

Systematic Review and Meta-Analysis

Does Vitamin E Improve the Outcomes of Pediatric Nonalcoholic Fatty Liver Disease? A Systematic Review and Meta-analysis

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ABSTRACT

Background and Aims: To systemically evaluate the efficacy of adjuvant vitamin E on the outcomes of nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH) in children. **Materials and Methods:** We searched MEDLINE, PUBMED, EMBASE, the Cochrane Central Register Controlled Trials, and the Cochrane Database of Systematic Reviews over the period between January 1980 and September 2012 for the studies that examined the role of adjuvant vitamin E given at any dose or duration, alone or in combination with other interventions, on the outcome of pediatric NAFLD. The outcomes are alanine aminotransferase (ALT) normalization and histological improvement. **Results:** Five randomized trials were eligible to be included in our analysis, with a total of 270 participants. There was no statistically significant difference in the effect of adjuvant vitamin E on normalizing serum ALT [risk ratio (RR) =1.18, confidence interval (CI) =0.92-1.53, $P = 0.77$ for heterogeneity, $I^2 = 0\%$]. Sensitivity analysis showed that using higher doses of vitamin E, a longer duration of therapy or adding vitamin C did not change the effect on the measured outcome. Only two studies looked at histological changes as an outcome. We observed substantial heterogeneity between the two studies. **Conclusions:** Our meta-analysis did not find a significant effect of adjuvant vitamin E over placebo in normalizing serum ALT. Data on the long-term effect of adjuvant vitamin E on histological improvements in NAFLD patients are still lacking. Larger, well-designed randomized controlled trials (RCTs) in children with histological endpoints are still needed to answer this question.

Key Words: α -Tocopherol, antioxidants, steatohepatitis, steatosis

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Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver diseases that ranges from simple hepatic steatosis to steatohepatitis, which may subsequently progress to cirrhosis.^[1] The prevalence of NAFLD has increased significantly in children along with the increasing prevalence of obesity. It has become the most common form of chronic liver disease in children in developed countries.^[2]

NAFLD is recognized to be a multifactorial disease, which includes genetic, metabolic, and environmental factors; however, the precise etiology of NAFLD is poorly understood.

The condition starts with the accumulation of triglycerides and free fatty acids within hepatocytes (steatosis), which is induced by insulin resistance (first hit). When the adaptive mechanisms of the hepatocytes fail to accommodate the accumulated free fatty acids, lipotoxicity occurs, which induces oxidative stress and inflammatory changes that progress to nonalcoholic steatohepatitis (NASH). This condition causes injury to the hepatocytes, fibrosis, and subsequently, cirrhosis.^[3,4] As such, oxidative stress is increasingly considered to be the therapeutic target in NAFLD management through the use of antioxidant agents.

Currently, there is no definitive treatment for NAFLD; however, lifestyle changes (dietary and exercise programs) are the mainstay of the therapeutic interventions in NAFLD management, which have been shown to improve liver disease and resolve NASH changes in adults and children.^[5,6] However, because of the issues regarding the long-term adherence to a strict lifestyle program, other

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pharmacological treatments have been attempted. These treatments include antioxidants, such as vitamins E and C, and insulin sensitizers, such as metformin, and lipid-lowering agents. Vitamin E is the most common antioxidant agent evaluated in NAFLD management. Its antioxidant activity is thought to be secondary to its effect on stabilizing the cell membranes by protecting the unsaturated fatty acids from lipid peroxidation and subsequent free radical generation which induce cell injury.^[7]

Randomized controlled trials (RCTs) in adults and children have not uniformly demonstrated the beneficial effects of vitamin E on the long-term outcomes of NAFLD patients. The PIVENS trial, the largest adult trial, showed significant improvements in the aminotransferase levels, hepatic steatosis, lobular inflammation, and the total NAFLD activity score in the vitamin E group compared to the placebo group.^[8] The TONIC trial, the largest pediatric trial, did not find significant differences between the vitamin E and placebo groups in improving alanine aminotransferase (ALT) levels. However, resolution of NASH was observed more in the vitamin E group compared to the placebo group, which was attributed primarily to the improvement in hepatocytes ballooning, but there were no differences in steatosis or lobular inflammation between the two groups.^[9] Both these trials did not show any improvement in fibrosis. Mixed results were also reported from other small, open-labeled, and uncontrolled studies.

