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Non-alcoholic Fatty Liver Disease Assessed by FIBROSCAN-AST (FAST) Score and Its Association with Colorectal Carcinoma Risk: A Case-Control Study

Jishnu J^{a++*}, Kandasamy Kumar E^{a#}, Poppy Rejoice^{a†}, Shafique A^{a‡} and Geetha D^{a‡}

^a Department of Medical Gastroenterology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is an entity with its evident role in systemic inflammation and tumorigenesis. Notably, the association between colorectal cancer (CRC) and NAFLD identified by the FibroScan-AST (FAST) score allows the assessment of steatosis and fibrosis as risk factors in CRC patients. However, there is a lack of studies to date.

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⁺⁺ Senior Resident;

[#] Professor and Head;

[†]Associate Professor;

[‡] Assistant Professor;

^{*}Corresponding author: E-mail: jj.pbhavan@gmail.com;

Objective: To determine whether NAFLD, identified by FAST scoring, is associated with an increased risk of colorectal cancer.

Methods: This was a case-control study where the case group involved patients with colorectal cancer before initiating chemotherapy, and the elapsed time between the Vibration-controlled transient elastography exam by Fibroscan and blood collection was less than 6 months, whereas the control group was without any documented chronic liver disease or prior malignancies. The demographic and clinical details of the patients were recorded for each patient. The quantitative values of steatosis and fibrosis were measured according to the controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), both measured using FibroScan and the FAST score calculated. Statistical analyses were performed using SPSS software version 25.

Results: The mean age was 53.7 years in the case group and 50.8 years in the control group. There were 37 males and 16 females in the case group, whereas there were 33 males and 20 females in the control group. About 77.3% of CRC and 71% of the control population had steatosis, defined by CAP \geq 238 dB/m (p=0.23). About 68% of the case group and 50% of the control group had fibrosis, defined by LSM \geq 7.5 kPa (p=0.02). With rule-in cut off value for FAST score as \geq 0.78, 63.4% of the case group and 36.6% of the control group had NASH with a statistically significant p-value of 0.045. Overall, it was found that 61.9% of the CRC patients were positive for NAFLD, whereas 30.1% of the patients found negative for NAFLD. The odds ratio for the association of NASH in CRC patients was 3.12, Cl 1.27-7.58, p=0.0015.

Conclusion: It was noted that there was a relationship between NAFLD and CRC, with 61.9% of the colorectal cancer patients shown positive for NAFLD detected by FAST scoring.

Keywords: Non-alcoholic fatty liver disease; colorectal carcinoma; FibroScan; FAST scoring; correlation.

1. INTRODUCTION

"The entity of non-alcoholic fatty liver disease (NAFLD) comprises a spectrum from simple to non-alcoholic steatohepatitis steatosis (NASH), and it can progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The global prevalence of NAFLD is estimated to be 25%" [1], with a higher prevalence seen in the Middle East and South America and the lowest in Africa. The prevalence of NASH is estimated to be 1.5%-6.5% [1]. "The prevalence of adult NAFLD in India has been reported between 6.7% and 55.1% [2]. Out of all cases with an asymptomatic elevation of liver enzymes, NAFLD may be responsible for almost one-third" [3].

"The concept that several pathways cause carcinogenesis and the development of HCC in NAFLD is supported by clinical, observational, and epidemiological studies. According to molecular studies on human HCC tissue and animal models of HCC associated with NAFLD, the development of carcinoma has been linked to substantial alterations in hepatocyte biology as well as systemic and local immunologic, endocrine. and metabolic pathways" [4]. Furthermore, "explant histology data from liver transplant centres suggest that two-thirds of the 'cryptogenic' patients with cirrhosis had NAFLD" [5].

