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THE STUDY OF THE SYMPATHOVAGAL RELATIONSHIP AS A SCREENING METHOD

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LIST OF ABBREVIATIONS

2D-PPG	Derivata a doua a fotopletismogramei
ADC	Convertor analogic-digital
ApEn	Entropia aproximativă
APT-Drăgan	Coloraţia albastru policrom tanin Drăgan
ASR	Aritmia sinusală respiratorie
CorDen	Dimensiunea corelaţiei
DF α1	*Detrended fluctuations α1*
DF α2	*Detrended fluctuations α1*
DZ	Diabet zaharat
ECG	Electrocardiogramă
FiA	Fibrilaţie atrială
HE	Coloraţia hematoxilină-eozină
HF	Puterea spectrală la frecvenţă înaltă
IDE	*Integrated development environment*
IMA	Infarct miocardic acut
Index SDNN	Media deviaţiilor standard ale mediilor intervalelor în segmente de 5 minute
LF	Puterea spectrală la frecvenţă scăzută
LF/HF	Raportul puterilor spectrale
Media HR	Media frecvenţelor bătăilor cardiace momentane
Media NN (RR)	Media tuturor intervalelor dintre bătăile cardiace succesive
NDA	Neuropatie diabetică autonomă
NN50	Numărul de bătăi cardiace succesive între care există o diferenţă mai mare de 50 ms
OMS	Organizaţia Mondială a Sănătăţii
pNN50	Procentul de bătăi cardiace succesive între care există o diferenţă mai mare de 50 ms
PPG	Fotopletismogramă
RMSSD	Rădăcina pătrată a mediei de suma pătratelor diferenţelor dintre intervale adiacente
SampEn	Entropia eşantionului
SD 1	Deviaţia standard 1 a diagramei Poincaré
SD 2	Deviaţia standard 2 a diagramei Poincaré
SDANN	Deviaţia standard a mediilor intervalelor calculate pe toate segmentele de câte 5 minute din întreaga înregistrare
SDHR	Deviaţia standard a bătăilor cardiace succesive
SDNN (SDRR)	Deviaţia standard a tuturor intervalelor dintre bătăile cardiace succesive
SEC	Sistemul excitoconductor nodal al inimii
VFC (HRV	Variabilitatea frecvenţei cardiace *Heart Rate Variability*)

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Studies on heart rate variability

Comparison between electrocardiogram and photoplethysmogram as methods for recording intervals used in analyzing the heart rate variability

Introduction

Theoretically, any method which allows the registration of intervals between successive heart beats (ms) is suitable for studies on heart rate variability (HRV). But practically, every method has advantages and disadvantages for both subject and examiner.

The **purpose of this research** was to compare HRV parameters obtained thorough these two methods, on simultaneous electrocardiography and photoplethysmography recordings.

Methods and materials

Being a pilot study, it has included a number of 10 participants aged 22-25 (6 females). For capturing the signals, there have been used two devices:

- An analogical photoplethysmograph based on light wavelengths found on the infrared spectrum;
- A mono-derivation electrocardiograph (D I) which uses an AD620 operational amplifier and a 30 Hz *low-pass* filter.

Both devices were connected to the microphone input of the sound card of a personal computer, which played the role of an analog-to-digital converter (ADC). The connection was made through a jack splitter cable, each device being linked to a channel of the analog input.

The recording instruments were concurrently turned on for a period of 10 minutes, while the trails for the two channels (photoplethysmographic and electrocardiographic, respectively) were separately interpreted.

Results

The biggest technical impediment encountered was the presence of artefacts on some of the photoplethysmographic recordings. These artefacts were due to the voluntary or uncontrolled movements of the test subjects, determining aberrant inflexions on the photoplethysmographic track with higher amplitude than the rest of the trail, exceeding the detecting threshold for heart beats and interfering with the accurate detection of them.

