

# Efficacy of water-based vitamin E solution versus placebo in the prevention of retinopathy of prematurity in very low birth weight infants

## A randomized clinical trial

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### Abstract

**Background:** Vitamin E has antioxidant properties, which help in scavenging free radicals, thereby reducing oxidation of lipids and proteins. This study aims to evaluate the efficacy of oral vitamin E supplementation in preventing retinopathy of prematurity (ROP) in very low birth weight (VLBW) infants with respiratory distress syndrome (RDS) and decreasing oxidative stress 15 and 28 days post-intervention.

**Methods:** Ninety VLBW infants were randomly assigned to two groups:

1. Treatment (treatment group (T), n=48) or
2. Placebo (control group (C), n=42).

Each group received 25 IU of vitamin E (T) or placebo (C).

**Results:** The incidence of ROP in groups T and C was 12.5% (n=6) and 31% (n=13), respectively (RR: 0.40; 95% CI: 0.10–0.96). There were no differences in mortality between groups. As expected, the vitamin E concentration was significantly increased 28 days post-intervention in group T.

**Conclusion:** Oral supplementation with vitamin E may effectively prevent ROP development in VLBW infants with RDS. Oxidative damage markers were significantly lower, whereas total antioxidant capacity was increased in group T. However, levels of other antioxidants as vitamin A and C were not measured in two groups.

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The datasets generated during and/or analyzed during the present study are not publicly available, but are available from the corresponding author on reasonable request.

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**Abbreviations:** CIs = Confidence Intervals, CPAP = Continuous Positive Airway Pressure, INPerIER = Instituto Nacional de Perinatología. Isidro Espinosa de los Reyes, LHP = Lipohydroperoxide, MDA = Malondialdehyde, PO<sub>2</sub> = Partial Pressure of Oxygen, RCTs = Randomized Controlled Trials, RDS = Respiratory Distress Syndrome, ROP = Retinopathy of Prematurity, ROS = Reactive Oxygen Species, RR = Relative Risks, TAC = Total Antioxidant Capacity, UCIREN = Newborn Intermediate Care Unit.

**Keywords:** antioxidant vitamins, oxidative damage, retinopathy of prematurity, total antioxidant capacity, vitamin E

## 1. Introduction

The survival rate of very low birth weight (VLBW) infants has markedly increased in the last two decades.<sup>[1]</sup> Retinopathy of prematurity (ROP) continues to be prevalent among VLBW infants.<sup>[1]</sup> ROP can be mild, with no visual defects, or aggressive, with retinal detachment and blindness.<sup>[1]</sup>

Ludwig<sup>[2]</sup> reported a frequency of ROP of 30.2% in newborns with birth weights between 750 and 999 g in the USA versus ROP incidence in Switzerland, which was only 9.3% in VLBW infants.<sup>[3]</sup> In Mexico, the reported incidence is 59.1% among premature neonates weighing 751–1000 g at birth.<sup>[4]</sup>

In addition to prematurity, oxidative stress contributes to the pathogenesis of ROP.<sup>[5–7]</sup> Oxidative stress is associated with tissue damage caused by excessive generation of reactive oxygen species (ROS).<sup>[6,7]</sup> The delivery of a fetus from a relatively hypoxic intrauterine environment, with a partial pressure of oxygen (PO<sub>2</sub>) of 20–25 mm/Hg, to a normal extrauterine environment, with a PO<sub>2</sub> of nearly 100 mm/Hg, can increase oxidative stress.<sup>[5,6]</sup> It has been suggested that ROP could result from different types of ROS and/or high concentrations of polyunsaturated fatty acids in photoreceptors.<sup>[8]</sup> The initial stimulus that activates the inflammatory process in very low birth weight and ROP infants may be the increased free radicals induced by oxygen therapy, barotrauma, volutrauma, infectious agents, antioxidant deficiency, and/or other stimuli.<sup>[9–11]</sup> The association between oxygen therapy and ROP has been recognized for a long time.<sup>[4,7,8]</sup>

