Contemporary Clinical Trials Communications 8 (2017) 175–180

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

Comparative assessment of methylcobalamin and ascorbic acid on cognitive function in post-menopausal women - A randomized, double-blind trial

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ARTICLE INFO

Keywords:
Cognitive decline
Menopause
Dementia
β-amyloid
Neurological health

ABSTRACT

Introduction: A decline in cognitive function occurs as women progress through the menopausal transition.
Objective: The present study was designed to compare the effect of Methylcobalamin and Ascorbic Acid on Cognitive Function in post-menopausal women.
Methods: A randomized, double-blind trial was conducted in postmenopausal women with mild to moderate cognitive dysfunction. Eligible 56 subjects were randomized, the effect of ascorbic acid (500 mg OD) and methylcobalamin (50 mcg OD) was compared after 12 weeks of treatment. MMSE Questionnaire was used to assess the cognitive function, and β-amyloid42 was estimated in serum by enzyme-linked immunosorbent assay (ELISA).
Results: In MMSE score, delayed verbal recall ($P = 0.027$), naming ($P = 0.042$) and repetition ($P = 0.031$) scores were significantly improved in ascorbic acid group when compared to baseline. The β-amyloid42 level was decreased significantly in subjects receiving ascorbic acid ($P = 0.04$) when compared to Methylcobalamin group ($P = 0.31$). The inverse relationship between β-amyloid42 levels and the MMSE score was found in ascorbic acid treatment ($r = 0.6324$, $P = 0.0004$).
Conclusion: Based on MMSE and β-amyloid42 results, ascorbic acid showed improvement in cognitive function among post-menopausal women when compared to methylcobalamin supplement.

1. Introduction

Cognitive decline is a recurrent complaint during the menopause transition and among post-menopausal women [1]. Changes in memory link with reduced estrogen production. Further, many post-menopausal women report sleep concerns, depression, and hot flashes, and these factors may contribute to cognitive decline [2]. Cognitive function is defined as any mental process that involves symbolic operations - e.g., perception, memory, the ability to learn new information, speech, the creation of imagery and thinking. It incorporates awareness and capacity for judgment. If cognitive decreases, it may lead to dementia which results in Alzheimer's disease (AD) [3]. Around the time of the menopausal transition, many women report problems with memory, perhaps suggesting that hormonal changes associated with menopause are linked to memory complaints. This is a potentially worrisome symptom because the inability to learn and consciously recall new information can be a very early sign of AD or other forms of dementia [4]. Methylcobalamin (Vitamin B_{12}) is an essential cofactor in several biochemical reactions including the conversion of homocysteine (Hcy) to methionine and the synthesis of SAMe (S-adenosylmethionine). These reactions are believed to be crucial in maintaining neurological health [5]. Many studies are confirming an association between vitamin B_{12}/folate deficiency and cognitive impairment [6]. Higher plasma ascorbic acid (Vitamin C) is associated with a better cognitive function, or lower risk of cognitive impairment and plasma ascorbic acid concentration tends to be lower in AD patients [7]. Even though, studies have shown both Methylcobalamin and Ascorbic acid plays a major role in cognitive function or dementia, no research has been carried out to determine the effect of Methylcobalamin and Ascorbic acid on cognitive function in post-menopausal women comparatively. Hence, the aim of the present study is to compare the effect of Methylcobalamin and Ascorbic Acid on Cognitive Function in post-menopausal women through the determination of serum β-amyloid and Mini-Mental State Examination (MMSE).

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http://dx.doi.org/10.1016/j.conctc.2017.10.006
Received 19 June 2017; Received in revised form 14 September 2017; Accepted 13 October 2017
Available online 16 October 2017

2. Methods

2.1. Study design and settings

This 12-week, randomized, double-blind, controlled, parallel-group trial was conducted in the community settings in and around kattankulathur in association with Urban Health Training Centre (UHTC), Department of Community Medicine, SRM Medical College Hospital and Research Centre, SRM University. The institutional human ethics committee approval for the trial protocol was formally obtained from the SRM Medical College Hospital and Research Centre, SRM University Board of Ethics (Grant No: 874/IEC/2015). This trial was conducted according to the tenets laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants before study entry. The participants were free to withdraw from the study at any time without compromising their relationship with their health care provider. The trial was registered at the clinical trial Registry of India (CTRI No: REF/2016/02/010726).