The aim of our study is to complete a systematic review of the literature and perform a meta-analysis to determine the efficacy of adjuvant vitamin E supplementation (adjuvant to lifestyle changes program) on the outcomes of NAFLD and/or NASH in children.

MATERIALS AND METHODS

Study selection

The databases, MEDLINE, PUBMED, EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews, and clinicaltrials.gov, were searched systematically for the time period between January 1980 and September 2012 by two independent reviewers. The search was limited to human studies and included both RCTs and observational studies that have at least two comparison arms. The search was limited to the pediatric age group (patients less than 18 years of age). The search was conducted using a combination of MeSH subject headings and text words as follows:

- Firstly, MeSH terms and text words including fatty liver, nonalcoholic fatty liver disease, NAFLD, nonalcoholic steatohepatitis, NASH, steatosis, and steatohepatitis were entered
- Secondly, we searched using the MeSH terms and text words, liver enzymes, aminotransferases, ALT and AST

- Finally, we searched using the MeSH terms and text words, vitamin E, alpha-tocopherol, and antioxidants
- We then combined the three searches
- At the end, we used the “age filter” function in each database to limit our search results to the pediatric age groups (1-18 years).

In addition, we searched the reference lists of retrieved articles to find other potentially relevant articles. Also, we contacted experts in the field to inquire about any more published or unpublished trials.

Inclusion criteria

Types of studies

RCTs and observational studies that have at least two arms of comparison.

Types of participants

Children or adolescents younger than 18 years of age with a radiological and/or histological diagnosis of NAFLD/NASH that is not attributed to other causes of hepatic steatosis or steatohepatitis, such as viral hepatitis, autoimmune hepatitis (AIH), Wilson’s disease, metabolic disease, or exposure to drugs that are known to induce steatosis, such as total parenteral nutrition (TPN).

Types of interventions

Vitamin E given at any dose or duration, alone or in combination with other interventions, versus a placebo or no intervention. Lifestyle interventions were considered as a co-intervention if applied equally in both arms.

Types of outcomes

Primary outcomes of interest included the following:

- Effect of adjuvant vitamin E on the normalization of ALT levels
- Effect of adjuvant vitamin E on histology improvement.

We decided to choose the percentage of patients with normalized ALT levels as an outcome (categorical outcome: yes/no) rather than the numerical ALT level changes (continuous outcome) because this more accurately reflects the effect compared to the level changes that tend to fluctuate over time and may just reflect the phenomenon of regression to the mean.

Secondary outcomes included any reported adverse events.

Data extraction and risk of bias assessment

Two independent authors (AS, AA) checked the titles and abstracts identified from the searches. We obtained the full texts of all the potentially relevant studies for the assessment. We selected the trials that satisfied our inclusion criteria and graded their methodological quality. Any disagreement was

resolved by consensus. We excluded non-human studies, adult trials, observational studies that did not have a comparison group, and studies that did not report the outcomes of interest.

One author performed data extraction (AS) using a standardized form. This process was checked by the second author (AA). Data on the study design, sample size, participant characteristics [number of patients randomized, gender, age, and body mass index (BMI)], intervention characteristics (dose, duration, and route of administration) and the outcomes of interest were extracted.

The methodological quality of the studies was assessed using the Cochrane Risk of Bias Tool.^[10] Each domain of the sex-domain tool was categorized as one of the following: Adequate, if low risk of bias; inadequate, if high risk of bias; or unclear, if uncertain risk of bias. Trials with adequate assessments in all the bias risk domains were considered as having low risk of bias and classified as high methodological quality. If one or more domains were judged as high risk of bias, then the study was regarded as a trial with high risk of bias and classified as low methodological quality.

Data analysis

We presented dichotomous outcomes as risk ratios (RR) with their 95% confidence intervals (CI). We used a fixed-effects model to calculate the pooled RR and 95% CI when the studies were sufficiently similar; however, we used a random effects model in the case of significant heterogeneity (i.e., having an I^2 of 50% or more), as this model represents a more conservative approach.^[11]

Because tests of heterogeneity may be insufficient to detect heterogeneity between studies when the number of studies is small, we also explored heterogeneity graphically and quantitatively using the I^2 statistic. An I^2 value of less than 25% was considered to have good homogeneity, a value of 25-50% to have reasonable homogeneity, and a value of >50% was considered to have significant heterogeneity. Then, we decided not to combine the results statistically.

