ENZYME ACTIVITY AND DE RITIS RATIO IN ALCOHOLIC AND NON ALCOHOLIC FATTY LIVER PATIENTS BASED ON ULTRASONOGRAPHY

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ABSTRACT

INTRODUCTION

Fatty liver disease (FLD) refers to a wide clinical and histological spectrum from simple hepatic steatosis to steatohepatitis or cirrhosis, and FLD has been classified as nonalcoholic FLD (NAFLD) and alcoholic FLD (AFLD) based on etiology and ultrasonography (USG).

MATERIAL AND METHODS

This Cross-sectional study was undertaken in the Department of Radiology, Universal College of Medical Sciences (UCMS), Bhairahawa, Nepal from March 2019 to February 2020. A total of 100 subjects involved where ultrasonographically graded for fatty liver and enzyme activity were assessed to observe their association.

RESULTS

In 100 cases, 66% were male and 34% were female. Ultrasonography (USG) guided FLD grade 1 patients were 81%, FLD grade 2 patients were 19% where as no cases was observed for FLD grade 3. Fifty Six percent (56%) of the total cases presented with AFLD while remaining 44% with NAFLD. There was significant difference in serum glutamate oxaloacetate transaminase (SGOT) (p-value: 0.003), serum glutamate pyruvate transaminase (SGPT) (p-value: 0.011) and alkaline phosphatase (ALP) (p-value: 0.003) in AFLD and NAFLD. However, there was no significant association of enzyme activity with FLD grade (p-value >0.05). There was significant difference in SGOT (p-value: 0.004), SGPT (p-value: 0.025) between AFLD grade 1 (AFLDG1) and NFLD grade 1 (NAFLDG1), SGOT (p-value: 0.016) between AFLDG2 and NAFLDG2 and ALP (p-value: 0.01) between AFLDG1 and NAFLDG1. However, De Ritis ratio was not significantly associated with fatty liver disease.

CONCLUSION

USG is a non-invasive simple tool for early detection of fatty liver in asymptomatic patients enabling clinicians to achieve early detection in conjunction with hepatic enzymes.

KEYWORDS Alcoholic, Non Alcoholic, De Ritis ratio, Enzyme activity, Fatty liver, Ultrasonography

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INTRODUCTION

Ultrasonography (USG) is a very efficient and widely available technique for the detection of fatty liver. The overall sensitivity and specificity of ultrasound in detection of moderate to severe fatty liver have been shown to be accurate and comparable to gold standard test liver biopsy.[1-3] Fatty liver in non-alcoholic individuals is rapidly becoming a major public health challenge.[4] Severe complications can occur because of the risk of progression to more advanced stages making early non invasive detection of fatty liver disease by USG very clinically important.[2-5] Alcoholic liver disease (ALD), induced by excessive alcohol consumption, and non alcoholic fatty liver disease (NAFLD), caused by obesity and insulin resistance are the most common diseases associated with hepatic steatosis.[6]

Under invasive procedures comes liver biopsy and non-invasive includes radiological tests like USG and various biochemical tests serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP).[7,8] USG which is non-invasive, simple tool, can be used for the early detection of NAFLD in asymptomatic patients and is the single best tool in the evaluation of focal liver lesions, unbeaten by any other imaging modality, due to real-time, dynamic nature, high resolution and good safety record.[9] The survival of patients with ALD and NAFLD depends on various disease-associated conditions.[10]

This present hospital-based study was undertaken to evaluate the relation of enzyme activity, De Ritis ratio, haemoglobin and albumin as an indicator of chronic liver disease with fatty liver disease in patients diagnosed based on USG.

MATERIAL AND METHODS

This cross-sectional study was undertaken in the Department of Radiology with collaboration of Department of Medicine and Department of Biochemistry, Universal College of Medical Sciences (UCMS), Bhairahawa, Nepal from March 2019 to February 2020.

Study population and sample size was determined as follow:

$$n = \frac{z^2PQ}{D^2}$$

$n = (1.96)^2 \times 0.07 \times 0.93/ (0.0025) = 100$ where, n = sample size, z = critical value = 1.96, P = prevalence of disease = 7%, Q = without disease (1-P), D = allowance error (5%).

AFLD Group comprised of excessive alcohol consumption 40 g/day in men (20 g/day in women), usually >5 years and / or ongoing daily consumption > 80 g in two weeks.

NAFLD group comprised of history of alcohol consumption or alcohol intake less than 140 g in men (70 g in women) on average per week in last 12 months.[5] Patients who have been diagnosed with fatty liver disease based on USG finding of GE LOGIQ6 PRO ultrasound scanner were included in the study. Ethical approval was taken from Institutional Review Committee with Registration number UCMS/IRC/046/19.