2.2. Trial participants

Eligible female participants, aged 50–75 years with average education of 8 years, who had attained menopause for a period extending over 4–10 years at the time of inclusion into the study and obtained MMSE Score 21–24. Exclusion criteria were as follows: History of recent or current Estrogen or hormonal replacement therapy (HRT), MMSE Score 25–30, Known hypertensive, diabetic, renal disease, endocrine disorders, overt nutritional deficiency states, anemia, liver disease, estrogen-secreting ovarian tumors, Women, who were physically unable to hear, read or understand written or explained instructions properly, or may have a motor deficit that affects writing and drawing skills and Women, who were already in any of the antioxidant or dietary supplement therapy.

2.3. Interventions

Eligible patients randomly received either 500 mcg Methylcobalamin once daily or 50 mg Ascorbic acid once daily in the same manner for 12 weeks. Medication adherence was measured using weekly capsule counts justified against participant reports of drug intake to calculate the proportion of dispensed medication doses that were actually ingested. Any adverse effects of these oral medications were monitored and documented on an adverse effects sheet in the patient folder. CONSORT flow chart was shown in Fig. 1.

2.4. Allocation concealment

Concealment of the randomization code was done to avoid selection bias. Third party randomization is the gold standard for concealment. Computer generated randomization list was prepared by a third person who was not involved in the recruitment of patients. Each allocation was written on paper and concealed in a serially numbered, opaque envelope.

2.5. Sample size calculation

This pilot study was undertaken to address several process issues before implementation of a full scale randomized clinical trial. The pilot study is essential to plan an adequate full-scale trial because a number of critical issues need to be resolved before time and more significant funding are committed. This study was conducted in a practice-based setting and tested all of the components of a full-scale trial but without the necessary sample size.

2.6. Physical examination

A questionnaire was completed at each health centre by trained interviewers. A questionnaire achieves demographic information. Height and body weight were measured without shoes and with the study subjects wearing light clothes. Height was measured to the nearest 0.1 cm, and weight was measured to the nearest 0.1 kg. Measurements were carried out using portable calibrated electronic weighing scale and inflexible measuring bars. BMI was calculated as weight/height2 (kg/m^2). Subjects were classified into: (i) normal (ii) overweight; (iii) obese, based on a definition of obesity for the population in India. Constant tension tape was used to measure waist circumference (WC) at the midpoint between the inferior costal margin and the highest point of the hip bone across the mid-axillary line, with arms relaxed at the sides. Hip circumference (HC) was measured to the nearest 0.1 cm at the level of the trochanters. Waist to hip (WHR) was calculated as the ratios of their respective components. Blood pressure was measured using a mercury-free LCD sphygmomanometer (Diamond, BPDG 234 LCD Super Deluxe); two readings were taken at 10-min intervals after subjects had been seated for at least 10 min. The two readings were averaged. Systolic Blood pressure (SBP) $\geq$140 mmHg and Diastolic Blood Pressure (DBP) $\geq$ 90 mmHg consider as high blood pressure.

2.7. Folstein Mini-Mental State Examination (MMSE) questionnaire

The Mini-Mental State Examination (MMSE) [8] was widely taught and used among health care professionals, serving as a universal indicator of the severity of impairment among persons with cognitive impairment among persons with cognitive disorders in the various disease state. In this study, we used MMSE as a screening tool for assessing the influence of antioxidants on cognitive function in Post-menopausal women.

