Risk factors for Non-Alcoholic Fatty Liver disease among the patients admitted to a Teaching Hospital in Sri Lanka: Case Control study

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Research article

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Abstract

Background: The burden of Non-Alcoholic Fatty Liver Disease (NAFLD) was rising globally. It is the disease condition where there is an increased fat deposition (≥5%) in the hepatic cells. The objective of this study was to determine the risk factors for NAFLD among the patients admitted to a Teaching Hospital in Sri Lanka.

Methods: A case control study was conducted among the 106 cases with NAFLD and 212 unmatched hospital-based controls without having NAFLD. A case was defined based on the presence of fatty liver, according to the diagnostic ultrasonographic criteria. Three factors were used in diagnosing a patient with NAFLD. They include, increase echogenicity of the liver compared to kidney and spleen, obliteration of vascular architecture and deep attenuation of ultrasonic signals. Presence of at least two out of the above was considered as NAFLD. The data was collected by using an interviewer-administered questionnaire. The risk factors were determined by performing a multiple logistic regression and the results were expressed as adjusted odds ratios (AOR) and 95 % confidence interval (95% CI).

Results: Risk factors for NAFLD include consumption of fast food ≥2 time per week (AOR=9.34, 95% CI= 4.47-19.5), infrequent coffee consumption (AOR=2.78, 95% CI= 1.32-5.88), family history of liver disease (AOR=12.2, 95% CI= 2.16-67.9), methotrexate usage (AOR=32.0, 95% CI=1.45-tet707.3), inadequate physical activity (AOR=8.10, 95% CI= 4.04-16.3), having a high BMI value more ≥23Kg/m^2 (AOR=6.17, 95% CI=2.93-13.0) and being a Sinhalese (AOR=12.7, 95% CI=2.03-79.3).

Conclusion: Most of the risk factors were modifiable such as inadequate physical activity, overweight, infrequent coffee consumption and frequent consumption of fast-food. Primary preventive strategies for those modifiable risk factors should be implemented to prevent NAFLD.

Background

The fatty liver disease is a condition that results due to the accumulation of fat in the liver cells. The rate of fat accumulation depends on the balance between fat deposition and the removal of fat from the liver cells. Non-alcoholic fatty liver disease (NAFLD) is the condition where fat accumulates in hepatic cells without damaging those cells. It was not due to the causes for secondary fat accumulation such as alcohol consumption, uses of steatogenic medications and hereditary disorder such as hemochromatosis. The active form of this NAFLD is Steatohepatitis. It may progress to cirrhosis or its complications and may lead to an increase in premature deaths due to cardiovascular diseases.[1]

A review reported that the global prevalence of NAFLD is 25.24%.[2] The prevalence of NAFLD was 13.5% in Africa, 30. 4% South America whereas in Middle east it was 31.8%.[1] Another study in the United States showed that the NAFLD prevalence varied from 27% to 34%.[3] The prevalence of NAFLD among the Asian population was ranged from 5% to 40% based on ultra-sonographic study.[4] One Sri Lankan study

reported that the prevalence of NAFLD was 18% in 2011.[5] However, the true prevalence is still unknown due to the absence of accurate tests for the diagnosis.[6]

The major risk factors for NAFLD are metabolic factors such as obesity, dyslipidemia, hypertension and diabetes Mellitus.[7–11] Further, the socio-demographic risk factors such as old age, ethnicity, and being male, a higher level of education, higher income level, low physical activity were also recognized as risk factors for NAFLD.[4,5,8,10] One study indicated that the hematocrit level is significantly associated with fibrosis in NAFLD in patients in Europe.[12] There is also a hypothesis that states high level of haemoglobin is a factor associated with NAFLD.[13]

Most of the patients with fatty liver disease were asymptomatic and they were only diagnosed by ultrasonography. Recent disease patterns in Sri Lanka has shown the fact that there is an increase in the prevalence of non-communicable diseases among Sri Lankans in contrast to communicable diseases. Lifestyle pattern of Sri Lankans along with their unhealthy dietary patterns which has been a major cause for the above observation, may also serve as a contributory factor for NAFLD. As the cost of medical care for NAFLD is very high, especially at the diagnosis[14] aiming on prevention of NAFLD tends to be a much successful move than focusing on curative measures. Therefore, the objective of the study was to determine the risk factors for NAFLD among the patients admitted to a tertiary care Hospital in Sri Lanka.

