Neurally mediated syncope: Is it really an endothelial dysfunction?

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Abstract

Objective: Syncope is a common problem in children and adolescents. Neurally mediated syncope is the most frequent form of this disorder. Although several studies have evaluated the pathophysiology of neurally mediated syncope, it is still not completely understood.

Methods: We performed a cross-sectional study that included 27 patients aged 5–20 years with unexplained syncope and 30 healthy subjects as a control group. All subjects in both groups were assessed for endothelial function by investigating the following physical and chemical factors: flow-mediated dilation (FMD), intima-media thickness (IMT), circulating vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM)], and endothelial leucocyte adhesion molecule (E-selectin), as well as epinephrine and norepinephrine. The data were statistically analyzed utilizing the SPSS 20.Significant differences between the groups in terms of mean scores were assessed using an independent sample t-test.

Results: Mean FMD was significantly higher in the syncope case group than in the control group (p=0.028). There was no significant difference in IMT between the two groups; however, mean levels of ICAM (p=0.02) and VCAM (p=0.008) were significantly higher in the case group than in the control group. The levels of E-selectin also increased in the case group, but not to a statistically significant extent. The mean levels of epinephrine (p=0.01) were significantly lower in the case group than in the control group, and the level of norepinephrine serum decreased slightly, but not significantly, in the syncope patients.

Conclusion: Our results showed that an endothelial dysfunction or augmented endothelial function might exist in patients with neurally mediated syncope. *(Anatol J Cardiol 2016; 16: 701-6)*

Keywords: neurally mediated syncope, augmented endothelial function

Introduction

Syncope is defined as a transient, self-limited loss of consciousness with an inability to maintain postural tone that is followed by spontaneous recovery (1). Up to 15% of children experience at least one episode of syncope before the end of adolescence. In a population-based study, neurally mediated syncope (NMS), previously known as vasovagal syncope, was reported as the most frequent type of syncope (75%) (2). Specifically, NMS is a common form of orthostatic intolerance (OI) in which symptoms are worsened by an upright posture and improved when supine. When the brain receives too little blood flow due to long periods of standing, patients may experience symptoms such as lightheadedness, dizziness, dimming of vision, nausea, or vomiting. Other subtypes of OI include orthostatic hypotension (OH) and postural orthostatic tachycardia syndrome (POTS). OH is defined as a drop in systolic blood pressure of at least 20 mm Hg or a drop in diastolic blood pressure of 10 mm Hg within 3 min of being upright compared with the relative measures when lying flat. POTS is defined as an exaggerated increase in heart rate (by 30 beats per min for adults or 40 beat per min for adolescents) within 10 min of standing. Palpitation is common in patients with POTS (3, 4). These signs and symptoms may result in syncope or fainting if patients experience an episode of transient loss of consciousness. A tilt table test, which is used to produce and observe these signs and symptoms, can be applied for diagnosis and also to specify and simplify treatment.

Sympathetic responses in subjects susceptible to NMS are heterogeneous (5). Systemic blood pressure is determined by cardiac output and total peripheral vascular resistance, and a fall in either can cause syncope; however, a combination of both mechanisms is often present, which causes vasodilatation and bradycardia manifesting as vasodepressor, mixed, or cardioinhibitory types of NMS (6). Shear stress is the main physiological stimulus that causes the endothelium to release vasoactive factors and regulate vascular tone. The endothelium responds dynamically to this stimulus by releasing Nitric Oxide (NO) and thus causing flow-mediated vasodilation (FMD). FMD can be evaluated using ultrasound imaging. This includes the measure-

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ment of the change in the diameter of an artery (typically brachial) in response to rise in blood flow induced by a period of ischemia applied to the distal part of the limb. The brachial artery dilation due to increased shear stress has been demonstrated to significantly correlate with invasive testing of brachial and coronary endothelial function, as well as carotid artery intima-media thickness (IMT) (7).

An alternative method for evaluating endothelial function involves measurement of biomarkers for endothelial activation and dysfunction, namely circulating vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and endothelial leucocyte adhesion molecule (E-selectin) (8).

