

**The Effect of Vitamin E (Mixed Tocotrienol) on The Liver Stiffness
Measurement Measured by Transient Elastography (FibroScan) among
NAFLD patients**

ABSTRACT

BACKGROUND

Vitamin E has been shown to slow down progression or cause regression of fibrosis stage among NAFLD patients.

Transient Elastography (FibroScan) is a non-invasive tool that has been used to determine the stage of fibrosis based on Liver Stiffness Measurement (LSM) among NAFLD patients and may be used for treatment monitoring. This study aims to determine the effects of vitamin E taken once a day on the LSM.

METHODS

NAFLD patients diagnosed by ultrasound who met the inclusion criteria were enrolled in the study. Liver Stiffness Measurement (LSM) measured by FibroScan at base line and at the end of 3 months. A change in the LSM was the primary objective. Chi Square analysis was used to measure the change of LSM pre and post treatment. P value less than 0.05 was considered significant.

Patients were assigned to either the Life style Modification Advice Group (LMAG)—with nutritional counseling and advise to exercise—or the Treatment Group (Vitamin E as Mixed Tocotrienol 100 mg daily for 3 months plus lifestyle modification advise).

RESULTS

Fifty-seven percent (38/67) of patients enrolled in both arms of the study improved --with decrease in their LSM measurements -- but 43% (29 of 67) did not.

Of those who improved 79% (30 / 38) were from the Treatment Group (Vitamin E) and 21% (8 / 38) were from the LMAG.

Twenty -nine (29) patients did not improve: 79% (23/29) from LMAG and only 6/29 (21%) from the Treatment Group. Chi-square analysis showed that treatment with Vitamin E had a significant effect ($p = <0.05$) on improvement of LSM.

CONCLUSION

Vitamin E (mixed Tocotrienol) 100 mg daily for 3 months could decrease the LSM among NAFLD patients.

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INTRODUCTION

Non Alcoholic Fatty Liver Disease (NAFLD), defined as fatty infiltration of the liver exceeding 5% to 10% by weight¹, is an increasingly recognized histopathology condition that may progress to end stage liver disease.^{2,3} The spectrum of NAFLD ranges from simple fatty liver (hepatic steatosis) to fat accumulation plus necro-inflammatory activity with or without fibrosis (Non Alcoholic Steatohepatitis or NASH), to fibrosis and to cirrhosis (Fibrosis stage 4).⁴

The "two hit" theory is the pathogenic mechanism used to explain why simple fatty liver can lead to steatohepatitis and can progress to liver fibrosis and cirrhosis. The first "hit", insulin resistance, leads to accumulation of fat within the hepatocytes, and second "hit", production of mitochondrial reactive oxygen radicals, leads to lipid peroxidation, cytokine induction, and the induction of apoptotic or necrotic signals which ultimately results in increased collagen formation in the extracellular matrix (fibrosis)⁵. Progressive fibrosis leads to cirrhosis.

Large-scale surveys in the United States (NHANES III) and Europe (Dionysus study in Italy) have shown that the prevalence of NAFLD in the general population is about 20%^{3,6-8}. While data obtained from large surveys in China, Japan, and Korea show very similar prevalence rates to those described in Western surveys.⁹⁻¹³ A study by De Lusong et al at the Philippine General Hospital showed a prevalence rate of 12.2%.¹⁴

NAFLD is usually diagnosed by abdominal ultrasound (US) either as an incident finding or as the most common diagnostic procedure to screen asymptomatic patients with an incidental elevation of liver enzymes.¹⁵ Ultrasound has a sensitivity of 89% and a specificity of 93% in detecting steatosis and a

sensitivity and specificity of 77% and 89% in detecting increased fibrosis. However, US cannot detect small amounts of hepatic steatosis and cannot establish the diagnosis of NASH or stage of hepatic fibrosis.¹⁶ A local study done by Sanchez JK et al of 29 NAFLD patients diagnosed as mild fatty liver on ultrasound who underwent elective cholecystectomy and intra-operative liver biopsy revealed that only 34.5% had a Metavir fibrosis score of F0 (no fibrosis or purely simple hepatic steatosis). The rest had varying degrees of fibrosis-- 24.1% had F1 (portal fibrosis without septa), 20.7% in F2 (portal fibrosis with few septa), 20.7% in F3 (numerous septa without cirrhosis) and 6.9% already had F4 (cirrhosis).¹⁷