Methods

This was a hospital-based unmatched case control study, conducted in the largest tertiary care hospital in Gampaha district during the period of 1st of June to 31st of August 2019. The hospital has four medical wards, five surgical wards, and three obstetrics and gynecology wards. The case group included the patients admitted to Medical, Surgical, and Gynecology wards, and who were newly diagnosed to have fatty liver disease or diagnosed with a fatty liver disease within the last six months or less by ultrasonography.

NAFLD was diagnosed based on established ultrasound findings which included increased echogenicity of the liver compared to kidney and spleen, obliteration of vascular architecture, and deep attenuation of ultrasonic signals. An individual was diagnosed as a NAFLD patients when he/she showed at least two of the above mentioned findings.[15] Four well-trained medicals officers at the radiology department in the hospital were involved in the diagnostic process. The control group included patients who were not diagnosed to be having fatty liver disease by ultrasonography. The exclusion criteria for both cases and controls were the patients who consumed alcohol above the safe limit including both current and former drinkers, pregnant women, pediatric age groups (≤14 years of age), patients who had been diagnosed with diseases such as hepatitis, cirrhosis, liver metastasis and liver cancers, cholestasis, alcoholic liver disease, and dengue hemorrhagic fever.

Sample size calculation was done considering odds ratio as 1.96 for elevated triglyceride for NAFLD,[11] the anticipated proportion of elevated triglyceride among controls as 46%, power as 80%, and with 5%

significant level. The control per case ratio was 2:1. According to the calculation, a minimum of 101 cases and 202 controls were needed. After adding 5% for the non-responders, it was decided to recruit 106 cases and 212 controls. Those who fulfilled inclusion criteria were recruited consecutively until the required sample size was achieved.

The study instruments were, a pre-tested interviewer-administered questionnaire (IAQ) and a record sheet. The IAQ has three parts; part 1- assessed socio-demographic factors, part 2- assessed medically related factors, and part 3- behavioral factors (Tea, Coffee, and Fast food consumption, Physical activity level, Alcohol consumption). The face and content validity of the questionnaire was assured. The validated global physical activity questionnaire (GPAQ) was used to access the physical activity levels.[16] The validity of the GPAQ ranged from low to moderately high (r =0.25-0.63).[17] Pretesting was done at a radiology unit of another hospital with the patients who were subjected to an abdominal ultrasound scan. The record sheet was used to record the hemoglobin level, height, and weight. The height was measured by using a standard measuring tape. The weight was measured by using a calibrated scale.

Data collection was done at the Radiology department of the hospital by the first author. Before the data was collected prior permission was obtained from the consultant radiologist. The ultrasound scan was done using 5MHz 50mm convex probe which was made in china.

The safe limit of alcohol consumption was assessed based on Asian standard values which were 14 units per week for men and 7 units per week for women.[18] One unit of alcohol is included in 25-30 ml of arrack or whiskey, 50ml of illicit alcohol, a half-pint of beer or toddy, 175ml glass of wine. Those were taken into account for calculating units of alcohol per week.[18] Hypertension, diabetic Mellitus, and dyslipidemia status were cross-checked with the Bed Head Tickets.

According to Body Mass Index (BMI) cut off values specified for Asians, an individual with a BMI value ≥ 23 kg/m2 was considered as overweight.[19] The physical activity level was assessed by using three types of activities. They include activity at work, physical activity involved travelling to and from places, and physical activities during recreational activities. It was calculated by using Metabolic Equivalent (MET). Moderate intensity physical activity of 150 minutes or Vigorous-intensity activity of 75 minutes or combination of moderate and vigorous-intensity physical activity achieving at least 600 MET minutes a typical week is essential to be regarded as an adequate level of physical activity. MET minutes were calculated in a case of combined activity as follows.