Monitoring endothelial dysfunction has diagnostic, preventive, and therapeutic values. A multifaceted approach should be applied when assessing endothelial dysfunction; limiting the diagnosis of endothelial dysfunction to one or two markers may under represent the diversity of endothelial functions leading to false conclusions (9).

Prolonged orthostatic stress induces major changes in hemodynamic and autonomic nervous system response. However, neurohormonal response to acute and prolonged orthostatic stress that may induce NMS is currently unclear (10).

The aim of this study was to address these research gaps by evaluating endothelial function in patients with NMS using both physical and biochemical markers, as well as by measuring the serum levels of epinephrine and norepinephrine in these patients to assess catecholamine changes relative to those in a control group.

Methods

The study was approved by our local ethics committee, and all subjects (or their parents) gave written informed consent before any procedure was initiated.

We performed a cross-sectional study that included 27 patients (12 males and 15 females) with a mean age of 12.24±4.37 years (range: 5–20 years) who were referred for the evaluation of unexplained syncope and 30 healthy subjects (15 males and 15 females) with a mean age of 12.83±6.12 years (range: 5–20 years) as a control group. The groups were matched in age, sex, and body mass index (BMI).

After the patients' history was taken and physical exams were conducted, the patients underwent a head-up tilt table test. Those who had a positive result in this test were selected as the syncope case group.

Patients with a history of true NMS or seizure, abnormal physical exam results, abnormal ECG, EEG, or echocardiography, psychological problems and those with a negative tilt test result or any associated diseases that may influence the study goals were excluded from the study.

Assessment of brachial artery FMD

The brachial artery FMD was assessed as previously described (11, 12) after approximately 6 h of fasting. Briefly, it was assessed using an ultrasound system (MEDISON EKO 7 with a 7-Hz transducer) equipped with vascular software for two-dimensional imaging based on the guidelines of the International Brachial Artery Reactivity Task Force (13).

To create a flow stimulus in the brachial artery, a blood pressure cuff was first placed on the forearm. A baseline rest image was acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume. Thereafter, the cuff was inflated to at least 50 mm Hg above the systolic pressure to occlude arterial inflow for 5 min. Subsequently, the cuff was deflated. A mid-artery pulsed Doppler signal was obtained after 60 s cuff release to assess hyperemic velocity. The brachial artery diameter was measured at the same time in the cardiac cycle using ECG gating during image acquisition. The onset of the R-wave was used to identify end diastole and to optimize the accuracy of measurements; an average derived from multiple diameter measurements determined along a segment of the vessel was used.

FMD percentage was calculated using the following formula:

FMD% = (D2–D1)/D1x100

where D2 is the maximum diameter of the artery and D1 is the measured baseline diameter (14).

Determination of IMT

To assess IMT, the anterior (near) and posterior (far) walls of the carotid artery were displayed as two bright white lines separated by a hypoechogenic space on a longitudinal, two-dimensional ultrasound image of the carotid artery. The distance between the leading edge of the first bright line of the far wall (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) indicated IMT. We took three measurements of IMT of the right common carotid artery just before the bifurcation; the mean IMT value was then used in our study.

Measurements of endothelial biomarkers

Five milliliters of blood were drawn in the fasting state from both the case and control groups when the patients were in a supine position. In the case group, blood was taken before the initiation of the tilt test. The collected blood samples were centrifuged (10,000 g), and the resulting plasma was aliquoted and stored at -70°C. The fasting plasma levels of ICAM, VCAM, and E-selectin were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Boster Immunoleader, Pleasanton, CA, USA), with the same procedure used in all three tests. Briefly, the working dilution was set as 1:100 (1 µL of sample added to 99 µL of diluent buffer). Subsequently, 100 µL of standards or samples were added to wells and incubated at 37°C for 1 h. After removing the well contents, 100 µL of biotinylated anti-human antibody was added to the wells, and the plates were incubated again at 37°C for 1 h. After three washes, 100 µL of avidin–biotin–peroxidase complex working solution was added to the wells and incubated at 37°C for 30 min. The wells were then washed, 90 µL of

