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Research Article

WAVELET-BASED TECHNIQUE TO DETECT GESTATIONAL COMPLICATIONS BY ANALYZING NON-STATIONARY HEART RATE VARIABILITY

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ABSTRACT
In order to study non-stationary heart rate variability (*HRV),* we developed a new approach of processing arhythmogram signal. It involves simulating the rhythmogram as a frequency-modulated signal, applying double continuous wavelet transformation (*DCWT*), as well as considering a large set of special diagnostic parameters related to frequency restructuring in *HRV* due to external non-stationary disturbance (oral glucose tolerance test - *GTT*), calculating and analyzing spectral integrals in various spectral bands. We found the critical values of diagnostic parameters and, using the Mann-Whitney non-parametric statistics, separated all the tested women into two clusters with weak and strong *GTT* impact on *HRV*. We registered the correlation of spectra behavior and glycemic parameters at different *GTT* stages. The changes in the balance of sympathetic and parasympathetic *ANS* in pregnant women during *GTT*, which we identified by mathematical analysis of special *HRV* parameters, can serve as markers of hidden sympathicotomia and predictors of complications in gestation and childbirth. Thus, the proposed technique provides opportunities for monitoring women at early stage of gestation. The approach also offers the tools to study the physiological mechanisms and risk factors of gestational diabetes, as well as the effect of obesity, true diabetes mellitus and other factors significant in the process of childbearing.

Keywords: *Continuous Wavelet Transform, Gestation, Heart Rate Variability, Rhythmogram*

INTRODUCTION
The recommendations of (Diagnosis, 2013) discuss the main aspects of the most common diseases of the cardiovascular system in women of reproductive age, which complicate the gestational period and negatively affect the fetus and the woman's organism. Prenatal diagnostics based on the analysis of the heart-rate variability (*HRV*) during pregnancy provides the possibility to conduct an objective and non-invasive assessment of the way various factors influence gestational complications. The relationship between sympathetic and parasympathetic activity of the autonomic nervous system (*ANS)* in pregnant and non-pregnant women, as well as the changes taking place in different trimesters of pregnancy have been considered in many papers.
The authors of a review (Kleshchenogov and Fleishman, 2006) state that the role of the systemic *ANS* reactions in the pathogenesis of pregnancy complications has not been studied enough, although the potential to reduce maternal and perinatal morbidity and mortality can be significant. Currently, there is a sufficient number of studies supporting the association of *HF* range (0.15-0.4 Hz) of the heart rate with parasympathetic (vagal) *ANS* activity (Appel *et al.,* 1989; Malliani *et al.,* 1991).
In many cases, the fluctuations in *HF* band are associated with the respiratory sinus arrhythmia (*RSA)*. Under various experimental conditions, the low-frequency range *LF* (0.04-0.15 Hz) in heart rate associated with the blood pressure regulation corresponds both with the sympathetic and parasympathetic activity (Kleshchenogov and Fleishman, 2006; Malliani *et al.,* 1991; Swansburg *et al.,* 2005). The *VLF* range (0.003-0.04 Hz) has been studied less profoundly. In the paper (Kleshchenogov and Fleishman,

2006) it is suggested that *VLF* relates to hormonal function and energy metabolism; besides, the concept of the integrated indicator of autonomic balance as a ratio of *HF/VLF* (named "vagosympathetic index") is introduced. Such indicators as *LF/HF, LF/(TP-VLF), HF/(TP-VLF),* where *TP* is the total spectral power, are also used in *HRV* analysis of pregnant women (Yang *et al.,* 2000; Kuo *et al.,* 2000; Weissman *et al.,* 2006).

Current *HRV* research of pregnant women (Yang *et al.,* 2000; Kuo *et al.,* 2000; Weissman *et al.,* 2006; Khlybova *et al.,* 2008; Dmitrieva *et al.,* 2013; Hudkov *et al.,* 2001; Tejera *et al.,* 2011) shows a significant change in heart rate spectra: a reduction in *HF* and an increase in *LF/HF*. These changes indicate the growing influence of *ANS* sympathetic division.

HRV studies under hyperglycemia (Singh *et al.,* 2000, Schroeder *et al.,* 2005) show decline in *HRV* and significant increase in sympathetic activity. The studies (Straznicky *et al.,* 2005; Montani *et al.,* 2001) establish the relationship between insulin resistance, which leads to different disorders of carbohydrate metabolism, and sympathetic activity.

Hyperglycemia and associated metabolic disorders (obesity, diabetes) are aggravating factors during pregnancy. Changes in the balance of sympathetic and parasympathetic *ANS* in pregnant women during the glucose tolerance test, which we identify by mathematical analysis of *HRV* parameters in different trimesters, can serve as predictors of complications in gestation and childbirth. The paper (Weissman *et al.,* 2006) presents the spectral analysis of *HRV* in the 100-g *GTT* in pregnant women, in the normal state and with gestational diabetes mellitus (*GDM*). Within 60 min. after glucose administration, both groups showed a decrease in *HF* activity and an increase in *LF/HF*, which was greater in women with gestational diabetes.

The reduction of *HRV* in women with *GDM* was confirmed in (Ruozi-Berretta *et al.,* 2004). Thus, most of the researchers believe that pregnancy in itself, especially aggravated by obesity, hyperglycemia and insulin resistance, triggers a cascade of reactions resulting in the activation of the sympathetic nervous system.

The majority of *HRV* studies in pregnant women use the method of spectral analysis such as Fast Fourier Transform (*FFT*) (Yang *et al.,* 2000; Faber *et al.,* 2004). Based on *HRV* data and the neural network simulation method, (Tejera *et al.,* 2011) suggests dividing all cases into three groups: normal pregnancy, gestational hypertension and pre-eclampsia. In (Salazar *et al.,* 2004) the *HRV* analysis was carried out with the help of non-linear methods of the Chaos Theory by calculating the correlation dimension and the largest Lyapunov exponent. The study showed a more regular character of *HRV* patterns in the case of pre-eclampsia. According to Salazar *et al.,* (2004), the classical Fourier transform does not reveal any significant difference in the groups of women with normal and complicated pregnancies, because ordinary spectral analysis does not work in the case of non-stationary processes. By using *FFT*, the authors of (Hossen *et al.,* 2013) revealed the decrease in *HF* power fluctuations in women with preeclampsia. Calculating the *LF/HF* ratio made it possible to identify the difference between the normal state and pre-eclampsia in 76% of cases. Despite the obvious advantages of applying wavelet transform to solve similar problems, it has been used mainly in filtration problems, in particular, to separate the mother and fetus rhythmograms (Almeida *et al.,* 2013), but not to obtain the relevant information for detecting the signs of a pathological course of gestation.

