

Prevalence and associations of liver diseases among Sri Lankan adults: An autopsy study

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Abstract

Background:

Most chronic liver diseases are subclinical until advanced, delaying their detection and management. Population screening should be guided by prevalence, which determines cost-effectiveness. Autopsy studies are a valuable source to estimate population prevalence and associated factors, particularly for conditions that need invasive screening methods. No large-scale post-mortem studies on liver diseases have been reported from Sri Lanka previously.

Aims to determine the prevalence and associated factors of liver diseases in an autopsy series of Sri Lankan adults.

Methods:

A cross-sectional autopsy study was performed on a series of post-mortems performed in the Institute of Forensic Medicine and Toxicology, Colombo between 2014 and 2016. Liver specimens were examined macroscopically and microscopically with Haematoxylin and Eosin staining under the light microscope. Clinical and demographic data were collected from the next-of-kin and/or by review of medical records whenever available.

Results:

Of 441 autopsies performed (men 77.8%, age at death 52.5 ± 17.2 years), 431 specimens were examined macroscopically. Microscopic findings were available from 382 liver specimens. Fatty liver (56.8%), cirrhosis (7.1%) and granulomatous hepatitis (2.1%) were the main histological findings. Prevalence was not different between men and women. Only one specimen showed a neoplasm, which was an adenoma. Diabetes and alcohol use independently predicted the severity of fatty liver. Age, sex, diabetes and alcohol did not predict the presence of fatty liver or cirrhosis.

Conclusions:

Fatty liver and cirrhosis are common in this post-mortem series of urban-dwelling Sri Lankan adults. Diabetes and alcohol use independently predicted more severe fatty liver.

Keywords: Fatty liver, Cirrhosis, Steatosis, Steatohepatitis, Hepatoma, Post-mortem study, Sri Lanka

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Background

Chronic liver diseases are common among adults. Most such diseases remain clinically occult until the disease is advanced or has progressed to cirrhosis. The global burden of liver diseases has increased over time. For example, cirrhosis contributed to 1.9% of deaths in 1990. By 2017, it had increased to 2.4%. [1]. The Asia-Pacific region largely contributes to the global burden of liver diseases accounting for 4.6% of liver-related deaths in 2015, compared with 2.7% in the USA and 2.1% in Europe [2]. Increased alcohol consumption, ageing population and rising burden of obesity may have contributed to this rise.

With the rising prevalence of metabolic syndrome, its close association, metabolic dysfunction associated fatty liver disease is also becoming increasingly prevalent. Its impact may only become apparent in the future with an exponential rise in cirrhosis and liver malignancies, hence the need to institute preventive measures. However, the cost-effectiveness of screening programmes depends on the prevalence of the target disease. Post-mortem studies provide crucial information on the prevalence of clinically occult diseases. Several autopsy studies have been reported from other parts of the world [3-14]. However, to the best of our knowledge, no large-scale autopsy study has been performed in Sri Lanka to examine liver diseases. Therefore, we aimed to determine the prevalence of liver diseases and their associated factors in an autopsy series of Sri Lankan adults.

Methods

A cross-sectional descriptive study was conducted on consecutive post-mortem examinations performed at the Institute of Forensic Medicine and Toxicology, Colombo Sri Lanka from December 2014 to December 2016. Details of the study methods were reported previously [15]. In summary, liver specimens and endocrine tissues were sampled from consecutive post-mortem studies performed at the above institution, after obtaining written informed consent from the next of kin. Visibly traumatized organs, externally or histologically apparent putrefaction of bodies or tissues and the presence of medico-legal reasons that preclude organ retrieval and/or storage were the key exclusion criteria. Ethical clearance was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo. Sociodemographic and clinical data were collected using a structured interviewer-administered

questionnaire. Answers were sought from the next of kin and when available by reviewing past medical records. Abnormalities in the endocrine glands were reported previously [15].