"Colorectal cancer is the third most prevalent cancer and the second most common cause of cancer-related mortality worldwide, respectively" [6]. "Colorectal adenoma is 1.5 times higher in patients with NAFLD than in the general population" [7, 8]. "This implies that the importance of NAFLD in colorectal tumorigenesis is increasing. A previously postulated mechanism of the relationship between NAFLD and colorectal tumorigenesis is that the pro-inflammatory status, presence of metabolic syndrome. increased insulin and decreased adiponectin in resistance. patients with NAFLD may be associated with an increased risk of colorectal adenoma" [9]. "The NAFLD is regarded as a chronic systemic inflammatory state characterised by increased inflammation. This chronic inflammatory condition or secretory molecules such as reactive oxygen species, interleukin (IL)-6, and tumour necrosis factor (TNF)- α could be associated with the occurrence of colorectal adenoma" [7]. However, the exact mechanism underlying the close association between NAFLD and colorectal tumorigenesis remains unclear.

"Liver biopsy is considered the gold standard diagnostic tool for evaluating hepatic steatosis and fibrosis. However, it is an invasive technique and can cause complications, such as massive bleeding. Abdominal ultrasonography is the standard modality for evaluating NAFLD: however, it is a subjective procedure and thus has limitations due to inter-observer variation" "FibroScan is a novel modality for [10]. evaluating fatty liver disease and has the advantages of convenience, reproducibility, and non-invasiveness" [11]. "It measures a novel, non-invasive value called the controlled attenuation parameter (CAP) by acquiring a considerable part of the liver volume. The accuracy of CAP in the evaluation of hepatic steatosis has been examined in many studies, including the studies of NAFLD" [12].

"Recently, Newsome et al. derived the FibroScan-AST (FAST) score in a cohort of patients with NAFLD from England and validated it in multiple international cohorts. The FAST score clearly demonstrated an impressive AUROC of 0.74–0.95 for detecting patients with NASH. elevated NAFLD activity score (NAS \geq 4). and significant fibrosis (≥F2) on liver biopsy. By incorporating serum AST with FibroScan-derived parameters of LSM and CAP, the FAST score allows the simultaneous assessment of steatosis (CAP), inflammation (AST), and fibrosis (LSM)" [13]. However, no studies on the relationship between NAFLD diagnosed by FibroScan-AST (FAST) score and colorectal cancer have been conducted to date. Thus, this study aimed to determine the relationship between the incidence of colorectal cancer and NAFLD detected by FAST scoring as a risk factor.

2. METHODOLOGY

This was a case-control study that was conducted in the Department of Medical Gastroenterology at the Government Superspecialty Hospital, Tirunelveli, Tamil Nadu from January 2023 to January 2024. The inclusion criteria were individuals over 18 years of age with biopsy-proven colorectal cancer before initiating chemotherapy and the elapsed time between the VCTE exam by Fibroscan and blood collection is less than 6 months. The exclusion criteria were the presence of liver metastasis, history of inflammatory bowel disease and history of chronic hepatitis B and C, liver cirrhosis of any cause, alcoholic liver disease, or autoimmune hepatitis. The control group included adults over 18 years of age attending the Gastroenterology outpatient ward without any documented chronic liver disease or prior malignancies. The scientific and ethical committee of the institution approved the study

and all the patients included in the study provided written informed consent.

The sample size was calculated in openepi.com using Fleiss/ Kelsay sample size formula for case control studies. Keeping the alpha error as 0.05 (confidence level = 95%), power as 80%, ratio of cases to control as 1 and with Odd ratio of NAFLD in Colorectal CA as 3.31 (from previous study [13]), the total sample size calculated was 106 (53/group).

"The demographic details such as age, sex, body mass index (BMI), and presence of hypertension, diabetes. and hypercholesterolaemia were recorded for each patient. Smoking and drinking habits were documented. The quantitative values of steatosis and fibrosis were measured according to CAP and liver stiffness measurement (LSM). respectively, both measured using FibroScan 502 Touch device equipped with an M probe (Echosens, Paris, France). Patients were asked to fast for at least 3 hours before the examination. Patients were placed in the supine position with their right arm fully abducted, and measurements were done by scanning the right liver lobe through an intercostal space. CAP is an average estimate of ultrasound attenuation at 3.5 MHz and is expressed in dB/m. LSM by Vibration-controlled transient elastography (VCTE) is an average estimate of stiffness (Young's modulus) at a shear wave frequency of 50 Hz and is expressed as kPa. Only examination results with at least ten valid individual measurements were deemed valid" [10].

