Association of Vitamin B12 and Folate Deficiency with Vasovagal Syncope: A Case-Control Study

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Abstract

Aims: There is some clinical evidence of the association between vitamin B12 deficiency and vasovagal syncope (VVS) in pediatric patients. We aimed to investigate the possible association of vitamin B12 and folate deficiency with VVS in adults. **Methods:** In this case-control study, we included adult patients with VVS who presented to our tertiary syncope unit for a head-up tilt table test as the case group. We selected age- and sex-matched individuals without any history of syncope from the population-based Tehran Cohort Study as the control group. The exclusion criteria included but were not restricted to taking vitamin B supplements, carbamazepine, or phenobarbital, and sleeve gastrectomy. We measured and compared serum levels of vitamin B12, folate, and homocysteine. **Results:** From February 2020 to February 2021, we included 44 patients in the case group, matched with 44 controls, with a mean age of 37.9 years and 23 (52.3%) females in each group. There was no statistically significant difference between the study groups regarding vitamin B12 or folate deficiency, or their serum levels. The serum level of vitamin B12 was remarkably lower in patients with frequent VVS ([?]3 lifetime episodes) compared to patients with infrequent VVS (<3 lifetime episodes) [233.8 (80.7) versus 305.2 (118.1) pg/mL; P=0.042] and this association remained significant after adjustment for possible confounders (P=0.026). **Conclusion:** We found no association between vitamin B12 or folate deficiency, or their serum levels and VVS; nevertheless, frequent VVS, compared to infrequent VVS, was associated with a lower serum vitamin B12.

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ABSTRACT

Aims: There is some clinical evidence of the association between vitamin B12 deficiency and vasovagal syncope (VVS) in pediatric patients. We aimed to investigate the possible association of vitamin B12 and folate deficiency with VVS in adults.

Methods: In this case-control study, we included adult patients with VVS who presented to our tertiary syncope unit for a head-up tilt table test as the case group. We selected age- and sex-matched individuals without any history of syncope from the population-based Tehran Cohort Study as the control group. The exclusion criteria included but were not restricted to taking vitamin B supplements, carbamazepine, or phenobarbital, and sleeve gastrectomy. We measured and compared serum levels of vitamin B12, folate, and homocysteine.

Results: From February 2020 to February 2021, we included 44 patients in the case group, matched with 44 controls, with a mean age of 37.9 years and 23 (52.3%) females in each group. There was no statistically significant difference between the study groups regarding vitamin B12 or folate deficiency, or their serum levels. The serum level of vitamin B12 was remarkably lower in patients with frequent VVS ([?]3 lifetime episodes) compared to patients with infrequent VVS (<3 lifetime episodes) [233.8 (80.7) versus 305.2 (118.1) pg/mL; P=0.042] and this association remained significant after adjustment for possible confounders (P=0.026).

Conclusion: We found no association between vitamin B12 or folate deficiency, or their serum levels and VVS; nevertheless, frequent VVS, compared to infrequent VVS, was associated with a lower serum vitamin B12.

Keywords: Syncope, Vasovagal; Folic Acid; Folic Acid Deficiency; Vitamin B 12; Vitamin B 12 Deficiency; Homocysteine

INTRODUCTION

Vasovagal syncope (VVS) is a common and potentially debilitating condition with limited treatment strategies, particularly for frequent VVS.[1, 2] VVS, the most common type of syncope,[1, 2] accounts for 0.8-3.0% of emergency room visits and 1% of hospital admissions.[3-6] VVS can be a life disturbing condition,[7] and the quality of life of patients with recurrent VVS can be as impaired as the quality of life of patients with chronic diseases like rheumatoid arthritis[8, 9] and chronic low back pain[9]. Furthermore, 33% of patients with VVS incur injuries due to their episodes, with more fragility among older patients.[10] In addition to the lost productivity and other indirect costs, VVS directly imposes 2.4 billion dollars on the United States

health system for hospitalization of patients with VVS annually.[8, 11] Despite this enormous psychosocial and financial burden of VVS, there are scarce treatment options, especially for patients with frequent VVS.