The MMSE is a brief, quantitative measure of cognitive status in adults. It is used to screen for cognitive impairment, to estimate the severity of cognitive impairment at a given point in time, to follow the course of cognitive changes in an individual over time, and to document and to document an individual's response to treatment. It contains 14 items and six scale levels. The total score of 6 scales gives the overall cognitive function of each patient. The score was recorded using a denominator of 30 unless the patient was unable to complete the test due to physical handicap (e.g., blindness). The MMSE begins with a graded assessment of orientation to place and time, for which 10 points is possible. This is followed by testing two aspects of memory. The first is the immediate recall for three objects presented orally, followed by a serial sevens task which is interposed to assess attention, concentration, and calculation, and also to prevent the individual from rehearsing the three objects previously learned. A maximum of 11 points may be obtained in this section of the test. The final section surveys aphasia by testing functions of naming, repetition, understanding a three-stage command, reading, writing and copying a drawing. There is a maximum of 9 points which may be obtained in this section, for a total possible MMSE score of 10 points. A score of 24–30 indicates no cognitive impairment, a score of 18–23 indicates mild cognitive impairment and score 0–17 indicates severe cognitive impairment.

2.8. Estimation of serum β-amyloid42

Blood samples of 3 ml were obtained from the subjects by venous puncture and were transferred into vacutainer, and centrifugation separated the serum for β-amyloid estimation, the process was done through centrifuge-5430 R (Eppendorf). After that the samples were aliquoted into Eppendorf tubes and stored at −20 °C until analysis. Serum β-amyloid concentrations were measured through a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) by the Microplate reader (Thermo Scientific Multiscan) using the Human Aβ42

