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RESEARCH ARTICLE

Prevalence of non-alcoholic steatohepatitis in a general population of North Karnataka

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Abstract

This study aims to investigate the prevalence of non-alcoholic steatohepatitis (NASH) in the general population of North Karnataka. A cross-sectional population-based study was conducted in the city of Belagavi, where in total of 730 subjects were included. The subjects were screened for the presence of liver disease with a detailed history, anthropometric measurements, and blood tests including liver function tests, lipid profile, and blood sugars, liver stiffness was measured by using the fibroscan. Out of 730 subjects, 228 (31.23%) had significant fibrosis suggestive of NASH on fibroscan. Of these 138 (60.52%) were males and 90(39.47%) were females. Of the total number of subjects having NASH 52 (22.80%) were diabetics and 64 (28.07%) were hypertensive. The study also revealed as the body mass index (BMI) increases the proportion of subjects with abnormal liver enzymes and significant liver fibrosis increased proportionately suggestive of NASH. Also, a small number of subjects (22.6%) with low or normal BMI had features suggestive of lean NASH. The incidence of NASH in the general population of North Karnataka is 31.23%. This high incidence could be due to the prevailing growing epidemic of diabetes mellitus and obesity in the community.

Keywords: Cirrhosis, Fibroscan, Liver function enzymes, Non-alcoholic fatty liver disease, Non-alcoholic fatty liver disease, Liver Fibrosis.

Introduction

Hepatic steatosis, often known as fatty liver, is the medical term for the intrahepatic accumulation of fat that accounts for 5 to 10% of the liver weight. This results either due to excessive intake of alcohol or excessive calories. When fatty liver results without a significant history of alcohol intake, it is labeled as Non-alcoholic fatty liver disease (NAFLD) (Nassir *et al.*, 2015). NAFLD is commonly associated with excessive intake of calories, obesity, hypertension, type 2 diabetes mellitus, dyslipidemia, cardiovascular disease, chronic kidney disease and sleep apnoea (Glass *et al.*, 2019). Non-alcoholic fatty liver disease causes the entire spectrum of liver disease ranging from benign fatty liver, to non-alcoholic steatohepatitis (NASH), cirrhosis, decompensated cirrhosis with portal hypertension and hepatocellular carcinoma (Ghevariya *et al.*, 2014; Adams and Feldstein, 2010, Sheka *et al.*, 2020).

The prevalence of NAFLD grew globally from 25.5% in 2005 to 35.5% in 2016 due to increased sedentary behavior and lifestyle changes, with the highest incidence in the West and Middle East and the lowest in Africa. NASH is expected to be present in 59.10% of NAFLD patients, with a global prevalence of 6.4% (Riazi *et al.*, 2022; Pedrosa *et al.*, 2018). NAFLD is diagnosed by simple imaging of the liver like ultrasonography/computed tomography (CT) of the liver. Over the years liver biopsy was the gold standard for diagnosing NASH with classical features of steatohepatitis on biopsy. However, liver biopsy has numerous drawbacks, including the risk of bleeding, high procedural costs, and inaccurate sampling as it only represents 1/50000 of the liver volume and does not precisely reflect the whole liver's anatomy (Wai *et al.*, 2003; Danaf *et al.*, 2022). To overcome these limitations the diagnosis of NASH and its progression is recently being monitored using non-invasive, highly sensitive tests with consistent results such as fibrosis (FIB) score, aspartate aminotransferase-to-platelet ratio index (APRI) score (using liver function test and platelets counts), and

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measuring liver stiffness (LSM) with fibroscan or magnetic resonance imaging (MRI) elastography (Papagianni *et al.*, 2015; Piazzolla and Mangia, 2020; Paul, 2020; Sasso *et al.*, 2012).

In India, the prevalence of NAFLD/NASH ranges from 6.7 to 55%. Most patients have liver enzyme elevations and many are asymptomatic (Duseja *et al.*, 2015). However, the prevalence varies with the geographical locations (urban or rural areas), age of the population, and incidence of comorbidities in that place. According to a National Family Health Survey, in Karnataka state (India), the prevalence of non-communicable diseases, including type 2 diabetic mellitus (14.8%) and hypertension (25.9%) were found to be high which are risk factors for developing NAFLD/NASH (James *et al.*, 2022). Hence this study was designed to estimate the prevalence rate of NAFLD/NASH in North Karnataka regions using liver function tests and non-invasive fibroscan (Liver stiffness i.e. LSM score) of the liver.