The current guidelines suggest that improving our knowledge about the pathophysiology of VVS is critical in developing novel preventive and therapeutic strategies to reduce its burden.[1, 2]Therefore, it is encouraged to investigate the possible mechanisms as the basis of possible future interventions for treating VVS or at least a subgroup of these patients.[12, 13] Increased serum levels of catecholamines might play a role in the pathophysiology of VVS,[14-19] and vitamin B12 deficiency may cause increased catecholamines through biochemically plausible mechanisms.[20, 21] Moreover, there is some evidence on the association of vitamin B12 deficiency and VVS in pediatric patients;[22, 23] however, no methodologically rigorous study has ever investigated this possible association in adult or elderly patients with VVS.[24] Furthermore, concurrent evaluation of vitamin B12 and folate, as two vitamins with interwoven biochemical pathways, is encouraged.[22]

In this case-control study, we aimed to investigate the possible association of vitamin B12 and folate deficiency with VVS in adults from the syncope unit of a tertiary referral hospital and a population-based cohort.

METHODS

Data Sources

We evaluated patients with VVS who presented to the syncope unit[12, 13, 25] of Tehran Heart Center,[26] Tehran, Iran, for a head-up tilt table test (HUTT) from February 2020 to February 2021 as the case group. The control group was selected from the population-based Tehran Cohort Study (TeCS) on adult inhabitants of Tehran.[27] The demographic and baseline data were retrieved from the syncope registry of Tehran Heart Center[25] for the case group and the database of TeCS[27] for the control group. The protocol of this study was designed according to the Declarations of Helsinki 2013 and approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1397.856). Informed consent was obtained from all participants after a detailed discussion on the study protocol and before inclusion.

Study Population

The case group consisted of adult patients with VVS aged 18 to 70 years. These patients received this diagnosis after a comprehensive history taking, physical examination, and guideline-indicated diagnostic workups,[1, 2] including electrocardiogram, echocardiogram, electrocardiogram Holter monitoring, and laboratory data in the syncope unit.[25] The control group comprised age- and sex-matched individuals without any history of syncope from TeCS. The exclusion criteria were: 1) Taking midodrine or fludrocortisone; 2) Taking oral or parenteral vitamin B supplements, including but not restricted to folic acid, vitamin B12, vitamin B complex, or hair supplements in the last six months; 3) Gastric, intestinal, or bariatric surgeries; 4) Alcohol use disorder; 5) Taking antiepileptic medications interfering with vitamin B12 or folate metabolism, including phenytoin, phenobarbital, or carbamazepine; 6) Taking methotrexate or isoniazid; 7) History of malabsorption, including celiac disease, Crohn's disease, or ulcerative colitis; and 8) History of malignancy.

Study Exposures

Our hypothesis was the greater vitamin B12 or folate deficiency in patients with VVS than in controls. Therefore, we measured serum levels of vitamin B12, folic acid, and homocysteine, a metabolite that its increase indicates functional vitamin B12 or folate deficiency. Blood samples were drawn from the case group after inserting the intravenous line as a routine preparation for HUTT and before conducting the test. Blood samples of the control group were drawn during standard procedures of TeCS. Participants were instructed to fast for at least eight hours prior to blood sampling. All blood samples were handled in dark containers designed for light-sensitive biochemicals, centrifuged a few hours after sampling, and stored at -80@C. The analyses were done using Abbott Diagnostic chemiluminescence kits.

Vitamin B12 deficiency was defined as serum vitamin B12<187 pg/mL, and folate deficiency was defined as serum folate<3.1 ng/mL. Increased serum levels of homocysteine may indicate a functional deficiency of either vitamin B12 or folate.[20] Thus, we defined another variable named vitamin B12/folate deficiency

defined as serum homocysteine>16.2 and >13.6 μmol/L in males and females, respectively, or vitamin B12 deficiency, or folate deficiency.

