

## RESEARCH ARTICLE

### Case–control study evaluating relationship of blood pressure and anthropometric parameters with non-alcoholic fatty liver disease

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#### ABSTRACT


**Background:** The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased over the last decade. India is one of the countries with highest prevalence of NAFLD. High prevalence of lifestyle disorders among Keralites and their association with NAFLD necessitates studies on the subject. **Aims and Objective:** Owing to the scarcity of studies evaluating the association of NAFLD with anthropometric and clinical parameters in Kerala, the present study was conducted. **Materials and Methods:** Our case–control study enrolled 81 cases (with NAFLD) and 79 controls (without NAFLD) who were undergoing voluntary health checkup over a period of 2 years. Institutional Ethics Committee approved the study and written informed consent was obtained from all the study participants. Sociodemographic, anthropometric parameters and blood pressure (BP) were recorded and categorized based on gender, body mass index (BMI), and BP (JNC-8). Data were analyzed using free software R™. Independent sample *t*-test, Mann–Whitney U-test, and Chi-square test were used for statistical analysis and  $P < 0.05$  was considered statistically significant. **Results:** 15% and 11% of the study participants were having normal BMI and BP, respectively. Cases were significantly taller ( $P = 0.003$ ), were having higher BMI ( $P = 0.04$ ), weight ( $P < 0.001$ ), waist circumference (WC) ( $P = 0.02$ ), and systolic BP (SBP) ( $P = 0.02$ ) compared to controls. Significant association was observed between NAFLD and stage 2 hypertension ( $P = 0.04$ ). Our study did not demonstrate any association between NAFLD and BMI ( $P = 0.2$ ) and between NAFLD and WC ( $P = 0.7$ ). **Conclusion:** High prevalence of weight-related and BP disorders were seen in participants undergoing voluntary health checkup. NAFLD was associated with a significantly higher weight, BMI, height, SBP, and WC. Significantly higher odds of NAFLD were seen in participants with Stage 2 hypertension.

**KEY WORDS:** Non-alcoholic Fatty Liver Disease; Stage 2 Hypertension; Weight-Related Disorders; Lifestyle Disorders; Pre-hypertension

#### INTRODUCTION

From the inception of the term non-alcoholic steatohepatitis (NASH), this perplexing and incomprehensible entity has

been a favorite area of research. Hepatic steatosis in non-alcoholic individuals is considered as non-alcoholic fatty liver disease (NAFLD)<sup>[1]</sup> and the term encompasses simple steatosis to end-stage liver failure.<sup>[2]</sup> Incipiently, NAFLD was considered a disease of the developed, but with the pandemic of lifestyle diseases<sup>[3]</sup> the global prevalence has increased to 25%<sup>[4]</sup> and encounters with NAFLD and its complications are alike in both developing and developed nations. Diagnosis of NAFLD is overlooked due to the absence of symptoms, oftentimes, NAFLD is an incidental finding during ultrasonography (US) of the abdomen.

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NAFLD is bidirectionally associated with metabolic syndrome (MS) and has strong association with components of MS such as obesity, central obesity, insulin resistance (IR), diabetes, dyslipidemia, and hypertension.<sup>[5]</sup> Increase in body mass index (BMI) has been shown to increase the risk of NAFLD by 5–14 fold,<sup>[6]</sup> a unit increase in waist circumference (WC) has been shown to increase the risk of NAFLD by 0.07,<sup>[7]</sup> and hypertension has been shown to have a 1.8 higher odds of developing NAFLD.<sup>[8]</sup> Patients with at least one allele of the gene palatin-like phospholipase 3 display a favorable metabolic profile with normal insulin sensitivity<sup>[5]</sup> and these individuals are considered as having lean NAFLD. Being the most common disease of the liver, high risk of developing MS among Asians, pandemic of lifestyle disorders, reduced survival among patients with NAFLD<sup>[9]</sup> and the risk of hepatocellular carcinoma (HCC) associated with NASH, and simple NAFLD<sup>[10]</sup> warrants the question - why study NAFLD?