Materials and Methods

Study Design

A cross-sectional, population-based study was conducted at Jawaharlal Nehru Medical College and KLES Dr Prabhakar Kore Hospital and Medical Research Center, Belagavi, Karnataka. The study received approval from the Institutional Ethics Committee on Human Subjects (Ref.No.KAHER/EC/21-22/014). A total of 730 subjects were included in the study. Subjects aged between 18 to 80 years without a prior history of liver diseases were included in the study. All the subjects below the age of 18, or who had a prior history of heavy alcohol consumption or had liver diseases associated with alcohol consumption and subjects diagnosed with hepatitis B and C were excluded from the study. All the selected subjects were screened for liver diseases with detailed history, anthropometric measurements (height, weight, body mass index), liver function tests, blood sugars, hypertension and ultrasound abdomen and liver fibrosis. Those subjects with evidence of fatty liver on USG imaging were labeled as having NAFLD. Those patients with abnormal LSM on fibroscan with or without abnormal liver function tests were labeled as NASH.

Laboratory Analysis

Serum samples of the selected subjects were collected and analyzed using a Hitachi Cobas C analyzer. The following parameters were analyzed- blood sugar, total cholesterol, triglycerides, ALT/SGOT and AST/SGPT. Serum alanine aminotransferase (ALT) is a specific indicator of liver inflammation with a standard range of 0 to 40 U/L. Serum aspartate aminotransferase (AST) is an indicator of liver damage or injury with a normal range of 14 to 20 U/L (males) and 10 to 36 U/L (females).

Fibroscan

Fibroscan/ultrasound elastography is primarily used to assess liver stiffness/hardness and fatty alterations. It is mainly used for determining the NAFLD and NASH stages. The Fibroscan 430 MINI (Echosens/Device ID-03662264001192;18/08/2021) instrument was employed in the current investigation to conduct the analysis (Figure 1). It operates by assessing the speed of shear waves. In this procedure, a tiny transducer on the end of an ultrasonic probe transmits a 50-MHz wave into the liver. The probe also incorporates a transducer that can monitor the shear

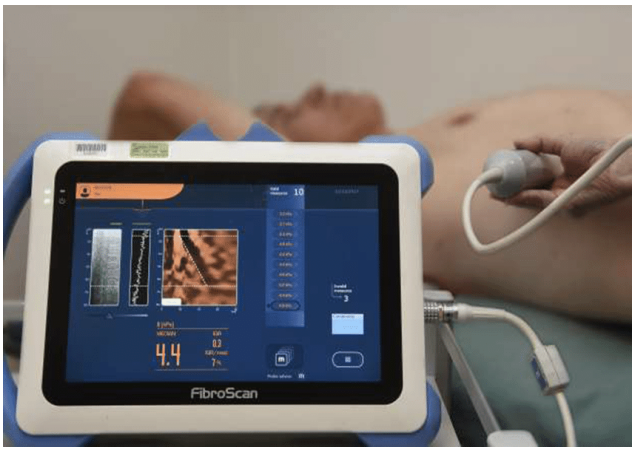

Figure 1: Fibroscan 430 MINI

Table 1: Baseline demographic characteristics of the study (n = 730)

Variables		*Sub category*	*Number of subjects (%)*
Age	< = 20	6	0.8
	21–30	114	15.6
	31–40	216	29.6
	41–50	188	25.8
	51–60	146	20.0
	61–70	51	7.0
	71–80	6	0.8
	> 80	3	0.4
Sex	Male	353	48.4
	Female	377	51.6
BMI	< 18.5	32	4.4
	18.5–24.9	268	36.7
	25.0–29.9	305	41.8
	> = 30	125	17.1
DM	No	640	87.7
	Yes	90	12.3
HTN	No	616	84.4
	Yes	114	15.6
LFT	Normal	586	80.3
	Abnormal	144	19.7

Note: BMI -Body Mass Index, DM -Diabetes Mellitus, HTN - Hyper Tension, LFT - Liver Function Test.