Table 1. Baseline characteristics of case and control groups

	Case (n=27)	Control (n=30)	*P*
Age, years	12.24±4.37	12.83±6.12	*0.744
Gender (M/F), n	12/15	15/15	*0.678
Weight, kg	47.48±19.79	44.56±21.65	*0.514
Height, cm	149.68±19.79	146.74±18.54	*0.728
BMI, kg/m^2	20.22±5.24	19.93±5.86	*0.918
SBP, mm Hg	101.48±6.76	103.33±10.11	*0.846
DBP, mm Hg	58.15±7.74	68.16±5.64	*0.325
Hemoglobin, g/dL	12.75±1.12	12.65±2.13	*0.843
Hematocrit, %	38.21±3.54	37.42±3.86	*0.926
FBS, mg/dL	90.92±7.19	88.73±8.42	*0.664
Triglyceride, mg/dL	76.92±28.04	80.21±30.23	*0.641
TC, mg/dL	144.96±28.52	140.02±31.24	*0.537
LDL-c, mg/dL	82.32±21.18	85.54±26.65	*0.848
HDL-c, mg/dL	45.24±7.54	42.86±6.42	*0.755

All variables are reported as the mean±standard deviation
*Not significant (p>0.05). The normality of all data was assessed with the Kolmogorov–Smirnov test for normally distributed data and the nonparametric Mann–Whitney U test for non-normal data.
BMC - body mass index; DBP - diastolic blood pressure; FBS - fasting blood glucose; HDL-c - high density lipoprotein cholesterol; LDL-c - low density lipoprotein cholesterol; SBP - systolic blood pressure; TC - total cholesterol

3,3',5,5'-Tetramethylbenzidine (TMB) color developing agent was added to each well, and the plates were incubated at 37°C for 30 min. Finally, after the addition of 100 µL of stop solution, the absorbance of each well was read at 450 nm. The amounts of ICAM, VCAM, and E-selectin were calculated based on standard curves (15).

Determination of norepinephrine and epinephrine levels

The fasting serum levels of norepinephrine and epinephrine were measured using a BI-CAT ELISA Kit (DLD Diagnostika GmbH, Adlerhost, Hamburg, Germany), with measurements taken from patients in both groups (before the initiation of the tilt test in the case group) in the supine position. The test was conducted in two separate steps. First, samples were prepared. Briefly, 300 µL of sample and 50 µL of extraction buffer were added to an extraction plate. After 1 h incubation and washing steps, 150 µL of acylation buffer and 50 µL of acylation reagent were added to the wells. After 20 min incubation at room temperature and a washing step, 200 µL of 0.025 M HCL was added to the wells. After another 20 min incubation at room temperature, the supernatant was removed for the second step, i.e., the ELISA procedure described below.

ELISA procedure

For the measurement of norepinephrine, 20 µL of enzyme mix and 15 µL of standards or samples were added to the wells. After 30 min incubation at room temperature, 100 µL of adrenaline–antiserum was added to the wells, and they were incubated overnight at 4°C. After four washes, 100 µL of POD-conjugate was added to the wells, and they were incubated for 30 min at room temperature. Subsequently, 100 µL of ELISA substrate was added to the wells, and following color development (20 min), 100 µL of stop solution was added. The optical density was then immediately read at 450 nm. For the measurement of epinephrine, 20 µL of enzyme mix and 100 µL of standards or samples were added to the wells. After 30 min incubation at room temperature, 100 µL of adrenaline–antiserum was added to the wells and they were incubated overnight at 4°C. After four washes, 100 µL of POD-conjugate was added to the wells and they were incubated for 30 min at room temperature. Subsequently, 100 µL of ELISA substrate was added to the wells, and following color development (20 min), 100 µL of stop solution was added. The optical density was then immediately read at 450 nm. The amount of norepinephrine and epinephrine were calculated based on standard curves (16).

Statistical analysis

The data were statistically analyzed utilizing the Social Package of Statistical Science software (SPSS version 20; IBM, Chicago, IL, USA). All quantitative data were summarized using mean±standard deviation, and qualitative data using numbers. Significant differences between the groups in terms of mean scores were assessed using an independent sample t-test. The normality of all data was assessed with the Kolmogorov–Smirnov test for normally distributed data and the nonparametric Mann–Whitney U test for non-normal data. Statistical significance was set at $p<0.05$.