Like any other non-communicable disease (NCD), India stands tall in the prevalence ( $\geq 30\%$ ) and burden of NAFLD<sup>[11]</sup> with reports of higher prevalence in rural<sup>[12,13]</sup> than the urban population.<sup>[14]</sup> Indian studies show a prevalence of NAFLD between 9 and 32%,<sup>[15]</sup> and 60% prevalence has been reported among obese children from Kerala.<sup>[16]</sup> The exact prevalence of NAFLD in our population is unclear since the population undergoing voluntary health checkup is small and assessing community prevalence is laborious financially since diagnosis requires US abdomen. The prevalence and association of various lifestyle disorders with NAFLD in our setting has not been evaluated. Hence, this study was conducted to determine the association of anthropometric and clinical parameters with NAFLD in participants who undergo voluntary health checkup.

## MATERIALS AND METHODS

The present cross-sectional case-control study enrolled 160 participants (81 cases and 79 controls) undergoing voluntary health checkup in General Medicine Department of Sree Gokulam Medical College and Research Foundation, Trivandrum during 2014 - 2016. Cases were participants with the US evidence of NAFLD (Grades 1-3 fatty infiltration of the liver) and controls were participants with no sonological evidence of NAFLD (Grade 0). Participants excluded were those unwilling to participate, history of alcohol use or clinical evidence of alcoholic liver disease, history of use of drugs causing steatosis (e.g., corticosteroids), history of jejunoileal bypass and extensive small bowel resection, suspected or confirmed HCC, anemia, and known hemoglobinopathies. Sample size was calculated as 160 (80 cases, 80 controls) to detect a minimum odds of 0.2 assuming  $\alpha$  as 0.05 and  $\beta$  as 0.2, case to control ratio of 1 and 10% attrition rate. The study commenced after approval from the Institutional Ethics Committee (SGMC IEC No: 16/117/01/2015) and written informed consent was obtained from all the study participants.

Sociodemographic (age, gender), anthropometric (height, weight, BMI, WC, and waist-to-height ratio [WHtR]), and examination findings (blood pressure BP and US) were recorded in structured case record forms. Data were categorized based on gender, BMI (for Asians<sup>[17]</sup>), and BP (according to Joint National Committee 8 [JNC-8] guideline). Data were analyzed using free to use software R<sup>TM</sup>; nominal variables were compared using independent sample *t*-test (normal distribution) and Mann-Whitney U-test (not normally distributed, Shapiro-Wilk  $P < 0.05$ ) and association between categorical variables were checked using Chi-square test. Odds ratio and confidence interval were used for describing association in  $2 \times 2$  table. Values were rounded off to one decimal point and are expressed as mean (standard deviation) (normal distribution) and median (interquartile range) (not normally distributed).  $P < 0.05$  was considered statistically significant.

## RESULTS

A total of 160 participants (81 cases, 79 controls) were enrolled, of which 89 (55.6%) were male and 71 were (44.4%) female. The mean age, height, weight, BMI, WC, WHtR, and BP of the study participants were  $46.3 \pm 11.7$  years,  $162.7 \pm 11.1$  cm,  $71.2 \pm 13.1$  kg,  $27.3 \pm 3.7$  kg/m<sup>2</sup>,  $95.7 \pm 10.2$  cm,  $0.6 \pm 0.07$ , and  $127.6 \pm 12.9/82.4 \pm 8.5$  mmHg, respectively. Among the study participants, 25 (15.6%) were in the normal BMI range, 21 (13.1%) were overweight, 72 (45%) were obese 1, and 42 (26.3%) were obese 2 according to BMI categorization of Asians. Only 18 (11.3%) study participants were having normal BP, 87 (54.4%) were having pre-hypertension, 38 (23.8%) were having stage 1 hypertension, and 17 (10.6%) were having stage 2 hypertension. The baseline parameters of the study participants are demonstrated in Table 1.

