


Vasoactive Biomarkers in Patients With Vasovagal Syncope During Head-Up Tilt Test: A Case-Control Study

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ABSTRACT

BACKGROUND: Vasovagal syncope (VVS) is the most common cause of syncope. Some stages of its pathophysiological mechanisms remain unclear. Vasoactive substances such as nitric oxide metabolites (NOx) and endothelin (ET) may be involved during acute orthostatic stress.

OBJECTIVE: To analyze plasma changes in NOx and ET and heart rate variability (HRV) in the supine positions (T1) and during the head-up tilt test (HUTT) (T2), in patients with VVS (case group) and control group.

METHODS: Thirty-seven patients (17 in the case group and 20 in the control group), matched for age and sex (mean aged 31.8 years) underwent HUTT with simultaneous HRV recording and venipuncture. Blood samples were collected during phases T1 and T2 and the analysis was performed without knowledge of the HUTT result.

RESULTS: In the total sample, there was an increase in NOx values ($P = .014$), however there was no increase in ET values from phase T1 to phase T2. Patients with VVS tended to increase plasma NOx values ($P = .057$) and had significantly higher plasma values compared to ET ($P = .033$) between phases T1 to T2. In the control group, there was no significant change in the values of these vasoactive substances. Regarding HRV, there were a decrease in the component HF (high frequency) and increased of the LF (low frequency)/HF ratio during HUTT.

CONCLUSIONS: There was an increase in ET during HUTT occurred only in the case group. These patients are more likely to have an imbalance between antagonistic vasoactive biomarkers during orthostatic stress.

KEYWORDS: Vasovagal syncope, nitric oxide, endothelin, vasoactive agents, head-up tilt test, autonomic nervous system

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Introduction

Syncope is defined as a sudden transient loss of consciousness (TLOC) due to cerebral hypoperfusion, characterized by short duration and spontaneous complete recovery.¹ The vasovagal syncope (VVS) is triggered by orthostatic stress and/or emotional factors (eg, pain, medical settings, and sight of blood). Its incidence is markedly evident by the age of 11 years, and most people report their first vasovagal event before age 40.² Despite its benign outcome, VVS is associated with loss of quality of life^{3,4} due to its high rate of recurrence and higher healthcare costs, including emergency department care, hospitalization, medical examination, and treatment.⁴ The pathophysiology of syncope is complex and not completely understood. Orthostatic posture causes subdiaphragmatic gravitational blood pooling within the venous system, resulting in a decrease in central blood volume. Consequently, it reduces venous return to the thorax following a decrease in stroke volume and cardiac output despite an

increase in heart rate (HR), in the absence of an active veno-muscular pumping of the lower limbs. Blood pressure (BP) is stabilized by compensatory mechanisms, such as baroreceptor control of peripheral vascular resistance, passive venous elastic retraction, increased HR, and splenic venoconstriction.^{5,6}

Other complementary tools besides the participation of the autonomic nervous system (ANS) have been studied in specialized literature in order to contribute to the diagnosis, prognosis, and pathophysiology of syncope. Some studies have described the involvement of biomarkers in the pathophysiology of syncope.⁷⁻¹⁴ It is unclear whether biomarkers participate directly in the pathophysiology and hemodynamic changes of syncope and/or are released in response to acute orthostatic stress. The behavior of some endothelial vasoactive substances, such as endothelin (ET)¹⁵⁻¹⁹ and nitric oxide metabolites²⁰⁻²⁴ have been studied as a result of orthostatic stress during head-up tilt test (HUTT).