Moderate intensity activity MET min/week = 4.0 x Moderate intensity activity minutes x Moderate activity days. Vigorous-intensity activity MET min/week = 8.0 x Vigorous intensity activity minutes x Vigorous-intensity activity days and Walking/cycling MET min/week equals 3.3 x walking/cycling minutes x Walking/Cycling days.[16] If the participant has not fulfilled the above criteria it was taken as an inadequate level of physical activity .

The frequency of tea consumption was divided into two groups. Consumption of >4 cups per day and consumption of ≤4 cups per day. Coffee consumption was classified as ≥1 cups per day or none use per day.[11,20] Hamburgers, hot dogs, pizza, fried chicken, french fries, processed fish, instant noodles, and pastries were classified under fast food. The frequency of fast food consumption was divided as ≥2 time and the <2 time per week.[21] The average hemoglobin value among Asian women was 11 to 13 g/dl and among men, it was 13 to 15 g/dl. In females, hemoglobin level greater than 13g/dl is considered as High Blood Hemoglobin Level whereas in males hemoglobin level greater than 15g/dl is considered as High Blood Hemoglobin Level . Familial risk was determined based on the self-reported health history for first- and second-degree relatives. A patient was considered to be having a family history for a liver disease if any family member either from maternal or paternal side had suffered or is having an any kind of a liver disease condition such as fatty liver, cirrhosis, NASH, etc.

The statistical analysis was done by using SPSS 16 version. The results were expressed as odds ratios (OR), 95% confident intervals (95% CI), and the p values. The variables with p-value <0.2 in the bivariate analysis were selected for the multiple logistic regression. The purposeful selection was used. Hosmer and Lemeshow test was used as the test for goodness of fit and it was observed as satisfactory (chi-square 4.48, p value=0.61)

The permission was taken from the Director of the hospital and the consultants and the interview was conducted after obtaining the informed written consent of the patients. Further, measures were taken to minimize the disturbances to the routine ward works. The data collection was done by avoiding the routine daily ward round times. Ethical clearance was taken from the Ethics Review Committee at the Postgraduate Institute of Medicine, Colombo.

Results

The mean age of the study population was 46.46 years with a SD of 15. The range was 58 years which spanned between a minimum age of 17 years to a maximum age of 75 years. Percentage of males among the cases were 60.4% (n=64) and among the controls, it was 45.8% (n=97). Most of the participants were Sinhala (n=297, 93.4%) and Buddhist (n=210, 66.0%). Among the study population, 250 (78.6%) participants were married and 68 (21.4%) were single.

Being male, being Sinhalese, having higher income level and higher educational level showed a statistically significant positive association with NAFLD. (Table 1)

Table 1 Association between socio-demographic factors and NAFLD

		Cases		Controls		OR	
		n	%	n	%	(95% CI)	p value
Male		64	60.4%	97	45.8%		
Female		42	39.6%	115	54.2%	1.81 (1.12-2.90)	0.014
Age >45 Years		57	53.8%	119	56.1%		
Age ≤45 Years		49	46.2%	93	43.9%	0.91 (0.57-1.45)	0.69
Paid Employed		64	60.4%	105	49.5%		
Unemployed		42	39.6%	107	50.5%	1.55 (0.97-2.50)	0.068
Sinhalese		104	98.1%	193	91.0%		
Non-Sinhalese		2	1.9%	19	9.0%	5.12 (1.17-22.4)	0.017
Buddhist		74	69.8%	136	64.2%		
Non-Buddhist		32	30.2%	76	35.8%	1.92 (0.78-2.13)	0.315
Income Rs.							
≥10,000	<10,000	92	86.8%	164	77.4%		
		14	13.2%	48	22.6%	1.92 (1.01-3.68)	0.045
Married		86	81.1%	164	77.4%		
Unmarried		20	18.9%	48	22.6%	1.26 (0.70-2.25)	0.439
Education							
> Ordinary Level		38	35.8%	52	24.5%		
≤ Ordinary Level		68	64.2%	160	75.5%	1.72 (1.04-2.85)	0.035
Total		106	100%	212	100%		