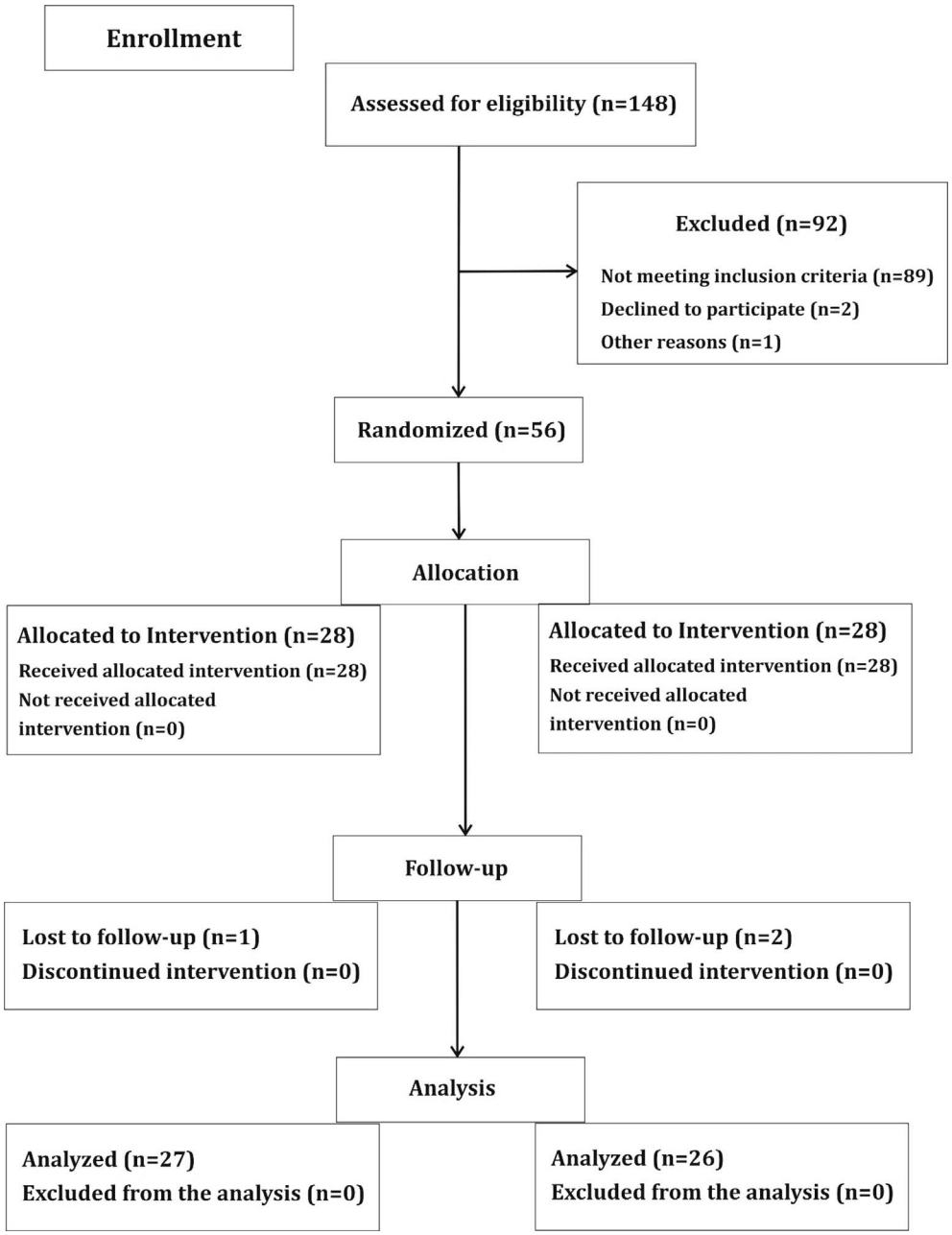

Fig. 1. CONSORT Flow chart.

Table 1
Baseline characteristics.

Variables	Methylcobalamin	Ascorbic acid
Age	57.56 ± 7.72	55.88 ± 6.01
Height	159.27 ± 11.21	150.42 ± 3.60
Weight	58.44 ± 4.64	60.91 ± 6.55
BMI	25.52 ± 4.27	27.70 ± 4.69
Systolic BP	119.96 ± 7.24	115.26 ± 6.63
Diastolic BP	82.96 ± 7.24	86.24 ± 4.36
Waist Circumference	38.17 ± 3.08	38.43 ± 3.67
Hip Circumference	40.78 ± 2.54	41.02 ± 3.37
Waist Hip Ratio	0.93 ± 0.049	0.93 ± 0.06
MMSE Total Score	19.13 ± 4.36	20.64 ± 2.03

Values are expressed in Mean ± Standard deviation. $P < 0.05$* Significant, Adjusted SBP.

kit (Catalog number: ab120301).

2.9. Statistical analysis

Two-sample t-tests were used to examine the association between treatment and the follow-up MMSE score or change in MMSE score from baseline to follow-up. All hypotheses tests were two-sided and p-values less than 0.05 were considered statistically significant. Two sample *t*-test was performed to compare the change in serum β-amyloid42 level between two treatment arms. The Pearson's correlation coefficients were calculated as well as tested for significance of the linear relationship between MMSE and serum β-amyloid42 levels. All the Statistical analysis was performed using GraphPad Prism software (Version 6.01.

3. Results

Demographic characteristics of Methylcobalamin and an Ascorbic

Table 2
Effect of Methylcobalamin and Ascorbic acid on patients with mild and moderate cognitive decline using MMSE.

MMSE Parameters	Methylcobalamin		Ascorbic Acid	
	Baseline	After Treatment	Baseline	After Treatment
Orientation to Time	4.148 ± 1.064	3.48 ± 1.15	4.29 ± 0.75	4.20 ± 0.83
Orientation to Place	3.48 ± 1.014	3.81 ± 1.11	3.66 ± 0.70	3.87 ± 0.90
Immediate Recall	2.74 ± 0.66	1.66 ± 1.11*	3.00 ± 0.29	1.91 ± 1.21*
Attention	1.85 ± 1.32	1.81 ± 0.76	1.62 ± 0.77	1.72 ± 0.75
Delayed Verbal Recall	0.85 ± 0.98	1.02 ± 1.17	0.97 ± 0.64	1.81 ± 1.10*
Naming	2.00 ± 0.00	0.55 ± 0.50	2.04 ± 0.20	2.00 ± 0.00*
Repetition	0.51 ± 0.50	2.00 ± 0.00	0.58 ± 0.58	1.50 ± 0.51*
3 Stage Command	2.25 ± 0.65	2.03 ± 0.51	2.37 ± 0.49	2.08 ± 0.58
Reading	0.59 ± 0.50	0.59 ± 0.50	0.70 ± 0.46	0.62 ± 0.49
Writing	0.66 ± 0.48	0.55 ± 0.50	0.54 ± 0.50	0.62 ± 0.49
Copying	0.11 ± 0.32	0.44 ± 0.50	0.08 ± 0.28	0.29 ± 0.46
Total	19.13 ± 4.36	18.77 ± 2.47	20.64 ± 2.03	19.43 ± 2.26