Respiratory distress syndrome (RDS) is a risk factor for ROP.<sup>[4–7]</sup> Respiratory management of RDS includes oxygen therapy and surfactant administration.<sup>[12]</sup> This therapy can increase the risk of ROP. In this regard, several approaches have been implemented to decrease ROP, particularly the use of antioxidants. Vitamin E is an important antioxidant that can scavenge free radicals, reducing lipid peroxidation and oxidant injury in VLBW infants.<sup>[11]</sup> However, the results of trials evaluating the efficacy of vitamin E in the prevention of ROP have not been encouraging.<sup>[13–17]</sup> In fact, research on the roles of vitamins and antioxidants, including vitamin E, in preventing ROP were halted because of complications involving sepsis and necrotizing enterocolitis.<sup>[18]</sup> These complications were attributed to excipients of oral vitamin E products, which included polyethylene glycol, propylene glycol, ethanol and, polysorbate 80.<sup>[11]</sup> Since these substances, may induce adverse effects in premature newborns.<sup>[11,18]</sup> In a meta-analysis by Fang et al. (2016),<sup>[19]</sup> the use of vitamin E supplementation at any stage did not affect ROP rates.

In Mexico, pediatricians and neonatologists advocate supplementation with vitamin E free of possibly toxic vehicles for ROP prevention in premature newborns. However, to date, there have been no reports on the efficacy of this intervention. Thus, the present study's objective was to evaluate the effect of vitamin E oral supplementation at a dosage of 12.5 IU every 12 h (25 IU/

day) from 72 h after birth to 28 days in the prevention of ROP in VLBW infants with RDS.

## 2. Methods

### 2.1. Study design and participants

The study was a randomized, double-blind clinical trial conducted in the intensive care unit of the Instituto Nacional de Perinatología (INPer) in Mexico City, from March 1, 2013, to October 1, 2015. The study protocol was approved by the Internal Review Board of INPer (Registration ID: 212250-10231), and signed informed consent was obtained from the children's parents. This trial was retrospectively registered as NCT03274596 at <http://clinicaltrials.gov/>.

The inclusion criteria were VLBW infants with an RDS diagnosis defined as labored breathing, such as tachypnoea, nasal flaring, chest retractions, or grunting.<sup>[20]</sup> All VLBW infants included were treated with the intubation-surfactant-extubation technique. Subsequently, they received continuous positive airway pressure (CPAP). Some neonates required mechanical ventilation, after which they received CPAP via the nasal route or a nasal cannula. All the patients were continuously monitored using a transcutaneous oxygen monitor to maintain O<sub>2</sub> saturation between 90 and 93%. At all times from birth, a controlled oxygen supply was provided using an air-oxygen blender in accordance with the institution's neonatal resuscitation guidelines.

The exclusion criteria were preterm newborns with congenital malformations, Rh incompatibility and isoimmunization, non-immune or immune hydrops fetalis, or grade III/IV intraventricular hemorrhage, and neonates born to mothers with thrombocytopenic purpura and/or any immunodeficiency.

### 2.2. Randomization and allocation concealment

The VLBW infants were randomly assigned to a treatment group (group T) or a placebo control group (group C) using a random digit table. Pharmacy staff not involved in the patients' care were responsible for allocating, enrolling, and assigning participants to interventions. Group T received orally administered vitamin E (25 IU) in two 12.5 IU doses every 12 h. Group C received a placebo comprising 0.1 mL of distillate water every 12 h. Both groups received the assigned treatment from 72 h after birth until 28 days of age. Both interventions were presented in the same packaging, appearance, and solution (0.1 mL) amount, administered in a sterile and graduated plastic syringe, which was protected from light using aluminum foil. The research team and participants' parents were blinded to the group allocation until after the final analysis.

Nutritional management in both groups was similar. From birth, the infants received a protein solution (2.4 g/kg/day). Then, 12–24 h later, they received total parenteral nutrition (3.5 g/kg/day protein, 3 g/kg/day lipids and 4 mg/kg/min glucose, up to 10

mg/kg/min). Trophic feeding of the VLBW was initiated 36–48 h after birth.

