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Heart rate variability analysis during normal and hypertensive pregnancy

Eduardo Tejera Puente

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Supervisors

Professor (a) Maria Irene Rebelo
Professor José Manuel Nieto Villar

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Abstract

The heart rate variability or essentially, the continuous changes of heart beats, measured through electrocardiographic procedure is, in general, influenced by the sympathetic and parasympathetic nervous activity. However, several other underlying mechanisms are also involved as respiratory rhythms, hormonal changes, inflammatory response and blood pressure regulation; all of them are significantly modified during normal or pathological pregnancy. Heart rate variability results from the sum of all these process responses, and therefore, should be plausible to expect several heart rate variability behaviours during normal or pathological pregnancies.

This study investigates, through a longitudinal approach, the changes in the HRV fluctuation during normal and hypertensive pregnancy, focused in the maternal HRV complexity changes and considering beside normal state, hypertensive pregnancy and preeclampsia.

To reach our goal, several ECG measurements were performed during the pregnancy period of several women considering other concomitant factors like maternal age, body mass index, systolic and diastolic blood pressure, smoke habit, parity, drug treatment and personal and familiar history of hypertension, diabetes or preeclampsia. Otherwise we also considered, for some women, the common biochemical markers, routine hemogram, as well as uric acid and creatinine blood levels, in order to study correlations between these biochemical markers and the indexes obtained for HRV analysis.

Several mathematical methods are available for HRV analysis and within this wide spectrum of possibility we essentially applied, inside the linear procedures, the mean and heart rate standard deviation as well as the spectral signal analysis. On the other hand, considering the nonlinear, we applied approximated and sample entropy, detrended fluctuation analysis and Lempel-Zip complexity indexes. All these methods are capable for quantifying, at some level, HRV signal properties.

Both, normal and pathological pregnancies were associated with a reduction of the parasympathetic activity, a reduction of mean RR intervals and standard deviation as well as a reduction of HRV complexity during gestational age, however, all these parameters reveal a deep and progressive reduction through normal, hypertensive and preeclamptic pregnancies. In this sense, the lowest complexities and parasympathetic activity indexes

were obtained for the preeclamptic women. On the other hand, during normal pregnancy the sympathetic activity seems to increase until 2nd trimester of pregnancy followed by a progressive reduction. This profile was also observed in the hypertensive group but a polemic sympathetic activity reduction was identified for the preeclamptic group.

This sympathetic activity reduction in preeclamptic women was related, at least partially, to the drugs treatment, however, even in the non-treated group, the parasympathetic activity seems to be lower. Our results, also demonstrated that general autonomic balance is modified by parity effect. Primigravid women presented lower complexity values and reduced parasympathetic activity in comparison with two-parous women, which suggest, some adaptative process. Other factors like maternal age, personal and familiar history of hypertension and diabetes were also related with different obtained indexes. On the other hand, in the preeclamptic group, the increment of complexity was related with an increment of hemoglobin concentration and a reduction of platelet count, while the low frequency region was associated with a reduction of uric acid concentration.

Using the obtained HRV indexes we were capable to classify around 80% of the preeclamptic cases and, even higher sensibility values for hypertensive and normal women were obtained, using a neural network model. We also tested an alternative method for HRV analysis improving the differentiability capacity between normal and preeclamptic groups and, therefore, potentially increase the classification power already obtained.

This study comprises a wide kind of HRV modification during normal and pathological pregnancies, exploring some aspects related to physiological significance and clinical applicability. However, also reveal some aspects that could inspire future research studies.

Resumo

A variabilidade da frequência cardíaca (VFC), ou principalmente, as contínuas mudanças dos batimentos cardíacos, medidas através do electrocardiograma são em geral influenciadas pela actividade do sistema nervoso simpático e parassimpático. No entanto, vários outros mecanismos subjacentes também estão envolvidos como os ritmos respiratórios, as alterações hormonais, a resposta inflamatória e a regulação da pressão arterial, todos eles se apresentam significativamente alterados durante a gravidez normal ou patológica. A variabilidade da frequência cardíaca resulta do somatório de todas essas respostas e, portanto, deve ser plausível esperar vários comportamentos durante a gravidez normal ou patológica.

Este estudo analisa através de uma abordagem longitudinal, as alterações na flutuação da VFC durante a gravidez normal e hipertensa, com realce nas mudanças da complexidade da frequência cardíaca materna VFC da gravidez normal, da hipertensa e da pré-eclâmptica.

Com este objectivo, medidas de ECG foram realizadas durante o período de gestação em várias mulheres, considerando outros factores concomitantes como idade materna, índice de massa corporal, pressão arterial sistólica e diastólica, hábito de fumar, paridade, tratamento com fármacos e história pessoal e familiar de hipertensão, diabetes ou pré-eclampsia. Em algumas mulheres foram também avaliados marcadores bioquímicos comuns, hemograma, concentração sérica de ácido úrico e creatinina a fim de identificar possiveis correlações entre esses marcadores bioquímicos e os índices obtidos através da análise da VFC.

Entre os numerosos métodos matemáticos disponíveis para análise da VFC aplicamos os de análise linear, o desvio médio padrão da frequência cardíaca assim como a análise do sinal espectral. Por outro lado, considerando os não-lineares, aplicamos a entropia aproximada e a entropia da amostra, os índices de complexidade Lempel-Zip entre outros. Com estes métodos é possível quantificar, em algum nível, propriedades do sinal da VFC.

Ambas, gravidez normal e patológica, foram associadas a uma redução da actividade parassimpática, uma redução do intervalo promedio RR e do desvio-padrão, assim como uma redução da complexidade da VFC durante as diferentes idades gestacionais. No entanto, todos estes parâmetros revelam uma profunda e progressiva redução durante a gravidez normal, a hipertensa e a pré-eclâmptica. Nesta análise, as baixas complexidades e

a menor actividade parassimpática foram obtidas para as mulheres pré-eclâmpticas. Por outro lado, durante a gravidez normal a actividade simpática parece aumentar até ao 2º trimestre de gestação seguido por uma redução progressiva. Este perfil também é observado no grupo de hipertensas, mas uma redução polémica da actividade simpática foi identificada para o grupo de pré-eclâmpticas.

Esta redução da actividade simpática nas mulheres pré-eclâmpticas foi relacionada, pelo menos parcialmente, com o tratamento com fármacos; no entanto, mesmo no grupo não tratado, a actividade parassimpática parece ser inferior. Os nossos resultados também demonstraram que o equilíbrio autonómico é em geral modificado pelo efeito da paridade. As mulheres primigrávidas apresentavam valores de menor complexidade em comparação com as mulheres grávidas do 2º filho, o que sugere um certo processo de adaptação. Outros factores como idade materna, história familiar e pessoal de hipertensão e diabetes também foram relacionados com os diversos índices identificados. Por outro lado, no grupo de mulheres com pré-eclampsia, o aumento de complexidade estava relacionado com o aumento da concentração de hemoglobina e com a redução dos níveis de plaquetas no sangue, enquanto a região de baixa frequência foi associada com uma redução da concentração de ácido úrico.

Através dos índices de VFC obtidos, fomos capazes de classificar cerca de 80% dos casos de pré-eclampsia e, valores de sensibilidade ainda mais elevados foram obtidos para as mulheres com gravidez normal e hipertensa, utilizando uma rede neural. Também testamos um método alternativo para a análise da VFC de modo a melhorar a capacidade de diferenciação entre o grupo de mulheres com gravidez normal e com pré-eclampsia com o intuito de aumentar potencialmente o poder de classificação já obtido.

Este estudo compreende um amplo tipo de modificação da VFC durante a gravidez normal e patológica, explorando alguns aspectos relacionados com o significado fisiológico e a aplicação clínica. No entanto, também revela alguns aspectos que poderiam servir de base para futuros trabalhos de investigação.

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Acronyms Notation

HRV: heart rate variability

PRE: preeclampsia

HT: hypertensive

ECG: electrocardiogram

RR: interval between consecutive R peaks in the electrocardiographic records

ANS: autonomic nervous system

AV: atrioventricular node

SNS: sympathetic nervous system

PNS: parasympathetic nervous system

CRP: C-reactive protein

IL: interleukin

TNF-alpha: alpha tumor necrosis factor

RRm: mean RR intervals

RRstd: RR standard deviation

PSD: power spectral density

ULF: ultra low frequency

VLF: very low frequency

LF: low frequency

HF: high frequency

LFnu: low frequency in normalized units

HFnu: high frequency in normalized units

LZ: Lempel-Ziv complexity

ApEn: approximated entropy

SE: sample entropy

MSE: multiscale entropy

DFA: detrended fluctuation analysis

mHRV: maternal heart rate variability

fHRV: fetal heart rate variability

SBP: systolic blood pressure

DBP: Diastolic blood pressure

"That which is Below corresponds to that which is Above, and that which is Above corresponds to that which is Below…"

H.T. Esmerald Tablet

Chapter I

The study of heart rate variability

I.1 Bases of Heart Rate Variability

The bases of the HRV analysis is the RR time interval obtained for electrocardiographic records (ECG). As suggested by the name, the RR interval is the time difference between two consecutive R peaks of the ECG wave complex (Fig.1). The sequences of these consecutives time differences generate a time series with several interesting and particular properties.

Fig.1. Top) A normal ECG with the location of the wave complex. Bottom) An RR time series obtained by consecutive RR time interval.

The nature of the ECG signal is well known and has already been fully described elsewhere [1-2]. To our propose is only important to remark that, in general, the RR signal is only composed by normal sinus beats with roots in the sinoatrial (SA) node located in the right atrium of the heart.

In the study of arrhythmias, some authors consider the inclusion of other beats (extrasystoles o ectopic beats) that are not generated in the SA node and may increase the RR time series information [3]. However, usually these beats are interpolated or removed in the HRV analysis [1-2, 4-5].

The main influence on the heart rate variability (HRV) is due to the interaction of the two branches of the autonomic nervous system (ANS): sympathetic and parasympathetic control. In the SA node several specialized cells act as pacemakers generating intrinsic action potentials with a spontaneous frequency of around 60-100 beats/ minute [1-2]. This electrical impulse is propagated by the specialized conductor system to the atrioventricular node (AV). If the SA node is dysfuntional or if the impulse is blocked, then the AV node becomes the main pacemaker with a lower frequency (40-60 beats/min) [2].

The sympathetic nervous system (SNS) is activated during stressful situations as a “fight or flight” response mechanism. The rapid release of noradrenalin increases the SA firing and, therefore, the heart rate. The SNS activity is complemented by the parasympathetic nervous system (PNS) response that acts through the heart by means of vagus nerve. In

this case, the release of acetylcholine in the nerve terminals results in a decrement of heart rate.

The SA node has a higher content of acetylcholinesterase and, therefore, any vagal stimulus acts very quickly, however, under resting conditions the vagal tone prevails [1-2]. Parasympathetic influences exceed sympathetic effects probably via two independent processes: a cholinergically induced reduction of norepinephrine released in response to sympathetic activity, and a cholinergic attenuation of the response to an adrenergic stimulus [2]. This prevalence of the parasympathetic activity leads to a reduced heart rate in contrast with intrinsic pacemaker and provides a better capability to response fast to different physiological or pathological situations.

Fig.2. ANS ramifications. Sympathetic (red) and Parasympathetic (blue) nervous system. (From Gray´s Anatomy [6])

As can be observed, several other organs are connected with the ANS (Fig.2) and therefore, the overall response will be a consequence of the global balance.

The wide ramification of the ANS leads to the necessity of considering the HRV as a complex and multifactorial phenomena. This multifactorial bases of the normal HRV as the result of many other physiological interactions, is a major advantage and at the sametime, a big problem in the HRV analysis and interpretation.

The normal RR variability interval is not stationary, this means, RR intervals naturally change across the time by several reasons and under different scales. In fact, some known physiologic processes interact under several time periods [2, 7-10]. The normal RR interval is around 1 sec (mean heart rate of 60 beats/min), the respiratory rhythm (major PNS activity) that

considerably affects the HRV is around 4 seconds period, the blood pressure control (Mayer waves) as baroreceptors and chemoreceptors modulations is around 10 seconds period, while other processes like hormonal changes and circadian rhythms are superior to 1 minute oscillations or even days and could also affect the HRV. Beside all these underlying mechanisms, the complexity and fluctuation of heart rate is beyond the fact of several inputs under different time scales [8].

Because HRV is an overall response, it seems plausible to think that some measurements could be altered under different physiological or psychological states. This hypothesis has been confirmed by several authors and is briefly represented in Table I. However, more difficult results the connection between HRV measurement and biochemical markers. Evidently, catecholamines levels or any other biochemical markers directly connected with ANS activity also would be related with HRV indexes; however, it is surprising the wide kind of metabolic species that have been correlated with the HRV as briefly represented in Table I.

Table I. Some review connecting HRV measurements under different conditions.

Pathologies or general states	**Biochemical Markers**	**References**
Chronic kidney disease	Hemoglobin concentration	[11]
Ageing		[12-14]
Multiple organ dysfunction syndrome		[15]
Cardiac related diseases	CRP concentration	[13, 16-22, 45-46]
Diabetes and Diabetic neuropathy	Glucose levels	[23-26]
Cardiac transplantation		[27, 28]
Drugs effect		[29-33, 42, 50]
Exercise training		[34-37]
Psychological conditions	Catecholamines levels	[38-41, 49]
Hypertension		[42-44]
Inflammation	IL-6, CRP, TNF-alpha, Fibronectin levels	[45-48, 50]

Notes: CRP: C-Reactive protein, IL-6: interleukin 6, TNF: tumor necrosis factor.

Beside the pathophysiological states reported in Table I, other application of HRV analysis could be found in areas like epilepsy, Parkinson disease and cancer [51-53]. Actually, the full representation of all scientific branches, where HRV analysis was applied, would be very extensive and far form the present scope.

The mechanisms behind HRV modifications under normal or pathological conditions and the relationship with some of the reported biochemical markers are not completely understood; however, these studies reveal and suggest not only the several processes involved in HRV modification but also, the applicability and importance of continuing this area of investigation. This fusion of mechanisms and responses that would point out the heart as an adaptive organ, induces several doubts to HRV analysis: How we can separate the ANS components? Will it be possible to extract and identify all the implicit information? Could the HRV be useful to predict pathological events? These questions and many others could arise from the nature itself of the control mechanism involved in the HRV fluctuations and some of them remain under discussion in present times, mainly, those aspects related to the clinical usefulness.

I.2. Methods for Heart Rate Variability Analysis

This section could be entitled as “Method for biological signal analysis” because, in fact, most of the methodologies are used in other signal analysis beside the RR time series and in any case, the RR is a particular case of biological signals. Other examples are electroencephalographic and blood pressure records, but in a more general sense, the methodologies that we will discuss are valuable for any measurement performed in a biological (or not) system during different periods of time.

Traditionally and in a very global approach, the mathematical tools applied in the HRV analysis could be separated in two general branches: I) Linear and II) Non-linear methods based on the nature of the interactions or, alternatively, could be rearranged as [1-2, 54]: I) time or II) frequency domain and III) non-linear methods. In any case, the goal of any of these methods is to extract the information content of the RR signal and transform to a unique quantitative pattern (single number or not). Evidently, behind any of these methods or mathematical indexes some mathematical or physiological model is implicit that would be helpful to explain the results while, at the same time, impose the respective limitations. For example, the spectral analysis assume stationary signal, therefore, we can use the method and extract useful information about the frequency structure but the

nature or the model leads to a very careful application in RR signal recorded during a very long period of time.

Nowadays we could have a higher number of methodologies with the subsequent mathematical description for the RR signal analysis and evidently, is impossible to embrace all of them in the present study. For this reason we will discuss some of the more applied methods in the HRV analysis and particularly those used in our study.

I.2. 1. Time Domain Methods

In this group we have the conventional mean (RRm), standard deviation (RRsd) and variance of the RR signal. Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N:

$$RRm = \frac{1}{N}\sum_{i=1}^{N} X_i \qquad \text{eq.1}$$

$$RRsd = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(X_i - \overline{X}\right)^2} \qquad \text{eq.2}$$

Obviously, the variance (used as a HRV descriptor) will be related to the RRsd. These two indexes are very useful by their simple calculation and interpretation. Other indexes, NN50 and pNN50, represent the number (or percent) of pairs of successive RR intervals with more that 50 ms (with respect to the total number of interval in the pNN50 case) [55]. The NN nomenclature indicates normal beats. The method was further generalized to NNx and pNNx considering $4 \leq x \leq 100$ milliseconds (ms), with useful results to discriminate some pathology chiefly for $x \leq 20$ instead of 50 ms [55-57].

There are other methods that transform the RR interval sequence into a graphic representation (called geometric methods [1,2]) like the recurrence plot and some variation of the Poincaré maps, but the indexes usually extracted from this representation have linear behaviors [58-60]. In the last years the Wavelet methods have been used widely in the HRV analysis as preferred time-domain methods. The Wavelet methodology opens the possibility to explore different temporal pattern in a qualitative and quantitative way and even provide some link with the non-linear indexes [61-65], but we don't use them in the present study.

I.2.2 Spectral Domain Methods

This kind of method is, undoubtedly, the most used in the linear approaches because as we already discussed, different physiological mechanisms act under different times. Several methods for the spectral analysis are available [1-2, 9] and, in general, could be classified as parametric or non-parametric with comparable results. The spectral analysis is a fast procedure (mainly using the Fast Fourier Transform –FFT-). The major information is the power spectral density (PSD) that means how the power is distributed in the frequency space.

In the FFT the measurement needs to be performed in equal intervals [1-2, 9]. However, the RR intervals are not at the same time intervals because the RR interval itself is variable. To avoid this problem some considerations are available as using the succession order of the RR interval as "time variable", this mean: if $\{X_1, X_2, X_3 \ldots X_N\}$ is the RR interval sequence, the "time variable" will be 1, 2, 3,...,N instead $t_i = \sum_{k=1}^{i} X_i$ or alternatively, an interpolation is performed by resampling under a constant time interval. Both methods present some limitation concerning to information loss or even information creation [1, 68]. However, the problem could be actually solved using the Lomb-Scargle periodogram (LS) method [1, 66-68]. The LS method performs a linear least square regression of unevenly spaced data to a sine/cosine series of different frequencies. If we define $\{X_1, X_2, X_3 \ldots X_N\}$ as the measure of variable X sampling at unequal time ($\Delta t = t_{i+1} - t_i \neq$ constant) with a mean $\overline{X}$ and variance σ^2 then the normalized LS periodogram (PSD) is:

$$PSD(w_i) = \frac{1}{2\sigma^2}\left\{\frac{\left[\sum_j (X_j - \overline{X})\cos w_i (t_j - \tau_i)\right]^2}{\sum \cos^2 w_i (t_j - \tau_i)} + \frac{\left[\sum_j (X_j - \overline{X})\sin w_i (t_j - \tau_i)\right]^2}{\sum \sin^2 w_i (t_j - \tau_i)}\right\} \quad \text{eq.3}$$

Where the sums over j are from 1 to N and the constant τ_i is defined for a given angular frequency (w) as:

$$\tau_i = \frac{1}{2w_i}\arctan\left[\frac{\sum_j \sin 2w_i t_i}{\sum_j \cos 2w_i t_i}\right] \quad \text{eq.4}$$

In the evenly sampled limit, the eq.3 could be reduced to the conventional periodogram definition. Even when other approaches are available to deal with the unevenly sample data, the LS methods is usually preferred because solve the problem at sufficient level and, on the other hand, the routine codes as well as software are freely available.

In the HRV analysis four major frequency bands are usually separated [2] (Fig.3):

- Ultra low frequency (ULF): ≤ 0.003 Hz
- Very low frequency (VLF) : 0.003 - 0.04 Hz
- Low frequency (LH) : 0.04 - 0.15 Hz
- High frequency (HF) : 0.15 - 0.4 Hz

The frequency quantification is generally obtained by the sum of the power of these bands and/or forming ratios between them (i.e. the LF/HF ratio). In the short records analysis the ULF band should not be considered and the VLF band is therefore ≤ 0.04 Hz. The reason of this change is a direct consequence of the sample size. If the record is performed during 5 min or 300 sec (the minimum size recommended) the minimal frequency that can be resolved is 1/300 = 0.003 Hz that, as we can note, is the borderline between ULF and VLF and therefore, the ULF has not sense.

