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THE INFLUENCE OF AUTONOMIC NEUROPATHY ON COUGH REFLEX SENSITIVITY IN CHILDREN WITH DIABETES MELLITUS TYPE 1

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Diabetic autonomic neuropathy (DAN) is manifested by dysfunction of one or more organ systems. Its subclinical form (sDAN) can be recognized with the use of noninvasive cardiovascular reflex tests. As the cough reflex is mediated *via* autonomic nervous system, there is a reason to suppose that it can also be changed due to presence of sDAN. The aim of the present study was to assess cough reflex sensitivity (CRS) in diabetic children with and without sDAN. A CRS test was performed in 35 children suffering from diabetes mellitus type 1 and the results were compared with those from age-matched 27 healthy children. Cough was induced by inhalation of capsaicin aerosol in doubling concentrations (0.61-1250 $\mu\text{mol/l}$) for 400 ms each. CRS was defined as the lowest capsaicin concentration that evoked 2 or more coughs (C2 parameter) and 5 or more coughs (C5 parameter). We found that CRS in the whole group of diabetic children was not significantly different from that in healthy children [diabetic children - C2: 75.1 $\mu\text{mol/l}$ (95% CI: 42.0-134.2 $\mu\text{mol/l}$) vs. healthy children - C2: 72.4 $\mu\text{mol/l}$ (95% CI: 75.7-644.8 $\mu\text{mol/l}$)]. However, a significant decrease ($P=0.005$) in CRS was found in diabetic children with sDAN [$n=12$; C2: 221.0 $\mu\text{mol/l}$ (95% CI: 75.7-644.8 $\mu\text{mol/l}$)] compared with diabetic children without sDAN [$n=23$; C2: 42.7 $\mu\text{mol/l}$ (95% CI: 23.1-79.0 $\mu\text{mol/l}$)]. We conclude that testing cough reflex sensitivity might be a way to establish the presence of diabetic neuropathy.

Key words: *autonomic neuropathy, children, cough reflex sensitivity, diabetes mellitus*

INTRODUCTION

Diabetes mellitus type 1 is a metabolic disease that affects each organ system in the body. Its prevalence is highest in children and young people. One of the

most serious chronic complications of diabetes is neuropathy. It involves sensory, motor, and autonomic innervation. Diabetic autonomic neuropathy (DAN) can affect any visceral organ of the body. It is associated with increased morbidity and mortality risk (1-3). DAN is characterized by widespread neuronal degeneration of sympathetic and parasympathetic nerve fibres. It may manifest clinically early in the course of diabetes, but more often DAN starts as a subtle functional alteration of an organ detectable only by specialized tests, as is the case with the cardiovascular system.

Impaired neural control of breathing in diabetics can also be related to autonomic neuropathy (4). Diabetics suffer from sleep-related breathing disorders more frequently than healthy people (5). Although some authors report normal pulmonary function (6), most of them find abnormalities in lung volumes (7-9), breathing pattern (9), diffusing capacity (7, 8, 10-13), and respiratory system reactivity to different stimuli in diabetics (14-20).

Efferent autonomic dysfunction can be revealed by noninvasive cardiovascular tests (21) as early as within two years from the onset of diabetes. It is unknown whether dysfunction of the afferent part of the cardiovascular system could also be revealed so early. Information on respiratory function in the early phase of diabetes is scanty. It is well known that airways and lungs are abundantly supplied by afferents from the vagus nerves, including those coming from „airway cough receptors“. Vagal afferents belong to the longest in the autonomic nervous system. As DAN manifests first in longer nerves (21, 22), we suppose that impairment of the cough reflex can be present from early on in the pathogenesis of diabetes. Therefore, in the present study we tested the hypothesis that cough reflex could be down-regulated in diabetes. We assessed cough reflex sensitivity (CRS) in a population of diabetic children with and without subclinical signs of diabetic autonomic neuropathy (sDAN).