According to institutional guidelines, prophylactic surfactant was administered to premature infants less than 28 weeks of gestation that had not received prenatal steroids. Rescue surfactant was administered to newborns < 32 weeks of gestation who received antenatal steroids but had risk factors for developing RDS. Caffeine was started in all newborns < 32 weeks of gestation, within the first 24 h of life, with an impregnation dose of 20 mg/kg and a maintenance dose of 5 mg/kg per day until term-corrected gestational age (38 weeks of gestation). All infants with patent ductus arteriosus with hemodynamic repercussions received Ibuprofen treatment orally at 10 mg/kg per day as initial dose and subsequently 5 mg/kg every 24 h for two days. Neither group received erythropoietin. All infants who remained intubated on day 14 of life received only one steroid scheme as follows: Dexamethasone 0.075 mg/kg/dose every 12 h for three days, continued with 0.05 mg/kg/dose every 12 h for three days, then 0.025 mg/kg/dose every 12 h for two days and finally 0.01 mg/kg/dose every 12 h for two days and it was suspended.

### 2.3. Vitamin E preparation

The pharmacologist team at the institution prepared a vitamin E formulation at a 25 IU/0.2 mL concentration under laminar flow hood and sterile conditions. The preparation was as follows: 2800 IU of vitamin E contained in a volume of 1.75 mL (7 capsules of 400 IU, vitamin E (dl-alpha-tocopherol); Eternal, Bayer, Mexico) and 1.75 mL of glycerol at 1% were mixed in a 1:1 ratio and gauged to 22.5 mL of sterilized water. The solution was mixed until a uniform preparation in a sterilized Erlenmeyer flask, which contained 224 doses of vitamin E (12.5 IU/0.1 mL per dose), was attained. Only the required doses for each day were placed in a sterile and graduated plastic syringe protected from light (stored at 4°C) using aluminum foil. Until the administration, the rest of the prepared solution was discarded. This process was repeated every day in order to maintain the stability of vitamin E.

### 2.4. Primary endpoint

The primary endpoint was the efficacy and safety of vitamin E in preventing ROP in VLBW infants with RDS. ROP was defined according to the international ROP classification.<sup>[20,21]</sup> An ophthalmological evaluation by a blinded-to-treatments ophthalmologist was performed 28 days after birth, and the incidence of any grade of ROP was compared between groups. The safety of vitamin E administration was evaluated according to the incidence of morbidity, including sepsis, necrotizing enterocolitis, gastrointestinal intolerance (defined as transitory dysfunction (<24 h) to ingest and digest the prescribed enteral feeding), and mortality. Additionally, each participant's evolution was evaluated by an external clinical monitor from epidemiological department of our institution that did not take part in this research, with constant comparison with our institutional endemic channel for each complication reported among participants of the study. As security criteria, any complication outside the endemic channels in both groups could be used to stop the study.

### 2.5. Secondary outcomes

As secondary outcomes, markers of oxidative stress were evaluated in both groups. A blood sample (0.3 mL) was collected

by venipuncture before the administration of the intervention. Subsequent blood samples were obtained in both groups 15 and 28 days after vitamin E or placebo supplementation. Clinical status was monitored, and demographic variables, including gestational age, sex, weight, height, days of oxygen therapy, prenatal care, treatment with prenatal and postnatal steroids, type of mechanical ventilation, and surfactant administration, were recorded.