ET is continuously synthesized and released from endothelial cells, which are influenced by physiologic and pathophysiologic factors such as hypoxia, thrombin, shear stress, vasoactive factors, nitric oxide, and prostaglandins. In addition to its role in controlling vascular tone, it has inotropic and mitogenic properties, participates in salt and water homeostasis, and stimulates the renin-angiotensin-aldosterone system, potentiating central and peripheral sympathetic activity.²⁵

Nitric oxide (NO), a free radical present mostly in the vascular system, has potent vasodilator action. Due to its vasodilator role, NO and its metabolites (NO_x), nitrite and nitrate, have been the focus of studies on orthostatic intolerance syndromes, including neurally mediated events.^{9,20-24,26,27} The sympatholytic action of NO, which attenuates the vasoconstrictor effect of the ANS, has already been cited in the literature.^{28,29}

There is an interaction between the ANS and the endothelial factors since sympathetic stimulation releases ET, while parasympathetic stimulation increases NO synthesis, as already demonstrated in previous studies.^{22,28-31} Through HR variability measures (HRV), the participation of endogenous and exogenous NO in the modulation of baroreflex-dependent cardiac vagal control in humans has been demonstrated.²²

In addition, changes in the balance between vasoconstriction and vasodilation may have a significant impact on the regulation of the vascular tone during orthostatic stress, resulting in an inappropriate hypotension that may exceed the compensatory mechanisms of blood pressure and cerebral perfusion maintenance, contributing to the pathophysiology of syncope.^{12,30} Therefore, these vasoactive substances can be potential candidates for circulating biomarkers and useful in the diagnosis, prognosis, and possible specific treatment of syncope subgroups. Some previous studies used different methods^{10,12,13,31-35} and mostly included patients with orthostatic intolerance syndrome or unexplained syncope, not VVS exclusively, demonstrated the behavior of several vasoactive biomarkers during HUTT.

In this study, the main objectives were to analyze and compare the behavior of vasoactive neurohormones, ET and NO_x, and HRV, both measured in the in the supine position and during HUTT, in patients with a history of VVS and in healthy participants without history of VVS.

Methods

Participants and study design

This study was approved by the local and national research ethics committee. An initial sample size of 28 patients was calculated in this case-control study, considering the estimated prevalence of positive HUTT (60%) in the case group, 95% power, and a 5% significance level. All patients signed an informed consent form before entering the study. Patients were divided into a case group (with a clinical history of VVS based on the Calgary Score and positive HUTT) and a control group

(volunteers with no previous syncope and negative HUTT). Patients between 18 and 50 years of age, of both genders, in sinus rhythm and able to tolerate HUTT were included. Pregnant patients, heart transplant recipients, patients with cardiac implantable electronic devices, antiarrhythmic drug users, and patients with acute or chronic conditions that could interfere with the results of plasma biomarker values were excluded. Patients were instructed to follow a nitrate-free diet for 48 hours before HUTT, in addition to not consuming caffeine and alcohol, and a fasting period of 2 to 4 hours before the test according to guidelines.¹

HUTT was performed in the morning, in an air-conditioned room, using an appropriate tilt table with a footrest. After an initial 20-minute resting period, the table was tilted at 70° for the initial passive phase, which lasted 20 minutes. If there were no symptoms and/or clinical signs of TLOC, the next phase of HUTT was a provocative pharmacological phase with a 1.25 mg isosorbide tablet, administered sublingually. In our country, sublingual nitroglycerin has not been marketed since 2002; therefore, the vasodilator used during HUTT is a dose of 1.25 mg nitrate tablet, as recommended by national guideline.³⁶ The HUTT was interrupted in case of syncope or at the end of the provocative phase if no symptoms of TLOC occurred. Positivity criteria of HUTT were defined as the occurrence of syncope accompanied by bradycardia (HR < 40 bpm) or asystole (pause > 3.0 s) and/or hypotension (systolic BP < 70 mmHg). If this response was observed, the patient was immediately returned to the Trendelenburg position in order to recover the patient's basal status. Hemodynamic parameters, HR and BP, were monitored continuously and intermittently, respectively, throughout the HUTT, using a Hewlett Packard Omnicare 24C monitor. After the initial rest, a blood sample was collected at the 15th minute in the supine position (T1) and at the 15th minute after the inclination or before, at the moment of prodromes, or if the test resulted positive (T2). The laboratory methods used for measuring the ET and NO_x plasma concentrations are shown below in the next section.