Sensitivity analysis was performed to look for the effect of different doses of vitamin E (600 IU/day or more vs. less than 600 IU/day) and the effect of duration of the therapy (more or less than 1 year) on the outcomes of interest to explore if there is a dose- and/or duration-response effect. In addition, we did a sensitivity analysis to examine the effect of vitamin E alone or in combination with other antioxidants on the outcomes of interest. Finally, we did sensitivity analysis excluding the studies with “high risk of bias” to assess their influence on the outcomes. We planned to perform the quantitative analysis on an intention-to-treat basis. We had planned to examine the publication bias if an adequate number of studies were identified.

The meta-analysis was performed using RevMan V5® software.

RESULTS

Search results

In total, we identified 156 references: 115 through MEDLINE, PUBMED, and EMBASE; 31 through the Cochrane Central Register of Controlled Trials in The Cochrane Library; and 10 through the Cochrane Database of Systematic Reviews. Of these references, we identified 22 studies that examined the role of vitamin E in pediatric patients with NAFLD. Finally, we identified five completed trials^[9,12-15] that fulfilled our inclusion criteria and included these in the analysis [Figure 1].

Nobili *et al.*, published two trials.^[13,14] The second trial was an open-label extension of the first, when the study was extended for another year to examine the effect of vitamin E on histological changes. We decided to extract the data about the first outcome (normalization of the ALT levels) from the first trial because it was of better quality with low risk of bias compared to the extension arm which was an open label. However, we decided to include only the histological data from the extension trial, recognizing its risk of bias. These five trials that satisfied our inclusion criteria included a total of 270 participants.

Description of the included studies

Two trials identified NAFLD/NASH cases based on elevated aminotransferases and ultrasound changes,^[12,15] while the other two trials identified NAFLD/NASH based on elevated aminotransferases, ultrasound changes, and histology.^[9,13]

All the included trials compared vitamin E to at least one control group. There were 2 three-arm studies: One compared vitamin E to metformin and a placebo,^[9] while the other study compared non-blind vitamin E administration plus lifestyle intervention to a group with lifestyle intervention alone without medication (camp group) and a third group with no interventions at all (neither lifestyle program nor medication).^[15]

The vitamin E dose and duration of the therapy varied considerably among the included studies [Table 1]. All the included studies, apart from the Nobili *et al.*, trials, used vitamin E as a single antioxidant agent; however, Nobili *et al.*, used vitamin C in combination with vitamin E as an adjuvant antioxidant agent.

Most of the included studies were conducted over a year or less. Nobili *et al.*, extended their first trial^[13] for the second year to assess the histological changes, as an open-label extension.^[14] The TONIC trial ran over 2 years.^[9]

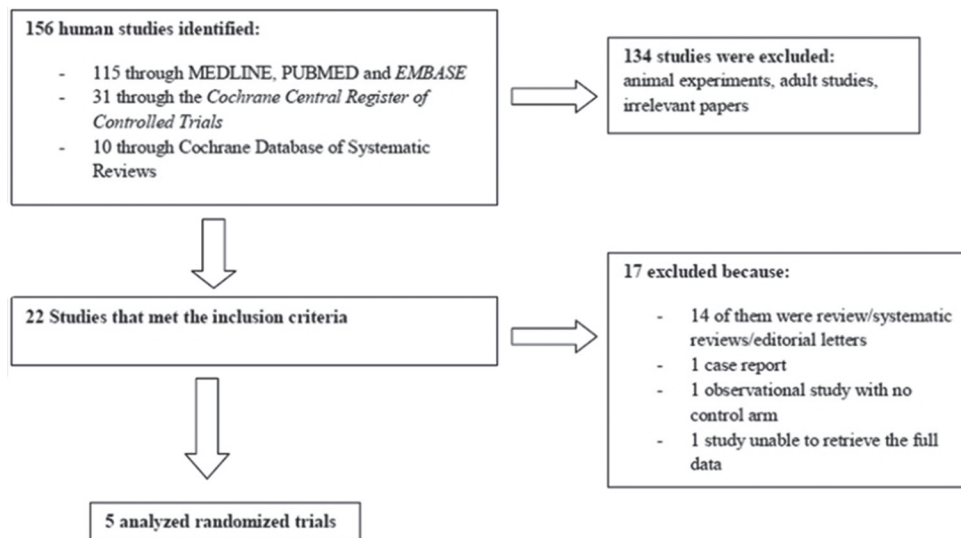


Figure 1: Flowchart of the search results

All the trials implemented a lifestyle intervention program (diet and exercise program) as a co-intervention that was applied for both the treatment and the control groups; however, these programs were not uniform among the included studies, with variable degrees of weight loss obtained at the end of each study.

