Original Communication

Positive Association of Vitamin E Supplementation with Hemoglobin Levels in Mildly Anemic Healthy Pakistani Adults

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Received: January 27, 2015; Accepted: May 5, 2015

Abstract: *Background:* Hemoglobin levels slightly below the lower limit of normal are common in adults in the general population in developing countries. A few human studies have suggested the use of antioxidant vitamins in the correction of mild anemia. The objective of the present study was to investigate the association of vitamin E supplementation in mildly anemic healthy adults with post-supplemental blood hemoglobin levels in the general population of Karachi, Pakistan. *Method:* In a single-blinded and placebo-controlled randomized trial, 124 mildly anemic subjects from the General Practitioners' Clinics and personnel of the Aga Khan University were randomized into intervention (n = 82) and control (n = 42) group. In the intervention group, each subject was given vitamin E (400 mg) everyday for a period of three months, while control group subjects received a placebo. Eighty six subjects completed the trial. Fasting venous blood was collected at baseline and after three months of supplementation. Hemoglobin levels and serum/plasma concentrations of vitamin E, vitamin B12, folate, ferritin, serum transferrin receptor (sTfR), glucose, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, creatinine, total-antioxidant-status and erythropoietin were measured and analyzed using repeated measures ANOVA and multiple linear regression. *Results:* The adjusted regression coefficients (β) and standard error [SE(β)] of the significant predictors of post-supplemental hemoglobin levels were serum concentration of vitamin E (0.983[0.095]), gender (–0.656[0.244]), sTfR (–0.06[0.02]) and baseline hemoglobin levels (0.768[0.077]). *Conclusion:* The study showed a positive association between vitamin E supplementation and enhanced hemoglobin levels in mildly anemic adults.

Key words: anemia, haemoglobin, Pakistani population, randomized trial, vitamin E supplementation

Introduction

Anemia is one of the major health problems of the developing countries of the world [1]. According to the WHO reference criteria, an adult is labeled as anemic if their blood hemoglobin concentration falls below 13.0 g/dL in adult men, or to less than 12.0 g/dL in non-pregnant women [2]. Hemoglobin concentrations below the lower limit of normal are a common laboratory finding in apparently healthy people in general populations all over the world [3–5]. Many of these mildly anemic individuals are not investigated sufficiently to establish the probable cause of their anemia and thus may end up with morbidity and health problems, especially young women in developing countries [4].

Some studies in humans over the past few years have suggested the possible use of antioxidant vitamins in the correction of anemia [6]. Vitamin E is an essential lipophilic vitamin with a variety of antioxidant and non-antioxidant functions [7]. It has been shown that treatment with vitamin E results in an increased number of colony forming units of erythroid precursors in experimental animals and protects their bone marrow against drug-induced toxicity [8]. In some clinical trials in patients with hematological disorders, vitamin E supplementation resulted in enhanced blood hemoglobin levels due to decreased red cell fragility [9, 10]. Although the clinical benefits of vitamin E on increasing hemoglobin levels in some disease states have been published, [6, 9–11], its effect on a presumably healthy adult population with no abnormality other than mild anemia has rarely been reported. The objective of the present study was to investigate the association of vitamin E supplementation in mildly anemic healthy adults with post-supplemental blood hemoglobin levels in the general population from Karachi, Pakistan.

Subjects and Methods

Ethics statement

The study received approval by the Ethics Review Committee of the Aga Khan University, dated July 31, 2008 [RE: 1009-BBS/ERC-08] and prior written informed consent was obtained from all the participants included in the study. The authors confirm that all ongoing and related trials for this drug/intervention are registered. There was a delay in registration due to uncertainty about whether clinical trials that began in 2008 would require registration.

Trial design

A single-blinded, placebo-controlled, randomized intervention trial was carried out on human volunteers using parallel assignment to investigate the effectiveness of vitamin E supplementation on hemoglobin levels. The allocation ratio between the intervention and control arms was 2:1.