When there are few artefacts (caused by ectopic heart beats or subject movement), they can be easily removed from the recording without changing the values of the parameters, but in some cases (the situation of the 5th patient enrolled in the research), the artefacts

outnumbered by 50% the normal heart beats, therefore these recordings were entirely excluded from the study. The artefacts can be visually recognized based on two criteria:

1. The triangle-like aspect of the Poincaré diagram, with collinearity between some points and the point of origin;
2. The chaotic aspect of the tachogram, with the identification of the normal trail, but which has numerous either positive or negative deviations.

As a result, the measurements had to be repeated until the complete elimination of these artefacts.

Contrasting between the mathematical parameters (statistical descriptors) collected from the two methods

Parameters from all three categories (time, frequency and Poincaré diagram) have been taken into account. The calculation and processing of them were conducted by using *Kubios HRV Analysis* software. These parameters have showed a nearly perfect correlation, with an index R^2 value higher than 0,95, meaning the methods are identical in terms of obtaining RR intervals used in HRV assessment.

The results indicate the fact that PPG can serve as an alternative for ECG in the evaluation of heart rate variability. All types of parameters have presented major similarities between the methods, these also been confirmed by the statistics. Therefore, we conclude that PPG can be a precision tool in the mathematical and geometrical evaluation of HRV. Still, a meticulous management of the artefacts is highly necessary, as well as the prevention of them as much as possible, which can, indeed, create a significate discomfort for both the investigator and the patient (who has to keep his hand, respectively head still).

The study of nonlinear heart rate variability parameters on a group of patients with recent acute myocardial infarct

The used **recording device** was built up by the author of this thesis, from an operational amplifier functioning as a nucleus, having the amplifying and filtering characteristics calculated based on the necessity to get a free-bugged ECG signal, without any muscular influences.

The ECG signal **registrations** from which HRV parameters are determined, were obtained from DI derivation, using electrodes connected to the wrists. The neutral electrode was

connected to the right ankle. For every subject, the registration had a length of 7 minutes, supine, while asked not to talk or move.

Results

Patients with AMI had a much lower average heart rate than the subjects from the control group ($p = 0,002$). This can be due to the administration of beta-blocker drugs, mandatory for these patients, in order to bring down arterial tension and to prevent myocardial remodeling after AMI. Also, there has been a decrease of SDRR in patients with AMI, comparing with the control group ($p = 0,001$). This decrease is because of the pathological history of the patient, on one hand, and the beta-blockers drug therapy, on the other hand.

The assessment of entropic parameters (the novelty of this study) has revealed interesting information regarding the cardiac rhythm complexity of the subjects with AMI condition. Hence, the majority of the parameters (ApEn, SampEn, DF $\alpha 1$, CorDen) had lower values in patients with AMI, the differences being statistically significant just in the case of CorDen ($p = 0,001$). This means that the complexity of cardiac rate in these patients is lower, so the sympathovagal alternation is more unbalanced than it is in subjects from the control group. The only parameter which had a higher value on the AMI patient group was DF $\alpha 2$ ($p = 0,003$), the reason for this difference being unknown.

Among these parameters, CorDen was the only one that correlated with SDRR based on regression testing, with an R^2 of 0,824. This was concordant with previous results obtained in the same study on diabetic and healthy subjects, where it was as well concluded that CorDen parameter alone correlates, in some way, with SDRR (considered the “golden” standard for appreciating heart rate variability).

Considering the fact that the analyzation of entropic parameters is the novelty of this study, we wanted to evaluate the link between them and consecrated parameters for globally HRV analysis, with SDRR (standard deviation of successive RR intervals) being the most relevant. This connection was evaluated using regression testing. Thereby, it has been noticed that amongst entropic parameters the Correlation dimension (CorDen) was the one to present a good equivalence with SDRR, the relation between them being linear. However, this category of parameters demands supplementary research, on a larger group of subjects, under various pathological conditions.

Challenges on measuring heart rate variability in a family doctor's office

The office of a family doctor represents the place where both patients with the most varied pathologies and healthy people come either for screening purposes or for obtaining different medical documents (such as medical certificate for good health and non-chronic conditions). Also, I considered this type of doctor's office a good starting point in testing the reliability of implementing HRV as a routine checkup. The diversity of pathological states has allowed to appreciate the usability of testing HRV in real situations, in a doctor's office (in an alert working environment, under the pression of solving a high number of medical cases while respecting the appointments), not only under laboratory conditions.