Table 2: Fibroscan scores in all subjects

Variables	*Subcategory*	*Fibroscan*	
		Normal	*Abnormal*
		Number of subjects (%)	
Fibrosis Score	F0	502 (68.76%)	0
	F1	0	99 (43.4%)
	F2	0	70 (30.7%)
	F3	0	38 (16.7%)
	F4	0	21 (9.2%)
		502 (68.76%)	228 (31.23%)

wave's velocity as it travels through the liver (in m/sec). The liver stiffness, which is measured in kilopascals, can then be obtained by converting the shear wave velocity. The fibrosis score F0 (0–7 kPa) indicates no scarring on the liver, F0-F1 (7–8.2) indicates mild scaring (mild fibrosis), F2 (8.3–9.7 kPa) indicates moderate scarring (significant fibrosis), F3 (9.7–13.6 kPa) indicates severe scarring with obstructive blood flow (advanced fibrosis) and F4 (> 13.6 kPa) indicates cirrhosis of the liver. The fibroscan reports were reviewed by the gastroenterologist.

Statistical Analysis

Data is analyzed using SPSS software version 21 and Excel. Categorical variables are given in the form of a frequency table. The Chi-square test analyses categorical variables. The Shapiro-Wilk test and QQ plot check the normality of the variable. Mann Whitney U test compares the distributions of variables over fibroscan. *p-value* less than or equal to 0.05 indicates statistical significance.

Results

Baseline demographic characteristics of the study (n = 730) is shown in Table 1.

Distribution of Subjects Based on Fibroscan

Table 2 indicates that, out of 730 subjects, 502 subjects were found to have normal fibroscan with fibrosis score of

Table 3: Association of fibroscan scores with different variables

Variables	*Subcategory*	*F0*		*F1*		*F2*		*F3*		*F4*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
		Chi-square (d.f. = 7) = 84.759, p < 0.001											
	< = 20	5	1	0	0	1	1.4	0	0	0	0	6	0.8
	21–30	105	20.9	6	6.1	3	4.3	0	0	0	0	114	15.6
	31–40	149	29.7	31	31.3	23	32.9	10	26.3	3	14.3	216	29.6
Age	41–50	141	28.1	24	24.2	11	15.7	10	26.3	2	9.5	188	25.8
	51–60	61	12.2	31	31.3	26	37.1	13	34.2	15	71.4	146	20
	61–70	35	7	6	6.1	5	7.1	4	10.5	1	4.8	51	7
	71–80	5	1	0	0	1	1.4	0	0	0	0	6	0.8
	> 80	1	0.2	1	1	0	0	1	2.6	0	0	3	0.4
		Chi-square (d.f. = 1) = 19.664, p < 0.001											
Sex	Male	215	42.8	58	58.6	39	55.7	27	71.1	14	66.7	353	48.4
	Female	287	57.2	41	41.4	31	44.3	11	28.9	7	33.3	377	51.6
		Chi-square (d.f. = 3) = 86.97, p < 0.001											
	< 18.5	30	6	0	0	2	2.9	0	0	0	0	32	4.4
BMI	18.5–24.9	219	43.6	26	26.3	16	22.9	5	13.2	2	9.5	268	36.7
	25.0–29.9	206	41	46	46.5	31	44.3	16	42.1	6	28.6	305	41.8
	> = 30	47	9.4	27	27.3	21	30	17	44.7	13	61.9	125	17.1
		Chi-square (d.f. = 1) = 33.679, p < 0.001											
DM	No	464	92.4	83	83.8	50	71.4	33	86.8	10	47.6	640	87.7
	Yes	38	7.6	16	16.2	20	28.6	5	13.2	11	52.4	90	12.3
		Chi-square (d.f. = 1) = 39.022, p < 0.001											
HTN	No	452	90	75	75.8	58	82.9	25	65.8	6	28.6	616	84.4
	Yes	50	10	24	24.2	12	17.1	13	34.2	15	71.4	114	15.6
		Chi-square (d.f. = 1) = 29.416, p < 0.001											
LFT	Normal	430	85.7	73	73.7	47	67.1	23	60.5	13	61.9	586	80.3
	Abnormal	72	14.3	26	26.3	23	32.9	15	39.5	8	38.1	144	19.7

Note: - d.f. = degree of freedom, BMI -Body Mass Index, DM -Diabetes Mellitus, HTN - Hyper Tension, LFT - Liver Function Test.