*OR-Odds ratio **CI-Confidence Interval

Presence of a family history of liver diseases, use of methotrexate, higher hemoglobin level also showed a statistically significant positive association with NAFLD. (Table 2)

Table 2 Association between medically related factors and NAFLD

Medically related factors	Cases		Controls		OR	
	n	%	n	%	(95% CI)	p value
Hypertension						
Yes	24	22.6%	52	24.5%	0.90 (0.52-1.56)	0.710
No	82	77.4%	160	75.5%		
Diabetes Mellitus						
Yes	28	26.4%	45	21.2%	1.33 (0.77-2.29)	0.03
No	78	73.6%	167	78.8%		
Hyperlipidemia						
Yes	26	24.5%	35	16.5%		
No	80	75.5%	177	83.5%	1.64 (0.93-2.91)	0.087
Family History of liver diseases Yes						
No	13	12.3%	2	0.9%		
	93	87.7%	210	99.1%	14.68 (3.25-66.3)	<0.001
Haemoglobin level						
Higher	37	34.9%	55	25.9%		
Lower	69	65.1%	157	74.1%	1.53 (0.92-2.53)	0.097
Used methotrexate						
Yes	10	9.4%	1	0.5%		
No	96	90.6%	211	99.5%	21.9 (2.77-174.1)	<0.001
Total	106	100%	212	100%		

*OR-Odds ratio **CI-Confidence Interval

Consuming >4 cups of tea per day, ≥1 cup of coffee per day, consumption of fast food ≥2 times per week, inadequate levels of physical activities, BMI ≥23Kg/m2, and consuming alcohol within the safe limit showed a statistically significant positive association with NAFLD. (Table 3)

Table 3 Association between behavioral factors and NAFLD

Behavioral factors	Cases		Controls		OR (95% CI)	p value
	n	%	n	%		
Physical activity						
Inadequate	84	79.2%	53	25%	11.45 (6.52-20.1)	<0.001
Adequate	22	20.8%	159	75.0%		
No of Teacups						
>4 cups per day	12	11.3%	1	0.5%	26.9 (3.45-210.1)	<0.001
≤4 cups per day	94	88.7%	211	99.5%		
No of Coffee Cups						
Not consumed	77	72.6%	110	51.9%	2.46 (1.48-4.08)	<0.001
≥1 cups per day	29	27.4%	102	48.1%		
Fast food Consumption ≥2 time per week	76	71.7%	49	23.1%	8.42 (4.96-14.3)	<0.001
<2 time per week	30	28.3%	163	76.9%		
Alcohol within the safe limit						
Never used	49	46.2%	64	30.2%	1.98 (1.23-3.21)	0.005
	57	53.8%	148	69.8%		
Body Mass Index						
≥23Kg/m^2	73	68.9%	78	36.8%		