Fig.3 Example of an estimated PSD obtained from entire 24 h record. (Adapted [2]).

On the other hand, another frequency limit should be considered (Nyquist frequency) [1, 68]. The average time interval for N points over a time T is $\Delta = T/N$, consequently the average Nyquist frequency constrain ($0 \leq f \leq 1/2\Delta$) is: $fc = N/2T$. Usually, the upper frequency in the HRV analysis is around 0.4 Hz and therefore, $N/2T \geq 0.4$ suggesting that for a record of 5 min the minimum N should be around 240 points and hence the mean RR interval should be around 1.25 seconds. This clearly reveals that if in 5 min there are not RR intervals with less than 1.25 seconds, the contribution to the HF is poor. On the other hand, as we previously discussed the LS method applies a linear least square regression procedure and so the number of points is important. This mean that the estimation and significance of the HF power estimation will be dependent of the number of points associated with this frequency region [1, 68].

The spectral methods have two important and intrinsic considerations: the signal is stationary (a common problem for many not spectral methods) and the signals at each frequency are independent [1,2,68]. The condition of stationarity is extremely important

in the same way that we increased the recording time. Holter procedures (24 h) of recording give the possibility to study very low frequencies like the circadian rhythm but the entire signal spectral analysis should be carefully analyzed or fragmented in smaller time intervals.

The frequency independent assumption is very polemic, because as we can presume, the mechanisms involved in the HRV regulation don't have to be independent in all cases. There are several models that circumvent this problem, even so, there are beyond the scope of the present study.

Another important aspect in the spectral analysis of the HRV could be presented with the following related questions: Why to separate the frequency region in three or four bands? What are the physiological meanings of these regions? In fact, there is not an objective reason to split the spectral region as previously described. The presented bands lie in the common believe that (at least) main mechanisms involved in the HRV regulation are located approximately in that frequency range [1,2]. However, this is only a "conviction" because, in fact, the evidence suggests that one region could be associated with more than one mechanism or even worst, one mechanism could affect several regions [2, 69-74].

Traditionally the HF region is associated almost exclusively with parasympathetic activity, while the LF is generally assumed as a mixture between parasympathetic and sympathetic activity [2, 69]. The physiological association of VLF and even ULF is more polemic without a clear interpretation. On the other hand, the LF/HF ratio is scale independent and is generally interpreted as a sympathovagal balance, changing in several physiological and pathological conditions. The number of studies trying to explain or associate the spectral bands to clear physiological conditions is higher [69-74] and we will reduce this space just to the HRV modifications associated with pregnancy state. However, it is important to explain that some of the contradiction in the results of various experiments referring the LF band could be, at least partially, explained by the measurement differences.

Some physiological conditions beside reducing the LF or HF bands tend to decrease the total power spectrum and, consequently some authors suggest the use of normalized LF and HF band (LFnu and HFnu) instead of the absolute values (expressed as ms^2) [2, 75]. Some evidence has showed that vagal activity (mainly HF) is in fact the major contributor of the absolute value of LF with some contribution from the sympathetic activity (76). Thus, the standard procedure suggests that LF should be normalized by HF (LF/HF) or

total power to represent sympathetic activity [77-79]. In fact, the reproducibility of the spectral indexes in normalized units is higher as well as the convergence between measurement in different labs [75]. A central reason of this homogeneity is that using normalized units the values tend to follow a normal distribution. However, there is a big problem with this normalization associated with redundant information: the normalized procedure is generally as [75]: LFnu = LF/(VLF+LF+HF) and LHnu = HF/(VLF+LF+HF) or in a general form: LFnu = LF/constant, HFnu = HF/constant. It is important to notice that the calculation is performed after HF and LF power calculation and consequently: HFnu + LFnu = 1. This mean that they are inversely related and if LF increases the HF will decrease by the mathematical formulation instead of a system property. If we consider the HFnu as parasympathetic and LFnu as sympathetic activity, then if experimentally we find an increment of the parasympathetic control we will obtain a collateral reduction of the sympathetic control and LF/HF index in the same proportion and obviously, this should not be precisely true in physiological sense. In other words, both HFnu and LFnu are informative redundant variables as well as the ratio LFnu/HFnu (that evidently is the same as LF/HF with no normalized unit). In this sense, the general conclusion is that the variables: LFnu, HFnu and LF/HF give the same information content and therefore, only one should be required.

I.2.3. Nonlinear Methods

The nonlinear methods are, in fact, a big family of mathematical indexes and procedures focused in several signal properties like [9]: fractality, chaotic behaviour, periodicity or regularity, entropic modifications and so on. In any case and, in a very general point of view, the goal is to describe patterns in the signal beyond the spectral structure capable to extract another set of information. On the other hand, in the non-linear indexes some words like: physiological complexity, short and long-term correlation and many others are frequently used and therefore, need to be clearly defined.

Fig.4 Four different kind of signals: I) periodic, II) Brownian noise, III) chaotic system and IV) RR signal. All of them present similar means and standard deviations.

As can be observed in Fig.4, several signals could have similar mean and standard deviations and still, we can obtain signals with the same power spectrum (using the same autocorrelation function [80]) although, they are very different signals and are undifferentiated with common linear methods. In these cases, the use of nonlinear methods could be a helpful tool [81-83].

If we consider a system under a constant stimuli-response dynamics and if this dynamic is characterized by different scales, it means, the system has to respond quickly or slowly under several circumstances by different time-dependent mechanisms, then our questions about the system stability, adaptability, memory or dynamic patterns, are very logical. All these aspects that could describe the system dynamic, characterize the basic ideas of the nonlinear methods, however: How real are supported chaos and fractality HRV properties under physiological and metabolic bases?

This question can't be satisfactorily answered; however the fractal structures are present in physical and biological pathways, proteins, interaction networks, vasculature and the His-Purkinje network of the heart [82-83,100]. On the other hand, fractals are also observed in time processes, such as HRV, blood pressure and encephalographic response. Alternatively the HRV could present several dynamic patterns, from periodic to random behaviour under different external/internal conditions [84-85]. Such known mechanisms under HRV control are sufficient to suggest several kind of response under different time scales not always independents. Obviously, the integration of all these processes and structures in a unified knowledge is not yet possible but investigations continue under several scientific branches increasing the evidence level and our understanding.

The physiological complexity is characterized by the presence of one or more of the following aspects [86]: I) non-linearity, II) non-stationarity, III) time irreversibility and

IV) multiscale variability. These aspects facilitate system adaptation to external/internal stimuli and the necessity to respond across different temporal scales; both these aspects are the bases of the physiological complexity concept [86, 87] or, in other words, the reflection of the system robustness [88]. As can be observed, the complexity as described is not synonymous of complication that is more used in terms of the number of variables. In this sense, a system with only three variables that could be very simple, can generate a very complex dynamic involving transitions from periodic to chaotic behaviour.

Even when most of the cited studies concerning aging and/or disease, using physiological signals, reveal a physiological complexity reduction [12, 86, 89-95], others point to the possibility of the increment in the physiological complexity depends on the task in progress [96], postural changes and other conditions [95, 96-99]. This bi-directional complexity hypothesis, however, remains under open discussion [13].

In general, system complexities could be modified, by the transition to a more random or periodic dynamic. If we measure the complexity on the bases of "unpredictability" or irregularity (mathematical complexity) of the time series, an uncorrelated white noise would indicate a high complexity value in contrast to Brownian or chaotic dynamic behaviour. Following the irregularity complexity criteria in the analysis of physiological signals, the maximal complexity may correspond, for example, to some cardiovascular pathological conditions like atria fibrillation or arrhythmias where erratic rhythms appear in contrast to normal healthy rhythm that is regulated by multiple interconnected mechanisms. The previous discussion means, in fact, that an increment of irregularity or unpredictability does not involve an increment in the physiological complexity [93-94], this has been the main base to criticize the bi-directional complexity hypothesis.

The nonlinear methods are subject to criticism based on two principal premises: usefulness and interpretability. The first is related to the spectral indexes: Are the nonlinear indexes better than the linear? Or in other words: Do the non-linear indexes really give new information with respect to the linear indexes? In this sense, several comparisons have been performed and now the debate still continues [101]. In the prediction of sudden death, for example, both lineal and nonlinear indexes reveal similar independent results and, in fact, there is a very well know correlation between some complexity indexes and the LF or HF band of the spectral analysis under different pathological and physiological conditions [90, 101-102]. However, some important observations should be made. The nonlinear indexes have some important limitations: signal length, stationarity, noise influence and signal artefacts (missing beats or

arrhythmia inclusion). As we can note, are in general the same factors that affect the linear methods however, some nonlinear methods avoid the stationarity problem (i.e., detrended fluctuation analysis) and even provide some information about the scale-pattern modifications [86, 94]. These types of scale-methods provide better results in the classification of patients with cardiovascular heart failure and, in general, are the best successfully approaches within the nonlinear tools instead of those that only provide a single overall quantification [103-104].

Other important problem in HRV analysis concerns to the reproducibility of the calculated indexes. Recently, several authors described that the time-domain and complexity indexes present a better reproducibility (RRsd) compared to spectral indexes in extremely short time series [105-106] however, the spectral and complexity indexes are more sensible to the missing beats or outliers (by arrhythmia) than other time-domain methods (like RRm and RRsd) [107-109]. Some results suggest that in some cases, complexity indexes are better descriptors than spectral analysis. [110-111]

The so-called multiscale methods like the multiscale entropy have shown better results but remain under a very polemic discussion concerning their physiological meaning. As we previously discussed, is generally accepted that in ageing or disease the complexity decreases by rupture of one or more of the discussed properties but, obviously, it is not enough for a proper physiological meaning. However, it is the only thing that we can say with some kind of confidence about the physiological meaning of complexity.

There is another important aspect that we believe should be discussed. There are several wellknown methods (mathematically) for the study of the complexity and chaos related properties in time series: Why should we use or develop new methodologies instead to using the conventionals? The answer is not simple but is supported in the time series length and stationary. The conventional methods (i.e., Lyapunov exponent, time delay, embedded dimension or correlation dimension) require time series with high number of points and under stationary conditions, however, the increment in the number of points increase the non stationary properties of the signal, therefore they have a poor implementation in the HRV analysis.

I.2.3.1 Lempel-Ziv complexity

The Lempel-Ziv complexity [112-113](LZ) is an useful tool to measure complexity by quantifying the randomness in a sequence. As many other quantification procedures, it is

supported in methods of symbolic dynamic. In this sense and, similar to other approaches, the time series (the RR signal) suffer some kind of codification being the core of the LZ calculation the determination of different patterns contained in the finite sequence.

In this kind of procedure two important parts should be considered: how to transform RR signal to symbols and the further mathematical index definition. The RR signal is transformed by some discretization procedure; in this case, it is usually considered the increment or decrement with respect to the mean value or with respect to the precedent RR interval forming a binary code (i.e., 1 or 0 for increment or decrement, respectively). Other alternative encoding can be done by the use of more than two codes; however, the number of codes (alphabet size) is an important aspect that should be considered in any discretization procedure.

In the LZ complexity procedure after codification, the goal is to count the number of different substring in the sequence. If S(N) is the original sequence (obtained for RR signal transformation) with alphabet size n and c(N) is the number of different substring in S(N), then:

$$LZ(N) = \frac{c(N)}{b(N)} \qquad \text{eq.5}$$

Where $b(N) = N/\log_n(N)$ is a normalization term that describes the asymptotic behaviour of LZ(N) for a random sequence. Therefore, the LZ range varies between 0 and 1 indicating the complete deterministic pattern (ex: sine function) and uncorrelated sequence (ex: white noise), respectively. As we previously discussed the signal length is an important parameter to consider in all the methods and LZ is not an exception, however, beside a wide application in HRV analysis, several studies described the LZ as useful in series with a reduced number of data and also good stability and reproducibility.

I.2.3.2 Approximated, sample and multiscale entropies

The ApEn [114] (ApEn) has been used in several time series analysis and, in general, is a measure of irregularity or unpredictability of the time series. On the other hand, the ApEn is similar to the sample entropy [115] (SE). Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N, we can define the vector $Y_m(i)= \{X_i, X_{i+1}, X_{i+2},\ldots X_{i+m-1}\}$; if we define $n^m_i(r)$ as the number of vectors $Y_m(j)$ that are close to $Y_m(i)$ ($d[Y_m(i),Y_m(j)] \leq r$, $i \neq j$, where d is the Euclidian distance and r the distance cutoff) then:

$$ApEn(m,r,N) = \frac{1}{N-m} \ln \frac{n_i^m}{n_i^{m+1}} \quad \text{eq.6} \qquad SE(m,r,N) = \ln \frac{\sum_{i=1}^{N-m} n_i^{'m}}{\sum_{i=1}^{N-m} n_i^{'m+1}} \quad \text{eq.7}$$

where the differences between n'^m and n^m are associated with the inclusion of self-matched elements. We can note that both indexes are very similar and this similarity could be related to the Renyi entropy [94]. The difference with respect to the multiscale entropy (MSE) [86, 94] is that the calculation is not performed over the original time series but instead it is used an average coarse graining time series obtained as:

$$y_j^\tau = \frac{1}{\tau} \sum_{i=(j-1)\tau+1}^{j\tau} X_i \quad \text{eq.8}$$

where τ represent a scaling factor and $1 \leq j \leq N/\tau$. Smaller values of ApEn and SE imply a time series with similar pattern of measurements and consequently more "regular". We can note that in MSE, the irregularity (or informational content) expressed as SE is analysed across different scales and, therefore, it is more suitable to study short and long-term correlations. In our calculation, the conditions were ApEn(2, 0.2, N) and SE(2, 0.15, N) because are the common values used in the HRV analysis, however, theoretically each system could present an optimal value of m (that is related to the time-delay and embedding dimension) and r.

The use of coarse graining procedure, require a signal with a big number of points because in the scaling process the real signal (y_j) has N/τ points. For example, if the original signal has 1000 points it is transformed to a time series with 500 point to SE calculation for τ=2. This method of scaling, as we can expect, even when reduce the noise of de signal by local averaging, can not be applied in short time series using scale intervals above to 3 or 4.

It is important to note that the ApEn, SE and LZ complexity are measures of irregularity and, in this sense, the complexity is increased in uncorrelated noise [116]. Therefore, one more time, we must discuss complexity modifications. There is, some debate regarding complexity reduction, the use of LZ, ApEn or SE in a random signal result in higher complexity values, however, in a random signal no correlation or fractal structures are present and therefore, the properties of nonlinearity and multiscale variability are absent reducing the complexity.

To solve this contradiction some authors suggested the idea (previously discussed) of physiological and mathematical complexity which are the background of criticism of the

bidirectional hypothesis. The physiological complexity is maximal in systems with the discussed properties, while the mathematical complexity is maximal in uncorrelated signal. As can be noted, both methods converge in the transition to periodic behaviour because in this path both, mathematical and physiological complexities should be minimal. However, if the dynamic shifts to a more random dynamic (by random addiction or surrogation), then the difference in both approaches emerges, the physiological complexity should be minimal and the mathematical complexity will be higher.

Fig. 5. Left) Plot of MSE average signature for different age groups in healthy conditions. Right) Plot of MSE average signature for extreme age groups in healthy/disease conditions. Adapted from [13]

For example, in Fig.5 Left, we can observe a reduction of the complexity from younger age groups to the elderly in healthy conditions, however, in Fig. 5 Right, the reduction of complexity between healthy and pathological condition (i.e. arrhythmia), is only significant for younger groups. This simple result indicates that the hypothesis of complexity reduction with aging could be valid but the reduction of complexity by disease condition is not valid, at least for groups of older.

There is not a mathematical index capable to describe completely all the properties of the physiological complexity. As we proved in precedent studies, the MSE even when could increase our understanding of the signal structure, can not describe in all cases a maximal complexity in health and/or disease [13]. The “healthy” approach to define the transition pathway is made by combining several nonlinear methods based on complexity (entropic or not) and fractal (or short and long-term correlation) structure.

I.2.3.3 Detrended fluctuation analysis (DFA).

The DFA [117] is a method that gives information about short and long-term correlations (fractal-like type) and can be applied in non stationary time series to detect some apparent self-similarities. The first part of computation is the integration of the signal as follows:

$$Y_j = \sum_{i=1}^{j} \left(X_i - \overline{X} \right) \qquad \text{eq.9}$$

The integrated signal is divided into boxes of equal length n. In each box a linear or polynomial function is fitted to the data. This fitted curve is, in fact, the trend of the signal inside this box with values $\hat{y}_n$. The detrend procedure is:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} \left(Y(k) - \hat{y}_n(k) \right)^2} \qquad \text{eq.10}$$

where N is the number of points. As we can note, detrend procedure is the difference between the real and the predicted (trend) value of the signal. The root mean square fluctuation (F(n)) will increase with the box length n increment. However, if scaling properties are present then F(n) ~ n^{α} with scaling exponent α.

This power exponent α has a closer relationship with Hurst exponent. Values of α=0.5 represent a white noise, α=1 represent 1/f noise and α=1.5 indicate a Brownian noise. However, in the RR interval time series, α exponent could not be constant in all the scale interval and for this reason α is usually divided into intervals α_1 and α_2 representing the short (4-13 beat) and long-term (>13 beat) coefficient, respectively [89, 117, 118].

Evidently that separation is an approximation, in fact, is well known that in general a multifractal structure is defined by a set of scaling exponents. The DFA method was generalized to multifractal systems (MDFA) [119] with further applications in HRV analysis proving that in some pathological conditions, the multifractal structure is modified by reducing the scale exponent variations [120].

Chapter II

Heart rate variability in pregnancy

II.1. Common changes of heart rate variability indexes during pregnancy

Pregnancy is an state that trigger changes both at physiological and psychological levels. Inflammatory, immunologic, hormonal and hemodynamic changes during pregnancy are remarkable and modifications in all these systems are somehow connected with the autonomic control and consequently with the HRV. Besides the intrinsic metabolic modifications some other "controllable" variables like maternal weight, alimentary regimen and daily exercise changes are ver heterogeneous which may increase the internal variability of the data.

As already discussed, HRV is a sum of different mechanisms and if pregnancy is a state of change, seems plausible to think that some of these modifications could be extracted from HRV analysis. Obviously maternal HRV analysis is not a new area, however, several topics like: pathologic events predictability, sympathetic and parasympathetic balance as well as the influence of several factors like maternal age, smoke habit, parity, fetal sex and familiar history of hypertension or diabetes [121-126] (two common gestational diseases) remain open for discussion. Moreover, although there are several studies related to maternal HRV, the tools for complexity analysis are not commonly explored contrarily to fetal HRV studies [127-128].

Most of the HRV analyses performed during pregnancy are made in the fetus because during pregnancy several ecographic recording of fetal heart beat are obtained as practice routine and, on the other hand, fetal development and their wellbeing is obviously a central problem. In this sense, several studies point out the usefulness of the fetal HRV (fHRV) analysis as a prognostic tool in normal and pathological pregnancies. However, the maternal HRV (mHRV) analysis has several advantages over fHRV: is possible to obtain ECG records from early states of pregnancy, the recording time could be very long by the accessibility of the maternal ECG and is possible to combine mHRV indexes with maternal biochemical markers following less invasive procedures.

As was previously described, a major influence on the HRV is the respiratory rhythm. Throughout pregnancy the respiratory rhythm increases during early stages followed by a progressive reduction with no significant differences at late stage with respect to non-pregnant women [129]. However, decreased HRV during pregnancy cannot be explained only by pregnancy-induced respiratory changes. The tidal volume (the quantity of air exhaled during normal breathing) increases around 40% during pregnancy as well as the minute volume (volume of air leaving the lung each minute) in pregnancy [130, 131], but

consequently the respiratory rate remains almost unchanged (those changes are associated with the fact that the progesterone act as respiratory stimulant as well as with the anatomic changes [130, 131]). In pathological states like preeclampsia even when the coupling mechanism between respiratory rhythm, cardiovascular system and blood pressure control remains similar to health conditions, the respiratory rhythm seems to be significantly increased [132]

On the other hand, changes in blood volume, cardiac output (CO) and stroke volume from the beginning of pregnancy are a phenomenon well known as a response mechanism to propitiate fetus development. The cardiac output increases 30-50% during normal pregnancy mainly during the first trimester [130] and it is strongly related with an increased stroke volume [130] by increasing the left ventricle mass and blood volume [130]. For these reasons pregnant women in left-lateral position reveal small declines in cardiac output [130]. However, during the third trimester CO remains almost unchanged (or even decrease as well as stroke volume [133]), but heart rate continue to decrease. The systemic vascular resistance decreases, and the supine hypotension syndrome become more frequent (in late pregnancy) by aortocaval compression at the supine position resulting in a decreased return to the heart and decreased stroke volume and CO.

