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Η επίδραση της λήψης συμπληρωμάτων βιταμίνης Ε στην πρόοδο και εξέλιξη της μη-αλκοολικής λιπώδους νόσου του ήπατος: μια συστηματική ανασκόπηση και μετα-ανάλυση

ΣΤΑΜΑΤΙΝΑ Α. ΒΟΓΚΛΗ

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ΠΕΡΙΛΗΨΗ

Η μη αλκοολική λιπώδης νόσος του ήπατος αποτελεί τη συχνότερη αιτία τελικού σταδίου ηπατικής νόσου στον δυτικό κόσμο και ο επιπολασμός της στους ενήλικες παγκοσμίως υπολογίζεται πως αγγίζει το 30%, με αυξητικές τάσεις και στον παιδιατρικό πληθυσμό. Το οξειδωτικό στρες διαδραματίζει κομβικό ρόλο στην παθογένεση της νόσου και αρκετοί αντιοξειδωτικοί παράγοντες έχουν εξεταστεί θεραπευτικά τα τελευταία έτη. Η βιταμίνη Ε αποτελεί αντιοξειδωτικό παράγοντα του οποίου η επίδραση στην πρόοδο και εξέλιξη της μη αλκοολικής λιπώδους νόσου του ήπατος έχει μελετηθεί σε ικανό αριθμό κλινικών δοκιμών. Η παρούσα διπλωματική εργασία αποτελεί μια συστηματική ανασκόπηση της βιβλιογραφίας έως τον Ιούνιο 2022 με μετα-ανάλυση των τυχαιοποιημένων κλινικών δοκιμών που εξέτασαν την επίδραση της βιταμίνης Ε στη συγκεκριμένη νόσο συγκριτικά με εικονικό φάρμακο ή παρεμβάσεις στον τρόπο ζωής (συμβουλές για ισορροπημένη διατροφή και άσκηση). Ως καταληκτικό σημείο επιλέχθηκε η μεταβολή των επιπέδων αμινοτρανσφερασών ορού [αλανινική και ασπαρτική αμινοτρανσφεράση, Alanine (ALT) and Aspartate (AST) aminotransferase]. Η εγκυρότητα των κλινικών δοκιμών αξιολογήθηκε βάσει του “Risk of bias tool” που έχει δημοσιευθεί από τον οργανισμό Cochrane. Συνολικά αναλύθηκαν 763 ασθενείς με μη αλκοολική λιπώδη νόσο του ήπατος από 12 τυχαιοποιημένες κλινικές δοκιμές και φάνηκε πως η λήψη συμπληρωμάτων βιταμίνης Ε προκάλεσε σημαντική μείωση των επιπέδων AST σε σχέση με τους ασθενείς που έλαβαν εικονικό φάρμακο ή μόνον δέχθηκαν συμβουλές για τον τρόπο ζωής τους. Τα επίπεδα της ALT, αντιθέτως, δεν μειώθηκαν στο σύνολο των κλινικών δοκιμών, όμως σημείωσαν σημαντική μείωση σε μελέτες στις οποίες χορηγήθηκε εικονικό φάρμακο στην ομάδα ελέγχου και στις μελέτες στις οποίες χορηγήθηκε βιταμίνη Ε αποκλειστικά. Σε κλινικές δοκιμές μεταξύ Ασιατικών πληθυσμών, η βιταμίνη Ε προκάλεσε συνολικά σημαντική μείωση στις αμινοτρανσφεράσες ορού των ασθενών. Συνοψίζοντας, η χορήγηση συμπληρωμάτων βιταμίνης Ε φαίνεται πως μπορεί να ωφελήσει ασθενείς με μη αλκοολική λιπώδη νόσο του ήπατος που διαθέτουν συγκεκριμένα χαρακτηριστικά, ως προς τη μείωση των αμινοτρανσφερασών ορού. Στο μέλλον, χρειάζεται να διεξαχθούν περισσότερες κλινικές δοκιμές υψηλής εγκυρότητας που θα βοηθήσουν να καθοριστούν επακριβώς τα χαρακτηριστικά των κατάλληλων υποψήφιων ασθενών, η βέλτιστη δοσολογία και διάρκεια χορήγησης καθώς και οι πιθανές ανεπιθύμητες ενέργειες των συγκεκριμένων σκευασμάτων.

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ABSTRACT

Background and Aim: Non-alcoholic fatty liver disease (NAFLD) has a global prevalence of 30% among adults with rising tendency among children too and is estimated to be the most common cause of end-stage liver disease in developed countries. Oxidative stress plays a key role during the course of the NAFLD pathogenesis and vitamin E supplementation has shown to have beneficial effects possibly due to its antioxidative properties. The aim of the present systematic review and meta-analysis is to investigate the effects of vitamin E supplementation in biochemical parameters in patients with NAFLD.

Methods: We performed a literature search in three major electronic databases (MEDLINE, CENTRAL, and Embase) for all randomized clinical trials that examined the effect of vitamin E versus placebo or lifestyle modification (dietary modification with or without encouragement for exercise) in patients with NAFLD, published until June 2022. Changes in serum aminotransferases were considered as the outcomes of interest while the quality of evidence was assessed through risk of bias according to the Cochrane risk of bias tool.

Results: A total of 763 patients from 12 randomized clinical trials were included in the present meta-analysis. Vitamin E did not reduce the values of alanine aminotransferase (ALT) compared with placebo or lifestyle modification but it effectively reduced aspartate aminotransferase (AST) serum values. ALT showed a significant reduction in studies that used vitamin E alone and placebo as a comparator, as well. Additionally, the analysis of Asian patients indicated greater and significant reductions in serum aminotransferases compared to non-Asian studies.

Conclusions: Vitamin E can improve serum aminotransferases in specific groups of NAFLD patients. Serum aminotransferases are among the commonest means of follow-up of these patients and our results indicate that vitamin E can be considered as a treatment option although further investigations are needed.

INTRODUCTION

Researchers estimate that non-alcoholic fatty liver disease (NAFLD) will affect up to one-third of the population globally in the next few years, becoming one of the most important global public health issues of the twenty-first century **[Lazarus 2020]**. NAFLD is a term used to describe a spectrum of clinical entities ranging from simple liver fat accumulation or steatosis to non-alcoholic steatohepatitis (NASH), progressing to cirrhosis and even to hepatocellular carcinoma (HCC) **[Chalasani 2018]**. The pathogenesis of NASH remains obscure, although several mechanisms have been proposed, from the traditional "two-hit hypothesis" to the "multiple parallel-hit hypothesis" **[Buzzetti 2016]**. In all pathophysiologic theories, oxidative stress is considered as one of the important key factors for the progression to NASH **[Ashraf 2015]**. Moreover, observations of depleted levels of vitamin E and other antioxidants in liver diseases provided the rationale of the use of antioxidants in clinical trials to examine their potent hepatoprotective effects **[Erhardt 2011]**. Given the fact that specific pharmacologic interventions for NAFLD are lacking, lifestyle interventions, such as lifestyle modification, weight loss and daily exercise, are the most common recommendation given to overweight and obese patients in an attempt to improve the clinical course of the disease.

Vitamin E has been thought to act as antioxidant agent and its efficacy on adult patients with NASH has been reported by a number of randomized clinical trials (RCTs) **[Sanyal 2010, Harrison 2003, Dufour 2006, Lavine 2011]**. New data from recent RCTs are available investigating the role of vitamin E in the treatment of NAFLD, with or without other interventions, in different age groups of patients but no firm conclusions have yet been reached. Further data regarding the duration and the dose of vitamin E administered, the effect of co-interventions, baseline variations, and potential associations between the age and the response to treatment could offer more information about the effect of vitamin E on NAFLD.

BACKGROUND

Early history and definition

NAFLD is a spectrum of liver disease ranging from simple deposition of adipose tissue in the liver to more progressive steatosis with associated steatohepatitis, fibrosis, cirrhosis, and in some cases HCC **[EASL 2016]**. Thomas Addison, broadly known by the eponymic disease of cortisol deficiency, was the first to describe fatty liver in 1836. In 1980, the term nonalcoholic steatohepatitis was used by Ludwig et al., to describe the progressive form of fatty liver disease histologically resembling alcoholic steatohepatitis though observed in patients who denied any alcohol abuse **[Lonardo 2020]**. The majority of these patients were obese women, and many were diabetic. Although, for decades, pathologists described the similarities of liver histology abnormalities seen in diabetic and morbidly obese individuals with those of alcoholics, Schaffner and Thaler were first to use the name "nonalcoholic fatty liver disease" in 1986 **[Schaffner 1986]**. Recommendations by scientific societies about NAFLD were notably delayed with the first NAFLD guidelines being released by the Asian Pacific Association Study of the Liver in 2007 **[Farell 2007]**. NAFLD is comprised of two distinct conditions with different prognoses: non-alcoholic fatty liver (NAFL) and NASH. NAFL is characterized by excessive liver fat accumulation and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a proton density fat fraction (providing a rough estimation of the volume fraction of fatty material in the liver) >5.6% assessed by proton magnetic resonance spectroscopy or quantitative fat/water selective magnetic resonance imaging (MRI), with no evidence of hepatocyte injury **[EASL 2016]**. The definition of NAFLD entails the absence of competing liver disease etiologies, such as chronic viral hepatitis, use of medications that induce steatosis such as amiodarone or tamoxifen, and other chronic liver diseases, such as autoimmune hepatitis, hemochromatosis, Wilson's disease, or significant alcohol consumption **[EASL 2016]**. According to the U.S. Guideline for NAFLD (endorsed as the American Association for the Study of Liver Diseases, American College of Gastroenterology, and American Gastroenterological Association NAFLD Guideline) significant alcohol consumption is defined as current or recent alcohol

consumption of >21 drinks/week in men and >14 drinks/ week in women **[Chalasani 2012]**. A smaller subgroup of these patients can develop NASH, which is a more progressive type of liver disease defined histologically by presence of hepatic steatosis with evidence for hepatocellular damage with hepatic fibrosis being the most important histological feature in NASH **[Benedict 2017]**.