<23Kg/m^2	33	31.1%	134	63.2%	3.80 (2.31-6.25)	<0.001
Total	106	100%	212	100%		

*OR-Odds ratio **CI-Confidence Interval

As shown in Table 4, the fast-food consumption ≥2 times per week is a risk factor for NAFLD and it has 9.34 times more risk of getting NAFLD to compare to those who consume fast foods <2 time per week. The none consumption of coffee was a risk factor for NAFLD and it has a 2.78 times more risk of getting NAFLD compared to the coffee consumers. Therefore, coffee consumption was a protective factor for NAFLD. The inadequate exercise level was also a risk factor for NAFLD. There was a 8.10 times more risk of getting NAFLD among the people with inadequate physical activity level in contrast to those who were engaged adequate exercise level. The BMI ≥23Kg/m^2 was a risk factor for NAFLD. Individuals with a BMI ≥23 has a 6.17 times more risk of getting NAFLD compared to individuals with a BMI <23Kg/m^2. The family history of liver disease was a risk factor for NAFLD. There was a 12.2 times more risk of getting NAFLD among those who had a family history of liver disease compared to those who had not a family history of liver disease. Methotrexate usage was a risk factor for NAFLD. It has a 32 times more risk of getting NAFLD among the methotrexate users to compare to none users. Being a Sinhalese also a risk factor for NAFLD. There was a 12.0 times more risk of getting NAFLD among the Sinhala ethnicity group compared to the non-Sinhala ethnicity group.

Table 4 The risk factors for NAFLD according to the multiple logistic regression

Risk factors	B co-efficient	SE*	AOR**	95% CI***	p value
Consumption of Fast Foods ≥2 times/week	2.23	0.37	9.34	4.47-19.5	<0.001
None use of coffee	1.02	0.38	2.78	1.32-5.88	0.007
Inadequate physical activity	2.09	0.35	8.10	4.04-16.3	<0.001
≥23Kg/m^2 Body Mass Index	1.82	0.38	6.17	2.93-12.9	<0.001
Family history of Liver Disease	2.50	0.88	12.22	2.16-68.9	0.005
Methotrexate usage	3.46	1.57	32.02	1.45-707.3	0.028
Sinhalese ethnicity	2.54	0.93	12.70	2.03-79.33	0.007

*SE-Standard Error **AOR- Adjusted Odds Ratio ***CI-Confident Interval

Hosmer and Lemeshow Test (Chi square value=4.48 p value=0.61)

Discussion

The risk factors for NAFLD were increased frequency of fast food consumption, none usage of coffee, inadequate exercise level, high BMI value ≥ 23kg/m^2, having a family history of liver disease, usage of methotrexate, and being a Sinhalese.

In the present study, the increased frequency of fast food consumption had 9.4 times more risk of getting NAFLD to compare to less fast food consumers. The findings are compatible with past literature as well.[21] One study reported that fast foods induced NAFLD in mice.[22] A review had also reported that NAFLD patients have shown better outcomes with low carbohydrate and low-fat diet.[23]

There was a 2.8 times more risk of getting NAFLD among those who do not consume coffee compare to those who consume coffee. A systematic review revealed that coffee contains caffeine and it had a positive effect on the NAFLD.[20] Another two studies reported that coffee consumption is associated with a significant reduction in the risk of fibrosis among NASH patients.[24,25] Coffee reduces NAFLD prevalence.[26] Several possible mechanisms underlying in this is that coffee includes anti-oxidative, anti-inflammatory, and anti-fibrotic effects.[26,27]

There was 8.1 times more risk of getting NAFLD among the participants with less physical activity level compared to the participants who are having an adequate physical activity level. These findings are consistent with another study.[28] One study reported that longer sitting time was associated with a higher prevalence of NAFLD.[29] According to a randomized control trial, exercise programs reduced fatty liver index and NAFLD-fibrosis score among the NAFLD patients.[30] According to a meta-analysis, dose-dependent increase levels of physical activity leads to a reduced risk of NAFLD.[31] Another case-control study found that among men physical activity was inversely associated with the risk of NAFLD in a dose-dependent manner.[32] One review emphasized the independent effect of physical activity, including aerobic exercise in the treatment of NAFLD.[33] Exercise increases fatty acid oxidation, decreases fatty acid synthesis, and prevents mitochondrial and hepatocellular damage.[34]

Individuals with a high BMI (≥23 Kg/m^2) value has a 6.17 times more risk of getting NAFLD compared to the low BMI value. Therefore, this study finding is also consistent with the other findings where there was a statistically significant association of high BMI values with the NAFLD. [5,21,35–38]

