ORIGINAL ARTICLE

Reduction in Migraine and Headache Frequency and Intensity With Combined Antioxidant Prophylaxis (*N*-acetylcysteine, Vitamin E, and Vitamin C): A Randomized Sham-Controlled Pilot Study

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■ **Abstract**

Objective: To investigate the preventive effects of a combined antioxidant drug (*N*-acetylcysteine, vitamin E, and vitamin C [NEC]) on migraine outcomes. Migraine is characterized by increased oxidative stress and neurogenic inflammation in the brain; therefore, antioxidants may have a migraine preventive effect.

Design: Randomized, double-blind, sham-controlled pilot study.

Setting: Australian community.

Subjects: Adults reporting 2 to 8 migraines per month for at least a year.

Methods: After a 1-month baseline period, 35 subjects completed 3 months of treatment with NEC (n = 19) or sham (n = 16) capsules. The primary outcome was the difference in mean number of headaches per month between baseline and final month of the trial for NEC and sham groups; secondary outcomes are listed below.

Results: For NEC there was a significant decrease in mean number of headaches by 3.0 per month (P = 0.004) compared with 1.4 for sham (P = 0.073); there was no significant difference in these changes between the 2 groups (P = 0.052). Average monthly headache (P = 0.041) and migraine frequency (P = 0.018) were significantly less for NEC vs. sham. In NEC subjects, there was a significant decrease in average monthly migraine days (−3.1), moderate/severe headache days (−3.2), migraine duration, headache pain scores, and acute headache medication use.

Conclusions: This is the first randomized controlled trial to find that combined antioxidant therapy with NEC reduces headaches and migraines in adult migraineurs. Given the limitations of this pilot study, an adequately powered randomized controlled trial is planned to further investigate antioxidant prophylaxis in migraine. ■

Key Words: migraine, headache, antioxidants, *N*-acetylcysteine, vitamin E, vitamin C

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INTRODUCTION

Migraine is a complex neurovascular inflammatory brain disorder with multiple potential targets for treatment and prophylaxis.[1–3] Recent research has focused

on the key role of calcitonin gene-related peptide (CGRP) as a mediator of neurogenic inflammation and trigemino-cervical nucleus (TCN) sensitization in migraine. This has resulted in the recent development of CGRP molecule and receptor monoclonal antibodies as preventive drugs with significant improvements in migraine outcomes.[4–9]

However, over the past decade, research has shown that oxidative stress and the accumulation of oxygen and nitrogen free radical species (FRS) in the brain (in particular nitric oxide [NO]) also plays a major role in migraine pathophysiology.[10–25] FRS are chemically unstable and reactive oxygen or nitrogen moieties (eg, O_2^-, NO, H_2O_2) produced during normal cell metabolism, which may accumulate in tissues, causing damage to lipid membranes, proteins, and DNA via peroxidation.[26–28]

There is a higher than normal incidence of complex regional pain syndrome (CRPS), asthma, and inflammatory bowel disease in migraineurs, based on a shared pathophysiology of oxidative stress, FRS generation, and neurogenic inflammation.[28–30] There is also ample evidence of impaired oxidative-antioxidant balance in patients with migraine, with increased FRS and reduced levels of antioxidants in their blood and CSF.[11–13,31] The free radical NO is a potent migraine trigger and is used to precipitate migraine attacks in experimental research.[17–21] NO releases CGRP and substance P, activates platelets, and modulates the function of serotonin receptors, transient receptor potential cation channels ($TRPV_1$), potassium channels, and mitochondria. In the brain, these processes are linked to spreading cortical depression, cerebral vasodilation, and activation of the TCN, which are key processes in migraine generation.[10–25,32]

Free radical scavengers are antioxidants that chemically reduce FRS to counteract their effects.[26–28] Vitamin C (Vit C) and vitamin E (Vit E) are powerful antioxidants[33] that reduce NO levels in mouse nerve and muscle tissue[34,35] and enhance the neuro-inhibitory effects of gamma amino butyric acid in the brain, which may reduce cortical activation in migraine.[36] *N*-acetylcysteine (NAc) is an antioxidant moiety of cysteine used to treat CRPS, Parkinson's disease, and traumatic brain injury by reducing oxidative stress and FRS.[37,38]

