Journal of Applied Pharmaceutical Research
Volume 9, Issue 4, Year of Publication 2021, Page 29 – 38
DOI: 10.18231/j.joapr.2021.29.38

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ***ISSN: 2348 – 0335***

EFFECT OF VITAMIN E ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN REVERSING THE BIOCHEMICAL AND RADIOLOGICAL HEPATIC PATHOLOGY – AN INTERVENTIONAL STUDY

R. Divakar, P. Sugirda*, R. Lenin, T. Henry Daniel Raj

Article Information
Received: 25th January 2021
Revised: 17th September 2021
Accepted: 6th October 2021
Published: 31st December 2021

Keywords
Non-alcoholic fatty liver disease (NAFLD), Vitamin E (α-tocopherol), Serum transaminases

ABSTRACT
We aimed to assess the effect of Vitamin E on hepatic pathology in terms of liver enzymes and ultrasonographic (USG) findings in Non-alcoholic Fatty liver disease (NAFLD) patients. Vitamin E was administered as α-tocopherol for 12 weeks for 60 patients. Baseline and after 12 weeks of Vitamin E therapy values of anthropometric measures, fasting blood glucose, lipid profile were estimated apart from liver enzymes and USG based assessment of liver size and Hamaguchi score. NAFLD fibrosis score (NFS), a predictor of hepatic fibrosis was evaluated pre and post treatment with Vitamin E. Significant statistical difference was noted in the reduction of Triglycerides, Cholesterol, VLDL and LDL both diabetic and non-diabetic population in our study. ALT and AST normalization was observed and the mean reduction were -38.11 ($p < 0.001$) and -22.4 ($p < 0.001$) respectively due to Vitamin E. Mean liver size was also decreased from 16.05 (SD±1.2) to 13.36 (SD±2.0) after 12 weeks of Vitamin E therapy. However, no significant change in NFS score was noted [baseline – 0.248 (SD±0.78) and at the end of treatment – 0.535 (SD±0.87)], indicating morphological changes were not reversed with Vitamin E. No significant difference observed for mean weight, waist circumference, body mass index (BMI), serum albumin, bilirubin and HDL levels as well as platelet count from baseline to end of treatment. Hence, Vitamin E alone would be useful in the treatment of NAFLD patients even with diabetes and irrespective of biopsy status.

INTRODUCTION

Non-alcoholic fatty liver disease [NAFLD] is defined as fatty infiltration affecting greater than 5% of hepatocytes in the absence of excessive alcohol consumption (>20 g day for women and >30 g/day for men). It comprises of several causes except due to excessive alcohol intake and pathologically may range from steatosis to cirrhosis [1]. Overall prevalence of NAFLD to be in the range of 9-53% in India and there is a strong

*Department of Pharmacology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India, 605601

For Correspondence:** **sugimdpharm@gmail.com

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association of NAFLD with metabolic syndrome [2,3]. The majority of patients with NAFLD are asymptomatic; hence such diagnosis is either overlooked or made incidentally after an abnormal liver function test (LFT) report or fatty liver finding on imaging [4,5]. Most typical biochemical abnormalities found in NAFLD are elevated transaminases especially ALT levels (>40 U/L) and ALT ratio of more than one [alanine amino transferase (ALT) >aspartate amino transferase (AST)] and/or gamma-glutamyl transferase irrespective of their metabolic prolife status [6]. Still, liver biopsy is the gold standard method to confirm the diagnosis of NAFLD and several authors proposed histological criteria, mostly steatosis [7].

The usual course of NAFLD is benign with some exceptions and much depends upon the level of lipid peroxidation and secondary cellular injury in the transition from relatively stable hepatic steatosis to potentially progressive hepatitis or cirrhosis which operate at a faster pace in patients with diabetes [8,9]. Putative pathophysiology of NAFLD is best explained by "multiple hit" hypothesis, where the first "hit" is the development of hepatic macrosteatosis as a result of increased lipolysis and free fatty acid levels. Several possible "second hits" may be oxidative stress from reactive oxygen species in the mitochondria and cytochrome P450 enzymes, endotoxins, cytokines, adipokines and environmental factors [10].