Epidemiology of NAFLD

The prevalence of NAFLD is increasing throughout the world and NAFLD is currently the most common liver disorder in Western countries **[Gadiparthi 2020]**. Clinically, NAFLD patients tend to be obese, with insulin resistance and/or type 2 diabetes (T2D), dyslipidemia, hypertriglyceridemia, and hypertension, which are all risk factors for cardiovascular diseases (CVDs). Incidence of NAFLD has been shown to be 10% higher in overweight individuals compared to normal-weight persons in the United Stetes **[Younossi 2012]** and NAFLD has been reported in 55% of T2D patients worldwide **[Younossi 2019]**. Interestingly, although NAFLD parallels the prevalence of obesity and its comorbidities, it is also present in 7% of normal-weight persons, more frequently in females at a younger age and with normal liver enzymes **[Younossi 2012].** With regard to differences in the prevalence of NAFLD there appears to be a remarkable geographical variation throughout the world. According to a meta-analysis including 8,515,431 individuals, the overall global prevalence of NAFLD in adult population was estimated to be 25%, with the highest rates being in South America (31%) and in the Middle East (32%) whereas the lowest prevalence was estimated in Africa (14%). The prevalence was up to 27% in Asia, 24% in North America, and 23% in Europe **[Younossi 2015]**. Prevalence of the metabolic comorbidities associated with NAFLD was also increased and obesity was present in 51% of NAFLD patients; T2D in 22%; hyperlipidemia in 69%, hypertension in 39%; and metabolic syndrome in 42%. Fibrosis progression proportion, and mean annual rate of progression in NASH were 40% and 0.09% with liver-specific and overall mortality reported much greater among patients with NASH **[Younossi 2015]**. Although the true prevalence of biopsy-proven NASH is difficult to determine, as the majority of NAFLD patients do not undergo biopsy, NASH has been recognized as one of the leading causes of cirrhosis in adults in the United States **[Villerett 2022]**. Progression to cirrhosis is variable, being

influenced by genetic and environmental factors but it is estimated that 11% of patients with NASH progress to cirrhosis within 15 years and HCC may develop in up to 13% in patients with NASH and cirrhosis. Since NASH was first assigned as a diagnostic category by United Network for Organ Sharing (UNOS) in 2001, the prevalence of NASH as an indication for liver transplantation was unknown prior to 2001 and the majority of cases that were formerly classified as cryptogenic cirrhosis were most likely cases of NASH-related cirrhosis **[Kappus 2017]**. Currently NASH is the second indication for liver transplantation in the United States and NAFLD has been projected in the next few years to become the major cause of liver related morbidity and mortality as well as a leading indication for liver transplantation **[Villerett 2022]**.

Diagnosis and grading of NAFLD

As mentioned before, liver biopsy is essential in order to establish the diagnosis of NASH and it is the only procedure that differentiates NAFLD from NASH **[EASL 2016]**. In histology terms, NAFLD shows a wide range of manifestations from mild steatosis (5% or more of hepatocytes involved), to more aggressive forms with lobular and/or portal inflammation, ballooning hepatocytes, fibrosis, and ultimately cirrhosis **[Kleiner 2012]**. On the other hand, the diagnosis of NASH requires the joint presence of steatosis, ballooning and lobular inflammation **[Kleiner 2012]**. In adult patients, steatosis typically affects the centrilobular hepatocytes first, contrary to pediatric NAFLD in which disease is found mainly periportal **[Nobili 2019]**. For the purpose of grading, the affected parenchyma is usually divided into thirds: 5%-33%, 34%-66% and > 66% **[Takahashi 2014]**. The rule of thirds has allowed a three-tiered classification system with 5%-33% designated as mild, 34%-66% designated as moderate, and > 66% corresponding to severe steatosis. Noticeably, conventional imaging techniques are not supposed sensitive enough to detect hepatic steatosis when the percent involvement is less than 30% **[Li 2018]**. More advanced imaging techniques such as controlled attenuation parameter, magnetic resonance imaging-estimated proton density fat fraction, and 1H-magnetic resonance spectroscopy have been shown to correlate well with histologic steatosis assessment in both the adult and pediatric NAFLD populations **[Karlas 2014]**.

In order to provide a consistent and reproducible scoring system for assessment of NAFLD, NASH Clinical Research Network proposed NAFLD Activity Score (NASH CRN-NAS) **[Brunt 1999]** which uses numerical scores to develop an activity grade. This grading system includes steatosis (0-3 points), hepatocellular ballooning (0-2 points), acinar inflammation (0-3 points) and fibrosis stage (0-4) **[Kleiner 2005]**. Using a threshold of < 3, the NAS showed a good correlation with the absence of a histological diagnosis of NASH whereas a threshold of greater than or equal to 5 was correlated with having a diagnosis of NASH **[Kleiner 2005]**. In validation by Hjelkrem et al **[Hjelkrem 2011]**, a total of 386 liver biopsies were evaluated, the sensitivity and specificity were 57% and 95%, respectively, when using a NAS ≥ 5 (indicating NASH) and NAS < 5 (indicating no NASH). When using an activity score of ≥ 4, the sensitivity increased to 85% with a slight decrease in specificity to 81% **[Hjelkrem 2011]**. The ≥ 4 threshold has been recommended for any admission to an interventional trial for NASH. EASL guidelines propose that NAS should not be used for the diagnosis of NASH but rather for the evaluation of disease severity **[EASL 2016]** and although NAS is correlated with aminotransferase and homeostasis model assessment of insulin resistance (HOMA-IR), they have a low prognostic value **[Ekstedt 2015]**.

Risk factors for NAFLD development

In recent years, NAFLD is increasingly recognized as the liver disease component of metabolic syndrome and the two conditions are closely linked. Diagnosis of metabolic syndrome requires 3 of 5 of the following characteristics: elevated serum triglycerides, low high-density lipoprotein (HDL) cholesterol, hyperglycemia, an increased waist circumference, and hypertension. In addition to increasing rates of NAFLD parallelly with the rising rates of metabolic syndrome, it has been reported that NAFLD incidence increases with increasing number of criteria met. Moreover, given that T2D patients with NAFLD can have normal liver function tests, the prevalence of NAFLD in T2D patients may be much higher than estimated in this patient population **[Lonardo 2015].** Diet has been proposed as an independent risk factor for the development and progression of NAFLD. Data from the literature report that a diet high in carbohydrates might worsen the clinical course of NAFLD whereas the quality of dietary fats plays a key role **[Sanjaya 2015]**. The beneficial effect of mono- and poly-unsaturated fatty

acids has been demonstrated in a number of studies with the Mediterranean Diet being among the prudent dietary patterns that affect beneficially both the emergence and the progression of NAFLD **[Abenavolli 2019]**. Considering the race as a risk factor, the wide variation of NAFLD prevalence in different regions of the world can partially be explained by ethnicity differences apart from the different ethnic lifestyles. The rate at which NAFLD develops has been shown to be greatest in Hispanic patients who also have a higher prevalence of steatohepatitis and cirrhosis. In fact, the PNPLA3 allele, a gene that affects lipid metabolism, has been found to be most common in Hispanics **[Lonardo 2020]**. PNPLA3, also known as adiponutrin, is a member of the patatin-like phospholipase family and the rs738409 C>G single-nucleotide polymorphism (SNP), encoding the Ile 148Met variant protein of PNPLA3, is a well-described genetic determinant of hepatic steatosis. Hepatic fat content has been found more than two-fold higher in PNPLA3 rs738409[G] homozygotes than in noncarriers **[Sookoian 2011]**. The role of gender in predisposition of NAFLD has been evaluated in a number of studies but available data were inconclusive. According to Lonardo et al **[Lonardo 2020]**, NAFLD is more common in male patients and has been shown to increase in those who are younger to middle aged with a following decline after the age of 50-60 years. Contrary, in female patients who are pre-menopausal NAFLD has been shown to be less common. An Asian study found an increased prevalence of NAFLD in the late menopausal transition as well as postmenopausal stages **[Ryu 2015]**, and there is evidence that NASH is histologically more severe in women when compared to men of the same age. Polycystic ovary syndrome (PCOS) and obstructive sleep apnea (OSA) are among the recognized conditions linked to NAFLD progression. Body mass index and insulin resistance are important associated factors with both PCOS and NAFLD and in a study from United States NAFLD was found in 55% of women with PCOS and nearly 40% of them were normal-weight patients **[Carmina 2006]**. Regarding OSA, it is a comorbidity of NAFLD that affects 4% of the general population and 35 to 45% of obese individuals **[Young 1993]**. OSA has been proposed as an independent risk factor for NAFLD, suggesting pathophysiological alteration in gas exchange (repetitive hypoxemic and hypercapnic events) can lead to increased proinflammatory cytokine production, endothelial dysfunction, oxidative stress, metabolic dysregulation, and insulin resistance **[Mesarwi 2019]**.

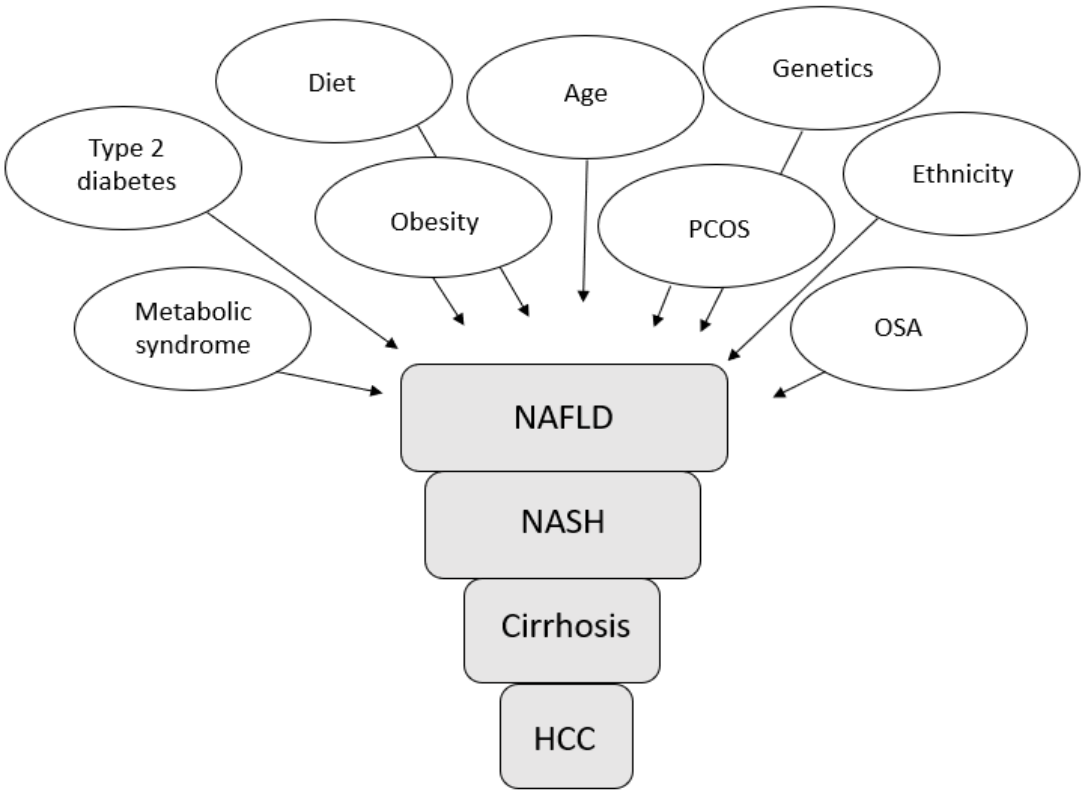

Figure 1. The risk factors for non-alcoholic fatty liver disease (NAFLD). NASH, non-alcoholic steatohepatitis; HCC, hepatic cell carcinoma; PCOS, polycystic ovary syndrome; OSA, obstructive sleep apnea.