Study Covariates

We included baseline demographic characteristics, past medical history, drug history, family history, and syncopal history using the abovementioned databases. Other than demographic characteristics and past medical history, we carefully selected the study covariates that may interfere with serum levels of vitamin B12 or folate to minimize their confounding effects. For this purpose, we specifically recorded the history of hypo-/hyperthyroidism, and taking proton pump inhibitors, H_2 receptor blockers, metformin, and oral contraceptive pills, which may interfere with levels of vitamin B12 or folate. We also investigated the family history of syncope, seizure, or sudden death. The evidence of possibly lower serum levels of thyroid-stimulating hormone (TSH) in pediatric patients with VVS and positive HUTT compared to negative HUTT[23] encouraged us to measure serum TSH as a possible confounder in this study. We did so using the Abbott Diagnostic chemiluminescence kit and the abovementioned protocol. In the case group, we inquired about the last year and the lifetime number of syncopal episodes. According to the number of lifetime syncopal episodes, we categorized patients into frequent VVS with [?]3 episodes versus infrequent VVS with <3 episodes in the lifetime. All patients in the case group underwent HUTT according to the Italian protocol.[28] According to the modified VASIS classification,[29] we categorized the hemodynamic response in a positive HUTT into 1) Type 1, mixed; 2) Type 2A, cardioinhibition without asystole; 3) Type 2B, cardioinhibition with asystole; and 4) Type 3, vasodepressor.

Sample Size Calculation

This is the first study to compare the serum level of vitamin B12 between patients with VVS and healthy controls in the adult age group. Hence, we calculated the sample size from a similar study on adolescents showing that 47.2% of patients with VVS had vitamin B12 deficiency compared to 18.0% of the control group.[22] To achieve a power of 80%, we need 39 patients in each group to reach a statistical significance level of 0.05.

Statistical Analysis

We described data as mean (standard deviation) or number (percentage) for continuous and categorical variables, respectively. We employed the Student t-test and Chi-squared or Fisher's exact tests to compare continuous and categorical variables between the study groups, respectively. For calculating odds ratio (OR) and 95% confidence interval (CI), we fitted a binary logistic regression model to the data. In the case group, we fitted a linear regression model to the data for predicting serum vitamin B12 in these patients. We included age, sex, TSH, and the frequency of lifetime syncopal episodes ([?]3 versus <3 episodes) as independent variables in the model. All analyses were done using R version 4.0.3 (2020-10-10). The significance level was set at 0.05 with two-sided tests for all hypotheses.

RESULTS

Baseline characteristics

We screened 157 patients with a definite diagnosis of VVS who were referred for HUTT to the syncope unit of Tehran Heart Center from February 2020 to February 2021. After exclusion of 107 patients due to taking vitamin B supplements, two due to taking carbamazepine, two due to taking phenobarbital, one due to a history of sleeve gastrectomy, and one due to a history of malignancy, we included 44 patients aged 37.9 (14.7) years consisting of 23 (52.3%) females, in the case group. For the control group, we had 44 age- and sex-matched individuals without any history of syncope from TeCS, aged 37.9 (13.9) years, comprising 23 (52.3%) females. The baseline characteristics of the participants are presented in Table 1. We found no significant difference between the study groups regarding characteristics that may potentially confound serum levels of vitamin B12 or folate, including smoking, alcohol consumption, related medication, and past medical history (Table 1). In the case group, 13 (29.5%) and 21 (47.7%) patients had >3 syncopal episodes in the last year and the lifetime, respectively. According to our definition, 28 (63.4%) patients had frequent

VVS versus 16 (36.4%) patients with infrequent VVS in the case group. Furthermore, 17 (38.6%) patients had a positive HUTT with vasodepressor response as the most common response, 7 (41.2%) patients, followed by the mixed response, 5 (29.4%) patients (Table 1).

Association of vitamin B12/folate and VVS

We compared serum levels of vitamin B12, folate, homocysteine and TSH, and the prevalence of vitamin B12 and folate deficiency between the study groups in Table 2. There was no statistically significant difference between the patients and the controls regarding the abovementioned parameters (Table 2). Although the difference in the prevalence of vitamin B12 deficiency was not statistically significant between patients with frequent and infrequent VVS [8/28 (28.6%) versus 2/16 (12.5%); P=0.283], this difference may be of clinical significance and reach statistical significance with a larger sample (OR=2.80, 95% CI: 0.52 to 15.23; P=0.233; Table 2).