Methods and materials

In order to obtain the signal needed to calculate HRV parameters, a heartbeat detection system based on photoplethysmography was used. A study which took place in 2012 (Mirescu & Harden, 2012), along with a series of other studies (Assad et al., 2012; Medeiros, 2010; Combatalade, 2010; Chevalier et al., 2011; Elgedi et al., 2011) state the possibility of using the photoplethysmogram (PPG) for HRV, under the following conditions:

- The excluding of patients with tremor condition, no matter the cause;
- The subject does not move;
- Ensure sensor-skin tightness.

There have been no statistically significant differences between the parameters obtained from the two signals (PPG and ECG), stating their equivalency for using them in determining the heart rate variability.

The obtained signal is processed under the same algorithm used for ECG signal, in order to calculate HRV parameters, while the detection of the peaks is automatically made.

The study has been made on 70 volunteer patients who presented themselves at the family doctor's office starting with October 2013 until February 2014 (39 females, 31 males: aged 19-70). 31 of the individuals did not have records for either chronic diseases or significant antecedents, therefore they have been included in the control group, upon which the normal values of some of the HRV parameters have been established.

Discussion and results

The recordings from the 65 remaining patients could have been used neither for the calculation of HRV nor their graphic representation.

Registrations made on healthy patients have allowed the calculation of normal values of the targeted HRV parameters.

Generally, these values are concordant with those found in the international scientific literature (Malik, 1996; Singh et al., 1998; Taelman, 2008; Tsai et al., 2014) and with data collected from a study on Romanian population (Țibre, 2007). Further more, these are the values considered normal in subsequent studies.

HRV and arterial hypertension

After excluding the patients with multiple comorbidities, there has been left a number of 16 registered patients with the single diagnosis of arterial hypertension (HTA). They were aged 40-71 and they had stage 2 HTA.

It is extremely difficult to make a clear distinction between HRV modifications due to arterial hypertension and those influenced by antihypertensive medication, considering the fact that all patients with HTA were under beta-blocker treatment, which significantly affects the sympathovagal balance, having a sympatholytic action. In the study, there was no identification of subjects with newly detected HTA, who were not receiving therapy.

According to the scientific literature, HTA affects the sympathovagal equilibrium and vice versa (Karim et al., 2006; Singh et al., 1998), contributing to maintain a vicious cycle meant to sustain the physiopathological mechanism and to facilitate the development of complications.

Acute effects of conventional cigarette smoking on sympathovagal balance, measured with heart rate variability

The current study's objective was to determine the acute effects of cigarette smoking on sympathovagal equilibrium.

Methods and materials

In this study, there have been included 25 healthy volunteers, habitual smokers for over 3 years, aged 20-22 (15 females). The device Neurosoft Poly-Spectrum-8 based only on the electrodes for the members (so, a total of 6 derivations: DI, DII, DIII, aVL, aVR and aVF), was used to record the ECG track.

The subjects were asked not to smoke at least 4 hours prior to the experiment, and also, to avoid psychostimulants, such as caffeine (found in coffee or energy drinks, highly consumed by young adults in the same age range as the subjects).

A ECG standard test (10 seconds) was made on every individual to eliminate the possibility of a cardiac condition, which could have interfered with the variability determinations.

Results

The ECG parameters were normal for all subjects with no detection of pathological modification (both rhythmologically, patients having a sinus rhythm, and morphologically).

Time parameters. The first thing noticed during smoking, also in a subjective manner by the patient himself, was an increase in the heart rate, the differences being statistically significant ($p < 0,001$). In the next period, the heart rate has started to continuously drop during the last 5 minutes-interval ($p < 0,001$).

Standard deviation of RR intervals (SDRR) is considered an independent descriptive item of HRV. During the 5 minutes smoking session, the SDRR has started to decrease signaling a global drop of HRV under the action of substances inhaled from the cigarette smoke. This reduction has continued in the next 5 minutes-interval, with an increase in the second interval until the end of the measurement, but without reaching the starting value. The differences are not statistically significant.