Table II summarizes several effects (in a general view) of pregnancy in the HRV indexes revealing some contradictory results mostly in the spectral analysis. Even when comparing non-pregnant/pregnant states some results reveal no significant differences, is generally accepted a reduction of, at least, one of the spectral components or of the total power.

Table II. Some review connecting HRV measurements under pregnancy

Indexes	**Normal Pregnancy**	**PIH**	**CH**	**PRE**
VLF	D: [129,130,136]	N: [134]	N: [134]	N: [134]
LF	D: [129,136,130,137]	N: [134,135,138]	N: [134]	N: [134,140,141,142]
HF	N: [129] D: [135,136,138,130,137]	N: [134]	N: [134]	N: [134, 140] D: [141,142]
RRsd	N: [137] D: [129,136,130,139]	N: [134]	D: [134]	N: [134] D: [142]
RRm	D: [129,130,137,139]			D: [142]

Notes: D, I, N: decrease, increase and no statistically significant differences, respectively. PIH: pregnancy induced hypertension, CH: chronic hypertension and PRE: preeclampsia.

However, we can assume that changes are not the same during all the pregnancy progress. In early pregnancy (<7 weeks) statistical differences in the spectral indexes tend to be smaller than in late pregnancy with major contradictions in the published data [129]. Some authors report no significant differences in early pregnancy (with respect to non-pregnancy) except for LF region and RRsd, and mainly, during the sleep time but no differences at all with respect to late pregnancy [129] while others reveal that only in obese women the differences between early and late pregnancy are significant with an increment of LF/HF, and a reduction of RRsd that in this case is not associated with changes in insulin and glucose blood levels but possibly related with leptin increment [143].

The contradictions do not concern only to different gestational age but also concern to not pregnant women and some pathologic conditions. Some authors report a reduction of LF/HF (in 24h records)[137] or no significant differences of LF/HF in short records at the third trimester of pregnancy as well as HFnu and LFnu compared to non-pregnant state [139] while others only report significant differences in HFnu and not in LFnu [136]. On the other hand, an increment of the LF/HF increase (30 min record) across non-pregnancy, normal pregnancy and PRE, respectively [147] has been noticed as well as an increment of the LFnu in pregnancy compared to non-pregnancy but not difference between PRE and normal pregnancy was observed [142].

During the orthostatic manoeuvre on preeclamptic women a higher increase of heart rate is noted compared to normotensive, non-pregnant and hypertensive women without significant changes in the SBP and DBP [147]. However, these results are not unified, even when there is some consensus about the reduction of the baroreceptor sensitivity during pregnancy (during orthostatic test) [133, 138],

In normal pregnancy some results are in agreement that the increment of the heart rate could be, at least, partially associated with the inhibition of resting parasympathetic activity connected with an increment of the sympathetic modulation. However, during the 3rd third trimester of pregnancy could be a parasympathetic deactivation instead of an increment of the sympathetic activity (under unstimulated conditions) even when the head-up tilt test induce changes in the parasympathetic activity and the sympathovagal balance [138].

As we can observe the contradictions in the parasympathetic/sympathetic control during pregnancy (assessed by HRV measurement) are higher during normal and pathological conditions even with alternative measurement procedures. The evaluation of

postganglionic sympathetic-nerve activity in blood vessels of skeletal muscle by intraneural microelectrodes; considering non-pregnant normotensive and hypertensive women as well as pregnant normotensive and preeclamptic women during the 3rd trimester of pregnancy, reveals significant increment only during preeclampsia without significant differences in the RRm and the Valsava manoeuvre [148]. This result could indicate an oversympathetic activity during pregnancy. However, more recent studies reveal some contradictions using similar procedure under longitudinal analysis. In women with history of preeclampsia there is a higher level of sympathetic activity during the beginning of pregnancy (without hypertension) but it doesn't change significantly in further preeclampsia [149]. Therefore, the autonomic control during preeclampsia seems to play a relevant roll but the oversympathetic activity could not be confirmed even using HRV evaluations, catecholamine in plasma or urine [151, 153, 154] or neuropeptide Y measurements [150].

At the complexity level of mHRV complexity, there are few articles available (in contrast with the fHRV). In general, an increment in α_1 [136,152], no statistically significant differences with respect to α_2 [136] and a reduction of the ApEn are observed [152]. In the analyzed literature authors didn't find correlations between these indexes and spectral analysis, however as was already discussed, several articles (during non-pregnancy condition) report different type of correlations between α-indexes (and other complexity measurement) and spectral indexes.

As previously discussed, the psychological factors could influence the HRV response. In a controlled group for stress situations, 2nd trimester as well as 3rd trimester women presented a reduced HF band with respect to the non-pregnant state (with not statistical differences under stress situations) [135]. On the other hand, the LF remains not statistically significant with small trend to be modified under stress only in the third trimester. Also, a reduction of the LF/HF is noted in both gestational ages compared to non-pregnant women [135].

A major problem concerns the influence of the body mass index (BMI), a generally accepted risk factor for gestational pathologies like hypertension or diabetes. However, in this direction some authors didn't find any correlation in the RRsd reduction with respect to insulin or glucose blood levels but a correlation with respect to leptin levels was identified [143], whereas some inverse correlation has been referred between HF and glucose blood levels [123].

The origin of the pregnancy-induced increment in leptin concentration is not known with confidence. There is strong evidence suggesting that placenta, rather than maternal adipose tissue, gives a significant contribution to the rise in maternal leptin concentrations [143, 144]. On the other hand, the increase in body weight during pregnancy cannot explain the increased sympathetic activity because obese women gain less weight than non obese pregnant women as reported by Schieve et al. (145) in over 266,000 women, probably related to insulin resistance. Furthermore, the relationship between leptin levels and sympathetic activity has already been reported [143, 146].

Why so much contradiction in the spectral analysis during normal or pathological pregnancy? There are several reasons: variations in the methodology and time series length, few studies perform the longitudinal analysis and even fewer consider some confounding factors during normal pregnancy. As we previously discussed, the differences in the consideration of spectral indexes (normalized or not) with the associated physiological interpretation could be an extremely important source of disagreement that can be extrapolated to pathological situations. On the other hand, studies with pathological events are composed of reduced sample size, and in preeclampsia the differences can be also associated with variations in the methodology used for diagnosis as well as with the influence of different degree of preeclampsia severity and even the drugs effect [147].

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Chapter III

Research Project

III.1 Project Objectives

The study of maternal HRV during pregnancy is a very polemic area in which some branches like maternal HRV complexity are poorly studied. These conditions worsen in pathologic conditions like hypertension or preeclampsia. In this context, the major problems of the HRV analysis are:

- The modification of the autonomic control (parasympathetic and sympathetic activity modulation) during normal pregnancy evolution and its assessment through HRV analysis.
- The comparison of maternal HRV changes between normal, hypertensive and preeclamptic pregnancy.
- The lack of physiological meaning of HRV indexes (mainly complexity) and, therefore, their relationships with respect to common biochemical markers.
- The clinical applicability of these indexes, as well as other non-invasive variables, in the classification of normal and hypertensive pregnancy conditions. In other words: the diagnostic capability.

I addition to these major problems, another situation is also important, in a more mathematical context:

- The development of a mathematical model to study HRV and provide a proper differentiability bringing the possibility of a multiscale analysis under short time records.

Evidently, there are other interesting problems in the analysis and interpretation of HRV, however, focusing on the presented ones, the major goal of the present study was:

- The maternal HRV analysis during normal and hypertensive pregnancy.

However, we also intend to achieve other objectives:

- To study the relationship between HRV indexes and biochemical markers.
- To explore the classification capabilities between normal and hypertensive pregnancies considering HRV indexes and general non invasive measurements.
- The development of a new family of indexes through alternative mathematical modelling for HRV study.

To reach our goals, the following studies were performed:

1. *Blood pressure and heart rate variability complexity in normal pregnancy. Influence of age, familiar history and parity.*
2. *Blood pressure and heart rate variability complexity analysis in pregnant women with hypertension*.
3. *Relationship between heart rate variability indexes and common biochemical markers in normal and hypertensive third trimester pregnancy.*
4. *Artificial neural network for normal, hypertensive and preeclamptic pregnancy classification using maternal heart rate variability indexes.*

Concerning to the mathematical aspects related to complexity problem and alternative HRV modelling other three studies were developed:

5. *Network centrality and multiscale transition asymmetry in the heart rate variability analysis of normal and preeclamptic pregnancies.*
6. *Heart rate variability complexity in the aging process.*
7. *Unexpected heart rate variability complexity in the aging process of arrhythmic subjects.*

However, for simplicity reasons, only the fifth study is fully presented in the current dissertation. The last two, were partially described during presentation of Chapters I and II.

III.2. Sample description

The sample was regrouped for specifics studies and for this reason it will be described very globally in this topic. In the subsequent studies, the characteristics of the sample will be better specified.

We obtained a total of 772 short (10-15 min) ECG (at 1000 Hz) records from 305 pregnant women in different gestational ages. The records with several extrasystoles were completely removed from this sample. For a tight study of the extrasystoles, this means, a proper count for statistical analysis, the periodicity and other aspects, a Holter procedure must be performed for long time recordings. Short records are not appropriated for extrasystoles analysis and even when we observed that the extrasystoles tend to be more frequent in hypertensive women, further studies would be designed in this direction.

All the women were informed of the procedure and their assigned consent was obtained following an interview to gather information about familiar and personal history of hypertension and diabetes either, before and after pregnancy, preeclampsia history as well as the smoking, drugs or alcohol habits, parity, age, weight, height and the use of therapeutic drugs.

III.3. Statistical Analysis

In some women we performed several ECG records during different gestational ages while in others only one or two records are available and, in general, gestational ages are not the same. In a longitudinal approach the time is usually a regular or periodic variable where under some defined period a measurement is obtained from the system. In our study the measured frequency was unequal and, consequently, we can use two approaches: considering interval time groups (i.e., 1st, 2nd and 3rd trimester) or considering the time as a continuum variable. Under the first approach, we can note that ideally we need at least one measure in three groups for each woman (balanced design) that is not the case leading to a non balanced design. With this approach the differentiability between groups should be improved with respect to the longitudinal variation. We used the second approach, and therefore, we will study the variation of the independent variable with respect to time, both, as continuum variables, and consequently the general longitudinal profile and its variation with respect to confounding variables and pathologic states that is our statistical goal.

We used the MIXED method in the SPSS statistical package that is a generalization of the general linear regression where the residual should be normally distributed but may not be independent or has constant variance. Is important to note that these models are linear in the parameters and, therefore (as we will se later), to consider some kind of nonlinear pattern is necessary to perform some variable modifications. In this sense, the general approach is to transform the independent variable (i.e. time) by subtracting the mean. This grand-mean centre variable is further included in the model as linear and/or quadratic term. One objective with the mean subtraction is to remove the colinearity between the linear and the quadratic terms.

The normality condition, even when is important, in this procedure is in some way less influent thant in conventional ANCOVA, however, the slope heterogeneity is an aspect to carefully consider (like in general linear models), even more when longitudinal modifications are possible by including pathologic groups.

Chapter IV

Results

Blood pressure and heart rate variability complexity in normal pregnancy. Influence of age, familiar history and parity.

Authors: E. Tejera[1]; J. M. Areias[2], A. Rodrigues[2], J. M. Nieto-Villar[3]; I. Rebelo[1].

1. Biochemical Department, Pharmacy Faculty Porto University. Portugal / Institute for Molecular and Cell Biology (IBMC), Porto, Portugal. R. Aníbal Cunha n.º 164, 4050-047 Porto - PORTUGAL ou 4099-030 Porto – PORTUGAL. Tel +351 222 078 900.
2. Maternal Hospital "Julio Dinis", Porto, Portugal.
3. Chemical-Physics Department. Havana University. Cuba.

Abstract

Objectives) Blood pressure and heart rate variability analysis during normal pregnancy. Study Design) A total of 285 short ECG records (10 min) were obtained from 135 normal pregnant women during several gestational ages. The records were studied with the spectral analysis, and several complexity indexes. We used a mixed unbalanced model for the longitudinal statistical analysis. Results) Significant differences were found in the blood pressure, spectral and complexity indexes changes during pregnancy and in the effects of parity, maternal age, familiar history of hypertension and diabetes. Conclusion) Pregnancy evolution is characterized by a complexity and vagal stimulation reduction while the sympathetic stimulation increase at least in the first half of the pregnancy. The systolic blood pressure remains almost unchanged while the diastolic blood pressure presented a non-linear behaviour during pregnancy. The effect of parity, maternal age, familiar history and other factors are also discussed.

Keywords: heart rate variability; complexity; autonomic control; parity; pregnancy

Introduction

The study of heart rate variability (HRV) during pregnancy commonly assessed by the RR time interval analysis of the electrocardiographic (ECG) records is a very complex field that involves a multidisciplinary approach. The HRV modification is a consequence of the balance between sympathetic and parasympathetic control, however, other aspects like respiratory and circadian rhythms as well as psychological states and hormonal changes could affect this balance [1-3]. Some of the general accepted modifications of maternal

HRV during pregnancy (normal or pathological) are [4-6]: reduction of mean heart rate (RRm), RR standard deviation (RRstd) and some indexes obtained by spectral analysis of RR time series.

Although maternal HRV analysis is not a new area, several topics like: the pathologic events predictability, sympathetic/parasympathetic balance as well as the influence of several factors like maternal age, smoke habit, parity, fetal sex and familiar history of hypertension or diabetes [5-10] (two common gestational diseases) remain open to discussion. On the other hand, even when several works related to maternal HRV could be found, the tools for complexity analysis are not commonly explored contrarily to fetal HRV studies [11,12]. In the present work we analyse blood pressure and maternal HRV modification during pregnancy, using spectral and complexity indexes, considering several factors that modify autonomic modulation during pregnancy evolution.

Methods

We select as complexity indexes: the approximated entropy (ApEn), the Lempel-Ziv complexity (LZ), the sample entropy (SE) and the detrended fluctuation analysis (DFA). All these methods have been widely used in the HRV analysis and, therefore, there are very well described in the literature.

Lempel-Ziv complexity

The Lempel-Ziv complexity [13,14] (LZ) is an useful tool to complexity measurement that characterizes the degree of order/disorder in a sequence. In any case, the sequence is transformed into a binary code followed by the determination of different patterns contained in the sequence. The LZ complexity ranges between 0 and 1 indicate the complete deterministic pattern (ex: sine function) and uncorrelated sequence (ex: white noise), respectively.

Approximated and sample entropy

The ApEn [15] (ApEn) and sample entropy [16] (SE) have been used in several time series analysis and, in general, are measures of irregularity or unpredictability [17]. Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N, a vector $Y_m(i)= \{X_i, X_{i+1}, X_{i+2},\ldots X_{i+m-1}\}$ is defined. If $n^m{}_i(r)$ is the number of vectors $Y_m(j)$ that are close to $Y_m(i)$ ($d[Y_m(i),Y_m(j)] \leq r$, $i \neq j$, where d is the Euclidian distance and r the distance cutoff) then:

$$ApEn(m,r,N) = \frac{1}{N-m}\ln\frac{n_i^m}{n_i^{m+1}} \quad (1) \qquad SE(m,r,N) = \ln\frac{\sum_{i=1}^{N-m} n_i^{'m}}{\sum_{i=1}^{N-m} n_i^{'m+1}} \quad (2)$$

The differences between $n^{'m}$ and n^m are associated with the inclusion of self-matched elements. Smaller values of ApEn and SE imply a time series with similar pattern of measurements and, therefore, more "regular". In our calculation, the conditions were ApEn(2, 0.2, 800) and SE(2, 0.15,800). The ApEn, SE and LZ complexity are measures of irregularity and therefore, the complexity is increased in uncorrelated noise [18].

Detrended fluctuation analysis (DFA).

DFA [19] is a method that gives information about short and long-term correlations and can be applied in non stationeries time series to detect some apparent self-similarities. The original signal is integrated and detrended. The root mean square fluctuation calculated in several segments follow a power law relationship with respect to the segment size. However, in the RR interval time series, the power exponent α could not be constant in the entire scale interval and for this reason α was divided into intervals α_1 and α_2 representing the short (4-13 beat) and long-term (>13 beat) coefficient, respectively [20].

Spectral indexes

The total power spectrum was separated in three major components [3]: very low frequency (VLF≤0.04 Hz), low frequency (0.04<LF≤0.15 Hz) and high frequency (0.15< HF ≤ 0.4 Hz). The effect of vagal activity is predominant in the HF while LF has been considered as a mixture between sympathetic and vagal stimulation [3]. In general, the factors that could affect the LF are polemics [3,21-23]. On the other hand, the physiological aspects of the VLF are hard to describe and have been associated with thermal regulation and some blood pressure control process [3,11].

Sample and Data collection

A total of 285 short ECG (10 min) records were obtained in the sitting position from 135 normal pregnant women during several gestational ages (GA). At the beginning of the study all women were interviewed to obtain information about smoke habit, familiar history of hypertension and diabetes (restricted to direct parenthood: mother/grandmother, father/grandfather). Simultaneously with the ECG records, other

variables were measured like blood pressure and maternal weight. The average values and some global characteristics of the sample are presented in Table I. All the selected women were primiparous or two-parous with normal pregnancy evolution in the current and precedent gestations. The mathematical indexes were calculated using the RR interval of normal sinus beats with 800 points.

Table I. Sample description

Parameters	**Mean values (min-max)**
Gestational Age (GA) (weeks)	24.4 (6 - 40)
Systolic Blood Pressure (SBP) (mmHg)	116.0 (88 - 148)
Diastolic Blood Pressure (DBP) (mmHg)	63.0 (35 - 85)
Body Mass Index (BMI)	27.45 (18.38 - 41.22)
Maternal Age	27.0 (16 - 39)
Smoke	25.93 %
Number of Children (NCh) = 1 / 2	60.00 / 40.00 %
Sex =Male (M) / Female (F)	49.63 / 46.67 %
Familiar History of Hypertension (FHT)	39.26 %
Familiar History of Diabetes (FD)	45.93 %

The statistical analysis was performed using unbalanced linear mixed models with the SPSS package [24]. To obtain final models we consider the parsimony principle as well as the 2 restricted Log Likelihood and Schwarz's Bayesian Criterion [25]. The maternal age was categorized according to four age groups: ≤20 (1), 21-25 (2), 26-34 (3) and >34 (4) for a better analysis. To study of GA the original values in weeks were mean grand centered (GA-mean(GA)). This approach simplifies the intercept analysis and the correlation between linear and quadratic terms (GA and GA^2) [26]; however, even when the GA was treated as scale variable we will represent the GA by interval in some graphs for better illustrative purposes. On the other hand, we used the fixed predicted values for the graphic representations (indicated by "Pred" in the graph). These predicted values don't include the random effects and, therefore, the factor influences and the global trends of the model are better illustrated.

Results and Discussion

DBP reveals a clear nonlinear behaviour (Table II) while SBP remains almost unchanged during gestational time with only $p<0.1$ for GA^2. On the other hand, the BMI is only significantly different for the SBP with a positive coefficient. The fetal sex, FHT and age

influence are not statistically significant. The variation of the blood pressure across the pregnancy period is quite polemic, in part, by the nature itself of the measurement procedure [23,27,28]. The independent SBP behaviour during pregnancy in contraposition to the nonlinear behaviour of DBP with respect to the GA was already reported [27,29,30].

Table II. Analysis of variables with respect to SBP and DBP.