There have only been 5 small clinical studies of antioxidants in the treatment of migraine. Two randomized controlled trials (RCTs) found the antioxidants curcumin and coenzyme Q10 decreased migraine frequency[39,40] and a single RCT reported that Vit E decreased menstrual migraines.[41] Finally, 2 uncontrolled studies reported a decrease in migraine symptoms with an antioxidant combination of pine bark extract, Vit C, and Vit E.[42,43]

Given the compelling evidence for oxidative stress in migraine pathophysiology, the efficacy of Vit C and NAc in treating CRPS and related neuro-inflammatory disorders,[44–47] and the lack of clinical research in the field,[10,31,48] this pilot study was undertaken to investigate the preventive effects of a combined antioxidant (NAc, Vit E, and Vit C [NEC]) on migraine outcomes in patients with episodic migraines. As a proof-of-concept study, we tested a triple antioxidant combination to improve the odds of detecting any effects on migraine outcomes. The primary experimental hypothesis to be tested was that twice daily administration of NEC (NAc 600 mg, Vit E 250 IU, and Vit C 500 mg) for 3 months would significantly decrease the mean number of headache episodes per month (between baseline and the final month of the trial) compared with a sham control group.

METHODS

Trial Design

This pilot study was a prospective, double-blind, randomized, sham-controlled, 2 parallel group trial. Trial design was based on the "Guidelines for Controlled Trials of Drugs in Migraine; Third Edition. A Guide for Investigators"[49] and "Guidelines of the International Headache Society for Controlled Trials of Preventive Treatment of Chronic Migraine in Adults"[50] of the International Headache Society (IHS). The study was performed in the community throughout Australia over a period of 14 months between February 1, 2016, and April 1, 2017. Subjects participated for 4 months (16 weeks or 112 days), including a 1-month (4 weeks) baseline measurement phase and a 3-month treatment phase. Ethics approval was obtained from our University Human Research Ethics Committee in accordance with the Declaration of Helsinki, and the trial was registered with the Australian and New Zealand Clinical Trial Registry (ACTRN: 12615001339549p) and ClinicalTrials.gov (ID: NCT02629536).

Study Population

Female and male patients with migraine who were 18 to 65 years of age were recruited as a convenience sample from throughout Australia via television and social

media advertising and website postings. Interested subjects contacted the researchers via e-mail and, after providing informed consent, completed an online screening questionnaire via SurveyMonkey (San Mateo, CA, U.S.A.) to identify inclusion and exclusion criteria as per IHS guidelines.[49,50]

Inclusion criteria included migraine (with-or-without aura) of at least 1 year's duration with onset before 50 years of age; 2 to 8 migraine episodes or migraine days and less than 6 other headache types per month, averaged over 3 months prior to recruitment; subjects able to distinguish between migraine and other headache types; and cognitive and English language skills allowing completion of headache diaries and self-administration of trial drugs.

Exclusion criteria included participation in a concurrent research trial; chronic daily headaches, medication-overuse headache, and/or other primary headache disorders; change in migraine treatment in the 3 months prior to, or during, the study; taking more than 2 migraine prevention drugs; taking NAc, Vit E, or Vit C supplements in the 3 months prior to the study; pregnancy, or risk of pregnancy, during the study, women of childbearing age not using contraception, or breast feeding; adverse reactions to NAc, Vit E, or Vit C preparations; Vit C deficiency, clinical reports of renal or liver dysfunction, clinical risks associated with bleeding, coagulopathy, or warfarin therapy; hemochromatosis; glucose-6-phosphate dehydrogenase deficiency; daily opioid use, substance abuse, dependence, or addiction in the 3 months prior to, or during, the study; or psychosis or bipolar affective disorder.

Subjects stabilized on up to 2 migraine prevention drugs were not excluded because cessation and washout of drugs would hinder recruitment and retention and does not reflect real-life clinical practice. If after randomization subjects did not report 2 to 8 migraines or migraine days during the baseline month, they were withdrawn from the trial at that time.