### 2.6. Biochemical analysis

In order to detect oxidative damage, biochemical analysis of plasma and red blood cell lysates was performed, as described previously.<sup>[22]</sup> Briefly, to detect the total antioxidant capacity (TAC) of plasma, we used the cupric ion reducing antioxidant capacity spectrophotometric method, with absorbance measured at 450 nm.<sup>[23]</sup> Malondialdehyde (MDA) concentrations in erythrocyte lysates were measured using the spectrophotometric method at 586 nm,<sup>[24]</sup> and the resulting values were expressed as pmol of carbocyanine/mg dry weight. The assay described by El-Saadani was used to assess lipid peroxides in the erythrocyte lysate, and lipid peroxidation was measured spectrophotometrically at 360 nm.<sup>[25]</sup> Protein damage was evaluated by determining the carbonyl content in plasma following treatment with 2,4-dinitrophenylhydrazine, which reacts with carbonyl groups to form stable hydrazones.<sup>[26]</sup> These were then measured spectrophotometrically at 370 nm. All experiments were performed in duplicate. Unless otherwise specified, all reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Vitamin E in human serum was measured using high-performance liquid chromatography (Flexar LC, PerkinElmer, Norwalk, U.S.).<sup>[27]</sup>

### 2.7. Sample size

Thirty-nine participants per group were required to probe the hypothesis that vitamin E decreased the incidence of any stage of ROP in VLBW infants with RDS from 40% to 15%, with a power of 80% and an alpha error of .05 in a one-tailed test.

### 2.8. Statistical analysis

Mann–Whitney *U*-tests were conducted to compare continuous variables according to the distribution of the variables. Chi-square tests were performed to determine significant differences in categorical values between groups. Student's *t*-tests were used when needed. Significance was defined at  $P < .05$ . Relative risks (RRs), with 95% confidence intervals (CIs) were calculated. SPSS, version 15 (SPSS, Chicago, IL, USA) was used for the statistical analyses. Oxidative damage data were expressed as the mean  $\pm$  standard deviation (SD), and values were examined using Prism 5.0 software (GraphPad, San Diego, CA, USA).

## 3. Results

### 3.1. Flowchart of the participants in the study and clinical characteristics of the mothers and newborns

During the study period, 240 VLBW infants were born. Of these, 140 patients were eligible to participate in the study. Parents declined participation before randomizing the 42 newborns; therefore, 98 patients whose parents consented to participate in the study were randomly allocated (Fig. 1).