Values are expressed in Mean ± Standard deviation. $P < 0.05$* Significant

acid group was shown in Table 1. In each group 28 participants were enrolled according to inclusion and exclusion criteria in a randomized, double blind fashion. All the baseline characteristics of the study population were similar between groups, and there was no statistically significant difference except Systolic BP ($P = 0.041$). In the present study, the repeated measure analyses of mean and standard deviation of MMSE variables revealed the significant difference between two groups: Methylcobalamin and Ascorbic acid has shown improvement in immediate recall $P = 0.038$ and $P = 0.035$ respectively from baseline. Delayed verbal recall ($P = 0.027$), naming ($P = 0.042$) and repetition ($P = 0.031$) scores were significantly improved in an Ascorbic acid group when compared to baseline (Table 2).

Influence of Methylcobalamin and Ascorbic acid on cognitive function was determined by using serum β-amyloid levels in post-menopausal women. The serum β-amyloid42 level was decreased significantly in subjects receiving Ascorbic acid ($P = 0.042$) when compared to Methylcobalamin group ($P = 0.310$) (see Table 3). Effect of Methylcobalamin and Ascorbic acid after 12 weeks of treatment on cognitive function in post-menopausal women was compared using MMSE Scores (Fig. 2). From the MMSE components, orientation to time and delayed verbal recall was significantly improved in ascorbic acid treatment ($P < 0.05$) when compared to Methylcobalamin.

Pearson's correlation coefficient was used to correlate the Serum β-amyloid42 with MMSE Score after 12 weeks treatment of Methylcobalamin and Ascorbic acid (Figs. 3 & 4). We found that there was no correlation between serum β-amyloid 42 and MMSE Score in Methylcobalamin treated group ($r = 0.037$, $P = 0.852$) but in ascorbic acid treatment, a significant reduction in the serum β-amyloid 42 levels when improvement in the MMSE score ($r = 0.6324$, $P = 0.0004$) (inverse correlation between serum β-amyloid42 and MMSE score).

4. Discussion

The present study was designed to compare the effect of Methylcobalamin and ascorbic acid on cognitive function in post-menopausal women. An assessment of cognitive function was conducted using MMSE tests and serum β-amyloid42 levels. The MMSE is a modified screening tool used to evaluate cognitive deficits and assessments are simple and can be applied in primary health care with low cost [9,10].

Although the mechanisms for the relationship between cognitive function and β-amyloid are not entirely clear, the current study demonstrates an inverse relationship between serum β-amyloid42 levels and MMSE score in ascorbic acid treatment, but in a case of methylcobalamin group, there was no correlation. Vitamin C is thought to be the most effective antioxidant in plasma, in part due to its water solubility and to the wide range of reactive oxygen species (ROS) that it can scavenge. If found to be protective against age-related and neurological diseases, vitamin C supplements would provide an intervention of low cost and toxicity [11].