Important changes to method after trial commencement

At the start of the trial, WHO criteria for mild anemia (hemoglobin: 11.6–13.0 g/dL in males and 9.1–11.0 g/dL in females) was used [2], however, we were informed by the hematologist that Aga Khan University Hospital (AKUH) had adopted the following criteria for mild anemia in the Pakistani population (hemoglobin: 10.0–13.9 g/dL for males and 8.4–11.2 g/dL for non-pregnant females). These criteria were therefore used after of the first two months of recruitment. During this initial two month period, only four subjects were found to be mildly anemic according to the WHO criteria, but had not yet been subject to intervention. They were included in the trial for intervention along with others who had been identified as mildly anemic following the AKUH criteria.

Study participants

Eligibility Criteria: All the subjects included in the trial were mildly anemic (on the basis of screening) Pakistani adult males, and non-pregnant and non-lactating females who had given written informed consent. They were normolipidemic, normotensive and had no history of diabetes, hyperlipidemia, obesity, cancer, cardiovascular disease, sexually transmitted disease or any major illness due to respiratory, urogenital, hepatic, musculoskeletal, endocrine or psychiatric problems during the last five years. They also had not experienced any acute or chronic blood loss during the last six months; did not smoke cigarettes, chew tobacco or use alcohol, and had not taken vitamin E, vitamin B_{12}, folate, iron or antioxidants supplements in the six months prior to enrollment.

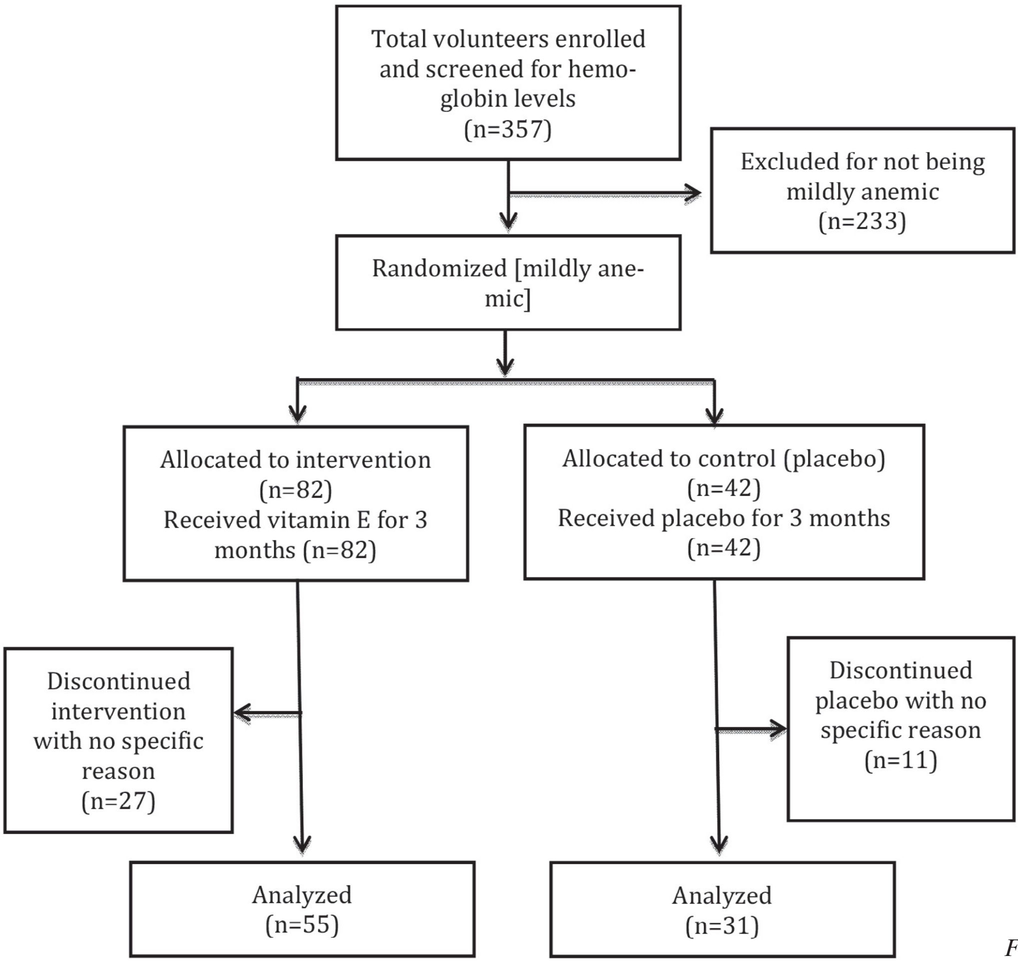

Figure 1: Trial profile.