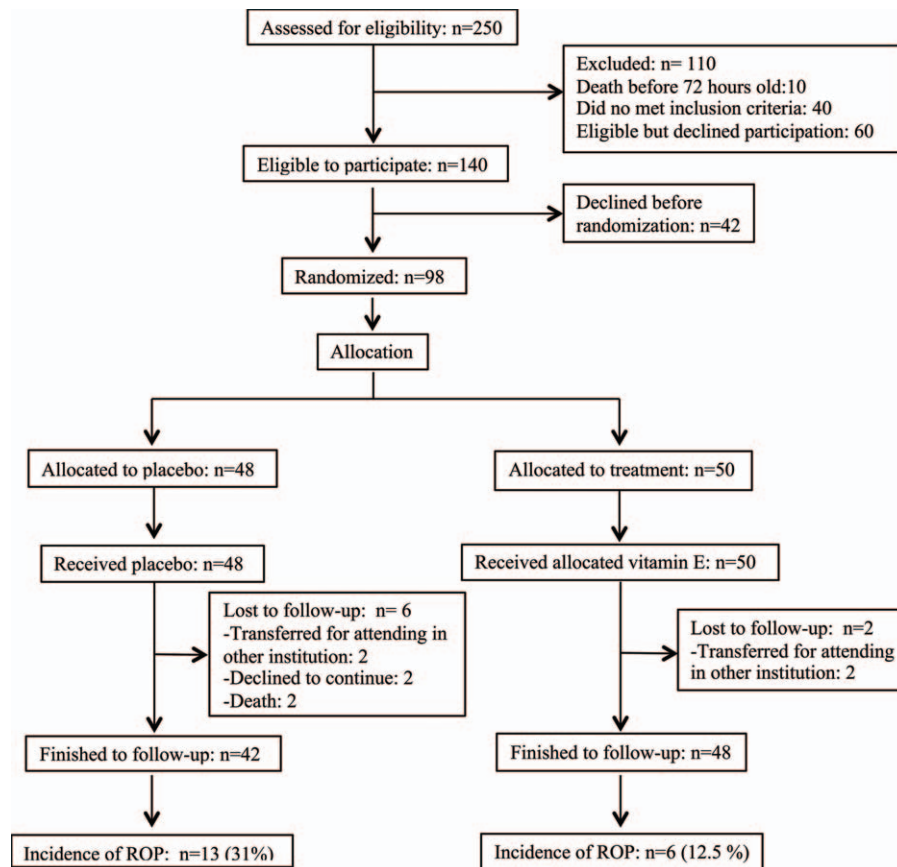


Figure 1. Flowchart of the participants in the study.

The clinical characteristics of the mothers and newborns at the time of admission to the study are presented in Table 1. There were no significant between-group differences in maternal or neonatal morbidity or demographic characteristics and pharmacologic interventions upon admission to the study.

### 3.2. Vitamin E supplementation was associated with a lower incidence of ROP

The clinical outcomes of group T and group C are shown in Table 2. The total incidence of ROP was 21.1% (19/90), and the incidence of ROP was significantly lower in group T (12.5%; 6/48) than group C (31%; 13/42) in the per-protocol analysis. However, the reduced incidence was observed only in stage I of ROP. In the analysis based on intention to treat, there were no significant differences between-group in the incidence of ROP; C group  $n=13/48$  (27%) and T group  $n=6/50$  (12%)  $P=.10$  relative risk; 0.44 (CI 95% 0.18 – 1.07). There were no between-group differences in other clinical outcomes, including sepsis or necrotizing enterocolitis, which were considered markers of vitamin E supplementation safety. Additionally, there were three cases of intrahepatic cholestasis in each group.

The basal vitamin E concentration was similar in group T and group C ( $1.03 \pm 1.0$  vs  $1.09 \pm 1.5$ ,  $P=.97$ ). On day 15, the vitamin E concentration was higher in group T than group C, but the difference was not significant ( $1.52 \pm 0.7$  vs  $1.20 \pm 1.5$ ,  $P=.27$ ). On day 28, the vitamin E concentration was significantly higher in group T than group C ( $1.71 \pm 1.1$  vs  $1.37 \pm 1.5$ ,  $P=.05$ ).

In addition, there was no significant difference in calorie intake ( $130 \pm 10$  kcal in group C and  $127 \pm 18.4$  kcal in group T,  $P=.18$ ).

### 3.3. Oxidative stress markers

In terms of oxidative damage, group T showed a significant decrease as compared to group C. TAC was higher in group T than in group C ( $P < .05$ ) (Fig. 2).

Lipohydroperoxide (LHP) formation was lower, approximately 76.3% in group T ( $P < .0001$ ) than in group C, as shown in Figure 3.

The production of a highly reactive MDA molecule, a consequence of the breakdown of LHP during the lipoperoxidation process, is 53.3% lower ( $P < .0001$ ) in group T than in group C (Fig. 4).

MDA generation is associated with protein injury, favoring new carbonyl formation groups (carbonylation) in the protein molecules.

Figure 5 shows a significant decrease ( $P < .001$ ) in protein carbonylation in group T ( $2.87 \pm 0.93$  nmol DNPH/mg protein) as compared with that in group C ( $3.96 \pm 1.32$  nmol DNPH/mg protein).

## 4. Discussion

This study is the first to report the efficacy of vitamin E in ROP prevention among Mexican VLBW infants. The results showed