Another independent descriptor of HRV is pNN50, the percentage of successive heart beats with at least a period of 50 ms between them, from the total of recorded heart beats on the entire trail. As it was expected (due to the strong connection between pNN50 and SDRR), this parameter has significantly dropped during smoking ($p < 0,001$), continuously decreasing in the next 5 minutes, and then slowly increasing in the second interval.

Frequency parameters were not relevant for this study because of the high value of standard deviation for every group of subjects. However, regardless this aspect, it has been observed that during smoking, the ration LF/HF has increased, extending in the first 5 minutesinterval, while decreasing during the second interval.

According to a part of the scientific literature, this increase of the ratio could be relevant regarding the inflation of sympathetic tone, which is concordant with a raise in heart rate. However, there are several studies, based on mathematics, which refute the relationship between LF/HF increasing ration and increasing of the sympathetic tone. In conclusion, the author is limiting himself to consider the increase of this ratio being relevant only for the global decrease of variability.

Nonlinear parameters. The most visually outstanding modifications were the ones of the nonlinear parameters. The Poincaré diagram was made along with the calculation of SD1 and SD2 parameters, for the each of the 4 tracks of every subject. **Discussion**

The present study draws attention to the acute modifications induced by smoking and noninvasively determined by HRV parameters. We demonstrated the existence of a serious alteration of sympathovagal balance, taking place during smoking and persisting at least 5 minutes after.

Although, the chronic effects of smoking on human organism are known and fully comprehended, the study answers the need to understand the acute effects of this habit.

Acute effects of electronic cigarette smoking on sympathovagal balance, measured with heart rate variability

The purpose of research was to evaluate the acute effects of electronic cigarette smoking on sympathovagal balance.

Methods and materials

In this study, there have been included 15 healthy volunteers, habitual smokers for over 3 years, aged 20-22 (8 females). The device Neurosoft Poly-Spectrum-8 based only on the electrodes for the members (so, a total of 6 derivations: DI, DII, DIII, aVL, aVR and aVF), was used to record the ECG track.

The characteristics for e-cigarette Vapez® were:

- 1000 mAh battery;
- Clearomizor atomizer with resistance 2,1±0,2 Ω;

- Liqua 10 ml Berry Mix nicotine solution, with a nicotine concentration of 24 mg/ml.

Results

All subjects included in the study have presented a sinus rhythm, without abnormalities detection of the electrocardiogram.

Time parameters have expressed discordant variations, at least compared with the values obtained in the case of conventional cigarette smokers. The average heart rate has increased in the 5 minutes smoking period ($p < 0,05$), continuing to increase in the first 5 minutes-interval after the smoking session, with a slight tendency of returning to the initial value maintained until the end of the third interval, the modifications being concordant with the ones suffered by the RR intervals mean.

On the other hand, standard deviation of RR intervals (SDRR) concordant with standard deviation of heat rate (SDHR), considered variability independent descriptors, has increased in the period specific for smoking, stating a global HRV increase, and so, a balancing of the sympathovagal ($p < 0,05$). This modification is opposite to the one found in the case of conventional cigarette smoking.

After the specific smoking interval, both SDRR and SDHR have dropped, reaching the starting value and continuing dropping, until the end of the recorded track. Based on this parameter, we can practically state that the heart rate variability has counterintuitively register an increase while inhaling the nicotine solution, followed by the drop under the initial value.

These results are also being supported by the pNN50 variation, which has presented an approximately 20% increase out of the initial value, subsequently decreasing to the half of this value and being steady until the end of the measurement.

Nonlinear parameters have showed a boost of LF/HF ratio, reaching its peak in the first 5 minutes after the smoking interval (doubling compared to the original value, $p = 0,02$), dropping in the next two intervals, but without coming back to the starting value. As some part of the scientific literature (the one which considers the LF/HF ratio as being relevant for the evaluation of the sympathovagal balance) (Gondim, 2015; Karakaya, 2007; Middlekauff, 2014) states, electronic cigarette smoking has the tendency of increasing the activity of the sympathetic tone.