The current study did not detect any benefits of methylcobalamin supplementation over 12 weeks on cognitive function in post-menopausal women except immediate recall from MMSE Score. In ascorbic acid treatment, 32.14% of subjects were shown improvement in MMSE score from baseline, but in case of methylcobalamin, it was 21.42%. Van der Zwaluw et al., 2014 [12] reported that two-year folic acid and vitamin B12 supplementation did not beneficially affect performance on four cognitive domains in older adults with elevated homocysteine levels. It may slightly slow the rate of decline of global cognition, but the reported small difference may be attributable to chance. Our present trial contributes robust evidence on the effect of ascorbic acid on cognitive function by improving MMSE components such as immediate recall, delayed verbal recall, naming, and repetition. Riviere et al., 1998 [13] demonstrated that even in the healthy elderly, a higher level of plasma ascorbic acid is associated with better cognitive functioning and La Rue et al., 1997 [14] have also demonstrated a positive association between vitamin C intakes and cognitive performance in healthy elderly subjects. Also, from our study, 12 weeks treatment of ascorbic acid decreases the serum β-amyloid42 levels. Lim et al., 2005 [15] reported that, dehydroascorbic acid modulates Aβ precursor protein (APP) processing by reducing C-terminal fragment products of both α-amyloid protein and APP and full-length APP, and that adequate dietary dehydroascorbic acid could be protective against Aβ accumulation.

No significant differences were observed between ascorbic acid and methylcobalamin groups in a number of reported adverse events, and no subjects were removed from the study for a treatment-related adverse event.

Some limitations in the present study design need to be considered in the interpretation of the findings. First, we were unable to recruit a larger number of subjects with menopause and cognitive impairment group due to the unwillingness of many of the patients. Second, the MMSE test used to assess the cognition of post-menopausal women was

Table 3
Effect of Methylcobalamin and Ascorbic acid on patients with mild and moderate cognitive decline using serum β-amyloid levels.

Treatment	Serum β-amyloid42		*P Value*
	Baseline	After Treatment	
Methylcobalamin	43.17 ± 2.31	43.90 ± 2.57	0.31
Ascorbic acid	45.33 ± 4.91	38.18 ± 4.20	0.04*