Transient Elastography (FibroScan, EchoSens, Paris) is a recently developed technique for the detection of fibrosis by measuring liver stiffness in patients with chronic liver disease. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness: the stiffer the tissue, the faster the shear wave propagates.¹⁸⁻²⁰ The results are expressed in kilopascals and vary from 2.5 to 75 KPa, corresponding to the median value of 10 successful acquisitions in the same patients.^{21,22}

Liver Stiffness Measurement (LSM) using transient elastography can determine the hepatic fibrosis stage in NAFLD. Several studies done^{23,24} have shown that the Liver Stiffness Measurement (LSM) of NAFLD patients with Fibrosis Stage 0 (no fibrosis) was from 0-5.8, F1 (portal fibrosis without septa) was from 5.9-7, F2 (portal fibrosis and few septa) was from 7.1-7.9, F3 (numerous septa without cirrhosis) was from 8-10.3 and F4 (cirrhosis) was >10.3.²⁴

Liver Stiffness Measurement (LSM) may also be used as a modality to monitor treatment response. Several studies have proved its use in treatment monitoring of chronic liver disease as in chronic Hepatitis B and C.²⁵⁻²⁷ However, there is no published data yet regarding LSM monitoring in NAFLD treatment.

Treatment for NAFLD is directed toward to its cause and pathogenesis.²⁸ Vitamin E is an antioxidant with anti-fibrogenic properties (anti-TGF β) and has been used in the treatment of NAFLD. Sanyal et al. randomly assigned 247 adults with NASH non-diabetes to receive pioglitazone (80 subjects), vitamin E (84 subjects), or Placebo (83 subjects), for 96 weeks. Patients had liver biopsy at baseline and at the end of the study. The results showed that Vitamin E therapy, as compared with Placebo, was associated with a significantly higher rate of improvement in nonalcoholic steatohepatitis (43% vs. 19%, $P= 0.001$).²⁹ A study conducted by Magosso et al showed half of subjects diagnosed as NAFLD by ultrasound taking Mixed Tocotrienol (family of vitamin E) for one year had complete remission, compared with only 23 percent in the control group ($P<0.05$).³⁰

This study is done to determine the effect of Vitamin E (Mixed Tocotrienol) on Liver stiffness among NAFLD patient using Transient Elastography to measure response to treatment.

METHODOLOGY

Study Oversight

This study was reviewed and approved by ethics committee of the hospital where it was conducted. All subjects gave written informed consent. Data were gathered by using Microsoft Excel program.

Study design

Our study is an Experimental research pre-test post-test design with control group. The rationale for this study has been described previously. This study focused on adults (>18 years old) without diabetes or other comorbidities and non-alcoholic person, who are diagnosed as Fatty Liver (NAFLD) based on ultrasound finding.

Exclusion criteria were age less than 18 years old, alcohol consumption of more than 20 g per day in the case of women and more than 30g per day in the

case of men, diabetes, heart failure, creatinine clearance < 50 $\mu\text{mol/l}$, ALT more than 2 times upper normal limit, viral hepatitis, BMI more than 30 kg/m^2 , liver stiffness more than 10.3 kPa, ascites, and those who refused to give consent.

Liver stiffness was measured using Transient Elastography (FibroScan®, Echosens) and correlated to Fibrosis stage for sub analysis. A decrease of liver stiffness or decreasing fibrosis stage at the end of study is considered improvement and no change or increased liver stiffness measurement (LSM) or fibrosis stage) was considered as no improvement. A change in the liver stiffness measurement is the primary objective.

All subjects who met the inclusion criteria were assigned to Treatment group (VITAMIN E group) and Lifestyle Modification Advice Group (LMAG). The sample was not randomized due to financial constraints.

Patients who refused treatment were included in the Lifestyle Modification Advice Group.

Patients in the treatment group received Vitamin E (Mixed Tocotrienol) 100mg 1 cap daily for 3 months.

Both groups were given similar lifestyle modification advice that included nutrition counseling, advise to limit alcohol consumption and regular aerobic exercise at least 2 times a week.