Setting and Locations: Three hundred and fifty seven healthy volunteers (235 males and 122 non-pregnant females of age 18–45 years) were recruited and enrolled with written informed consent, through general practitioner (GP) clinics from various localities in Karachi and from the personnel of the Aga Khan University, Karachi. Recruitment started in November 2008 and the trial completed in October 2011. The clinical examination of study subjects was carried out by a general physician. The criteria defining the participants as healthy is described above. They were screened for the presence of mild anemia (hemoglobin: 10.0–13.9 g/dL, in males and 8.4–11.2 g/dL, in non-pregnant females) using the criteria adopted at AKUH for Pakistani population.

Interventions

After screening, 124 subjects (80 males and 44 females) who were found to be mildly anemic and fulfilled the criteria were included in the intervention trial. After randomization, 82 subjects were placed in the intervention (Vitamin E) group and 42 in the control (Placebo) group (Figure 1).

Intervention (Vitamin E) Group: Each subject was provided with 400 mg oral capsules of vitamin E (Evion, containing dl-alpha tocopheryl acetate, manufactured by Merck (Pvt.) Ltd, Karachi, Pakistan) and was asked to take one capsule daily with breakfast continuously for three months. The rationale for selecting this dose of vitamin E was based on the evidence that four months of supplementation with this vitamin up to 727 mg/day had no adverse effect on healthy older adults [12].

Control (Placebo) Group: Each subject was provided with placebo (edible oil) capsules (manufactured by Merck (Pvt.) Ltd, Karachi, Pakistan) to be taken once daily with breakfast for three consecutive months.

The compliance of participants in both the groups was monitored by counting the number of capsules consumed after every four weeks.

Primary outcome

The change in hemoglobin levels in mildly anemic adults was determined at baseline, and after three months of intervention (vitamin E or placebo) in all participants in intervention and placebo groups. Blood samples at these two time points (baseline and end-line) were analyzed in an automated hematology analyzer (Sysmex XE 5000, Sysmex Asia Pacific PTE Ltd, Singapore) at the Clinical Laboratory of AKUH.

Blood sampling and measurement of biomarkers

Ten mL of fasting blood was collected from each participant before the start of intervention and another sample (10 mL) was obtained after three months of treatment. Each blood sample was divided into two equal parts; one part of blood was transferred to a tube containing EDTA, while the other part was transferred to a plain tube for obtaining serum. Blood samples were analyzed for blood hemoglobin levels in an automated hematology analyzer as mentioned above. Serum/plasma samples were kept frozen at –70°C for the analysis of other biomarkers. The serum concentration of vitamin B_{12} was determined using a radio assay [13], and plasma/serum levels of folate, ferritin, soluble transferrin receptor (sTfR), total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, creatinine and glucose were measured using kits obtained from Roche Diagnostics, USA. Serum concentrations of total antioxidant status (TAS) and erythropoietin were determined using kits obtained from RANDOX Laboratories Ltd, UK and R & D Systems, USA, respectively. Kit numbers for these biomarkers are shown in Table I. Serum levels of vitamin E (α-tocopherol) were determined at the Merck's Laboratories (Pvt.) Ltd, Karachi using a modification of the HPLC method [14]. Briefly, vitamin E was extracted from the serum by mixing 0.2 mL of serum with 0.2 mL of absolute ethanol and 1.0 mL of n-hexane. After gentle mixing for 30 seconds, the contents were subjected to centrifugation at 1500 *g* at 4 °C. The upper n-hexane layer containing vitamin E was transferred to a brown microfuge tube and dried with the help of nitrogen gas. At the time of analysis, the sample was reconstituted in HPLC grade methanol and analyzed on a HPLC [VWR Hitachi, Darmstadt, Germany] using a C18 Chromlith column (100 × 4.6 cm) and UV-visible photo diode array detector (L-2420) at 280 nm. The minimum detectable amount of vitamin E using this procedure was 15 ng per injection (20 μL). The minimum concentrations of detection for serum/plasma vitamin B_{12}, folate, ferritin, sTfR, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, creatinine, glucose, TAS and erythropoietin were 50 pg/mL, 0.64 ng/mL 0.5 ng/mL, 0.068 μg/mL, 9.7 mg/dL, 8.9 mg/dL, 3.9 mg/dL, 3.0 mg/dL, 0.2 mg/dL, 2 mg/dL, 1.3 mmol/L and 2.5 mIU/mL, respectively.