	SBP		**DBP**	
Variables	**Coeff**	**p-value**	**Coeff**	**p-value**
Sex [M]	-0.072 (1.27)	0.955	0.924 (1.39)	0.509
FHT [No]	-1.601 (1.29)	0.216	-2.212 (1.43)	0.124
BMI	0.593 (0.15)	0.000	0.088 (0.17)	0.598
GA	-0.004 (0.06)	0.948	0.178 (0.06)	0.002
GA^2	0.011 (0.01)	0.089	0.022 (0.01)	0.001
Age [1]	-2.603 (3.38)	0.443	3.537 (3.68)	0.338
Age [2]	-4.634 (2.96)	0.119	4.630 (3.20)	0.150
Age [3]	-3.934 (2.79)	0.161	5.605 (3.04)	0.068
Smoke[0] * NCh [1]	1.527 (2.08)	0.464	9.285 (2.26)	0.000
Smoke[0] * NCh [2]	0.509 (2.24)	0.820	10.324 (2.40)	0.000
Smoke[1] * NCh [1]	4.947 (2.60)	0.059	10.810 (2.81)	0.000

Notes: [...] represents the reference group, for this reason age groups are between 1-3 while the two values categorical variables (Sex, Smoke and NCh) are referred to the baseline value. The values are reported as coefficient (standard error).

The simultaneous effect of NCh and smoke habit reveal some interesting behaviours. The effect of parity and smoke habit is stronger in DBP (Fig.1) where all the coefficient are statistically significant while only the Smoke[1]*NCh[1] condition seems to be relevant in SBP. SBP in smoker primigravid women tend to be higher than in two-parous women (p=0.059) while in the DBP the results clearly indicate that smoker two-parous women present the lowest DBP levels (Fig.1).

Fig.1. DBP (Left) and SBP (Right) variation with respect to gestational time separated according to smoke habit and parity.

The obtained significant effect of BMI increment in the SBP is in agreement with other authors [6,29,30], however, no significant differences were found in BMI with respect to DBP. On the other hand, the change in DBP during pregnancy is influenced by the parity and smoke habit indicating a lower blood pressure in smoker two-parous women. The protective effect of maternal smoking has been reported by other authors in cases of pregnancy induced hypertension and preeclampsia either in primigravid or in multiparous women [38,39]. However, our results refer to normal pregnancy mainly in the two-parous group.

The mean RR interval (RRm), RRstd and HF spectral power decrease (Table III). The factor effects in the spectral indexes as well as the RRmean and RRstd are very different. Even when the GA is significantly different in all the indexes except for lnLF (were the GA^2 is significant), the BMI is only significant for the RRstd.

Table III. Analysis of spectral and time related indexes under several factor effects.

	RRm	lnRRstd	lnVLF	lnHF	LH/HF
NCh[1]	-0.016 (0.011)	-0.038 (0.061)	0.011 (0.059)	-0.050 (0.086)	0.134 (0.289)
Smoke[No]	-0.023 (0.011)*	-0.061 (0.064)	0.072 (0.062)	-0.074 (0.09)	0.191 (0.306)

Age[1]	-0.002 (0.026)	-0.058 (0.145)	-0.223 (0.141)	0.677 (0.205)*	-2.651 (0.712)*
Age[2]	0.002 (0.023)	-0.062 (0.124)	-0.102 (0.121)	0.356 (0.165)*	-1.625 (0.624)*
Age[3]	0.001 (0.021)	-0.026 (0.117)	-0.088 (0.113)	0.333 (0.165)*	-1.483 (0.597)*
FHT[No]	0.001 (0.01)	0.018 (0.058)	-0.112 (0.055)*	0.059 (0.082)	-0.17 (0.276)
FD[No]	0.018 (0.01)**	0.035 (0.058)	-0.068 (0.055)	0.158 (0.081)**	-0.522 (0.277)**
Sex[M]	0.001 (0.01)	-0.042 (0.056)	-0.027 (0.054)	-0.017 (0.079)	0.155 (0.264)
GA	-0.002 (0.001)*	-0.005 (0.002)*	0.009 (0.002)*	-0.007 (0.003)*	0.03 (0.011)*
BMI	0 (0.001)	-0.014 (0.006)*	0.001 (0.006)	-0.002 (0.009)	-0.033 (0.031)

Note: All the units are ms or ms^2. Notations * and ** refer to p-value less that 0.05 and 0.01, respectively. The lnLF present significant differences with respect to GA^2 with p-value = 0.04 but no statistical significant coefficients.

On the other hand, the variation with respect to GA is linear except for lnLF (Fig.2 Left). We can corroborate that until around the second trimester the sympathetic stimulation (LF) tends to increase (Fig.2). The reduction of the parasympathetic control during pregnancy is age-mediated (significant differences of age in lnHF an LF/HF ratio (Table III)) (Fig.2 Right). FHT is only significant in VLF while FD shows relative significant values for HF, RRmean and LF/HF ratio (p-value <0.1). The significance level at 90 % could be a consequence of unbalance modelling; this means that increasing the number of women or the number of measurements, the significance can be improved.

During pregnancy the sympathetic modulation reacts increasing the general autonomic control and, therefore, is logical to found a reduction of RRm and HF [6,23]. The reduction of HF and the consequent increment of the LF/HF ratio point out to the well known reduction of the parasympathetic control, however, we could notice that after the second trimester this reduction could be not directly related to an increment of the sympathetic stimulation as have been suggested by other authors [33]. On the other hand, during pregnancy the increment in the BMI itself can't explain the sympathetic stimulation [23,34] and this could be the reason of the BMI significance lack. The same

explanation can be applied to the DBP, were the BMI was not significant and therefore, could be mediated by the sympathetic activity. Alternatively, the quadratic behaviour of lnLF as well as the FHT influence on the VLF could be related to the blood pressure mechanism as have been reported elsewhere [3,11,32].

Fig.2. Left) Predicted spectral indexes with respect to gestational ages. Right) The predicted lnHF variation with respect to gestational ages separated by maternal age and familiar history of diabetes.

The smoke effect on heart rate is apparently contradictory, because the negative coefficient suggests that during pregnancy heart beat increases more in non smoker women. We should expect that in smoker women the mean HR could be lower due to nicotine stimulation; however, the periodic nicotine stimulation could have a regulatory effect and, therefore, could muffle the intrinsic sympathetic exaltation at the heart rate level. On the other hand, an explanation of the FD influences is difficult. The positive coefficient indicates that women without FD presented a smaller reduction of HF compared to women with FD (Fig.2 Right). There are several studies of HRV analysis in diabetic patients and is always noted the dramatic reduction of HRV [35]; in pregnant women (normal or diabetic) a reduction of HF after glucose administration have been found [7], that is in agreement with our results, if we assume that in general the familiar history of diabetes predefine an abnormal glucose metabolism that is enhanced during pregnancy.

Table IV. Analysis of complexity indexes under several factor effects.

	LZ	SE	ApEn	α_1	α_2
NCh[1]	-0.036 (0.017)*	-0.059 (0.041)	-0.012 (0.017)	0.022 (0.026)	0.008 (0.021)
Smoke[No]	-0.022 (0.017)	-0.008 (0.043)	-0.013 (0.017)	0.007 (0.026)	0.019 (0.022)

Age[1]	0.142 (0.04)*	0.207 (0.097)*	0.092 (0.04)*	-0.274 (0.061)*	-0.028 (0.051)
Age[2]	0.082 (0.036)*	0.131 (0.084)	0.076 (0.036)*	-0.138 (0.054)*	-0.013 (0.044)
Age[3]	0.085 (0.033)*	0.106 (0.079)	0.069 (0.033)*	-0.125 (0.05)*	-0.02 (0.041)
FHT[No]	0.01 (0.015)	0.021 (0.038)	0.008 (0.015)	-0.016 (0.023)	-0.042 (0.02)*
FD[No]	0.022 (0.015)	0.02 (0.038)	0.003 (0.015)	-0.032 (0.023)	-0.024 (0.02)
Sex[M]	-0.008 (0.015)	-0.039 (0.037)	-0.017 (0.015)	0.024 (0.023)	-0.019 (0.019)
GA	-0.003 (0.001)*	-0.006 (0.002)*	-0.003 (0.001)*	0.003 (0.001)*	0.003 (0.001)*
BMI	0.002 (0.002)	0.004 (0.004)	0.004 (0.002)*	0 (0.003)	0 (0.002)

Note: Notations * and ** are referring to p-value less that 0.05 and 0.01, respectively

Complexity indexes (Table IV) are in general more age-dependent and in all cases presented significant variation with respect to the gestational age. LZ, SE and ApEn decrease during pregnancy evolution. With respect to α_1 and α_2, is possible to note that the age is significantly different in α_1 but not in α_2 while FHT is only significant in the α_2 index. Short and long-term correlations, in general, increase with GT, however, the reduction of maternal age will be associated with a reduction in short-term correlation increment across pregnancy and, similarly, women without FHT present a smaller increment in the long-term correlation across pregnancy evolution (Fig.3 Right).

Fig.3. Left) Predicted LZ complexity and Right) Predicted α_2 variation with respect to gestational time separated by parity and familiar history of hypertension, respectively. Error bar corresponds to standard error.

Short and long-term correlations increase with gestational age [20], however, the differences in α_1 and α_2, with respect to maternal age and FHT are important for two main reasons: 1) the influence of the age should be related to short-term correlation modifications and 2) the effect of the FHT could be associated with long-term correlation modification. These results could explain why the age is present in almost all the analysed indexes instead of FHT.

Another aspect is the dependence of LZ complexity with parity beside maternal age (Fig.3 Left and Table IV). Primigravid women presented a higher reduction in the complexity with respect to the two-parous women. The results clearly indicate that pregnancy evolution is associated with a complexity reduction but this reduction is smaller in the two-parous women suggesting some kind of body adaptation even at early gestational age. Women with successfully history of pregnancy present, in general, a reduced rate of spontaneous abortion and a reduced predisposition to gestational diseases and, in general, some kind of adaptability [31,36,37]. This "maternal memory" could have several physiological and psychological factors and should be more exhaustively studied even in multiparous women. The reduction of LZ, MSE and ApEn together with the increment of α_1 and α_2 are stronger indicators of the regularity increment in the RR signal and "order" increment could be a consequence of the increment in sympathovagal balance (LF/HF).

Conclusion

Several changes are involved in HRV behaviour during pregnancy evolution and these changes are influenced by a multifactorial process. SBP remains almost unchanged during pregnancy, however, is influenced by BMI changes while the DBP is affected by parity and smoke habit. The SBP is lower in smoker two-parous women while in the primigravid group the smoke effect on SBP and DBP is reduced.

Mean heart rate decreases during gestation and this decrement is higher in non-smoking women. VLF increase and is influenced by familiar history of hypertension. On the other hand, LF values reveal a non-linear variation with maximal value around the second trimester of gestation. The pregnancy is characterized by a HF reduction related to familiar history of diabetes and maternal age but can't be associated with a simultaneous increment of the sympathetic stimulation mainly after the second trimester. Women with increased maternal age or primigravid presented a higher LZ complexity reduction while the short and long-term correlations are significantly modified by maternal age and familiar history of hypertension, respectively.

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Blood pressure and heart rate variability complexity analysis in pregnant women with hypertension.

Authors: E. Tejera[1], J. M. Areias[2], A. Rodrigues[2], J. M. Nieto-Villar[3], I. Rebelo[1].

1. Biochemistry Department, Pharmacy Faculty Porto University. Portugal / Institute for Molecular and Cell Biology (IBMC), Porto, Portugal.
2. Maternal Hospital "Julio Dinis", Porto, Portugal.
3. Chemical-Physics Department, Havana University, Cuba.

Abstract

In the present work we perform a comparative analysis of blood pressure and heart rate variability complexity during pregnancy between normal, hypertensive, and preeclamptic women. A total of 563 short ECG (10 min) records were obtained from 217 pregnant women (137 normal, 53 hypertensive and 27 preeclamptic) during several gestational ages in sitting position. We used a mixed unbalanced model for the longitudinal statistical analysis and beside the conventional spectral analysis we applied Lempel-Ziv, sample entropy, approximated entropy and detrended fluctuation analysis in the complexity measurement. The obtained results revealed significant differences between pathological and normal states with important consideration related to pregnancy adaptability and evolution as well as the relationship of complexity and blood pressure with factors like maternal age, familiar history of diabetes or hypertension and parity.

Introduction

Heart rate variability (HRV) study during pregnancy commonly assessed by RR time interval analysis of the electrocardiographic (ECG) records is a very interesting field that involves necessarily a multidisciplinary approach. HRV modification is a consequence of the balance between sympathetic and parasympathetic control, however, other aspects like respiratory and circadian rhythms as well as psychological states and hormonal changes could affect this balance[1-3].

Some of the general modifications of maternal HRV during normal pregnancy are the reduction of mean RR interval (RRm), RR standard deviation (RRstd) and reduction of some spectral indexes obtained by spectral analysis of the RR time series[4,5]. On the other

hand, in the case of gestational hypertension (HT) and preeclampsia (PREE) some contradictions are found. Some works didn't reveal any differences in HRV spectral analysis in preeclamptic women or differences mainly related to vagal control[9,10] while other works point out an oversympathetic activity and a parasympathetic control reduction in hypertensive and PREE women[6,7,8]. Even when few HRV complexities studies have been performed during pregnancy, in all cases a complexity reduction is recognized for PREE women[11,12] but differentiability between normal, HT and PREE is still poorly explored.

Although maternal HRV analysis is not a new area, several topic remain open to discussion: pathologic events predictability, sympathetic/parasympathetic balance as well as the influence of several factors like maternal age, smoke habit, parity, fetal sex and familiar history of hypertension or diabetes[5,6,13-16]. On the other hand, even when several works related to maternal HRV could be found, the tools for complexity analysis are not common contrarily to the fetal HRV studies[17-18]. In the present work we explore blood pressure and maternal HRV modification during normal and pathological pregnancy process, using spectral and complexity indexes, considering several factors that, as our results reveal, are significant in the autonomic modulation during pregnancy evolution.

Methods

Several indexes have been proposed to complexity analysis of physiological signals, however, some of them are not appropriated in short records analysis[19]. We select as complexity indexes approximated entropy (ApEn), Lempel-Ziv complexity (LZ), sample entropy (SE) and detrended fluctuation analysis (DFA). All these methods have been widely used in HRV analysis (short and long records) and, therefore, they are very well described in the literature; however, we will briefly expose each of the methodological bases.

Lempel-Ziv complexity

The Lempel-Ziv complexity[19,20] (LZ) is an useful tool to complexity measurement that characterizes the degree of order/disorder in a sequence. This sequence could be a time series or a string array. In any case, the sequence is transformed to a binary code and the core of the LZ calculation is the determination of different patterns contained in the finite sequence. LZ complexity ranges between 0 and 1 indicating the complete deterministic pattern (ex: sine function) and uncorrelated sequence (ex: white noise), respectively.

Approximated and sample entropy

ApEn[21] (ApEn) has been used in several time series analysis and, in general, is a measure of irregularity or unpredictability of the time series. On the other hand, the ApEn is similar to the sample entropy[22] (SE). Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N we can define the vector $Y_m(i)= \{X_i, X_{i+1}, X_{i+2},\ldots X_{i+m-1}\}$. If we define $n^m{}_i(r)$ as the number of vectors $Y_m(j)$ that are close to $Y_m(i)$ ($d[Y_m(i),Y_m(j)] \leq r$, $i \neq j$, where d is the Euclidian distance and r the distance cutoff) then:

$$ApEn(m,r,N) = \frac{1}{N-m} \ln \frac{n_i^m}{n_i^{m+1}} \quad (1) \qquad SE(m,r,N) = \ln \frac{\sum_{i=1}^{N-m} n_i^{'m}}{\sum_{i=1}^{N-m} n_i^{'m+1}} \quad (2)$$

where the differences between n'^m and n^m are associated with the inclusion of self-matches elements. We can note that both indexes are very similar and this similarity could be related to Renyi entropy[23]. Smaller values of ApEn and SE imply a time series with similar pattern of measurements and, therefore, more “regular”. In our calculation, conditions were ApEn(2, 0.2, 800) and SE(2, 0.15,800). It is important to note that the ApEn, SE and LZ complexity are measurements of irregularity and, in this sense the complexity is increased in uncorrelated noise[24].

Detrended fluctuation analysis (DFA).

DFA[25] is a method that gives information about short and long-term correlations (fractal like ones) and can be applied in non stationary time series to detect some apparent self-similarities. The first part of the computation is integration of the signal, followed by a detrended procedure. The root mean square fluctuation calculated in several segments follows a power law relationship with respect to the size of the segment. This power exponent α has a closer relation to Hurst exponent. Values of α=0.5 represent a white noise, α=1 represent 1/f noise and α=1.5 indicate Brownian noise. However, in the RR interval time series, α exponent could not be constant in all time interval, for this reason α was divided into intervals α_1 and α_2 representing short (4-13 beat) and long-term (>13 beat) coefficient, respectively[26].

Spectral and other indexes

Total power spectrums were separated in three major components[3]: very low frequency (VLF≤0.04 Hz), low frequency (0.04<LF≤0.15 Hz) and high frequency (0.15< HF ≤ 0.4 Hz). The effect of vagal activity is predominant in HF while LF has been considered as a mixture between sympathetic and vagal stimulation[3,8]. In general, factors that could be described by LF like blood pressure regulation are polemics[3,27-29]. On the other hand, the physiological aspects of the VLF are hard to describe and have been associated with thermal regulation and some blood pressure control process[3,17]. The ratio LF/HF is widely used as indicator of the sympathetic/parasympathetic balance.

Sample and Data collection

A total of 563 short ECG (10 min) records were obtained from 217 pregnant women in several gestational ages (GA) in sitting position. The sample was classified according to: normal, hypertensive (HT) and preeclamptic (PREE) groups based on the current pregnancy. In this sample, 135 women didn't reveal any gestational disorder (and no personal history of hypertension or diabetes), 55 women presented hypertension during pregnancy or had some history of hypertension and 27 women presented preeclampsia.

At the beginning of the study all women were interviewed to obtain information about familiar history of hypertension (FHP) and diabetes (FD) (restricted to direct parenthood: mother/grandmother, father/grandfather) as well as smoke, drugs or alcohol addiction. All selected women for this study are primiparous or two-parous and, therefore, gestational history (G. History) of hypertension, diabetes and preeclampsia were also considered in the two-parous group. Simultaneously with ECG records other magnitude were measured like blood pressure and maternal weight. Average values and some global sample characteristics are presented in Table I. In the hypertension (HT) and preeclamptic (PREE) groups, medication was also considered however, we will not regroup according to drug type because almost all pregnant women with medication are under administration of methyldopa or methyldopa/Cartia combination.

All mathematical indexes (spectral, complexity, etc) were calculated using RR interval of normal sinus beats with 800 point.

Table I. General sample description

Parameters	Normal	HT	PRE
GA (weeks)	24.4 (6 - 40)	24.6 (7 - 40)	27.2 (7 - 40)
Maternal Age	27.2 (16 - 39)	31.7 (22 - 42)	29.5 (20 - 40)
SBP (mmHg)	116.0 (88 - 148)	131.2 (99 - 230)	138.7 (118 - 184)

DBP (mmHg)	63.0 (35 - 85)	74.4 (46 - 100)	84.7 (67 - 107)
Body Mass Index (BMI)	27.5 (18.4 - 41.2)	32.6 (21.7 - 49.0)	31. 8 (22.5 - 44.9)
Drugs Treatment (DT)	0.00 %	36.36 %	59.26 %
Sex = Male (M)/ Female (F)	46.67 / 49.63 %	45.45 / 50.9 1%	62.96 / 37.04 %
Parity =1/2	60.00 / 40.00 %	40.00 / 60.00%	74.07 / 25.93%
Smoke	25.93 %	10.91 %	11.11 %
G. History of HT	0.00 %	14.55 %	3.70 %
G. History of Diabetes	0.00 %	7.27 %	0.00 %
G. History of PREE	0.00 %	25.45 %	14.81 %
Familiar History of HT	39.26 %	78.18 %	66.67 %
Familiar History of Diabetes	45.93 %	60.00 %	29.63 %

Note: The values are presented as mean (min-max) and percent. Notations: Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Gestational Age (GA), Personal gestational history (G. History) and HT as well as PREE are always referred to hypertension and preeclampsia, respectively.