All patients had Liver Stiffness Measurement by FibroScan and ALT at base line and repeated at the end of 3 months.

Subjects were followed up according to a predetermined schedule for assessment of the safety, tolerability and compliance of the study drugs. Subjects were discouraged from adding other drugs that are used to their regimen.

The planned sample size was 41 subjects in each group. With this estimated sample size, the study would have 90% power to detect a difference in the rate of improvement.

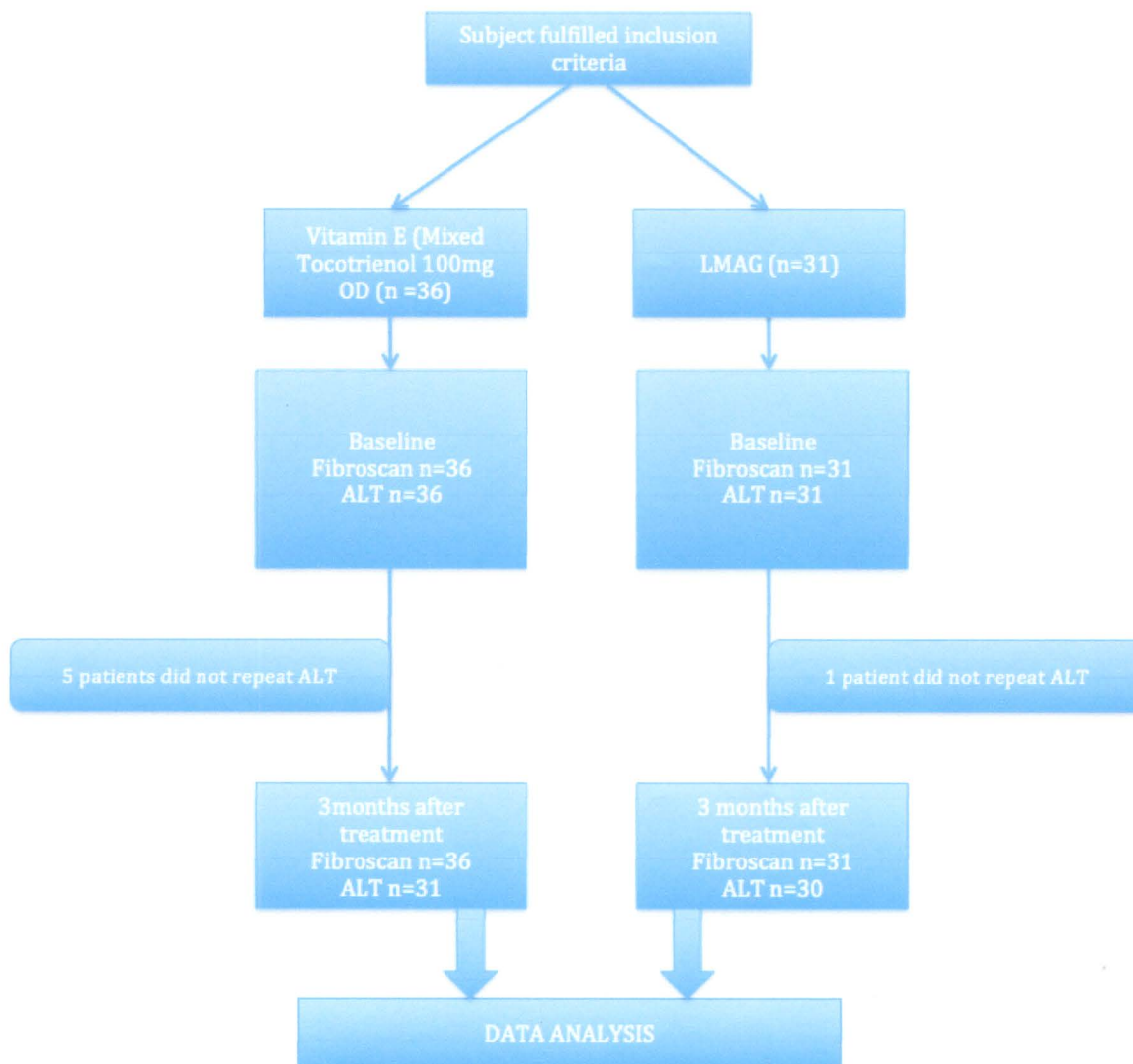
Statistical analysis

Data was analyzed using SPSS program.