Measurement of biomarkers

In the periods T1 and T2, 5 mL blood samples were collected by venipuncture into EDTA-coated tubes and immediately centrifuged for 15 minutes at 3000 rpm at 4°C to extract the plasma samples and then stored at -80°C. All samples were anonymized and coded to laboratory co-workers. The measurements of ET and NO_x were performed using ELISA from R&D System (Catalog no KEG 100, Minneapolis, MN, USA) according to the manufacturer's instructions. The concentrations of nitrite and nitrate were determined using a procedure based on the Griess reaction. All analyses were performed in duplicate. Standard curve and ET/NO_x concentrations were determined using the GraphPad Prism 5.0 software.

HRV analysis

A 3-channel ECG record (leads V1, V5, and aVF) was recorded using a Holter recorder (DMS 300-7, Compact Flash Card Holter Recorder, DMS, Stateline, NV, USA) during the HUTT phases. Recordings were obtained in a quiet and controlled temperature room, during spontaneous breathing in supine and upright positions. The stored data were imported into the program for HRV analysis (CardioScan 12 software, DMS). After automatic and manual editing to eliminate abnormal beats and artifacts, the 5-minute RR intervals were analyzed. The results of the spectral analysis of HRV were expressed in ms^2 using the software. The values in the normalized unit (nu) were obtained by applying the formula $[\text{HF or LF (nu)} = \text{HF or LF} / (\text{total power} - \text{VLF}) \times 100]$ according to the literature.³⁷ The parameters of the frequency domain of HRV in short-term recordings (5 minutes) are: very low frequency (VLF; 0.003-0.04 Hz), low frequency (LF; 0.04-0.15 Hz), and high frequency (HF; 0.15-0.40 Hz) bands.

Statistical analysis

Statistical analysis was performed using the SPSS software package (Statistical Package for Social Science) version 16.0. The results were expressed as numbers and proportions, for categorical variables, and in means \pm standard deviation or medians and interquartile ranges (Q1: 25th; Q3: 75th percentile) for continuous variables. Comparisons of proportions were analyzed using the chi-square test or Fisher's exact test. For quantitative data, the differences between 2 groups (control and case) were analyzed using Student's *t*-test or Mann-Whitney test according to normal or non-normal distributions. Normality of data distribution was assessed by the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The Wilcoxon test was used to compare the 2 periods (T1 and T2) of biomarkers and HRV spectral components. Pearson's correlation coefficient was used to examine the statistical relationship between 2 variables. A *P* value $< .05$ was considered statistically significant.

Results

Characteristics of the study group

The study population included 37 patients (mean age 31.8 ± 8.7 years; range 19-50 years) and 56.7% were female. There were 20 patients in the case group and 17 in the control group. The hemodynamic characteristics, analysis of HRV and biomarkers are shown in Table 1.

Case group

The mean age was 34.3 years. The median time interval since the first syncope was 360 days, and the mean time since the last syncope episode and HUTT was 138.8 days. The mean

Table 1. Baseline characteristics: study population (n=37).

HEMODYNAMIC DATA	
SBP T1 (mmHg)	131.0 \pm 18.5
DBP T1 (mmHg)	66.0 \pm 9.7
SBP T2 (mmHg)	128.4 \pm 19.8
DBP T2 (mmHg)	68.3 \pm 10.8
HR T1 (bpm)	71.1 \pm 11.8
HR T2 (bpm)	86.4 \pm 12.2
HRV	
LF T1 (nu)	84.3 \pm 133.1
LF T1 (ms^2)	965.7 \pm 889.7
LF T2 (nu)	94.2 \pm 108.4
LF T2 (ms^2)	799.2 \pm 588.5
HF T1(nu)	41.9 \pm 40.6
HF T1 (ms^2)	590.0 \pm 617.6
HF T2 (nu)	20.6 \pm 13.3
HF T2 (ms^2)	267.1 \pm 345.1
LF/HF T1	3.1 \pm 4.9
LF/HF T2	7.6 \pm 9.3
Neurohormone data	
NOx T1 (mmol/L)	0.6 \pm 0.1
NOx T2 (mmol/L)	0.6 \pm 0.2
ET-T1 (pg/mL), Q2 [Q1; Q3]	2.1 [0.5; 2.9]
ET-T2 (pg/mL), Q2 [Q1; Q3]	2.4 [1.7; 15.2]

