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Diagnostic value of alcoholic liver disease (ALD)/ non alcoholic fatty liver disease (NAFLD) index (ANI) in differentiating alcoholic liver disease from non alcoholic fatty liver disease

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Abstract
Background and objectives
There are limited tools to differentiate alcoholic liver disease (ALD) from non-alcoholic fatty liver disease (NAFLD). Liver biopsy despite being the gold standard is invasive while the easily available biochemical indices lack sensitivity and specificity. ANI (ALD/NAFLD Index) developed in 2006 by Dunn *et al*. proved to be an efficient yet non-invasive diagnostic tool to differentiate ALD from NAFLD. The aim of this study is to validate the efficiency of this index in the Indian population.
Methods
A Cross sectional observational study was carried out on a group of 48 inpatients of a tertiary care hospital in Vadodara, Gujarat out of which 24 patients were diagnosed with ALD and 24 with NAFLD. Case study forms were filled with relevant clinical history, examination results and investigations from their recent blood samples. Ultrasonography reports were accessed. Using this data the ANI score was calculated on an online calculator and appropriate statistical tests were applied.
Results
The value of ANI in ALD patients is significantly higher than that in NAFLD patients (Median of ALD= 9.2485 vs Median of NAFLD= -2.651) (P<0.001). The cut-off value for ANI was calculated to be >1.702, for which the index shows high sensitivity (95.83%) and specificity (83.33%).
Interpretation and conclusions
ANI scoring system proved to be an efficient diagnostic tool in the Indian population with a high sensitivity and specificity at the cut-off value of >1.702. Although it cannot replace liver biopsy as the gold standard, it has a better diagnostic performance compared to currently used serological markers like AST/ALT and MCV in differentiating ALD patients from NAFLD patients.

Keywords: alcoholic liver disease, ANI score, AST/ALT ratio, MCV non-alcoholic fatty liver disease

Introduction
Fatty liver disease is a condition in which fat builds up in the liver cells. It is one of the most frequently seen liver pathologies. It can manifest either as alcoholic liver disease (ALD) which is caused by excessive alcohol consumption or non-alcoholic fatty liver disease (NAFLD) which is mainly caused by obesity/ insulin resistance [1].

With changes in the lifestyle, the prevalence of alcoholic and non-alcoholic fatty liver is on the rise, with 20-30% of adult population suffering from NAFLD [2] and 9-32% of the Indian population being affected with NAFLD [3]. The incidence of alcohol related liver injury is also alarming in India especially in recent times [4]. It becomes mandatory to differentiate between ALD and NAFLD because of the different therapeutic approaches to these diseases [5].

Various serological markers were independently used to differentiate between ALD and NAFLD including aspartate transaminase/ alanine transaminase (AST/ALT) ratio [6, 7], mean corpuscular volume (MCV) [8], and new parameters including Mitochondrial aspartate transferase isoenzyme (mAST), serum carbohydrate- deficient transferrin (CDT) and serum gamma-glutamyl transpeptidase assey (GGT) [9-11]. However, they proved to have low sensitivity and specificity. Moreover, Ultrasonography is a reliable technique to detect the degree of fatty changes in the liver, but it cannot differentiate the etiology of hepatic steatosis [12]. Liver biopsy is the gold standard is differentiating ALD from NAFLD [13]. However, being an invasive procedure, it has a limited application. It can cause several complications especially in patients with risk of bleed [14].

Hence, in view of finding a single, non- invasive, yet efficient diagnostic tool to differentiate ALD from NAFLD, a new model called ALD/NAFLD index (ANI) was developed in 2006 by Dunn *et al*. [15] using four parameters: MCV, AST/ALT ratio, Body Mass Index (BMI), and gender. The cut- off of the scoring system was

calculated to be zero in the American population such that values above zero indicated the presence of ALD and values below zero favoured the diagnosis of NAFLD [15]. However, literature regarding the efficiency and the cut-off value of this score in the Indian population is limited.