Chi Square analysis was used to measure the change of liver stiffness pre and post treatment. P value less than 0.05 was considered significant in this study.

Liver stiffness measurement will be correlated to Fibrosis stage in the sub analysis.

To determine if gender will influence the results data from treatment group and lifestyle modification group will be analyzed separately according to sex. The homogeneity of both groups (M and F) will be analyzed using Breslow-Day and Tarone's tests.



RESULTS

Demographics

There were 67 patients enrolled in the study: Thirty-six (36) in the Vitamin E and life style modification advice group (treatment group) and thirty-one (31) in the lifestyle modification advice only group.

Twenty-nine were females (29/ 67 or 43.3%) and 38 were males 38/67 or 56.7%). Seventeen of the females (17/29 or 58.6%) were in the lifestyle modification advice group and 12/29 or 41.4% were in the treatment group. Fourteen of the 38 males (36.8%) were in the lifestyle modification advice group (LMAG) while 24 (63.2%) were in the treatment group.

The mean age, BMI, ALT levels, and pre-treatment Liver Stiffness Measurements of patients in the treatment group and in the lifestyle modification advise groups did not differ significantly.

The descriptive statistics of the subjects are shown in table 1.

Table 1. Descriptive statistics of subjects*

Characteristic	LMAG (n = 31)	Vitamin E (n = 36)	Total (n = 67)
Female	17	12	29
Male	14	24	38
Age	42.6±0.11	48.1±0.12	45.6±11.6
BMI (pre-treatment)	25.7±2.25	26.02±2.32	25.88±2.28
BMI (post-treatment)	25.85±1.99	25.84±2.33	25.84±2.16
Alt (pre-treatment)	53.07±5.90	54.47±.104	55.75±13.43
Alt (post-treatment)	56.13±.011	51.70±8.0	53.78±9.840
Fibrosis (pre-treatment)	5.78±1.41	6.3±1.92	6.06±1.71
Fibrosis (post-treatment)	5.91±1.63	5.70±1.50	5.8±1.56

*Plus and minus signs are mean ± standard deviation.

Comparison of the LSM among Treatment Group and LMAG

The pretreatment LSM was deducted from the post treatment LSM in both groups. Improvement was defined as decreasing LSM or decreasing fibrosis stage at the end of study duration compared to baseline, and no improvement was defined as no change or increasing liver stiffness measurement or fibrosis stage at the end of the study compared to baseline.

The results (table 2) showed that 38 of 67 patients (57%) improved while 29 of 67 (43%) did not improve. Thirty (30) of the 38 (79%) who improved were from the treatment group (Vitamin E) and only 8 of the 38 (21%) were from the Lifestyle Modification Advice group. Twenty-nine patients did not improve, 23 (79%) from Lifestyle Modification Advice group and only 6/29 (21%) from the treatment group (Vitamin E).

Chi-square analysis showed that treatment had a significant effect ($p < 0.05$) on improvement of fibrosis.

Table 2. Frequencies of subjects cross-tabulated for medication by outcome for liver stiffness measurement *

		Outcome		Total
		Improvement	No improvement	
Medication	Vitamin E	30	6	36
	Life Style Modification Advice	8	23	31
	Total	38	29	67

*Pearson chi square ($p = 2.1520579301898186E-6$) and Fisher's Exact Test ($p = 2.23021650285871E-6$) are significant at 0.05.

Any significant relationship may be influenced by other factors that are intrinsic to the units of analysis. In this study it may be possible that gender or

sex distribution may confound the analysis. Thus, the sample was analyzed using Mantel-Haenszel Test and produced separate contingency tables for males and females.

Fourteen out of the 29 females in this study (48%) improved and 15 of 29 (52%) did not improve (table 3 on appendix). Of the 14 who improved, 9 (64%) were from the treatment group (vitamin e) and 5 (36%) were from the LMAG. Of Twelve of the 15 patients who did not improve (80%) were from the LMAG. Only 3 of the 15 patients who did not improve (20%) were from the treatment group (vitamin E).