Abbreviations: bpm, beats per minute; DBP, diastolic blood pressure; ET, endothelin; HF, high frequency; HR, heart rate; HRV, heart rate variability; LF, low frequency; NOx, nitric oxide metabolites; SBP, systolic blood pressure; T1, supine position; T2, at the 15th min after the inclination or before, at the moment of prodromes, or if the test resulted positive. All values are given as mean \pm SD (standard deviation), except for ET (median and interquartile range [Q1; Q3]).

number of episodes was 3.0 ± 2.6 . The Calgary Score ranged from -4 to +6 points (mean: +0.4). None of the patients had heart disease or were using cardiovascular medications. Three patients reported injury due to syncope episodes in previous clinical history. The positive HUTT responses were vasodepressor (n=05) and mixed (n=15). Nine patients presented syncope during the passive phase of HUTT. Eleven patients presented positive HUTT during the pharmacological provocative phase. Syncope occurred at a mean time of 20.8 minutes after the upright position.

Table 2. Hemodynamic variables between the control and case groups.

HEMODYNAMIC VARIABLES	CONTROL GROUP (MEAN ± SD)	CASE GROUP (MEAN ± SD)	P VALUE
SBP T1 (mmHg)	139.7 ± 21.9	123.6 ± 10.8	.006
SBP T2 (mmHg)	137.8 ± 21.0	120.5 ± 15.1	.006
DBP T1 (mmHg)	67.1 ± 9.1	65.2 ± 10.4	.55
DBP T2 (mmHg)	69.5 ± 8.3	67.3 ± 12.6	.54
HR T1 (bpm)	72.9 ± 14.3	69.6 ± 9.3	.40
HR T2 (bpm)	87.5 ± 14.5	85.5 ± 10.6	.62

Abbreviations: DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure.

Comparison between control group and case group

There was no significant difference between the case and control groups in terms of age and sex. There were 12 women in the case group and 9 in the control group ($P=.66$). The mean ages were 34.3 and 29.0 years, respectively, for the case and control groups ($P=.06$). Systolic BP (SBP) was lower in the case group, both in the supine and in the upright positions. There was no statistically significant difference in SBP and diastolic BP (DBP) between the resting position and upright phases in the control group ($P=.37$ for SBP and $P=.45$ for DBP). The data comparing hemodynamic variables between groups are shown in Table 2.

Spectral analysis of HRV components

The results of the spectral analysis of HRV components are listed in Table 1 (study group), and Table 3 (each group separately). The Wilcoxon parametric test was used to compare the values of the spectral analysis of HRV components during T1 and T2. There was a decrease in the HF (nu) component ($P=.001$) and an increase in the LF/HF ratio ($P<.0001$) in the entire study population, with no significant difference in relation to the LF (nu) with the change in position ($P=.72$) (data in Table 1).

Similar behavior was observed in each group separately. In the case group, with the change from the supine to the upright position, there was no change in the LF (nu) ($P=.63$), but there was a decrease in the HF (nu) ($P=.012$) and an increase in the LF/HF ratio ($P=.02$). In the control group, P values were .26 for LF (nu), .003 for HF (nu), and $P<.001$ for LF/HF ratio with position change (data in Table 3).