Treatment of NAFLD is based on guidelines which vary across the globe and rest upon the pathogenetic aspects rather than etiological ones. Though lifestyle management strategies like diet modification and physical activity are recommended; in practice, pharmacological interventions are widely used. Among the host of drugs for NAFLD like Statins to Metformin; almost all drugs found insufficient evidence as per the standard guidelines [11,12]. While American Association for the Study of Liver Diseases (AASLD) recommends Vitamin E only in non-diabetic, biopsy-proven NASH patients; National Institute for Health and Care Excellence (NICE) guidelines state that it can be considered irrespective of diabetic status [13].

Vitamin E also known as α-tocopherol, is a chain-breaking antioxidant in free radical reactions that is of particular importance in lipid peroxidation and membrane stabilization due to its lipid solubility. Apart from antioxidant effects, Vitamin E might attenuate cytokine stimulation of stellate cells by decreasing transforming growth factor-β (TGF-β) levels [14]. The molecular mechanisms of Vitamin E are supporting its role in halting the progress of not only fatty liver but also fibrosis and based on experimental evidence, it should be effective in NAFLD [15,16]. Yet, it is not recommended as a first-line drug by most of the guidelines [11,12,13]. The reasons could be incomplete translation of animal studies in real life scenario and its potential side effects but most of the clinical trials were not head-to-head; rather Vitamin E was used as an adjuvant or a combination drug, leading to invalid conclusions [17]. Given the scarcity of studies on Vitamin E use in NAFLD in India, we conducted this study [1]. Specifically, we wanted to determine the changes in the level of serum transaminases and grade of fatty infiltration of liver assessed by ultrasonography (USG) in NAFLD patients after Vitamin E therapy.

MATERIALS AND METHODS

This was a prospective, open-label, non-randomized single arm, single center study conducted in a medical college hospital in Tamil Nadu. The study was approved by the Institutional Ethics Committee. The study period was from September 2016 to June 2017 and duration of Vitamin E therapy for an individual patient was 12 weeks (84 days). Patients above 18 years of age irrespective of gender who were diagnosed as Non-Alcoholic Fatty Liver Disease (NAFLD) by USG were taken up for this study [18].

Patients were evaluated initially for diabetes (Fasting Blood Glucose >126mg/dl) and hypertension (BP >140/90 mmHg) in addition to the liver function tests and anthropometry [19,20]. Patients aged less than 18 years and those with history of excessive alcohol consumption (>20 g day for women and >30 g/day for men) and patients who were already diagnosed with chronic viral hepatitis, autoimmune liver disease and haemochromatosis, chronic renal disease were excluded. Pregnant and lactating women and patients on steatogenic medications like oral contraceptive pills were also excluded from the study along with those who were unwilling to participate.

A total of 60 patients (male – 26; female -34) were finally selected for this study after applying inclusion and exclusion criteria and after getting written informed consent from each. All of them were given Vitamin E 600 mg orally once daily for 12 weeks in capsule form after breakfast. The capsule form of Vitamin E (Evion 600) manufactured by Procter & Gamble

Hygiene and Health Care Ltd, was given free of cost to the participants. No specific instructions on diet or lifestyle change like exercises were given and no change in the drug regime for diseases like diabetes, hypertension and obesity they were already on were made. All the patients were reviewed at 4th, 8th and 12th week and during each visit, drug compliance, subjective improvements and adverse events were monitored.

The primary outcome measures were the change in level of serum transaminases, grade of fatty infiltration of liver in USG and NAFLD score at the end of 12 weeks of treatment with Vitamin E. Other outcome measures were changes in weight, waist circumference, body mass index (BMI), Lipid profile (Total Cholesterol, Triglycerides, VLDL, LDL, HDL) and liver size by USG. The scoring system developed by Hamaguchi et al., to assess visceral obesity and hepatic steatosis in the context of metabolic syndrome was used (it consists of four USG findings viz., hepatorenal echo contrast, bright liver, deep attenuation, and vessel blurring) and the score goes from 0 to 3 [21]. NAFLD Fibrosis Score (NFS) was calculated non-invasively by the following formula: NFS = -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×109/L) - 0.66 × albumin (g/dL) [22]

Safety evaluation is based on reported as well as observed adverse events. We divided the group into diabetic and non – diabetic patients, based on their history and medical records and compared the changes of parameters between them. For data collection and analysis, Microsoft Excel and SPSS (V.20) were used respectively. The continuous data was presented as mean (standard deviation -SD) and median (range) while categorical data was presented as frequency and percentages. Mean difference between two groups and pre and post treatment of parameters were determined by paired ***"t"*** test. For all the statistical results and outcome measures, $p < 0.05$ was considered to be statistically significant.