Time variation in the conditions of many functional tests leads to heart rate rearrangements, which show strong non-stationarity of the signal (Hossen *et al.,* 2013). The fact is that according to(Guidelines, 1996) one can use statistic parameters describing *HRV (RRNN, SDNN, RMSSD*), spectral characteristics of cardio intervals obtained by Fourier transformation and histogram methods only in stationary situations, when spectral properties of the signal do not change in time. In this paper, we present the processing of non-stationary rhythmogram records during *GTT* (Glucose tolerance test) by the techniques worked out by the authors in (Bozhokin and Shchenkova, 2008; Bozhokin and Suvorov, 2008; Bozhokin *et al.,* 2012; Bozhokin and Suslova, 2013; Bozhokin and Suslova, 2014-a; Bozhokin and Suslova, 2014-b) on the basis of double continuous integral wavelet transformation. The special quantitative characteristics of pregnant women's physiologic regulation obtained in the study can serve to monitor the early stages of gestation.

MATERIALS AND METHODS

Methods

Research Design

The investigation involved *N* =39 pregnant women. We formed the group of pregnant women on the criterion of having one living fetus with gestation less than 12 weeks. All the participants signed two copies of informed consent forms approved by the local ethics committee at the Kirov Military Medical Academy, Protocol N 121 of 31.01.2012. The participants received one copy each, with one copy remaining at the medical center.

Before testing, we identified in the women involved in the test the factors which complicated gestation, such as obesity, true diabetes mellitus, essential hypertension, etc. We monitored the women during the first and second trimesters of gestation. To study transients in cardio rhythm we used the functional test with positive sympathomimetic activity, namely, 75-g oral glucose tolerance test *(GTT)* (Negrusha, 2013; Negrusha *et al.*, 2012; Negrusha *et al.*, 2010). We chose this test due to its being highly standardized and safe for the mother and fetus.

The continuous interval of the rhythmogram record included the following three stages:

1. Stage *A* (the state of relative rest during approximately 30 min.) involved the rhythmogram record in each of the tested subjects on empty stomach in a relaxed position of lying horizontally on the left side in the state of relative emotional calm. $(0 < t \le t_A)$.

2. Stage *B* started directly after taking 75g. of glucose and lasted 30 min. This period ($t_A \le t \le t_B$) was characterized by the transients in the heart rhythm related to hyperglycemia. After stage *B*, the Research design required a break in the rhythmogram registration for about 30 minutes.

3. Stage *C* $(t_B \le t \le t_C)$ was related to the changes in autonomous regulation of the heart rate with the decrease in blood glucose levels.

The duration of each recording averaged 30 minutes. The total duration of rhythmogram (the duration of stages *A, B, C*) was about 1.5 hours.

Rhythmogram registration was carried out by the certified device "Maternal/fetal monitor series 120" manufactured by «GE Medical System Inc.»(USA) in one standard lead, in the morning, in a quiet warm room, after 5-10 minutes of adaptation, with the women lying on the left side, breathing quietly. The heart rate and blood pressure on each hand were fixed throughout the test in the lying, sitting and standing positions. According to the standard oral glucose test protocol, the laboratory measurements of the venous blood glucose level were taken first in the state of empty stomach, then one hour and two hours after receiving75 g. of glucose. We interpreted the results according to H.A.P.O. recommendations (Metzger *et al.*, 2008).

Double Continuous Wavelet Transformation (DCWT) Method and Characteristic Parameters of Non-Stationary Rhythmogram

According to (Bozhokin and Shchenkova, 2008; Bozhokin and Suslova, 2013), we simulate therhythmogram as a superposition of Gaussian peaks with the constant amplitude equal to the characteristic width of the *QRS* complex. The center of *n*-peak, occurring in heart beat interval RR_n at time t_n, is located on the non-uniform time-scale, where $t_n = t_{n-1} + RR_n$, $n = 1,2,3..N-1$, $t_0 = RR_0$, N is the total number of heart beats in the test. Non-stationary rhythmogram simulation as a frequency-modulated signal was suggested in (Bozhokin and Shchenkova, 2008). The advantage of this model is that it allows to obtain the analytical expression for the continuous wavelet transform $V(\nu, t)$. This, in turn, makes it possible to calculate the local frequency $F_{\max}(t)$ related to the maximum value of $|V(\nu, t)|$. In medical practice, $F_{\max}(t)$ determines the contribution of respiratory waves related to the influence of sympathetic and parasympathetic *ANS* on a heart rhythm in transient periods. We repeat the wavelet transformation, this time applying it to the signal $F_{\max}(t)$. Let us denote the result of the double wavelet

transformation as $V_{DCWT}(\nu,t)$. The use of double wavelet transformation *(DCWT)* gives the opportunity to analyze both a periodic and oscillatory movements of the local frequency $F_{\max}(t)$ relative to the trend. Thus, the application of *DCWT* is caused by the non-stationary character of the process under study. Then we calculate the skeletons and spectral integrals $E_\mu(t)$ in different spectral bands $\mu=\{VLF, LF, HF\}$making some sort of filtration by summing the contributions of local density $\varepsilon(\nu,t)$ of the power spectrum only in the frequency band μ. The local density of the power spectrum in the case of wavelet transformation can be determined through the analog of the Parseval equality. The formulas for the continuous wavelet transform, the Morlet mother wavelet function and spectral integrals are given in (Bozhokin and Shchenkova, 2008; Bozhokin and Suvorov, 2008; Bozhokin *et al.*, 2012; Bozhokin and Suslova, 2013; Bozhokin and Suslova, 2014-a; Bozhokin and Suslova, 2014-b).

When analyzing *HRV* of pregnant women during *GTT*, we used a special technique to assess the variation of different spectral components $\mu=\{VLF, LF, HF\}$ and, consequently, the variation of *ANS* response. By calculating the duration of the transition periods, we can estimate the adaptive capacities of the patients (the characteristics of *ANS* control).