Table I: Biomarkers, kit numbers and the manufacturers.

Biomarker	Kit number	Manufacturer
Folate	04476433190	Roche Diagnostics, USA
Ferritin	03737551190	Roche Diagnostics, USA
sTfR*	12148315122	Roche Diagnostics, USA
Total Cholesterol	04718417190	Roche Diagnostics, USA
LDL-Cholesterol	05401682190	Roche Diagnostics, USA
HDL-Cholesterol	05401488190	Roche Diagnostics, USA
Triglycerides	04657594190	Roche Diagnostics, USA
Creatinine	05401755190	Roche Diagnostics, USA
Glucose	04657527190	Roche Diagnostics, USA
Total antioxidant status	NX2332	RANDOX Laboratory, UK
Erythropoietin	DEPOO	R&D Systems, Minneapolis, USA

*sTfR: Serum transferrin receptor

Change in trial outcomes

Since TAS and body iron status can modulate the effect of vitamin E on post-supplemental levels of hemoglobin, these parameters were included after the commencement of the trial.

Sample size

A total of 117 mildly anemic healthy individuals (78 in the intervention and 39 in the control groups) were acquired by assuming an increase of 20 % in the mean hemoglobin levels after intervention and variability in the hemoglobin levels of 30 % with 95 % confidence level; 80 % power; 30 % loss to follow up and ratio of intervention and control groups of 2:1. A total of 357 healthy subjects were screened for identification of the required number of mild anemia subjects (Figure 1).

Randomization and blinding

A simple randomization technique was used to allocate subjects to different groups. Randomization was undertaken with the help of a statistician, and vitamin E or placebo capsules were packed in envelopes with the help of a pharmacist, and the list was provided to the principal investigator.

Envelopes containing capsules (vitamin E or placebo) were numbered according to randomization. The sealed envelopes containing capsules of vitamin E or the placebo were provided to participants by the Principal Investigator at the time of intervention. Participants did not know whether they received vitamin E capsules or placebo capsules. They were simply provided with sealed envelopes. The placebo capsules were identical to the vitamin E capsules in size, shape, color, appearance and taste.

Duration of subject recruitment, intervention and follow-up

November 4, 2008 until November 7, 2011.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences® (SPSS) software version 19 for Windows® (Apache Software Foundation, USA). Continuous variables such as age, BMI, vitamin E, hemoglobin, folate, vitamin B_{12}, ferritin, sTfR, sTfR/ferritin ratio, erythropoietin, TAS, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, creatinine and glucose were expressed as mean ± SD. Overall and gender-specific differences in mean ages and BMIs between intervention and control groups were assessed using independent samples t-tests. Pre- and post-intervention values were compared via repeated measures ANOVA within subjects, between subjects and in their interaction. Multiple linear regression analysis was used to determine the effect of various parameters (such as those for the vitamin E supplemented group, gender, ferritin, sTfR, HDL-cholesterol and hemoglobin) on hemoglobin levels post-supplementation with vitamin E. The forward selection method was employed for the inclusion of significant parameters in the linear regression model. The Kolmogorov-Smirnov test of normality was used to assess the distribution of post-supplemental hemoglobin levels and later for the error terms of the final model. $P < 0.05$ was considered significant.