RESULTS

Out of 60 patients, 47(78%) were aged 40 and above. At the time of recruitment, 53.3 % of patients had diabetes and 68.3 % patients had hypertension already and these co-morbidities were presented in Fig.1. Baseline as well as at the end of 12 weeks measurements and values of body mass index (BMI), Fasting Blood Glucose, serum lipid profile and serum transaminases (AST & ALT), liver size along with NAFLD score of the participants were presented in Table 1. No significant differences in all the parameters studied between males and females on both occasions. Mean weight at baseline and at the end of 12 weeks were 82(SD±23) Kg and 82.8(SD±22.6) Kg respectively. Mean waist circumference at baseline and at the end of 12 weeks were 102.2 (SD±26.2) cm and 103.5 (SD±26.) cm respectively.

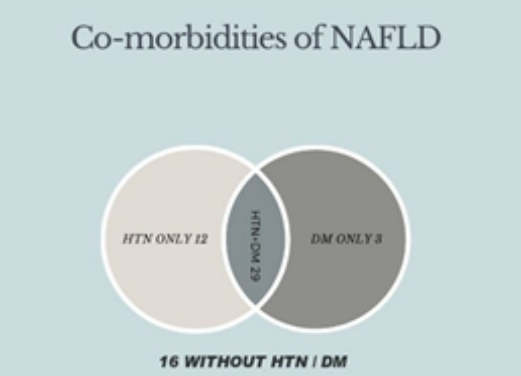

Fig. 1. Co morbidities of NAFLD – DM & HTN

Baseline fasting blood glucose (mean) of patients was 95 mg/dl (SD±14.5) and at the end of 12 weeks it was 96.4mg/dl (SD±12.6) and as expected there was a significant difference between diabetic vs non-diabetic population (Table. 2). No significant change in the level of serum bilirubin [baseline – 0.51mg/dl (SD±0.2) & at end point – 0.62mg/dl (SD±0.2)], albumin [baseline – 4.2mg/dl (SD±0.7) & at end point – 4.6mg/dl (SD±0.6)] and platelet count [baseline – 18x109/ L (SD± 76) & at end point – 178x109/ L (SD± 84)] noted by us. Negligible amount of reduction of mean BMI and blood glucose level in diabetic and similar amount of gain in same parameters in non-diabetic study population noted for treatment period. Overall, no significant difference observed for mean weight, waist circumference, body mass index (BMI), serum albumin, bilirubin and HDL levels as well as platelet count between males *vs* females as well as between diabetic *vs* non-diabetic patients from baseline to end of treatment. Mean difference and percentage of significance for rest of the parameters between diabetic and non-diabetic population presented in Table 2.

Measurements of liver size by ultrasonogram at baseline as well as at the end of 12 weeks of Vitamin E therapy were presented in Figure.2. Pre and post treatment Hamaguchi scoring based on USG findings were presented in Table.3 and in Fig.3. NAFLD Fibrosis Score (NFS) based on the calculation as mentioned in methodology was sketched in Table 1 & 2.

Table 1. Baseline and at the end of study period values *[+ indicates gain and – indicates reduction from previous level*] * ligands explained