Results

In this study, 57 subjects aged 5–20 years, 27 patients with NMS and 30 healthy control subjects, were matched by age, sex, and BMI. All 57 patients were checked for anemia, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia to exclude other causes of endothelial dysfunction. All participants lived in a similar environment. Table 1 summarizes the baseline characteristics of the included subjects.

Biophysical characteristics

FMD, D1 (the vessel diameter at rest), and D2 (the vessel diameter after hyperemia) were measured as biophysical characteristics. Statistical analysis showed that mean D1 was higher in patients with syncope than in patients in the control group, but the difference was not statistically significant (2.89±0.51 mm vs. 2.71±0.40 mm, p=0.138). However, the mean D2 was significantly higher in the case group than in the control group (p=0.008). Additionally, mean FMD was significantly higher in the case group than in the control group (p=0.028). There was no significant difference in IMT between the two groups.

Biochemical characteristics

ICAM, VCAM E-selectin, epinephrine, and norepinephrine were measured as biochemical characteristics. Mean levels of ICAM (p=0.02) and VCAM (p=0.008) were significantly higher in the case

Table 2. Biophysical and biochemical characteristics of endothelial function from subjects with and without neurally mediated syncope

	Case (n=27)	Control (n=30)	*P*
D1, mm	2.89±0.51	2.70±0.40	0.128
D2, mm	3.29±0.50	2.95±0.44	0.008*
FMD, %	8.58±3.62	5.26±2.26	0.001*
IMT, mm	0.285±0.04	0.298±0.05	0.327
ICAM, ng/mL	93.64±52.01	58.95±42.85	0.022*
VCAM, ng/mL	115.32±62.36	72.22±51.23	0.008*
E-selectin, ng/mL	39.90±4.25	39.93±2.87	0.413
Epinephrine, ng/mL	0.36±0.3	0.76±0.55	0.013*
Norepinephrine, ng/mL	1.05±0.96	1.04±1.17	0.961

All variables are reported as the mean±standard deviation
*P<0.05, statistically significant. Significant differences between the groups in terms of mean scores were assessed using an independent sample t-test. D1 - Vessel diameter at rest; D2 - Vessel diameter after hyperemia; FMD - flow mediated dilation; IAM - intracellular adhesion molecule; IMT - intima media thickness; VCAM - vascular cell adhesion molecule

group than in the control group; however, although levels of E-selectin increased in the case group, the increase was not statistically significant. Mean levels of epinephrine (p=0.01) were significantly lower in the case group than in the control group; norepinephrine serum levels were also slightly lower, but not significantly so. Table 2 shows the biophysical and biochemical characteristics of endothelial function from subjects in both groups.

Discussion

The main findings of this study included evidence for augmented FMD in association with significantly increased serum levels of biochemical markers of endothelial function (ICAM and VCAM) and significantly decreased serum levels of epinephrine in patients with NMS. In addition, we found that resting brachial artery diameters were increased in patients with NMS compared with those in control patients.

FMD is known to increase in response to orthostatic stress. The augmented endothelial function and the abnormal vasodilation of peripheral arteries play an important role in the development of NMS and POTS in young subjects (17-21).

There is still controversy regarding the normal values for FMD in children and adults. There are currently few large-scale studies published on FMD in children; however, in the largest population-based FMD study, which comprised 333 British children, the mean FMD was 4.7±4.3%, whereas the mean maximal FMD was 7.7±4% in a sample of 105 healthy Finnish children (7). Similar to our results, in a recent study by Sabri et al. (11), the mean FMD was 6.53±2.36 in healthy Iranian children from the same geographical area.

Environmental factors, such as air pollution and living in urban areas or large cities, may affect FMD (22), and this may explain the wide normal range for FMD in different populations. Santini et al. (23) evaluated endothelial function in 17 patients with NMS aged 35.8±16.0 years and concluded that NMS is characterized by a marked and sustained endothelial-independent vasodilation. FMD was within the normal range in the NMS subjects their study, and values were not significantly different from those measured in their control group. They also found that the resting values of brachial artery diameter were lower in syncope patients than in controls. The different results observed in our study and that of Santini et al. (23) might have arisen because of the lower number of cases and/or the older patients evaluated in their study, or perhaps because of the dissimilarity between populations and environmental factors.