In the present study, the family history of liver disease was found as a risk factor for NAFLD. It had a 12.2 times risk of getting NAFLD among the participants who had a family history of liver disease compared to the participants who did not have a family history of liver disease. Also these findings were supported

with other findings as well.[39,40] The methotrexate is a drug used to treat arthritis. Several studies showed that there is a statistically significant association between methotrexate usage and NAFLD.[41,42]

In the present study the high hemoglobin value, increased frequency of tea consumption, consumption of alcohol above the safe limit, high-income level, high education level, and being a male showed a statistically significant association with NAFLD according to the bivariate analysis. In this present study, high hemoglobin value is not a risk factor for NAFLD. However, several studies reported that high hemoglobin value had a statistically significant association with NAFLD.[43] In Sri Lanka most people don't consume plain tea, they usually add milk and sweeteners to the tea. One study had showed that adding milk or sweeteners to the tea would reduce the antioxidant capacity of the tea and thus was not protective against NAFLD.[44] This could be the reason for the observation of frequent tea consumption as a significant positive association with NAFLD according to the bivariate analysis. However, the wider confidence interval indicates an inadequacy of the sample size. According to the present study, higher income and higher educational levels are not risk factors for NAFLD. Based on two previous findings, there had been a statistically significant association of high-income level with NAFLD [5,38] and higher education with the NAFLD.[38]

The gold standard method to diagnose NAFLD was a liver biopsy. The sensitivity of a liver biopsy to diagnose fatty liver disease is 90% and the specificity is as high as 90%. However, in the present study diagnosis of the NAFLD mainly depended on the ultrasound scan[44] even though the sensitivity of ultrasound diagnosis of fatty liver disease is 71.7% and the specificity is 75.9%.[45] This is a major limitation of the study. Even though the most suitable representative sample for controls could be selected from the community, it was costly and time-consuming. This is another limitation. Recall bias is a another inherent limitation of the case-control studies when assessing the exposures especially when assessing physical activities and past history of liver diseases. Even though we excluded patients who consumed alcohol above the safe limit, their accuracy of the information may not be acceptable. The alcohol consumers who consume above the safe limit could be misclassified under safe drinkers as they might not tell the truth. In the present study hypertension, diabetic mellitus, and dyslipidemia were diagnosed by using the recent health records of the patients. Therefore, it might lead to misclassification of the disease conditions. Further it had been better to if the values were measured without using health the records. The confidence intervals are wider for some risk factors even though we found that results were statistically significant, which indicate the sample size is not adequate.

Conclusions

Most of the risk factors for NAFLD are modifiable. These include fast food consumption, inadequate physical activity, high BMI value, infrequent consumption of coffee, and usage of methotrexate. Consumption of fast food is rising especially among the younger generation in Sri Lanka. Higher academic competition among students in Sri Lankan schools can be a major reason for the inadequacy in physical activity level. Further, case control and cohort studies with a larger sample size is required to

determine the effect of tea consumption and hemoglobin level for NAFLD. Also awareness programs could be implemented at school, workplace, and community level as primary preventive measures.

List Of Abbreviations

BMI - Body Mass Index

CI - Confidence Interval

GPAQ - Global Physical Activity Questionnaire

IAQ - Interviewer-Administered Questionnaire

MET - Metabolic Equivalent

NAFLD - Non-Alcoholic Fatty Liver Disease

OR - Odds Ratios

SD – Standard Deviation

Declarations

Ethics approval and consent to participate – The Ethics Review Committee of the Postgraduate Institute of Medicine, Colombo granted ethical clearance. Informed written consent was obtained before data collection.

Consent for publication – Not applicable

Availability of data and materials - The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest – “The authors declare that they have no competing interests" in this section.

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Authors' contributions – AN participated in the design of the study, coordinated data collection, and helped to draft the manuscript. CA participated in the design of the study, performed the statistical analysis, interpreted the data, performed the statistical analysis, and drafted the first version of the manuscript. All authors read and approved the final manuscript.

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