We found that the serum level of vitamin B12 was remarkably lower in patients with frequent VVS compared to patients with infrequent VVS [233.8 (80.7) versus 305.2 (118.1) pg/mL; P=0.042]. This difference remained significant after considering possible confounders, including age, sex, and TSH (Table 3). The linear regression model for predicting serum level of vitamin B12 in the case group demonstrated that patients with frequent VVS were more likely to have lower serum vitamin B12 than patients with infrequent VVS (coefficient=-73.97, 95% CI: -138.54 to -9.40; P=0.026; Table 3).

DISCUSSION

In this case-control study from a tertiary referral hospital and a population-based cohort, we found no remarkable difference in vitamin B12 or folate deficiency, or their serum levels between adult patients with VVS and controls; nonetheless, patients with frequent VVS are more likely to have lower serum levels of vitamin B12 compared to patients with infrequent VVS. Moreover, the prevalence of vitamin B12 deficiency is possibly higher in patients with frequent VVS compared to patients with infrequent VVS, albeit statistically non-significant, may be of clinical significance.

Biochemical mechanism of VVS in vitamin B12/folate deficiency

The pathophysiology of VVS is poorly understood, and there are ongoing efforts to address this gap in order to introduce novel treatment strategies for this potentially debilitating condition.[30] According to a hypothesis,[31] VVS is characterized by an over activation of the parasympathetic nervous system in response to an exaggerated activation of the sympathetic nervous system, demonstrated by increased serum levels of catecholamines.[14-19]Patients with VVS have normal resting serum levels of catecholamines; nevertheless, their serum catecholamines start to rise in response to head-up tilting, a similar situation to an actual syncopal episode. [14-19]Ineffective metabolism of released catecholamines is suggested to play a key role in the increased levels of these metabolites.[21] There are two main pathways for the metabolism of catecholamines, catechol-O-methyltransferase (COMT)- and monoamine oxidase (MAO)-dependent pathways. The COMT pathway requires S-adenosyl methionine (SAM), of which normal levels necessitate sufficient serum levels of vitamin B12 and folate.[20] In fact, vitamin B12 and folate are cofactors for the degradation of catecholamines. Hence, vitamin B12 or folate deficiency can decrease SAM, decrease COMT-dependent degradation of catecholamines, increase serum levels of catecholamines in response to VVS triggers, and lead to syncope.

Clinical evidence of VVS and vitamin B12/folate deficiency

There is some clinical evidence of the association between vitamin B12 deficiency and VVS in pediatric patients;[22, 23]nonetheless, there is scarce and weak evidence in adults and the elderly.[24] In a case-control study, pediatric patients with VVS (N=125) were more likely to have vitamin B12 deficiency (47.2% versus 18.0%; P<0.001) and lower serum vitamin B12 (352.8 versus 411.3 pg/mL; P<0.001) compared to healthy controls (N=50); however, there was no difference in serum levels of folate.[22] Another study revealed that pediatric patients with VVS and a positive HUTT (N=80) are more likely to have vitamin B12 deficiency (80.0% versus 52.5%; P=0.001) and lower serum vitamin B12 (282 versus 358 pg/mL; P=0.01) compared to HUTT negative patients (N=80).[23]

Despite this evidence in pediatric patients <18 years of age, there are only some descriptive studies in other age groups.[24, 32, 33] These studies showed that the prevalence of vitamin B12 deficiency might be as high as 70% in adult[24] or 23% in eldely[33] patients with VVS and a positive HUTT; nevertheless, no comparison was made with a control group. Notably, supplementation with intramuscular vitamin B12 in patients with deficiency reduced HUTT-induced syncope by 50-60% in a six-month follow-up.[24] Although these findings may imply the association of vitamin B12 and VVS in adults, the lack of a proper control group limits their generalizability. Furthermore, our results do not support this association, at least in a general population of patients with VVS who were referred to our syncope unit. This is in contrast with the current evidence in pediatric patients. This discrepancy may be attributed to the physiologic differences in dietary needs and growth between adults and adolescents, as most of the pediatric patients included in the abovementioned studies were older than ten years.[22, 23]

Implications for practice and research

Our findings may support measuring serum vitamin B12 in adult patients with frequent VVS and treating existing deficiencies, particularly when other treatment strategies with a class I recommendation like increased salt and fluid intake are not effective.[1, 2] The possible association of refractory and recurrent VVS with vitamin B12 deficiency, a potential clinical benefit of treating vitamin B12 deficiency,[24, 32] and also the safety of its measurement and administration, encourage this approach in daily clinical practice; nonetheless, future research is warranted to define the role of vitamin B12 deficiency in VVS clearly. The current evidence and our findings call for future studies to investigate the association of vitamin B12 deficiency with VVS in adult patients with frequent VVS. Furthermore, future randomized controlled trials will determine the effectiveness of supplementation with vitamin B12 in these patients. The current evidence of this intervention lacks an appropriate control group,[24, 32] which limits its authenticity and generalizability.