Table 4: Association of abnormal fibroscan scores (NASH) with different variables

Variables	*subcategory*	*Normal*		*Abnormal*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*
		Chi-square (d.f. = 7) = 84.759, p < 0.001					
Age	< = 20	5	0.68	1	0.13	6	0.8
	21–30	105	14.38	9	1.23	114	15.6
	31–40	149	20.41	67	9.17	216	29.6
	41–50	141	19.31	47	6.43	188	25.8
	51–60	61	8.35	85	11.64	146	20
	61–70	35	4.79	16	2.19	51	7
	71–80	5	0.68	1	0.13	6	0.8
	> 80	1	0.13	2	0.27	3	0.4
		Chi-square (d.f. = 1) = 19.664, p < 0.001					
Sex	Male	215	29.45	138	18.9	353	48.4
	Female	287	39.31	90	12.32	377	51.6
		Chi-square (d.f. = 3) = 86.97, p < 0.001					
BMI	< 18.5	30	4.1	2	0.27	32	4.4
	18.5–24.9	219	30	49	6.71	268	36.7
	25.0–29.9	206	28.21	99	13.56	305	41.8
	> = 30	47	6.43	78	10.68	125	17.1
		Chi-square (d.f. = 1) = 33.679, p < 0.001					
DM	No	464	63.56	176	24.1	640	87.7
	Yes	38	5.2	52	7.1	90	12.3
		Chi-square (d.f. = 1) = 39.022, p < 0.001					
HTN	No	452	61.91	164	22.46	616	84.37
	Yes	50	6.84	64	8.76	114	15.6
		Chi-square (d.f. = 1) = 29.416, p < 0.001					
LFT	Normal	430	58.9	156	21.36	586	80.26
	Abnormal	72	9.86	72	9.86	144	19.72

Note: - d.f. - degree of freedom, BMI -Body Mass Index, DM -Diabetes Mellitus, HTN - Hyper Tension, LFT - Liver Function Test.

F0, 228 subjects were found to be abnormal fibroscans with fibrosis scores ranging from F1 to F4. Among these subjects 99 (43.4%) were diagnosed with F1 score, 70 (30.7%) had F2 scores, 38 (16.7%) subjects had F3, and 21 (9.2%) subjects had F4 scores (Tables 3 and 4).

Fibroscan Scores with Different Variables

The majority of the subjects included in the study were between 21 to 60 years. Most of the subjects with abnormal fibroscan scores were between 30 to 60 years. Out of 228, 138 males and 90 females had abnormal fibroscan scores. The majority of the subjects included in this study had a normal BMI or were overweight. Most of the subjects with normal fibroscans were either having low BMI or normal BMI. Out of 144 subjects with abnormal liver function tests, 72 subjects had abnormal fibroscans suggestive of NASH and the remaining and out of 586 subjects with normal liver function tests only 156 had abnormal values suggestive of NASH (Tables 5 and 6).

Figure 2 shows that, fibroscan score's sensitivity was 74.6% and specificity was 74.3%. Area under the ROC curve is 0.816 (95% CI: 0.784–0.843; p <0.001).