Values are expressed in Mean ± Standard deviation. $P < 0.05$* Significant

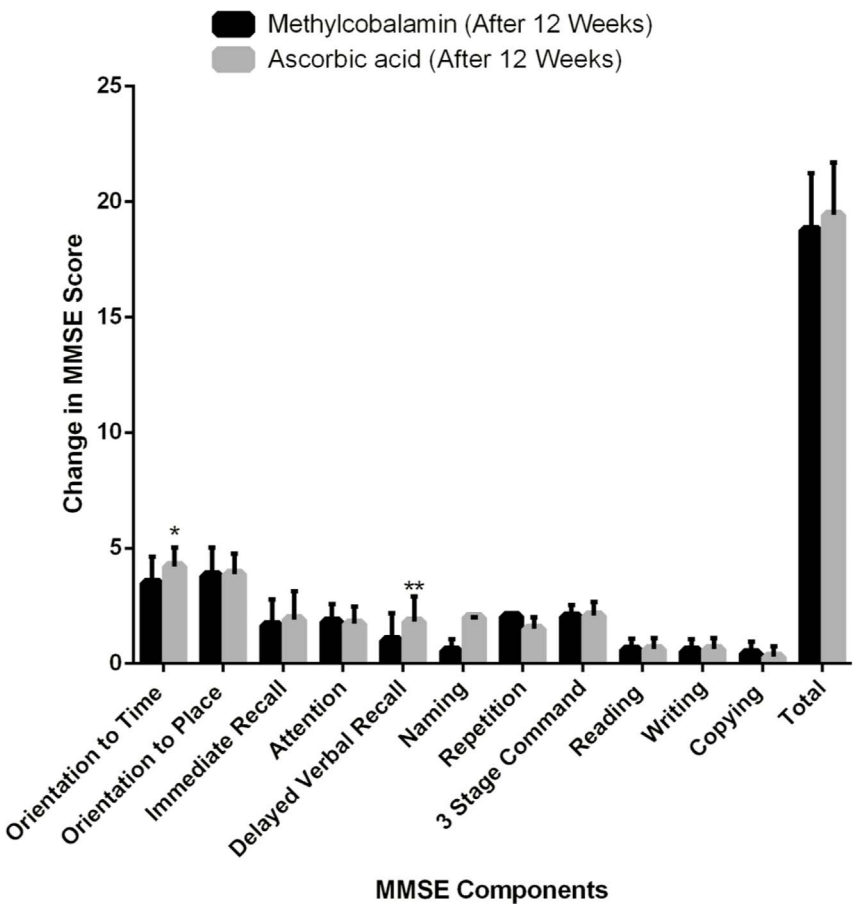

Fig. 2. Comparative effect of Methylcobalamin and Ascorbic acid on cognitive function using MMSE Scores in Post-menopausal women (Level of significance $*P < 0.05$, $**P < 0.01$).

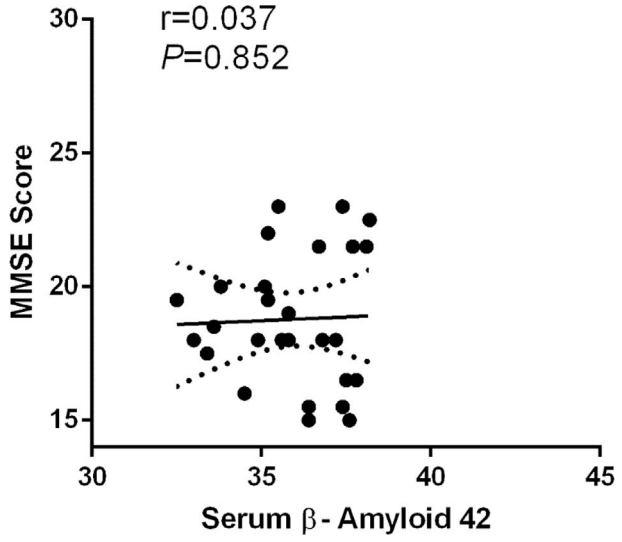

Fig. 3. Correlation of Serum β-amyloid 42 with MMSE Score after 12 weeks treatment of Methylcobalamin.

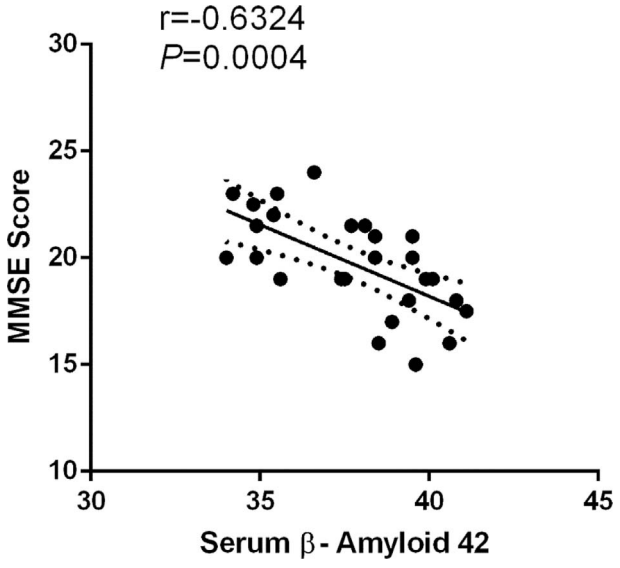

Fig. 4. Correlation of Serum β-amyloid 42 with MMSE Score after 12 weeks treatment of Ascorbic acid.

in the English version, and as a result, the generalizability of our conversion scores was limited.

5. Conclusion

In Summary, the present study provides evidence that ascorbic acid showed improvement in cognitive function among post-menopausal women when compared to methylcobalamin supplement. It is our hope that this preliminary data will be utilized to design large-scale longer-term trials with similar objectives in post-menopausal women.

Conflict of interest

The authors declare that none of them has any conflict of interests.

Acknowledgements

This study was funded by SRM College of Pharmacy, SRM University. The authors would like to thank Prof. K.S.Lakshmi, Dean, College of Pharmacy, SRM University for providing facilities. The authors would also like to thank all subjects, nurses, doctors, and research assistants who have contributed to this research.

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