Procedure for patients

Conventional B-mode USG is the most common technique used to assess the presence of fatty liver in clinical settings and population studies. Fatty liver is diagnosed based on the following ultrasound parameters: parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition.[11]

The patients were mostly examined with real-time sonography, after a 6-8 hour abstaining from food. Mostly supine and right anterior oblique views were obtained. Sagittal, transverse, coronal, and subcostal oblique views were also performed using both a standard abdominal transducer and a higher frequency transducer. In few cases intercostal views were also needed.

USG gradings of Fatty liver/ Diffuse hepatic steatosis

Mild (Grade1): Minimal diffuse increase in hepatic echogenicity with normal visualization of diaphragm and intrahepatic vessel borders.

Moderate (Grade 2): Moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of intrahepatic vessels walls and diaphragm.

Severe (Grade 3): Marked increase in echogenicity with poor penetration of posterior segment of right lobe of liver and poor or no visualization of hepatic vessels and diaphragm. The Patients with other causes of liver disease like viral or alcoholic hepatitis, on drugs therapy or any chemotherapy and patient's age less than one year and greater than 80 years were excluded from this study.

Biochemical test procedure

Serum was separated from the blood sample of patients diagnosed with fatty liver by the help of USG and the tests were carried out. The samples were subjected for measuring liver enzymes which will include SGPT, SGOT (Rietman's and Frankel's method) and ALP (Kind's and King's method). The Albumin was tested by Bromo Cresol Green (BCG) dye binding method. SGOT: SGPT ratio was calculated as De Ritis

Ratio. Hemoglobin level was obtained from hematology analyzer (Beckman coulter, DxH 520). All the biochemical assays were done in fully automated Humanalyzer-600, Germany.

All the data from cases were fed in MS Excel (Microsoft office 2007) and then analyzed by Statistical Package for Social Service (SPSS) for window version; SPSS 22, Inc., Chicago, IL). All the data were expressed in terms of percentage frequency, Median and compared by non-parametric tests Chi-Square test, Man Whitney U test, Kruskal Wallis test and Spearman's rho correlation etc. P-value <0.05 was considered to be statistically significant.

RESULTS

Table 1. General characteristics of the study subjects based on USG

Characteristics	FLD G1	FLD G2	Total	p-value
Median Age (IQR) in years	44(21-67)	45(24-66)	45(20-80)	0.812
20-40 years n (%)	37 (45.7)	9 (47.5)	46	
41-60 years n (%)	30 (76.9)	9 (47.4)	39	0.384
> 60 years n (%)	14 (93.3)	1 (5.3)	15	
Gender (Male: Female)	1.8:1	5.3:1	1.94:1	
Male n (%)	50 (61.7)	16 (84.2)	66	0.063
Female n (%)	31 (38.3)	3 (15.8)	34	
Alcoholism				
Alcoholics n (%)	42 (51.9)	14 (73.7)	56	0.084
Non-Alcoholics n (%)	39 (48.1)	5 (26.3)	44	
Median Hemoglobin (Hb) IQR	10 (5.7-14.3)	10 (7.1-12.9)	10 (3.3-17.7)	0.944
Hb (≤ 10 g/dl) n (%)	56 (69.1)	16 (88.2)	72	0.188
Hb (>10 g/dl) n (%)	25 (30.9)	3 (15.8)	28	
Albumin (Alb) IQR	3.3 (2.8 -3.8)	3.2 (2.3 -5)	3.2 (3.2 -3.5)	0.419
Alb (≤ 3.5g/dl) n (%)	62 (76.5)	16 (84.2)	78	0.468
Alb (>3.5 g/dl) n (%)	19 (23.5)	3 (15.8)	22	

Table 1 shows that the median age of the study subjects was 45 years with IQR of 20-80 maximum of the patients being aged between 20-40 years followed by 41-60 and least was more than 60 years. There was no statistical significance in age distribution (p-value: 0.384). Male to female ratio is 1.94:1 with no statistical significance difference in two groups (p-value: 0.063). The percentage of alcoholic and non-alcoholic patients with grade 1 FLD was 51.9% and 48.1% respectively and with grade 2 FLD being 73.7% and 26.3% respectively with no statistical significance difference (p-value: 0.084). The median hemoglobin concentration is 10 with over all IQR 3.3-17.7 g/dl.