Parameters studied	TOTAL (60)				Diabetes (32)				Non-Diabetic (28)			
B- Baseline E – End of study	Mean	SD(±)	Mean diff.	*p* value	Mean	SD	Mean diff.	*p* value	Mean	SD	Mean diff.	*p* value
BMI (B)	33.24	1.84	-0.118	<0.673	33.3	2.0	-0.78	.02	33.2	1.7	+0.64	0.13
BMI (E)	33.12	2.04			32.5	2.2			33.8	1.7		
FBS (B)	95	14.5	+1.4	<0.7	108	10.2	-6	0.4	82	12.4	+8.8	0.2
FBS (E)	96.4	12.6			102	10.8			90.8	11.6		
TCL (B)	206.9	63.25	-40.9	<0.001	202.7	65.2	-37.96	.005	211.8	61.7	-44.25	0.004
TCL (E)	166	33.1			164.7	32.4			167.5	34.4		
TG (B)	229.18	81.88	-74.25	<0.001	237.9	87	-78.50	.001	219.2	75.9	-69.39	<0.001
TG (E)	154.93	52.1			159.4	58			149.8	44.9		
VLDL (B)	57.23	36.67	-27.31	<0.001	58.1	35.8	-26.87	.001	56.2	38.3	-27.82	0.001
VLDL (E)	29.92	13.21			31.2	16			28.4	9.1		
LDL (B)	137.72	46.02	-28.31	<0.001	136.9	47.4	-30.43	.002	138.6	45.3	-25.89	0.014
LDL(E)	109.40	25.26			106.5	25.4			112.7	25.1		
HDL (B)	40.42	9.97	+2.43	<0.197	40.3	8.6	+2.15	.320	40.6	11.5	+2.75	0.398
HDL	42.85	10.21			42.4	9.6			43.3	11.0		
AST(B)	46.00	13.52	-22.4	<0.001	46.2	13.9	-24.12	.000	45.8	13.3	-20.42	<0.001
AST(E)	23.60	8.47			22.1	5.8			25.4	10.6		
ALT(B)	66.62	16.43	-38.11	<0.001	67.7	17.5	-41.28	.000	65.3	15.3	-34.5	<0.001
ALT(E)	28.50	16.46			26.5	12.1			30.8	20.3		
AAR(B)	.6902	.131	+0.27	<0.001	0.7	0.1	+0.27	.002	.7	0.1	+0.27	0.008
AAR(E)	.9670	.448			1	0.5			1.0	0.5		
Liver size(B)	16.05	1.249	2.69	<0.001	16.1	1.4	-2.68	.000	16.0	1.1	-2.71	<0.001
Liver size(E)	13.36	2.034			13.4	2.5			13.3	1.3		
NFS(B)	.248	.785	+0.28	<0.001	0.9	0.4	+0.27	.002	-.5	0.4	+0.3	.005
NFS(E)	.535	.872			1.2	0.6			-.2	0.5		

*BMI – Body Mass Index (kg/m^2), FBS – Fasting Blood Glucose (mg/dL), TCL – Total Serum, Cholesterol (mg/dL), TG – Triglycerides (mg/dL), VLDL – Very Low-Density Lipoproteins (mg/dL)

LDL - Low Density Lipoproteins (mg/dL). HDL - High Density Lipoproteins (mg/dL)

AST - Aspartate transaminase / SGOT (U/dL). ALT- Alanine transaminase / SGPT (U/dL)

AAR – AST/ALT Ratio. Liver size – in centimeters (cm)

NFS - NAFLD Fibrosis Score calculated by

NFS = -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×109/L) - 0.66 × albumin (g/dL)

Table 2. Analysis of Mean difference and significance between diabetic and non-diabetic population

Variable	Diabetic	Non-Diabetic	Percentage Difference
BMI	-0.787	+0.64	2.218
FBS	-6	-8.8	0.28
TCL	-37.96	-44.25	-0.141
TG	-78.5	-69.39	0.131
VLDL	-26.87	-27.82	0.034
LDL	-30.43	-25.89	0.175
HDL	+2.15	+2.75	0.216
AST	-24.12	-20.42	0.18
ALT	-41.28	-34.5	0.196
AAR	+0.278	+0.275	0.01
Liver size	-2.681	-2.7143	0.012
NFS	+0.27	+0.306	0.116

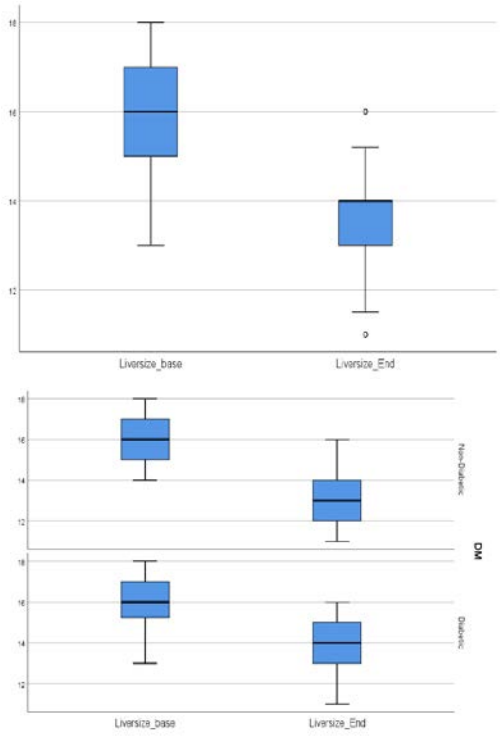

Figure 2. Comparison of Liver Size (in cm) before and after treatment with Vitamin E [Refer Tables. 1&2]