To study *HRV*, for every stage $S=\{A, B, C\}$ of rhythmogram registration and for every spectral band $\mu=\{VLF, LF, HF\}$, we introduce the following characteristics:

The mean values $<E_\mu(S)>$ and standard deviations $<\sigma_\mu(S)>$ of spectral integrals

$$<E_\mu(S)>=\frac{1}{t_f(S)-t_i(S)}\int_{t_i(S)}^{t_f(S)}E_\mu(t)\,dt, \qquad (1)$$

$$<\sigma_\mu(S)>^2=\frac{1}{t_f(S)-t_i(S)}\int_{t_i(S)}^{t_f(S)}\left(E_\mu(t)-<E_\mu(S)>\right)^2\,dt \qquad (2)$$

where $E_\mu(t)$is the spectral integral in μ-band, $t_f(S)$ and $t_i(S)$are the time moments, corresponding to the end and the beginning of stage *S*.

The nine parameters $C_{nst}(\mu,S)$characterizing non-stationary *HRV* in terms of percentage are:

$$C_{nst}(\mu,S)=\frac{100<\sigma_\mu(S)>}{<E_\mu(S)>} \qquad (3)$$

The values $C_{nst}(\mu,S)$increase considerably at stage S, together with the spectral integral $E_\mu(t)$.

We can take the relation of the mean spectral integral $<E_\mu(S)>$ to the total value of the spectral integral: $<E_{tot}(S)>=<E_{VLF}(S)>+<E_{LF}(S)>+<E_{HF}(S)>$as another characteristic of *HRV*.

Stage *B* (after receiving glucose) can be considered the most informative. Because of the developing hyperglycemia, we observe here the maximum value of spectral integral $E_{\mu\max}(t_{\max})$ in the μ-band at the time $t_{\max}$, $(t_i(B)<t_{\max}<t_f(B))$. Let $t_i(B)=t_A$and $t_f(B)=t_B$. The time lag of energy maximum $\tau_\mu(B)=t_{\max}-t_A$(in seconds) characterizes the process of glucose utilization, which is manifested in spectral integral growth.

We introduce the spectral integral peak width $w_\mu(B)$ at its half height in seconds as another characteristic of non-stationary *HRV*.

Next, we consider the coefficient of rhythm mastering, given by the relation

$$K_M(\mu)=\frac{<E_\mu(B)>}{<E_\mu(A)>}, \qquad (4)$$

Which shows how many times the mean value of $E_\mu(t)$ at stage *B* (the stage of glucose administration) exceeds the mean value of $E_\mu(t)$ at stage *A* (the stage of rest). Similarly, the coefficient of relaxation

$$K_R(\mu)=\frac{<E_\mu(C)>}{<E_\mu(A)>}, \qquad (5)$$

represents the ratio of the spectral integral at the stage of relaxation (*C*) to its value at the stage of rest (*A*). Apart from the parameters (1)-(5) describing *HRV* at various stages in a given spectral range, it is useful to introduce the factors which could represent the ratio of the mean values of spectral integrals at the same stage but in different spectral bands, such as $<E_{LF}(B)>/<E_{HF}(B)>, <E_{VLF}(A)>/<E_{HF}(A)>$, $<E_{LF}(A)>/<E_{HF}(A)>$, and others.

Further, we take into consideration parameters QSA, QSB, QSC representing blood glucose levels before glucose administration (stage *A*) and at the end of stages *B* and *C*, respectively. Finally, we have m=32 quantitative parameters for every pregnant woman. The number of women taking part in the test is N=39. The number of the correlation coefficients R_{ij} between various parameters is $m(m-1)/2$ =496.

Moreover, to study the heart rhythm bursts in details, we consider the functions describing the time variation of spectral integrals in different spectral bands $\mu=\{VLF, LF, HF\}$. We compare the functions $E_\mu(t)$ with the mean values $E_\mu(S)$ at different stages $S=\{A,B,C\}$ by introducing the function

$$d_{LF}(t)=E_{LF}(t)/<E_{LF}(A)>. \qquad (6)$$

This function shows the relation between $E_{LF}(t)$ in *LF* range and its mean value at stage *A*. If we consider the function which describes the instantaneous ratio of $E_{LF}(t)/E_{HF}(t)$ to its mean value $K_{LF/HF}(A)=<E_{LF}(A)>/<E_{HF}(A)>$ at stage *A*, we will obtain one more dynamic characteristic of heart rhythm

$$d_{LF/HF}(t)=\frac{E_{LF}(t)<E_{HF}(A)>}{E_{HF}(t)<E_{LF}(A)>}. \qquad (7)$$

Functions (6) and (7) describe time-fluctuations of spectral integrals over the entire interval $0<t<T$ of observation.

RESULTS AND DISCUSSION

Results

We classified the tested subjects by the influence of *GTT* on *HRV*. Let the main parameters of clustering be the rhythm-mastering coefficient $K_M(LF)$ (4) in *LF*-band and the value $K_{LF/HF}(B)$. The latter parameter shows how many times the average spectral integral $<E_{LF}(B)>$ in *LF*-band exceeds the average spectral integral $<E_{HF}(B)>$ in *HF*-band. We separated all the participants into two clusters: cluster H with weak influence of *GTT* on *HRV* and cluster I with strong influence. To determine the differences between H and I, we used the Mann-Whitney test with error probability α <0.01. Each tested subject H_j (j =1,2... N_H) from cluster H and I_s (s =1,2... N_I) from cluster I can be characterized by a pair of parameters $\{K_M^{(j)}(LF); K_{LF/HF}^{(j)}(B)\}$ and $\{K_M^{(s)}(LF); K_{LF/HF}^{(s)}(B)\}$.

