ABSTRACT

Introduction: The autonomic nervous system has been related to an increase in heart rate, blood pressure and cardiac output in situations of stress.

Objective: To determine the modifications that the non-linear parameters undergo in the autonomic cardiovascular regulation of the heart rate variability subjected to mental stress through the mental arithmetic test.

Material and Method: A non-observational quasi-experimental study of before-after type without control group was conducted in the Biomedical Basic Sciences Laboratory at Medical school 1, University of Santiago de Cuba, from March 2016 to August 2018. A group of 10 Medical Students constituted the population and sample.

Results: Statistically significant differences between means were found in Heart rate values (p = 0.000), SD1 (p = 0.002), SD2/SD1rate (p = 0.000), Sample Entropy (p = 0.026), Short-term fluctuations α 1 (p = 0.000), Correlation Dimension (p = 0.020), Max line length (p = 0.017),
Recurrence rate (p = 0.007), Determinism (p = 0.003), Multiscale Entropy minimum (p = 0.001), Multiscale Entropy maximum (p = 0.000) and Multiscale Entropy Average (p = 0.001).

Conclusions: The non-linear parameters in the cardiovascular autonomic regulation of Heart rate, SD1, SD2/SD1 rate, Sample Entropy, Short-term fluctuations α 1, Correlation Dimension, Max line length, Recurrence rate, Determinism, Multiscale Entropy Minimum, Multiscale Entropy Maximum and Multiscale Entropy Average experienced variations in patients subjected to mental stress through the arithmetic calculation test.

Key words: mental stress, heart rate variability, non-linear parameters, mental arithmetic test.

INTRODUCTION

The Heart Rate Variability (HRV) is the variation of the heartbeat frequency during a defined time interval, never greater than 24 hours in an analysis of consecutive circadian periods. Its changes and variations are controlled by the autonomic nervous system.

Traditionally, heart rate (HR) has been considered as a product of emotional response to stress, but the interval between beats is a marker of the capacity to regulate internal and external demands. The intervals are not constant, but they differ from beat to beat: basically a higher HRV indicates better general health. In essence, the multitude of ways in which different physiological mechanisms modulate each other’s state, makes that the study of one aspect in isolation limits our understanding.

The stress levels to which university students are subjected can be considered as high, so that anxiety levels before stressful situations should be lower in the order in which tolerance to such situations can be acquired as they are continually exposed to them.

In the last two decades, interventions based on cardiac coherence practice have been used to treat a variety of pain and anxiety-related conditions. As well, cardiac coherence helps to maintain parasympathetic cardiac modulation in stressful conditions.

The determination of the HRV parameters that undergo modifications during exposure to mental stress would imply the possible use of this as a tool to evaluate the response capacity of certain individuals in extreme situations.

Some studies have been done to assess the effect of mental stress on HRV as well, mainly observing the variability during the examination time for healthy university students. Under a mental stressor, the autonomic nervous system balance of the organism is disrupted, leading to an increase in the sympathetic activity and a decrease in the vagal activity of the heart. In examination conditions, students present lower beat-beat variability compared to normal non-academic situations, and subsequently present the highest HR during the examination.
Then it can be considered that a mental stressor could cause variations in the modulation of cardiac activity by the autonomic nervous system, which thanks to the techniques of digital collection of the electrical activity registers of the heart can be stored and analyzed by experts in the topic. Cognitive tasks (such as mental arithmetic test) have an impact on HRV. Individuals under mental stress show HRV components compared to a control group. During an attention task lower total HRV power have been observed, in addition to a change in heart rate, when compared to baseline. It is possible to use spectral analysis of HRV to predict the optimal work time under mental stress. (5)

**OBJECTIVE**

To determine the modifications that the nonlinear dynamics of the heart rate variability during the autonomic cardiovascular regulation undergo in patients exposed to mental stress through the mental arithmetic test.