Randomization and Blinding

Eligible subjects were computer randomized in blocks of 10 to 1 of 2 trial groups—an NEC treatment group or a sham control group—and allocated an identification number as per IHS guidelines.[49,50] Subjects and researchers were blinded, and an independent pharmacist dispensed trial drugs according to the randomization schedule.

Intervention

NEC and sham trial drugs were prepared as capsules and bottled in the same containers by a government-registered pharmacy (Oxford Compounding Pharmacy, North Perth, Western Australia). NEC capsules contained NAc 300 mg, Vit E 125 IU, and Vit C 250 mg. Identical sham capsules contained a filling agent (cellulose) and food coloring. The total daily dose of NEC ingredients was NAc 1,200 mg, Vit E 500 IU, and Vit C 1,000 mg.

Subjects took 2 capsules twice daily because the total daily mass of NEC ingredients (2,425 mg) could only be compounded into 4 capsules. Twice daily dosing was used because of the short plasma half-lives of the active ingredients following oral administration. Ingredients in the NEC capsules were all approved for human use by the Australian Government Therapeutic Goods Administration (TGA) and are available in Australia as over-the-counter nutraceuticals. Capsules were to be taken on at least 5 days each week for a minimum of 8 weeks during the treatment phase. Subjects could continue up to 2 migraine preventive medications and their usual acute migraine medications during the trial.

Outcome Measures

Outcomes were defined according to the IHS guidelines.[49,50] The subjects' age (years), sex, duration of migraine, age of onset (years), monthly migraine frequency, preventive medication use (number of drugs), menstrual status, and Migraine Disability Assessment Score (MIDAS) were recorded within a month of starting the trial.

The primary outcome measure was the difference in mean number of headache episodes per month, between baseline and the final month of the trial, in both trial groups. Secondary outcome measures were the difference in (1) mean number of migraine episodes per month; (2) mean number of migraine days per month; (3) mean number of moderate-or-severe headaches days per month; (4) mean total duration of migraines per month (hours); (5) mean duration of each migraine episode (hours) per month; (6) mean headache VAS pain score per month (scale of 0 to 100 mm); and (7) mean number of acute headache medication doses per month. The number and type of self-reported adverse events for each trial month were also recorded.

Data Management

A novel headache diary (HD) was developed for this trial based on the HD for preventive therapies of the IHS

guidelines.[49] Subjects were invited to use an online version of the HD via SurveyMonkey or a paper-based version sent by post. Subjects completed baseline HD entries for 1 month prior to starting the trial, then treatment diaries for each of the following 3 months. De-identified data were compiled into monthly data sets for each study group by a blinded research assistant using Microsoft Excel version 15.0 (Microsoft Corporation, Redmond, WA, U.S.A.).

Statistical Analysis

Analysis was performed by a biostatistician at the completion of the trial using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, U.S.A.). Data were analyzed on an intention-to-treat basis if at least 2 months of HD had been completed. If data from the final (third) trial month were missing, data from the second month were brought forward in substitution. Unless otherwise indicated, results were reported as a group mean ± standard deviation (SD).

Hypotheses were tested using nonparametric statistics due to the small sample size and deviation from a normal distribution of scores for most of the outcome measures. Differences in outcome measures (baseline - final month) within the NEC and sham groups were analyzed using Wilcoxon's matched-pairs signed-ranks test. Differences in outcomes between the 2 groups were analyzed using the Mann-Whitney *U* test. The chi-square test was used for demographic data. The criterion for statistical significance was $P < 0.05$. Due to the exploratory nature of this pilot study, no correction was made for multiple comparisons. Post hoc, the sample size of 35 subjects was considered adequate for a pilot study because (1) the number was similar to previous pilot studies of migraine prevention,[51–53] (2) it was greater than 10% of the estimated sample size for a powered RCT, and (3) it was within the range of 20 to 45 subjects, as recommended by Whitehead et al.[54] and Lancaster et al.[55]

RESULTS

Subject Disposition, Demographics and Migraine History

A total of 375 patients with migraine completed the screening questionnaire; 291 (77.6%) were ineligible, leaving 84 subjects who were randomized. Thirty-five (41.7%) of these subjects (19 NEC, 16 sham) completed the trial, with 31 (36.9%) dropouts due to incomplete HD entries, 17 exclusions (20.2%) due to inadequate baseline migraine frequency, and 1 exclusion (1.2%) due to incorrect trial drug use (Figure 1).