We found the numbers for N_H и N_I from the condition that the differences in H and I could be considered as trusted with a probability of error α =0.01, if we separated the participants both by

$K_M(LF)$ and $K_{LF/HF}(B)$. First, we separated all *N*-participants by the values of $K_M(LF)$. We included into cluster I those with small values of $K_M(LF) < K_{MH}$, while cluster I was characterized by $K_M(LF) > K_{MI}$. To find K_{MH} and K_{MI} we used the procedure of maximizing N_H and N_I. The algorithm gave N_H =10 participants belonging to cluster H ($K_M(LF) < K_{MH}$, where K_{MH} =5.5), and N_I =11 participants belonging to cluster I ($K_M(LF) > K_{MI}$, where K_{MI} =9.5). The limit values for $K_{LF/HF}(B)$ were: $K^{(\min)}_{LF/HF}(B)$=2.6, $K^{(\max)}_{LF/HF}(B)$=16.1 for cluster H, and $K^{(\min)}_{LF/HF}(B)$=5.5, $K^{(\max)}_{LF/HF}(B)$=48.9 for cluster I. By averaging $K_{LF/HF}(B)$ over all subjects (N_I =11) of cluster I, we obtained $<< K_{LF/HF}(B) >>_{(I)}$ =19.4, which considerably exceeded the value of this parameter $<< K_{LF/HF}(B) >>_{(H)}$ =9.2 for cluster H (N_H =10). At stage *A* we had $<< K_{LF/HF}(A) >>_{(I)}$ =13.6 and $<< K_{LF/HF}(A) >>_{(H)}$ =9.0.

The rest of the pregnant women taking part in the investigation ($N - N_H - N_I$ =18) can be divided into two clusters, namely, the clusters of prenosological and premorbid states (M_D and M_P) of the adaptation systems (Baevsky *et al.*, 2011). We characterize the state of the organism as prenosological assuming that the regulatory systems experience some stress to preserve the stability of the basic vital systems. In the case of a premorbid state, we assume a strong tension of the regulatory systems and the reduction of functional capacity of the organism.

The scatter plot for participants from clusters H, I and intermediate group M is shown in Figure 1.

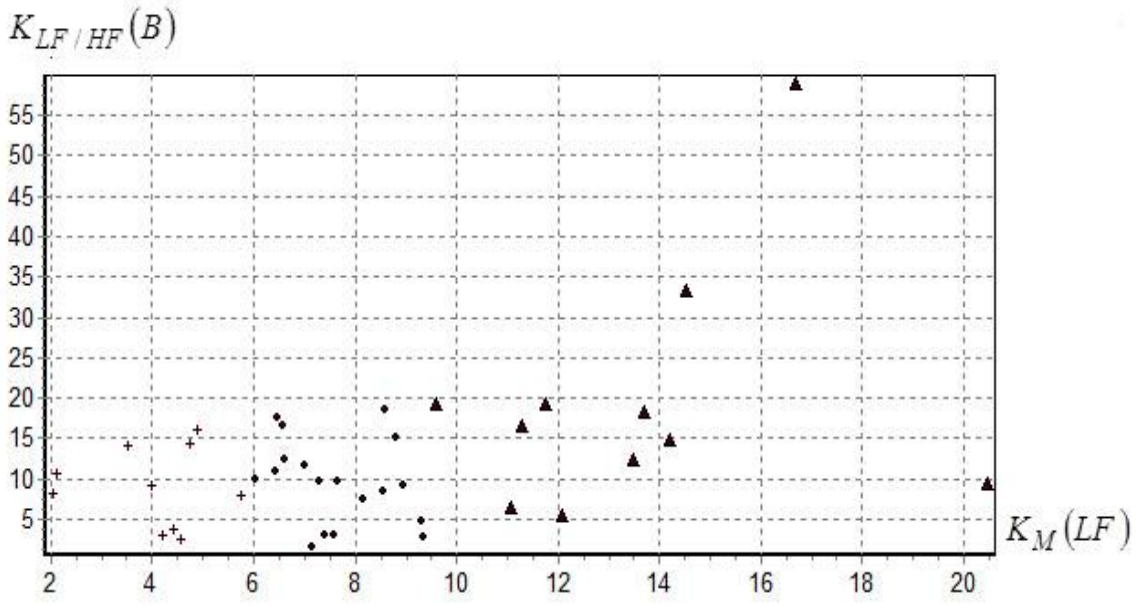

Figure1: Scattering diagram on the plane of $K_M(LF)$ and $K_{LF/HF}(B)$. The points corresponding to the tested subjects of cluster H are marked by crosses, those of the intermediate group – by circles, and those of cluster I - by triangles

Figure 1 shows that clusters H and I are separated in the space of features $K_M(LF)$ and $K_{LF/HF}(B)$. Let us divide intermediate group M into clusters M_D and M_P. The first cluster includes N_{MD}=9 tested subjects with the values of $K_M(LF)$ in the interval [K_{MH};7.5], where K_{MH}=5.5. The second cluster consists of N_{MP}=9tested subjects with $K_M(LF)$ within [7.5; K_{MI}], where K_{MI}=9.5. We can consider the difference in M_D и M_P as significant with the error probability α=0.01, using the parameter $K_M(LF)$ and Mann-Whitney test.

However, the differences in M_D and M_P become unreliable when taking $K_{LF/HF}(B)$ as the parameter of clustering. Nevertheless, we have an opportunity to classify the participants of the intermediate group (the mean impact of *GTT* on *HRV*) using another, more sensitive characteristics, such as, for example, those related to the heart rhythm rearrangements. We can choose them from the set of parameters presented in this paper.

For two diagnostic parameters $x=K_M(LF)$, $y=K_{LF/HF}(B)$, we have the Pearson's correlation coefficient $R_{xy}\approx$0.45.Some of the parameters introduced in this paper have a strong correlation, and we may interchange them to improve the quality of the results.

Let us examine non-stationary characteristics of heart rate in various groups of participants. To illustrate the data processing, we consider first the rhythmogram of a participant with weak *GTT* effect on *HRV* denoting her as subject H_j *(*cluster H).Stage *A* ends with the beat number n_A=*2511* ($t_A(H_j)$=*1915 s*). Stage *B* ends with the beat number n_B=*5072* ($t_B(H_j)$=*3827 s*).Note that visual analysis of the rhythmogram does not show any significant variation of RR_n at the stage of glucose administration (stage *B*) against stage *A*. However, the behavior of $|V_{DCWT}(\nu,t)|^2$ in Figure 2 demonstrates heart rate activity bursts in *VLF*, *LF* and *HF* bands, which happens due to strong non-stationarity of a rhythmogram during *GTT*.