Histological evaluation

Liver specimens were retrieved and preserved in 10% formalin. The liver was sliced into 5 mm slices and examined macroscopically for abnormalities. All macroscopically abnormal regions were examined microscopically. Two sections from the macroscopically normal liver tissue, from the right and left liver lobes were sampled for routine histopathological evaluation. Microscopic evaluation was made by examination of 3-5µm thick sections of liver tissue under light microscopy with Haematoxylin and Eosin stain. This was done by two independent specialist histopathologists. Standard histology criteria were used for diagnoses. The severity of steatosis was categorized semi-quantitatively as mild, moderate and severe. Data analysis was performed using SPSS version 20.0. Multinomial logistic regression was used to determine associated factors. Missing data were excluded pairwise from the analysis. The P-value of 0.05 or less was considered significant.

Results

Sociodemographic characteristics

The series includes 441 autopsy studies. The majority were males (77.8%). Thirty-five per cent of the deaths were related to accidents or trauma. Liver specimens were examined in 431 (10 were excluded due to gross traumatization or putrefaction). Macroscopic and microscopic data of the liver were available from 431 and 382 cadavers respectively. The demographic and clinical characteristics of the study population are summarized in table 1.

Macroscopic findings

Fatty liver, cirrhosis, nutmeg appearance and focal lesions were observed in 54 (12.5%), 19 (4.4%), 12 (2.8%) and 4 (0.9%) of the examined specimens (n = 431). The mean weight of the liver was 1302.7 (± 410.9) grams.

Histological findings

Among 382 liver specimens with microscopic examination findings, fatty liver was the commonest histological abnormality, found in 217 (57.8%) (see table 1). Cirrhosis, centrilobular necrosis, focal

fibrosis and granulomatous inflammation were less common, found in 7.2%, 4.3%, 2.9% and 2.1% respectively. Of the 12 specimens with nutmeg appearance in macroscopy, microscopic examination showed fatty liver in six and centrilobular necrosis in six. Only one specimen showed a neoplastic lesion

(adenoma, 5 x 6 x 8 mm). One of the four focal lesions observed macroscopically showed granulomatous changes on microscopy. The other three only showed steatosis. Among autopsies with microscopically confirmed cirrhosis, only 7.1 % were diagnosed before death.

Table 1 : Demographic characteristics of study participants included in the examination of liver tissue (N = 441)

Parameter	Number
Total number of autopsies	441
Age at death (years), mean (SD)	52.5 (17.2)
Males, n (%)	343 (77.8)
Co-morbidities*	
Diabetes, n (%)	42/220 (19.1)
Hypertension, n (%)	80/349 (22.9)
Ischemic heart disease, n (%)	48/350 (13.7)
Alcohol, n (%)*	116/218 (53.2)

* Data expressed as a proportion and percentage out of the available data

Table 2 : Prevalence of steatosis, cirrhosis and other microscopic abnormalities* (n = 382)

Abnormality	Males (n = 302)	Females (n = 80)	Total population
Steatosis, n (%)	172 (60.0)	45 (56.2)	217 (56.8)
Mild	110 (36.4)	31 (38.8)	141 (36.9)
Moderate	42 (13.9)	12 (15.0)	54 (14.1)
Severe	20 (6.6)	2 (2.5)	22 (5.8)
Cirrhosis, n (%)	22 (7.3)	5 (6.3)	27 (7.1)
Centrilobular necrosis, n (%)	8 (2.6)	8 (10.0)	16 (4.2)
Focal fibrosis, n (%)	10 (3.3)	1 (1.3)	11 (2.9)
Granulomatous inflammation, n (%)	4 (1.3)	4 (5.0)	8 (2.1)
Neoplasms, n (%)	0	1 (1.3)	1 (0.3)

* Data shown are the number and percentages (in columns)