To assess the possibility of abnormal or augmented endothelial function in NMS, we investigated other markers that are indicators of endothelial dysfunction. Our results showed that there are augmented serum levels of biochemical markers.

Ponthieux et al. (24) showed that serum ICAM-1 and E-, P-, and L-selectin levels are age-dependent in childhood and sex-dependent in adulthood. Their findings emphasize the necessity to use age- (for children) and sex-matched (for adults) controls to evaluate the circulating concentrations of adhesion molecules. Thus, we matched our patients in the case and control groups for age, sex, and BMI.

To the best of our knowledge, our study is the first to evaluate the biochemical markers ICAM and VCAM in syncope patients as indicators of endothelial function.

Several studies have suggested that the vasodilatation seen in NMS results from a withdrawal of sympathetic tone. There is evidence of reduced cardiac and renal norepinephrine overflow in patients who fainted during cardiac catheterization (5).

Our data also showed markedly decreased serum levels of epinephrine and slightly decreased level of norepinephrine in children with syncope compared with the levels observed in the normal healthy children. Several studies have examined neurohormonal and catecholamine changes during syncope in adult patients with syncope (5, 25-27). Leonelli et al. (28) examined differences in the mechanisms of head-up tilt-induced syncope between normal controls and patients with NMS with a mean age 30 years: eight HUT-negative volunteers, eight HUT-positive volunteers, and 15 patients with NMS. Epinephrine and norepinephrine plasma levels were measured at baseline and at regular 8-min intervals during the test. Epinephrine and norepinephrine values were similar in the three groups at baseline, and their plasma levels increased during HUT in every subject. Patients with NMS showed a six-fold increase in epinephrine above the baseline value at the onset of bradycardia and hypotension (28). However, as the authors acknowledged, their conclusions were based on a relatively small preselected sample of subjects. We measured epinephrine and norepinephrine levels during rest and not during the stress-inducing tilt test, because of the reduced venous return causing catecholamine secretion.

In another study by Perry et al. (29), formal autonomic function testing was performed in 22 patients (aged 7–18 years) with

recurrent syncope; 14 of the 22 patients had reproduction of syncope or symptoms during testing. Similar to our results, serum norepinephrine levels were significantly lower in patients with a positive test than in those with a negative test in both in the supine and standing positions, but there were no differences in serum epinephrine levels between the two groups.

Balaji et al. (30) evaluated 162 subjects aged 1–20 years with syncope and reported a significant difference in the supine epinephrine level, with the orthostatic positive group exhibiting a higher level than the orthostatic negative group. According to the authors, this (discrepancy between these two latest studies) may reflect a difference in the autonomic tone at rest between the two groups (30). None of these studies compared their results with normal healthy children.

Saar and Gordon (31) evaluated the variability of plasma catecholamine levels. Age and posture were important determinants for epinephrine and norepinephrine levels, whereas the time of day was less important. Furthermore, the secretion of epinephrine and norepinephrine are known to increase with smoking and decrease with increased BMI (32). Thus, we performed our study using similar conditions, such as fasting and the supine position, in both groups, as well as matching of their BMI and ensuring that all patients were non-smokers. Variations in age, posture, and/or BMI can be responsible for the dissimilarity of epinephrine and norepinephrine levels in different studies.

Von Kanel et al. (33) showed the modulation of circulating ICAM levels via the sympathetic nerve and adrenergic function, and serum ICAM levels have been shown to increase with acute stress. Low levels of serum catecholamines were observed among our patients, although serum biochemical levels increased significantly in the case group. Hence, it seems that these increments are due to this endothelialopathy.

Study limitations

Although we evaluated biochemical markers and FMD in patients with NMS, we did not check them again in a follow up or check their response to treatment. Additionally, there might be a lower catecholamine level at rest in patients with NMS. Thus, more studies should be conducted to observe these parameters in a follow-up period for the tested patients.

Conclusion

Our findings provide some evidence for endothelial dysfunction or augmentation in children and adolescents with NMS; specifically, increased basic vascular dimensions and FMD of peripheral arteries were observed in association with increased serum levels of ICAM and VCAM.

Conflict of interest: None declared.

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