Whilst the changes in the Poincaré diagram cannot be observed, its statistical descriptors do present variations, some of them highly significant.

As for SD1, this parameter increases during smoking, while decreasing under the original value on the entire 3 intervals following smoking ($p < 0,05$ when comparing the values from *Before* – control group and *Smoking* – primary experimental group with the corresponding secondary experimental groups *After 1, After 2* and *After 3*). The same variation pattern can be noticed in the case of SD2 parameter.

From the previous data compilation, it can be concluded that, on the studied experimental groups, the heart rate variability has raised during electrical cigarette smoking, while considerably dropping for at least 15 minutes afterwards (until the ending moment of the track).

Heart rate variability in diabetes patients: graphic representations and statistical descriptors

The study has been made on a group of 25 diabetes subjects (aged 22-70, 12 females), hospitalized in Clinical Section of Diabetes and Metabolic Disorders of County Clinical Emergency Hospital Cluj, during 01/09/16 – 21/12/16. There have been selected those patients who did not present rhythm disorders or any other electrocardiographic abnormalities, which could have interfered with the analysis of the cardiac rate. 12 from the total patients have been suffering from type I diabetes for 8-16 years, while the remaining 13 had been having type II diabetes for 10-22 years. Six of the patients presented already known neuropathic complications.

In order to set the reference values, 50 healthy subjects (aged 22-57, 23 females) registered to a family medicine's private office, have been taken into consideration. They have presented themselves to the family doctor either for *screening* investigations or for the purpose of obtaining a medical certificate of good health.

Results and discussion

One of the most valuable visual interpretation method of HRV is the tachogram. This ensures a rapid comparative appreciation of variability for two intervals. From the visual comparing of a healthy subject's tachogram with a diabetes patient's tachogram, it can be easily noticed that the latter has a much lower heart rate variability than the healthy one.

The mean of RR intervals and the average heart rate were similar for both studied groups, without statistically significant differences ($p = 0,5$, respective $p = 0,45$). However, the standard deviation of RR intervals (SDRR) was reduced in diabetes patients comparing with the healthy

subjects ($p < 0,001$), signifying a diminished heart rate variability in the case of diabetics. NN50 and pNN50 have also presented considerable lower values in diabetes patients in contrast to the healthy individuals ($p = 0,04$, respectively $p = 0,01$). However, in the present study there has been a great inter-individual variability of these parameters, materialized into high values of standard deviation, making them less relevant for comparing purposes between two studied groups of subjects.

Nonlinear parameters. Another valuable visual descriptor of HRV is the Poincaré diagram (obviously used while there is another control diagram). This offers fast information, based on the area described by the point dispersion on graphic. This area is narrower in the case of diabetes patients in correlation with the one described for a healthy subject, showing a low variability. This fact along with the shape of the tachogram, constitute the valuable visual instruments in the appreciation of variability.

The novelty of this study was the interpretation of entropic HRV parameters, which correspond to the complexity of the signal, their values being at least surprising. So, it has been found that ApEn ($p > 0,05$), SampEn ($p > 0,05$) and DFA $\alpha 2$ ($p < 0,01$) had higher values in diabetics. This contradicts the reduced variability recorded by all other parameters, from all three categories. Practically, looking at these three parameters (from which one has statistically significant differences) it can be said that the sign has a higher complexity in patients with diabetes, considering the fact that the variability is much lower. The reference articles have confirmed the existence of some research papers which attested this discrepancy, suggesting that these parameters can reveal hidden data to classic HRV parameters (Weippert, 2014). According to these studies, the predominantly sympathetic action determines an increase in both entropy of cardiac rhythm complexity, despite the dropping heart rate variability.

The other two entropic parameters, CorDen and DFA $\alpha 1$ have registered decreases of average values in diabetics in contrast to healthy subjects ($p < 0,01$ for CorDen, $p = 0,83$ for DFA $\alpha 1$).