Limitations

Our findings should be interpreted in the light of the following limitations: 1) This is an observational case-control study with its inherent drawbacks; however, we selected the control group from a population-based cohort to minimize the risk of bias. 2) We excluded more than two-thirds of our patients due to a history of taking vitamin B supplements in the last six months, which might bias our findings; nonetheless, more than half of the screened individuals from TeCS were excluded for the same reason as well. This eye-catching use of supplements may be attributed to the concurrency of the coronavirus disease 2019 (COVID-19) pandemic and our study because the usage of vitamin supplements dramatically increased during the pandemic.[34, 35] 3) The sample size of this study is small; nevertheless, it should be noted that the non-COVID-19 healthcare utilization dropped by one-third during the pandemic, which substantially limited the number of eligible participants for this study.[36] 4) It is more sensitive to screen for vitamin B12 or folate deficiency by measuring serum levels of methylmalonic acid and homocysteine,[37] while we did not measure methylmalonic acid in this study.

CONCLUSIONS

In this study from a tertiary referral hospital and a population-based cohort, we found no difference in vitamin B12 or folate deficiency, or their serum levels between adult patients with VVS and controls; nevertheless, frequent VVS was associated with a lower serum vitamin B12 compared to infrequent VVS. Future studies are warranted to investigate the role of vitamin B12 deficiency in frequent VVS and the efficacy of supplementation with vitamin B12 in these patients.

REFERENCES

1. Shen W-K, Sheldon RS, Benditt DG, et al. 2017 ACC/AHA/HRS Guideline for the Evaluation and Management of Patients With Syncope. *A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society* . 2017;70(5):e39-e110. doi:10.1016/j.jacc.2017.03.003

2. Brignole M, Moya A, de Lange FJ, et al. 2018 ESC Guidelines for the diagnosis and management of syncope. *European Heart Journal* . 2018;39(21):1883-1948. doi:10.1093/eurheartj/ehy037

3. Ruwald MH, Hansen ML, Lamberts M, et al. The relation between age, sex, comorbidity, and pharmacotherapy and the risk of syncope: a Danish nationwide study. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* . Oct 2012;14(10):1506-14. doi:10.1093/europace/eus154

4. Sun BC, Emond JA, Camargo CA, Jr. Characteristics and admission patterns of patients presenting with syncope to U.S. emergency departments, 1992-2000. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine* . Oct 2004;11(10):1029-34. doi:10.1197/j.aem.2004.05.032

5. Ruwald MH, Hansen ML, Lamberts M, et al. Accuracy of the ICD-10 discharge diagnosis for syncope. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* . Apr 2013;15(4):595-600. doi:10.1093/europace/eus359

6. Olde Nordkamp LR, van Dijk N, Ganzeboom KS, et al. Syncope prevalence in the ED compared to general practice and population: a strong selection process. *The American journal of emergency medicine* . Mar 2009;27(3):271-9. doi:10.1016/j.ajem.2008.02.022

7. van Dijk N, Sprangers MA, Colman N, Boer KR, Wieling W, Linzer M. Clinical factors associated with quality of life in patients with transient loss of consciousness. *Journal of cardiovascular electrophysiology* . Sep 2006;17(9):998-1003. doi:10.1111/j.1540-8167.2006.00533.x

8. Sun BC. Quality-of-life, health service use, and costs associated with syncope. *Progress in cardiovascular diseases* . Jan-Feb 2013;55(4):370-5. doi:10.1016/j.pcad.2012.10.009

9. Linzer M, Pontinen M, Gold DT, Divine GW, Felder A, Brooks WB. Impairment of physical and psychosocial function in recurrent syncope.*Journal of clinical epidemiology* . 1991;44(10):1037-43.