Gender-based comparison of variables showed significant difference in age ( $P = 0.002$ ), height ( $P < 0.001$ ), weight ( $P < 0.001$ ), and WC ( $P < 0.001$ ). There was no significant difference in BMI ( $P = 0.6$ ), systolic BP (SBP) ( $P = 0.1$ ),

**Table 1:** Baseline parameters of the study participants

Parameter	Median (IQR)	Mean (SD)
Age (years)	-	46.3 (11.7)
Height (cm)	161.5 (155-170)	-
Weight (kg)	68 (61-80)	-
BMI (kg/m <sup>2</sup> )	27.7 (23.8-30)	-
WC (cm)	94 (88-100)	-
WHtR	0.58 (0.54-0.63)	-
SBP (mmHg)	120 (120-138.5)	-
DBP (mmHg)	80 (80-90)	-

DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BMI: Body mass index, WC: Waist circumference, WHtR: Waist-to-height ratio, IQR: Interquartile range, SD: Standard deviation

diastolic BP (DBP) ( $P = 0.2$ ), and WHtR ( $P = 0.2$ ) between male and female participants. Gender-based comparison of variables is demonstrated in Table 2.

There was no significant difference in gender distribution between cases and controls ( $P = 0.2$ ). The participants with NAFLD were significantly taller ( $P = 0.003$ ), were having significantly higher weight ( $P < 0.001$ ), higher BMI ( $P = 0.04$ ), higher SBP ( $P = 0.02$ ), and WC ( $P = 0.02$ ). The comparison variables between cases and controls are demonstrated in Table 3.

No significant association was observed between NAFLD and BMI ( $P = 0.2$ ) as demonstrated in Tables 4 and 5. Participants were also categorized into those with normal BMI (18.5–22.9 kg/m<sup>2</sup>) and those with elevated BMI ( $\geq 23$  kg/m<sup>2</sup>) and were checked for association with NAFLD ( $P = 0.5$ ) and is demonstrated in Table 5.

We did not find any association between BP and NAFLD when participants were categorized into normal BP and elevated BP ( $P = 0.9$ ). We found significant association between BP categories (JNC 8) and NAFLD ( $P = 0.04$ ) and significant association was observed between stage 2 hypertension and NAFLD ( $P = 0.04$ ). We did not find any association between abdominal obesity and NAFLD ( $P = 0.7$ ). These are demonstrated in Tables 6-9.

Among participants with normal BMI ( $n = 25$ ), NAFLD was seen in 44% ( $n = 11$ ) of the participants with normal BMI ( $n = 25$ ) were having NAFLD and 53.8% ( $n = 14$ ) of the

participants without abdominal obesity ( $n = 26$ ) were having NAFLD.

## DISCUSSION

Our study enrolled 160 participants who underwent voluntary health checkup during a period of 2 years. The mean age of the study participants (~46 years) was considerably low for those undergoing voluntary health checkups which could be due to the higher literacy rate and awareness among Keralites regarding NCD and due to the egalitarian health-care policies of the state government. This could also be an indicator of the high prevalence of lifestyle diseases such as obesity among Keralites which could motivate them to undergo health checkup early in life. The age of female participants (~49 years) were significantly higher compared to males (~43 years), probably be due to the easier accessibility of hospitals to males and the stigma among females for undergoing health checkup even though female literacy is par with males in Kerala which would also explain the enrollment of higher proportion of male participants in our study. Only 15.6% of the study participants' BMI was in the normal range, 84.4% of the study participants were having higher than normal BMI. This alarming finding could be explained on the basis of the obesity pandemic claiming its share of Indians<sup>[18,19]</sup> and Keralites.<sup>[20,21]</sup> Kerala has even been described as one of the overweight states of India owing to high prevalence of weight-related disorders. Among the study participants, 13.1% ( $n = 21$ ) were overweight, 45% ( $n = 72$ ) were obese 1, and 26.3% ( $n = 42$ ) were obese