Among the studied cases, 72% were having hemoglobin ≤10 g/dl and 28% were having normal >10 g/dl hemoglobin concentration. The median serum albumin level was 3.2 with over all IQR of 3.2-3.5 g/dl. Lower levels of serum albumin was seen in 78% of the cases, while 22% had normal levels. There was no statistical significance in distribution of hemoglobin concentration and serum albumin with different grades of FLD p-value being 0.419 and 0.468 respectively.

Table 2. Status of enzyme activity and De Ritis ratio in the study subjects based on USG

Enzyme	Status	FLD Type		Total	p-value	FLD Grade		Total	p-value
		Alc.	Non Alc.			1	2		
SGOT	Normal	28	19	47	0.003	39	8	47	0.78
	High	16	37	53		42	11	53	
SGPT	Normal	27	20	47	0.011	40	7	47	0.32
	High	17	36	53		41	12	53	
ALP	Normal	39	35	74	0.003	61	13	74	0.530
	Low	30	31	61		20	6	26	
De Ritis	< 1	17	19	36	0.351	31	6	37	0.568
	≥2	12	6	18		13	5	18	
	1-1.99	27	19	46		37	8	45	

Table no 2 shows the association of enzyme activities results with alcoholic FLD. There was statistical significant difference in SGOT (p-value: 0.003), SGPT (p-value: 0.011) and ALP (p-value: 0.003). There was no significant association enzyme activity with grade of FLD (p-value>0.05).

Table 3. Association of enzyme activity, De Ritis ratio, hemoglobin and albumin level with grades of alcoholic and non-alcoholic FLD based on USG

Lipid	AFLG1	AFLG2	NAFLG1	NAFLG2	Total	p-value
SGOT	71*^{c}	207*^{b}	40*^{c}	25*^{b}	50	0.001
SGPT	61.5*^{d}	77*^{a}	32*^{d}	33*^{a}	41.5	0.007
ALP	263*^{e}	214	221*^{e}	220	228	0.099
De Ritis	1.2	1.3	1.08	0.81	1.17	0.447
Hb	10	9.5	9.98	10.1	10	0.954
Alb	3.3	3.2	3.2	3.1	3.2	0.790

Man Whitney U test p-value: *^{a}0.004, *^{b} 0.016. *^{c}0.007, *^{d}0.025, *^{e}0.01

There was statistical significant difference in median value for overall groups of SGOT (p-value: 0.001) and SGPT (p-value: 0.007). There was statistical significant difference in SGOT (p-value: 0.007), SGPT (p-value: 0.025) between AFLG1 and NAFLG1, SGOT (p-value: 0.016), SGPT (p-value: 0.004) between AFLG2 and NAFLG2, ALP between AFLG1 and NAFLG1.

Table 4. Association of liver enzymes with hemoglobin and albumin status

Liver enzymes		Hb		p-value	Alb		p-value
		Normal	Anemic		Normal	Low	
SGOT	Normal	14	33		13	34	
	High	14	39	0.70	9	44	0.19
	Total	**28**	**72**		**22**	**78**	
SGPT	Normal	12	35		13	34	
	Low	16	37	0.62	9	44	0.19
	Total	**28**	**72**		**22**	**78**	
ALP	Normal	18	56		15	59	
	High	10	16	0.16	7	19	0.48
	Total	**28**	**72**		**22**	**78**	

Table 4 shows that the association of enzyme activity with hemoglobin status and albumin status which were statistically non-significant.

Table 5. Spearman's rho correlation of study variables

Variables	FLD	A/N FLD	De Ritis	Hb	Alb
SGOT	0.113	-0.327**	0.335**	0.031	-0.021
	0.261	0.001	0.001	0.759	0.838
SGPT	0.102	-0.280**	-0.211*	0.039	0.056
	0 .313	0.005	0.035	0.702	0.578
ALP	-0.16	-0.225*	0.006	0.229*	0.065
	0.875	0.024	0.954	0.022	0.520
De Ritis	0.076	-0.092	1.000	0.037	-0.173
	0.453	0.363	-	0.718	0.085
Hb	-0.007	0.054	0.037	1.000	-0.026
	0.944	0.597	0.718	-	0.795
Alb	-0.065	-0.083	-0.173	-.026	1.000
	0.520	0.414	0.08	0.795	-

*p-value at <0.01, **p-value at <0.001

Table 5 shows the Spearman's correlation of the enzyme activity with different variables. Alcoholic and non-alcoholic FLD have statistically negative correlation with SGOT (p-value: 0.001), SGPT (p-value: 0.005) and ALP level (p-value: 0.024). Serum SGOT and SGPT have significant correlation with De Ritis ratio (p-value of 0.001 and p-value: 0.035) respectively. Similarly, ALP shows significant correlation with hemoglobin (p-value: 0.022). As compared with the alcoholic and non-alcoholic fatty liver, the statistical significance was observed with SGOT (p-value: 0.001), SGPT (p-value: 0.005) and ALP (p-value: 0.025).