Vasoactive neurohormones (NOx and ET)

There was no difference in NOx and ET plasma values between case and control groups referring to each phase. Regarding the T1 phase, P values were .56 for NOx means and 0.41 for ET

Table 3. Spectral analysis of heart rate variability (HRV) components between the control and case groups.

HRV	CONTROL GROUP (MEAN ± SD)	CASE GROUP (MEAN ± SD)	P VALUE
LF T1 (ms ²)	1255.2 ± 887.8	734.1 ± 841.9	.10
LF T1 (nu)	64.4 ± 16.4	101.3 ± 180.8	.40
LF T2 (ms ²)	1537.8 ± 2130.0	752.0 ± 512.7	.14
LF T2 (nu)	108.8 ± 158.1	81.9 ± 29.8	.46
HF T1 (ms ²)	729.9 ± 797.0	478.2 ± 414.3	.26
HF T1 (nu)	32.3 ± 14.5	50.1 ± 52.9	.18
HF T2 (ms ²)	299.6 ± 322.4	241.0 ± 368.4	.67
HF T2 (nu)	19.9 ± 12.7	21.2 ± 14.2	.78
LF/HF T1	4.1 ± 7.1	2.3 ± 1.9	.31
LF/HF T2	6.7 ± 7.3	8.0 ± 10.8	.68

Abbreviations: HF, high frequency; LF, low frequency.

medians. And as for T2 phase, P values were .85 for NOx means and 0.12 for ET medians. However, there was a difference in the NOx mean in the total study group when comparing the 2 phases (data in Table 4). There was a borderline increase in the mean NOx in the case group, with no significant difference in the control group during HUTT (Data in Table 4 and boxplot representation in Figure 1).

Regarding the medians of plasma ET values (non-normal distribution), there was an increase during phase 2 only in the case group (Data in Table 4 and boxplot representation in Figure 2).

No correlation between spectral components of HRV and vasoactive neurohormones was demonstrated, in either supine or upright position.

Discussion

In this study, we reported changes in vasoactive neurohormones (NOx and ET), spectral components of HRV, and hemodynamic parameters in a group of patients with HUTT positive response and previous syncope compared to healthy subjects without syncope. Plasma NOx values increased after tilting in this study population. However, this biomarker showed a borderline increase in the case group and there was no significant change in the control group between phases T1 (supine position) and T2 (during the HUTT). ET plasma concentration was elevated only in the case group after tilting, indicating a vasodilation/vasoconstriction imbalance in patients with a history of syncope and positive HUTT.

In response to orthostatic stress, an adequate hemodynamic response is the result of interaction between the integrity of cardiovascular and autonomic nervous systems associated to

Table 4. Vasoactive neurohormones data according to phases and groups.

NEUROHORMONE DATA	T1 PHASE	T2 PHASE	P VALUE
NOx (mmol/L)			
Total study group	0.63	0.68	.014
Case group	0.65	0.69	.057
Control group	0.61	0.67	.10
ET (pg/mL), Q2 [Q1; Q3]			
Total study group	2.15 [0.50; 2.96]	2.49 [1.73; 15.25]	.17
Case group	1.29 [0.18; 2.83]	10.14 [1.81; 15.30]	.033
Control group	2.49 [2.02; 3.69]	1.96 [1.02; 9.03]	.52

Abbreviations: ET, endothelin; NOx, nitric oxide metabolites.