**Table 2: Gender-based comparison of variables**

Parameter	Gender	<i>n</i>	Mean (SD), median (IQR)	<i>P</i>
Age (years)	Male	89	43.8 (12)	0.002*
	Female	71	49.4 (10.6)	
Height (cm)	Male	89	168.8 (9.9), 170 (164–175)	<0.001#
	Female	71	154.9 (6.8), 155 (148–160)	
Weight (kg)	Male	89	76.2 (13.5), 78 (66–87)	<0.001#
	Female	71	64.9 (9.7), 64 (60–72)	
BMI (kg/m <sup>2</sup> )	Male	89	27.1 (3.9), 28 (23.5–30)	0.6**
	Female	71	27.6 (3.6), 27.5 (25.6–30)	
DBP (mmHg)	Male	89	83.4 (8.4), 80 (80–90)	0.1**
	Female	71	81.3 (8.7), 80 (70–90)	
SBP (mmHg)	Male	89	128.2 (10.8), 130 (120–130)	0.2**
	Female	71	126.9 (15.2), 120 (120–140)	
WC (cm)	Male	89	98.5 (10.1), 96 (90–107)	<0.001#
	Female	71	92.1 (9.2), 90 (86–95)	
WHtR	Male	89	0.6 (0.08), 0.6 (0.5–0.6)	0.2**
	Female	71	0.6 (0.07), 0.6 (0.6–0.6)	

\*Indicates significant difference between the groups using independent sample *t*-test, #indicates significant difference between the groups using Mann–Whitney U-test, \*\*indicates no significant difference between the groups using Mann–Whitney U-test. DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BMI: Body mass index, WC: Waist circumference, WHtR: Waist-to-height ratio, IQR: Interquartile range, SD: Standard deviation

**Table 3:** Comparison of variables between cases and controls

Parameter	NAFLD	N	Mean (SD), median (IQR)	P
Age (years)	No	79	46.2 (11.7)	0.9*
	Yes	81	46.4 (11.7)	
Height (cm)	No	79	160 (10.8), 160 (149–167)	0.003**
	Yes	81	165.3 (10.8), 166 (159.8–172)	
Weight (kg)	No	79	66.8 (12.5), 64 (60–78)	<0.001**
	Yes	81	75.5 (12.4), 74 (66–84)	
BMI (kg/m <sup>2</sup> )	No	79	26.7 (3.8), 27.2 (23.5–29.1)	0.04**
	Yes	81	27.9 (3.5), 28.3 (25.7–30.3)	
DBP (mmHg)	No	79	81.4 (7.3), 80 (80–90)	0.1 <sup>#</sup>
	Yes	81	83.5 (9.5), 80 (80–90)	
SBP (mmHg)	No	79	125.2 (10.5), 120 (120–130)	0.02**
	Yes	81	129.9 (14.6), 130 (120–140)	
WC (cm)	No	79	93.9 (9.9), 93 (86–98)	0.02**
	Yes	81	97.4 (10.3), 96 (88–106)	
WHtR	No	79	0.59 (0.07), 0.6 (0.5–0.6)	0.9 <sup>#</sup>
	Yes	81	0.59 (0.08), 0.6 (0.5–0.6)	

\*Indicates no significant difference between the groups when compared using independent sample *t*-test, \*\*indicates significant difference between the groups when compared using Mann–Whitney U-test, <sup>#</sup>indicates no significant difference between the groups when compared using Mann–Whitney U-test. NAFLD: Non-alcoholic fatty liver disease, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BMI: Body mass index, WC: Waist circumference, WHtR: Waist-to-height ratio, IQR: Interquartile range, SD: Standard deviation

**Table 4:** Association between NAFLD and BMI

BMI categorization of Asians	NAFLD		Total
	No	Yes	
Normal (18.5–22.9 kg/m <sup>2</sup> )	14	11	25
Overweight (23–24.9 kg/m <sup>2</sup> )	14	7	21
Obese 1 (25–29.9 kg/m <sup>2</sup> )	35	37	72
Obese 2 (30–40 kg/m <sup>2</sup> )	16	26	42
Total	79	81	160

No significant association was observed between groups (Chi-square  $P=0.2$ ). NAFLD: Non-alcoholic fatty liver disease, BMI: Body mass index

**Table 5:** Association between NAFLD and elevated BMI

BMI category	NAFLD		Total
	No	Yes	
Normal BMI (18.5–22.9 kg/m <sup>2</sup> )	14	11	25
Elevated BMI ( $\geq 23$ kg/m <sup>2</sup> )	65	70	135
Total	79	81	160