MATERIAL AND METHODS

Study population

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia. Informed consent was obtained from the parents of all children participating in the study. Children found to be eligible for inclusion in the study were informed of the investigators' intention to study CRS for scientific purposes. The parents of the selected children obtained the same information, too. Nobody of them was aware of the hypothesis underlying the study nor was given any details about the possible results.

The study sample contained 35 children (mean age 15.3 ± 2 yr; 12 boys, 23 girls) suffering from diabetes mellitus type 1. All the patients were recruited from diabetics that regularly attended an outpatient clinic of a teaching hospital in Martin, Slovakia. As the first step, the patient's file was screened by an investigator for the inclusion criteria in the study, as defined below. This investigator was blinded to all information related to the aims and supposed results of the study. The children were excluded from the study group if they had severe comorbidity (e.g., bronchial asthma,

congestive heart failure, history of allergic diseases), symptoms of acute respiratory diseases during last two weeks before CRS testing, acute diabetic complications (e.g., ketoacidosis, hypoglycaemia), history of any other condition possibly associated with cardiovascular autonomic neuropathy (e.g., vitamin B12 deficiency) or were receiving ACE inhibitors. The children who agreed to participate in the study were tested for cardiovascular autonomic neuropathy, as defined below, and assigned to the group positive or negative for subclinical form of it. A number of metabolic parameters related to the level of diabetes compensation (glycosylated haemoglobin - HbA_{1c}, serum cholesterol, serum creatinine), body mass index (BMI), age at the onset of diabetes and its duration were recorded. None of the children had symptoms that could be related to overt autonomic neuropathy.

Twenty seven healthy, age-matched non-diabetic volunteers (mean age 13.2 yr) were recruited from primary and high schools in the Žilina and Martin regions in Slovakia.

Cough reflex sensitivity test (CRS)

CRS was tested with capsaicin according to the method of Chang et al (23), with some modifications elaborated in our laboratory (24). Each subject inhaled aerosol of a control solution (0.9% saline), followed by inhalation of 12 capsaicin aerosol concentrations in doubling doses (0.61, 1.22, 2.44, 4.88, 9.76, 19.53, 39.06, 78.12, 156.25, 312.5, 625, and 1250 $\mu\text{mol/l}$) at 1 min intervals. Aerosol of inhaled solutions was created by a Provo Jet nebulizer (Ganshorn Medizin Elektronik, Niederlauer, Germany) driven by compressed air, and connected to a breath-actuated dosimeter set. Time of aerosol inhalation was set for 400 ms. The end-point of the test was when 5 coughs were induced or when the maximum concentration of capsaicin (1250 $\mu\text{mol/l}$) was used. CRS was defined as the lowest capsaicin concentration that evoked 2 coughs (parameter C2) or 5 coughs (parameter C5). During inhalation of control solution and each concentration of capsaicin, we counted and recorded the number of coughs evoked during 30 s after actuation of the dosimeter. If the child did not cough 2 or 5 and more times during the testing, the double of the last concentration, i.e., 2500 $\mu\text{mol/l}$, was considered as the C2 or C5 parameter (25). CRS test was done on the same day as the cardiovascular autonomic function test was performed.

Cardiovascular autonomic function test

For the purpose of this study we decided to determine changes in heart rate variability (HRV) to deep breathing as a test of cardiovascular autonomic function (26, 27), which was performed in all diabetic children. In the children resting in the supine position, at least 2 h after a meal, heart rate was recorded and interpreted by VariaPuls TF3 system (Sima Media, Czech Republic). Children were instructed to breath deeply at a frequency of 6 cycles min^{-1} (a rate that produces maximum variation in heart rate) paced by a metronome.

In each respiratory cycle, the longest R-R interval during expiration and the shortest one during inspiration were selected. The results of this test were expressed as the ratio between the longest and the shortest R-R intervals averaged over three consecutive respiratory cycles (E/I ratio). The values of the E/I ratio in healthy age-matched population were taken from the study that was done by Tonhajzerova et al (28). The children from that study came from the same region as the diabetic children used in the present study. An abnormal result (subclinical DAN) was defined as HRV to deep breathing below that of the 10th percentile of the normal age-matched population.