The trial cohort had an average age of 44.7 years (range: 24 to 65 years), and 30 (86%) were female. Twelve subjects (34%) reported migraine with aura, with onset of migraines before 50 years of age in all cases. Each subject experienced between 2 to 8 migraines per month for at least 1 year and were taking either 0 or 1 (8 subjects, 23%) migraine preventative medication. Twenty-seven subjects (77.1%) had moderate or severe MIDAS. There was no significant difference between the trial groups for these data (Table 1).

Outcome Measures

Results are detailed in Table 2 and Figure 2.

Primary Outcome. In the NEC group, the mean number of headache episodes per month decreased by 3.0 ± 3.5 between baseline and the final month ($P = 0.004$) and by 1.4 ± 2.7 in the sham group ($P = 0.073$); however, there was no significant difference in these changes between the 2 groups ($P = 0.052$). During the final month of the trial, the mean number of headache episodes per month was significantly lower in the NEC group compared with the sham group ($P = 0.041$; Table 2, Figure 2A).

Secondary Outcomes. In the NEC group, the mean number of migraine episodes per month decreased from 3.7 ± 2.2 at baseline to 2.0 ± 2.5 during the final month ($P = 0.007$) but did not change significantly in the sham group. The decrease from baseline was significantly greater in the NEC group than in the sham group ($P = 0.018$; Table 2, Figure 2B).

In the NEC group, there was also a significant decrease in mean number of migraine days and total duration of migraine hours per month from baseline to final month, but not in the sham group (Table 2, Figure 2C,E).

The mean number of moderate or severe headaches days per month from baseline to final month decreased significantly in both groups, whereas the mean duration of each migraine episode (hours) did not change significantly (Table 2, Figure 2D,F). In the NEC group, the mean monthly headache VAS pain score decreased from 55.5 ± 17.9 mm at baseline to 34.7 ± 30.7 mm