Factors associated with liver abnormalities

Among 382 autopsies with liver histology data available, 115 had consumed alcohol and 41 had diabetes. Prevalence of fatty liver (of any severity), severe fatty liver and cirrhosis among individuals who had consumed alcohol were 57.4% (66/115), 7.8% (9/115) and 11.3% (13/115) respectively. Among people who had diabetes, prevalence rates were 63.4% (26/41), 0 (0/41) and 14.6% (6/41) respectively. Among individuals who had not

consumed alcohol, 62.4% (54/85) had fatty liver and 3.5% (3/85) had cirrhosis. The prevalence of cirrhosis was significantly higher among those who had diabetes and consumed alcohol compared to those with one of these two risk factors (see table 3). Multinomial logistic regression analyses with age, sex, diabetes status and alcohol consumption failed to predict the presence of fatty liver (Likelihood ratio test Chi-Square = 2.32, p = 0.68), severe fatty liver (Likelihood ratio test Chi-Square = 10.3, p = 0.11) or cirrhosis (Likelihood ratio test Chi-Square = 10.1,

p = 0.12). None of the above factors was independently associated with increased risk of fatty liver, severe fatty liver or cirrhosis. However, a multi-nomial regression model inclusive of the same factors was predictive of the severity of fatty liver (Likelihood Ratio Test Chi-Square 23.04, p = 0.027). Alcohol consumption (Chi-Square 7.55,

df = 3, p = 0.05) and diabetes (Chi-Square 12.61, df = 3, p = 0.006) independently predicted worsening severity of fatty liver.

The only hepatic adenoma was found in a female who died at 46 years. Other clinical data of this individual were not available.

Table 3 Prevalence of fatty liver and cirrhosis in selected sub-populations according to diabetes status and consumption of alcohol (n = 192)*

			Diabetes	
			Yes	No
Alcohol consumption	Yes	Fatty liver of any severity	12/23 (52.2)	49/84 (58.3)
		Severe fatty liver	0	9/84 (10.7)
		Cirrhosis	5/23 (21.7)	8/84 (9.5)
	No	Fatty liver of any severity	14/14 (100.0)	39/71 (54.9)
		Severe fatty liver	0	4/71 (5.6)
		Cirrhosis	1/14 (7.1)	2/71 (2.8)

* Autopsies of individuals in whom histological diagnosis and clinical data on diabetes status and alcohol consumption were available.

Numbers are indicated as a proportion and percentage

Discussion

This is the first large autopsy series to examine the liver diseases among adults in Sri Lanka, and among the largest cohorts reported from around the world, to the best of our knowledge. High prevalence of fatty liver (56.8%), severe fatty liver (5.8%) and cirrhosis (7.1%) were observed in the studied population. Age, sex, presence of diabetes or alcohol consumption did not predict the presence of fatty liver or cirrhosis. However, diabetes and alcohol consumption were independent risk factors for worsening severity of fatty liver. Individuals with diabetes who consumed alcohol had the highest prevalence of cirrhosis (21.7%).

Previous population-based studies from Sri Lanka reported the prevalence of non-alcoholic fatty liver disease to be 32.6% among urban-dwelling adults [16] and 18.0% among adults from a rural community [17]. The rate was 62.6% among adults with diabetes [18]. The prevalence of cirrhosis and liver tumours in Sri Lanka is not known. However, alcohol was the aetiology of cirrhosis among 54.7%

of adults with cirrhosis in a tertiary hepatology clinic [19].

Most prevalence studies on liver disease are based on non-invasive clinical assessment, biochemical investigations, abdominal imaging. With advances in imaging methods, liver biopsy is seldom performed for clinical and research indications in today's practice. Moreover, histologic features including hepatocyte ballooning of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) may not be uniformly distributed in the liver [20], resulting in sampling errors of liver biopsies. Therefore, autopsy studies are useful to determine the prevalence of liver diseases. However, post-mortem studies on liver diseases are limited. Table 4 summarizes the results of several such studies. Our findings indicate a higher prevalence of fatty liver and cirrhosis compared to most previous studies. Our series is the most recent of all reported studies. Therefore, the high prevalence may represent the globally rising prevalence of the fatty liver disease. However, sampling bias and recall bias may also have contributed to this observation.