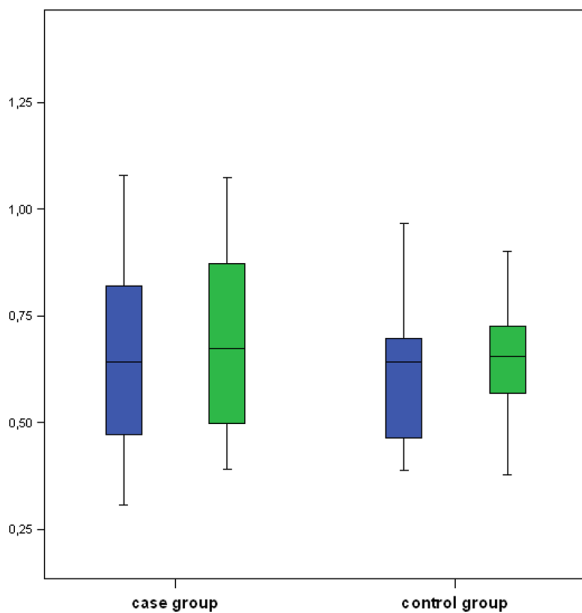


Figure 1. Nitric oxide metabolites (NOx) plasma values in case group and control group during phases T1 (in blue) and T2 (in green). Y-axis: NOx plasma values (mmol/L).

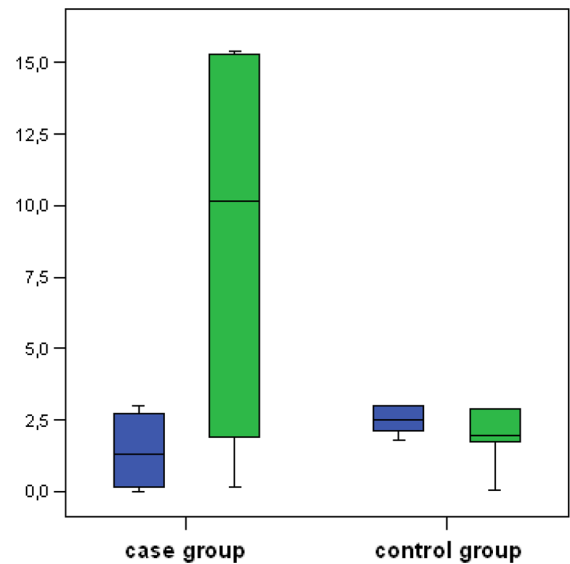


Figure 2. Endothelin (ET) plasma values shown as median and interquartile range in case group and control group during phases T1 (in blue) and T2 (in green). Y-axis: ET plasma values (pg/mL).

neurohumoral and endothelium responses. The disruption of this balance secondary to any impairment of the compensatory cascade results in sudden and transient symptoms of cerebral hypoperfusion such as syncope. Previous studies using noninvasive tools have demonstrated the sequential changes in hemodynamics and sympathetic nervous system, but the occurrence of disproportional vasodilation have raised questions about additional pathways.^{38,39} That one of the reasons why some authors have been studying the endothelial vasoactive substances involved in exaggerated decrease in peripheral vascular resistance during the orthostatic stress resulting in syncope.¹⁵⁻²⁴ The most important endothelial role in vasodilation is played by NO. Therefore, in this study, both this vasodilator, NOx, and ET, a potent vasoconstrictor, were analyzed.

Few studies evaluated the behavior NOx alone or in association with other biomarkers exclusively in patients who presented VVS.^{23,24,40} Only the study carried out with 10 children with VVS demonstrated an increase in NOx during HUTT compared to the group of 20 healthy children.²³ The other 2 studies with a sample of 12 and 9 patients including adolescents (and a control group of 13 and 23 healthy volunteers, respectively) showed no differences in serum NOx values.^{24,40} These contradictory findings are explained by the influence of diet on the plasma NOx level and the fact that plasma NOx levels are not a reliable indicator of nitrite oxide production.²⁴ In the present study, there was a strict control of the diet, avoiding this bias in NOx dosage. In addition, this dosage was performed without the interference of isosorbide dinitrate, which could have contributed to a decrease in the plasma NOx level. The dosage was made at the 15th minute after the inclination

or before, at the moment of prodromes and, if there was a provocative pharmacological phase, the administration of isosorbide occurred after the 20th minute.