Chi-square analysis showed that medication had a significant effect (p value < 0.05) on whether fibrosis would improve. This means that the proportion of females that improved to those that did not is significantly different in the two groups.

Twenty four of 38 males (63%) improved and 14 (37%) did not (Table 4 on appendix). Twenty-one of the 24 who improved (87.5%) were from the treatment group while 3 of the 24 (12.5%) were from the LMAG. Eleven of the 14 males (79%) who did not improve were from the LMAG while 3 of 14 males (21%) were from the treatment group.

Chi-square analysis showed that medication had a significant (p value < 0.05) effect on whether fibrosis would improve. This means that the proportion of males that improved to those that did not is significantly different in the two groups.

The odds of Liver Stiffness improvement in females in the treatment group were 7.2 greater than the odds of Liver Stiffness improvement in females in the LMAG (table 5 on appendix).

In males, the odds of Liver Stiffness improvement for those in the treatment group was 25.67 times greater than those in the LMAG.

However, the results of Breslow-Day and Tarone's homogeneity tests for odds ratios in males and females showed that there was no significant difference (p >0.05). This means that the odds ratio of improvement in males in the

treatment group is the same as the odds ratio of improvement in females the treatment group.

Therefore, the odds ratios of females and males were combined in the Mantel Haenszel test. A significant Mantel Haenszel test ($p < 0.05$) means that there is a significant relationship between medication and outcome when the effect of sex is controlled or removed. It also means that when sex is disregarded the odds of Liver Stiffness improvement were 13 times greater than the odds of Liver Stiffness improvement for those in the LMAG.

Comparison of LSM correlated with Fibrosis Stage among Treatment Group and LMAG.

Liver Stiffness measurement can be correlated with Fibrosis Stage. Based on FibroScan (Echosens); the correlation for NAFLD are as followed: F0 (0-5.8), F1 (5.9-7), F2 (7.1-7.9) F3 (8-10.3), F4 (>10.3).²⁴

Pre-treatment Fibrosis stage of both groups are shown in Table 6.

Majority of the 31 patients in the LMAG, (18/31 or 58.1%) were in Fibrosis Stage of 0 while 9 /31 (29%) were F1, 2/31 (6.45%) were F2 and 2 /31 (6.45%) were F3 stage.

In the treatment group 15/36 (42%) were in F0, 9/36 (25%) were in F1, 6/36 (16.7%) were in F2 and 6/36 (16.7%) were in F3.

Table 6. Descriptive statistics for pre-treatment Fibrosis Stage.

Fibrosis Category	LMAG		Vitamin E	
	N	Mean±Standard Deviation	N	Mean±Standard Deviation
F0	18	4.8333±0.63059	15	4.5200±0.65705
F1	9	6.5444±0.34319	9	6.3556±0.36780
F2	2	7.4000±0.14142	6	7.5500±0.29496
F3	2	9.2000±1.4142	6	9.4167±1.13739
Total	31	5.78±1.41	36	6.3±1.92

Table 7 (at appendix) showed Fibrosis Stage category from Lifestyle Modification Advice group (LMGA) analysis. Of the 18 patients in the F0 category, none (100%) showed improvement at the end of study. Eight of the 9 patients (89%) in F1 did not improve while 1 (11%) improved (F1 to F0). None of the 2 patients with F2 (100%) showed improvement and between the 2 patients in F3 category, 1 (50%) improved (F3 to F2) and 1 (50%) did not improve.

Chi square analysis however did not show that this was significant ($p > 0.05$).

Table 8 (at appendix) showed Fibrosis Stage category from the Treatment group (Vitamin E). Fifteen of 36 (42%) were in F0, 9 of 36 (25%) were in F1, 6 (17%) were in F2, and 6 (17%) were in F3.

All 15 patients in F0 (100%) showed no improvement (still F0 in the end of study). Three of 9 (33%) patients in F1 showed improvement (F1 to F0) and the remaining 6 (67%) did not improve. 5 of 6 patients (83%) in F2 showed improvement (F2 to F1) while only 1 (17%) did not improve. Two of 6 patients (33%) in F3 improved but 4 subjects (67%) did not improve.