The role of age

The prevalence of NAFLD has been found to increase with age and Yanossi et al estimated it 22% in people age 30-39 and 34% in those older than 70 years of age **[Yanossi 2015]**. The incidence of NASH and cirrhosis also increases with age especially in patients who are 50 years of age or greater compared with younger age groups **[Wang 2013]**. Similar to adults, the increasing burden of NAFLD in children is alarming. Obesity is a leading risk factor for the development of NAFLD in children and according to the Study of Child and Adolescent Liver Epidemiology, approximately one-third of obese children have NAFLD **[Schwimmer 2006]** making it the most common liver abnormality in this age group. Its prevalence increases with age, ranging from 0.7% for ages 2 to 4 years and up to 17.3% for ages 15 to 19 years **[Schwimmer 2006]** and in regard to that seen in adulthood, there is also an association of pediatric NAFLD and CVDs with higher levels of total cholesterol, LDL,

triglycerides, and systolic blood pressure reported **[Goyal 2016]**. The diagnosis of NAFLD in children is still challenging and caution is required not to miss alternate liver conditions. Thus, the presence of overweight or obesity does not mean that a child with steatosis and/or fibrosis does not have an alternative diagnosis including most usually infectious hepatitis, Wilson disease, inborn errors of metabolism, coeliac disease or alpha-1 antitrypsin deficiency **[Fitzpatrick 2019]**. Using magnetic resonance spectroscopy, liver steatosis has been found in infants born to mothers with gestational diabetes **[Goyal 2016]** and in a study using hepatic fat fraction performed in neonates born to normal mothers compared to those with gestational diabetes, neonates born to obese mothers with diabetes had a mean fraction that was 68% higher than those born to normal weight mothers **[Brumbaugh 2013]**. These results suggest that NAFLD is a condition that may begin in utero explaining partially why a small proportion of patients may present during childhood with significant fibrosis although, generally, the course of the disease is over 40–50 years before the end-stage **[Benedict 2017]**. The rapid physical and psychological changes that occur during adolescence also denote a critical period in which metabolism is regulated and may contribute to NAFLD progression **[Fitzpatrick 2019]**. Though it shares many features with adult-onset disease, NAFLD in children and adolescents has a number of differences. The distribution of lesions is usually different from adult disease with steatosis, inflammation and fibrosis being preferentially periportal (type 2 NAFLD) **[Molleston 2014, Schwimmer 2005]**. Ballooning is less common in children with type 2 disease and as a result children tend to score lower on the NAS because of less lobular inflammation and ballooning. A specific histological score (Paediatric NAFLD Histological Score – PNHS) has been proposed and validated for better classification of children with and without NASH **[Alkhouri 2012]**.

Pathogenesis of NAFLD

The pathogenesis of NAFLD is complex, multifactorial and not totally elucidated yet. The pathogenic drivers are not likely to be identical among all patients and as a result, both the mechanisms leading to disease and their clinical manifestations are highly heterogeneous **[Friedman 2018]**. Although understanding is still incomplete, it has been recognised that cellular and molecular mechanisms along with factors that

promote steatosis, hepatic inflammatory responses and fibrogenesis may play a role in a parallel or sequential way during the natural course of the disease **[Parthasarathy 2020]**. One of the first proposed pathophysiologic mechanisms was the "two-hit hypothesis" **[Buzzetti 2016]** which considered steatosis as a first hit to the liver that then would require a second hit to progress to NASH/fibrosis. Nowadays, this hypothesis is thought to be too simplistic and a multiple-hit hypothesis that implicates numerous factors acting in a parallel and synergistic manner in patients with genetic predisposition is an accepted explanation of the different NAFLD phenotypes and clinical courses **[Buzzetti 2016]**. In both models the primary insult is likely to be lipid excess and steatosis development. The first pathophysiologic step in this cascade is triglycerides (TG) accumulation that may result from a dysregulation between TG synthesis and utilization **[Arab 2018]**. Obesity and impaired action of insulin in adipocytes play a central role in this step and can lead to a failure in lipolysis suppression, adipocyte stress, and recruitment of macrophages in the adipose tissue. The excess lipolysis of TG leads to non-esterified fatty acid (NEFA) release into the circulation that bound with albumin and are delivered to the liver **[Arab 2018]**. The most strongly associated genetic variant with NASH is PNPLA3 gene mutation, which encodes a lipid droplet protein that is involved at this lipolytic step **[Romeo 2008]**. Apart from quantity, the type of NEFAs that accumulate in NAFLD are also altered, with significantly more saturated than monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively). In addition, other types of NEFA involved in NAFLD pathogenesis include diacylglycerols, ceramides, lysophophatidyl choline (LPC), and free cholesterol and all of them are considered lipotoxic **[Musso 2018]**. Since its first description of by Roger Unger in 1994 **[Lee 1994]**, lipotoxicity is increasingly being recognized as a factor contributing in NASH development. When toxic lipids accumulate in liver, the physiologically adaptive mechanisms may be overwhelmed leading to reactive oxygen species (ROS) formation, disruption of endoplasmic reticulum (ER) homeostasis (referred to as ER stress), lysosomal dysfunction, activation of inflammasome and inflammatory responses and cell death **[Friedman 2018]**. This continuum results in dysfunction and injury of hepatocytes and nonparenchymal liver cells, as well **[Parthasarathy 2020]**. Several extrahepatic factors, such as intestinal dysbiosis and adipokines, modulate lipotoxic exposure to the liver and subsequent injury and inflammation, with variable contributions across patients and different stages of the disease **[Parthasarathy 2020, Arab 2018]**. As in

most chronic liver diseases, ongoing liver injury in NASH is associated with the occurrence of hepatic fibrogenesis, ultimately leading to liver cirrhosis. Liver scarring is the consequence of a wound-healing response of the liver to injury and consists of the deposition of high-density extracellular matrix proteins that distort the liver architecture and form regenerative nodules **[Arab 2018]**.

The role of oxidative stress

Oxidative stress and ER stress, with activation of the unfolded protein response, are two well-characterized pathways that promote hepatocellular injury and cell death in NASH **[Ashraf 2015, Bravo 2013]**. The production of ROS in all eukaryotic cells is a highly regulated process that deeply affects cellular function and homeostasis in all organisms. Increased and dysregulated ROS formation is known to cause lipid peroxidation followed by inflammation and activation of hepatic stellate cells leading to fibrogenesis and both procedures are mediated by metabolic changes and pro-inflammatory transcription factor expression **[Friedman 2018]**. NAFLD and NASH patients have been found to present increased levels of ROS and lipid peroxidation products and decreased levels of anti-oxidant enzymes like superoxide dismutase, catalase and anti-oxidant compounds such as glutathione **[Videla 2004]**. The levels of α-tocopherol have also been found significantly decreased in NASH patients as compared to healthy subjects **[Erhardt 2011]**.

Vitamin E and NAFLD

Recently, there has been significant interest in examining the therapeutic effects of anti-oxidants supplementation in NAFLD/NASH. Vitamin E is a lipid soluble anti-oxidant that performs its function in the glutathione peroxidase pathway and protects cell membranes from oxidation by reacting with lipid radicals produced in lipid peroxidation chain reactions **[Herrera 2001]**. The term "vitamin E" covers at least eight different forms of the vitamin that are produced by plants alone (the natural vitamin E sources are vegetable oils) and have similar chromanol structures. Trimethyl (α-), dimethyl (β- or γ -) and monomethyl (δ-) tocopherols contain a saturated phytyl tail, while the corresponding tocotrienols have an unsaturated side chain **[Singh 2005]**. The

term Vitamin E describes both the tocopherols and tocotrienols and though it is generally believed that there are only four tocopherols and four tocotrienols in nature, in fact there are at least 12 known vitamin E regiments and this number is likely to increase in the future **[Asbagi 2020]**. The structure of each form governs its biological activity: the antioxidant properties are due to the chroman head while the tail largely determines mobility through lipid membranes. The double bonds of tocotrienols indicate a state of electron unsaturation, meaning it can accept electrons readily as an antioxidant resulting in superiority of tocotrienols as antioxidants **[Pacana 2012].** Once ingested, all forms of vitamin E are taken up by intestinal cells and released into the circulation in chylomicrons. The vitamins reach the liver via chylomicron remnants where a specific liver protein, α-tocopherol transfer protein (α-TTP), selectively targets α-tocopherol for incorporation into very-low-density lipoprotein while the other forms are less well retained and are excreted via the bile, the urine or other routes **[Herrera 2001, Singh 2005]**. Several clinical trials have studied the administration of vitamin E at different doses, both as monotherapy or combination therapy, in patients of all ages with NAFLD **[Sanyal 2010, Harrison 2003, Dufour 2006, Lavine 2011]**. There is evidence that vitamin E results in improvement in steatosis, inflammation, and ballooning and resolution of steatohepatitis in a proportion of nondiabetic adults with NASH and recently American guidelines for NAFLD suggest that Vitamin E be administered at a daily dose of 800 IU/day in nondiabetic adults with biopsy-proven NASH, without cirrhosis **[Chalasani 2018]**.

AIM AND OBJECTIVES

Clinical trials have investigated the role of vitamin E in the treatment of NAFLD/NASH, but no definite conclusion has yet been reached. In fact, Vitamin E is the most evaluated antioxidant agent in NAFLD with newer data from recent clinical trials being available since the latest meta-analysis **[Vadarlis 2020]**. The purpose of this study was to perform a systematic review of the literature and a meta-analysis to evaluate the effect of vitamin E on liver dysfunction including the most commonly used biochemical indices in patients with NAFLD/NASH, serum aminotransferases (ALT, AST). In doing so, the aim is to provide better treatment approaches for NAFLD patients with fewer patients progress to end-stage liver disease. Specifically, the main

objective of this study is to compare the effect of vitamin E with a placebo, a lifestyle modification, or a no-intervention in NAFLD patients. For this purpose, we consider baseline variations, potential associations between specific patient characteristics and vitamin E efficiency along with the effect of co-interventions. We hope that our research would assist clinicians in better assessment of the benefits of vitamin E in NAFLD/NASH in adults and children and would help to draw up clinical guideline for NAFLD/NASH with robust evidences.

METHODS

Literature search strategy

Our study was conducted according to a predetermined protocol which prespecified the research objective, search strategy, study eligibility criteria, and the methods of statistical analysis. The conduction of this systematic review and meta-analysis and the reporting of its findings were in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement as well as the standards of the Cochrane Handbook for Systematic Reviews of Interventions **[Higgins 2019]**. A systematic literature search was conducted in three major electronic databases including MEDLINE, CENTRAL, and Embase up to June 20, 2022. The following keywords were used: “NASH”, “NAFLD”, “liver steatosis “, “fatty liver”, “nonalcoholic steatohepatitis”, “nonalcoholic fatty liver disease”, “vitamin E”, “alpha-tocopherol”, “tocotrienol”. All published manuscripts (full-text and conference) were considered, with no language or publication year restrictions. We also searched for unpublished trials and those in progress using the database of the National Institute of Health (ClinicalTrials.gov). Authors and investigators of relevant trials whose results were not published, were contacted via email but no eligible study was added through this process. Furthermore, the bibliography of the included studies was searched manually to identify additional relevant records that were not retrieved during the literature search.