Table 3. Hamaguchi score – at baseline and at the end of treatment (Total & DM vs NDM)

Hamaguchi Score		**Total *n* (%)**	**DM *n* (%)**	**Non-DM *n* (%)**	***p* value**
Baseline	0	0	0	0	
	1	24 (40)	12 (37.5)	12 (42.8)	0.153
	2	32 (53)	16 (50)	16 (57.2)	
	3	4 (6.7)	4 (12.5)	0	
After 12 wks of Vit E	0	17 (28.3)	8 (25)	9 (32.1)	0.555
	1	42 (70)	23 (71.9)	19 (67.9)	
	2	1 (1.6)	1(3.1)	0	
	3	0	0	0	
Total number (%) of patients with change in the score					
Hamaguchi Score	**0**	**1**	**2**	**3**	***p* value**
Baseline	0	24 (40.0%)	32 (53.3%)	4 (6.7%)	**<0.001**
End Point	17 (28.3%)	42 (70.0%)	1 (1.7%)	0	

Table 4. Change in category-wise probability on NFS score from baseline to end of treatment

Probability		**Total *n* (%)**	**DM n (%)**	**Non- DM n (%)**	***p* value**
Baseline	Low	1 (1.6)	0	1 (3.6)	<0.001
	Intermediate	35 (28.3)	8 (25)	27 (96.4)	
	High	24 (40)	24 (75)	0	
End of Treatment	Low	0	0	0	<0.001
	Intermediate	33(55)	7 (21.9)	26 (92.8)	
	High	27(45)	25 (78.1)	2 (7.2)	

The classification to predict the probability of fibrosis based on NFS score by Treeprasertsuk S et al., was categorized into three as follows: NFS < -1.5 for low probability, > -1.5 to < 0.67 for intermediate probability, and > 0.67 for high probability and change from one category to another after Vitamin E therapy was presented in Table 4.

DISCUSSION

Studies on the effectiveness of Vitamin E in NAFLD patients had been done either alone or in combination with or without randomization [23 – 27]. While some authors studied all age groups; some did so with only children and adolescents or adults only and we studied only adult population [28]. While authors like Singh et al., and Das et al., noted predominant younger population; Foster et al., studied more of elderly people presented with NAFLD [6,29,30]. Some investigators recruited only diabetic patients whereas some others took only non-diabetic patients with NAFLD in their study for Vitamin E therapy [31 – 33]. Our study was a non-randomized one; including patients with as well as without diabetes and we didn't alter the other drug regimen of the patients for co morbidities like diabetes, obesity and hypertension.

Slight preponderance of females (56.7% compared to 43.3% of males) in our study was in concordance with Sanyal et al., and Hoofinagle et al but no significant gender difference was noted by Das et al [6,26,34]. On the contrary greater number of male patients were observed by Foster et al and Bril et al., [30,31]. Almost all participants in our study were obese (96.7%) [remaining 3.3% were overweight] and this should be taken into consideration as NAFLD was viewed as the hepatic manifestation of metabolic syndrome by many across the world. Even in India, which was believed to be having lesser load of metabolic syndrome as we are a developing nation, but this notion had been challenged by many and our study had provided the data for the rising trend like developed countries [1,6].

53.3% of our NAFLD patients were known case of diabetes at the time of recruitment and we compared the results in the change of parameters studied in diabetic and non-diabetic population with NAFLD. Pooled data showed no significant changes were noted in the BMI, HDL and waist circumference after 12 weeks course of Vitamin E in both genders and in both set of patients (with or without diabetes). Apart from HDL, other parameters of lipid profile, viz., LDL, VLDL, Total Cholesterol and Total Triglycerides which were "bad" based on the available evidence, significantly reduced after Vitamin E treatment. Though, lipid lowering ability of Vitamin E had been observed by some, by and large we lack rigorous evidence to support it [35,36]. Since Vitamin E reduced these dangerous lipids both in diabetic and non-diabetic patients, it would be prudent not to just brush aside the lipid lowering action of Vitamin E [37].