All the studies reported data on aminotransferase changes; however, only two studies reported the data on pre-and post-treatment histology: The TONIC trial^[9] and the extension arm of the Nobili trial.^[14]

Table 1 summarizes the characteristic features of the included studies.

Excluded studies

A total of 17 studies were excluded from the analysis. Fourteen of them were reviews/systematic review articles. One study was excluded because there was no control arm and another study was a case report. We did not have access to the full text of one paper written in Chinese in which vitamin E was compared to a Chinese herbal medication.^[16]

Assessment of the methodological quality of the included studies

The two investigators independently rated the methodological quality of the selected studies using the Cochrane Risk of Bias Tool. Only two trials were graded as high methodological trials.^[9,13] These two trials met the criteria for random sequence generation, adequate allocation concealment, and adequate blinding of outcome assessment. All the included studies reported a full description of study withdrawals (see online Appendix A for the quality assessment of the included studies).

Effects of the interventions on the primary outcomes

Effect of adjuvant vitamin E on normalization of serum ALT level

All the included trials reported changes in ALT. We included the data from the first trial performed by Nobili *et al.*,^[13] because it was of an adequate methodological quality compared to the open-label extension. The trial by Nobili *et al.*, and the TONIC trial showed a significant drop in the ALT levels in both the vitamin E and the placebo groups; however, this drop was similar in both arms. The study by Wang *et al.*, showed a significant drop in the ALT levels in the vitamin E group and the lifestyle intervention group (camp group) compared to the no interventions group (no medication or lifestyle changes). This drop was more evident in the camp group compared to the vitamin E group. This study was the smallest and the shortest among the included studies. In addition, it had the highest risk of bias among all the included trials in the methodology assessment. Vajro *et al.*, reported a comparable ALT drop in both groups; however, the beneficial effect of vitamin E was observed more in the patients who showed a better adherence to the lifestyle intervention programs compared to those who did not.

We pooled the data from these studies using the fixed-effects model. We found no significant difference in the proportion of the participants with normalized ALT levels between the vitamin E and the placebo groups (RR = 1.18, CI = 0.92-1.53, $P = 0.77$ for heterogeneity, $I^2 = 0\%$) [Figure 2].

Sensitivity analysis

We performed sensitivity analysis to examine the effect of the dose of vitamin E (below and above 600 IU/day),

Table 1: Characteristics of the included studies

Study name, country	Study design and duration	Participants' characteristics at baseline	Inclusion criteria	Intervention (participations number and dose)	Comparator/control group (number)	Co-interventions	Outcomes		
							ALT normalization	Fatty liver changes by US	Histology improvement (percentage)
Vajro <i>et al.</i> 2004 Italy	RCT 5 months	N=28 patients Age 5.9-14.15 y Male=21/28 (75%) BMI (μ) = 24.6 kg/m ² % of NASH/ borderline NASH-NA (no histology)	Chronic asymptomatic hypertransaminases (\geq 1.5 UNL (40U/L) for >6 months) and bright liver on US	N=14 vitamin E 400 mg/day for 2 months then 100 mg/day for 3 months	N=14 Placebo	Life style intervention program (dietary restriction+ exercise) 30 kcal/kg/day	Vitamin E=7/14 (50%) Placebo=6/14 (42.8%)	Liver brightness disappeared in 3/14 patients of vitamin E group vs. 6/14 patients in placebo group	Not done
Nobili <i>et al.</i> 2006 Italy	RCT 12 months	N=88 patients (No cirrhotic patients) Age=9-15.5 years Male=28/88 (31.8%) BMI (μ) =25.9 kg/m ² NASH or borderline NASH 59/88 (67%) at baseline	hypertransaminases+ ultrasound changes+histology diagnosis of NAFLD	N=45 Vitamin E (600 IU/day)	N=43 Placebo	Life style intervention program (dietary restriction+ exercise) Balanced caloric diet (25-30 kcal/kg/day) and physical exercise and Vitamin C (500 mg/day)	Vitamin E=32/45 (71%) Placebo=26/43 (60.5%)	Liver brightness disappeared in 3/45 patients of vitamin E group compared to 3/43 patients in placebo group	Not done
Nobili <i>et al.</i> 2008 Italy	RCT 24 months An open label-extension of the previous study from 2006	N=53 (35 patients were excluded from the first study because they refused post intervention biopsy) Age 5.7-18.8 yrs Male 37/53 (70%) BMI (μ) 25.2 kg/m ² NASH or borderline NASH 43/53 (81%) at baseline	hypertransaminases+ ultrasound changes+histology diagnosis of NAFLD	N=25 Vitamin E (600 IU/day)	N=28 Placebo 1 patient had diabetes mellitus	Life style intervention program (dietary restriction+ exercise) and Vitamin C (500 mg/day)	Placebo 22/28 (79%) Vitamin E 13/25 (52%)	Not reported	Placebo 19/28 (68%) Vitamin E 17/25 (68%)