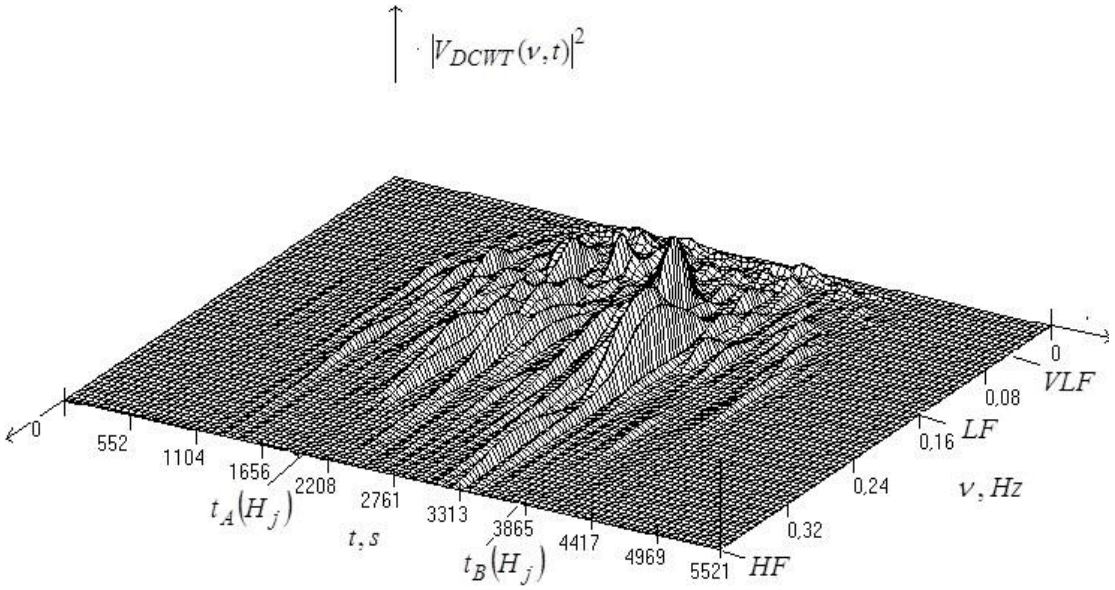

Figure2: Dependence of $|V_{DCWT}(\nu,t)|^2$ on frequency ν, Hz and time t, s for participant H_j

Let us analyze the dynamics of $d_{LF}(t)$ (6) using the mean value $K_M(LF)$ (4).

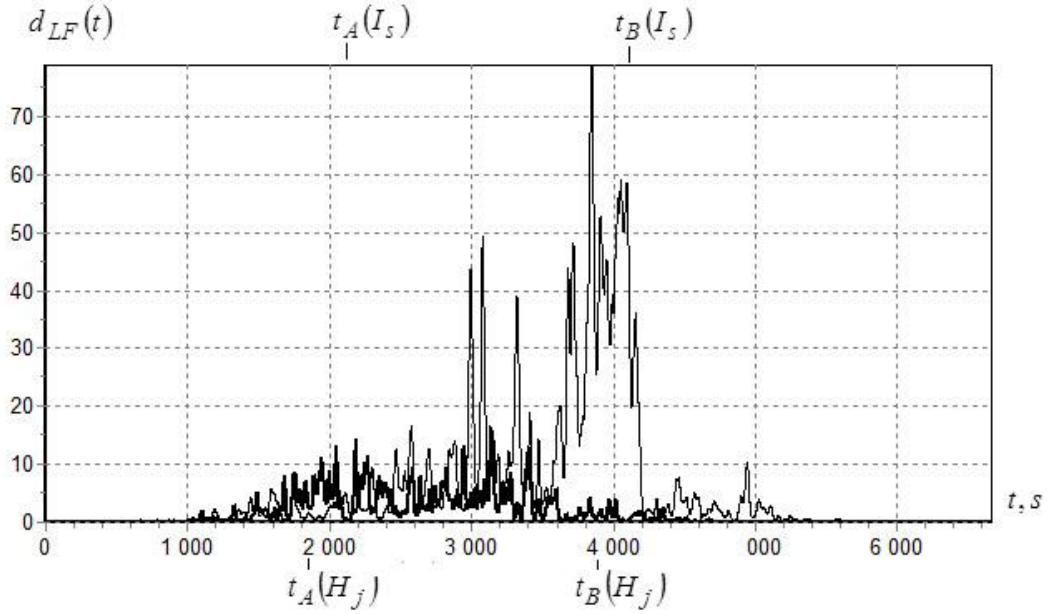

Figure 3: Dependence of characteristic function $d_{LF}(t)$ (6) on time *t, s* for participant H_j (bold line) and for participant I_s (thin line)

Figure 3 shows the increase of activity in *LF*–band for participant H_j (bold line) at stage *B*($t_A(H_j) < t < t_B(H_j)$). The *LF* activity bursts have the form of peaks with the characteristic width $w_{LF}(B)$≈33 *s*. The time lag of the maximum peak from the start of stage *B* is $\tau_{LF}(B)$=1243 *s*. For subject H_j from the group of the weak *GTT* effect on *HRV*, the coefficient of rhythm mastering $K_M(LF)$ is approximately equal to 4.24. This value is less than the critical $K_{MH}(LF)$=5.5, which demarcates the cluster of relatively healthy women (N_H =10). Figure 3 shows that there are time moments when $d_{LF}(t)$ (6) exceeds the critical value $K_{MH}(LF)$=5.5. The total time of observation for subject H_j is 5518 *s*. $K_M(LF)$ becomes larger than the critical value within the relative time interval $t_> / T$ =0.11. For the level of $K_M(LF)$=9.5, we have much smaller value of time interval $t_> / T$ =0.02. Thus, participant H_j remained in the state near the lower boundary of cluster *I* (strong reaction to *GTT*) approximately two percent of the entire observation time.