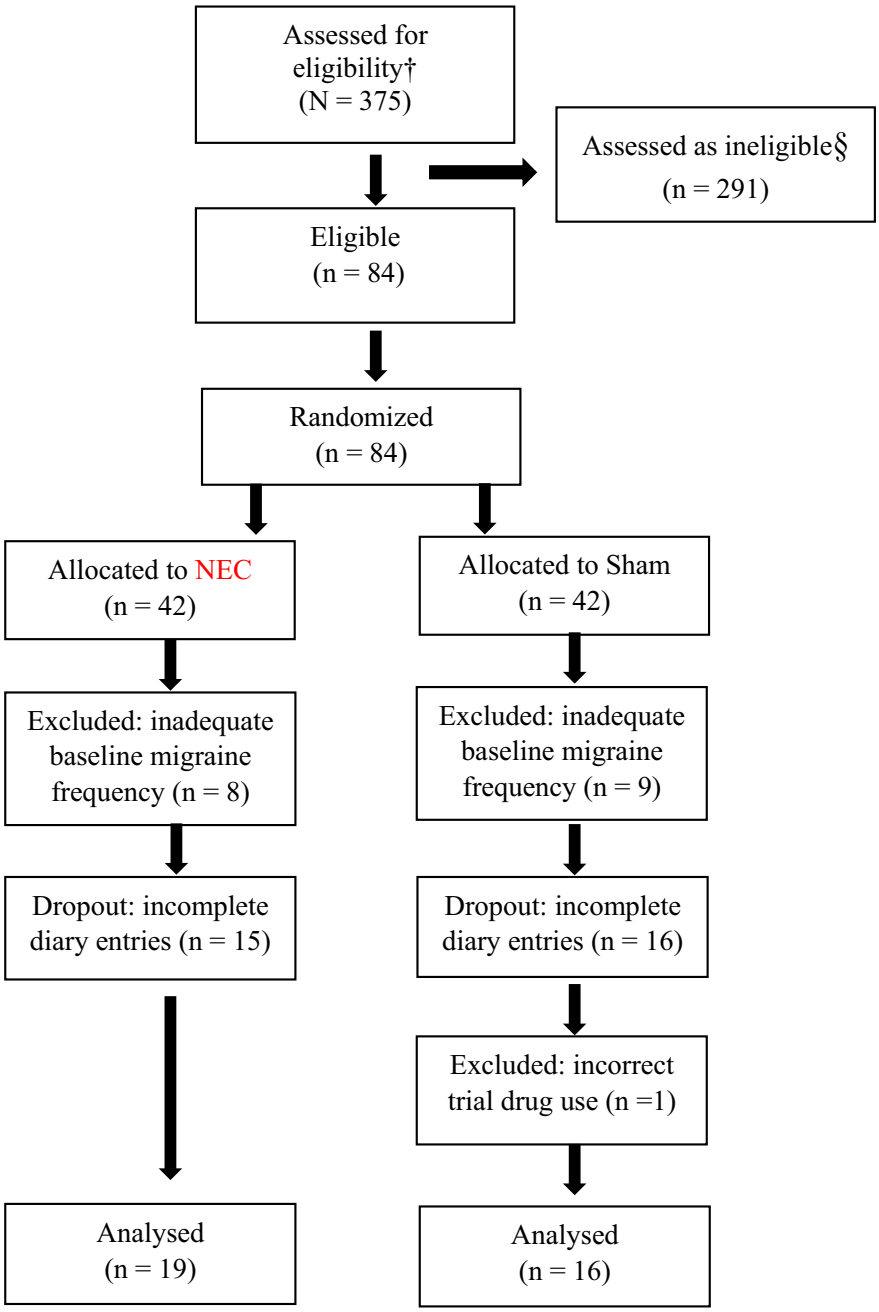

Figure 1. Participant disposition. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. †Potential subjects who completed the online screening questionnaire after reply to recruiting. §Ineligible due to exclusion criteria, incomplete data, loss of contact. NEC, *N*-acetylcysteine, vitamin E, and vitamin C.

in the final month ($P = 0.013$), but did not change significantly in the sham group (Table 2, Figure 2G). Furthermore, in the NEC group, the mean number of acute headache medication doses per month decreased by 5.9 ± 6.3 in the final month ($P = 0.002$), which was significantly more than the decrease in medication use in the sham group ($P = 0.007$; Table 2, Figure 2H). There was one self-reported adverse event during the trial, in which a subject consumed twice the recommended dose of NEC capsules for 1 month, without adverse consequences.

DISCUSSION

The results of this trial support the hypothesis that treatment with NEC significantly reduces headache and

Table 1. Demographics and Migraine History for NEC and Sham Groups

Variable	NEC (n = 19)	Sham (n = 16)	P value*
Female, n (%)	17 (89%)	13 (81%)	0.642
Age, mean years ± SD	44.6 ± 11.5	44.8 ± 10.0	0.909
Migraine with aura, n (%)	6 (32%)	6 (37%)	0.736
Average age of onset, mean years ± SD	22.7 ± 9.0	24.4 ± 8.2	0.161
Average number of migraines per month at baseline ± SD	3.7 ± 2.2	2.7 ± 1.1	0.385
Preventative medication, n (%)	4 (21%)	4 (25%)	1.000
MIDAS, mean ± SD	50.8 ± 37.3	26.4 ± 14.6	0.191
Women in menopause, n (%)	7 (41%)	4 (31%)	0.708

*Chi-square or Mann-Whitney U test. MIDAS, Migraine Disability Assessment Score; NEC, N-acetylcysteine, vitamin E, vitamin C; SD, standard deviation.

Table 2. Baseline and Final Month Trial Outcome Measures for NEC (n = 19) and Sham (n = 16) Groups