Contd...

Table 1: Contd...

Study name, country	Study design and duration	Participants' characteristics at baseline	Inclusion criteria	Intervention (participations number and dose)	Comparator/control group (number)	Co-interventions	ALT normalization	Fatty liver changes by US	Histology improvement (percentage)
Wang <i>et al.</i> 2008 China	3-arm non blinded prospective study. (Camp group=life style changes) vs. vitamin E group vs. no intervention group=no life style changes nor medications) 1 month	N=76 Age 10-17 years Male 52/76 (68%) BMI (μ) 29.5 kg/m ² % of NASH/ borderline NASH-not known (no histology)	Chronic asymptomatic hyper-transaminases (1.5 UNL) and bright liver on US	Vitamin E group=19 Vitamin E 100 mg/day (equivalent to 100-120 IU/day)	Camp group=19 (diet and exercise program in a summer camp=Low caloric diet (1300-1600 kcal and aerobic physical exercise (3hrs/day Vitamin E group with low intensity aerobic exercise (30 minutes of exercise, 2-3 times/week) Group 3 (control) N=38 pts (no interventions at all)	low intensity aerobic exercise for 30 minutes, 2-3x/wk in vitamin E group	Camp group 10/19 (52.6%) Vitamin E 9/19 (47.4%)	No US changes in all of the included patients	Not done
Lavine <i>et al.</i> 2011 (TONIC) USA	3-arms RCT (vitamin E vs. metformin vs. placebo) 24 months	N=173 (Non-diabetic, non-cirrhotic patients) Age 8-17 years Male 140/173 (80.9%) BMI (μ)=33.6 kg/m ² NASH or borderline NASH at baseline=121/173 (70%)	Persistently elevated ALT (> 60 U/L for 1-6 months) with biopsy-confirmed NAFLD	N=58 Vit E 800IU/day (400 IU bid)	N=58 in the placebo arm N=57 in the metformin arm (500 mg bid)	Life style intervention program (dietary restriction+ exercise) Details were not given	Placebo 10/58 (17%) Vitamin E 15/58 (26%)	Not reported	Placebo 11/39 (28%) Vitamin E 25/43 (58%) (as per treated analysis)

NAFLD: Non-alcoholic fatty liver disease, NASH: Non-alcoholic steatohepatitis, BMI: Body mass index, ALT: Alanine aminotransferases, NA: Not available, UNL: Upper normal limit