In this context, we designed a study to validate the performance of ANI and to find its cut-off value in the Indian population. The study also aims to compare the diagnostic efficiency of ANI with existing serological markers (AST/ALT, MCV) which are currently used to differentiate ALD from NAFLD patients.

Materials and Methods

Study Design: A cross-sectional observational study was carried out in the Department of Medicine at Sir Sayajirao General Hospital, Vadodara over a period of 2 months (June 2019- August 2019). 48 patients were included in this study out of which 24 were ALD patients and 24 were NAFLD patients, as diagnosed using relevant clinical history and ultrasonography by a qualified physician. Patients with viral hepatitis, autoimmune fatty liver disease, drug-induced fatty liver disease and metabolic liver disease were excluded from the study. Institutional Ethics Committee approval was obtained. A written informed consent was obtained from all the patients.

This sample population of 48 was calculated on MedCalc version 19.1 using the difference in mean and standard deviation of ANI measured in ALD and NFALD patients (7.11 ± 5.77 vs. –3.09 ± 3.89) as taken from a reference study [16], with 95% confidence interval and 80% power of study

Procedure: The diagnosed patients were selected by random sampling. A case study form was filled using the patient's relevant history, examination findings, and investigations which were recorded from the recent blood samples of the patients. The ultrasonography reports of the patients were also accessed. Data recorded in the case report form included- name, age, gender, history of alcohol consumption, height, weight, waist circumference, BMI, and the values of AST, ALT and MCV.

Using the values of AST (IU/l), ALT (IU/l), MCV (fl), weight (kg), height (m) and gender, the ANI score was calculated for all the patients on an online calculator provided by the Mayo clinic (Rochester, Minnesota, USA) [17] which used the formula (i).

$$ANI = -58.5 + 0.637 (MCV) + 3.91 (AST/ALT) – 0.406 (BMI) + 6.35 \text{ for male gender} \quad (i)$$

Statistical analyses of the data was done using MedCalc 19.1 (MedCalc, Mariakerke, Belgium). Significance was determined using Chi-square test, student *t* test and Mann-Whitney test. The efficacy of the ANI scoring system and its cut-off value for the Indian population was calculated using the area under the receiver operating characteristic curve (AUROC). Furthermore, the diagnostic efficiency of ANI was compared with that of serological markers including AST/ALT and MCV by plotting a Receiver Operating Characteristic Curve (ROC) for each marker and comparing the AUROC between the groups using the Z test.

Results

The demographic, clinical and biochemical characteristics of patients are displayed in Table I. According to this study, the clinical parameters including BMI and waist circumference were significantly higher in NAFLD ($P<0.01$ in both). However, average values of biochemical parameters like AST/ALT ratio and MCV were significantly higher in ALD patients ($P<0.01$ for both) (Table I).

The calculated ANI of ALD patients was higher than that of NAFLD patients with statistical significance [Median of ALD=9.2485, (95 % Confidence Interval,CI = 6.1312 to 10.0731) vs Median of NAFLD= -2.651, (95% CI=-6.6383 to -1.1333)]($P<0.001$) as calculated using Rank-Sum Mann-Whitney's test (Figure 1).

The cut-off value for the study population as calculated by Youden's Index (J Value) was found to be >1.702 which means that values greater than 1.702 signify a probable diagnosis of ALD while values equal to or below 1.702 yield a higher chance of diagnosing NAFLD.

For this cut-off, ANI shows an AUROC of 0.968 (95% CI= 0.872 to 0.998) and a high sensitivity (95.83%) and specificity (83.33%), as displayed in Table II. ANI values greater than 1.702 are seen in 95.83% of ALD patients, while only 16.66% of NAFLD patients show ANI values above this cut off.