Eligibility criteria and study selection

We searched for randomized controlled trials using vitamin E supplementation in NAFLD/NASH patients, regardless of number of participants. Specifically, we included all studies meeting the following criteria: (1) population: patients regardless of age, gender and ethnic origin diagnosed with NAFLD/NASH according to the international definitions **[EASL 2016, Chalasani 2012]**, (2) intervention: vitamin E as tocopherol or tocotrienol form, regardless of dosage, duration of supplementation, or route of administration. Co-interventions were also considered eligible if used equally in both intervention arms, (3) comparator: placebo, lifestyle modifications (dietary modification with or without encouragement for exercise) or no intervention, (4) outcomes: changes in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). We excluded observational and non-randomized trials, in vitro and animal studies, and studies whose data were not adequately published or accessed for a meta-analysis (conference abstracts). Other reasons for exclusion were secondary causes of liver steatosis or NAFLD and the usage of changes in vitamin E intake through diet (and not supplementation) as an intervention. Retrieved references were screened in two steps: the first step was to screen titles/abstracts for matching our inclusion criteria, and the second step was to screen the retrieved full-text articles for eligibility to meta-analysis.

Data extraction and quality assessment

Information extracted included: (A) name of the author, year of publication, country of origin, intervention groups, number of participants in each group, age of participants, dosage of Vitamin E, frequency per day, co-interventions and study duration; (B) risk of bias (ROB) domains including: randomization, allocation concealment, blinding of participants, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias; and (C) treatment outcome measures. Biochemical outcomes (ALT, AST) were the outcome measures that were extracted as mean differences and standard deviations (SDs) to indicate the effect related to the treatment groups.

The risk of bias within each included study was assessed using the Cochrane ROB assessment tool **[Higgins 2019]**. ROB domains included randomization (selection bias); allocation concealment (selection bias); blinding of participants (performance bias); blinding of outcome assessment (detection bias); incomplete outcome data

(attrition bias), selective reporting (reporting bias), and other sources of bias including extreme baseline irregularity, unreliable study design, or trial termination shortly due to data-dependent considerations. We classified RCTs in each domain as low, high, or unclear ROB. We further used funnel plots in order to assess the risk of publication bias. Finally, in order to assess the effect of trial quality on the effect size, sensitivity analysis was performed by excluding studies that did not comply with certain quality criteria and were classified as high or unclear ROB.

Statistical analysis

Analysis was conducted with the use of RevMan 5.4. A meta-analysis of the mean differences (MD) of the ALT and the AST between the treatment and control group was conducted for the studies included. All data were continuous (MD and standard deviations "SD") and were pooled as weighted MD with 95% confidence intervals (CI). The data were extracted after thorough inspection of the included studies and the information provided. In cases where the inclusion criteria were fulfilled and the risk assessment led to inclusion of the study on the analysis but the MD reported was not followed by the related SD of the change, we followed the methodology suggested by the Cochrane handbook for "Imputing standard deviations for changes from baseline" **[Higgins 2019]**, supported by a sensitivity analysis on the choice of the different values of correlation, to determine whether the overall result of the analysis is robust to the use of imputed correlation coefficients. The analysis was carried out for the total of the included studies followed by a subgroup analysis for children and adults separately as well as for the kind of therapy, kind of comparator and origin of the studies (Asian and non-Asian). Our analysis aimed to estimate the pooled effect size of therapy on these two specific biomarkers of the participants in the total as well as for each subgroup and the subgroups were tested for statistical differences between the effects using the Chi Square test, assessing for heterogeneity across subgroup results. A sensitivity analysis was performed to examine the effect of each study on the outcomes, for the total, but also for each subgroup. Heterogeneity was assessed with Cochran's Q statistic and quantified using the I^2 statistic, which indicated the proportion of variability across studies that was due to heterogeneity rather than sampling error. Values of 0−40%, 30−60%, 50−90%, and 75−100% represented low, moderate, substantial, and

considerable heterogeneity; respectively. We anticipated the presence of clinical heterogeneity, based on the findings that the effects of vitamin E seemed to vary depending on various treatment factors. Participant characteristics and clinical settings differed greatly between studies and the conceptual background of the studies along with the calculations of the Heterogeneity statistic I^2 showed that estimates should be based on random effect models and the inverse variance method in all cases. Results were regarded as statistically significant if $p<0.05$.

RESULTS

Results from the study search and characteristics of included studies

Our search retrieved 4,553 unique citations from searching three electronic databases. Following title and abstract screening, 67 full-text articles were retrieved and screened for eligibility. Of them 55 articles were excluded, and 12 RCTs of 12 articles (n=763 patients) were reviewed in detail and included in this meta-analysis (PRISMA flowchart; Figure 2).

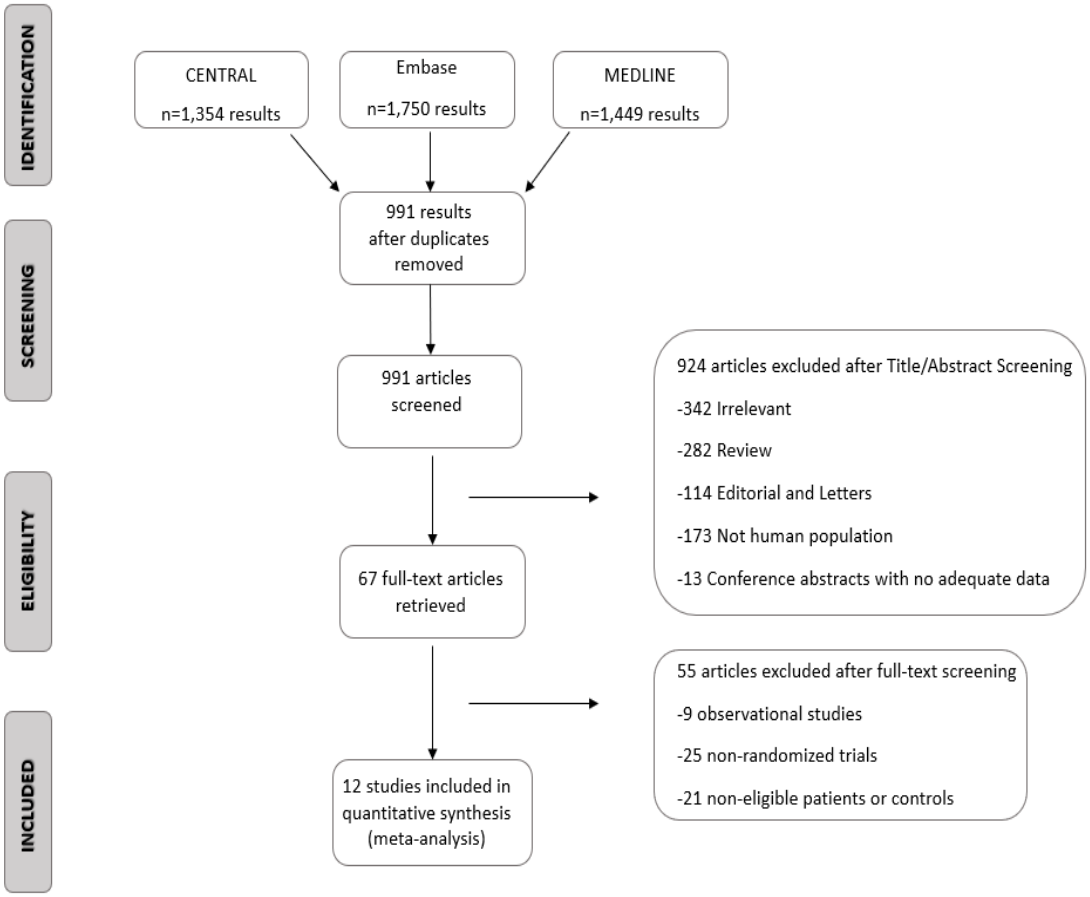

Figure 2. PRISMA flowchart and reasons of exclusion of studies.

The references of the included RCTs were searched manually, but no further records were added. All the included studies were performed between 2008 and 2020; six studies in Europe **[Aller 2015, Cerletti 2019, Curcio 2020, Sorrentino 2015, Nobilli 2018, Zohrer 2017]**, one study in U.S.A. **[Lavine 2011]**, and five studies in Asia **[Anushiravani 2019, Ekhlasi 2016, Pervez 2018, Ghergherehchi 2013, Wang 2008]**. A total of 8 studies compared vitamin E with placebo **[Anushiravani 2019, Cerletti 2019, Ekhlasi 2016, Pervez 2018, Lavine 2011, Nobilli 2018, Wang 2008, Zohrer 2017]** and 4 studies compared vitamin E with lifestyle modifications **[Aller 2015, Curcio 2020, Sorrentino 2015, Ghergherehchi 2013]**. The follow-up period ranged from 1month to 24months. Five studies included a cointervention to vitamin E, namely silymarin **[Aller 2015, Sorrentino 2015]**, Medronys epato® **[Curcio 2020]**, hydroxytriosol **[Nobilli 2018]** and Steatolip plus® **[Zohrer 2017]**. All cointerventions were given in equal doses to both groups (experimental and placebo) (Table 1). The majority of the studies reported a daily dose of Vitamin E of 400-800 IU and five studies reported their dosage in mg. Vitamin E is currently listed on the new Nutrition Facts and Supplement Facts labels in mg and the U.S. Food and Drug Administration (FDA) required manufacturers to use these new labels starting in January 2020. The conversion rule between mg and IU is as follow: 1 mg of alpha-tocopherol is equivalent to 1.49 IU of the natural form or 2.22 IU of the synthetic form **[ODS]**. Seven studies included adult populations and the other five included pediatric populations (Table 1). Two studies were conducted in mixed NAFLD and NASH population **[Aller 2015, Lavine 2011]** but numeric data regarding each population were inadequate. Two other studies **[Wang, Zohrer]** reported that were performed in children with NASH but in only one **[Zohrer]** the diagnosis of NASH had been established with histologic examination, according to current guidelines.

Table 1. Characteristics of Randomized Controlled Trials assessing the effect of vitamin E supplementation to outcome related to NAFLD/NASH.