**Table 1****Maternal and newborns characteristics at the time of admission to the study.**

Characteristic	Group C N=42	Group T N=48	P-value
Maternal age (years)	30.1 ± 8.1	28.6 ± 6.4	.32
Maternal infections	29 (69%)	34 (70.8%)	.37
-Urinary tract infections	20 (47.6%)	19 (36.6%)	.30
-Cervico-vaginal infections	9 (21.4%)	12 (25.0%)	.30
Chronic hypertension	1 (2.4%)	1 (2.1%)	1.0
Preeclampsia	14 (33.3%)	19 (39.6%)	.90
Prenatal care	40 (95.2%)	43 (89.6%)	.42
Prenatal steroids	26 (61.9%)	31 (64.6%)	.82
Male	23 (54.8%)	22 (45.8%)	.52
Female	19 (45.2%)	26 (54.2%)	.52
Weight (g)	1164 ± 203	1087 ± 194	.07
Gestational age (weeks)	30.1 ± 2	30.0 ± 2	.78
Nosocomial sepsis	28 (66.7%)	29 (60.4%)	.66
Nosocomial pneumonia	1 (2.4%)	2 (4.2%)	.90
Apnea of prematurity	26 (61.9%)	30 (62.5%)	.92
Barotrauma	2 (4.8%)	5 (10.4%)	.44
Renal failure	1 (2.4%)	3 (6.2%)	.62
Neonates managed only with Continuous positive airway pressure	19 (45.2%)	29 (60.4%)	.21
Mechanic ventilation (days)	5.0 ± 7.1	4.5 ± 7.6	.74
FiO <sub>2</sub> maximum concentration (%)	51.6 ± 10	52.3 ± 10	.75
Oxygen requirements (h)	512.9 ± 29	517.3 ± 23	.94
Parenteral nutrition (days)	14 ± 5.5	13.2 ± 6.3	.50
Hospital stay (days)	38.5 ± 11.6	42.6 ± 19.4	.20
Prophylactic surfactant	16 (38.0%)	13 (27.0%)	.37
Early surfactant	6 (14.2%)	6 (12.5%)	.95
Ibuprofen	13 (30.9%)	12 (25%)	.69
Steroid scheme	28 (66.7%)	31 (64.5%)	.83

that oral supplementation of 12.5 IU of vitamin E every 12 h, free of polyethylene glycol, propylene glycol, ethanol, or polysorbate 80, from 72 h after birth until 28 days of age in VLBW infants with RDS was effective in preventing ROP. They also revealed a significant decrease in carbonylation and lipid peroxidation (i.e., oxidative damage markers), as well as higher TAC in VLBW infants supplemented with vitamin E.

Emerging evidence suggests that lung and retinal injury that leads to broncho pulmonary dysplasia and ROP occur within hours to a few days after delivery.<sup>[28]</sup> During pregnancy, placental transfer of vitamin E into the fetus is limited,<sup>[29,30]</sup> and antioxidant enzymes are expressed at high concentrations only at the end of gestation, in preparation for the relatively hyperoxic extrauterine environment.<sup>[1,51]</sup> In premature newborns,

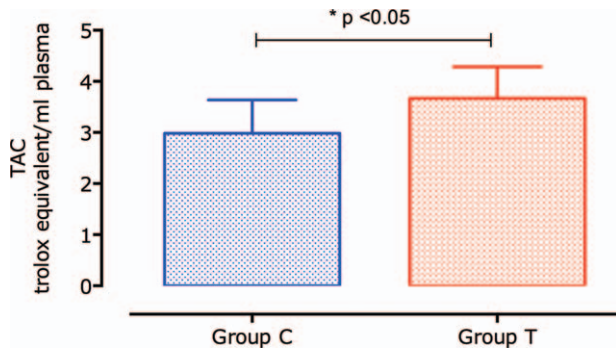
antioxidant defenses are not appropriately expressed, and vitamin E is a good alternative to protect against peroxidation.<sup>[30]</sup> As a lipophilic compound, vitamin E accumulates in circulating lipoproteins, cell membranes, and fatty deposits, and it reacts with free radicals and molecular oxygen.