Statistical analysis

Data analysis was performed using SYSTAT 11. The values of C2 parameter were normally distributed after natural logarithm (ln) transformation. The differences in C2 between the studied groups were analyzed using a *t*-test. Results of the C2 parameter were given as geometric means

(95% confidence interval). Not normally distributed data (C5 parameter) were analysed using Mann-Whitney nonparametric test and were given as medians and 25-75% interquartile range. Differences in CRS between the studied groups were considered significant at a value of $P \leq 0.05$.

The relationship between the parameters of the CRS test (C2 and C5 parameter) and E/I ratio was tested using Spearman's nonparametric rank correlation coefficient (r_s). The following correlation coefficients were considered significant: moderate if the value of r_s was 0.3-0.5, strong when r_s was 0.5-0.7, very strong with r_s between 0.7-0.9, and nearly perfect if r_s was 0.9-1.0 (29).

RESULTS

Assessment of diabetic autonomic neuropathy

According to the results of the E/I ratio, diabetic children were divided into two subgroups: „noDAN“ group (n=23) and „subclinical DAN“ (sDAN) group (n=12). Clinical characteristics of all diabetic children are shown in *Table 1*. There were no substantial differences between the two subgroups in the levels of HbA_{1c}, serum cholesterol, and serum creatinine as well as in the duration of diabetes and age at which the disease began. A significant difference was observed in BMI between the two subgroups of diabetic children. BMI in the sDAN group was 19.6 (95% CI: 17.9-21.2), which was lower ($P=0.027$) than in the noDAN group - 22.2 (95% CI: 20.4-24.0).

Cough reflex sensitivity

Parameter C2 was similar when compared between the whole diabetic [75.1 $\mu\text{mol/l}$ (95% CI: 42.0 – 134.2 $\mu\text{mol/l}$)] and healthy children [72.4 $\mu\text{mol/l}$ (95% CI: 25.7 – 204.4 $\mu\text{mol/l}$)] groups.

Table 1. Characteristics of diabetic children.

	All children	noDAN	sDAN
n	35	23	12
Sex (M/F)	12/23	8/15	4/8
Age at onset of diabetes (yr)	9.4 \pm 4.7	9.2 \pm 4.9	9.8 \pm 4.3
Duration of diabetes (yr)	6.5 \pm 4.3	6.5 \pm 4.4	6.5 \pm 4.2
Age (yr)	15.3 \pm 2.0	15.1 \pm 2.0	15.7 \pm 1.9
Height (cm)	165.0 \pm 11.1	164.7 \pm 11.8	166.4 \pm 10.1
Weight (kg)	58.4 \pm 14.4	60.9 \pm 16.1	54.0 \pm 9.6
BMI (kg/m ²)	21.3 \pm 3.7	22.2 \pm 4.0	19.5 \pm 2.6*
HbA _{1c} (%)	11.3 \pm 2.1	11.3 \pm 1.9	11.9 \pm 2.4
Serum cholesterol (mmol/l)	4.9 \pm 1.1	4.9 \pm 1.5	4.7 \pm 0.7
Serum creatinine ($\mu\text{mol/l}$)	73.7 \pm 11.9	75.1 \pm 13.0	71.2 \pm 9.3

* $P= 0.027$ - statistically significant difference between the noDAN and sDAN groups.

Comparison of CRS in the two subgroups of diabetic children showed (Fig. 1) that the C2 parameter in the „sDAN“ subgroup was significantly higher [221.0 $\mu\text{mol/l}$ (95% CI: 75.7 – 644.8 $\mu\text{mol/l}$)] than that in the noDAN group [42.7 $\mu\text{mol/l}$ (95% CI: 23.1 – 79.0 $\mu\text{mol/l}$)] ($P=0.005$). However, there was no significant difference in the C2 value between the control group and either the sDAN or noDAN diabetic subgroup (Fig. 1).