With respect to changes in liver enzymes, it should be apparent from the results that both serum aspartate and alanine transferases levels reduced drastically after Vitamin E treatment for 12 weeks. Several researchers agreed upon the pathophysiology of elevated liver enzymes in NAFLD patients and its implication in the progress of the disease [38]. ALT normalization with Vitamin E either alone or with combination and its relevance in the context of NAFLD pathology had been elucidated by majority though some authors could not spot the change and the reasons could be smaller sample size [23,26,28,33,39 – 41]. The finding of loss of response of ALT after discontinuation of Vitamin E bolsters the direct and proximate therapeutic effect of Vitamin E in reducing the hepatic inflammation as evidenced by the significant decrease in liver enzymes [28,34].

Hence, the effect of Vitamin E in decreasing the elevated liver enzymes, especially ALT in NAFLD patients would be undoubtedly a matter of acceptance. The association of weight loss and reduction in the level of transferase were not consistently observed except a few [28,42,43]. The assertion that elevation of transaminases occur only in nonalcoholic steatohepatitis category (NASH) doesn't translate well in clinical practice and many authors found underlying pathology for non-alcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) were same and amenable for treatment, especially with drugs like Vitamin E [7,11,44].

Hepatic steatosis, inflammation and fibrosis were studied in NAFLD patients and various methods to assess and estimate the pathology using scoring systems had been in vogue circumventing the need for liver biopsy, which is an invasive procedure with its attendant complications and the feasibility of repetition in any clinical trial [17,45]. We used non-invasive methods of assessment, viz. ultrasonogram for assessing the structural abnormalities and grading it with Hamaguchi scoring system and NAFLD Fibrosis Scoring system. The shuffling of

number of patients from higher scoring to lower scores after Vitamin E therapy ostensibly indicate its anti-oxidant, anti-inflammatory, anti-apoptotic actions for NAFLD patients [21,46].

Regarding fibrosis, which is a histopathological entity, we have evidence for as well as against Vitamin E, calling for rigorous scrutiny by reviews and metanalysis. While Sato et al., in their metanalysis showed improvements in all domains like enzymes, steatosis, inflammation and fibrosis, Usman et al. didn't find it useful in fibrosis [47,48]. In our study, we could not find any significant improvement in NFS score after Vitamin E therapy rather there was worsening of probability score for fibrosis derived from NFS [22]. As cautioned by Amanullah et al., larger sample size along with pre and post treatment biopsy should be needed to link the usefulness of Vitamin E in cases of hepatic fibrosis due to NAFLD [49].

Despite various guidelines differ with the use of Vitamin E either alone or as an adjuvant and recommending it as a first-line drug or not at all or with some prerequisites like only in non-diabetic, biopsy proven NAFLD patients; we feel it would be worth to use Vitamin E in patients with clinically established NAFLD as a first-line agent, specifically in patients with metabolic syndrome [12,13,50,51]. Regarding the adverse effects, we found it safe except meager number of patients reported gastrointestinal symptoms (Nausea: 0.05%. Diarrhea: 0.03%. Flatulence 0.01%) and no loss of follow up for adverse events noted. Maybe long-term studies with larger sample size might throw more light on this contentious issue [52 – 54]

LIMITATIONS

Since it was a non-randomized trial for a short period, we can't make generalizations and long term, randomized trial with larger sample size using blinding would help us to understand the role of Vitamin E in NAFLD better.

CONCLUSION

We advocate Vitamin E in NAFLD patients especially in cases with metabolic syndrome and in patients for whom elevated transaminases and radiological findings are suggestive of steatosis even without biopsy.

ACKNOWLEDGEMENT

The authors acknowledge all the patients who participated in the study.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

R. Divakar and P. Sugirda designed the study whereas R. Lenin and T. Henry Daniel Raj collected the data. R. Divakar and P. Sugirda analyzed the data while R. Lenin and T. Henry Daniel Raj interpreted the data. All authors contributed to the preparation of the manuscript. All authors read and approved the final manuscript.

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