The severity of steatosis was graded as normal, mild, moderate, and severe according to the CAP score defined by the cut-off value of FibroScan; these were marked as normal, S1, S2, and S3, respectively. The cut-off values of the CAP score were taken as ≥ 238 dB/m, ≥ 260 dB/m and ≥ 290 dB/m for S1, S2 and S3, respectively. Under the value of S1 grades were described as normal. The grade of fibrosis was defined by the cut-off values of LSM by Fibroscan: the values are ≥ 7.5 kPa, ≥ 10 kPA and ≥ 14 kPa for F2, F3 and F4, respectively. The value of <7.5 kPa was described as F0-1.

All patients underwent routine blood tests including a complete haemogram, liver function test, lipid profile and viral markers. USG/CT abdomen reports were included in the data. The FAST score was calculated using the equation:

 $\frac{[e^{-1.65+1.07 \times ln (LSM)+2.66*10^{-8} \times CAP^{3}-63.3 \times AST^{-1})]}{[1+e^{-(-1.65+1.07 \times ln (LSM)+2.66*10^{-8} \times CAP^{3}-63.3 \times AST^{-1})]}$

as carried out by Newsome et al. [12] The optimal rule-out (FAST: ≤ 0.55) and rule-in (FAST: ≥ 0.78) cut-offs were applied.

Statistical analysis: Continuous variables were expressed as the means with standard deviations or as percentages, as appropriate. Categorical variables were expressed as numbers or as percentages and were compared using the student 't' test or Fisher's exact test, as appropriate. The comparison of the association between CAP, LSM, AST and FAST score values between the case and control groups was done by the Mann Whitney U test. All statistical analyses were performed using the Package for Statistical Social Sciences (SPSS) software of version 25. Statistical significance was defined as a two-sided p-value of <0.05.

3. RESULTS

Basic characteristics and risk factors of study participants: The mean age was 53.7 years in the case group and 50.8 years in the control group. Also, the mean height and weight

in the case group were 162.3 cm and 63.3 kg while it was 160.6 cm and 63.2 kg in the control group respectively. In addition, the mean BMI was 24.1 in the CRC group and 24.5 in the control group (Table 1). There were 37 males and 16 females in the case group, whereas 33 males and 20 females in the control group. The presence of comorbidities such as hypertension, diabetes mellitus, dyslipidemia and social history were described in Table 2.

Comparison of CAP, LSM, AST and FAST score values between the case and control groups: The median CAP value was 283 dB/m in the case group and 290 dB/m in the control group (Fig. 1). Further, the median LSM values were 13 kPa and 8 kPa for the case and control groups, respectively which was statistically significant (P=0.002) (Fig. 2). The median AST was higher in the case group of 76 IU/L and 60 IU/L in the control group which was also statistically significant (p=0.013) (Fig. 3). There was an increased median FAST score in the CRC group of 0.78 and for the control group of 0.52 which was statistically significant of P value of 0.004 (Table 3).