We can also identify the activity burst in *LF*–band ($d_{LF}(t)$>8) lasting 12 *s* within the interval $t = [2818\ s; 2830\ s]$. Such burst of *LF* activity correlates with the sharp decrease in *HF*-activity. Now let us consider the graph of function $d_{LF}(t)$ for participant I_s (thin line). Stage *B* corresponds to the time interval $t_A(I_s) < t < t_B(I_s)$, where $t_A(I_s)$=2164 *s*, $t_B(I_s)$=4180 *s*. Figure 3 shows a strong increase in *LF* activity in participant I_s ($K_M(LF)$=16.7) compared with H_j ($K_M(LF)$

=4.24). The maximum of $K_M(LF)$≈20.4 corresponds to participant $I_{\max}$ (cluster I). It is approximately 4.8 times higher than the rhythm mastering coefficient $K_M(LF)$=4.24for participant H_j. We can calculate the values of relative time intervals for participant I_s when $K_M(LF)$exceeds certain critical values, namely, we have $t_>/T$ =0.22 for the level of $K_{MH}(LF)$=5.5, and $t_>/T$ =0.16 for $K_{MI}(LF)$=9.5. The values of relative times $t_>/T$ for participant I_s are much higher than for H_j. All in all, the analysis of Figure 3 shows many more *HRV* rearrangements at stage *B* for participant I_s (strong reaction to glucose test)over against participant H_j (weak reaction to glucose test). The strong heart rhythm bursts in *VLF*, *LF* and *HF* bands are clearly visible in the graphs of $|V_{DCWT}(\nu,t)|^2$ (see Figure 2) and in the graphs for all the spectral integrals.

Figure 4 shows the time-dependence of $d_{LF/HF}(t)$for H_j.

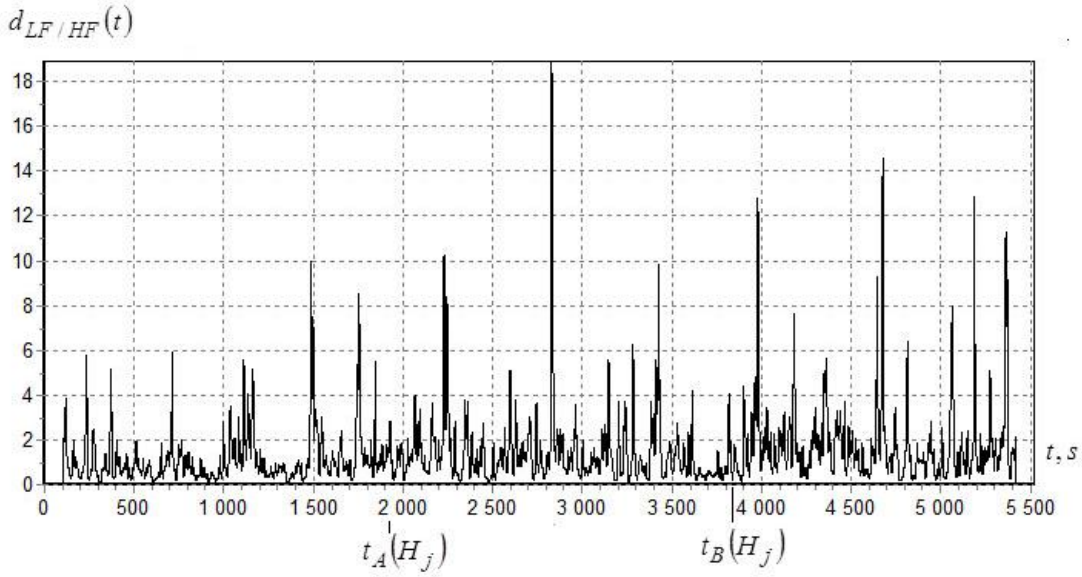

Figure 4: Dependence of $d_{LF/HF}(t)$(7) on time t, s for participant H_j.

The analysis of Figure 4 shows a strong fluctuation in time of $d_{LF/HF}(t)$(6) for subject H_j (weak reaction to glucose). For example, at t ≈3200 s (stage *B*) the instantaneous value of $E_{LF}(t)/E_{HF}(t)$ for subject H_j is about 19times higher than the mean value of this parameter $K_{LF/HF}(A)=<E_{LF}(A)>/<E_{HF}(A)>$=3.63 at stage *A*. Such strong fluctuations of $K_{LF/HF}(t)$occur due to very short-time fluctuations of spectral integral $E_{HF}(t)$.The value of $d_{LF/HF}(t)$for participant H_j, exceeds 3.63within the relative time interval $t_>/T$ =0.03. The strongest activity burst ($d_{LF/HF}(t)$>10) occurs in the interval $t=[2826;2831\,s]$and lasts nearly *5s*.Averaging $d^{(H)}_{LF/HF}$ over all participants of cluster H, we obtain $<d^{(H)}_{LF/HF}>$=1.49.Forthis level

of $d_{LF/HF}$, we obtain $t_> / T$ =0.05, i.e. participant H_j has the parameter $d_{LF/HF}$ higher than the average value in cluster H within relative time interval 0.05.
Figure 5 shows function $d_{LF/HF}(t)$ for participant I_s.

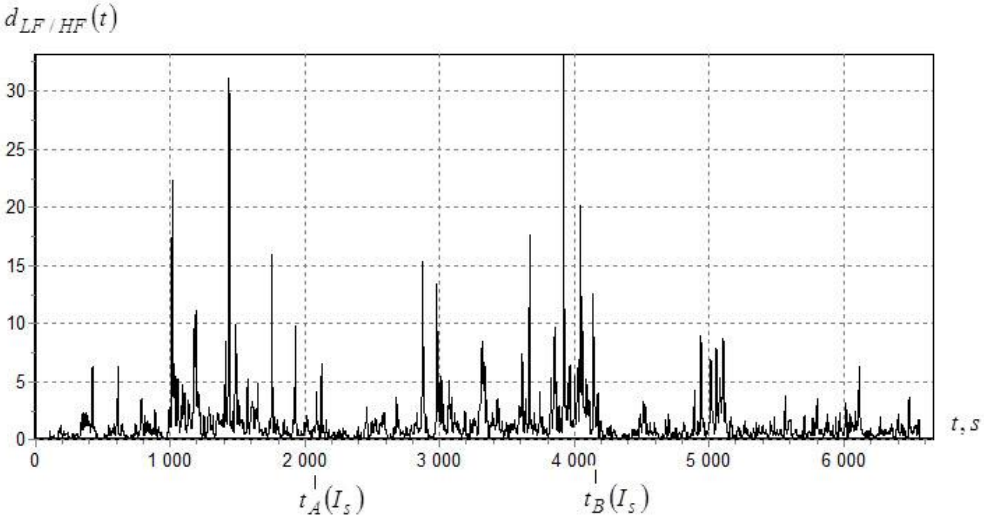

Figure 5: Dependence of $d_{LF/HF}(t)$(7) on time t, s for participant I_s

Time dependence $d_{LF/HF}(t)$ for participant I_s (Figure 5) reveals greater fluctuations in time than that for participant H_j (Figure 4). For participant I_s we have $d_{LF/HF}(t)$ at the level of $d^{(H)}_{LF/HF}$ during the time interval $t_> / T$ =0.83, which is much more than $t_> / T$ =0.05for participant H_j.
Figure 6 represents the dependence between $d_{LF/HF}(t)$ and $d_{LF}(t)$ for participant H_j characterized by weak *GTT* influence on *HRV*.