Table 4. Selected autopsy studies reporting liver diseases

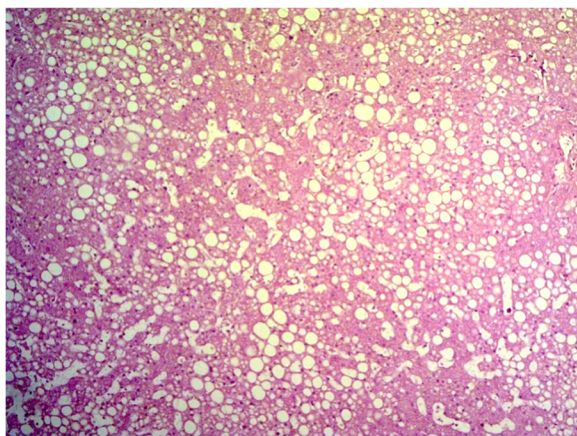
Reference	Setting	Population	Fatty liver	Cirrhosis	Neoplasm	Remarks
Zois et al, 2010 [3]	Greece 2006-08 PMs to determine CoD	N=498 Men 68.5% Age 64.5± 17.8	31.3	1.2	NR	H&E staining Reticulin and Masson's trichrome method for staining in selected specimens
Sotoudehmanesh et al, 2006 [5]	Iran 2002-2004 PMs to determine CoD	N=896 Men 86.7% Age 43.8 yrs	31.6	0.8	NR	Single deep slice from either lobe. H&E and reticulin and Masson's trichrome stains in selected specimens
Patel et al, 2016 [6]	India 2008-2013	N=202 Men 60.0% Age NR (2.5% were < 20y, 48.5% were 21-40y)	19.8	3.0	1 specimen contained a carcinoid	H&E and other stains
Fiel et al, 2012 [7]	USA	N=306 Men 48.4% Age (median) 71y	51.6	NR	NR	Modified Brunt classification for grading of steatosis. Included irrespective of alcohol and diabetes status.
Alagarsamy et al 2012 [8]	India PMs to determined CoD	N=50 Men 74% Age 16-60y	20.0	16.0	NR	-
Reis-Junior et al 2019 [9]	Brazil 2011-2013 PMs to determine COD	N=224 Men 81.7% Age 39.7 (±16.6)	48.2	NR	NR	6 samples from 2 lobes. H&E and other stains
Selvi et al, 2012 [11]	India 2009-2011	N=108 Men 75.9% Age f 46+/-9.52	26.9	7.4	1.9	3 wedge necropsies (1 each from left and right lobes, Centre of the liver) H&E stain. Recruited irrespective of diabetes or alcohol consumption status

*CoD: Cause of Death, H&E: Haematoxylin and Eosin stain, PM: Post-Mortem

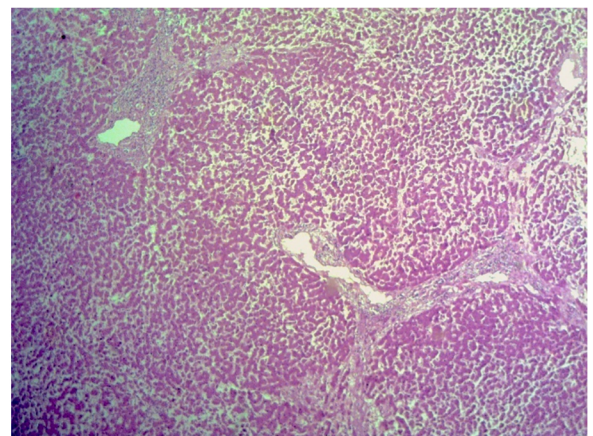
Age, sex, diabetes status and alcohol consumption did not predict the presence of fatty liver. However, previous studies showed a significant correlation with age and fatty change [3,5,11], whereas a substantial correlation with steatosis was not seen with the sex [3,5], history of diabetes and alcohol use [5] In the same studies. Although the findings from an early study suggested cirrhosis to have a poor association with diabetes mellitus [14], our findings suggest diabetes to be an independent risk factor for aggravating the condition of liver disease.