To compare the diagnostic efficiency of ANI against the efficiency of basic serological parameters including AST/ALT and MCV, the Receiver Operating Characteristic Curve (ROC Curve) was plotted for ANI, AST/ALT and MCV (Figure 2). The cut-off value for ANI, AST/ALT and MCV were calculated and corresponding to the calculated cut-off value, the AUROC, sensitivity, specificity, positive and negative predictive test values, positive and negative likelihood ratios were calculated for each of the above mentioned diagnostic indicators as displayed in Table II. The AUROC of ANI was significantly higher than AST/ALT and MCV (all $P<0.01$) (ANI vs. AST/ALT, $Z=2.603$, $P=0.0093$; ANI vs. MCV, $Z=3.903$, $P=0.0001$)

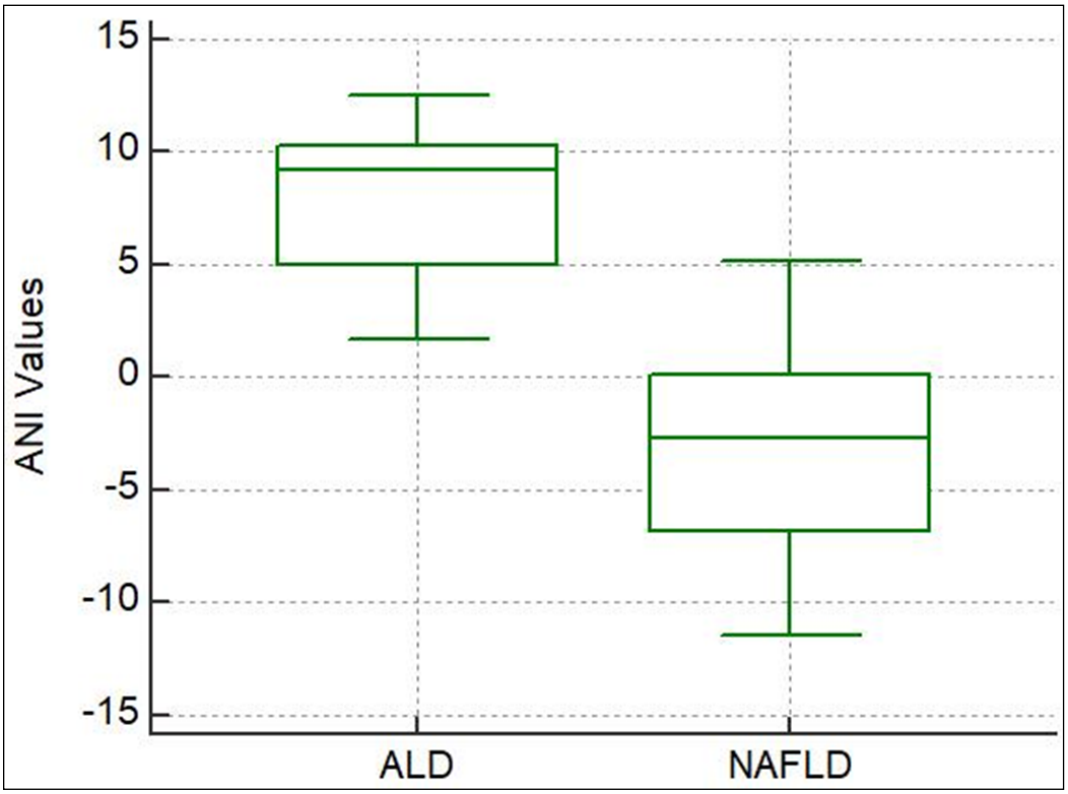

Fig 1: Box and whisker plot showing the value of ANI in ALD and NAFLD patients as calculated using Rank-Sum Mann-Whitney's test.