Outcome Measures	Baseline Month (mean ± SD)	Final Month‡ (mean ± SD)	P Value*	Difference From Baseline (mean ± SD)
Mean no. of headache episodes/month				
NEC	5.5 ± 2.5	2.5 ± 2.8	**0.004**	−3.0 ± 3.5
Sham	5.1 ± 2.6	3.7 ± 1.9	0.073	−1.4 ± 2.7
P value†	0.545	**0.041**		0.052
Mean no. of migraine episodes/month				
NEC	3.7 ± 2.2	2.0 ± 2.5	**0.007**	−1.7 ± 2.9
Sham	2.8 ± 1.1	2.4 ± 1.7	0.352	−0.4 ± 1.8
P value†	0.385	0.243		**0.018**
Mean no. of migraine days/month				
NEC	5.9 ± 4.3	2.8 ± 3.1	**0.009**	−3.1 ± 5.0
Sham	4.2 ± 2.1	3.4 ± 2.6	0.232	−0.8 ± 3.0
P value†	0.317	0.403		0.117
Mean no. of moderate/severe headache days/month				
NEC	6.2 ± 4.5	3.0 ± 2.9	**0.017**	−3.2 ± 4.8
Sham	5.7 ± 3.61.7	4.1 ± 2.4	**0.016**	−1.6 ± 2.5
P value†	0.731	0.142		0.117
Mean total duration of migraines/month (hours)				
NEC	77.4 ± 102.9	42.8 ± 67.6	**0.004**	−34.6 ± 54.7
Sham	48.5 ± 41.5	39.5 ± 40.3	0.408	−9.0 ± 47.6
P value†	0.589	0.385		0.071
Mean duration of each migraine episode (hours)				
NEC	21.7 ± 23.3	15.8 ± 23.8	0.306	−5.9 ± 26.5
Sham	18.3 ± 16.1	17.0 ± 19.4	0.816	−1.4 ± 23.5
P value†	0.987	0.333		0.659
Mean headache VAS pain score (0 to 100 mm)				
NEC	55.5 ± 17.9	34.7 ± 30.7	**0.013**	−20.8 ± 31.1
Sham	60.8 ± 11.7	53.9 ± 19.2	0.379	−6.9 ± 21.8
P value†	0.441	0.061		0.205
Mean no. of acute medication doses/month				
NEC	9.5 ± 9.1	3.6 ± 4.1	**0.002**	−5.9 ± 6.3
Sham	6.7 ± 4.5	5.2 ± 4.1	0.108	−1.5 ± 3.7
P value†	0.102	0.117		**0.007**

*Wilcoxon matched-pairs signed ranks test compared baseline and final trial month outcome measures within NEC and sham groups. †Mann-Whitney U test compared outcomes measures between the groups. ‡Final month = third month of treatment. NEC, N-acetylcysteine, vitamin E, vitamin C; SD, standard deviation; Statistically significant P values are highlighted in bold.

migraine frequency and pain intensity in adults with episodic migraines. After 3 months of treatment, the average number of headaches, migraine days, and moderate or severe headache days decreased by approximately 3 per month. There were also significant reductions in average monthly migraine episodes, headache pain scores, acute medication use and monthly migraine duration in the NEC treatment group. The results are equivalent to those reported in other migraine prophylaxis trials.[4–9,56–64] However, any conclusions regarding the role of antioxidants in migraine should be interpreted within the limitations of this pilot study design.

To our knowledge, this is the first randomized, sham-controlled trial of a combined antioxidant based on NEC and the only study of NAc in migraine prevention. In 2010, Visser[65] proposed trialing Vit C and NAc for migraine prevention based on their effectiveness in treating CRPS and suggested that migraine might be considered a form of "CRPS of the brain." As outlined in the introduction, there is a higher incidence of CRPS and inflammatory diseases such as asthma and inflammatory bowel disorders in migraineurs, based on a shared pathophysiology of oxidative stress, FRS generation, and neurogenic inflammation.[10–28] Research has established that these processes, and in particular the generation of NO in the brain, plays a major role in migraine.[17–25] Limited data from clinical trials suggest that antioxidants such as Vit C and Vit E may improve migraine outcomes; however, further studies are required.[39–43] Our study adds to the body of clinical evidence that antioxidants might have a preventive effect in migraine.

Reductions in the number of headache episodes, moderate or severe headaches, and migraine days by approximately 3 per month with NEC are clinically meaningful improvements and equivalent to the results of migraine prophylaxis trials with anti-CGRP antibodies,[4–9,56] beta blockers,[57,58] topiramate,[59–62] botulinum toxin,[63,64] and nutraceuticals such as curcumin and coenzyme Q,[39,40] riboflavin,[66–68] and melatonin.[69] There were also decreases in all outcome measures in the sham treatment group consistent with the strong placebo effect reported in many migraine trials.[70] However, none of these results were statistically significant except for a reduction in moderate to severe headache days.