Author, year, country	Population	Number of patients	Intervention[1]	Daily dosage	Study duration	Comparison	Outcomes
Aller 2015, Spain	Adults with NAFLD and NASH	36	Vit E + silymarin[2] + hypocaloric diet	72 mg	3 months	Hypocaloric diet	AST, ALT, GGT, BMI, histologic[3]
Anushiravani 2019, Iran	Adults with NAFLD	150	Vit E	400 IU	3 months	Placebo	AST, ALT, BMI, metabolic†
Cerletti 2019, Italy	Adults with NAFLD	126	Vit E (α-tocopherol)	20 mg	3 months	Placebo	AST, ALT, GGT, BMI, metabolic
Curcio 2020, Italy	Adults with NAFLD	81	Vit E + Medronys epato® ǂ + hypocaloric diet and exercise	40 mg	3 months	Hypocaloric diet and exercise	ALT, AST, GGT, metabolic, ultrasonographic
Ekhlasi 2016, Iran	Adults with NAFLD	60	Vit E (α-tocopherol)	400 IU	8 weeks	Placebo	AST, ALT, metabolic
Pervez 2018, Pakistan	Adults with NAFLD	71	Vit E (δ-tocotrienol)	600 IU	24 weeks	Placebo	AST, ALT, GGT, BMI, metabolic
Sorrentino 2015, Italy	Adults with NAFLD	78	Vit E + silymarin + Dietary modification	60 IU	90 days	Dietary modification	AST, ALT, BMI, metabolic
Ghergherehchi 2013, Iran	Children 4-15yo with NAFLD	33	Vit E + Dietary modification and exercise	400 IU	6 months	Dietary modification and exercise	AST, ALT, BMI, metabolic
Lavine 2011, U.S.A.	Children 8-17 yo with NAFLD and NASH	173	Vit E (α-tocopherol)	800 IU	96 weeks	Placebo	AST, ALT, BMI, metabolic, histologic
Nobili 2018, Italy	Adolescents with NAFLD	80	Vit E (α-tocopherol) + Hydroxytyrosol	20 mg	4 months	Placebo	AST, ALT, BMI, metabolic, ultrasonographic
Wang 2008, China	Children aged 10-17 yo with NASH	76	Vit E	100 mg	1 month	Placebo	AST, ALT, BMI, metabolic
Zohrer 2017, Italy	Children 4-16 yo with NASH	43	Vit E + Steatolip Plus®**	40 IU	6 months	Placebo	AST, ALT, BMI, metabolic, histologic, ultrasonographic

[1]Vitamin E administered as α-tocopherol or δ-tocotrienol. If blank, the type of vitamin E was not specified in the study, [2]Silymarin: mixture of flavonolignans isolated from the milk thistle plant Silybum marianum, [3]Histologic outcomes: NAFLD activity score (NAS) – covering steatosis, lobular inflammation, hepatocellular ballooning, †Metabolic outcomes contain at least one of the following: fasting blood glucose (FBG) and fasting blood insulin (FBI) levels, homeostatic model assessment of insulin resistance (HOMA-IR),

total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), ǂ Medronys epato®: each pill contains silymarin, vitamin C, coenzyme Q10, selenomethionine, ** Steatolip Plus®: each pill contains 250 mg of docosahexaenoic acid (DHA) and 201 mg of choline

Risk of bias assessment

Using the Cochrane ROB tool, three of the twelve trials in our study were at high risk of bias, because of insufficient description in most of the parameters of the ROB tool and many concerns about the randomization processes (open-label process through distribution of participants to interventions) **[Curcio 2020, Sorrentino 2015, Wang 2008]**. Three trials **[Aller, Ghergherehchi, Zohrer]** were judged to be in some concerns, and finally, six trials **[Anushiravani 2019, Cerletti 2019, Ekhlasi 2016, Pervez 2018, Lavine 2011, Nobilli 2018]** were evaluated to be at low risk of bias. Funnel plots of the standard errors versus the mean differences were examined for all outcomes; no publication bias was detected visually for ALT and AST.

Outcomes

Alanine aminotransferase (ALT)

The overall effect showed a non-significant difference between the two groups in ALT levels. The MD of the values of ALT that was recorded after the completion of the intervention was 1.41 IU/L favoring the control group according to the results of the data synthesis [MD=1.41, 95% CI (−9.31, 12.14), p=0.80] (Figure 3). It is therefore expected that patients receiving vitamin E will have a similar reduction in the ALT levels to the one observed in the control group. The pooled estimate was derived under a random effects model using the inverse variance method. The I^2 heterogeneity index was equal to 97%.

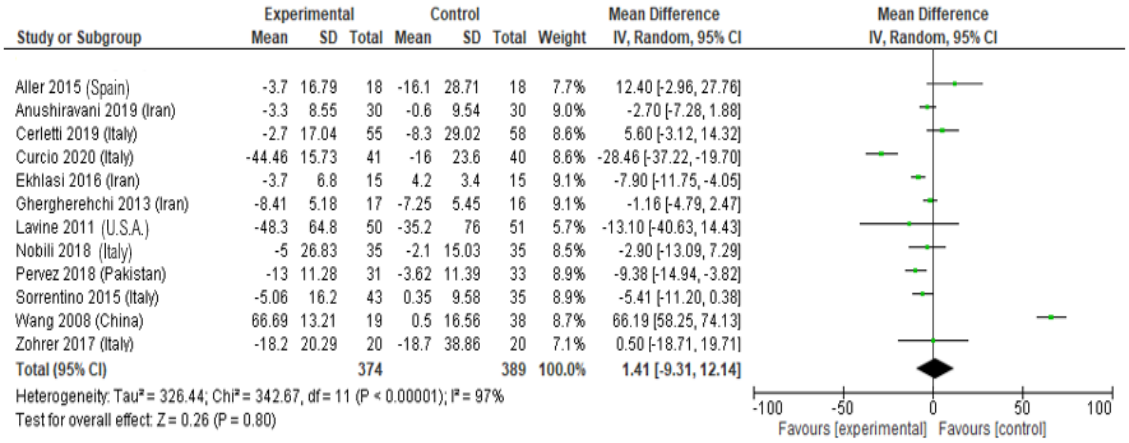

Figure 3. Forest plot of the effect of Vitamin E supplementation on alanine aminotransferase (ALT) changes assessed through RCTs.

In an attempt to explain the high heterogeneity, we repeated the analysis excluding the studies of Curcio (2020), Sorrentino (2015) and Wang (2008) which were of particular concern regarding their methodology and ROB scores. The values of ALT changes regarding the total of studies of low or moderate risk of bias were on average no different to the ones recorded for the control group with a MD= -2.94 IU/L favoring Vitamin E [95% CI (-6.69, 0.81), p=0.12)]. The I^2 heterogeneity index after the exclusion of the three studies was lower and equal to 60%. The sensitivity analysis that followed showed that the inference remains the same regarding almost all studies, while the conclusion changed when either the study of Aller (2015) or the study of Cerletti (2019) were excluded. Specifically, the exception of the study of Aller leads to a statistically significant greater change in the experimental group with an estimated mean reduction of -3.78 IU/L [95% CI (-7.26, -0.29), p=0.03]. In a similar way, the exclusion of the study of Cerletti leads to a significantly higher difference in the experimental group with an estimated MD of -4.04 IU/L [95% CI (-7.64, -0.43, p=0.03)]. The risk of bias assessment showed that some concern could rise for the study of Aller since it is the only study among the included ones with high risk regarding selection bias, performance bias and detection bias, while there is no reason to consider excluding the study of Cerletti.

Effect of Vitamin E in ALT in patients of different age and ethnicity

Subgroup analyses were performed according to the age group of the patients (adults and children/adolescents) and their ethnicity (Asian and non-Asian). In five studies **[Aller 2015, Anushiravani 2019, Cerletti 2019, Ekhlasi 2016, Pervez 2018]**, patients were adults and vitamin E administration had an effect similar to that presented for the total number of patients with a more robust result indicating no difference between the experimental and control group. It is therefore expected that patients receiving vitamin E will have a similar change in the ALT levels to the one observed in the control group with a MD equal to -2.78 IU/L [95% CI (-8.35, 2.79), p=0.33] and I^2 heterogeneity index equal to 75% (Figure 4). Including only the four studies that involved children and adolescents **[Ghergherehchi 2013, Lavine 2011, Nobilli 2018, Zohrer 2017]**, the effect of Vitamin E supplementation remains non-significant with a MD in ALT levels of -1.47 IU/L [95% CI (-4.82, 1.87), p=0.39] (Figure 4). We performed a sensitivity analysis within the subgroups that resulted in no differences. The statistical comparison between the two subgroups was not statistically significant with a $X_1^2 = 0.16$, p=0.69 (Figure 4) and the two subgroups did not differ even under the sensitivity analysis, where the excluded studies led to a different inference in the group of studies regarding adult patients. Furthermore, we estimated the effect of Vitamin E supplementation on patients of different ethnicity. Four trials were conducted in Europe **[Aller 2015, Cerletti 2019, Nobili 2018, Zohrer 2017]**, one trial in USA **[Lavine 2011]** and four trials in Asia **[Anushiravani 2019, Ekhlasi 2016, Ghergherehchi 2013, Pervez 2018]**. The results showed that in non-Asian studies, the effect of Vitamin E on ALT levels was not significant, causing a MD=2.58 IU/L favoring the control group [95% CI (-3.59, 8.75), p=0.41] with an estimated I^2=10% whereas when we analyzed the Asian studies, a significant reduction of ALT level [MD=-5.08, 95% CI (-8.98, -1.18), p=0.01] was demonstrated, favoring Vitamin E group. The sensitivity analysis within the studies of both subgroups resulted in no differences in all cases but the comparison between the two subgroups demonstrated that they differed significantly ($X_2^2 = 4.22$, p=0.04) (Figure 4).