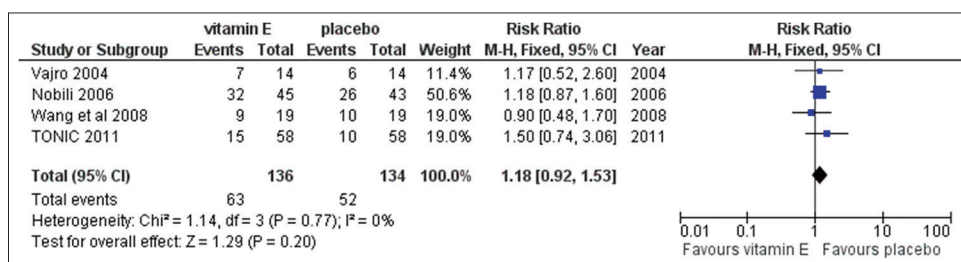


Figure 2: Analysis comparing the effect of vitamin E to the placebo for the proportion of participants with normalized ALT

duration of the therapy (2 years/1 year), and addition of other antioxidant agents versus the placebo. We did not find any beneficial effect when a higher dose of vitamin E was used (600 IU/day or more) or when vitamin C was added. Moreover, there was no difference when a longer duration of therapy (2 years or more) was used. Excluding the studies with a high risk of bias did not change the results.

Effect of adjuvant vitamin E on histological improvement

We found only two studies that looked at the histological changes pre- and post-intervention.^[9,14] Nobili *et al.*, reported NASH or borderline NASH in 81% of their patients at baseline, whereas the TONIC trial reported an incidence of 70%.

When we pooled the data from these two trials for meta-analysis, looking for the proportion of participants with histological improvement, we observed a substantial heterogeneity between the two studies ($I^2 = 80\%$). Therefore, it was not appropriate to combine their results statistically for this outcome.

Each trial had a different definition of histological improvement. The Nobili *et al.*, trial defined histology improvement as an improvement by ≥ 2 points in the NAFLD activity score, whereas the TONIC trial defined it as a resolution of the NASH changes at the end of the study. Nobili *et al.*, reported similar percentages of patients with histology improvement in both the placebo and vitamin E groups (68%), whereas the TONIC trial reported a higher percentage of histological improvement in the vitamin E group (58%) compared to the placebo group (28%). Table 2 summarizes the results of the NAFLD histological changes in both trials.

Secondary outcomes: Adverse events of vitamin E

No significant side effects were reported in the included studies. Vajro *et al.*, reported one patient who had a significant increase in his ALT level (a five-fold increase) after starting vitamin E. This change was resolved after stopping the treatment.^[12] The TONIC trial reported one patient who had mood changes and another patient who committed suicide; however, similar events were also observed in the placebo group.^[9] Nobili *et al.*, did not report any significant

adverse events in the first trial or in the extension.^[13,14] There were no reports of increased incidences of bleeding disorders in these trials, as had been reported previously in adult studies.^[17]

Publication bias

We initially planned to use a funnel plot to assess the publication bias. However, because of the small number of the included studies, the funnel plot was inappropriate to assess for publication bias. It has been shown previously that up to five studies are too few to allow the detection of funnel-plot asymmetry.^[18]

DISCUSSION

We identified five trials that reported the use of adjuvant vitamin E in pediatric patients with NAFLD. Changes in aminotransaminase levels have been shown to fluctuate over time and to poorly correlate with histological changes.^[19,20] However, because it is a non-invasive and cheap surrogate marker for liver injury, ALT has been used in several studies as a primary outcome. Our meta-analysis did not find any significant beneficial effect of vitamin E in normalizing ALT, compared to the placebo. A longer duration of treatment, a higher dose of vitamin E, or the addition of vitamin C did not change the results in our sensitivity analysis.

There are no previously published systematic reviews or meta-analyses that examined the effect of adjuvant vitamin E on NAFLD using only pediatric data. To the best of our knowledge, our systematic review and meta-analysis is the first published study in the pediatric field. Our meta-analysis results are consistent with the results from other meta-analyses performed on adult patients, where no significant effect of vitamin E was found on the ALT levels among NAFLD/NASH patients.^[21,22] Socha *et al.*, published a meta-analysis that included data from two pediatric trials in addition to adult trials. They found no significant effect of vitamin E on the normalization of ALT levels. This meta-analysis did not include any studies that examined the effect of vitamin E on histological changes.^[22]

The natural history of NAFLD in pediatric patients is not well understood because most of the studies on this disease