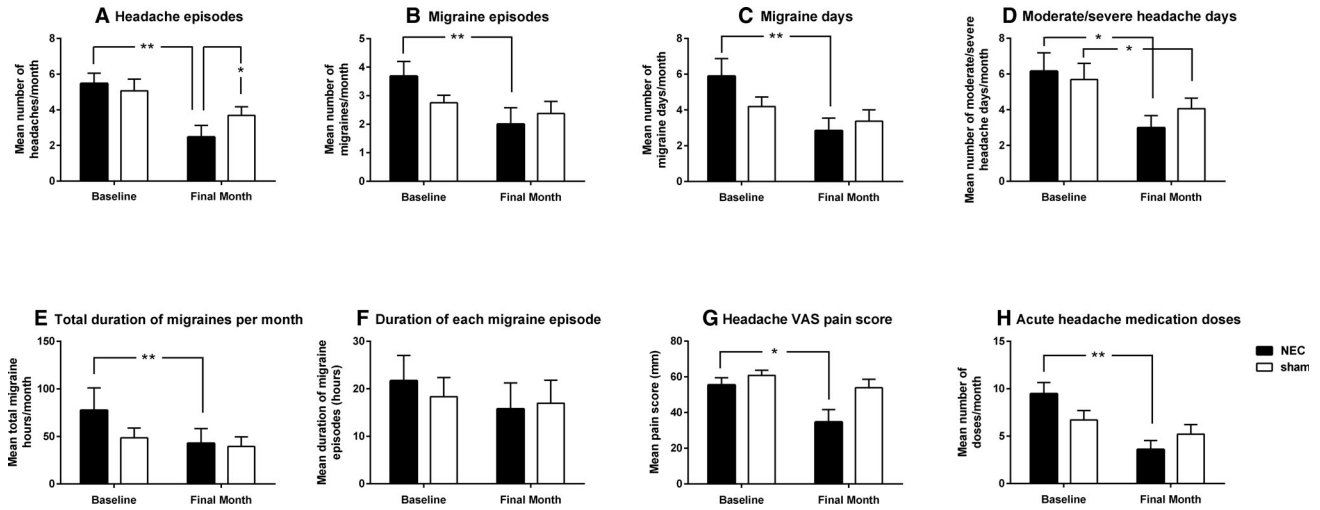

Figure 2. Column graphs (with standard error of the mean [SEM] bars) comparing outcome measures for *N*-acetylcysteine, vitamin E, and vitamin C (NEC) (n = 19) and sham (n = 16) groups for baseline and final month of the randomized controlled trial. Wilcoxon matched-pairs signed ranks test compared outcomes for baseline vs. final month of trial. Mann-Whitney *U* test compared data between the 2 groups. Subjects took either NEC or sham capsules (control group) for up to 3 months. (A) A significant decrease in mean monthly headache episodes (−3.0; P = 0.004) was seen between baseline and final month in the NEC group but not in the sham group (−1.4; P = 0.073); however, there was no significant difference in these changes between the groups (P = 0.052). During the final trial month, the mean number of headaches per month was significantly less in the NEC group (2.5 ± 2.8) compared with the sham group (3.7 ± 1.9) (P = 0.041). (B) A significant decrease in mean migraine episodes per month (−1.7 ± 0.7) was seen for the NEC group vs. the sham group (−0.4 ± 0.5) (P = 0.018). For the NEC group, there were also a significant decreases in mean monthly migraine days (−3.1 ± 1.1) (C), moderate/severe headache days (−3.2 ± 1.1) (D), total duration of migraines (−34.6 ± 12.6 hours) (E), headache pain scores (−20.8 ± 7.1 mm) (0 to 100 mm VAS) (G), and number of acute headache medication doses used (−5.9 ± 1.4) (H), during the final trial month. There was no significant difference in mean duration of migraine episodes between the groups. Considering the limitations of a small pilot study, these data suggest that taking NEC for up to 3 months may significantly reduce the frequency, total duration, and intensity of headaches and migraines in adult migraineurs experiencing 3 to 8 episodes per month. Significance levels on graphs: *P < 0.05; **P < 0.01. SEM, standard error of the mean.