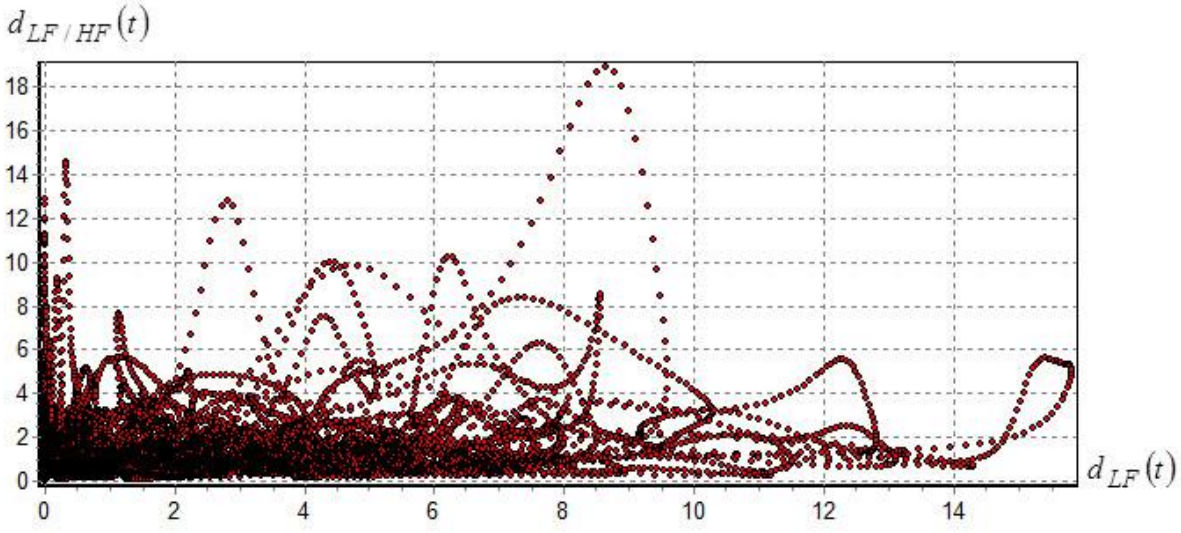

Figure 6: Scattering diagram on the plane $d_{LF/HF}(t)$(7)and $d_{LF}(t)$ (6) for participant H_j

The analysis of Figure 6 shows that within the interval $t=[2826\,;2831\ s]$ two parameters satisfy the relations $d_{LF/HF}(t)$>10 and $d_{LF}(t)$>8 simultaneously. During relative time interval $t_{>}/T\approx$ 0.001, we detect an activity burst for $E_{LF}(t)$ in *LF*- band and minimum of activity for $E_{HF}(t)$ in *HF*-band. In other time intervals, we have either small values of $d_{LF/HF}(t)$ and $d_{LF}(t)$ or the excess of one of the parameters over its critical value. For participant H_j we obtain respectively small value $t_{>}/T$ =0.014, when two conditions $d_{LF/HF}(t)>d^{(H)}_{LF/HF}$ and $d_{LF}(t)>K_{MH}$ are fulfilled simultaneously ($d^{(H)}_{LF/HF}$ =1.49 and K_{MH} =5.5).

Figure 7 represents the dependence between $d_{LF/HF}(t)$ and $d_{LF}(t)$ for participant I_S characterized by strong *GTT* influence on *HRV*.

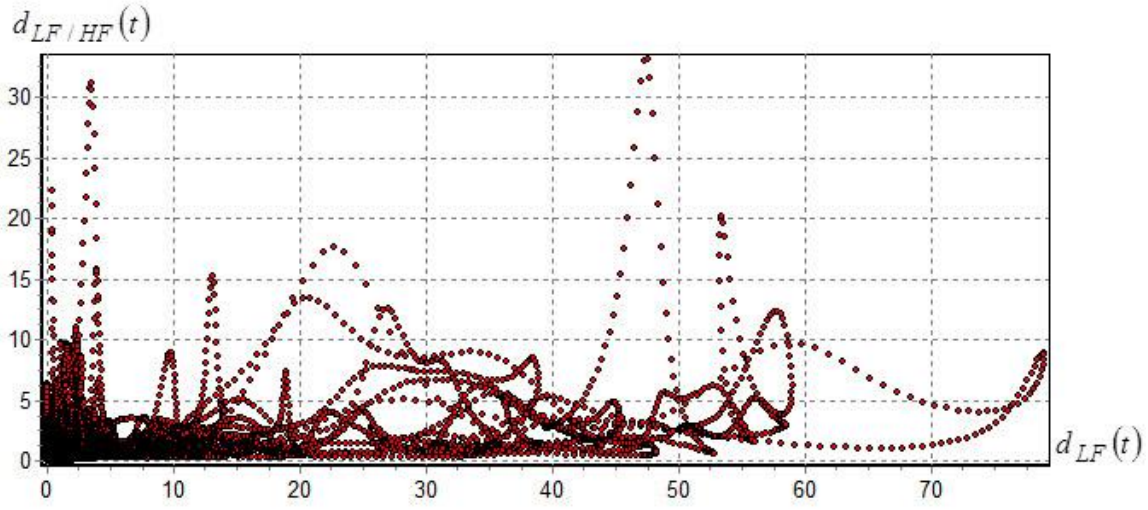

Figure 7: Scattering diagram on the plane $d_{LF/HF}(t)$ (7) and $d_{LF}(t)$ (6) for participant I_S

For participant I_S, we have the relative time interval $t_{>}/T$ =0.2. It means that she remained in the overcritical state ($d_{LF/HF}(t)>d^{(I)}_{LF/HF}$ and $d_{LF}(t)>K_{MI}$) for about twenty percent of the total time of observation. This duration is much larger than in the case of participant H_j under similar conditions ($d_{LF/HF}(t)>d^{(H)}_{LF/HF}$ and $d_{LF}(t)>K_{MH}$).