**METHODS**

A non-observational, quasi-experimental study without a control group, of before and after type was conducted in the period from February 2016 to August 2018, in the Laboratory of Basic Biomedical Sciences, Medical School 1 of the University of Medical Sciences of Santiago de Cuba. The study population was made up of first- and second-year medical students, in the ages between 17 and 19 years, at the University of Medical Sciences of Santiago de Cuba (N = 10). The sample coincided with the population and this was analyzed in two moments:

**Stress Status:** The electrocardiographic record was made by an 8-channel PowerLab polygraph to each subject, simultaneously inducing a certain degree of mental stress through the mental arithmetic test.

**Basal Status:** The subject’s records was registered in the same way, without inducing any type of physical or mental stress.

The individuals were selected in a simple random way.

**Exclusion criteria**

§ Individuals who were under some drug treatment at present or in the last month.
§ Individuals who suffer of Diabetes Mellitus and / or High Blood Pressure.
§ Individuals who suffer of diseases with known autonomic affection.
§ Individuals who do not agree in participating in the study.
Definition of the mental arithmetic test

A holter record of 20 minutes (control) and subsequently another recording of 20 minutes during the provocation of mental stress (experimental) was performed at rest. The registration was made in Fowler's position in a seat intended for this purpose. A simple arithmetic calculus was used as a psychological stressor, as was a subtraction task (subtracting backwards from 7 in 7 units from 100) typical described in the literature.

Variables

Heart rate (in beats per minute) (beats / min), Standard Deviation 1 (SD1), Standard Deviation 2 (SD2), Relationship Standard Deviation 2/Standard Deviation 1 (SD2 / SD1 rate), Approximated Entropy, Sample Entropy, Short- term fluctuations α 1, Long-term fluctuations α 2, Correlation Dimension, Mean line length (in Beats), Recurrence rate (in percentage), Determinism, Shannon Entropy, Multi-Scale Entropy Minimum, Multiscale Entropy Maximum, Multi-Scale Entropy Average and exposure to the mental arithmetic test.

Experimental procedure and collection of data

Data were collected during February and March 2016 in the Laboratory of Basic Biomedical Sciences, Medical School 1 of the University of Medical Sciences of Santiago de Cuba, through the 8-channel PowerLab® polygraph (ADInstruments), and stored by Kubios ® Software version 3.0.4 Premium.

Each measurement was recorded by the same person in the Body-Level Measure section of the already mentioned laboratory of Basic Biomedical Sciences, with the purpose of minimizing the errors of methodology.

At the beginning of the session of the electrocardiographic recordings in the morning (08:30-12:00 hours), the subjects were placed on a comfortable stretcher, located in a room with controlled temperature between 24 and 27 degrees Celsius and dim light. Under these conditions, they were allowed to rest for 10-15 minutes until achieving a better adaptation to the conditions of the room.

The electrodes corresponding to the limb leads were placed to record the electrocardiographic tracing for 5 minutes. The electrical signal was collected by the 8-channel PowerLab® polygraph of Australian production by the company ADI instruments (2016); then it was digitized at a sample rate of 1000 samples/second (1 kHz) using the Software Kubios®, version 3.0.4 Premium (2018), of Finnish production. This software package enables the tabulation and export of the records to the MatLab 2016® programming package of the MathWork Company, and its file in PDF format for easier analysis and interpretation of the information.

Statistical Analysis

The data obtained were stored and processed in the statistical package "StatisticalPackage for Social Sciences" (SPSS) version 21 for Windows. The means were compared through the Wilcoxon test for related samples. The results are shown as mean plus standard deviation (Mean
+ SD). It was decided that there were statistically significant differences between the means of each variable when the value of p < 0.05.

Ethics
The research was approved by the ethics committee of the lead institution. All ethic principles were rigorous accomplished according to the Helsinki principles. Each individual signed an informed statement and personal identification data was not published.

RESULTS
Table 1 shows the comparison of the means of the non-linear parameters of HRV analyzed in each individual in basal and stress status according to the Wilcoxon test. There were significant differences between the two groups in the variables Heart Rate, Standard Desviation 1 (SD1), SD2/SD1 rate, Sample Entropy, Short-term fluctuations α 1, Correlation Dimension, Max line length (%), Recurrence rate (%), Determinism, Multiscale Entropy Minimum, Multiscale Entropy Maximum and Multi-Scale Entropy Average.