Statistical Analysis

Statistical analysis was performed using unbalance linear mixed models (MIXED) with the SPSS package[30]. The MIXED method is a generalization of the usual generalized linear models (GLM) where residual should be normally distributed but may not be independent or have constant variance. With the MIXED method is possible to change the structure of the covariance matrix and in general is less affected by normality distribution criteria, however, remain influenced by the slope homogeneity assumption[31,32]. To obtain the final models we consider parsimony principle as well as 2 restricted log Likelihood and Schwarz's Bayesian Criterion[31]. To compare main effects we applied the Bonferroni method, implicit in the SPSS MIXED procedure.

To study GA variation, the original GA in weeks was mean grand centred: (GA-mean(GA)). This approach simplifies the intercept analysis and the correlation between the linear and quadratic terms (GA and GA^2)[32]. However, even when in the performed analysis the gestational age was considered as scale variable, for a better visual, in some presented graphs the data was regrouped in three or four gestational groups. On the other hand, we will use the fixed predicted values for graphic representation. These predicted values are the adjusted response for the included factors without incorporating random effects and, therefore, factor influences and global trends of the model are better illustrated. The maternal age was categorized according to four age groups: ≤20, 21-25, 26-34 and >34 for a better analysis. All the variables and factors are distributed according to their time dependency. In this sense, the GA (and GA^2) and BMI are used as covariates while fetal sex, smoke habit, familiar or gestational history of hypertension or diabetes, maternal age,

parity, drugs treatment and the group coding variable (Normal, HP or PREE) are considered as factors (more precisely all the maternal gestational history was considered as confounding variables). The SBP, DBP and HRV derived indexes are considered as independent variables.

Results

For a better understanding of information the results and discussion will be separated in a first topic related to blood pressure changes and a second one related to heart rate indexes.

SBP and DBP across the pregnancy evolution

The results presented in Table II correspond to a very global model and we can observe an increase in SBP and DBP from normal to PREE women, as expected (Fig. 1).

Table II. Analysis of variables with respect to SBP and DBP.

	SBP		DBP	
Variables	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Group [HT]	14.51 (1.43)	0.000	9.81 (1.22)	0.000
Group [PREE]	19.09 (1.77)	0.000	16.98 (1.54)	0.000
Sex [M]	-2.14 (0.94)	0.024	-0.15 (0.83)	0.858
GA	-0.013 (0.052)	0.806	0.076 (0.045)	0.089
GA^2	0.011 (0.006)	0.053	0.013 (0.005)	0.011
BMI	0.3 (0.11)	0.005	0.186 (0.087)	0.032
Maternal Age [≤20]	-2.8 (2.5)	0.259	-1.37 (2.20)	0.535
Maternal Age [21-25]	-4.2 (1.9)	0.028	1 (1.65)	0.546
Maternal Age [26-34]	-3.2 (1.6)	0.041	1.41 (1.30)	0.281
Smoke [No]	-2.4 (1.2)	0.045	1.74 (1.07)	0.106
Parity [1]	1.012 (1.1)	0.368	0.59 (0.98)	0.546
DT [No]	-3.087 (1.7)	0.064	-5.57 (1.40)	0.000
FHT [No]	-4.225 (1.1)	0.000	-5.1 (0.94)	0.000
FD [No]	-0.038 (0.99)	0.969	-0.27 (0.84)	0.749

Notes: The [...] indicates the reference groups and the missing class corresponds to the baseline reference. For this reason the age groups [>34] is missing, denoting the age baseline reference. Similarly the Group variable [Normal] is not present because is the Group reference level. Drugs treatment (DT), gestational age (GA), familiar history of hypertension (FHT) and diabetes (FD). In all cases the [No] is denoting the negative condition (i.e., no drugs treatment, no familiar history and no smoke habit) taking the positive as reference level. Values are reported as: coefficient (standard error).

The statistical significance values for the coefficients (Table II) are referred to the baseline levels that correspond to missing classes, for these baselines, the coefficient is fixed to zero. The coefficients for normal (fixed to zero), HT and PRE pregnancy reveal a progressive and statistically significant increment that can be confirmed in Fig.1 (Left), indicating that around the 24 week of gestation (mean centred value for GA) an increment of around 15 mmHg in the SBP is noted for HT group and 19 mmHg for the PREE group in comparison with the normal pregnancy group keeping all the other factors at the baseline level. The coefficients are also significant for DBP but with reduced coefficients mainly for HT group with increased difference between HT and PRE.

Fig. 1. Left) Predicted DBP and Right) SBP values with respect to gestational age and body mass index (BMI), respectively. The non-lineal behaviour of DBP with respect to GA and the BMI linearly respect to SBP is noted for normal and pathological conditions.

The BMI also remain statistically significant for both SBP and DBP with a positive coefficient suggesting a positive linear dependency (Fig. 1 Right), however with stronger influence in the SBP. The fetal sex, maternal age and smoke habit are only statistically significant in the SBP. Smoke habit and fetal sex coefficient suggest that SBP tend to be lower in non smoker women and in male fetus, while remain no significant for DBP. On the other hand, the maternal age has a significant effect with general decrement (negative coefficients) respect to the oldest group (reference group) only for SBP and not statistically significant coefficient for the youngest group (p-value >0.05).

The negative and statistically significant coefficients for SBP and DBP, associated with the familiar history of hypertension for the group without this background, suggest that women without FHT tend to present lower values of SBP and DBP. However contradictory

results are noted with respect to drug treatment. The DT is only statistically significant for DBP and the positive coefficient indicate that women without medication tend to present higher values of DBP.

Fig.2. The SBP (Left) and DBP (Right) variations with respect to the gestational weeks separated by maternal parity.

Women with medication presented increased values of SBP and DBP with respect to no treatment in the hypertensive or PREE groups. This could be associated with two reasons: parity influence and sample bias. The parity effect even when is not statistically significant (Table II), in the primigravid women tend to present higher values of SBP and DBP values mainly at the end of pregnancy (Fig.2). Statistically it is a consequence of the slope heterogeneity and can be solved applying interaction analysis (data not shown) where actually only remains the contradiction for the PREE group.

Heart rate variability analysis

All indexes are lineally related to GA (the lnSTD should reveal some quadratic effect maybe in increased sample) with an increment for the VLF and LF/HF ratio (Table III). As we can observe, LF is only different for HT group but not for PREE and an opposite behaviour is noted for HF component. Only HF significantly decreases during pregnancy (negative coefficient).

The drugs effect is noted by the statistically significant coefficient mainly with respect to lnLF. The negative coefficient for the group without drugs administration suggest that this group have an increased lnLF compared to women under drugs treatment and therefore the LF/HF ratio should increase too (Table III). The drugs effect is clearly represented in Fig. 3 where we can notice a predominant influence on the LF band instead of the HF. On the other hand, the LF/HF values tend to be higher in HT and PREE in comparison with the normal group (Fig. 3 Right).

Table III. Analysis of HRV spectral and time related indexes.

	RRm	**RRstd**[1]	**lnVLF**	**lnLF**	**lnHF**	**LF/HF**
Group [HT]	-0.055 (0.012)*	-0.13 (0.044)*	0.008 (0.057)	0.211 (0.046) *	0.029 (0.067)	0.544 (0.24)*
Group [PREE]	-0.048 (0.015)*	-0.267 (0.057)*	0.061 (0.074)	-0.031 (0.059)	-0.198 (0.088)*	0.646 (0.315)*
DT [No]	-0.021 (0.012)**	0.018 (0.044)	0.02 (0.058)	0.123 (0.047)*	0.011 (0.068)	0.46 (0.249)**
Maternal Age [≤20]	-0.038 (0.022)**	0.066 (0.082)	-0.261 (0.106)*	0.071 (0.085)	0.755 (0.125)*	-1.814 (0.437)*
Maternal Age [21-25]	-0.031 (0.016)**	0.071 (0.061)	-0.114 (0.079)	0.162 (0.064)*	0.412 (0.094)*	-1.075 (0.333)*
Maternal Age [26-34]	-0.036 (0.013)*	0.050 (0.048)	-0.068 (0.064)	0.18 (0.051)*	0.281 (0.075)*	-0.546 (0.272)*
Parity [1]	-0.013 (0.0097)	-0.143 (0.036)*	0.135 (0.047)*	-0.06 (0.038)	-0.16 (0.056)*	0.226 (0.197)
GA	-0.002 (0.0004)*	-0.0041 (0.0017)*	0.008 (0.002)*	0.002 (0.002)	-0.01 (0.003)*	0.044 (0.01)*
BMI	0.0005 (0.0009)	-0.012 (0.0032)*	0.002 (0.004)	-0.008 (0.003)*	0.007 (0.005)	-0.027 (0.018)

Notes: Notations * and ** represent p-value<0.05 and p-value<0.1, respectively. We use RRm, RRstd and spectral indexes in ms^2 units. The variables: Smoke, fetal sex, FHT and FD were not statistically significant for any indexes even when some of them reported some p-values <0.1 for RRm, RRstd, HF and VLF. (1) The p-value for GA^2 was equal to 0.064. Values are reported as: coefficient (standard error).

Fig.3. Left) Predicted LF and HF spectral indexes with respect to gestational age with and without medication in hypertensive and preeclamptic women. The PREE group has the lower LF even without drugs administrations. The drugs treatment reduces the LF but HF almost remains unchanged. Right) The predicted LF/HF variation with respect to gestational age for the different groups. The HF decrease during pregnancy but the differentiability between HT and PREE is low.

To study the sympathovagal balance behaviour we create a regression model when we predict LF using the mean centred HF (HF–mean(HF)) considering the three major groups and, therefore, to evaluate possible correlation patterns (Fig. 4 Left). The presented model in the Fig. 4 (Left) is obtained without adjustment for any factors, however, the inclusion of maternal age, drug treatment and gestational history don't change considerably the general profile.

The non-linear behaviour of the LF-HF correlation is quite evident, however, several important distinctions need to be made. The normal group clearly shows that an increment of HF is associated with a reduction of LF, but a reduction of HF could be associated with an increment of LF until the mean value and, after that, is possible to identify a small reduction of the LF. The mean HF value is generally around 20-30 weeks of gestation and, therefore, before this time (highest values of HF) the sympathetic control is predominant in the normal group. On the other hand, HT group presents little and quite symmetric modification with respect to HF, so, an increment of LF could be or not associated with a reduction of HF, while in the PREE group the dependency of HF-LF is almost linear for lower values of HF.

Fig.4. Left) Relationship pattern between LF and HF modelled for normal, HT and PREE groups. For PREE group the LF-HF relationship is almost linear for lower values of HF. Right) The HF decreases with maternal age, however, this decrement is reduced in the two-parous compare to primigravid women increasing the differences with age.

The parity is another important significant factor that we evaluated in the blood pressure and remains as a "positive" effect (Fig. 4 Right). Two-parous women tend to present higher HF and RRstd, both, with negative coefficients for primigravid women (Table III). The reduction of the parasympathetic control during pregnancy is age-mediated (significant differences of age in lnHF and LF/HF ratio) however, the differences in this control between two-parous and primigravid women increase with the maternal age (Fig. 4 Right).

Table IV. Analysis of HRV complexity indexes

	LZ	SE	ApEn	α_1	α_2
Group [HT]	-0.016 (0.016)	-0.097 (0.033)*	-0.056 (0.017)*	0.046 (0.022)*	-0.041 (0.022)**
Group [PREE]	-0.056 (0.021)*	-0.211 (0.043)*	-0.100 (0.022)*	0.053 (0.030)**	0.044 (0.027)
DT [No]	-0.031 (0.019)	-0.091 (0.042)*	-0.049 (0.02)*	0.036 (0.027)	-0.013 (0.024)
Maternal Age [≤20]	0.101 (0.030)*	0.107 (0.067)	0.046 (0.031)	-0.192 (0.043)*	-0.072 (0.041)**
Maternal Age [21-25]	0.046 (0.022)*	0.057 (0.05)	0.027 (0.024)	-0.066 (0.031)*	-0.052 (0.029)**
Maternal Age [26-34]	0.028 (0.018)	0.015 (0.039)	0.004 (0.019)	-0.023 (0.025)	-0.057 (0.025)*
Parity [1]	-0.043 (0.013)*	-0.084 (0.028)*	-0.026 (0.014)**	0.043 (0.019)*	0.045 (0.018)*
GA	-0.004 (0.001)*	-0.006 (0.002)*	-0.004 (0.001)*	0.004 (0.001)*	0.002 (0.001)*
BMI	0.001 (0.001)	0.004 (0.002)**	0.004 (0.001)*	-0.001 (0.002)	0.002 (0.002)

Notes: Notations * and ** represent p-value<0.05 and p-value<0.1, respectively. The variables: Smoke, FHT and FD were not statistically significant for any indexes. Values are reported as: coefficient (standard error).

The complexity indexes (Table IV) reveal, in general, more differentiability with respect to HT and PREE groups and in all cases presented significant variation with respect to gestational age (Fig. 5 Left). The LZ, SE and ApEn indexes decrease during pregnancy (negative coefficients) while α_1 and α_2 increase with gestational age (positive coefficients) but the capability to differentiate HT from PREE is very poor with only a tendency (p-value < 0.1) of α_1 and to increase from HT to PREE. On the other hand, the maternal age increment leads to a reduction of the positive coefficients of LZ and, therefore, a reduction of complexity. As we can observe the effect of medication, interestingly suggest that women without treatment present lower complexity values.

Fig.5. Left) ApEn complexity decrease in both, pathological and normal groups during pregnancy with a better differentiability between HT and PREE than obtained with spectral indexes. Right) Correlation between predicted ApEn and LF/HF. The correlation coefficient corresponds to the actual variables and not the predicted ones.

The LZ complexity index can't differentiate normal from HT, however, the ApEn and SE clearly show a significant complexity reduction from normal to PREE women. The reduction of the complexity is strongly related (R^2 = 0.443, $p<0.05$) with the sympathovagal modulation (LF/HF) (Fig. 5 Right) even when the last one revealed smaller differentiability. On the other hand, LZ, SE and ApEn coefficients associated to primigravid condition are negative revealing that the primigravid women presented a reduced complexity compared with two-parous women.

Discussion

SBP and DBP discussion.

Variation of blood pressure across pregnancy period is quite polemic, in part, by the nature itself of the measurement procedure[29,33,34]. In both cases BMI remains significant with a positive coefficient indicating that higher BMI is associated with higher SBP and DBP[6,35,36]. On the other hand, SBP has almost no significant variation across gestational period while DPB has a clearly non-linear behaviour. Considering the GA dependency without confounding factors by separated groups (data not shown) only significant difference was noted for the normal groups suggesting that in pathological cases the blood pressure profile is highly modified, and it is partially reflected in the curvature reduction for normal to PREE of the fitted model (Fig. 1 (Left)). This reveal that, even in the PREE group, there is a longitudinal change and, therefore, a response to pregnancy as have been suggested by other authors[16,8,37]. The fetal sex influence could be polemic and in general

could be related also with a maternal genetic susceptibility, as has been recently presented by Hocher et al, obtaining lower SBP values in women with male fetuses and AA variant of the PROGINS progesterone receptor[42]. However we can reject the idea of hormonal influence. On the other hand, the smoke habit clearly reveals an advantage for non smoker women with lower SBP while could be affected by the maternal age mainly in the group with age more than 35 years old.

As we should expect, women without familiar history of hypertension presented a negative and significant coefficient indicating reduced SBP and DBP values[38]. However, the contradictions noted for drugs treatment beside the possible relation with parity (as presented in Fig.2 and supported by HRV analysis) could have a biased component because groups under medication are treated precisely by higher SBP and DBP levels with a tendency to stabilize instead of reducing the blood pressure, principally in PREE women and in our sample several women with preeclampsia are under medication. These results introduce a sample limitation and even when it‘s partially solved considering interaction terms in the MIXED analysis (after that only remain the contradiction for the preeclamptic group), future experimental designs should take these considerations and include the drug dose that wasn’t included in our analysis.

HRV discussion

The sympathetic/parasympathetic balance (LF/HF) is increased for pathological conditions. However, our results reveal that in HT group this balance is shifted by a predominant oversympathetic activity according to precedent studies[6-8] while in the PREE group is a consequence of a pronounced reduction of vagal activity not directly related with an increment but with a reduction or, at least, unchanged sympathetic stimulation. The almost linearity between LF and HF for low HF values could indicate that a reduction of the HF will be associated with a predominant reduction of the LF, however, this could be a consequence of vagal influence on the LF component[3,8].

The drug administration predominantly affects the LF band by reducing the respective value. This could be a consequence of methyldopa inhibition of the sympathetic activity and, therefore, this result could indicate that the LF region, in both pathologic groups, is strongly related with the sympathetic activity. On the other hand, even without drugs treatment the PREE group presented lower LF values. It is important to consider that the majority of the women in the PREE group are under drugs treatment and, therefore, the stability of the LF or even the reduction could be related to the discussed drug effect under

LF band. This confounding effect could be the reason of the lower differentiability between HT and PREE groups using spectral analysis.

The well known reduction of the mean RR interval (RRm) and RRstd[6,29], is also supported by our results. According to the previous discussion in the HT group the increment of RRm could be related to a sympathetic stimulation, however, in the PREE group our results can't support the same idea, in fact the coefficients of RRm for PREE and HT could suggest that the RRm reduction is higher for the HT group. We have, in this sense, two concomitant factors: the drugs influence (majoritary in the PREE group) and the parasympathetic activity reduction (increased for PREE group).

The parity effect previously discussed for the blood pressure also remains in the spectral and lineal HRV indexes. The two-parous women presented higher RRstd and HF values suggesting a better autonomic adaptation and predominant for maternal age upper 25 years. After this age the differences between primigravid and two-parous women tend to decrease. However, the maternal age increment is clearly associated with a reduction of the parasympathetic activity. This muffled effect of the parity on maternal age could suggest that women with advanced age and several outcomes not necessarily present an elevated risk of adverse maternal prognosis as was recently showed by other authors[43].

The increment of α_1 and α_2 with respect to gestational age has been noted previously[26], however, in our result the capability of both indexes to differentiate HT from PREE is low, even when it seems to increase the values of α_1 from Normal to HT and PREE groups (that could be improved increasing the sample size). In this sense the ApEn and SE indexes are more sensible to HT and PREE conditions. The correlation between complexity and LH/HF has been pointed out before, under different condition[11] and is confirmed by us during normal or pathological pregnancy as well as the age-related complexity modification[39]. On the other hand, drug treatment reduces LF band and, therefore, in general LF/HF should decrease as previously discussed, consequently the complexity must increase in agreement with our results.

The results clearly reveal a reduction of LZ, SE and ApEn that, together with the increment of α_1 and α_2 is a strong indicator of the regularity increment in the RR signal. Pregnancy is a perturbation of the normal physiological condition and, therefore, the complexity variation (and other physiological indexes) reflects how the maternal-fetal system evolves or adapt to this perturbation. The complexity increment in two-parous women could reflect this type of body adaptation even at early gestational age. In general,

women with successfully history of pregnancy have a reduced rate of spontaneous abortion, and gestational diseases like PREE or HT are known to be more probably in older primigravid groups[38,40]. Gestational hypertension and preeclampsia have been associated with a pregnancy maladaptation[41] and our results, through complexity and spectral analysis of the RR signal as well as blood pressure modifications seem to support this idea.

Conclusion

The present work explored blood pressure, spectral and complexity analysis of heart rate variability during normal and pathological pregnancy. Our results lead to conclude that maternal parity can influence blood pressure, sympathovagal balance and complexity indicating some type of adaptation from primi to two-parous women. This "maternal memory" could have several physiological and psychological factors and should be also studied with multiparous women and combined with other approaches.

On the other hand, our results reveal a complexity reduction from normal to preeclamptic women related to the increment in LF/HF values. However, this increment of LF/HF in HT group, is mainly achieved by an oversympathetic stimulation while in the PREE group it is achieved by a parasympathetic and sympathetic activity reduction during pregnancy evolution. However, the drug treatment reduces LF but almost affect HF band and, therefore, can influence the LF analysis and comparison with the preeclamptic group. This reduction of LF by drug treatment should tend to reduce LF/HF and increase complexity. Even when this effect is not reflected in LF/HF itself, is clearly noted in the complexity indexes increment.

Other factors like maternal age, familiar hypertension history and fetal sex were evaluated and they affect in several ways the explored indexes. Our results could support the idea that strongest modification with respect to the non-pregnancy condition could reveal poor pregnancy adaptability and, therefore, a non favourable pregnancy evolution.