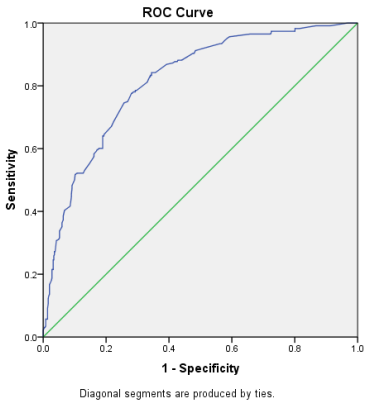

Figure 2: ROC curve

Table 5: Sociodemographic characteristics of the study participants

Variables	*N*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
Age (Years)	730	14.00	83.00	42.97397	12.39732
BMI	730	14.60	41.20	26.10628	4.542661
DM	730	0.00	1.00	0.123288	0.328993
HTN	730	0.00	1.00	0.156164	0.36326
SGOT	730	0.00	182.00	36.95753	26.04712
SGPT	730	0.00	142.00	35.00959	20.69668
CAP	730	165.00	393.00	260.0822	41.64727

Note: - BMI -Body Mass Index, DM -Diabetes Mellitus, HTN - Hyper Tension, SGOT - Serum Glutamic Oxaloacetic Transaminase, SGPT- Serum Glutamic Pyruvic Transaminase, CAP- Controlled Attenuation Parameter

Discussion

Given the enormous size of India's population, NAFLD is projected to become a major public health issue that will significantly influence the nation's meager medical resources. According to a recent study on NAFLD published in India, there are at least 25 million people with a high risk of developing liver disorders due to changing lifestyles. There is an urgent need to create more effective methods to identify this group of patients who are likely to have a progressive NAFLD and will go on to develop liver failure (Chalmers *et al.*, 2019).

In the present study, 730 subjects from the community were screened for the presence of NAFLD/NASH with the help of liver function tests and fibroscan scores. Those with NASH were identified and were further graded into those with mild fibrosis, moderate fibrosis, severe fibrosis and cirrhosis. Fibrosis was compared to different clinical variables of the subjects like age, sex, body mass index, diabetic mellites and hypertension. Out of 730 subjects studied, 228 (31.23%) that including 138 (60.52%) males and 90 (39.47%) females were had NASH with abnormal fibroscan scores reflecting a high prevalence of NASH in North Karnataka. It was similar to a study conducted by Singhai *et al.*, 2023.

In our study, when abnormal fibroscan scores were compared to liver function test (AST and ALT), 586 (80.3%) subjects were normal and 144 (19.7%) subjects were abnormal. According to Mofrad *et al.* (2003), when conducting laboratory tests, serum markers such as aminotransferases (AST, ALT) often show mild to moderate elevation. Nevertheless, AST and ALT levels can lack specificity in individuals with NAFLD or related conditions. In simpler terms, both AST and ALT levels may be elevated or

Table 6: Logistic regression analysis for predicting liver fibrosis

Variables		*Estimate*	*p-value*	*O.R.*	*95% C.I. for EXP(B)*	
				Lower	Upper	
Sex	Female			1		
	Male	-0.957	0	0.38	0.26	0.562
Age	> 80		0	1		
	< 20	-1.398	0.409	0.25	0.01	6.809
	21–30	-3.038	0.018	0.05	0	0.599
	31–40	-1.887	0.132	0.15	0.01	1.767
	41–50	-2.174	0.083	0.11	0.01	1.331
	51–60	-0.932	0.457	0.39	0.03	4.599
	61–70	-2.467	0.056	0.09	0.01	1.065
	71–80	-4.081	0.018	0.02	0	0.499
BMI (Kg/m^2)	> = 30 (Obese)		0	1		
	< 18.5 (Underweight)	-3.073	0	0.05	0.01	0.227
	18.5–24.9 (Normal)	-1.893	0	0.15	0.09	0.261
	25–29.9 (Overweight)	-1.298	0	0.27	0.17	0.45
DM	No			1		
	Yes	-0.759	0.009	0.47	0.27	0.827
HTN	No			1		
	Yes	-0.246	0.373	0.78	0.46	1.344
LFT	Normal			1		
	Abnormal	-0.897	0	0.41	0.26	0.633

Note: - S.E. - Standard Error, d.f. - degree of freedom, O.R. - Odds Ratio, BMI -Body Mass Index, DM -Diabetes Mellitus, HTN - Hyper Tension, LFT - Liver Function Test.

normal, and their values alone do not rule out the presence of NAFLD, as highlighted by studies conducted by Noguchi *et al.* (1995) and Charatcharoenwitthaya *et al.* (2012). Among patients with NAFLD, ALT elevations are more frequently observed than elevations in AST. Notably, ALT levels tend to be higher in NASH compared to simple steatosis, as indicated by the findings of Mofrad *et al.* (2003).