We did not find a significant difference in the C5 value (median and 25-75% interquartile range) between the whole diabetic [2500 $\mu\text{mol/l}$ (625-2500 $\mu\text{mol/l}$)] and healthy children groups [2500 $\mu\text{mol/l}$ (938 – 2500 $\mu\text{mol/l}$)]. A significant increase in the C5 value and thus a reduction in CRS ($P=0.003$) was observed in the children with sDAN [2500 $\mu\text{mol/l}$ (2500-2500 $\mu\text{mol/l}$)] compared with those in the noDAN group [1250 $\mu\text{mol/l}$ (625-2500 $\mu\text{mol/l}$)] (Fig. 2).

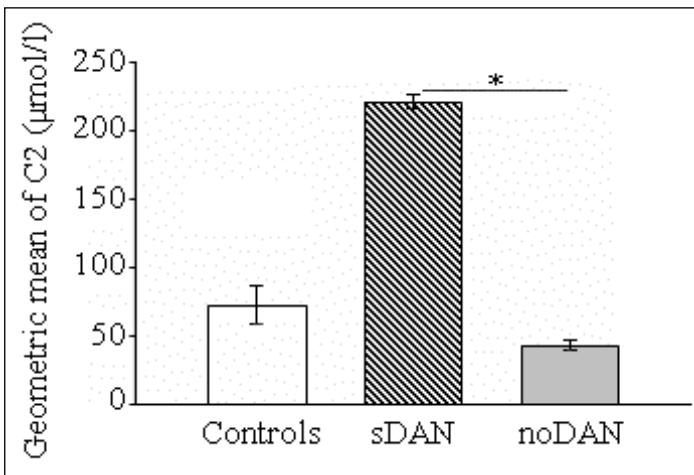


Fig. 1. Summary bar chart representing geometric means of C2 ($\mu\text{mol/l}$) of separate study groups; * $P=0.005$.

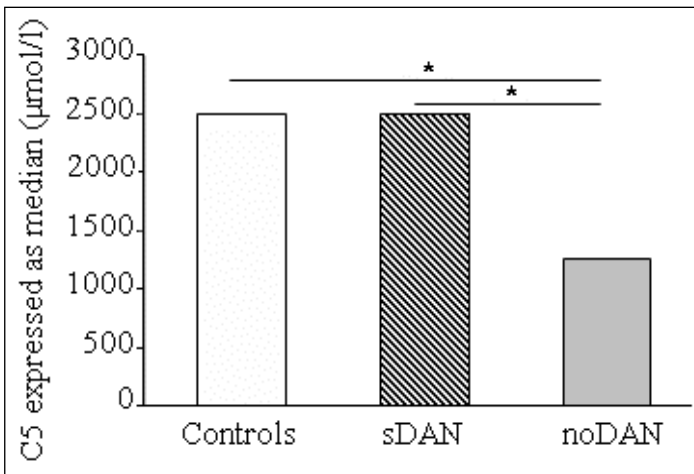


Fig. 2. Summary bar chart representing parameter C5 expressed as median ($\mu\text{mol/l}$) of separate study groups; * $P=0.003$.

Relationship between CRS and HRV parameters

A correlation was observed between parameters C2 and E/I ($r_s=-0.58$; $P=0.0007$) (Fig. 3). A moderate but significant negative correlation also was observed between parameter C5 and E/I ($r_s=-0.37$; $P=0.03$) (Fig. 4).

The C2 and C5 values correlated neither with the duration of diabetes nor with the quality of metabolic control, as determined by a concentration of glycosylated hemoglobin. Moderate, significant correlations were observed between C2 and BMI ($r_s=0.39$; $P=0.024$) and between C5 and BMI ($r_s=0.34$; $P=0.048$) (Table 2).