DISCUSSION

With the rising prevalence of obesity, the proportion of fatty liver has increased in both Eastern and Western countries.[4] ALD represents a broad range of histological changes ranging from simple steatosis to heavier forms of liver injury, including alcoholic hepatitis (AH), cirrhosis, or the parallel development of hepatocellular carcinoma (HCC).[12]

NAFLD is emerging as the most common chronic liver condition in the Western world. It is associated with insulin resistance and frequently occurs with features of the metabolic syndrome. Disease presentation ranges from asymptomatic elevated liver enzyme levels to cirrhosis with complications of liver failure and hepatocellular carcinoma.[13] Mahaling DU et al (2013), in their study out of 70 cases which were diagnosed as NAFLD on USG, grade 1 NAFLD cases were 47.15%, grade 2 were 42.85% and grade 3 were 10%. The mean age of the patients was found to be 49.14 years. Male to female ratio was 3:4.[14] In our study, out of 100 cases, 81% were diagnosed as grade 1 FLD while 19% as grade 2 FLD. 56% were AFLD while 44% were NAFLD. In the present study, the median age of the subjects was 45 years with IQR 20-80 years and male to female ratio was 1.8:1.

Amadol NL et al (2017), in their study found that the most frequent disorder was elevation of ALT levels (72.5%), higher than AST (25%) and ALP (45%).[15] In our study, SGOT, SGPT and ALP were showed correlation with AFLD and NAFLD but SGOT and ALP showed stronger correlation than ALT with p-value of 0.003 each, unlike their study.

In the study by Bhusal K, Simkhada R, Nepal P (2017), out of total 100 cases, mild nonalcoholic fatty liver disease was found in 83%, moderate in 17% and severe in none of the participants. Age of the participants ranged from 26 to 79 years with mean being 45 ± 11.99 years. Quite similar to their study, 88.6% of the non-alcoholic patients were having grade 1 (mild) fatty liver disease, 11.4% of them were having grade 2 (moderate) fatty liver disease and none of the participants had grade 3 (severe) fatty liver disease.[16] We studied patients with ALD in whom 81% were having grade I and 19% had grade II FLD. Mittal A et al in their study found that there was a significant correlation between sex as well as age group with Alcoholic liver disease. The mean ± SD value of De Ritis ratio was 1.59 ± 0.58 and it was significantly correlated with ALD.[17] Jwarchan B et al conducted a study in which total 85 patients were enrolled and they found that females (65.88%) were more affected by NAFLD than males (34.12%). The mean ± SD of only ALT showed statistical significant difference between grade 1 and grade 2 NAFLD (p-value = 0.027).[18] In our study, there was no such difference between males and

females were considered. The median age of the subjects was 45 years with inter quartile range of 20-80 years. The enzymes SGOT, SGPT and ALP were found to be statistically associated with the type of FLD, p-value being 0.003, 0.011 and 0.003 respectively. However, there was no association observed to exist with De Ritis ratio. When Man Whitney U test was used, there was statistical significant difference in median value in SGOT (p-value: 0.007), SGPT (p-value: 0.025) between AFLG1 and NAFLG1, SGOT (p-value: 0.016), SGPT (p-value: 0.004) between AFLG2 and NAFLG2, ALP between AFLG1 and NAFLG1. Ramesh et al in their case control study found that the increase in AST and ALT levels in AFLD were highly significant when compared to controls (p-value = 0.0001). Similarly, mean De Ritis ratio showed significant correlation with AFLD (p-value = 0.0001) while not with NAFLD.[19] But in our study, there was no correlation found between De Ritis ratio and type or the grade of FLD. But similar to their study, increase in SGOT and SGPT levels were significant with type and grade of FLD.

CONCLUSION

Although liver biopsy is the gold standard method for diagnosis of fatty liver, it cannot be performed in the entire general population. Ultrasonography, which is a non invasive and simple tool, can be used for the early detection of fatty liver in asymptomatic patients, where symptoms occur only in the advanced stages of the disease. This technique can also serve as an indicator of future comorbidities enabling clinicians to suggest blood investigations like SGOT and SGPT for proper counselling to patients regarding further management or preventive measures.

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