Table II: Baseline demographic characteristics of adults with mild anemia

Variable	Control Group (Placebo)	Intervention Group (Vitamin E)
	n (%)	n (%)
Gender		
Males	26 (83.9)	42 (76.4)
Females	5 (16.1)	13 (23.6)
Total	31 (100)	55 (100)
	Mean ± SD*	Mean ± SD*
Age (years)		
Males	31.31 ± 6.22	32.36 ± 7.97
Females	28.40 ± 2.3	36.31 ± 6.66**
Overall	30.84 ± 5.84	33.29 ± 7.81
BMI (kg/m^2)		
Males	27.80 ± 1.41	28.32 ± 1.86
Females	28.10 ± 2.72	27.96 ± 3.20
Overall	27.90 ± 1.63	28.23 ± 2.22

*Mean values of the two groups were compared using Independent samples t-test
**p = 0.02

Table III: Concentrations of biomarkers in serum/blood of adults with mild anemia before and after supplementation with vitamin E.

Biomarker	Control Group (n = 31)		Intervention Group (n = 55)		P-value*		
	Baseline	Endline	Before supplementation	After supplementation	Time[1]	Group[2]	Time x Group[3]
Vit E (μg/mL)	13.1 ± 5.5	12.7 ± 6.7	11.7 ± 5.9	15.3 ± 8.1	0.045	0.643	0.01
Hemoglobin (g/dL)	12.78 ± 1.32	12.38 ± 1.4	12.49 ± 1.43	13.09 ± 1.49	0.049	0.516	<0.001
Folate (ng/mL)	5.30 ± 3.15	5.88 ± 3.32	6.1 ± 3.48	8.37 ± 11.1	0.183	0.026	0.429
Vitamin B_{12} (pg/mL)	303 ± 133	284 ± 112	269 ± 140	310 ± 129	0.330	0.870	0.011
Ferritin (ng/mL)	51 ± 54	56 ± 59	48 ± 49	55 ± 56	0.396	0.612	0.747
sTfR[4] (μg/mL)	5.5 ± 3.8	4.9 ± 3.6	5.6 ± 4.9	4.8 ± 2.7	0.066	0.980	0.799
sTfR/Ferritin	450 ± 610	534 ± 960	1162 ± 5417	635 ± 1848	0.512	0.537	0.367
Erythropoietin (mIU/mL)	10.72 ± 7.13	12.54 ± 14.55	14.8 ± 13.6	13.7 ± 10.1	0.902	0.263	0.129
TAS[5] (mmol/L)	0.787 ± 0.32	1.02 ± 0.46	0.947 ± 0.32	0.99 ± 0.37	0.001	0.344	0.033
Cholesterol (mg/dL)	144 ± 37	144 ± 36	150 ± 40	146 ± 35	0.501	0.61	0.457
Triglycerides (mg/dL)	111 ± 68	104 ± 48	139 ± 86	125 ± 68	0.169	0.093	0.608
HDL-cholesterol (mg/dL)	41.2 ± 12.3	37.7 ± 11.2	35.9 ± 9.7	33.1 ± 8.5	0.04	0.013	0.683
LDL-cholesterol (mg/dL)	90 ± 34	92 ± 33	95 ± 33	90 ± 31	0.523	0.785	0.151
Creatinine (mg/dL)	0.7 ± 0.17	0.78 ± 0.15	0.63 ± 0.22	0.79 ± 0.2	<0.001	0.326	0.128
Glucose (mg/dL)	85 ± 10	83 ± 12.4	96 ± 30	95 ± 39.9	0.568	0.025	0.300

[1]Time: Within subject change in both intervention subjects and controls
[2]Group: Between subjects of intervention group and control group
[3]Time x Group: Interaction between time and group
[4]sTfR: Serum transferrin receptor
[5]TAS: Total antioxidant status
*P-value compares the pre-and post-supplementation values by repeated measures ANOVA for within subjects, between subjects and their interaction. A p-value < 0.05 was considered significant