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Relationship between heart rate variability indexes and common biochemical markers in normal and hypertensive third trimester pregnancy

Authors: E. Tejera[1], J. M. Areias[2], A. Rodrigues[2], A. Ramõa[2], J. M. Nieto-Villar[3], I. Rebelo[1].

1. Biochemical Department, Pharmacy Faculty Porto University. Portugal / Institute for Molecular and Cell Biology (IBMC), Porto, Portugal.
2. Maternal Hospital "Julio Dinis", Porto, Portugal.
3. Chemical-Physics Department. Havana University. Cuba.

Abstract

In the present study we explored the correlations between heart rate variability indexes and some biochemical markers during the third trimester of normal, hypertensive (HT) and preeclamptic (PRE) pregnancies. The obtained indexes are associated with complexity and spectral variables calculated from short ECG records. Including all the subjects in the analysis, we found that complexity indexes are positively related with hemoglobin concentration in the pathologic group and uric acid blood levels while low frequency (LF) was negatively correlated with uric acid and creatinine concentration as well as positively correlated with platelet levels. The low frequency (LF) was the only spectral region with significant correlation. Through an analysis of groups independently, only significant correlations were found in normal and PRE groups between LF and uric acid concentration and in normal and HT group for LF and creatinine blood levels.

Introduction

Heart rate variability (HRV) is a very interesting field that has been applied in many areas including pregnancy. HRV analysis during pregnancy is a very polemic area, in somewhat, because several physiological changes could affect maternal autonomic control. The common used methods in HRV analysis are divided in two wide branches: linear and non-linear. In the first group, the spectral analysis, the mean and heart rate standard deviation are usually applied [1], while in the second one the fractal and complexity derivate indexes are more commonly used [2].

The spectral indexes are (in short records) characterized by two major spectral bands: low frequency (LF) and high frequency (HF) powers [1]. HF is associated mainly with the parasympathetic activity, while LF is generally associated with both sympathetic and parasympathetic activities [1, 3]. On the other hand, HF is influenced by the respiratory rhythm, while LF is modified by several mechanisms including hormonal changes [3-6]. On the other hand, the complexity or fractal indexes are relatively "unknown" in biochemical terms and, in general, are usually interpreted as adaptability or response capability even when the relationship between these interpretations and the reduction or increment of the indexes are quite polemic [7-9].

Several studies have been performed correlating spectral or complexity changes with several biochemical markers, however, very few were performed during normal or pathological pregnancies [10-11]. The presented work explore the correlations between common biochemical markers like hemoglobin, platelets, globular volume, uric acid and others with several HRV indexes obtained during the third trimester of normal, hypertensive and preeclamptic pregnancies.

HRV indexes calculation

Several indexes have been proposed in complexity analysis of physiological signals; however, some of them are not appropriated to short records analysis. We select as complexity indexes the approximated entropy (ApEn), Lempel-Ziv complexity (LZ) and sample entropy (SE). All these methods are widely used in the HRV analysis (short and long records) and, therefore, there are very well described in the literature; however, we will briefly expose each of the methodological bases.

Lempel-Ziv complexity

Lempel-Ziv complexity [12, 13] (LZ) is an useful tool for complexity measurement that characterizes the degree of order/disorder in a sequence. This sequence can be a time series or a string array. In any case the sequence is transformed into a binary code with further determination of the different patterns contained in the sequence. LZ complexity interval is between 0 and 1 indicating the complete deterministic pattern (ex: sine function) and uncorrelated sequence (ex: white noise), respectively.

Approximated and sample entropy

ApEn [14] (ApEn) and sample entropy (SE) are entropy related measurements of irregularity or unpredictability of time series. On the other hand, ApEn is similar to the sample entropy [15] (SE). Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N we can define the vector $Y_m(i) = \{X_i, X_{i+1}, X_{i+2}, \ldots X_{i+m-1}\}$. If we define $n^m_i(r)$ as the number of vectors $Y_m(j)$ that are close to $Y_m(i)$ ($d[Y_m(i), Y_m(j)] \leq r$, $i \neq j$, where d is the Euclidian distance and r the distance cutoff) then:

$$ApEn(m,r,N) = \frac{1}{N-m} \ln \frac{n_i^m}{n_i^{m+1}} \quad (1) \qquad SE(m,r,N) = \ln \frac{\sum_{i=1}^{N-m} n_i^{'m}}{\sum_{i=1}^{N-m} n_i^{'m+1}} \quad (2)$$

where the differences between n'^m and n^m are associated with the inclusion of self-matched elements. We can observe that both indexes are very similar and this similarity could be related to Renyi entropy [16]. Smaller values of ApEn and SE imply a time series with similar pattern of measurements and, therefore, more "regular". In our calculation the conditions were ApEn(2, 0.2, 800) and SE(2, 0.15, 800). It is important to note that ApEn, SE and LZ complexity are measurements of irregularity and, in this sense, the complexity is increased in uncorrelated noise [17].

Spectral and other indexes

The total power spectrums were divided in two major components [1]: low frequency ($0.04 < LF \leq 0.15$ Hz) and high frequency ($0.15 < HF \leq 0.4$ Hz). Ratio LF/HF is widely used as an indicator of the sympathetic/parasympathetic balance.

Sample description

A total of 91 short ECG records (10 min and 1000 Hz) were obtained from 91 women during the third trimester of pregnancy, 28 were diagnosed with gestational hypertension, 21 were diagnosed with preeclampsia (PRE) and 42 presented a normal gestational evolution. At the beginning of the study all women were interviewed to obtain information about parity, smoke, drugs or alcohol addiction (Table I). Almost all the women under medication were treated with methyldopa (and in some cases with Cartia or methyldopa/Cartia).

Table I. Data description

	Normal	HT	PRE
Sex M/F	21.98 / 24.18 %	18.68 / 12.09 %	10.99 / 12.09 %
Parity =1/2/3	31.87 /14.29 /0.00 %	9.89 /18.68 /2.20 %	14.29 /7.69 /1.10 %
Smoke	9.89 %	2.20 %	3.30 %
DT	0.00 %	18.68 %	15.38 %
GA	32.43 (25-36)	32.14 (26-36)	31.19 (25-36)
Age	27.5 (18-39)	32.6 (22-40)	30.2 (20-40)
SBP (mmHg)	114.4 (95-131)	136.7 (105-230)	138.8 (114-184)
DBP (mmHg)	65.3 (46-83)	77.4 (57-105)	82.0 (46-105)
BMI	28.27 (21.11-39.56)	34.37 (23.53-49.01)	31.26 (25-41.32)

Notes: The nomenclature is: fetal sex (Sex), women with drug treatment (DT), maternal gestational age at the study moment (GA), maternal age (Age), systolic (SBP) and (DBP) diastolic blood pressure, body mass index (BMI).

The RR time series have 800 points and biochemical variables are: hemoglobin (HB), hematocrit (HTC), mean globular volume (MGV), mean globular hemoglobin (MGH), platelet (PLAT), leukocyte (LEU), blood glucose (GLU), uric acid (URI) and creatinine (CRE) levels. To study the correlation between HRV indexes and biochemical data it is important to adjust for the confounding variables and to consider the possibility of heterogeneous regression slope. Therefore, we applied a general linear model (ANCOVA analysis) in the SPSS software [18] for each biochemical-HRV index pairs.

Results

The complexity indexes are reduced from normal to PRE women but only the SE was statistically significant (Table II) while the lnLF decreases presenting a p-value <0.1. A similar result is obtained for biochemical levels where the only statistical significant values with a p<0.05 are the URI and CRE while the MGH and PLAT presented a p-value <0.1 (Table II).

Table II. Adjusted mean of HRV indexes and biochemical markers.

	Normal	HT	PRE
HB (g/dL)	11.78 (0.55)	11.879 (0.5)	12.3 (0.42)
HTC (%)	35.394 (1.39)	35.344 (1.26)	35.772 (1.04)
MGV (fL)	89.435 (1.6)	88.655 (1.44)	90.808 (1.2)
MGH[1] (pg)	29.991 (0.69)	29.612 (0.63)	31.331 (0.52)
PLAT[1] (10^9/L)	245.86 (30.64)	256.185 (27.71)	175.73 (23.01)
LEU (10^9/L)	10.15 (1.28)	8.65 (1.16)	11.07 (0.963)
GLU (mg/dL)	74.627 (7.63)	80.028 (6.9)	80.898 (5.73)
URI[2] (mg/dL)	3.261 (0.46)	3.213 (0.41)	4.909 (0.34)
CRE[2] (mg/dL)	0.629 (0.08)	0.447 (0.07)	0.726 (0.06)
LZ	0.66 (0.02)	0.64 (0.03)	0.63 (0.03)
SE[2]	1.54 (0.04)	1.49 (0.06)	1.37 (0.06)
ApEn	1.24 (0.02)	1.23 (0.03)	1.19 (0.03)

lnRRstd	-3.14 (0.06)	-3.2 (0.08)	-3.16 (0.08)
RRm (ms)	0.68 (0.012)	0.653 (0.015)	0.689 (0.015)
lnLF[1]	6.16 (0.07)	6.21 (0.09)	5.98 (0.09)
lnHF	5.12 (0.09)	4.94 (0.11)	4.97 (0.11)
LF/HF	3.41 (0.38)	4.1 (0.49)	3.4 (0.49)

Notes: 1) denote p-value < 0.1. 2) denote p-value < 0.05. Means are adjusted by BMI, GA, smoke, maternal age, fetal sex. RRm and RRstd correspond to the mean RR interval and RR standard deviation, respectively.

As previously discussed, the goal of the present study is the correlation analysis between the changes in biochemical level and HRV derivate indexes. For simplicity reasons, we first presented the statistically significance p-value for each relationship analysis (Table III) and the consequent model information was restricted just for the significant pairs (Table IV and Table V).

Table III. Obtained p-values in the ANCOVA analysis for each pair of indexes.

		SE	**LZ**	**EnAp**	**RRstd**	**RRm**	**lnLF**	**lnHF**	**LF/HF**
Independent Terms	HB	***0.014***	0.332	***0.037***	0.291	0.132	0.610	0.730	0.697
	HTC	***0.024***	0.568	0.056	0.284	0.224	0.566	0.855	0.928
	MGV	0.431	0.568	0.591	0.817	0.810	0.871	0.422	0.646
	MGH	0.165	0.942	0.150	0.762	0.427	0.532	0.962	0.699
	PLAT	***0.022***	0.989	0.146	0.182	0.093	***0.035***	0.823	0.404
	LEU	0.195	0.989	0.414	0.368	0.492	0.644	0.115	0.101
	GLU	0.211	0.989	0.473	0.737	0.632	0.120	0.904	0.207
	URI	***0.027***	0.344	***0.026***	***0.040***	***0.006***	0.076	0.733	0.127
	CRE	0.988	0.344	0.545	0.648	0.167	***0.006***	0.255	0.511
Drug Treatment Effect	HB	0.396	0.423	0.397	0.431	0.464	0.438	0.457	0.474
	HTC	0.956	0.980	0.397	0.980	0.976	0.986	0.996	0.997
	MGV	0.285	0.980	0.287	0.293	0.298	0.296	0.273	0.274
	MGH	0.080	0.083	0.077	0.081	0.086	0.081	0.085	0.092
	PLAT	***0.037***	0.054	***0.043***	0.051	0.062	0.057	0.056	0.074
	LEU	0.975	0.054	0.984	0.925	0.994	0.955	0.851	0.816
	GLU	0.316	0.054	0.322	0.337	0.334	0.319	0.347	0.360
	URI	***0.000***	***0.001***	***0.000***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
	CRE	***0.048***	***0.001***	***0.039***	***0.043***	***0.033***	***0.015***	0.058	***0.040***

Note: The spectral indexes as well as RRm and RRstd are expressed in millisecond (ms^2 and ms, respectively). The p-values < 0.05 are denoted by italic and bold characters.

PLAT is correlated with complexity and spectral indexes as well as URI levels with a statistically significant influence of drug treatment. However, only the complexity indexes revealed some correlations with the HB and HTC levels with no statistically significant differences by drug treatment effect. On the other hand, LZ and lnHF presented a poor or null correlation with biochemical markers. Because of the similarity between SE and ApEn in the obtained results we only considered for further analysis the first index.

Table IV. ANCOVA coefficients obtained in the regression analysis.

		HB	PLAT	URI	CRE
SE	SE.Coef	1.11 (0.40)	-59.39 (25.25)	0.90 (0.39)	
	DT	-0.28 (0.32)	40.31 (18.91)	-1.13 (0.30)	
RRm	RRm.Coef			3.63 (1.26)	
	DT			-1.06 (0.29)	
RRstd	RRstd.Coef			0.61 (0.29)	
	DT			-1.10 (0.30)	
lnLF	lnLF.Coeff		36.00(16.65)	-0.46 (0.26)	-0.12 (0.04)
	DT		37.03 (19.04)	-1.10 (0.30)	-0.13 (0.05)

Note: The (...) are associated with the standard error. The DT coefficient is relative to the treatment conditions.

Table V. ANCOVA coefficients obtained in the regression analysis of separated groups.

	Groups	HB	PLAT	URI	CRE
SE	Normal	0.313[1]	n.s	n.s	
	HT	1.408[2]	n.s	n.s	
	PRE	2.074[3]	n.s	n.s	
RRm	Normal	-0.361[1]		0.083[1]	
	HT	1.045[1]		-4.506[2]	
	PRE	7.391[3]		9.9[3]	
RRstd	Normal		41.552[2]	0.633[1]	
	HT		58.057[1]	-0.95[2]	
	PRE		-77.24[3]	1.754[3]	
lnLF	Normal		n.s	0.573[2]	-0.103[3]
	HT		n.s	-0.055[1]	-0.115[3]
	PRE		n.s	-1.765[3]	-0.147[1]

Notes: 1) not statistically significant coefficient. 2) p-value <0.1. 3) p-value -0.05. The values are adjusted for maternal age, gestational age, BMI and drug treatment. The notation n.s. corresponds to non significant correlation considering separated groups. Drug treatment was significant ($p<0.05$) for lnLF-URI, RRstd-URI and RRm-URI correlations in PRE group.

The SE is positively related with the HB suggesting an increment of the correlations from normal to PRE group (Fig. 1 Left) (Table IV and V) while a reduction of the PLAT is observed (Fig.1 Right), but only considering all groups data (Table IV). On the other hand, the relation between SE and lnLF with respect to PLAT contrary to HB and HCT could be influenced by the drug treatment (Table IV). In this sense, positive coefficient and p-value (Table III and Table IV) suggest that for the same levels of PLAT the group without medication presented increased values of SE and lnLF compared to group under medication. The differences observed between ANCOVA, considering all cases and separated groups, could be related with the data points increment. Considering all the groups together in the analysis, the number of points for the regression analysis increase and consequently could increase the analysis power.

Fig.1. (Left) Relationship between complexity and HB blood levels. Considering independent groups correlation, significant differences were obtained only for HT and PRE groups. Right) Relationship between LF and SE considering the different pathological groups.

Mean RR interval is positively correlated with URI concentration indicating an increment of the heart rate with URI concentration reduction; however, statistical significant differences were only observed in PRE group. In the HT group an inverse correlation could be present. The HR reduction could be associated with a reduction of the sympathetic activity that is corroborated by the negative coefficient of lnLF respect to URI (Fig. 2 Left and Table IV) in the PRE group (Table V); however, in the normal group a positive correlation between LF and URI could be identified. On the other hand, a positive correlation was also observed between RRm and HB levels for PRE group, suggesting that an increment of the HB blood concentration reduce the heart rate. Alternatively, all the variables related with URI and CRE also reveal a significant effect of the drug treatment.

Fig.2. (Left) Relationship between lnLF and blood levels of URI. Considering independent groups correlation, significant differences were obtained only for Normal and PRE groups. (Right) Relationship between lnLF and blood levels of CRE. Considering independent groups correlation, significant differences were obtained only for normal and HT groups.

A significant negative correlation was also observed between LF and CRE levels, however, in the group analysis the correlations were only significant in normal and HT groups (Table V, Fig. 2 Right).

Discussion

The LF component of the RR variability changes during normal or hypertensive pregnancy are somewhat controversy. Some authors reported a reduction of the LF and HF component of HRV while other found no significant differences with respect to hypertensive or PRE [19, 20]. The contradictions also remain with other procedures like the evaluation of postganglionic sympathetic-nerve activity [21, 22]. Our results suggest a trend to LF reduction. Methyldopa could reduce the sympathetic activity and explain our finding but previous works including the same type of patients (an increased sample) revealed that even without methyldopa, the LF is lower in PRE group [23]. On the other hand, in CARDIA and SAPALDIA studies the LF component of HRV was also reduced in hypertensive subjects and negatively correlated with levels of uric acid, C-reactive protein and other inflammatory markers [24, 25]. Even when these studies were not performed during pregnancy, similar results were obtained considering pregnancy hypertension [19, 20, 26]. Our findings also reveal a negative correlation of LF with respect to URI with the consequent increment of SE, that as we can observe (Fig.1 Right), are negatively correlated, at least, in the PRE group. This finding is in agreement with other authors

where a reduction in the uric acid by diet control was associated with an increment of the sympathetic activity [27].

The increment of URI seems to increase the complexity as well as to decrease the mean heart rate and increase the RRstd, however, the correlation analysis in separated groups suggests a more complex interpretation. During normal pregnancy the uric acid concentration increase after the 2nd trimester linked, at least partially, with a renal tubular reabsorption increment. On the other hand, normal pregnancy is characterized by a complexity reduction, mean heart rate increment and RRstd reduction [19, 20, 26, 28]; therefore, during normal pregnancy we should obtain a positive correlation between URI and LF as suggested by our results, however, in HT group, the URI increment tend to reduce the RRstd and to increase the mean heart rate. These changes are in agreement with the cardiovascular increased risk noticed by other authors in correspondence with the uric acid increment [29, 30]. On the other hand, in PRE group the URI increment tend to increase SE, RRstd and decrease the heart rate, suggesting a different response mechanism. We think that this change in the autonomic response associated with URI levels could be related as during preeclampsia, the inflammatory and oxidative stress mechanisms are enhanced [30, 31] and the URI levels increment reflects a natural antioxidant response [32] instead of a renal dysfunction (that could prevail under a prolongation of the pathologic condition), however, further analysis are required with large population.

Recently, several authors described that in preeclamptic women hemoglobin level is increased, while platelet count, at least in severe PRE, tend to be reduced [33-35].The relationship between hemoglobin concentration and general blood rheology with the autonomic and heart rate control have been reported by other authors [36-37] during the second half of pregnancy as well as in non-pregnant condition. The hemoconcentration had been observed during preeclampsia (and eclampsia) [38, 39] and, therefore, is not surprising the increment of the HB levels and consequently, the reduction of the heart rate. Preeclampsia is, however, characterized by a placenta perfusion problem (inducing hypoxia condition) whose goal could be the increment of oxygen circulation. Therefore, it seems plausible the increased positive correlation between complexity indexes and HB in the HT and PRE groups, if we consider the complexity indexes as a measurement related with the response capability.

With similar PLAT level, higher values of LF are observed in the non treated group. This could be a consequence of the methyldopa sympathetic inhibition effect. However, even

considering the drug influence, SE and the LF remain statistically significant. The PLAT levels during PRE decrease [35,40] (in agreement with our results) which suggest that pathogenesis of thrombocytopenia in preeclampsia (and even during normal pregnancy) is complex and not clear, even when could be due to endothelial damage and the further peripheral consumption [35]. On the other hand, inflammatory response tends to decrease the platelet count and consequently reduce the probability of thrombus formation. The positive correlation observed for the LF and the negative correlation for SE were not found in the internal group analysis. Moreover, a significant negative correlation is found with respect to RRstd, that could reveal the efforts of the system to reduce the sympathetic activity and increase the complexity, obviously, without reaching the overall stability.