Table 2: Effect of vitamin E on NAFLD histology

Study name, year of publication	NASH/borderline NASH percentage at enrolment (n/total (percentage))	Histology improvement definition	Percentage of patients with histology improvement	Effect of the vitamin E on NAFLD activity score compared to placebo	Effect of vitamin E on individual component of the NAFLD histology compared to placebo			
					Steatosis	Inflammation	Hepatocytes ballooning	Fibrosis
Nobili <i>et al.</i> 2008	43/53 (81)	Histology improvement defined as improvement by ≥ 2 points in the NAFLD activity score (1 point at least in inflammation or ballooning score)	Placebo 19/28 (68%) Vitamin E 17/25 (68%)	-	-	-	-	-
TONIC 2011	121/173 (70)	Histology improvement defined as resolution of NASH in those with NASH or borderline NASH)	Placebo 11/39 (28%) Vitamin E 25/43 (58%)	↓	-	-	↓	-

NAFLD: Non-alcoholic fatty liver disease, NASH: Non-alcoholic steatohepatitis

were of short duration.^[23] However, it is well documented that NAFLD in children has a distinct histological pattern compared to that seen in adults where more portal inflammation and less hepatocellular ballooning, lobular inflammation, and perisinusoidal fibrosis are observed. Consequently, data extrapolation from adult studies is not optimal for pediatric patients.^[24]

We found two pediatric trials that assessed the effect of vitamin E on histological changes.^[9,14] The Nobili *et al.*, trial did not find a significant effect on the histological changes between the vitamin E and placebo groups, whereas the TONIC trial found a beneficial effect on hepatocyte ballooning. Both the trials did not find any effect on fibrosis. Both these trials had a small sample size, varied methodological quality, and a relatively short duration of therapy. There was a significant heterogeneity between these two trials; therefore, we did not proceed with the statistical analysis and could not make a definite conclusion about the beneficial effect of adjuvant vitamin E on histological changes on the basis of the identified trials.

RCTs in adults showed some evidence that vitamin E may improve the early histological features of NASH but not the features associated with advanced disease.^[7] However, some of these meta-analyses reported significant limitations of the included trials, which have a clear impact on reaching a definite conclusion about the beneficial effect of vitamin E on histology changes.^[21,22] Even if we assume that the results from adult studies are encouraging, we think that extrapolating from the adult data may not be appropriate for pediatric patients, keeping in mind that NAFLD histological changes in children may have distinctive patterns compared to the changes seen in adults.^[24]

The trials that evaluated the effect of adjuvant vitamin E on pediatric NAFLD patients showed mixed results. These

mixed results are partially due to the several limitations that these studies had. These included limited methodological quality of most of the included trials, small sample sizes, and using surrogate markers such as aminotransaminase levels and ultrasound changes as the primary endpoints rather than the histological changes. In addition, there was a considerable degree of discrepancies among the included studies in regard to the treatment protocols, including the doses and implementation of variable lifestyle intervention programs. The short and significantly variable durations of therapeutic intervention (1 month to 2 years) used in these trials do not allow for the proper examination of the long-term efficacy and safety of vitamin E. Moreover, lifestyle intervention programs were not consistent among the included studies. Variable degrees of weight loss were achieved at the end of each study, which may have had a major effect on the outcomes and should not be ignored.

No significant side effects were reported in the included studies; however, it is important to recognize that these trials were small in size, short in duration, and were not sufficiently powered to look for adverse events.

It seems to be more logical to use combination therapy in managing these patients because NAFLD pathogenesis occurs in multiple steps. The beneficial effect of combination therapy has been observed in several adult trials.^[25-28] The Nobili *et al.*, trials used a combination of vitamin E and vitamin C, both of which acted primarily as antioxidants. However, there were no pediatric trials designed to examine the effect of combination therapy with different mechanisms, such as antioxidants and insulin sensitizers.

We acknowledge that our systematic review is not without limitations; our meta-analysis included a small number of

trials with limited methodological quality. We did not have enough trials with reasonable homogeneity to examine the effect of vitamin E on the histological outcomes; therefore, we do not have a firm conclusion about this important outcome.

CONCLUSION

Our meta-analysis did not find a significant effect of adjuvant vitamin E over the placebo in normalizing serum ALT. Data on the long-term effects of adjuvant vitamin E on histological improvements in pediatric NAFLD patients are still lacking. In future, larger well-designed RCTs with adequate power and duration concentrating on the histological endpoints in children are still needed to answer this question. Thus far, lifestyle interventions (diet and exercise changes) are the only proven therapeutic intervention for NAFLD in children.