Table 1 shows that the relative periods of time, when the characteristic parameters exceed their critical values, differ substantially for participants from clusters H and I. For example, if we consider the condition $d_{LF}(t)>K_{MH}(LF)$, then for all participants of cluster H we have $t_{>}(\min)/T$ =0.03, $t_{>}(\max)/T$ =0.14, while for cluster I they are much larger: $t_{>}(\min)/T$ =0.2, $t_{>}(\max)/T$ =0.34.We should emphasize that the difference in the parameter $t_{>}/T$ in H and I clusters can be considered as significant with the error probability α =0.01 for all three variants of separation conditions (Table 1).

Table 1: Minimal and maximal values of relative time intervals $t_> / T$,which determine the duration of remaining in overcritical regions for participants H_j and I_s, j =1,2… N_H , s=1,2… N_I.

Characteristic parameters, which take overcritical values	Limits of relative time intervals	Cluster H	Cluster I
$d_{LF}(t)$ is over critical level	$t_>(\min)/T$	0.03	0.20
	$t_>(\max)/T$	0.14	0.34
$d_{LF/HF}(t)$ is over critical level	$t_>(\min)/T$	0.06	0.59
	$t_>(\max)/T$	0.82	0.92
Both $d_{LF}(t)$ ***and*** $d_{LF/HF}(t)$ **are over critical values**	$t_>(\min)/T$	0.01	0.12
	$t_>(\max)/T$	0.11	0.20

Discussion and Conclusions

According to our approach, we characterized the physiological response of each pregnant woman during *GTT* by using approximately 32 parameters. The set of diagnostic parameters was calculated by *DCWT* method proposed by the authors for the study of non-stationary processes (Bozhokin and Shchenkova, 2008; Bozhokin and Suvorov, 2008; Bozhokin *et al.*, 2012; Bozhokin and Suslova, 2013; Bozhokin and Suslova, 2014-a; Bozhokin and Suslova, 2014-b). The aim was to identify the most important characteristics for clustering the subjects to diagnose the possible complications of pregnancy.

We divided all the parameters of heart rate used in this paper into two classes: the averaged parameters showing the average degree of glucose effect on *HRV*, and the dynamic characteristics associated with individual bursts of heart activity in different spectral bands $\mu = \{VLF, LF, HF\}$. The averaged quantities are the rhythm mastering coefficient $K_M(\mu)$(4), the rhythm relaxation coefficient $K_R(\mu)$ (5), their various interrelations, the characteristic peak width $w_\mu(S)$and the time lag of the peaks $\tau_\mu(B)$ measured from the moment of glucose administration. The dynamic parameters are $d_\mu(t)$ (6) and $d_{\mu/\nu}(t)$ (7). For all N=39 participants the correlation matrix of the parameters (496 values) was built, and the most significant correlations were identified. We found a strong correlation between the behavior of *LF* and *HF* spectra, as well as between the degrees of glycaemia for both *A* and *B* stages. Glucose administration (stage *B*) led to a sharp non-stationary response in *LF* and *VLF* spectra. Using non-parametric Mann-Whitney statistics, we formulated the clusterization algorithm. Based on two parameters $K_M(LF)$and $K_{LF/HF}(B)$, we identified three groups according to the reaction of their heart rhythms to glucose: weak reaction (cluster H), strong reaction (cluster I), mean reaction (intermediate group *M*).We also managed to separate participants of the intermediate group *M* into clusters of prenosological (M_D) and premorbid (M_P) states of physiological adaptation. However, it should be noted that the differences in the clusters M_D and M_P have been insignificant, if we used $K_{LF/HF}(B)$ as the parameter of clustering. *GTT* revealed the complex dynamics of the appearance and disappearance of rhythmic activity bursts in different frequency bands. We introduced the relative

time intervals $t_> / T$ during which every tested woman was in the overcritical areas of the phase space of dynamic parameters, namely, in the areas where these parameters were beyond their critical values. For participants with weak glucose effect (cluster H), the relative time was short: $t_> / T \approx 0.03$. This characteristic grew for those in prenosological and premorbid states (*M*-group) and became close to unity for the tested women with strong glucose effect on *HRV* (cluster I).

By calculating the time intervals when some of the parameters exceed their critical values, we can predict or assess the *ANS* stress state. We believe these estimates could facilitate early diagnostics in the area of pregnancy complications. We also emphasize that most of the diagnostic parameters presented here have not yet been used for *HRV* analysis. A more detailed classification of different physiological states using the new characteristic parameters can offer a prospect for further research.

Overall, the study of non-stationary *HRV* during *GTT* showed that the proposed characteristics of heart rate could not be unambiguously associated with standard quantitative health indicators, such as glycemic indexes, blood pressure and others. However, when we compared the most general expert opinion on the group under investigation and the results of the present study, we found that among eight pregnant women diagnosed with myocardial dystrophy with the threatened miscarriage at the early stage, six women were identified as belonging to clusters I and M_P, i.e. to the clusters with substantial shift to sympathicotomia. The reason for this is that the development of myocardial dystrophy in pregnant women is accompanied by strengthening of sympathoadrenal effects on cardiovascular system. Similarly, most of the pregnant women under investigation diagnosed with "preeclampsia" belonged to our “bad” clusters I and M_P (five out of six pregnant women). Among seven women with anthropometric factor $BMI > 35\, kg/m^2$, five belonged to “bad” clusters. It turned out that five women from the group of six pregnant women with chronic hypertension belonged to cluster I, and only one to cluster H. Thus, we can conclude that the proposed method gives the integral characteristics of the physiological state and can be used for the detection of disorders at early stages of gestation.

The approach to the analysis of non-stationary *HRV* developed in this paper and based on the study of rhythm restructuring can be applied in many studies devoted to revealing the abnormalities in the cardiovascular system.

The detailed calculation of rhythm characteristics in transient processes allows identifying a disease manifested in arrhythmias at an early stage. The proposed technique can be used in testing the adaptive capabilities of a person (an important problem in training), to study the dynamics of interaction between the sympathetic and parasympathetic divisions of the autonomic nervous system, as well as to analyze the rhythmogram during the biofeedback session.

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