The negative correlation noticed between lnLF and CRE is difficult to explain. During a progressive renal insufficiency we should expect an increment of the sympathetic activity as has been reported by others authors [41, 42], however some important observation should be made. The analysis of the correspondent groups only reveals significant correlation in the normal and HT groups, therefore, is possible that the correlation observed has no relation with a progressive renal insufficiency (or at least not totally). During normal pregnancy the blood CRE levels tend to decrease in connection with the increment of the creatinine clearance [43]. This normal process should increase, at least partially, the sympathetic activity measured by HRV with like the increment of mean heart rate, RRstd reduction and even reduce the complexity, that are the common changes during the 3rd trimester of pregnancy. The no statistical significant results obtained for PRE could indicate either a mechanism change similar to URI previously discussed or/and the necessity to increase the sample size in future research.

Conclusion

In the present study we explored the relationship between HRV indexes and some biochemical markers. The results indicate that the sympathetic autonomic activity is related with the change in the platelet count and uric acid blood levels in the preeclamptic women while the complexity indexes (mainly SE) are additionally related with HB and HCT levels that tend to increase the correlation from HT to preeclamptic women group. On the other hand, lnLF presented a negative correlation with the general CRE mainly in normal and HT group. On the other hand, the results indicate that PRE is a complexity reduced state with significant differences with respect to the HGM, PLAT, URI and CRE.

The correlation obtained for complexity, spectral and conventional mean RR and standard deviation with respect to URI suggest different response mechanisms in normal, HT and PRE groups with respect to the URI modification. However, this result and those obtained with CRE should be confirmed increasing the sample size and considering other factors like drug doses and pathology severity degrees.

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Artificial neural network for normal, hypertensive and preeclamptic pregnancy classification using maternal heart rate variability indexes.

Authors: E. Tejera[1], M. J. Areias[2], A. Rodrigues[2], A. Ramõa[2], J. M. Nieto-Villar[3], I. Rebelo[1].

1. Biochemistry Department, Pharmacy Faculty Porto University. Portugal / Institute for Molecular and Cell Biology (IBMC), Porto, Portugal.
edutp00@yahoo.com
irebelo@ff.up.pt
2. Maternal Hospital "Julio Dinis", Porto, Portugal.
3. Chemical-Physics Department. Havana University. Cuba.

Abstract

Objective: A model construction for classification of women with normal, hypertensive and preeclamptic pregnancy in different gestational ages using maternal heart rate variability (HRV) indexes.

Method and Patients: In the present work we applied the artificial neural network for the classification problem, using the RR signal (n=568) obtained for ECG records. Beside the HRV indexes, we also considered other factors like maternal history and blood pressure measurements.

Results and Conclusions: The obtained result reveals sensitivity for preeclampsia around 80 % that increases for hypertensive and normal pregnancy groups. On the other hand specificity is around 85-90%. These results indicate that the combination of HRV indexes with artificial neural networks (ANN) could be helpful for pregnancy study and characterization.

Keywords: HRV, artificial neural networks, pregnancy, preeclampsia, hypertension

Introduction

One of the main problems with clinical applications of heart rate variability (HRV) indexes derived from the fact that the heart beat signal fluctuation is a sum response of several underlying mechanisms. The HRV fluctuations are consequence of autonomic neuron system (ANS) modulations; however, several hormonal, respiratory and inflammatory

processes could affect this modulation in a complex and unfamiliar way [1-3]. The multifactorial nature in HRV analysis is, at the same time, the core of its powerfulness, validated by the risk evaluation in several cardiovascular complications [4-6]. The HRV analysis usually generate a relative higher number of indexes (spectral, linear or complexity related), and even when some of them could be correlated, in general, the problem of how information is shared have been poorly explored and seems to be dependent of specific physiological situations. For this reason, it could be plausible to combine several of these indexes in a single model to reach the maximal classification capacity. In this sense the artificial neural network (ANN) could be an excellent tool, because brings the possibility to create a model without previous information about variable relationships.

During pregnancy, HRV can be even more complex to understand because pregnancy state itself achieves several physiological and psychological modifications at maternal and fetal levels [7, 8]. Pregnancy is characterized, at the maternal HRV analysis level, by the reduction of RR complexity, RR mean and standard deviation as well as other modifications that can be potentially used as diagnostic tools of pathological events like pregnancy associated hypertension or, even, preeclampsia [7, 9, 10].

The early predictions of preeclampsia or gestational hypertension are central problems in pregnancy study. The underlying mechanism of preeclampsia and even pregnancy hypertension remains under debate and, until today there isn't any reliable biochemical marker available for an accurate prediction. Recently, some authors include the changes in several biochemical markers in a single model for preeclampsia prediction just about 75-80% of correct classification [11-13]. These results, besides indicating a potential predictive model, also reveal the usefulness of combined approaches instead of searching for a single biochemical marker. However, should it be possible to obtain similar or better results through non-invasive measurements?

The application of ANN for classification or prediction tasks in medicine is not a new area. Though, the combination of HRV indexes and ANN, to evaluate the capabilities to create a classification model to identify which RR signal obtained for electrocardiographic (ECG) record match to women with normal, hypertensive or preeclamptic pregnancy, is a novel approach and is the main goal of the present study.

Sample and Data collection

A total of 568 short ECG (10 min) records were obtained from 217 pregnant women in several gestational ages (GA) in sitting position. The sample was classified according to: normal, hypertensive (HT) and preeclamptic (PREE) groups based on the current pregnancy. In the population studied, 130 (272 records) women didn't reveal any gestational disorder (and no personal history of hypertension or diabetes), 59 (234 records) women presented hypertension or some history of hypertension during pregnancy and 28 (62 records) women presented preeclampsia. The preeclampsia was diagnosed in women with blood pressure higher than 140/90 and proteinuria (>300 mg/24h) after the 20th week of gestation, while pregnancy related hypertension appear generally before the 20th week, or after but without proteinuria.

Table I. General sample description

Parameters	Normal	HT	PRE
Gestational weeks	24.58 (6-40)	24.7 (7-40)	26.94 (7-40)
Maternal age	27.18 (16-39)	31.72 (22-42)	30.12 (20-40)
Systolic blood pressure (SBP)	116.02 (88-148)	131.67 (99-230)	136.75 (107-184)
Diastolic blood pressure (DBP)	63.03 (35-85)	74.33 (46-100)	82.55 (46-107)
Body mass index (BMI)	27.4 (18.4-41.2)	32.9 (20.0-49.0)	30.5 (20.6-44.9)
PHT	0.00 %	61.83 %	41.54 %
Drugs treatment (DT)	56.43 %	58.46	0.00 %
PPHT	0.00 %	22.82	3.08 %
APRE	0.00 %	26.14	26.15 %
FHP	42.81 %	78.84	67.69 %
FD	47.72 %	54.77	27.69 %
Fetal Sex (Male/Female)	50.88 / 47.37 %	60.17 / 38.17 %	47.69 / 52.31 %
Smoke	24.56 %	9.96 %	15.38 %

Note: APRE: history of preeclampsia; PHT: personal history of hypertension (no pregnant state); PPHT: personal pregnancy history of hypertension; FHT, FD: familiar history of hypertension and diabetes, respectively. The SBP and DBP are expressed in mmHg. The values are reported in percent or mean (min-max) values. The values are referred to the number of ECG records instead of the number of women in each group.

At the beginning of the study all women were interviewed to obtain information about familiar history of hypertension (FHP) and diabetes (FD) (restricted to direct parenthood: mother/grandmother, father/grandfather), personal pregnancy history of hypertension (PPHT) as well as smoke addiction. Simultaneously with ECG records other variables were also measured like blood pressure and maternal weight. Average values and some global

sample characteristics are presented in Table I. In the hypertensive (HT) and preeclamptic (PRE) groups, medication was also considered however, we will not regroup according to drug type because almost all pregnant women with medication are under administration of methyldopa or methyldopa/Cartia combination. All mathematical indexes (spectral, complexity, etc) were calculated using RR interval of normal sinus beats with 800 points.

Lempel-Ziv complexity

Lempel-Ziv complexity (LZ) [14, 15] is a useful tool to complexity measurement that characterizes the degree of order/disorder in a sequence. This sequence could be a time series or a string array. In any case, the sequence is transformed into a binary code and the core of the LZ calculation is the determination of different patterns contained in the finite sequence. LZ complexity ranges between 0 and 1 indicating the complete deterministic pattern (ex: sine function) and uncorrelated sequence (ex: white noise), respectively.

Approximated and sample entropy

Approximated entropy [16] (ApEn) has been used in several time series analysis and, in general, is a measure of irregularity or unpredictability of the time series. On the other hand, ApEn is similar to the sample entropy [17] (SE). Given a time series $\{X_1, X_2, X_3 \ldots X_N\}$ of length N we can define the vector $Y_m(i) = \{X_i, X_{i+1}, X_{i+2}, \ldots X_{i+m-1}\}$. If we define $n^m_i(r)$ as the number of vectors $Y_m(j)$ that are close to $Y_m(i)$ ($d[Y_m(i), Y_m(j)] \leq r$, $i \neq j$, where d is the Euclidian distance and r the distance cutoff) then:

$$ApEn(m,r,N) = \frac{1}{N-m} \ln \frac{n_i^m}{n_i^{m+1}} \quad (1) \qquad SE(m,r,N) = \ln \frac{\sum_{i=1}^{N-m} n_i'^m}{\sum_{i=1}^{N-m} n_i'^{m+1}} \quad (2)$$

where the differences between n'^m and n^m are associated with the inclusion of self-matched elements. We can note that both indexes are very similar and, this similarity could be related to Renyi entropy [18]. Smaller values of ApEn and SE imply a time series with similar pattern of measurements and, therefore, more “regular”. In our calculation, conditions were ApEn(2, 0.2, 800) and SE(2, 0.15,800). It is important to note that the ApEn, SE and LZ complexity are measurements of irregularity and, in this sense, the complexity is increased in uncorrelated noise [19].

Spectral and other indexes

Total power spectrum is separated in three major components [20]: very low frequency (VLF≤0.04 Hz), low frequency (0.04<LF≤0.15 Hz) and high frequency (0.15< HF ≤ 0.4 Hz). The effect of vagal activity is predominant in HF while LF has been considered as a mixture between sympathetic and vagal stimulation [20, 8]. In general, factors that could be described by LF like blood pressure regulation are polemic [20, 22-24]. On the other hand, the physiological aspects of the VLF are hard to describe and have been associated with thermal regulation and some blood pressure control process [20, 21]. The ratio LF/HF is widely used as indicator of the sympathetic/parasympathetic balance.

Network Construction

The multifactorial causes of HRV changes could indicate that the HRV signal contain a lot of information that we are not capable to isolate or identify even when several works have been carried out to elucidate these aspects. Powerful methods like ANN can be used to explore the applicability of the HRV indexes in more complex situations; in our case, pathological events associated to pregnancy. The ANN even though a “black box” provides a potent capability for classification, regressions and general data mining problems where the intrinsic model is unknown.

The neural network model was obtained with the SPSS statistical package [25]. Data was randomly fragmented in around 55 percent for model construction, 15 for the test group (a total of 70 percent for training) and 30 for external sample validation. This procedure leads to: 390 records for training (195 normal, 158 HT and 37 PRE) and 178 for validation (77 normal, 76 HT and 25 PRE). All the input variables were previously standardized. We used the hyperbolic tangent as activation function in hidden layer and the softmax function for the output layer. For the training we also used the batch method and the scale conjugate gradient as optimization algorithm.

Results

The final network obtained was composed by only one hidden layer with five neurons and the classification results revealed a very well differentiability capacity with a ROC curve areas higher than 0.95 in all the groups (Fig.1).

Fig.1. ROC curve of neural network classification output according for the training (including test group) (Left) and validation sets (Right). The values between parentheses are the ROC curve area.

The ROC curve for both, training and validation groups was similar (Fig.1). However, an area reduction is noticed in the validation group. The classification results considering the same values used for model construction or testing, tend to be too "optimistic" while the external validation is more realistic and, therefore, the sensitivity should be lower. We can note a sensitive reduction in the validation group mainly for the PRE group however, in both cases we obtained sensitivity values higher that 80% with a wrong assignment around 10-20%. This wrong assignment is the probability of erroneously allocate some RR records to PRE group.

Fig.2. Sensitivity and specificity variation with respect to the predicted pseudo-probability obtained for the training (Left) and validation (Right) sets.

For the classification task is necessary to impose a cutoff criteria based on the pseudo-probability generated by the network. We can note that with values around 0.4, in both, training and validation (Fig.2) sets, was possible to correctly classify more that 80 % for PRE and an even higher percentage for the normal and HT groups. The sensitivity is lower in the validation group mainly in the PRE group.

Fig.3. Normalized importance of the independent variables and factors in the obtained ANN.

The network analysis brings the possibility to study the variable influences in the global model, and in this sense, we can note that SBP and DBP were extremely important (Fig.3). An interesting result is the spectral and conventional indexes are, in general, more relevant than the complexity indexes while on the other hand BMI and GA, which we should expect as important variable, presented lower significance levels.

Discussion

For both validation and training we obtained sensitivity values higher that 80% with a wrong assignment around 10-20% with fixing the cut-off probability of 0.4. The lowest classification power is noticed for PRE group and, considerably, increases for normal and hypertensive pregnancy. This could be related to the small sample in the PRE group and/or even to the different stages of pathology. However, all the records that actually correspond to the PRE group although with an incorrect classification, are in fact classified as hypertensive cases, therefore, some alert could be provided.

The major effect of SBP and DBP is, in fact, an obvious result, since blood pressure and protein levels are the more common evaluated parameters for preeclampsia diagnosis, therefore, have to be present in the model. However the increased significance of spectral indexes over non-linear and, the poor significance of BMI or even GA are consequences of the co-linearity between the independent variables. We should expect higher correlations between LF/HF and HF, LF/HF and complexity indexes like ApEn or SE, and on the other hand, the complexity and HF reduction are related with gestational age. Actually, if we

remove the LF/HF, the value of LF increases (data not shown) even more than HF suggesting an important contribution of the sympathetic stimulation, what is in agreement with others [8, 21]. These indicate that ANN, undoubtedly, could be optimized using a reduced number of variables.

As previously discussed, other authors obtained around 75-80% of correct classification using a combined approach with conventional biochemical markers like uric acid and haemoglobin [10-11]; however, it seems possible to increase this percent with non-invasive methods. Alternatively the combination of both, HRV indexes as well as biochemical markers, could increase the specificity, but further works are required.

Conclusion

The obtained results reveal a sensitivity for preeclampsia classification around 80 % that increases for hypertensive and normal pregnant group with specificity (1-specificity) around 10-15 percent. The false positive classification as PRE group is generally obtained in records belonging to the HT group and not the normal pregnancy. Therefore, the false positive values for PRE are in some way "compensated" because in clinical terms, both cases (PRE and HT) require a particular attention. These results indicate that the combination of maternal HRV indexes and ANN could be helpful in pregnancy evaluation. However, the number of ECG records should be increased and mainly in the PRE groups.

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Network centrality and multiscale transition asymmetry in the heart rate variability analysis of normal and preeclamptic pregnancies.

Authors: E. Tejera[1], A. I. Rodrigues[3], M. J. Areias[3], I. Rebelo[1], J. M. Nieto-Villar[2]

[1] Biochemical Department, Pharmacy Faculty Porto University. Portugal / Institute for Molecular and Cell Biology (IBMC), Porto, Portugal.
[2] Chemical-Physics Department. Havana University. Cuba.
[3] Maternal Hospital "Julio Dinis", Porto, Portugal.

Abstract

In the present work we define and apply a new methodology for the analysis of time series that involves a network representation. In this methodology, time series are transformed into a network with the subsequent calculation of some centrality indexes as well as the asymmetry of the transition frequency matrix, across different scales. The properties of the proposed indexes were analyzed with short-length simulated and physiological time series. In this case, we use RR time intervals, obtained from electrocardiographic records. The proposed indexes are compared with common complexity indexes in the analysis of the RR time series in normal and preeclamptic pregnancies.

Keywords: HRV, complexity, asymmetry, network, pregnancy, preeclampsia.

Introduction

The complexity of the time series (TS) generated by physiological systems could be presented under two approaches: the presence/absence of self-organized structures and the roughness or unpredictability of the TS. For random TS, the complexity based on entropy measure (like approximated entropy [1], Shannon entropy or even Higushi fractal dimension [2]) is maximal, which means that irregularities as well as unpredictability are also maximal. However, in this kind of TS there are not organized or fractal structures and therefore, in this sense, the complexity is minimal [3]. For example, the complexity values of the RR intervals of healthy subjects and patients with previous myocardium infarct,

calculated using the approximated entropy (ApEn) or the sample entropy index, are smaller than the values obtained from subjects with atrial fibrillation. However, in the last case the autonomic control is reduced and almost no correlation or fractal structure is detected [3].

In the last decade, novel and innovated procedures have been developed for the TS analysis based on the TS-network translation with the subsequent network description [4-10]. The objective with the conversion of TS to network is to bring the possibility of apply the tools and mathematical knowledge of the graphs and networks theory to understand the correlation structure and dynamical properties of the TS. The major problem in this TS-network translation is, specifically, the methodology inherent to the transformation procedure. This means, the appropriated definitions of the nodes and edges in the generated network. The common approaches to networks construction consider as starting points: 1) the autocorrelation matrix of the TS [4-7], 2) the relative magnitude between the TS points [8,9], 3) the distance between the phase space points [9] and 4) the similarity between non-overlapping segments of specific length in the TS or general number sequence [10]. All these methods have been capable of showing different TS properties like periodicity, fractal or chaotic dynamical behaviours [11] and were useful in the differentiation between normal subjects and patients with coronary affectation through the analysis of electrocardiographic (ECG) records [4].

On the other hand, the physiological time series, like the R-R, are characterized by the presence of one or more of the following aspects [11]: I) non-linearity, II) non-stationarity, III) time irreversibility and IV) multiscale variability. These aspects facilitate the system adaptation to external/internal stimuli as well as the capability to respond across different temporal scales; both these aspects are the bases of the physiological complexity concept [11, 12] or, in other words, the robustness [13] of the system. These TS properties are better analyzed with methodologies like the multiscale entropy (MSE) [11, 14] or the multiscale asymmetry (MSA) [15] because they put forward the possibility to study the correlation patterns across multiple scales. However, these scaled-entropy indexes can't be applied to short-length TS because with the use of the coarse graining procedure, the number of points rapidly decrease with the scale increment (1000 point with scale=1 are reduced to 500 with scale=2).

In this study using a discretization process, a new methodology is proposed for the TS-network conversion considering different scales. In the obtained networks, the properties

of some centrality indexes as well as the asymmetry of respective transition frequency matrix were analyzed in short-length simulated and physiological TS.

Theoretical background

The discretization procedure used in this work is similar to SAX method [16-18] and was previously used in other TS analysis [19]. The TS recorded at regular times is considered as a numeric sequence $Y=\{y_1,y_2,\ldots,y_N\}$ with known minimal (y_{min}) and maximal (y_{max}) values. This sequence is divided in *m* not overlapping intervals of size: $\Delta Y = ((y_{max}-y_{min})/m)$. Each value of the Y is evaluated as follow: if $Y_i \in [y_{min}+(k-1)\Delta Y,\ y_{min}+k\Delta Y]$ with k=1,2,...,*m*, then $Y_i \Rightarrow S_k^i = \text{mean}([y_{min}+(k-1)\Delta Y,\ y_{min}+k\Delta Y])$. The original TS of length N is transformed in a sequence S of length N but with only *m* possible values (Fig. 1). In other words, any value of Y_i inside the interval $Y_i \pm \Delta Y$ will be considered equal and replaced by the interval average.

Fig.1. Left) Schematic representation of the discretization process. The original sequence $Y=\{y_1, y_2, y_3, y_4 \ldots y_{12}\}$ is divided in m=6 regular intervals of size ΔY. Each element of Y is replaced by the average value of the interval and therefore Y is transformed in a new sequence S with equal length but only six possible values: $S = \{S_4^1, S_2^2, S_6^3, S_3^4, S_6^5, S_5^6, \ldots, S_1^{12}\}$ with $\{S_k^i$ k=1...6; i=1...12}.

The discretization process creates a limited space of values (*m*) and each of them will be considered as the network nodes $n=\{n_1,n_2,\ldots,n_m\}$. Two of these nodes are connected ($n_a \rightarrow n_b$) for the scale τ if in S, the elements S_a^i and S_b^j are separated by $j=i+\tau$ at least one time in the sequence S (Fig. 2).