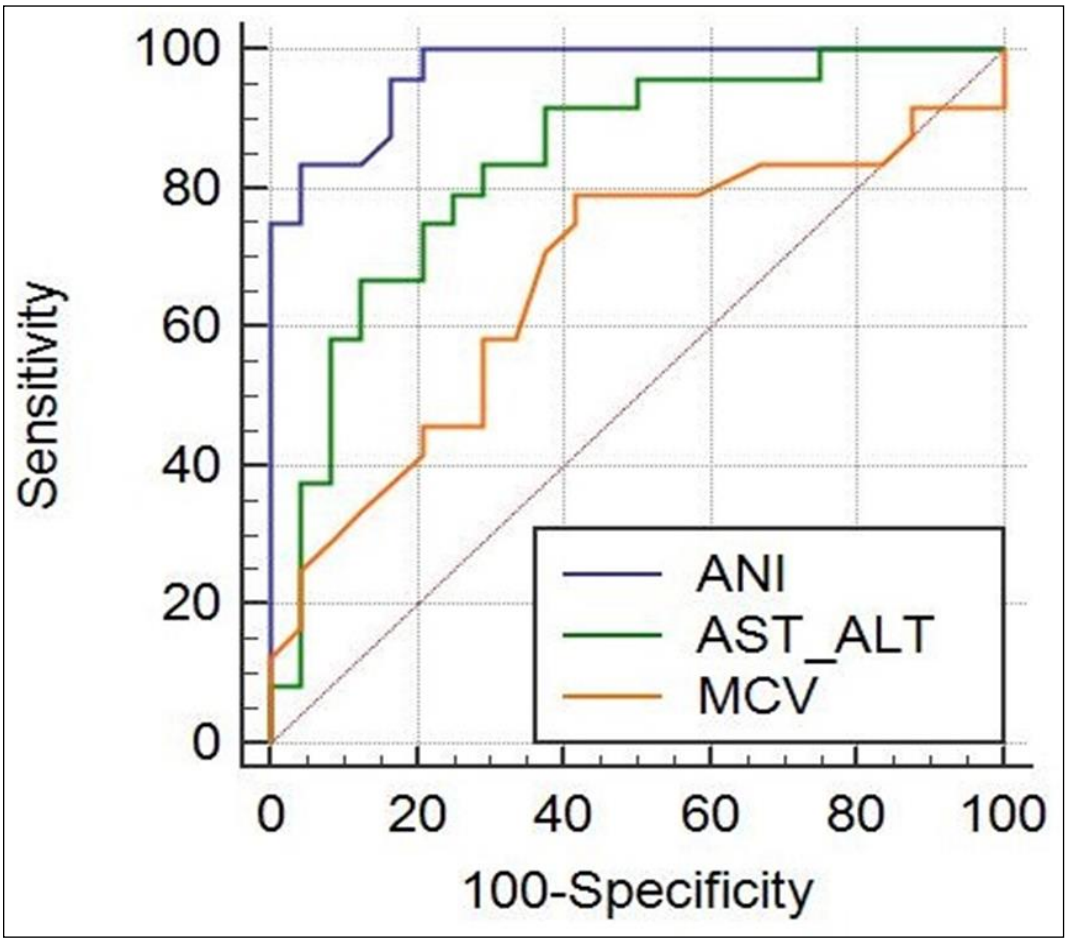

Fig 2: The receiver operating characteristic curve (ROC) plotted for ANI, AST/ALT and MCV to compare their efficiencies in diagnosing ALD.

Table 1: Demographic, clinical and biochemical characteristics of patients of ALD (n=24) and NAFLD (n=24).

Characteristic	Patients of ALD (n=24)*	Patients of NAFLD (n=24)*	*t*/*Z* /X^2 value	P value	Significance, Taking 95% Confidence
Age (Years)	40.875±14.405	50.291±13.687	*t*=2.321	0.0247	Significant
Gender (Male: Female)	22:2; 91.6%:8.3%	15:9; 62.5%:37.5%	X^2 =5.778	0.0174	Significant
BMI	19.754±2.779	30.758±5.215	*t*=9.123	<0.001	Significant
Waist Circumference(cm)	85.166±9.998	104.416±13.004	*t*=5.749	<0.01	Significant
AST/ALT	2.684±1.165	1.377±0.94	*t*=4.278	=0.0001	Significant
MCV (fl)	86.782±8.635	80.089±7.279	*t*=2.843	0.0067	Significant

*Values are presented as mean ± SD (Standard Deviation). ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; AST/ALT, aspartate aminotransferase/ alanine aminotransferase; MCV, mean corpuscular volume.