No significant association was observed between groups (Chi-square  $P=0.5$ ; OR: 1.4, 95% CI: 0.6–3.2). NAFLD: Non-alcoholic fatty liver disease, BMI: Body mass index, OR: Odds ratio, CI: Confidence interval

voluntary health checkup. This could also be explained on the basis of the recent BMI guidelines for Asians<sup>[17]</sup> which reduced the range of values for normal BMI (18.5–24.9 to 18.5–22.9 kg/m<sup>2</sup>), overweight (25–29.99 to 23–24.9 kg/m<sup>2</sup>), and obese ( $\geq 30$  to  $\geq 25$  kg/m<sup>2</sup>). which would result in enrollment of more participants into overweight and obese category who were previously considered as having normal BMI and overweight, respectively. 88.7% of the participants had BP-related abnormalities. This could draw attention to the previous report of 45% prevalence of pre-hypertension from South India,<sup>[23]</sup> which was considered coincidental by at least some. This could also be due to the inclusion of a new BP category (pre-hypertension) since JNC 7 of 2003 which categorizes participants who were considered previously as having normal BP to pre-hypertension or could be due to a very high prevalence of pre-hypertension among Keralites. The height, weight, and WC of male participants were significantly higher compared to females. This is a normal finding due to the effect of androgens leading to increased bone growth and bone mineralization leading to a taller stature and a higher value for weight.<sup>[24]</sup> Anatomically, the WC will also be higher in males. We did not find any significant difference in mean BMI, BP, and WHtR between males and females.

The participants with NAFLD were significantly taller, which is a new observation and requires further evaluation. This could only be explained by the enrollment of a non-significant ( $P = 0.2$ ), but higher number of male participants ( $n = 49$ ) among cases. The mean weight ( $P < 0.001$ ) and BMI ( $P = 0.04$ ) of the cases were significantly higher compared to

2. This is high compared to the state survey reports of 2015–2016<sup>[22]</sup> which reported a prevalence of obesity and overweight among males and females of 28.5% and 32.4%. This disparity could be due to the concerns of self and family members among obese and overweight individuals making them the predominant fraction of the population undergoing

**Table 6: Association between BP and NAFLD**

BP category	NAFLD		Total
	No	Yes	
Normal (SBP<120 mmHg and DBP<80 mmHg)	9	9	18
Pre-hypertension (SBP 120–139 mmHg/DBP 80–89 mmHg)	48	39	87
Stage 1 hypertension (SBP 140–159 mmHg/DBP 90–99 mmHg)	19	19	38
Stage 2 hypertension (SBP≥160 mmHg/DBP≥100 mmHg)	3	14	17
Total	79	81	160

BP: Blood pressure, NAFLD: Non-alcoholic fatty liver disease, DBP: Diastolic blood pressure, SBP: Systolic blood pressure

**Table 7: Association between NAFLD and elevated BP**

BP category	NAFLD		Total
	No	Yes	
Normal (SBP<120 mmHg and DBP<80 mmHg)	9	9	18
Elevated BP (SBP≥120 mmHg/DBP≥80 mmHg)	70	72	142
Total	79	81	160

No significant association was observed between the groups (Chi-square  $P=0.9$ ; OR: 1, 95% CI: 0.4–2.7). BP: Blood pressure, NAFLD: Non-alcoholic fatty liver disease, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, OR: Odds ratio, CI: Confidence interval

**Table 8: Association between NAFLD and Stage 2 hypertension**

BP category	NAFLD		Total
	No	Yes	
Normal (SBP<120 mmHg and DBP<80 mmHg)	9	9	18
Stage 2 hypertension (SBP≥160 mmHg/DBP≥100 mmHg)	3	14	17
Total	12	23	35

There was significant association between the groups (Chi-square  $P=0.04$ ; OR: 4.7, 95% CI: 1–22) indicating a 4.7 higher odds of hypertensive 2 to develop NAFLD. BP: Blood pressure, NAFLD: Non-alcoholic fatty liver disease, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, OR: Odds ratio, CI: Confidence interval

controls which is explained by the reports of strong correlation between obesity and NAFLD.<sup>[25]</sup> In obesity, there is an expansion of adipose tissue (subcutaneous and visceral) due to the accumulation of excess fat which promotes oxidative stress causing dysregulated adipocytokine production and increase in pro-inflammatory cytokines (tumor necrosis factor  $\alpha$  and interleukin 6) leading to an inflammatory state in the adipose tissue. These changes increase the release of free fatty acids (FFA) from adipose tissue into the circulation, which is the major source of FFA for the liver (65%).<sup>[25]</sup> Inflammation in adipose tissue also causes pro-inflammatory and profibrotic changes such as reduced adiponectin and increased leptin. Increased leptin levels also cause central leptin resistance leading to reduced anorexigenic effects of