Fig.2 Left) Network representation of Fig. 1 for two different scale (τ=1, 2). Each possible *m*-values in S is associated with a network node $\{n_1,n_2,...,n_m\}$ and two of these nodes are connected if they are separated by τ-1 element in S at least one time. Ex: the nodes ($n_2 \rightarrow n_3$) are connected for τ=2 because $S_2^i, S_3^{i+\tau}$ appears at least one time (there are two: $S_2^2 - S_3^4$ and $S_2^7 - S_3^9$) in S.

With the current methodology the networks (G) obtained presented the following properties:

1. G is directed and therefore could be symmetrical or not.
2. The existence of loops is allowed.
3. The network topology could change with the scale (τ) variation (G(τ)).

Some pairs of elements can be found in the sequence for a distance τ-1 more than one time and therefore we define the transition frequency matrix $T_{a\rightarrow b}(\tau)$ for the scale τ as the number of pairs: ($S_a^i, S_b^{i+\tau}$) in S. The multiscale transition asymmetry (MSTA(τ)) was defined as follow: If: $\Gamma_{a,b}(\tau) = \begin{cases} 1 & if \quad |T_{a\rightarrow b}(\tau) - T_{b\rightarrow a}(\tau)| > 0 \\ 0 & otherwise \end{cases}$ then:

$$MSTA(\tau) = \frac{2}{m(m-1)} \sum_{a}^{m-1} \sum_{b>a}^{m} \Gamma_{a,b}(\tau) \qquad \text{(eq.1)}$$

where 2/(*m*(*m*-1)) is a normalization term and *m*, as before, is the network number of nodes. In the case of MSTA = 0 or MSTA = 1, the matrix is symmetric or asymmetric, respectively. For uncorrelated time series the transition probability must be independent of node types and τ, therefore, MSTA(τ) should be constant and minimal. Using the Shannon formulation is possible to define the entropy as:

$$S(\tau) = -\sum P_{a,b}(\tau) \log P_{a,b}(\tau) \qquad \text{(eq. 2)}$$

Where $P_{a,b}(\tau)$ is the probability of each pair $S_a^i, S_b^{i+\tau}$ at scale τ. Several aspects of networks (ex: number of cycles, shortest and longest paths, eccentricity, clusters or degree distribution) could be analyzed [20]. However, in the present work we only consider two indexes related with graph centrality [21]. The selected indexes are: the mean network distance (MND(τ)) and the network efficiency (EF(τ))defined as:

$$EF(\tau) = \sum_{i.j}^{m} \frac{1/d_{ij}(\tau)}{m(m-1)} \qquad \text{(eq.3)}$$

where $d_{ij}(\tau)$ is the element of the distance matrix and represents the shortest path between the nodes i and j in the network obtained for the scale τ. The centrality indexes have been used in graphs topology analysis under a wide kind of problems [20-22] and in general, they are related to the compactness of the graph and to the connectivity degree. In a network with edges between any pairs of node, for example, the MND is 1 and NE is higher; while in a network where every node is connected only with the two neighbors the MND will be higher with low NE. For simplicity, in the present work the centrality indexes were calculated under two conditions: I) the loops in the networks were ignored and II) the distance $d_{ij}(\tau)$ corresponds to the topological one ignoring the edges weight.

Simulated and physiological time series

To explore the capabilities of the proposed indexes to capture the dynamical aspects and complexity degrees in TS, two types of simulations were performed using MIX processes with periodic and chaotic (LOR) behaviors. A MIX(p) process is defined as [1]:

$$MIX_i(p) = (1-Z_i)X_i + Z_i R_i \qquad \text{(eq.5)}$$

Where 0<p<1, $X_i = \left[\left(\frac{1}{12}\left(\sum_{j=1}^{12}\sin^2(2\pi\pi/12)\right)\right)^{-1/2}\sin(2\pi\pi/12)\right]$, R_i is a family of independent identically distributed (i.i.d) real random variable with uniform density on the interval $[-\sqrt{3},\sqrt{3}]$ and Z_i is a family of i.i.d random variable where Z_i = 1 with probability p and Z_i = 0 with probability 1-p. Informally, the MIX process is a sine wave with N points where, with a probability p, the points are replaced by a random signal, therefore MIX(p=0) is a sine wave while MIX(p=1) is a random signal.

In a similar way the LOR(p) process was produced replacing X_i of eq.5 by the numerical simulated y-values of Lorentz differential equation system:

$$\begin{aligned} dx/dt &= a(y-x) \\ dy/dt &= x(b-z)-y \\ dz/dt &= xy-cz \end{aligned} \qquad \text{(eq.6)}$$

Where a, b and c are the control parameters. In the present work, a= 10, b=28 and c= 8/3, under these conditions the generated TS presents a chaotic behavior. The integration time was 0.05 and y-values were recorded at intervals $\Delta t=0.2$. The R_i interval (eq.5) is restricted to the minimal and maximal value of X_i. All the generated TS have 1000 points.

The physiological data used in this article, correspond to 63 RR intervals time series obtained form short length (10 min) electrocardiographic records in preeclamptic (n=21) and normal (n=45) pregnant women, during the third trimester of gestation. Complementary information related to maternal age and body mass indexes (BMI) were also obtained.

Results and Discussion

The selection of the intervals number *m*, is an important point in the presented methodology. The maximal value of *m* is: $m_{max} = (y_{max}-y_{min})/|\Delta Y_{min}|$ where $|\Delta Y_{min}|$ is the minimal difference between any two points of Y. Any value of $m>m_{max}$ will produce the same number of nodes because no points of Y will be inside any interval lesser than $|\Delta Y_{min}|$. The selection of very small values of *m* lead to small number of nodes and therefore the network analysis will be faster, however important information could be lost and small fluctuation can be ignored. On the contrary, a higher number of nodes could improve the sensibility for small fluctuations but the computational cost for network analysis increases. In all the calculations we fix m=300 that is a higher value of node.

From periodicity to randomness analysis

Fixing m to higher values (*m*=300), the MSTA(τ) decrease with p=0.1-0.5 while a little increment is noted for p=0.6-1.0, but always lower that the periodic TS. This is a consequence of the relative conservation of the number of node for higher p.

For random signals (p=1) almost all the nodes are inter-connected and therefore, MND(τ) should be minimal, however theoretically the MSTA(τ) should be minimal too because the transitions are the same for all nodes. The peaks that we can note, principally in the MSTA(τ) and EF(τ) profile, correspond to the period (λ) and semi-period (λ/2) of the periodic signal (see eq.5) and as we can note its disappear with the increment of p as a consequence of the correlation break. When τ= kλ, (k is any integer) $Y_i = Y_{i+\tau}$ so, there are almost no transition between nodes: $T_{ij} = T_{ji} \approx 0$ for any i≠j and, therefore, the MSTA(τ) is minimal. Evidently the network centrality indexes are only calculated for p>0 because for exactly periodic TS (p=0), with τ= kλ the network is completely disconnected. A similar behavior is noted when τ= kλ/2 where the transition space is reduced.

Fig.3. Indexes values across different scales for MIX(p) process using several values of parameter p and fixing *m*=300. In all cases for each p, 10 TS were generated and therefore all the indexes correspond to average values.

The differences between the peaks for τ= kλ/2 and τ= kλ are noted principally for EF(τ) and MND(τ). The S(τ) is maximal for random signal and as the transition frequency is independent of the nodes type, if the TS has N points, the number of pairs-transition is N-1 and $S = -\log(1/(N-1))$. In our case N=1000 and S=-log(1/999)= 2.99 that is very close to the obtained results for p=1.0.

Analysis of time series with scale variation properties

The analysis of periodic to random variation in the TS is important to understand the indexes behavior under regularity or roughness modifications. However, in the periodic and random dynamic the correlation structures are almost scale independent and no fractal structure is available, therefore, the indexes (with peak exceptions as was discussed) are scale independents. On the other hand, in physiological conditions the TS are generally far from the periodic behavior, but conserving some correlation structure. For these reasons, to study the scale properties as well as complexity modification, the LOR(p) process was analyzed (Fig. 4).

Fig.4. Indexes values across different scales for LOR(p) process using several values of parameter p and fixing m=300. In all cases for each p, ten TS were generated and therefore all the indexes correspond to average values.

The scale profiles are very different with respect to the MIX process. The indexes in general are modified for low scales and the values tend to be constant for higher scales (Fig. 4). With the increment of p the indexes variation are similar to the Fig. 3 as we can expect. The EF profile is the inverse of the MND according to the eq.3 and for this reason the data was not showed. The peaks, mainly presented in the MND profile (for p=0), are the consequence of TS periodicity or recursivity (like the recursive percent index [29]) and in fact, reveal some internal long-term correlations undetectable if we use other no scale-complexity indexes.

With the increment of p, the subsequent additions of noise components reduce the short-term correlations (and quickly the long-term) and the indexes variation with respect to the scale tends to be null. On the other hand, the MSTA and EF values are higher in TS with increased internal correlations while the MND and S present a progressive reduction using higher *m*-values.

Physiological TS analysis

Pregnancy is a condition with effects in several metabolic, physiologic and psychological processes that modified the nervous system response. Some of these changes involve the reduction in the vagal and sympathetic stimulation, the increment in the heart rate and in general, a reduction of the adaptive capacity of the cardiovascular system [30-32]. The main goal in the application of the presented indexes to the TS obtained from pregnant women is to explore the differentiability potential as well as the complexity modification. The LZ complexity and EnAp are not statistically significant (Table I) while the SE reveal a significant complexity reduction. All the proposed indexes were statistically significant for almost the complete scale interval with short-term scale modifications (Fig. 5).

Table I. ANCOVA analysis for the analyzed indexes.

	NORMAL	**PREE**	**p-value**
MND(τ)	3.29 (0.08)	2.91 (0.11)	$p<0.05$ ($\tau \geq 3$)
EF(τ)	0.33 (0.01)	0.39 (0.01)	$p<0.05$ ($\tau \geq 1$)
MSTA(τ)	0.12 (0.01)	0.15 (0.01)	$p<0.05$ ($\tau \geq 1$)
S(τ)	2.86 (0.02)	2.76 (0.03)	$p<0.05$ ($\tau \geq 1$)
LZ	0.68 (0.03)	0.67 (0.03)	0.805
SE	1.53 (0.05)	1.37 (0.07)	0.034
EnAp	1.26 (0.03)	1.21 (0.04)	0.321

Note: Notation (...) corresponds to standard error. The p-values are restricted to the scale intervals in the multiscale indexes, outside the presented interval no significant differences were noted while the mean values were selected for τ =10 (mainly for representative purpose). The mean values are controlled for maternal age, body mass index and gestational time.

As we can observe, the PRE group present an increased MSTA and EF while a reduction of MND and S. These profiles strongly indicate an increment of “order” or correlations from normal to PRE. This is the same information that we can obtain with the SE reduction, however, for $\tau > 10$ approximately, the indexes remain constant but with significant differences between normal and PRE groups (Fig.5). In average, the structure of TS (for long scales) is more regular in the PRE suggesting that even when not scale variations, there are some correlation structures. For $1< \tau < 10$ approximately, a progressive decrement of the correlation is noted in both groups. On the other hand, there are some similarity between the physiological indexes profile and the obtained form LOR procedure with the important differences of the absence of peaks for higher scales (mainly in MND).

Fig. 5. Average indexes (and standard error bar) values for the normal and PRE groups across different scales.

Preeclampsia is characterized by a complexity reduction (order increment) that could be a consequence of the increment of the oversympathetic stimulation and therefore, a pregnancy maladaptation as proposed by other authors [33-35].

Conclusions

In the present work an alternative methodology is defined and applied for the complexity analysis of short-length TS. The mathematical indexes obtained with the proposed method are capable of differentiating between normal and preeclamptic pregnant women from the analysis of RR time series signals. On the other hand, are capable to reflect modifications in the short and long-term structure correlation in physiological and simulated time series. The presented results indicate that preeclamptic women reveal a reduced complexity by an increment of periodicity.

The differentiability between the normal and PRE groups is significant in almost the complete scale interval for the MSTA(τ), EF(τ) and S(τ) indexes while MND(τ) is only significant for τ>2.

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General discussion and conclusions

According to the objectives, several HRV modifications were identified during normal and pathological pregnancy. In this sense, the most important results obtained are:

I. Throughout normal, hypertensive and preeclamptic pregnancy HF progressively decreases during pregnancy evolution.
II. HF significantly decreases as: Normal > Hypertensive >Preeclampsia.

Both modifications indicate a reduction of the parasympathetic activity that is related with other factors like parity, maternal age and familiar history of diabetes. In normal and pathological groups, two-parous women presented higher values of HF while the increment in maternal age contributes to HF decrement. In the normal group, women with familiar history of diabetes presented reduced values of HF while the same effect was not confirmed in the hypertensive and/or preeclamptic women. On the other hand, no significant correlations were found between HF and any of the studied biochemical markers.

III. A significant reduction of the LF was noticed from normal to preeclamptic groups and an increment from normal to hypertensive group.
IV. In normal pregnancy the LF present a nonlinear behaviour with maximal values around the 2nd trimester.
V. A nonlinear correlation was found between HF and LF in the three studied groups.
VI. LF values decrease with methyldopa administration while HF remains almost unchanged.
VII. The LF/HF index increases during pregnancy evolution.
VIII. The LF/HF index is higher in pathologic groups in comparison to normal women but the differentiability between hypertensive and preeclamptic groups is low.

The LF is quite polemic as previously discussed. In this sense, we should remember that in general the LF region modification is associated with both sympathetic and parasympathetic activity. However, the selective reduction of the LF region (without HF changes) under methyldopa administration clearly indicates, at least, a dominant sympathetic activity in the LF region of pathological group. On the other hand, the nonlinear correlation between HF and LF reveal a complex autonomic control mechanism that result in an increment of the sympathovagal balance (LF/HF) during pregnancy. Even when the different LF profile between hypertensive and preeclamptic groups with respect

to normal women can explain the lack of differentiability between the pathological states, the underlying mechanism is unclear. The reduction of LF during preeclampsia is polemic (mainly in terms of sympathetic activity) and contradictory literature is easily found, however, the LF reduction obtained in our results is also confirmed by the complexity modifications and also by the biochemical markers.

IX. Throughout normal, hypertensive and preeclamptic pregnancy complexity progressively decreases during pregnancy evolution.
X. The complexity significantly decreases as: Normal > Hypertensive > Preeclampsia.
XI. Complexity indexes are related to maternal age, methyldopa administration and parity.

The complexity reduction clearly indicates an increment of RR periodicity and is related with LF/HF index. The complexity increment is generally associated with an increment on the autonomic control (like in aging); however, as previously discussed HF and LF tend to decrease. Therefore, LF/HF index increases suggesting that instead of an autonomic increment the reason of the complexity reduction could be mainly associated with an autonomic imbalance that, in some way, is amplified during pathological pregnancy. As we should expect, complexity decreases with maternal age, however, an interesting phenomenon is noticed with respect to maternal parity. Primigravid women presented lower complexity values than two-parous women. This parity effect (also noticed in HF) can be related to an adaptive process that can involve both physiological and psychological processes.

On the other hand, the group under methyldopa administration presented higher complexity values with respect to the group without medication. Even when the objective (and consequently the experimental design) was not conceived for a medication evaluation (and evidently deeper studies should be required), the reason of this modification could be explained by methyldopa effect on the reduction of the LF region, that consequently reduces the LF/HF index increasing complexity indexes.

XII. The LF is negatively correlated with URI blood levels change in the preeclamptic women and positively related in the normal group.
XIII. The LF presented a negative correlation with CRE in normal and HT groups.
XIV. Complexity indexes (mainly SE) are correlated with HB concentration and tend to increase the correlation from hypertensive to preeclamptic women.

XV. Mean RR interval is significantly correlated with HB concentration in preeclamptic group and with URI in both pathological groups. RR standard deviation is correlated with PLAT in normal and hypertensive groups while significant correlation was also found with URI concentration in both pathological groups.

XVI. Significant differences were found between normal and preeclamptic women with respect to HGM, PLAT, URI and CRE.

The correlation obtained for complexity, spectral and conventional mean RR and standard deviation with respect to URI suggests different response mechanisms in normal, HT and PRE groups. These mechanisms can involve oxidative-stress and inflammatory responses that are different in normal and pathological conditions. On the other hand, the correlation obtained with respect to HB concentration can be a consequence of the well known hemoconcentration process also elevated during preeclampsia.

The CRE correlation analysis of the correspondent groups only reveals significant correlation in normal and HT groups, therefore, it is possible that the correlation observed has no relation with a progressive renal insufficiency. Actually, during normal pregnancy blood CRE levels tend to decrease in connection with the increment of the creatinine clearance. Nevertheless this result and those obtained with URI concentration should be confirmed increasing the sample size and considering other factors like drug doses and pathology severity degrees.

XVII. Using HRV indexes and other non-invasive measurements is possible to obtain a sensitivity for preeclampsia classification around 80 %, which increases for hypertensive and normal pregnant group with a specificity around 85-90 percent.

The false positive classification as preeclamptic group is generally obtained in records belonging to the HT group and not to normal pregnancy. Therefore, the false positive values for PRE are in some way “compensated” because in clinical terms, both cases (PRE and HT) require a special attention. These results clearly indicate that the combination of HRV indexes and ANN could be helpful in pregnancy evaluation.

XVIII. The proposed methodology for time series analysis, through graph theory translation, brings the possibility to apply a multiscale approach for short-length time series.

XIX. The obtained graph-derived and multiscale asymmetry indexes were statistically significant between normal, hypertensive and preeclamptic groups.

The mathematical indexes obtained with the proposed method are capable of differentiating between normal and preeclamptic pregnant women from the analysis of RR time series signals. On the other hand, are capable to reflect modifications in the short and long-term structure correlation in physiological and simulated time series. The presented results also confirm the reduced complexity in preeclamptic women by an increment of periodicity.

Beside the conclusions obtained and briefly discussed (more related with the thesis objectives) other important results were also obtained:

I. SBP remains almost unchanged during pregnancy, however, is influenced by BMI changes. The SBP is lower in smoker two-parous women while in the primigravid group the smoke effect on SBP and DBP is reduced.
II. DBP presented a nonlinear behaviour during pregnancy evolution with a minimum value around the 2nd trimester of gestation.
III. Body mass index (BMI) is positively related with ApEn.
IV. In pathological pregnancy BMI is positively related with SBP and DBP.
V. Mean RR interval decreases during normal and pathological pregnancies and this reduction is higher in non-smoking women (in the normal group).
VI. Mean RR interval decreases in hypertensive and preeclamptic pregnancies compared to normal women.
VII. Mean RR interval is higher in women under methyldopa administration.
VIII. RR standard deviation is reduced during pregnancy evolution and is also reduced from normal to preeclamptic women.
IX. VLF increases during normal and pathological pregnancies.
X. VLF is influenced by familiar history of hypertension in normal pregnant women.
XI. During normal pregnancy LF/HF index is higher in women with familiar history of diabetes.
XII. Short (α_1) and long-term (α_2) correlation increase during normal and pathological pregnancy and is influenced by maternal age.
XIII. In normal pregnancy α_2 is higher in women with familiar history of hypertension.

The results suggest that HRV can be an interesting an powerful tool in pregnancy evolution analysis and even diagnosis, however, like any other research, many questions are answered but many others remain open or emerge as a results of the investigation creating the bases for future experimental designs.

Future Perspectives

In terms of experimental design and for a better clinical research, the sample should be increased mainly with preeclamptic women. On the other hand, other aspect like, methyldopa doses modification, nutritional balance and even psychological evaluation should be desirable.

We recommend for future studies:

- To consider the recording process before conception and until several months after delivery.
- The classification rate obtained with HRV and other indexes and the ANN suggest an effective method even for preeclampsia prediction. However, more cases should be included before the 2nd trimester to test the prediction capabilities, obviously together with a proper blinded experimental design.
- In order to understand in a deeper way the complexity indexes, can be important the analysis of the correlations with other biochemical markers associated with inflammatory and immunological responses. In this sense, the same can be performed to clarify the parity effect.

In a mathematical and statistical direction some aspects should be considered in further studies:

- Reconsideration or validation of the three (or four) spectral band analysis.
- Study of the multifractal modifications in normal and pathological pregnancies.