Table 2: Comparing the diagnostic efficiencies of ANI, AST/ALT and MCV in diagnosing ALD.

Criteria	ANI	AST/ALT	MCV
Cut-Off	>1.702	>1.5161	>80
AUROC (95%Confidence Interval)	0.968(0.872 to 0.998)	0.837 (0.702 to 0.928)	0.67 (0.519 to 0. 0.799)
Sensitivity %	95.83	83.33	79.17
Specificity %	83.33	70.83	58.33
PPV %	85.2	74.1	65.5
NPV %	95.2	81	73.7
PLR	5.75	2.86	1.9
NLR	0.05	0.24	0.36

AST/ALT, aspartate aminotransferase/ alanine aminotransferase; MCV, mean corpuscular volume; ANI, ALD/nonalcoholic fatty liver disease (NAFLD) index; ALD, alcoholic liver disease; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Discussion and Conclusion

According to our findings, the calculated ANI was significantly higher in ALD patients than NAFLD patients (Figure 1) making it a good diagnostic tool. This was similar to the conclusions drawn in other studies [16, 18]. This is because parameters using which the ANI is calculated takes into account the standard risk factors for NAFLD and ALD (high BMI is a risk factor of NAFLD [2, 19], male gender is a risk factor for ALD) [20] and laboratory abnormalities associated with ALD (high AST/ALT ratio [6, 7] and high MCV) [8].

The cut-off obtained in the study was >1.702 for the Indian population, for which the ANI proved to have a high sensitivity (95.83%) and specificity (83.33%) (Table II). In the model of ANI proposed by Dunn *et al*, the cut-off is taken to be 0 in the American population [15]; in the study by Wang *et al*. [16] the cut-off is -0.22 in Chinese population; and in the study by Cerovic *et al*. [18] the cut-off is -0.66 in Serbian population. This discrepancy may have arisen due to the complex interaction between environmental and genetic factors in the different populations [21].

ANI also proved to be a better diagnostic tool compared to using serological markers AST/ALT and MCV alone, indicated by the significantly higher AUROC value of ANI compared to AUROC of AST/ALT and MCV (Figure 2, Table II). The superiority of the ANI score over the existing markers can be attributed, firstly, to the adjustment for disease severity which was done in the derivation of the ANI [15]. Clinical predictors of ALD like AST/AST ratio are confounded by disease severity [22]. Secondly, ANI derived using logistic regression which may have facilitated appropriate weighting of the parameters included in ANI [15].

Although the ANI score can be used as an accurate and reliable tool, it presents with some limitations: (i) the power of study was limited due to the small sample size; (ii) other liver diseases should be excluded before applying ANI as they aren't included in this study; (iii) a positive ANI, though indicating the presence of ALD, doesn't exclude co-existing metabolic syndrome [15]; (iv) the cut-off of the ANI varies with different populations hence more research is needed to validate the cut-off value. The efficiency of the ANI scoring system can be further improved by adding other new parameters of liver function such as gamma-glutamyl transferase, carbohydrate deficient transferrin, or protein kinase C to its calculation [9, 11].

In conclusion, ANI is an efficient and non-invasive diagnostic tool with a high sensitivity (95.83%) and specificity (83.33%) at the cut-off value of 1.702. It has a better diagnostic performance compared to serological markers like AST/ALT and MCV in differentiating ALD patients from NAFLD patients. Although ANI cannot replace histopathology as the gold standard, it can help the clinician in prioritizing patients for liver biopsy and provide an alternative to biopsy for patients with risk of bleed.

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Footnotes
Conflicts of Interest: None.

Abbreviations
ALD: Alcoholic Liver Disease
NAFLD: Non-Alcoholic Fatty Liver Disease
ANI: Alcoholic Liver disease (ALD)/ Non-alcoholic Fatty Liver disease (NAFLD) Index
AST/ALT: Aspartate transaminase/ Alanine transaminase
MCV: Mean corpuscular Volume
BMI: Body Mass Index
WC: Waist Circumference

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