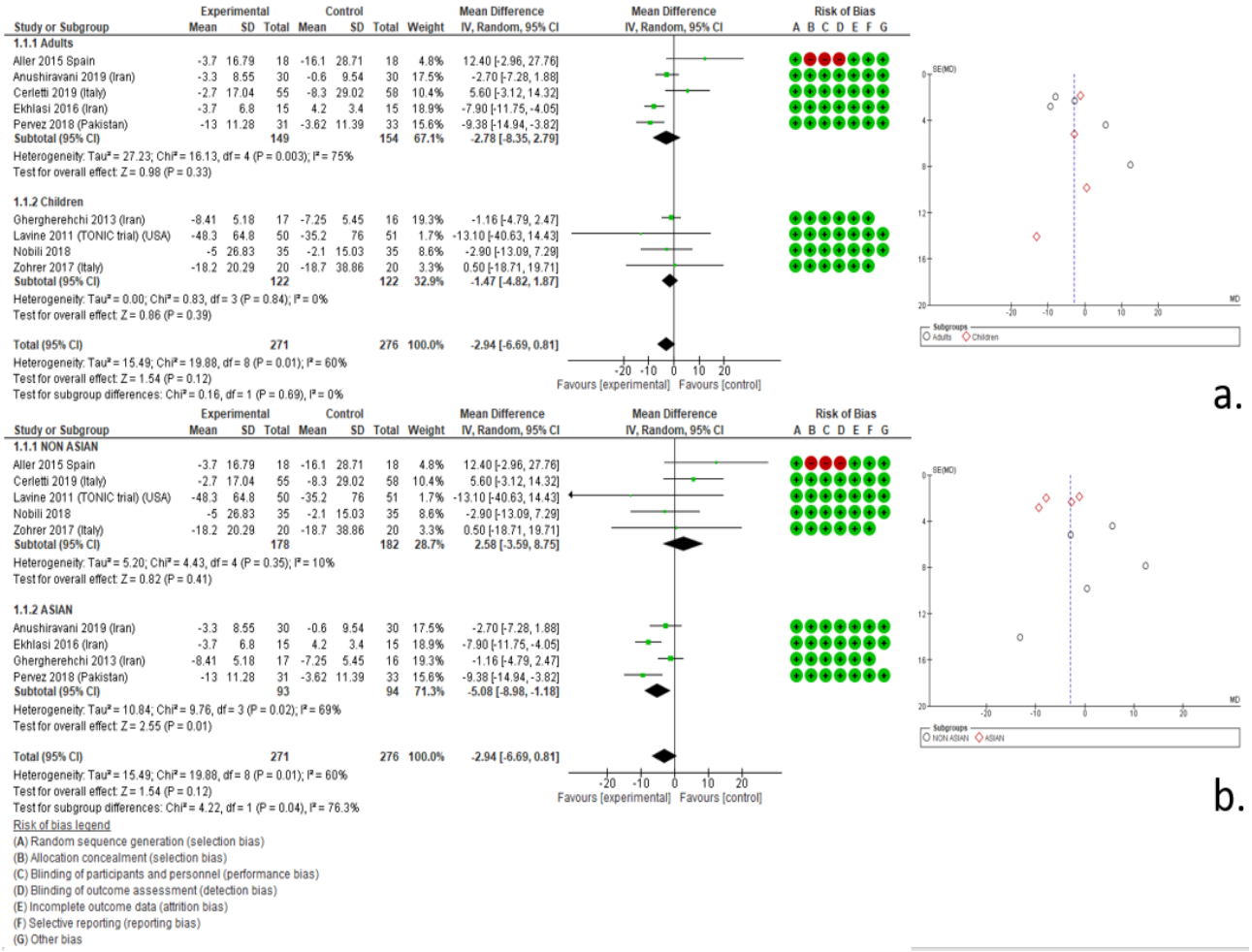

Figure 4. Forest plots of the effect of vitamin E in ALT a. in adults and children and b.in non-Asian and Asian patients and funnel plots of the studies included in the analysis. The funnel plots are symmetric, indicating mostly absence of publication bias.

Effect of different types of intervention in ALT

Among the finally included studies, two **[Aller 2015, Ghergherehchi 2013]** used as control group NAFLD patients who were given only lifestyle modification advices and the rest seven trials **[Anushiravani 2019 Cerletti 2019, Ekhlasi 2016, Lavine 2011, Nobilli 2018, Pervez 2018, Zohrer 2017]**, utilized placebo similar to Vitamin E supplements that were provided to the intervention arms. The analysis of the studies used placebo concluded that Vitamin E can cause a significant decrease in ALT level of NAFLD patients with a MD= -4.46 IU/L [95% CI (-8.46, -0.86), p=0.03] with a I^2 heterogeneity index of 50%. The mean changes of ALT in the experimental groups in the two studies that used only lifestyle modification advices were 12.40 IU/L [95% CI

(-2.96, 27.76), p=0.67] and -1.16 IU/L [95% CI (-4.79, 2.47), p=0.45] . With regard to the type of intervention used in each study, when we included these patients who received Vitamin E supplementation alone, from five studies **[Anushiravani 2019, Ekhlasi 2016, Ghergherehchi 2013, Lavine 2011, Pervez 2018]**, a statistically significant difference in ALT levels was observed, indicating a greater difference in the experimental group. The pooled estimate showed that patients who received vitamin E only will have a greater difference in the ALT levels comparing to the one observed in the control group with a MD= -5.20 IU/L [95% CI (-8.93, -1.48), p=0.006] (Figure 5) and the I^2 heterogeneity index 60%. The sensitivity analysis within the studies showed no changes in the inference in all cases with an exception regarding the study of Ekhlasi (2006), the exclusion of which leads to a borderline non significance (p=0.05). When we analyzed the data from the four studies **[Aller 2015, Cerletti 2019**, **Nobilli 2018, Zohrer 2017]** that used combinational therapy, the inference was similar to that presented for the total in the sense that the MD did not differ between the two groups [MD= 3.36 IU/L, 95% CI (-2.60, 9.33), p=0.27] (Figure 5) and the I^2 heterogeneity index was equal to 4%. The sensitivity analysis in this group showed that the inference does not change for any of the studies that could theoretically be omitted. When we compared the two subgroups, the differences were statistically significant with a chi-square equal to 5.70, p=0.02. It should be mentioned that when excluding the effect of the study of Aller (2015), with a p equal to 0.06 or when excluding the effect of the study of Cerletti (2019), with a p equal to 0.15, through the sensitivity analysis, the differences are alleviated.

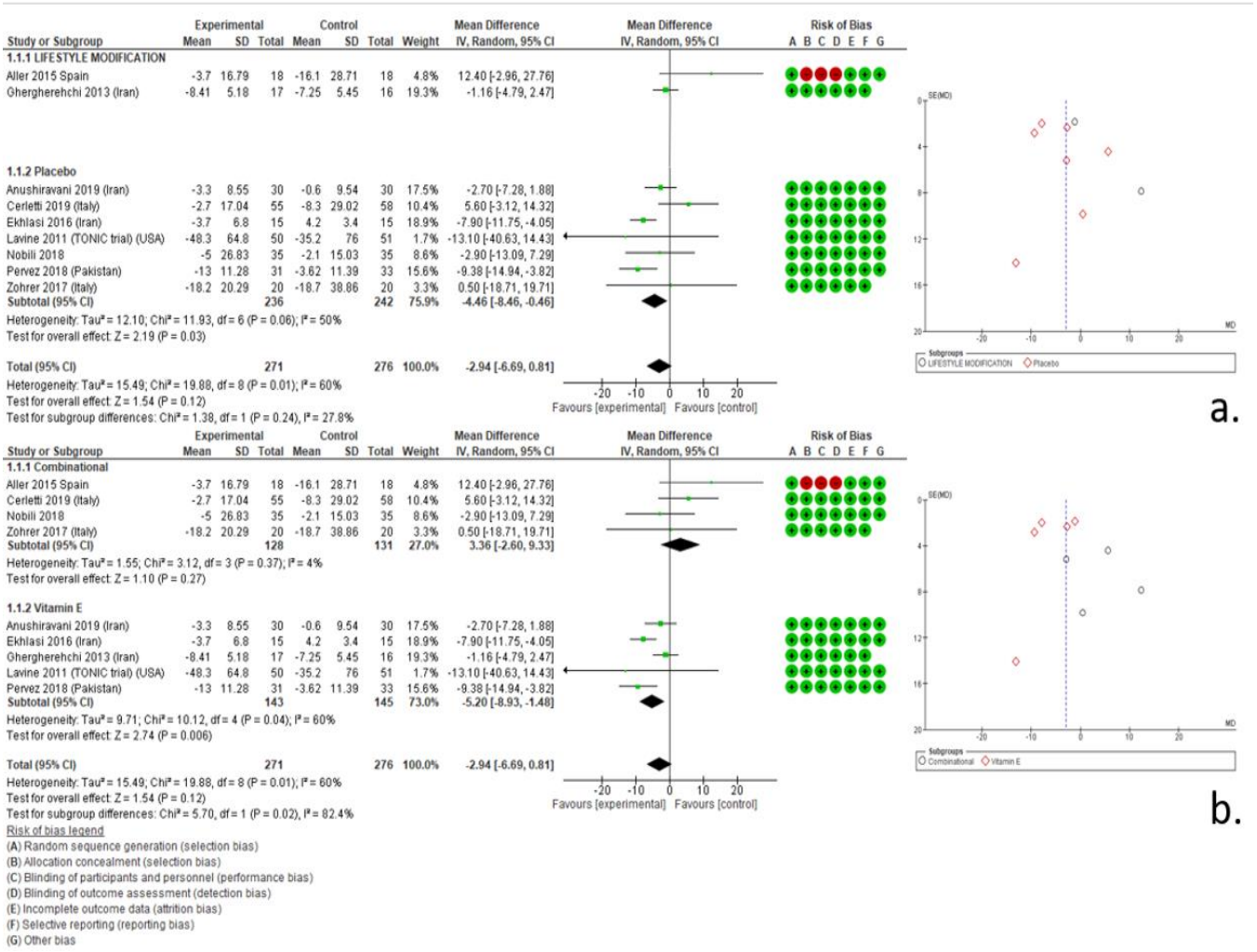

Figure 5. Forest plots a. of the effect of intervention in ALT when compared to lifestyle modification or placebo and b. the effect of vitamin E or combinational therapies in ALT. The funnel plots of the studies included in the analysis are symmetric, indicating mostly absence of publication bias.

Aspartate aminotransferase (AST)

The values of AST changes that were recorded after the completion of the intervention were on average different to the ones recorded for the control group according to the results of the data synthesis. The MD of AST levels was -6.88 IU/L and it is therefore expected that patients receiving vitamin E will have a greater reduction in the AST levels to the one observed in the control group [MD=-6.88 IU/L, 95% CI (-11.08, -2,69), p=0.001]. The I^2 heterogeneity index was equal to 87% (Figure 6).

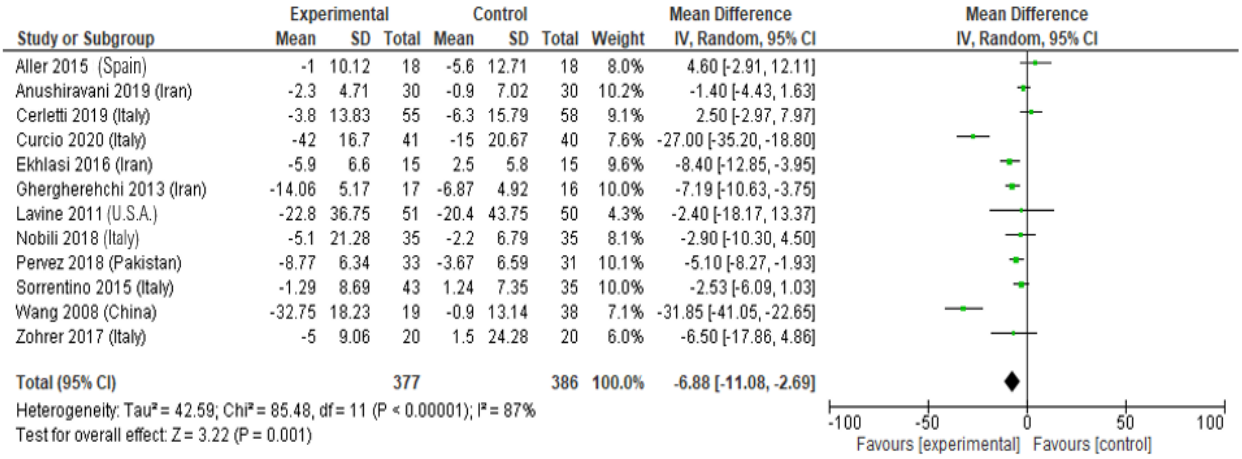

Figure 6. Forest plot of the effect of Vitamin E supplementation on aspartate aminotransferase (AST) changes assessed through RCTs.