We found only one neoplastic lesion in the examined 382 liver specimens. This may be due to the relatively young age of the study population., Autopsy studies reporting the incidence of hepatic neoplasms are limited. Population prevalence of hepatoma in Sri Lanka is not known. The lower prevalence of viral

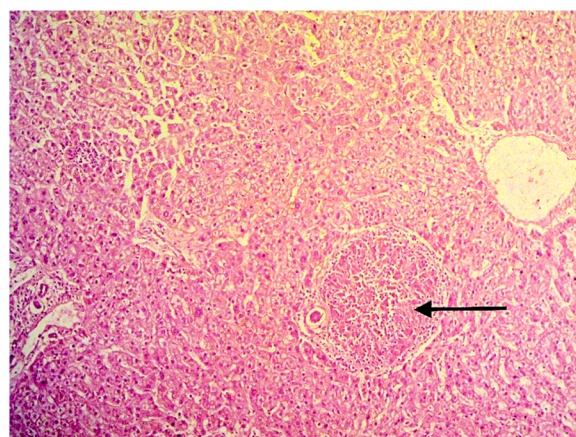
hepatitis may at least partly contribute to the lower incidence of hepatocellular carcinoma. It is uncertain whether genetic factors contribute to the lower rate of hepatomas in this population. There are several limitations to our study. First, the clinical data were obtained by the next of kin in most instances. This may have introduced recall bias. Furthermore, we could not obtain details to quantify the alcohol consumption or severity and duration of diabetes and other co-morbidities including obesity. These factors may determine the presence and severity of fatty liver and cirrhosis. We did not have biochemical and radiological data to define the functional status of the liver and compare it with histological findings. Secondly, the study was conducted in the Institute of Forensic Medicine and Toxicology, Colombo where most post-mortems were on urban or semi-urban dwellers affected by unnatural deaths.



(a)



(b)



(c)

Figure 1. Microscopic appearances of selected abnormalities.

- (a) Steatosis (x 100), H and E stain
- (b) Nodular regeneration and bridging fibrosis (x100), H and E stain
- (c) Non-caseating granuloma in low power (x100) H and E stain

This may have introduced a sampling bias with over-representation of men, younger age group and a higher proportion with alcohol consumption. Finally, as in any cross-sectional study, the causality cannot be determined with certainty. For instance, a higher incidence of fatty liver and cirrhosis among those with diabetes does not necessarily indicate diabetes caused by fatty liver. In fact, fatty liver and cirrhosis increase insulin resistance and may contribute to the onset of diabetes.

Nevertheless, our findings have several practical clinical and research implications. First, our data suggest that fatty liver is widely prevalent in Sri Lankan adults. This may at least in part be explained by genetic predisposition among South Asians to develop fatty liver [21]. Cirrhosis is also common and was undiagnosed in 92.9% at the time of death. The high prevalence of fatty liver suggests that cirrhosis can become a major public health problem in the future if no preventive measures are employed. Secondly, alcohol consumption among people with diabetes appears to significantly increase the risk of having cirrhosis. This disproportionate increase of risk may result from a synergistic effect of alcohol and diabetes on the liver. Therefore, people with diabetes should be strictly advised against alcohol consumption. Finally, our analyses failed to identify a single associated factor or a predictive model to predict the presence of fatty liver or cirrhosis. But diabetes and alcohol consumption was independently associated with worse severity of fatty liver. This indicates the need for further clinical, biochemical and radiological studies to determine optimal fatty liver screening strategies for the Sri Lankan population.

Conclusions

Fatty liver and cirrhosis are common in autopsies performed among adults from an urban setting in Sri Lanka. Diabetes and alcohol consumption was independently associated with greater severity of fatty liver.

Declarations

Author contributions

PK conceived the research question. The final study protocol was developed by PK, NPS, GWK, MVCS, AAHP, HDW, GGR and AT. AT conducted the post-mortem studies. GR, HW, AAHP, MVC were involved in the pathological evaluation. SA, SLG, AHNS, KTMPHS, NHJPA, HACLH conducted

data collection, data entry and cleaning. HAD, SC and TAS conducted the data analysis. HAD and TAS wrote the first draft of the manuscript. All authors read and approved the final manuscript for submission.

Ethical clearance

Ethical clearance was granted by the Ethics Review Committee of Faculty of Medicine, University of Colombo, Sri Lanka.

Data availability

The dataset is available with the principle investigator (PK) and can be shared upon written request.

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Conflicts of interests

All authors have no conflicts of interests to declare.

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