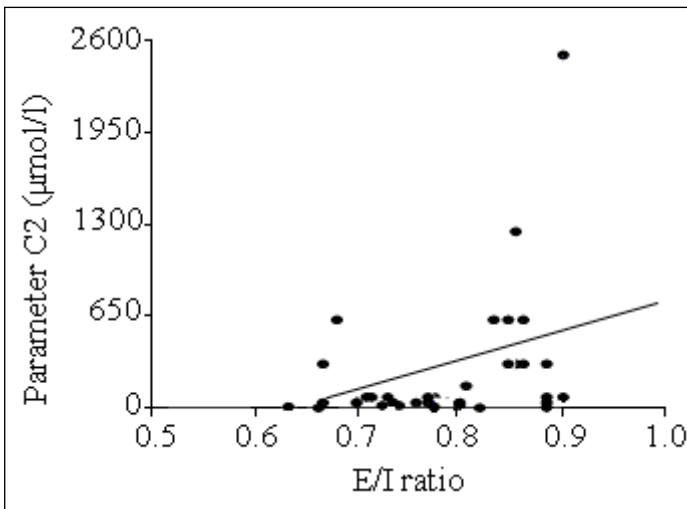


Fig. 3. Scatter plot for the relationship between parameter C2 and E/I ratio.

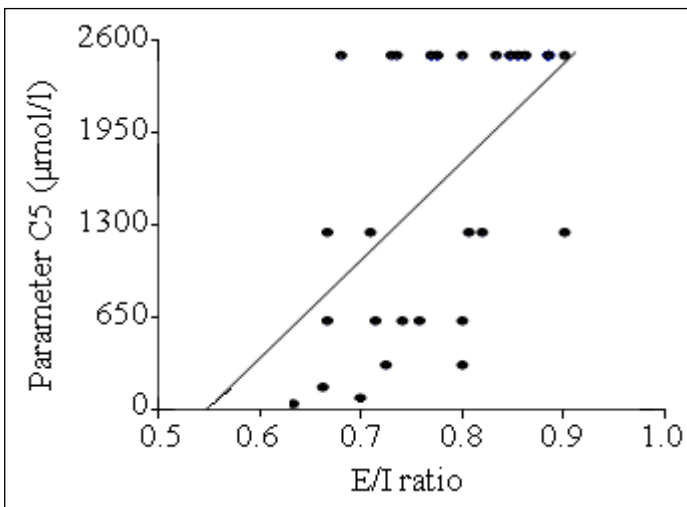


Fig. 4. Scatter plot of the relationship between parameter C5 and E/I ratio.

Table 2. Spearman's correlation coefficients and significance of correlations between CRS parameters and clinical characteristics of diabetic patients.

	C2		C5	
	r	P	r	P
Age at onset of diabetes (yr)	r=0.1	P>0.05	r=0.07	P>0.05
Duration of diabetes (yr)	r=-0.04	P>0.05	r=0.01	P>0.05
BMI (kg/m ²)	r=0.39	P<0.05	r=0.34	P<0.05
HbA _{1c} (%)	r=-0.08	P>0.05	r=0.06	P>0.05
Serum cholesterol (mmol/l)	r=0.29	P>0.05	r=0.03	P>0.05
Serum creatinine (μmol/l)	r=-0.28	P>0.05	r=-0.07	P>0.05

DISCUSSION

The most important result of the study is that the sensitivity of the cough reflex was, in general, decreased in diabetic children with subclinical autonomic neuropathy, as opposed to children without neuropathy. A second interesting finding is a good correlation between cough reflex sensitivity, expressed by C2 and C5 parameters, and heart rate variability, expressed by the ratio of the longest R-R intervals during expiration and the shortest ones during inspiration, the E/I index. The decreased cough reflex sensitivity confirmed our hypothesis that diabetic neuropathy downregulates cough in children. More than 10 years ago, two studies have been published (19, 20) which showed a reduction of CRS to citric acid in adult diabetics. Our findings showed that CRS also is reduced in diabetic children and the reduction is present at a time when DAN is at a subclinical level. The second finding shows that DAN influences respiratory system function, especially its defensive reflex mechanisms, to the extent similar as that present for the cardiovascular system.

It seems that testing the CRS in diabetic children has a potential to provide information on the starting dysfunction of the respiratory system, as is the case with the cardiovascular autonomic tests that point to the starting dysfunction of the heart. Possibly, in the future the importance of CRS testing for respiratory system autonomic neuropathy could be of similar importance as today are Ewing's tests for the diagnosis of heart autonomic neuropathy.