10. Jorge JG, Raj SR, Teixeira PS, Teixeira JAC, Sheldon RS. Likelihood of injury due to vasovagal syncope: a systematic review and meta-analysis. *EP Europace* . 2021;23(7):1092-1099. doi:10.1093/europace/euab041 %J EP Europace

11. Sun BC, Emond JA, Camargo CA, Jr. Direct medical costs of syncope-related hospitalizations in the United States. *The American journal of cardiology* . Mar 1 2005;95(5):668-71. doi:10.1016/j.amjcard.2004.11.013

12. Aminorroaya A, Tavolinejad H, Sadeghian S, et al. Comparison of Outcomes with Midodrine and Fludrocortisone for Objective Recurrence in Treating Syncope (COMFORTS trial): Rationale and design for a multi-center randomized controlled trial. *American Heart Journal* . 2021/07/01/ 2021;237:5-12. doi:https://doi.org/10.1016/j.ahj.2021.03.002

13. Tavolinejad H, Poopak A, Sadeghian S, et al. Compression stockings for treating vasovagal syncope (COMFORTS-II) trial: Rationale and design of a triple-blind, multi-center, randomized controlled trial.*American Heart Journal* . 2022/07/01/ 2022;249:57-65. doi:https://doi.org/10.1016/j.ahj.2022.04.002

14. Benditt DG, Ermis C, Padanilam B, Samniah N, Sakaguchi S. Catecholamine response during haemodynamically stable upright posture in individuals with and without tilt-table induced vasovagal syncope.*Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* . Jan 2003;5(1):65-70.

15. Goldstein DS, Holmes C, Frank SM, et al. Sympathoadrenal imbalance before neurocardiogenic syncope. *The American journal of cardiology* . Jan 1 2003;91(1):53-8.

16. Alboni P, Dinelli M, Gruppillo P, et al. Haemodynamic changes early in prodromal symptoms of vasovagal syncope. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* . Jul 2002;4(3):333-8.

17. Olgunturk R, Turan L, Tunaoglu FS, et al. Abnormality of the left ventricular sympathetic nervous function assessed by I-123 metaiodobenzylguanidine imaging in pediatric patients with neurocardiogenic syncope. *Pacing and clinical electrophysiology : PACE* . Oct 2003;26(10):1926-30.

18. Vanderheyden M, Goethals M, Nellens P, Andries E, Brugada P. Different humoral responses during head-up tilt testing among patients with neurocardiogenic syncope. *Am Heart J* . Jan 1998;135(1):67-73.

19. Sra JS, Murthy V, Natale A, et al. Circulatory and catecholamine changes during head-up tilt testing in neurocardiogenic (vasovagal) syncope. *The American journal of cardiology* . Jan 1 1994;73(1):33-7.

20. Richard Harvey DF. *Lippincott's Illustrated Reviews: Biochemistry* . fifth ed. 2011.

21. Huotari M, Gogos JA, Karayiorgou M, et al. Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *The European journal of neuroscience* . Jan 2002;15(2):246-56.

22. Oner T, Guven B, Tavli V, Mese T, Yilmazer MM, Demirpence S. Postural Orthostatic Tachycardia Syndrome (POTS) and Vitamin B-12 Deficiency in Adolescents. *Pediatrics* . Jan 2014;133(1):E138-E142. doi:10.1542/peds.2012-3427

23. Pektas A, Koken R, Koca HB. Serum vitamin B-12 in children presenting with vasovagal syncope. Article. *Asia Pacific Journal of Clinical Nutrition* . 2018;27(1):176-181. doi:10.6133/apjcn.022017.17

24. Parekh S, Sastry B, Narasimhan C, Arora HJJC. To study vitamin B12 deficiency and response to treatment in patients presenting with vasovagal syncope. 2018;2(5):000134.