Vit E protects polyunsaturated fatty acids from peroxidation.<sup>[30,31]</sup> Lipoperoxidation has been proposed as a crucial step in the pathogenesis of ROP, as the retina contains elevated levels of polyunsaturated fatty acids, such as docosahexaenoic acid and cis-arachidonic acid.<sup>[32,33]</sup> In addition to increased lipoperoxidation, protein changes may affect retinal functionality because of the consequences of oxidative damage.<sup>[28]</sup>

Results reported here demonstrate the antioxidant efficacy of supplementation with vitamin E in VLBW infants, resulting in a

**Table 2****Relative risks (RR), with 95% confidence intervals (CIs) for clinical outcomes in new-borns in the control (group C) and treatment (group T) groups at 28 days of age.**

Outcome	Group C n=42	Group T n=48	P-value	Relative risks	95% Confidence intervals
Retinopathy of prematurity	13 (31%)	6 (12.5%)	.03	0.40	0.17–0.97
Retinopathy of prematurity severity	12 = Stage I 1 = Stage II	5 = Stage I 1 = Stage II	.03 .5	0.40 0.87	0.17–0.97 0.05–13.5
Bronchopulmonary Dysplasia	27 (64.3%)	31 (64.6%)	.97	1.00	0.74–1.37
Ductus arteriosus	19 (45.2%)	16 (33.3%)	.28	0.78	0.51–1.2
Intraventricular haemorrhage	5 (11.9%)	4 (8.3%)	.72	0.81	0.38–1.75
Sepsis	22 (52.4%)	28 (58.3%)	.72	1.11	0.76–1.6
Gastrointestinal intolerance	3 (7.1%)	7 (14.6%)	.32	1.30	0.86–2.16
Necrotizing enterocolitis	0 (0%)	1 (2.1%)	.91	2.60	0.11–61.7



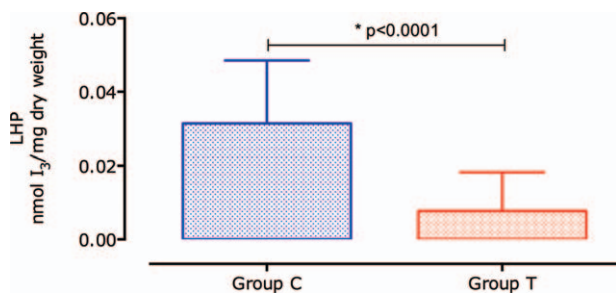
**Figure 2.** Total antioxidant capacity (TAC) comparison between group T and C. Data are expressed as mean  $\pm$  standard deviation (SD).

significant decrease in all oxidative stress biomarkers measured after 15 and 28 days of vitamin E supplementation.

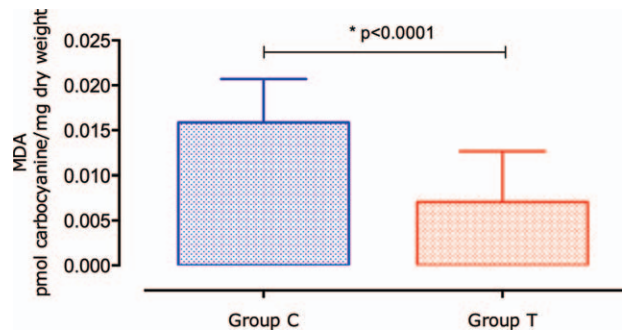
In the present study, there was no statistically significant difference in the basal concentrations of vitamin E between groups, which could be attributed to similar concentrations at birth, and that participants in both groups received vitamin E (7.5 IU) as parenteral nutrition from 12 to 24 h of life.

The concentrations of vitamin E administered in the present study are considered in normal ranges, according to the American Academy of Pediatrics.<sup>[6]</sup> After 15 days of treatment, there were still no differences in serum vitamin E concentrations. Only after 28 days of treatment did the concentration significantly increase in the treated group, although the serum vitamin E concentrations did not exceed 3.5 mg/dL. The low levels of vitamin E can be attributed to poor oral absorption due to immaturity of the digestive tract, resulting in poor fat absorption in premature neonates.<sup>[34]</sup> The low levels found in this study also can be attributable to the preparation of solutions. Previous research reported that vitamin E supplementation increased levels (>3.5 mg/dL) and could possibly reduce the risk of severe ROP but increase the risk of sepsis,<sup>[18]</sup> our results avoiding potentially dangerous solvents decrease the potential toxicity of commercial vitamin E preparations.