Results

Of 124 mildly anemic adults who were recruited in the intervention trial, 86 completed it, while 38 adults discontinued the use of vitamin E or placebo for no specific reasons (Figure 1). Baseline demographic characteristics of these adults are shown in Table II. Fifty five belonged to the intervention group (vitamin E supplementation), while 31 were control (placebo) subjects. These two groups of participants were subjected to analysis. The proportion of males compared to females was more than threefold in both groups. Except for the age of females, no significant difference was observed in the mean age and mean BMI values between the intervention and control groups. The mean age and mean BMI value of the 38 subjects who did not complete the trial were not significantly different from the mean values of these parameters in those who completed the trial, indicating the absence of any bias from the dropout ($p > 0.05$).

Concentrations of biomarkers in these anemic adults before and after vitamin E supplementation were compared using repeated measures ANOVA for change within subjects, between subjects belonging to the intervention and control groups at different time points (pre-post) and between the intervention and control groups (Table III). There was a significant increase in the concentrations of both vitamin E and hemoglobin after supplementation ($p = 0.045$ and $p = 0.049$, respectively). A significant increase in mean hemoglobin level post-supplementation was observed in intervention subjects; although mean hemoglobin level at baseline was lower compared to controls ($p < 0.001$). Similarly, mean vitamin E concentration at baseline was lower in intervention subjects compared to controls, however, it increased significantly after supplementation ($p = 0.01$).

We also analyzed the effect of gender on vitamin E and hemoglobin levels in both intervention subjects and controls at baseline and post-supplementation. A significant difference was found in mean hemoglobin levels between intervention subjects and controls at these two time points when adjusted for gender ($p = 0.008$), however, no significant difference in mean concentrations of vitamin E in intervention subjects and controls was observed at these time points when adjusted for gender ($p = 0.717$). A weak positive correlation was observed between changes in vitamin E concentration and changes in hemoglobin levels in both intervention subjects and controls [Pearson's correlation, "r" = 0.195; $p = 0.07$].

Table IV shows the effect of different factors, particularly vitamin E supplementation, on hemoglobin levels post-supplementation using multiple linear regression. Compared to the control group, the average hemoglobin level in the vitamin E supplemented group was 0.983 unit higher when adjusted for gender, serum concentrations of erythropoietin and sTfR and baseline hemoglobin levels. Compared to males, the hemoglobin level in females (post supplementation) was 0.656 gm/dL lower (on average) when adjusted for serum concentrations of vitamin E, erythropoietin and sTfR and baseline hemoglobin levels.

Similarly, with a one unit decrease in serum concentration of sTfR, there was an 0.06 unit average increase in hemoglobin level when adjusted for gender, serum concentrations of vitamin E and erythropoietin and baseline levels of hemoglobin. There was a 0.768 unit average increase in hemoglobin level post-supplementation relative to a one unit increase in hemoglobin level at baseline after adjusting for gender, serum concentrations of vitamin E, erythropoietin and sTfR. The regression model explains 92.3 % variation in hemoglobin levels post-supplementation due to vitamin E status, gender, serum concentrations of erythropoietin and sTfR and baseline hemoglobin levels. Serum levels of erythropoietin and sTfR were included in the model because of their potential role in change in hemoglobin levels.

Table IV: Effect of vitamin E supplementation on hemoglobin level using multiple linear regression.

Variable	Crude β[SE(β)]*	Adjusted[1] β[SE(β)]*
Vitamin E supplemented	0.702[0.263]	0.983[0.095]
Placebo (Ref)**		
Gender		
Female	−2.922[0.239]	−0.656[0.244]
Male (Ref)**		
Erythropoietin	−0.04[0.013]	−0.004[0.005]
sTfR[2]	−0.235[0.048]	−0.060[0.020]
Hb level (at baseline)	0.965[0.051]	0.768[0.077]
Constant		3.022[1.071]
R^2		0.923