25. Sadeghian S, Aminorroaya A, Tajdini M. The Syncope Unit of Tehran Heart Center. *European Heart Journal* . 2020;42(2):148-150. doi:10.1093/eurheartj/ehaa532 %J European Heart Journal

26. Poorhosseini H, Abbasi SH. The Tehran Heart Center. *European Heart Journal* . 2018;39(29):2695-2696. doi:10.1093/eurheartj/ehy369 %J European Heart Journal

27. Shafiee A, Saadat S, Shahmansouri N, et al. Tehran cohort study (TeCS) on cardiovascular diseases, injury, and mental health: Design, methods, and recruitment data. *Global Epidemiology* . 2021/11/01/ 2021;3:100051. doi:https://doi.org/10.1016/j.gloepi.2021.100051

28. Bartoletti A, Alboni P, Ammirati F, et al. 'The Italian Protocol': a simplified head-up tilt testing potentiated with oral nitroglycerin to assess patients with unexplained syncope. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* . Oct 2000;2(4):339-42. doi:10.1053/eupc.2000.0125

29. Brignole M, Menozzi C, Del Rosso A, et al. New classification of haemodynamics of vasovagal syncope: beyond the VASIS classificationAnalysis of the pre-syncopal phase of the tilt test without and with nitroglycerin challenge. *EP Europace* . 2000;2(1):66-76. doi:10.1053/eupc.1999.0064

30. Tajdini M, Aminorroaya A, Tavolinejad H, et al. Atomoxetine as an adjunct to nonpharmacological treatments for preventing vasovagal attacks in patients with recurrent vasovagal syncope: A pilot randomized-controlled trial. *IJC Heart & Vasculature* . 2021/06/01/ 2021;34:100789. doi:https://doi.org/10.1016/j.ijcha.2021.100789

31. Iwase S, Nishimura N, Mano T. Role of sympathetic nerve activity in the process of fainting. *Front Physiol* . 2014;5:343-343. doi:10.3389/fphys.2014.00343

32. Sharada K, Reddy G, Narasimhan C. Cobalamin deficiency presenting as refractory vaso vagal syncope. Letter. *International Journal of Cardiology* . 2012;156(1):e7-e8. doi:10.1016/j.ijcard.2011.07.093

33. Ghaznain M, Donnelly TM, Halpenny L. Tilt table test outcome in the diagnosis and prevalence of syncope in patients with vitamin d and vitamin B12 deficiency. *Age and Ageing* . Sep 2017;46doi:10.1093/ageing/afx144.60

34. Aysin E, Urhan M. Dramatic Increase in Dietary Supplement Use During Covid-19. *Current Developments in Nutrition* . 2021;5(Supplement_2):207-207. doi:10.1093/cdn/nzab029_008 %J Current Developments in Nutrition

35. Çimke S, Yıldırım Gürkan D. Determination of interest in vitamin use during COVID-19 pandemic using Google Trends data: Infodemiology study.*Nutrition* . 2021;85:111138-111138. doi:10.1016/j.nut.2020.111138

36. Moynihan R, Sanders S, Michaleff ZA, et al. Impact of COVID-19 pandemic on utilisation of healthcare services: a systematic review. 2021;11(3):e045343. doi:10.1136/bmjopen-2020-045343 %J BMJ Open

37. Oh R, Brown DL. Vitamin B12 deficiency. *American family physician* . Mar 1 2003;67(5):979-86.

Table 1. Baseline characteristics of the participants

Characteristic	**Case (N=44)**	**Control (N=44)**	**P value**
Demographics	**Demographics**	**Demographics**	**Demographics**
Age (year)	37.9 (14.7)	37.9 (13.9)	0.994
Sex (Female)	23 (52.3%)	23 (52.3%)	1.000
Smoking	7 (15.9%)	6 (13.6%)	0.764
Alcohol consumption	1 (2.3%)	1 (2.3%)	1.000*
Body mass index (kg/m^2)	27.0 (8.7)	25.3 (3.6)	0.251
Past medical history	**Past medical history**	**Past medical history**	**Past medical history**
Diabetes mellitus	0 (0%)	0 (0%)	-
Hypertension	7 (15.9%)	5 (11.4%)	0.534
Dyslipidemia	6 (13.6%)	9 (20.5%)	0.395
Coronary artery disease	1 (2.3%)	0 (0%)	1.000*
Hypothyroidism	4 (9.1%)	1 (2.3%)	0.360
Hyperthyroidism	1 (2.3%)	0 (0%)	1.000*
Medication history	**Medication history**	**Medication history**	**Medication history**
Proton pump inhibitors	4 (9.1%)	2 (4.5%)	0.676*
H2 receptor blockers	1 (2.3%)	1 (2.3%)	1.000*
Metformin	1 (2.3%)	1 (2.3%)	1.000*
Oral contraceptive pills	1 (2.3%)	0 (0%)	1.000*
Family history	**Family history**	**Family history**	**Family history**
Syncope	3 (6.8%)	0 (0%)	0.241*
Seizure	3 (6.8%)	1 (2.3%)	0.616*
Sudden death	2 (4.5%)	1 (2.3%)	1.000*
Syncopal history	**Syncopal history**	**Syncopal history**	**Syncopal history**
Last year's syncopal episodes			-
1	14 (31.8%)	-	
2	8 (18.2%)	-	
3	3 (6.8%)	-	
>3	13 (29.5%)	-	
Lifetime syncopal episodes			-
1	7 (15.9%)	-	
2	9 (20.5%)	-	
3	7 (15.9%)	-	