Our study findings show that, the age of the subjects were between 20 to 80 years. Most of the subjects with abnormal scan were between 21 to 30 years (n = 114; 15.6%) between 31 to 40 years (n = 216; 29.6%), 41 to 50 years (n = 188; 25.8%) and 51 to 60 years (n = 146; 20%). In a study conducted by Lin *et al.* (2011), it was observed that the majority of participants fell within the age range of 21 to 60 years. Among those without fibrosis, the predominant age group was 21 to 50 years. Subjects with abnormal FibroScan scores were mostly distributed between 30 to 60 years, with 67 (29.38%) participants aged 31 to 40 years, 47 (20.60%) in the 41 to 50 years, and 85 (37.28%) between 51 to 60 years. It can be seen that younger subjects have less fibrosis and fibroscan scores increase with age. However, age is insignificantly associated with fibroscan scores and fibrosis (Pradat *et al.*, 2002)

In our study, 90 (12.3%) subjects were diabetics. Of them, 52 had abnormal fibroscans and 38 were normal. Also, of the 640 non-diabetic subjects only 176 had abnormal fibrosis. Diabetes is significantly associated with NASH. Out of 228 subjects with NASH, 52 (22.80%) subjects had diabetic mellitus and 176 (77.19%) subjects were non-diabetics, which was statistically significant. Type II diabetes mellitus hastens the advancement of simple fatty liver to a more severe NASH (Tomah *et al.*, 2020; Dharmalingam and Yamasandhi, 2018). Additionally, our study also found that, 114 (15.6%) were hypertensive. Of them, 64 had abnormal fibroscans and 50 were normal. Of the 616 non-hypertensives, 164 had abnormal fibrosis and 452 were normal. Out of the 228 subjects with NASH, 64 had hypertension and 164 were normotensives. Hypertension is not significantly associated with the development of fibrosis (Tables 3 and 4).

The majority of the subjects included in this study had a normal BMI or were overweight. Few subjects were obese and those who were underweight were the least in number. Most of the subjects with normal fibroscans were either had low BMI 30 of 32 or a normal BMI 219 out of 268. The majority of subjects with abnormal fibroscan scores were either overweight 99 subjects out of 206 or obese 78 out of 125 subjects. Only 2 of the 32 subjects who were underweight with low BMI had abnormal fibrosis. As the BMI of the subjects increased they had more risk of fibrosis with increased fibroscan scores. As the BMI increases beyond normal that is >24.9 there will be the development of steatosis, hepatitis and also an increase in fibrosis This shows a significant association of abnormal liver fibroscan scores with BMI (Eilenberg *et al.*, 2021)

In our study, 144 had abnormal liver function tests. Of them, 72 subjects had abnormal fibroscans suggestive of NASH and the remaining 72 had normal fibroscans. 586 subjects had normal liver function tests, of them 430 had normal fibroscan scores whereas only 156 had abnormal values suggestive of NASH (Table 3).

In the present study, the sensitivity of the fibroscan score prediction with AUROC of 0.80 was found to be 74.6% and the specificity was 74.3%. Thus, it could be postulated that fibroscan along with liver function tests can be used to diagnose NASH from NAFLD. Also, different stages of NASH fibrosis can also be predicted after the fibroscan scores.

Conclusion

The study shows that the prevalence of NAFLD/NASH is relatively high in North Karnataka, that is 31.23%. The prevalence of diabetic mellitus and obesity with high BMI is a significant risk factor for the development of NASH. Fibroscan which is a simple and non-invasive test for assessing firmness of the liver along with liver function tests can be used to predict significant fibrosis of the liver that is NASH. Thus, fibroscan and liver function tests can be used to screen for significant liver diseases in the general population.

Policymakers can use these tests to estimate the burden of significant liver diseases in the population so that appropriate interventions can be planned to reduce the mortality and morbidity of the population leading to advanced liver disease.

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