueous formalin solution till the sections turn black.

11. Rinse in tap water.

12. Tone in 0.5% gold chloride for 1 minute if desired.

13. Rinse in tap water.

14. Treat slides with 5% sodium thiosulphate for 5 minutes.

15. Rinse in tap water.

16. Counterstain in eosin for 1 minute.

17. Dehydrate, clear

Mount sections in DPX

RESULTS:

--Reticulin fibres - black

--Nuclei - black or unstained.

--Other elements according to the counterstain.

Appendix V: criteria for diagnosis and describing granulomatous liver diseases adopted from Coash.M et al, Granulomatous liver diseases: A review

Granuloma etiologies	Granuloma characteristics
Autoimmune	
Sarcoid	Noncaseating epithelioid granulomas
Primary biliary cirrhosis	Noncaseating granulomas near portal triads
Infectious	
Mycobacterium tuberculosis	AFB inside epithelioid granulomas and giant cells often with ring of lymphocytes and histiocytes
M avium intracellulare	Aggregates of foamy macrophages in parenchyma and portal triads with +AFB stain
M leprae	Foamy histiocytes in portal tracts and lobules with multiple AFB found
Brucella	Noncaseating granulomas
Rickettsia	Fibrin ring surrounding vesicle of fat
Francisella	Suppurative microabscesses with surrounding macrophages
Listeria	Microabscesses with small granulomas
Bartonella henselae	Stellate abscesses with three distinct zones
Tropheryma whipplei	Epithelioid granulomas
Histoplasma	Macrophages and lymphocytes with histoplasma and epithelioid cells in center
Schistosoma	Eosinophils with fibrosis and collagen deposition in peri-portal and peri-sinusoidal areas often with egg at the center
Leishmania	Fibrin ring or epithelioid granulomas
Hepatitis C	Epithelioid granulomas
Drugs and Chemicals	Granulomas with eosinophils
Malignancy	Non-necrotic granulomas

AFB = Acid-fast bacilli.

Appendix VI a: staging of fibrosis

Stage	Histological findings
0	No fibrosis
1a	Zone 3, perisinusoidal fibrosis, special staining (i.e. EvG) required
1b	Zone 3, perisinusoidal fibrosis, can be detected with H&E
1c	Only periportal/portal fibrosis
2	Zone 3, plus portal/periportal fibrosis
3	As above, but with bridging fibrosis
4	Cirrhosis

Table 1. Degree of fibrosis (staging)

Appendix VI b: grading steatosis

Steatosis, inflammatory changes and hepatocytic injury can be semiquantified as a 'Brunt Score' (Brunt et al., 1999) (Table 2) or 'NAS' (NAFLD activity score; Table 3), providing the basis on which to decide whether or not steatohepatitis is present.

Activity	Steatosis	Ballooning	Inflammation
Mild: grade 1	1 – 2 (up to 66%)	Minimal	Lobular: 1 – 2 Portal: none to mild
Moderate: grade 2	2 – 3 (> 33%, occasionally > 66%)	Clear	Lobular: 2 Portal: mild to moderate
Severe: grade 3	3 (≥ 66%)	Marked	Lobular: 3 Portal: mild to moderate

Table 2. NASH activity grading. Steatosis grade 1: ≤ 33%; grade 2: > 33%, < 66%; grade 3: ≥ 66%

NAS	Steatosis (% fat deposition in hepatocytes)	Ballooning hepatocytes	Lobular inflammation
0	< 5% (0)	None (0)	None (0)
3	5 – 33% (1)	Few (1)	1– 2 foci per 200x field (1)
6	34 – 66% (2)	Many (2)	2 – 4 foci per 200x field (2)
8	> 66% (3)	Many (2)	> 4 foci per 200x field (3)

Table 3. NAFLD activity score (grading): The numbers in parentheses give the NAS for each histological criterion

Appendix VI c, evaluation and scoring by semi quantification for grading fat liver from Brunt et al protocol

Evaluation and semiquantitative analysis for grading (Brunt et al., 1999):

Grade of fatty degeneration:

< 5%	= grade 0
5 – 33%	= grade 1
34 – 66%	= grade 2
More than 66%	= grade 3

Grade of lobular inflammation:

Absent	= grade 0
Up to 2 foci per field of view (200× magnification)	= grade 1
2 to 4 foci per field of view	= grade 2
More than 4 foci per field of view	= grade 3

Lipogranulomas are included in the category of inflammation

Ballooning:

Absent	= grade 0
Few ballooned hepatocytes	= grade 1
Many ballooned hepatocytes	= grade 2

This scoring system is readily reproducible and can provide the basis for deciding whether steatohepatitis should be diagnosed or not:

0 – 2	definitely no steatohepatitis
3 – 4	questionable
5 or more	definite steatohepatitis

The scoring can also be applied to paediatric cases (Brunt EM, 2007; Schwimmer et al., 2005).

Summary definitions for decisions:

Not FLD (<5% steatosis, by definition); **NAFL**, not Steatohepatitis (SH) (>5% steatosis, with or without lobular and portal inflammation); **Borderline** steatohepatitis, zone 3 or Borderline steatohepatitis, zone 1 (most, but not all criteria for SH present, with accentuation of steatosis or injury in zone 3 or zone 1, respectively); and **Definite** steatohepatitis (all criteria present, including steatosis, hepatocellular ballooning, and lobular inflammation). Any of these diagnostic categories, including Not FLD, may have no fibrosis or any amount of fibrosis up to cirrhosis. Specifically, stage 1 is zone 3 (perivenular), perisinusoidal, or periportal fibrosis; stage 2 is both zone 3 and periportal fibrosis; stage 3 is bridging fibrosis with nodularity; and stage 4 is cirrhosis.