The question is why there was not a significant difference in the C2 value between the healthy children and the whole group of diabetes. As it is presented in *Fig. 1*, the C2 in diabetics without neuropathy, noDAN, tended even to be lower than that in healthy children. When these data are combined with the data from the diabetic neuropathy group, sDAN, then the difference between the whole diabetic group and healthy children becomes apparently blurred.

It is rather unexpected that the statistical evaluation did not show a significant difference in the C2 value between diabetics with sDAN and healthy children, although this value was evidently higher (*Fig. 1*), and thus cough reflex

sensitivity was lower, in the diabetics. This result cannot be readily explained. We can only offer the following speculative explanations: (i) the CRS test performed in our patients was limited by the concentration of capsaicin used; the highest was 1250 $\mu\text{mol/l}$, which was too low to determine the real value of C2 in some children, particularly in those with sDAN; and (ii) the sample size of children tested was not large enough to reveal a significant difference between the sDAN group and healthy children. Nevertheless, the biological significance of this difference seems important. We suggest that the scale of capsaicin aerosol concentrations used for testing CRS in patients with a suspected decrease in cough reflex sensitivity should be extended upward.

A clear difference between the diabetics with and without sDAN found in our study can be ascribed mainly to dysfunction of the afferent part of the cough reflex arc. We can summarize that there are structural changes in afferents coming from airway cough receptors due to metabolic changes which accompany diabetes. Studies with streptozotocine-induced diabetes in animals reveal peptide changes in autonomic nerves (30). A decrease in the cough response to capsaicin observed in our group of diabetic children with sDAN could be caused by decreased release of neuropeptides from tracheal and bronchial vagal afferents. These segments of airways are densely innervated by CGRP and substance P-containing afferents that originate from the vagus nerve and are involved in mediating the cough reflex.

Abnormality in HRV during deep breathing has been suggested to be a marker of the earliest stage of autonomic neuropathy (31, 32). HRV induced by deep breathing is almost exclusively mediated by the activity of parasympathetic nerve fibres (33), which is early reduced in cardiac neuropathy. Ample evidence indicates that abnormalities in the tests of parasympathetic function typically precede much those in the sympathetic nerves. As the E/I ratio is a reliable measure of the parasympathetic function (21, 34), it was reasonable to choose it for the detection of early stages of cardiac neuropathy.

In the respiratory tract, the CRS test could be a good alternative for testing the afferent part of the parasympathetic nervous system, as the afferent limb of the cough reflex arc goes via a parasympathetic branch of the vagus nerve (35, 36) and the sympathetic system is not directly involved in this reflex.

In view of moderate but significant correlations between HRV and CRS in diabetics with sDAN found in this study we can suggest that the process of autonomic nerve fibres degeneration in the respiratory and cardiovascular systems is likely simultaneous. Beside, we can also suggest that the process of DAN affects almost simultaneously the afferent parasympathetic fibres in the vagus nerve that mediate cough reflex and the efferent ones that mediate HRV during deep breathing. It would be interesting to know whether there is any time difference between the onset of neuropathy in the heart and in the respiratory system.

There are numerous respiratory complications in patients suffering from diabetes. Most of them are subtle and detectable only by sensitive functional tests.

Despite the subclinical intensity, respiratory changes may worsen metabolic compensatory mechanisms in diabetic patients and may further aggravate morbidity and mortality related to diabetes. It is generally accepted that such patients are prone to infectious diseases and diabetes is recognized as an independent risk factor for respiratory tract infections (37, 38). We suppose that downregulation of cough sensitivity, the most important reflex mechanism for cleaning airways, could participate in the genesis of increased susceptibility of diabetics to respiratory infections.

Acknowledgements: We thank Marta Ilovska, Lila Surinova, Lenka Mazurova, Tomas Zatko, Jana Javorkova, Jarmila Kecerova, and Janka Lettrichova for their outstanding assistance in the study realization and the manuscript preparation. This research was supported by Science and Technology Assistance Agency under contract No. APVT-20-005304.

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