**Table 9: Association between abdominal obesity and NAFLD**

Abdominal obesity (WC>80 cm in females and >90 cm in males)	NAFLD		Total
	No	Yes	
No	12	14	26
Yes	67	67	134
Total	79	81	160

No significant association was observed between groups (Chi-square  $P=0.7$ ; OR: 0.9, 95% CI: 0.4–1.9). WC: Waist circumference, OR: Odds ratio, CI: Confidence interval

leptin. Circulating FFA also gets deposited in other ectopic tissue (muscles) leading to increase in IR by the generation of lipid-derived second messengers such as diacylglycerol and ceramide which interfere with insulin receptors. In liver, FFA undergoes oxidation,  $\beta$ -oxidation and the remaining FFA is converted to triglycerides and is exported as very-low-density lipoprotein (VLDL). IR also cause defective VLDL assembly from the liver leading to triglyceride accumulation in liver and steatosis. Thus, IR and obesity potentiate each other in a vicious cycle. The WC of the cases was significantly higher which could also be explained by the above-said pathogenesis. WHtR has been suggested as a screening tool for NAFLD as it is considered a better tool for detecting visceral adiposity than WC,<sup>[26]</sup> but our study did not demonstrate any significant difference in WHtR between cases and controls ( $P = 0.9$ ). This finding requires further evaluation as to whether WHtR could be used as a screening tool for NAFLD among adult Keralites. Cases were having a significantly higher SBP than controls ( $P = 0.02$ ), and no significant difference was observed in DBP ( $P = 0.1$ ). The exact association between BP and NAFLD still remains elusive to the scientific community though association of pre-hypertension,<sup>[26]</sup> SBP and DBP,<sup>[25]</sup> and impairment in dipping of BP<sup>[27]</sup> has been shown to be associated with NAFLD. Obese insulin-resistant individuals are at high risk of developing hypertension due to the stimulation of endothelin-1 release and antidiuretic property of insulin<sup>[28]</sup> coupled with the overactive sympathetic nervous system results in hypertension.<sup>[28]</sup> MS also contributes to an increase in BP through oxidative stress, endothelial dysfunction, and increased inflammatory mediators. Animal studies have demonstrated hypertension in the presence of steatosis increases liver injury and fibrosis.<sup>[26]</sup> Our study did not demonstrate any significant association between NAFLD

and BMI when participants were categorized according to BMI for Asians<sup>[17]</sup> and when categorized as normal and elevated BMI. This could be an indicator of the magnitude of weight-related disorders among Keralites, or it could indicate that majority of the individuals who undergo voluntary health checkup have weight-related disorders. There was significant association between NAFLD and hypertension which would probably indicate the higher proportion of participants with Stage 2 hypertension having NAFLD. We did not find any association between NAFLD and other categories of BP abnormalities (pre-hypertension and Stage 1 hypertension), unlike the previous studies. We also did not find any association between NAFLD and abdominal obesity. This could indicate the high prevalence of pre-hypertension and Stage 1 hypertension and abdominal obesity among both cases and controls. Further community-based evaluation is required to assess the prevalence of weight-related disorders, abdominal obesity, and BP disorders among Keralites.

### Strengths and Limitation

Being a case-control study, it has its own merits and our study address one of the most relevant health issue faced by all age groups alike. Our study enrolled participants undergoing voluntary checkup, hence not a true representation of the community in terms of prevalence and burden.

### CONCLUSION

High prevalence of weight-related and BP disorders was seen in participants undergoing voluntary health checkup. NAFLD was associated with a significantly higher weight, BMI, height, SBP, and WC. Significantly higher Odds of NAFLD was seen with participants with Stage 2 hypertension.

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