Due to questions about the influence of NO on VVS, a study with young people aged between 15 and 27 years, 12 with a history of VVS and 12 healthy, were submitted to inhibition of NO synthesis by the infusion of monomethyl-L acetate-arginine.⁴¹ Hemodynamic measurements were performed under a lower body negative pressure protocol. Saline plus phenylephrine was used as volume and pressor control of the NO synthesis inhibitor. Thus, the authors concluded that the impaired splanchnic vasoconstriction in the VVS has been restored by inhibiting the synthesis of NO. Therefore, the contribution of this vasodilator to splanchnic hyporeactivity during the pre-syncope phase was demonstrated. However, NO modulation has not yet been defined in older adults with VVS. Our study included patients aged between 19 and 50 years, 20 with VVS and 17 in the control group, matched for age and sex. The borderline increase in NOx dosage in the case group may be due to part of the sample of young adults based on the results of the study by Stewart et al⁴¹

By adding the simultaneous analysis of another vasoactive endothelial substance, we demonstrated that ET could play a role in reproducing clinical syncope during HUTT. In agreement with the behavior of ET in our study, other authors demonstrated that its plasma concentration increases after postural change. Among healthy individuals, there was an increase in ET in parallel with a decrease in BP and this was reproducible in HUTT performed within a period of 14 days in 7 individuals who developed VVS during the HUTT.^{7,16} However, in another study with 46 healthy individuals without a history of syncope, among the 14 who had syncope during tilt, there was an increase in ET only in 3 individuals, with no clinical or hemodynamic difference with the others who also had syncope.¹⁸ Other authors who used the measurement of this neurohormone had different purposes such as identifying patients with VVS by asystole or comparing its dosage in the supine position among healthy individuals, patients with VVS and patients with epilepsy.^{15,19} Therefore, the results are different due to the characteristics of the participating individuals, objectives and research methods. Furthermore, there is a genetic influence on the susceptibility to VVS, since the 3A/4A polymorphism of the EDN1 gene affects the expression of ET. The 4A allele was significantly more frequent in patients with positive HUTT and it was associated with increased ET expression.¹⁷

A combined assessment of NO and ET, which have antagonistic vascular effects, was performed in one study, but with a population of children with primary arterial hypotension and healthy children.⁹ With a cannulated forearm vein for blood sample collection and after a 10-minute rest period in the supine position, the children underwent cardiac monitoring, periodic blood pressure checking and blood collection for

NO and ET measurement during active standing. There was a greater increase in NO/ET ratio in children with primary artery hypotension than in healthy children. However, the sample was not VVS patients and all participants were children.

Additionally, in the present study, the HRV analysis was performed. Sympathetic activation and decreased vagal action, with a resulting increase in the LF/HF ratio, have also been reported by other authors.⁴²⁻⁴⁴


Therefore, despite the evidence of changes in neurohormones or vasoactive substances during the event of VVS, their participation in the pathophysiology of this reflex syncope is not well elucidated.⁴⁵ Future studies, with a larger number of patients, may evaluate the inclusion of the measurement of vasoactive biomarkers to increase specificity and sensitivity in the diagnosis of VVS. In addition, they can provide additional information for specific therapies.

There are some limitations to this study. The genetic evaluation of patients was not performed, since the 4A allele is associated with increased ET expression. Additional measurements of non-invasive hemodynamic parameters such as cardiac output, stroke volume and peripheral vascular resistance were not performed. These measures could better differentiate whether the exaggerated vasodilation would be related to the imbalance of vasoactive biomarkers.

Conclusions

There was no difference between the case group and the control group, matched by gender and age, regarding biomarkers and HRV, in the supine position. There was an increase in plasma NOx values after tilting in the study group population, with no statistically significant increase in the case group. In contrast, there was an increase in ET during HUTT only in the case group. Therefore, this study demonstrated an imbalance between vasodilation and vasoconstriction in VVS patients undergoing passive orthostatic stress, expressed by comparative values of plasmatic vasoactive biomarkers with antagonist vascular effects.

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