In the same way as measuring the effect on ALT, we repeated the analysis excluding the studies of Curcio (2020), Sorrentino (2015) and Wang (2008) which were of high risk of bias regarding their methodology. The values of AST changes regarding the total of studies of low or moderate risk of bias were still different to the ones recorded for the control group but the MD was lower and equal to -3.35 [95% CI (-6.17, -0.52, p=0.02]. The MD was estimated under a random effects model using the inverse variance method and I^2 heterogeneity index was lower and equal to 62%. In the sensitivity analysis the inference remained the same regarding almost all studies, while the conclusion changed when either of the studies of Ekhlasi (2016), Pervez (2018) or the study of Ghergherehchi (2013) were excluded. The exception of the study of Ekhlasi led to a non-significant difference in the experimental group [MD= -2.58, 95% CI (-5.49, 0.33), p=0.08] and similarly, the exclusion of the study of Pervez [MD= -2.92, 95% CI (-6.36, 0.51, p=0,09] and Ghergherehchi [MD= -2.62, 95% CI (-5.67, 0.43), p=0,09] resulted in non-significant MD in the experimental group.

Effect of Vitamin E in AST in patients of different age and ethnicity

We performed subgroup analyses according to the age and the ethnicity of the patients to examine the effect of Vitamin E on AST levels in these groups. In the five studies that included adult patients **[Aller 2015, Anushiravani 2019 Cerletti 2019, Ekhlasi 2016, Pervez 2018]**, the inference indicated no significant difference between the experimental and control group [MD= -2.19, 95% CI (-6.08, 1.70, p=0.27)] while the

I^2 heterogeneity index was equal to 75%. The sensitivity analysis within the studies that regard adults only showed no statistically significant differences in the two groups in all cases. Regarding studies that involved only children **[Ghergherehchi 2013, Lavine 2011, Nobilli 2018, Zohrer 2017]**, the overall effect showed a significant difference between the two groups in AST levels favoring vitamin E with a MD= -3.35 IU/L [95% CI (-6.17, -0.52, p=0.02)] and I^2 heterogeneity index equal to 62% (Figure 7). The sensitivity analysis within the subgroup showed that when excluding the study of Ghergherehchi, the difference becomes no significant between the Vitamin E and control group regarding AST reduction [MD=-3.76, 95% CI (-9.53, 2.01), p=0.20]. The difference in reductions of AST levels was not significant when the two subgroups were compared (X_1^2 = 2.71, p=0.10). We also estimated the effect of Vitamin E on patients of different ethnicity and our analysis resulted that this is non-significant regarding non-Asian studies [MD=0.63, 95%CI (-2.92, 4.17), p=0.70] with an I^2 heterogeneity index equal to 1%. The result regarding Asian studies was different: the experimental group exhibited a MD= -5.3 IU/L in AST [95%CI (-8.35, -2.26), p<0.01] and the two subgroups differed significantly between them (X^2=6.19, p=0.01) (Figure 7). The sensitivity analysis in both subgroups showed that the inference did not change for any of the studies that could theoretically be omitted.

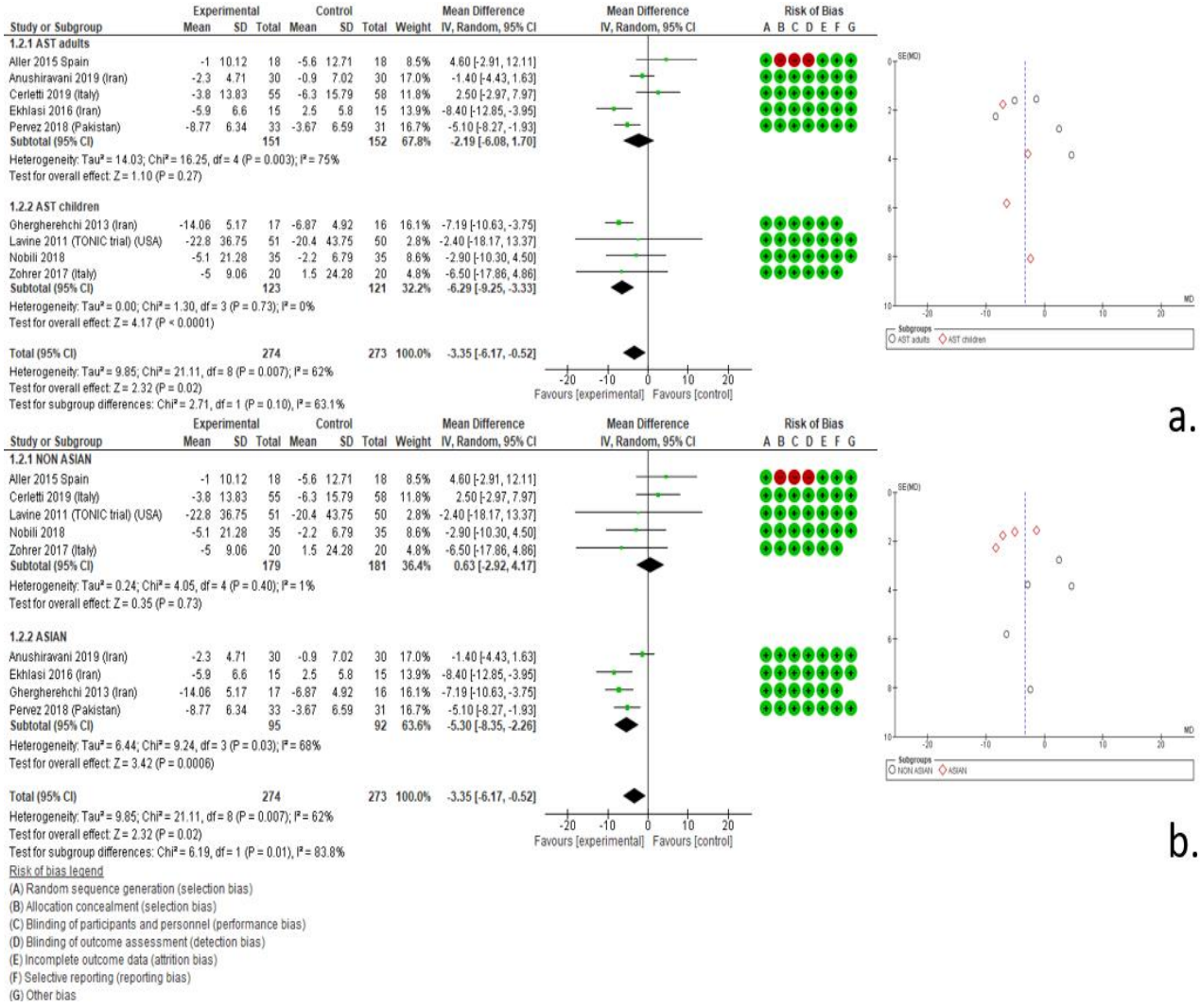

Figure 7. Forest plots of the effect of vitamin E in AST a. in adults and children and b.in non-Asian and Asian patients and funnel plots of the studies included in the analysis. The funnel plots are symmetric, indicating mostly absence of publication bias.

Effect of different types of intervention in AST

With regard to the two studies that provided only lifestyle modification advices to their control groups, the mean changes of AST in the experimental groups were 4.60 IU/L [95% CI (-2.91, 12.11), p=0.40] and -7.19 IU/L [95% CI (-10.63, -3.75), p<0.05] (Figure 8). When we analyzed the studies that used a placebo for their control group, the inference was different and the mean difference between Vitamin E and placebo group was significant. Specifically, patients who received supplementation are expected to experience a mean decrease of 3.43 IU/L in AST level [95% CI (-6.36, -0.51), p=0.02] with no study causing a change to the result when omitted in the

sensitivity analysis. The statistical comparison of the subgroups (lifestyle modification and placebo) showed that their inference is not significantly different (X_1^2=0.07, p=0.79) (Figure 8). Afterwards, we analyzed the studies in subgroups of different types of intervention and we resulted that when the investigators used mixed or combinational regiments of Vitamin E, the mean change of AST did not differ between the two groups. Typically, it is expected that patients in the experimental group will have a similar change in the AST levels to the one observed in the control group [MD=0.56, 95% CI (-3.67, 4.79), p=0.80, I^2 heterogeneity index 23%] (Figure 8). Regarding studies whose experimental group received only vitamin E, a statistically significant decrease of AST was observed. The pooled estimate showed that it is expected that patients receiving vitamin E only will have a greater mean difference in the AST levels equal to -5.19 IU/L [95% CI (-8.02, -2.37), p<0.001] with a I^2 heterogeneity index equal to 57%. The sensitivity analysis within the studies of both subgroups of interventions resulted in no change of the conclusion in all cases. The effects on the two subgroups were different in statistical means (X_2^2 = 4.91, p=0.03) but this difference is alleviated and loses its significance when excluding the effect of any of the following studies: Aller (2015), Cerletti (2019), Ekhlasi (2016) and Ghergherehchi (2013), through the sensitivity analysis.