In Figure 1 can be observed the values of the Multiscale Entropy in mental stress state increased with respect to the baseline. However, through the taking of the sample in both states a decrease of the values of the Multiscale Entropy was observed, showing a possible short-term adaptability of the autonomic regulation in presence of a stressful stimuli, as it is the case of the mental arithmetic test.

![Multiscale Entropy](image)

**Fig. 1.** Distribution of the Multi-Scale Entropy values in Basal and Stress Status.
Figure 1. Distribution of the Multi-Scale Entropy values in Basal and Stress Status.

Table 1. Non-linear parameters of heart rate variability in basal and stress status. Laboratory of Basic Biomedical Sciences. Faculty 1. University of Medical Sciences of Santiago de Cuba. 2016-2018

<table>
<thead>
<tr>
<th>Non-linear Parameters of HRV</th>
<th>Basal status</th>
<th>Stress status</th>
<th>Total</th>
<th>$p^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>74.5 ± 4.88</td>
<td>95.5 ± 12.51</td>
<td>85.00 ± 14.19</td>
<td>0.000*</td>
</tr>
<tr>
<td>SD1</td>
<td>42.45 ± 16.93</td>
<td>26.64 ± 15.33</td>
<td>34.54 ± 17.68</td>
<td>0.002*</td>
</tr>
<tr>
<td>SD2</td>
<td>63.36 ± 23.62</td>
<td>59.08 ± 27.10</td>
<td>61.22 ± 24.84</td>
<td>0.571</td>
</tr>
<tr>
<td>SD2/SD1</td>
<td>1.60 ± 0.42</td>
<td>2.53 ± 0.68</td>
<td>2.06 ± 0.73</td>
<td>0.000*</td>
</tr>
<tr>
<td>Approximated Entropy</td>
<td>1.90 ± 2.45</td>
<td>1.15 ± 0.08</td>
<td>1.52 ± 1.73</td>
<td>0.359</td>
</tr>
<tr>
<td>Sample Entropy</td>
<td>1.66 ± 0.30</td>
<td>1.43 ± 0.25</td>
<td>1.54 ± 0.30</td>
<td>0.026*</td>
</tr>
<tr>
<td>Short-term fluctuations α 1</td>
<td>0.83 ± 0.15</td>
<td>1.28 ± 0.16</td>
<td>1.06 ± 0.28</td>
<td>0.000*</td>
</tr>
<tr>
<td>Long-term fluctuations α 2</td>
<td>0.25 ± 0.05</td>
<td>0.38 ± 0.21</td>
<td>0.32 ± 0.16</td>
<td>0.080</td>
</tr>
<tr>
<td>Correlation Dimension</td>
<td>3.81 ± 0.47</td>
<td>2.41 ± 1.52</td>
<td>3.11 ± 1.31</td>
<td>0.020*</td>
</tr>
<tr>
<td>Mean line length (Beats)</td>
<td>8.91 ± 1.85</td>
<td>9.55 ± 1.81</td>
<td>9.23 ± 1.81</td>
<td>0.289</td>
</tr>
<tr>
<td>Max line length (%)</td>
<td>81.90 ± 32.30</td>
<td>190.1 ± 120.1</td>
<td>136.0 ± 102.0</td>
<td>0.017*</td>
</tr>
<tr>
<td>Recurrence rate (%)</td>
<td>22.56 ± 4.96</td>
<td>27.10 ± 5.26</td>
<td>24.83 ± 5.50</td>
<td>0.007*</td>
</tr>
<tr>
<td>Determinism</td>
<td>96.24 ± 1.46</td>
<td>97.61 ± 1.28</td>
<td>96.92 ± 1.51</td>
<td>0.003*</td>
</tr>
<tr>
<td>Shannon Entropy</td>
<td>2.92 ± 0.23</td>
<td>2.97 ± 0.19</td>
<td>2.95 ± 0.21</td>
<td>0.478</td>
</tr>
<tr>
<td>Multiscale Entropy Minimum</td>
<td>0.26 ± 0.10</td>
<td>1.41 ± 0.65</td>
<td>0.83 ± 0.74</td>
<td>0.001*</td>
</tr>
<tr>
<td>Multiscale Entropy Maximum</td>
<td>0.88 ± 0.64</td>
<td>2.08 ± 0.30</td>
<td>1.48 ± 0.79</td>
<td>0.000*</td>
</tr>
<tr>
<td>Multiscale Entropy Average</td>
<td>0.57 ± 0.36</td>
<td>1.14 ± 0.09</td>
<td>0.86 ± 0.39</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Source: statistical processing; $^1$ Bilateral asymptotic significance of the Wilcoxon test. * Statistically significant.
DISCUSSION