Chi square analysis showed no significant result ($p > 0.05$).

Since both groups received the same Lifestyle Modification Advice, to determine whether this affected the change in liver stiffness measurement, the BMI was used as a substitute marker.

To analyze the correlation between the BMI and the Liver Stiffness, the Pearson's correlation coefficient was used. The test revealed that there was no significant correlation between BMI and Liver Stiffness in pre treatment (Pearson's $r = 0.149$ $p = 0.230$) and post treatment (Pearson's $r = 0.098$, $p = 0.431$) when LMAG and treatment groups are analyzed together (figure 2 and 3 at appendix). This means that the improvement of Liver Stiffness is determined by treatment (Vitamin E (Mixed Tocotrienol)) only.

DISCUSSION

The '2-hit hypothesis' remains to be the pathogenic pathway of NAFLD – NASH-Fibrosis-Cirrhosis.⁵ Inflammatory cytokines, mitochondrial dysfunction, oxidative stress and growth factors e.g. NF-kappa B, TNF alpha, Interleukin 6, TGF beta play big roles in turning steatosis to steatohepatitis and fibrosis.³¹

Initially discovered in 1938 as a "fertility factor," vitamin E now refers to eight different isoforms that belong to two categories, four saturated analogues (**a**, **b**, **g**, and **d**) called Tocopherols and four unsaturated analogues referred to as Tocotrienols.³² Similar to Tocopherols, Tocotrienol has antioxidant, anti-inflammatory properties as well as anti proliferative activity of growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor-beta (TGF-**b**), HER2/neu, and interleukin-6 (IL-6).³³⁻³⁶ It also can inhibit the activation of Nf-Kappa B.³⁷ Theoretically Vitamin E either (whether Tocopherols or Tocotrienols) may be beneficial and able to slow down or to reverse progression of the NAFLD to fibrosis. This hypothesis was proven by studies conducted by Sanyal et al²⁹ and Magosso et al.³⁰

A study conducted by M Yoneda et al (2007) by using Transient Elastography in diagnosis patients with NAFLD, they revealed the areas under the receiver operating characteristic curves (AUROC)—which estimate the diagnostic performance of the elasticity measurements for hepatic fibrosis stage equal to or greater than F 1, F 2, F 3, and F 4 in NAFLD patient—were 0.881, 0.876, 0.914, and 0.997, respectively.²³ Similar result showed by Wong et al (2010), the AUROC for F3 or higher and F4 stage was 0.93 and 0.95, respectively, and was significantly higher than that of the aspartate aminotransferase-to-alanine aminotransferase ratio, aspartate aminotransferase-to-platelet ratio index, FIB-4, BARD, and NAFLD fibrosis scores (AUROC ranged from 0.62 to 0.81, $P < 0.05$ for all comparisons).²⁴ These study showed that Transient Elastography could also be used to determine the degree of liver

fibrosis among NAFLD patients. It also suggests the clinical utility and importance of using transient elastography in treatment monitoring as a substitute for liver biopsy.

Our study is the first study done using Transient Elastography as a monitoring tool determines the effect of vitamin E (Mixed Tocotrienol) on the liver stiffness measurement in NAFLD patients.

The study showed that vitamin E (mixed Tocotrienol) could decrease the liver stiffness measurement among NAFLD patients within 3 months using transient elastography to measure change in LSM. This study also showed a tendency of improving Fibrosis Stage within the treatment group even though not significant statistically. However the duration of our study is probably the main limitation since the studies conducted by Sanyal et al ran for 96 weeks²⁹ and that of Magosso et al for 1 year.³⁰ Extending the duration of the study may yield better results.

CONCLUSION

Compared to lifestyle modification advice only, Vitamin E (mixed Tocotrienol) 100mg once a day for three months can significantly reduce the liver stiffness measurement among patient with NAFLD.

Transient elastography, aside from measuring liver stiffness measurement and determining Fibrosis stage among NAFLD patients, can also be used as an effective monitoring tool to measure response to treatment.

However, these results cannot be generalized to patients with diabetes, heart failure or kidney failure.

The study has several limitations – need for more subject to decrease the sample margin of error, need for randomization, and also the short duration treatment and therefore it is recommended that these limitations should be addressed in the next study.

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