[1]The model was adjusted for vitamin E levels post-supplementation, gender, serum concentrations of erythropoietin and sTfR and baseline hemoglobin levels.
[2]sTfR: Serum transferrin receptor
*β[SE(β)] refers to regression coefficient with its standard error
**Ref. indicates the reference group category in regression

Discussion

Vitamin E deficiency is considered rare in most populations because this vitamin is abundantly present in vegetable oils, unprocessed cereal grains and nuts and also in moderate amounts in fruits, vegetables and meats [15]. No epidemiological data is available for the prevalence of the deficiency of vitamin E in the Pakistani population, however, it is conjectured that an insufficiency of vitamin E would not be uncommon in Pakistani people because of the cooking methods used in this part of the world. Several food items rich in vitamin E, when subjected to high temperature in the presence of air and fat, are likely to have decreased contents of this vitamin [16]. In South Asia including Pakistan, foods are generally cooked at high temperatures thereby destroying several essential vitamins including vitamin E. There could also be inadequate consumption of vitamin E-rich food by some of the people belonging to the lower socio-economic strata. We therefore embarked on investigating the role of vitamin E in correcting mild anemia in Pakistani adults.

While there have been reports about the association of a deficiency of fat soluble vitamins such as vitamin A and vitamin D with anemia [17–19], the relationship between an increased serum concentration of vitamin E and improved hemoglobin levels in mildly anemic adults is a unique finding of this study.

Mild anemia, which is quite common in developing countries such as Pakistan, is generally a neglected condition and often remains untreated in GP clinics because of an absence of apparent symptoms. Vitamin E supplementation could offer a convenient mode of treatment in such cases.

In a previous communication, we reported that vitamin E supplementation probably enhanced hemoglobin in mildly anemic adults through increased levels of erythropoietin [20], but that pilot study was based on only 30 intervention subjects. Our present study, involving 55 intervention subjects (who completed the intervention trial), not only confirmed our previous findings but also provided evidence that a host of factors such as gender, sTfR and baseline Hb levels, in addition to vitamin E, are responsible for increased levels of hemoglobin. No significant effect of erythropoietin was found on increasing the levels of hemoglobin, however. This is suggestive that vitamin E supplementation has no influence on the induction of erythropoietin.

The cut-off values used for mild anemia in this study merit some discussion. According to WHO reference criteria, an adult is classified as anemic if their blood hemoglobin level falls below 13.0 g/dL in men or 12.0 g/dL in non-pregnant women [2], however, it has been reported that the normal range for blood hemoglobin levels may vary in healthy individuals depending upon the age, gender, race, geographical location and food habits of the study population [21]. Based on this report, the criteria developed by the AKUH, for a mildly anemic Pakistani adult population is 10.0–13.9 g/dL for males and 8.4–11.2 g/dL for non-pregnant females. We used these criteria for screening apparently healthy adults for mild anemia for this intervention trial.

Although we excluded adults with microcytic anemia, the presence of mild iron deficiency in some cannot be discounted. sTfR is an index of tissue iron needs and is therefore considered a better biomarker for early iron deficiency anemia [22]. Analysis of our data revealed that 8.6 % of variation in hemoglobin levels post-supplementation with vitamin E was because of a change in sTfR concentration. This is suggestive that vitamin E in some manner improved the body iron status of these adults. Vitamin E is a synergistic vitamin to iron absorption. A deficiency of vitamin E, could thus lead to iron deficiency [23]. Vitamin E supplementation is therefore likely to improve its absorption and correct this deficiency. This is further supported by a study in which 300 mg of vitamin E supplementation for six weeks increased serum levels of iron in elite taekwondo athletes [24]. The precise mechanism for this increase in serum iron has not been defined, however, there is a possibility that because vitamin E is an antioxidant it might be facilitating iron absorption by keeping it in the ferrous state [25]. Alternatively, vitamin E might be conserving iron at the cellular level, thereby improving the body iron status.