The results of the present study show the parameters of heart rate variability that experienced variations when exposing a group of 10 medical students to the mental arithmetic test. There were statistically significant differences between the parameters of Heart Rate in both groups. There is scientific evidence that HR issues are a strong risk factor for cardiovascular mortality, while the HRV provides insights into general autonomic changes associated with different disease states.\(^6\) The authors of the present study consider that the state of mental stress maintained constitutes an important risk factor for the development of cardiovascular diseases. Hammoud et al\(^4\) comparing the nonlinear dynamics of HRV in university students before and after a test observed that SD1 and SD2 showed significant differences in the contrast of results before and after examination.

It has been shown that HRV is decreased in patients with cardiovascular diseases and in those exposed to high levels of stress. Lazo de la Vega et al\(^7\) in a study conducted in Havana, compared the response to acute mental stress in healthy patients and individuals with cardiovascular diseases, described that the HRV decreased due to mental effort. With greater mental demands, the decrease in heart rate variability was also greater, especially in the group of individuals with cardiovascular diseases, and baseline values in heart patients were also lower.

In the present study, the mean values of the Approximate Entropy compared in both groups was not significantly different, this coincides with that described by Sánchez Moya et al\(^8\) in a very similar study conducted in Spain, where it showed a certain tendency to lower complexity of the Entropy system with mental stress, but without statistical significance. Castaldo et al\(^9\) conducted a study in which they compared the values of HRV in a group of healthy individuals in basal status first and in a stress status later, induced by the Stroop Color Word Test (CWT), in which the nonlinear measures showed a general decrease during the acute mental stress.

The variations in the values of the non-linear parameters of HRV during the stress induced by the mental arithmetic test respond to the changes towards a more stable and periodic behavior of the heart rate under stress, which are associated with a greater regularity and, therefore, a partial deactivation of the nervous regulatory mechanisms of the cardiovascular system.

The results obtained by Cornforth et al\(^10\) show that Multiscale Entropy afforded no useful discrimination between stress categories. The results of the present study show that there were statistically significant differences between the means of the minimum, maximum and average values of the Multiscale Entropy, showing also that the values of this parameter decrease as the
mental arithmetic test progresses, which demonstrates what it could be a short-term adaptability of the non-linear dynamics of the HRV.

CONCLUSIONS

The nonlinear dynamics of the heart rate variability during the autonomic cardiovascular regulation of Heart Rate, Standard Desviation 1 (SD1), SD2/SD1 rate, Sample Entropy, Short-term fluctuations α1, Correlation Dimension, Max line length (%), Recurrence rate (%), Determinism, Multiscale Entropy Minimum, Multiscale Entropy Maximum and Multiscale Entropy Average experienced variations in patients under mental stress induced by the mental arithmetic test.

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Conflicts of interest: The authors declare no conflicts of interest.

Author Contributions
Elys María Pedraza-Rodríguez: conception and design of the study, analysis of the data, interpretation of the data, drafting the article.
Carlos Rafael Almira-Gómez: conception and design of the study, analysis of the data, interpretation of the data, critical revision of the manuscript.
Sergio Cortina-Reyna: interpretation of the data, critical revision of the manuscript.
David de J. Bueno-Revilla: collection of data, critical revision of the manuscript.
Erislandis López-Galán: collection of data, critical revision of the manuscript.
Miguel E. Sánchez-Hechavarria: conception and design of the study, collection of data, critical revision of the manuscript.
All authors have read and approved the final manuscript.
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