Total migraine hours per month decreased significantly with NEC, but there was no change in the average duration of each migraine episode. Many preventive trials do not record these measures.[59,60,64] Our results suggest that preventive treatment with NEC reduces migraine frequency but not their average duration.

There was also a significant decrease in mean monthly headache pain scores with NEC treatment, in a range similar to that of botulinum toxin,[63,64] which is relevant because not all preventive migraine studies report VAS headache pain scores.[59,60] Subjects treated with NEC also reported a significant reduction in average monthly medication use for acute attacks, which serves as a useful proxy measure for migraine frequency and severity, and is consistent with decreased rescue medication use in other trials of migraine prevention drugs.[60,61,63]

NAc, Vit E, and Vit C were chosen as our trial drug constituents because there is clinical and basic sciences evidence of their efficacy as antioxidants and therapeutic drugs.[37–47] They have a history of safe clinical use, including as over-the-counter nutraceuticals, and are registered for human use by the Australian TGA. No patient recorded adverse events on the self-reporting system for up to 6 months following this trial. One subject accidently took twice the dose of capsules for 1 month, but there were no adverse effects, and full blood picture, electrolytes, and liver function test results were normal on review.

As a proof-of-concept study, we decided to test a high-dose combined antioxidant as the most pragmatic way of detecting if antioxidants had any measurable effects on migraine outcomes. The components of NEC were selected because of their good oral absorption and brain penetrance, and the doses were based on previous therapeutic studies.[33,37,38,41–47] There is a plateau plasma concentration effect for Vit C with oral doses greater than 250 mg, explaining why this was chosen as our capsule dose.[71–74] Brain transfer and storage of Vit C is the highest of any organ[74] and oral absorption and brain penetrance of Vit E is high.[75,76] However, bioavailability of NAc is low at 15%, and new formulations are being developed to improve gut absorption.[77–79] Although there is clear evidence of a powerful

antioxidant effect with NAc administration in peripheral tissues, the effects on brain oxidation remain unclear.[37,38,78]

The total daily mass (≈2,500 mg) of NEC could only be administrated as 2 capsules, twice daily for pharmaceutic reasons. Twice daily dosing was also used because of the short plasma half-lives of the constituent drugs following oral absorption.[71–79] This may have affected compliance with taking the trial drugs and may compromise NEC's usefulness in the future as a migraine preventive drug. Clearly, further pharmacological research is required to determine the optimal oral formulation of NEC.

A major limitation of this pilot study was its small sample size of 35 subjects, due to a high dropout rate following randomization, which in turn decreased statistical power and required post hoc changes in data analysis. The study was run from a single site, with communications and monitoring of subjects and delivery of trial drugs managed over the large geographic area of Australia via internet and post. This made the logistics of data collection, diary completion, drug administration and safety monitoring difficult and contributed to the high diary noncompletion rate and data loss. Future studies will require a more robust oversight system, including trial drug auditing and monitoring for adverse effects.

An aim of this pilot study was to inform the design of future RCTs of antioxidants for migraine prevention. Our results suggest such trials should be powered to detect a decrease in average migraine and headache frequency of 2.5 per month in the treatment group and 1.0 per month in sham controls after 3 months of treatment, with an estimated dropout rate of 30% after randomization.

In conclusion, this RCT shows for the first time that combined antioxidant therapy with NEC significantly decreases headache and migraine frequency, total monthly duration, pain intensity, and acute headache medication use in adults with episodic migraines, compared with a sham control group. These results are clinically meaningful and equivalent to outcomes in other RCTs of migraine prevention. Conclusions regarding the role of antioxidants in migraine should be interpreted within the limitations of this pilot study, and our data are currently not applicable to the general migraine population. Nevertheless, our results support the growing body of evidence that oxidative stress, FRS, and neurogenic inflammation in the brain plays a major role in migraine pathophysiology, and that combined antioxidants such as NEC may have a role to play in migraine prevention and treatment.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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