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APPENDIX

The quality assessment for the included studies

Vajro <i>et al.</i> (2004): High risk for bias		
Bias	Judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Authors stated "were randomly allocated to one of the two single-blind treatments" but no mention of details of random sequence generation
Allocation concealment (selection bias)	Low risk	Randomization was made with the use of sealed envelopes containing the treatment assignments
Blinding of participants and researchers (performance bias)	High risk	Researchers were not blind (single-blinded trial). Only the participants and the US operator
Blinding of outcome assessment (detection bias)	Low risk	Outcome was assessed by monitoring liver enzymes and blinded ultrasound evaluation
Incomplete outcome data (attrition bias)	Low risk	All participants were followed up, no losses to follow up
Selective reporting (reporting bias)	Low risk	All prespecified outcomes were reported
Other bias		

Nobili <i>et al.</i> (2006): Low risk for bias		
Bias	Judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	A computer-generated randomization sequence assigned participants in a 1:1 ratio to treatment with alpha tocopherol 600 IU/day plus ascorbic acid 500 mg/day (vitamin group) or placebo (placebo group)
Allocation concealment (selection bias)	Unclear risk	Randomization was generated by computer but further description of allocation is not included
Blinding of participants and researchers (performance bias)	Low risk	Vitamins and placebo pills were of identical appearance and taste, and prepared by the same pharmacist
Blinding of outcome assessment (detection bias)	Low risk	Outcome was assessed by monitoring liver enzymes, insulin resistance, and blinded ultrasound evaluation
Incomplete outcome data (attrition bias)	Low risk	Almost all participants were followed up, two patients were lost follow up from the placebo group but included in ITT analysis
Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
Other bias		

Nobili <i>et al.</i> (2008): High risk for bias		
Bias	Judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	A computer-generated randomization sequence assigned participants in a 1:1 ratio to treatment with alpha tocopherol 600 IU/day plus ascorbic acid 500 mg/day (vitamin group) or placebo (placebo group)
Allocation concealment (selection bias)	Unclear risk	Randomization was generated by computer but further description of allocation is not included
Blinding of participants and researchers (performance bias)	High risk	The study was double-blinded at the first 12 months (Vitamins and placebo pills were of identical appearance and taste, and prepared by the same pharmacist) and open label at the second 12 months
Blinding of outcome assessment (detection bias)	High risk	Outcome was assessed by monitoring liver enzymes, insulin resistance, NAFLD activity score (scored by a single blinded pathologist)
Incomplete outcome data (attrition bias)	Low risk	Almost all participants were followed up, only 2 cases lost to follow up
Selective reporting (reporting bias)	Low risk	All prespecified outcomes were reported
Other bias		

Wang *et al.* High risk for bias

Bias	Judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Authors stated "were randomly allocated to one of 3 groups" but no mention of details of random sequence generation
Allocation concealment (selection bias)	High risk	No concealment of allocation
Blinding of participants and researchers (performance bias)	High risk	Open label
Blinding of outcome assessment (detection bias)	High risk	Open label/Outcome was assessed by monitoring liver enzymes, insulin resistance, and blinded ultrasound evaluation
Incomplete outcome data (attrition bias)	Low risk	All participants were followed up, no losses to follow up
Selective reporting (reporting bias)	Low risk	All prespecified outcomes were reported
Other bias		

TONIC trial: Low risk for bias

Bias	Judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	Eligible patients were randomized in permuted blocks of treatments stratified by clinical center. Patients were assigned in a 1:1:1 ratio to 1 of 3 groups
Allocation concealment (selection bias)	Low risk	Eligible patients were randomized in permuted blocks of treatments
Blinding of participants and researchers (performance bias)	Low risk	All measures used to blind trial participants and researchers from knowledge of which intervention a participant received
Blinding of outcome assessment (detection bias)	Low risk	Outcome was assessed by monitoring ALT at 48 and 96 months, and NAFLD activity score (scored by a single blinded pathologist to the treatment assignment)
Incomplete outcome data (attrition bias)	Low risk	Eighty-seven percent of patients completed 96 weeks of treatment and had end-of-study clinical parameters and liver biopsy obtained. All enrolled patients were included in analysis of the primary outcome; sustained reduction in ALT level. The analysis was conducted using a modified intention-to-treat analysis
Selective reporting (reporting bias)	Low risk	All prespecified outcomes were reported
Other bias		