Characteristic	Case (N=44)	Control (N=44)	P value
>3	21 (47.7%)	-	
Positive HUTT	17 (38.6%)	-	-
HUTT response			-
Mixed	5 (29.4%)	-	
Cardioinhibition without asystole	1 (5.9%)	-	
Cardioinhibition with asystole	4 (23.5%)	-	
Vasodepressor	7 (41.2%)	-	

Data are presented as mean (standard deviation) or number (percentage). Abbreviations: HUTT, head-up tilt table test.

* Fisher's exact test was done for comparison.

Table 2. Comparison of laboratory data and prevalence of vitamin B12/folate deficiency between patients and controls, and between patients with recurrent VVS and patients with infrequent VVS

Characteristic	Case (N=44)	Control (N=44)	P value	Frequent VVS (N=28)	Infrequent VVS (N=16)	P value
Vitamin B12 (pg/mL)	259.7 (100.8)	238.0 (82.7)	0.271	233.8 (80.7)	305.2 (118.1)	**0.042**
Folate (ng/mL)	7.8 (3.7)	7.4 (3.0)	0.526	8.1 (4.1)	7.4 (2.9)	0.530
Homocysteine (μmol/L)	15.6 (10.0)	13.5 (5.1)	0.234	14.6 (7.1)	17.2 (13.8)	0.505
Vitamin B12 deficiency	10 (22.7%)	15 (34.1%)	0.237	8 (28.6%)	2 (12.5%)	0.283*
Folate deficiency	2 (4.5%)	0 (0%)	0.494*	1 (3.6%)	1 (6.3%)	1.000*
Vitamin B12/Folate deficiency	18 (40.9%)	22 (50.0%)	0.392	13 (46.4%)	5 (31.3%)	0.325
TSH (U/mL)	2.3 (1.4)	2.3 (1.7)	0.947	2.1 (1.2)	2.6 (1.7)	0.254

Data are presented as mean (standard deviation) or number (percentage). Abbreviations: TSH, thyroid-stimulating hormone.

* Fisher's exact test was done for comparison.

Table 3. Linear regression model for predicting serum vitamin B12 based on syncopal history

Characteristic	Serum vitamin B12	Serum vitamin B12	Serum vitamin B12
	Coefficient	**95% CI**	**P value**
Age (year)	0.18	-1.91 – 2.28	0.862
Sex	**Sex**	**Sex**	**Sex**
Male	Reference	Reference	Reference
Female	36.83	-25.10 – 98.75	0.236

Characteristic	Serum vitamin B12	Serum vitamin B12	Serum vitamin B12
TSH	2.89	-19.90 – 25.69	0.799
Lifetime syncopal episodes	**Lifetime syncopal episodes**	**Lifetime syncopal episodes**	**Lifetime syncopal epi**
Infrequent (<3 episodes)	Reference	Reference	Reference
Frequent ([?]3 episodes)	-73.97	-138.54 – -9.40	**0.026**

Abbreviations: CI, confidence interval; TSH, thyroid-stimulating hormone.

FIGURE LEGEND

Figure 1. Comparison of laboratory data and prevalence of vitamin B12/folate deficiency between (**A**) patients and controls, and between (**B**) patients with recurrent VVS and patients with infrequent VVS