Alleviation of oxidative stress by vitamin E has been suggested as a mechanism for increased erythropoiesis in uremic children on hemodialysis [26], however, in the present study, there was no significant change in the TAS of mildly anemic adults after three months of supplementation with vitamin E. Our results conform well with those reported by El-Azab et al. [27], who have shown significant improvement in indices of oxidative stress but no significant change in hemoglobin levels after 12 weeks of vitamin E supplementation in hemodialyzed patients with renal anemia. The role of vitamin E in enhancing hemoglobin levels in mildly anemic adults is therefore not likely to be through alleviation of oxidative stress.

In addition to body iron status, plasma concentrations of folate and vitamin B_{12} are among the main predictors of hemoglobin levels in South Asian populations [28–30], however, there is no evidence in the literature that the plasma levels of these vitamins undergo a change as a result of vitamin E supplementation. In the present study, vitamin E supplementation had little effect on serum levels of folate and vitamin B_{12}, therefore improved hemoglobin levels cannot be attributed to any change in the status of these vitamins. The precise mechanism by which vitamin E could increase hemoglobin levels in these mildly anemic subjects requires further investigation, especially focusing on the effect of vitamin E on erythroid progenitor cells. Some of the intervention trials using vitamin E in different hematological conditions have shown decreased rates of hemolysis leading to an improved hemoglobin concentration in these patients [9–11]. In another trial on healthy middle-aged and elderly Chinese people, four-month supplementation with vitamin E significantly reduced erythrocyte hemolysis [31]. Vitamin E as an antioxidant would therefore prevent free radical-induced lipid peroxidation in the erythrocyte membrane, thereby decreasing the hemolysis and increasing the survival of red blood cells [32]. It is plausible that in mildly anemic adults vitamin E could be increasing the stability of erythroid progenitor cells. While anemic adults in this trial have benefited from vitamin E supplementation at a dose of 400 mg/day for three months, the potential risks of high vitamin E doses should not be ignored [33]. These include increased pro-oxidative activity leading to excessive generation of reactive oxygen species, especially in the presence of copper and iron [34], decreased systemic vitamin A stores [35] and bleeding tendencies in patients on anticoagulant therapy [36]. According to the guidelines provided by the Institute of Medicine healthy individuals should not be using more than 1000 mg vitamin E per day, which is the upper limit of safety for the use of this vitamin [37].

Our results need to be viewed in the context of certain limitations of the study. Since no previous studies were available on the relationship of vitamin E concentration with hemoglobin levels, the sample size was calculated using percentage change in mean and standard error hemoglobin levels instead of absolute changes in these two parameters due to intervention. The intervention trial was completed only by 86 mildly anemic adults due to 38 drop-outs from the trial. Although we asked the study participants to continue with their routine diet, it would have been better to administer a food frequency questionnaire to ascertain their dietary habits and exclude those who were consuming a vitamin E rich diet. In spite of these limitations, the study had ample power to detect the difference between the intervention and control arms, and provided sufficient evidence of the positive role of vitamin E in improving hemoglobin levels in mild anemia.

Conclusion

In a placebo-controlled and single blinded trial, supplementation with vitamin E for three months was found to be associated with improved hemoglobin levels in mildly anemic adults. Vitamin E supplementation may offer a simple and inexpensive mode of treatment in mild anemia.

Acknowledgements

The study had been supported by a grant from the University Research Council (Code: 70206) from the Aga Khan University. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The study was registered with Clinical Trials.gov with the identifier: NCT02181348. TJ, MPI and BM conceived and designed the project, interpreted the data and wrote the paper. IA analyzed and interpreted the data, and NM contributed to analysis tools and interpreted the data.

We gratefully acknowledge Merck (Pvt.) Ltd, Pakistan for providing vitamin E (Evion) and placebo capsules and making their HPLC available for the analysis of vitamin E. In this regard, we are grateful to Mr. Abdul Baqy Khan, Dr. Ahmed Nawaz, Mr. Akhtar Hussain, Mr. Muhammad Ashraf and Mr. Tajamal Hussain for their support. We also thank Professor Amirali Gulamhusein, formerly Director of Clinical Anatomy, Department of Medical and Social Care Education, University of Leicester Medical School, Leicester, UK for copyediting the manuscript.

Conflicts of Interest

None.

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