In the present study, supplementation with vitamin E was not sufficient to prevent BPD, similar to findings reported by Brion et al.<sup>[11]</sup> We found no evidence of complications associated with sepsis or necrotizing enterocolitis. These findings agree with a previous study that examined the efficacy of vitamin E in preventing ROP, as well as the safety of vitamin E supplementation in 287 newborns <1,500 g (adjusted to plasma levels of 3 to



**Figure 3.** Concentration of Lipid hydroperoxide. The generation of lipid hydroperoxide (LHP) was 4.2 times higher in group C as compared with group T. Data are expressed as mean  $\pm$  standard deviation (SD).



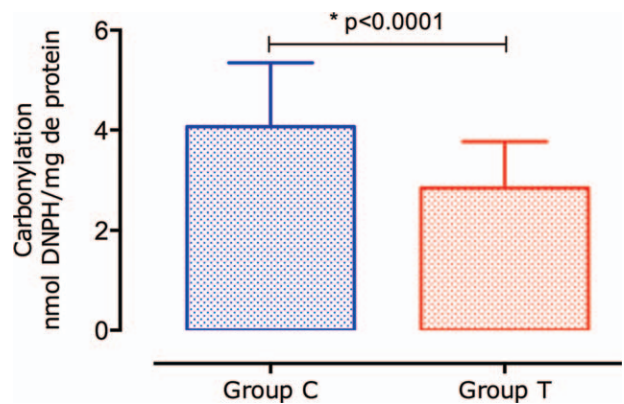
**Figure 4.** Concentration of malondialdehyde. The malondialdehyde (MDA) concentration was significantly higher in group C as compared with that in group T. Data are expressed as mean  $\pm$  standard deviation (SD).

3.5 mg/dL),<sup>[33]</sup> and with a recent meta-analysis showing that the risk of any/severe ROP was significantly reduced with vitamin E (RR 0.30, 95% CI 0.14–0.62).<sup>[35]</sup>

In the present study, there were six intrahepatic cholestasis cases (three per group), which could be associated with parenteral nutrition.

The present study had several limitations. It was performed in a single center and consisted of a limited sample size of only Mexican VLBW infants with RDS. Future prospective and multi-center studies with larger sample sizes are required to corroborate these findings. Another limitation of the study was vitamin E's ability to prevent ROP only in the per-protocol analysis. Vitamin E showed a trend in the efficacy of intention-to-treat analysis, which was not significant. Another limitation was that this study used a vitamin E formulation prepared by our pharmacologist team rather than an available commercial formulation. It is necessary to adjust doses with this type of water-based preparations to reach higher serum concentrations. Other limitation was that levels of other antioxidants, as vitamin A and C have not been measured in two groups that could be confounding factors and results should be interpreted with caution.

Infants at risk of ROP suffer from numerous conditions, and are exposed to many forms of polypharmacy which likely add to the etiology of the disease. Pharmacologic interventions for treatment and/or prevention of ROP have predominantly



**Figure 5.** Protein carbonylation values. Carbonylation was significantly lower in group T than in group C. Data are expressed as mean  $\pm$  standard deviation (SD).

targeted oxidants and VEGF, however given the multifactorial characteristic of ROP and complexity of the disease, the use of a single therapeutic agent may not be prudent.<sup>[36]</sup>

Antioxidant therapy may be useful in the management of neonates with problems caused by oxidative stress, but further biochemical investigations are required to identify the most effective antioxidant therapy. We propose that future studies explore the ability of antioxidants mixtures with different redox potentials to prevent ROP.

## 5. Conclusions

Oral supplementation with vitamin E may help prevent ROP development in VLBW infants with RDS without increasing adverse effects. Oxidative damage markers were significantly lower, whereas total antioxidant capacity was increased in the vitamin E group. However, levels of other antioxidants as vitamin A and C were not measured in two groups. More randomized clinical trials are necessary to confirm our findings.

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