Table 1. Comparison of baseline characteristics between the case and control groups

Variables	Contr	ol (n=53)	Cas	Case (n=53) t value		p value
	Mean	SD	Mean	SD		
Age (years)	50.8	10.1	53.7	10.2	1.46	0.147
Height (cm)	160.6	4.7	162.3	5.5	1.72	0.087
Weight (Kg)	63.2	5.2	63.3	6.3	0.05	0.96
BMI (Kg/m ²)	24.5	2.2	24.1	1.8	1.36	0.176

Variables		Control		(Case	Fisher's	p value
		n	%	n	%	statistic	-
Gender	Female	20	55.6	16	44.4	0.673	0.539
	(n=36)						
	Male (n=70)	33	47.1	37	52.9		
Diabetes	No (n=67)	31	46.3	36	53.7	1.01	0.421
	Yes (n=39)	22	56.4	17	43.6		
Hypertension	No (n=71)	37	52.1	34	47.9	0.384	0.681
	Yes (n=35)	16	45.7	19	54.3		
Dyslipidemia	No (n=61)	33	54.1	28	45.9	0.965	0.432
	Yes (n=45)	20	44.4	25	55.6		
Smoking	No (n=67)	32	47.8	35	52.2	0.365	0.687
	Yes (n=39)	21	53.8	18	46.2		
Alcoholism	No (n=62)	30	48.4	32	51.6	0.155	0.844
	Yes (n=44)	23	52.3	21	47.7		

Parameters	Control (n=53)		Cas	e (n=53)	Mann	p value
	Median	IQR	Median	IQR	Whitney U test	-
CAP (dB/m)	290	217 - 332	283	241 - 315	1273.5	0.408
LSM (kPa)	8	5 - 11	13	7 - 49	1895.5	0.002*
AST (ÌU/L)	60	46 - 93	76	62 - 92	1796.5	0.013*
FAST score	0.52	0.32 – 0.78	0.78	0.5-0.9	1861.5	0.004*

Table 3. Comparison of CAP, LSM, AST and FAST score values between the case and control groups

*P<0.05, significant



Fig. 1. Comparison of median CAP value between case and control group



Fig. 2. Comparison of median LSM value between case and control group



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Fig. 3. Comparison of median AST values between case and control group





Table 4. Comparison of hepatic steatosis and fibrosis between case and control groups

Parameter		С	Control		Case	Fisher's	df	p value
		n	%	n	%	statistic		
Hepatic	Normal (n=28)	15	53.6	12	44.4	4.3	3	0.23
steatosis	S1 (n=9)	2	22.2	8	77.8			
	S2 (n=19)	9	47.4	10	52.6			
	S3 (n=50)	27	54	23	46			
Hepatic	F0-F1 (n=43)	26	60.5	17	39.5	9.42	3	0.02*
fibrosis	F2 (n=19)	12	63.2	7	36.8			
	F3 (n=7)	4	57.1	3	42.9			
	F4 (n=37)	11	29.7	26	70.3			

*P<0.05, significant

Fast score category	Control		Case		Fisher's statistic	p value
	n	%	n	%		
NAFLD negative	27	64.3	15	35.7	6.42	0.045*
Indeterminate	11	47.8	12	52.2		
NAFLD positive	15	36.6	26	63.4		

Table 5. Comparison of NAFLD occurrence (based on FAST score category) between case and control groups

*P<0.05, significant

Severity of hepatic steatosis and fibrosis between case and control groups: About 77.3% of CRC (41/53) and 71% of control population (38/53) had steatosis, defined by CAP \geq 238dB/m (p=0.23). About 68% of the case group (36/53) and 50% of the control group (27/53) had fibrosis, defined by LSM \geq 7.5kPa (p=0.02) (Table 4).

Association of NAFLD in colon cancer: With the rule-in cut-off value for FAST score as ≥ 0.78 , 63.4% of the case group and 36.6% of the control group had NASH with a statistically significant of p-value of 0.045. Also, 52.2% of the patients in the case group had intermediate NASH while 47.8% of patients in the control group had intermediate NASH (Table 5). Overall, it was found that 61.9% of the CRC patients shown positive for NAFLD whereas 30.1% of the patients shown negative for NAFLD (Fig. 4). The odds ratio for the association of NASH in CRC patients was 3.12, CI 1.27-7.58, p=0.0015 which was statistically significant.

4. DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide. Non-alcoholic steatohepatitis (NASH) is the more active form of NAFLD and is characterised by the presence of hepatic steatosis. inflammation, and hepatocyte ballooning. NASH is emerging as one of the causes of cirrhosis, leading cirrhotic complications, hepatocellular carcinoma (HCC) and liver-related death [14]. "Studies have shown that, apart from HCC, NAFLD is also associated with other types of gastrointestinal cancers, including colorectal cancer (CRC) [15]. Around 18%-24% and 9%-20% of advanced colon neoplasm occurs in NAFLD men and women respectively compared to 9%-15% and 3%-8% in non-NAFLD men and women respectively" [8].

"In this study, the mean age in the case group and the control group were 53.7 years and 50.8 years which highlighted that disease prevalence across the middle age groups. Further, there were majority of male patients in both groups which showed the male predominance in this study. According to FibroScan, the population was first classified as having steatosis. The steatosis group consisted of people with S1 or more steatosis, whereas the normal group consisted of subjects with normal values. Age, sex, height, weight, smoking, comorbidities, smoking and alcohol intake did not significantly differ across groups. This was in line with Kim KW et al., however, they found significant differences noted in BMI and alcohol intake among CRC patients with fatty liver disease" [16]. In addition, there were statistically significant higher median AST levels in the case group which correlates with the existence of NASH.

Moreover, one of the major constraints in understanding NAFLD is the absence of a disease-specific biomarker. By incorporating serum AST with fibroscan- derived parameters of LSM and CAP, the FAST score allows the simultaneous assessment of steatosis. inflammation and fibrosis. In our study, with rulein FAST score of ≥ 0.78 . 63.4% of the colorectal cancer group and 36.6% of the control group had NASH with a statistically significant p-value of 0.045. "It suggests that steatohepatitis may be associated with colorectal tumorigenesis. It is evident from the previous studies that fat accumulation in hepatocytes can lead to an inflammatory state in the liver" [17]. "Hepatic steatosis can further accelerate or induce disarray of the gastrointestinal tract via proinflammatory cytokines that promote tumorigenesis in the colon" [16].

Fibroscan also has the advantage of providing more information than liver biopsy by acquiring images of a larger liver volume and being noninvasive, requiring only a probe to touch the skin. It helps in early detection of the disease and identifying patients with NASH who are at high risk of progressive disease and who are likely to benefit by the emerging therapies in NASH [18]. "Fibroscan and FAST scoring system can be utilised as a good screening test for NAFLD in the general population and thereby, identify patients in their early disease course. This may help to actively intervene before they develop disease complications, including various malignancies" [19]. Also, FibroScan requires relatively little operator training when compared to other elastographic procedures [20].

Our study points towards the importance of NAFLD diagnosis in its earlier stages and considers the utilisation of Fibroscan and its derivative Scoring systems in the screening programs. However, our study had some limitations. First, this was a single-centre study, and thus, the results may have limited generalizability. Secondly, the study population was not large enough and we could not evaluate other confounding factors such as abdominal visceral adiposity and show more clear statistical values in various aspects of colorectal neoplasia. Third, there was very few data about the stages of fibrosis and its statistical significance in the tumorigenesis process. Finally, we chose only one of the reference values for Fibroscan that have been previously reported in the validatory study. There are various reference values of CAP and LSM for assessing hepatic steatosis and fibrosis. It means that the cut-off values could vary due to many factors such as countries, ethnicity, statistical methods, underlying clinical characteristics and so on. Further large-scale prospective studies are required to confirm our results and the effect of managing hepatic steatosis in reducing colorectal tumorigenesis.

5. CONCLUSION

In this study, it was found that 61.9% of the colorectal cancer patients showed positive for NAFLD detected by FAST scoring. Therefore, clinicians should be aware of the relationship between colorectal cancer and NAFLD and its clinical implications. The FAST score reduces the need for unnecessary liver biopsies in patients who are unlikely to have serious illness by offering an effective non-invasive method of identifying patients at risk of developing NASH for clinical trials or treatments when they become available.

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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