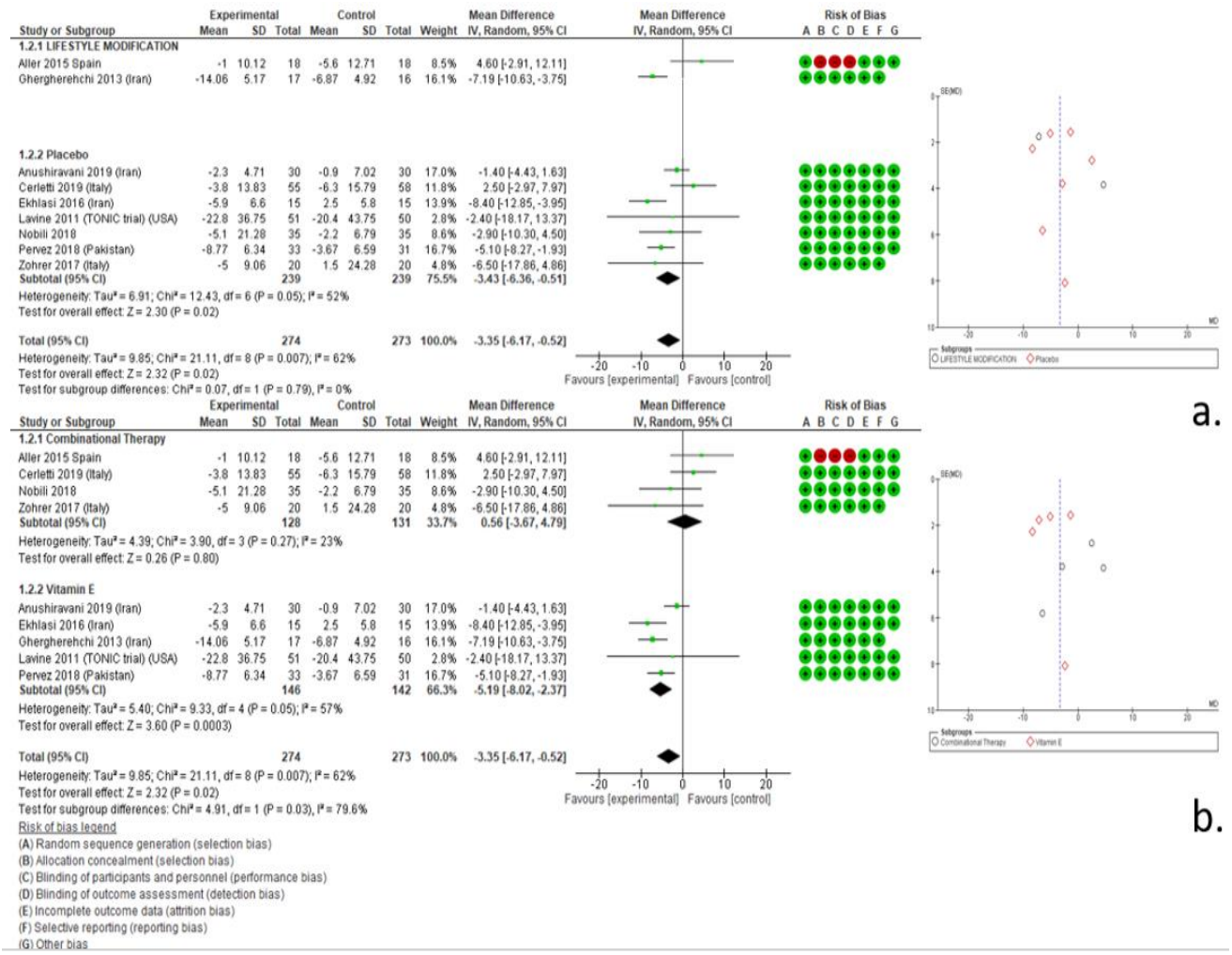

Figure 8. Forest plots a. of the effect of intervention in AST when compared to lifestyle modification or placebo and b. the effect of vitamin E or combinational therapies in ALT. The funnel plots of the studies included in the analysis are symmetric, indicating mostly absence of publication bias.

DISCUSSION

According to the results of our meta-analysis that included 763 NAFLD and NASH patients of all ages from 12 RCTs, vitamin E supplementation significantly decreases serum AST levels in children whereas ALT levels do not show a statistically significant reduction in children or adults. Four of our included studies reported superiority of vitamin E alone or combined over placebo or lifestyle modifications on ALT levels, however the overall effect in improving ALT remained insignificant even if the patients were analyzed according to their age group. Interestingly, when we analyzed separately

the studies from Asia, the reduction of ALT and AST became significant indicating a positive effect of Vitamin E on biochemical markers in these populations. Epidemiologic data suggest that except for the different incidence of NAFLD in Asian populations **[Sayner 2016]**, different pathophysiologic pathways are not excluded as well. NAFLD diagnoses in Asian populations are increasing, especially in normal BMI patients who have also been shown in some studies to have a more severe histologic picture **[Mohanty 2009]**. In view of that fact, an explanation for the discrepancies found in our analyses could be the distinct pathophysiologic mechanisms of NAFLD cascade in Asian populations.

Contrary to ALT, the pooled estimate for AST indicated that its values significantly declined after administration of Vitamin E and the improvement was prominent in pediatric population. A recently published meta-analysis **[Amanullah 2019]** that included both adult and pediatric patients did not result in a significant improvement in aminotransferases levels in the pediatric age group. Of note, although this review included patients of all ages, the meta-analysis was performed only in studies including children and one trial was included twice. In another systematic review **[Abdel-Maboud 2020]** of 15 RCTs with patients of all ages, the authors considered short, intermediate, and long-term follow-ups and concluded that the group who received vitamin E had a statistically significant improvement in ALT and AST among adults. Interestingly, in the pediatric population, the significant change in biochemical parameters started to appear at long-term follow-up (i.e. after 12 months). In our meta-analysis, the favorable effect of vitamin E on children could be partially explained by the important role of oxidative stress in pediatric NAFLD **[Nobili 2010]**. Several studies indicate increased biomarkers of oxidative stress in pediatric populations **[Soto-Méndez 2016]** and specifically, for pediatric NAFLD, Nobili et al **[Nobili 2010]** reported that a high proportion (83%) of children with NAFLD show signs of oxidative injury, as evaluated by elevated circulating levels of protein carbonyls. Children with elevated serum protein carbonyls also show an increased hepatocyte nuclear staining for 8-hydroxy-2-deoxyguanosine (8-OHG), a lipid peroxidation end-product and marker of oxidative DNA damage.

According to our analysis, both the types of control and co-intervention the studies have used cause a different effect on aminotransferases levels. The studies which used a placebo in their control group have a significantly greater mean decrease of ALT and

AST than the mean decrease of aminotransferases estimated for the studies that provided lifestyle modification advices alone to their control groups. Moreover, the group who received only vitamin E as a supplementation had a statistically significant decrease in ALT and AST levels compared with patients who received combinational therapies. Although a mixed intervention could raise queries if the effects on liver function regard particularly vitamin E or a synergistic effect of all supplements, our findings are in accordance with these of a recently published meta-analysis **[Vadarlis 2020]**. In this study, authors examined the effect of vitamin E alone in adult patients with NAFLD and the values of ALT and AST were decreased with statistical significance in patients treated with vitamin E. The regression model of Abdel-Maboud et al **[Abdel-Maboud 2020]** also concluded that co-interventions did not significantly modify the changes in ALT and AST implying that vitamin E alone can improve liver function outcomes not acting merely as a treatment adjuvant. The common denominator in these studies was the dose of vitamin E that was significantly higher in interventions considering vitamin E alone. The precise dose of vitamin E has long been under discussion, with a wide variation among trials. European and American associations suggest 800 IU as a daily dose of vitamin E for NAFLD patients **[EASL, Chalasani 2018]**, much higher than the daily recommended dose of 20-30 IU for healthy adults **[NIH-ODS]**. Since the ideal duration and the adequate intake of vitamin E supplements in order to derive the greatest benefit have not been studied and published yet, some still unsolved concerns should be considered regarding the safety of high doses of vitamin E administration. It has long been suspected that vitamin E could have a dichotomous suppressive and promoting activity with respect to tumorigenesis, possibly explained by host gene-supplement interactions. In an attempt to examine its protective role on cancer, two large trials (HOPE-TOO Trial and Women's Health Study) failed to prove a benefit in any tumor incidence **[Lonn 2005, Lee 2005]**. Interestingly, a large randomized controlled trial, called SELECT, using vitamin E with or without selenium supplementation which was initiated in 2001 and was discontinued in 2008 when in an analysis was found that the supplements not only did not prevent prostate cancer but the men who had taken vitamin E had a 17% increased risk of prostate cancer compared to men only taking placebo **[SELECT 2011]**. Furthermore, an extensive meta-analysis of 57 RCTs examining the effect of vitamin E on all-cause mortality revealed that vitamin E doses up to 5500 IU/day did not affect it **[Abner 2011]**. The data are still insufficient for a conclusive answer, making the safety profile

of vitamin E an important clinical question with an urgent call for further clinical trials with long term follow ups.

The term vitamin E describes both the tocopherols and tocotrienols, the lipid-soluble antioxidants naturally synthesized by plants that prevent the propagation of free radicals. With regard to the form of supplementation used, four of our included studies used α-tocopherol as a supplement and one study used δ-tocotrienol. In six studies the exact form of vitamin E utilized was not described adequately. As a result, in our meta-analysis we could not examine the role of different forms of vitamin E on liver function in NAFLD and this is a limitation of our meta-analysis if we consider that more than half of our studies did not mention which form of vitamin E was given to the patients. Meanwhile, a previous meta-analysis **[Vadarlis 2020]** indicated that when vitamin E was administrated as α-tocopherol caused a statistically significant decrease of the transaminases levels whereas the form of tocotrienol significantly decreased only ALT levels and not AST. Other limitations in the present analysis were the relatively small sample size of the included RCTs so that significant biochemical changes associated with vitamin E might not have been detected together with the differences in dose and duration of supplementation that might have also affected the accuracy of the results. Although elevated ALT levels correlate positively with NASH and advanced fibrosis, liver biopsy remains the gold standard method for evaluating the degree of steatosis, inflammation and fibrosis. Recently, a number of studies have focused on non-invasive, serological markers to assess NAFLD disease severity to avert the need for a liver biopsy and its potential complications. Patients with normal ALT present less frequently advanced steatosis and inflammation compared with elevated ALT group of patients and both the mean NAS score and percentage of patients with NASH have been found significantly lower in the normal ALT group compared with elevated ALT group of patients **[Verma 2013]**. Moreover, serum ALT has been found to correlate positively with liver triglyceride content, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and fasting insulin, making it a good predictor of NAFLD **[Martin-Rodriguez 2017]**. Along with BMI, waist circumference and serum triglyceride, ALT and AST have been widely used in NAFLD risk scoring models. For example, NAFLD liver fat score includes the presence of the metabolic syndrome and type 2 diabetes, fasting serum insulin, AST, and the AST/ALT ratio with an AUC (Area Under the Curve) of 0.86 in the validation group **[Kotronen 2009].** However, abnormal liver

function tests and their changes remain both insensitive and nonspecific markers of NAFLD although they represent the most frequently used serum biomarkers for NAFLD evaluation in every-day clinical practice.

Our study features a number of strengths. We have included in our review all available RCTs regarding the effect of vitamin E on liver enzymes, and it is the most up-to-date systematic review and meta-analysis of the topic. Furthermore, we have tried to detect every possible source of biases, including deficiencies in the design, conduct, analysis, and interpretation of research, as well as publication biases. Nevertheless, since the quality of a systematic review and meta-analysis rests upon its included studies, our included studies presented relatively high quality in terms of ROB scores. The mixed choice of ages, study origins and Vitamin E administration methods or control group interventions were a limitation of previous meta-analyses in this field. As a result, our analysis examines the effect of Vitamin E on serum aminotransferases accompanied by extensive subgroup analysis concerning patients age and ethnicity, co-administration of different regimens and usage of a placebo or lifestyle modification advices in the control groups.

CONCLUSION

This meta-analysis confirms the known therapeutic potency of vitamin E on NAFLD biochemical parameters, using one of the largest NAFLD/NASH patient samples to date. Specifically, we demonstrate that vitamin E is superior than placebo or lifestyle modification in lowering levels of AST in adult and pediatric patients while the administration of vitamin E alone, on higher doses, effectively decreases AST and ALT levels. Although age, ethnicity and types of interventions are associated with different biochemical outcomes, further studies are needed to elucidate the mechanisms governing these observations. Overall, the current evidence indicates that vitamin E alone is efficient in improving commonly used biochemical outcomes in adult and pediatric patients with NAFLD. Further multi-center, large sample RCTs are needed to investigate the safety of daily vitamin E alone or combined with other regimens, as well as to further evaluate the accuracy of aminotransferases measurement in NAFLD follow-up.

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