In order to establish a possible connection between global HRV parameters and entropic parameters, the linear regression analysis has been performed between SDRR (considered by most authors a relevant global descriptor of HRV) and those five statistical parameters which were taken into consideration, the results being presented such as the correlation coefficient R^2.

The significance of oral brush cytology in diagnosing oral mucosa changes in diabetes mellitus

The purposes of research have been the following:

- Evaluation of the morphological alterations of oral squamous exfoliated cells using three different staining assays: Papanicolaou, APT (polychrome tannin blue)- Drăgan and Hematoxylin-Eosin (H&E);
- Comparing the reliability of the three staining methods and establishing the specific elements which can be highlighted through each of these methods;
- Correlation of the observed changes with DM (type I or type II) and duration of disease;
- Establishing some modifications of the oral microbial flora in DM.

Methods and materials

Subjects. A number of 30 adult patients (aged 19-87) have been selected among the patients from the Center of Diabetes, Nutrition and Metabolic Disorders of County Clinical Emergency Hospital Cluj and investigated: 10 potentially prediabetic, without clinical signs of DM (glycemic index ≥120 mg/dL and family history of DM) and 20 patients with DM (10 with type I DM, 10 with type II DM), 5 cases with complications (especially microvasculation) and 5 cases without complications from each type of DM. These patients were under insulin treatment (the case of those with type I DM), oral anti-diabetic medication or a mix of these two therapeutic methods (the case of those with type II DM). The duration of disease had a range between 3 to 30 years.

The control group was made out of 30 healthy subjects, with glycemic indexes <120 mg/dL and without family history of DM.

Exclusion criteria:

- Smoking due to possible modification induced to cells from the oral mucosa; Alcoholism;
- Subjects with gum disease or periodontitis. **Results**

According to Bethesda 2001 criteria for cytological reporting (Solomon & Nayar, 2004), all smears have been considered satisfactory in terms of cell number. The predominant types of cells were the superficial and intermediate ones, with rare parabasal cells. All staining has

correctly displayed the cytoplasm and the nucleus of squamous cells. For interpretation purposes, the changes of oral squamous cells have been gathered based on color.

Papanicolaou staining has separated the cells by the germ layer: the majority had a superficial origin (orange, due to high level of keratin), a small number of them were intermediate cells (green) and some extremely rare basal cells (blue-green).

There has been no difference between the cells harvested from the control group and group of prediabetics. In both situations, the nuclei were small, with compacted chromatin and with a normal nucleocytoplasmic ratio. Mild DM cases were characterized by an increased number of keratohyalin granules with a perinuclear distribution, while the complicated forms had a lower number of granules.

A large number of superficial binucleated cells it has been observed, sometimes showed as clusters. Also, in the majority of DM cases, no matter the presence or absence of complications or the type of DM, some moderately atypia has been noticed, materialized through the increased nucleocytoplasmic ratio and dispersed chromatin, with hyperchromasia.

Also, all DM cases have presented lipid vacuole in the cytoplasm; in three complicated cases of type I DM it has been noticed the presence of halo perinuclear cells and rare events of karyorrhexis and karyolysis.

APT-Drăgan staining has showed similar modifications to the ones in Papanicolaou staining. For subjects of the control group as well as for the ones with prediabetic glycemic indexes, the cytological aspects were normal, with small and compact nuclei, and usual nucleocytoplasmic ratio.

Mild DM were characterized by cells with peripheric cytoplasmic granules, fatty degeneration of the cytoplasm, binucleated cells and nucleocytoplasmic ratio slightly increased, along with rare small dystrophic nuclei.

In the complicated type II DM forms, a high number of beta and alpha cells underwent cytolysis. Also, the intracytoplasmic lipid vacuoles were visible in these patients, while having variant dimensions (from small one to vacuole who occupies the entire cytoplasm). Cytoplasmic changes were accompanied by modifications of the cellular membrane aspects (looking paler) or focal nuclear hyperchromasia.

Regardless the fact that **hematoxylin-eosin staining** does not allow to differentiate cells based on their germ layer, this method has proven its utility in appreciating chromatin changes with a much higher resolution than the other two staining methods, making it highly important in recognizing subtle changes which can appear at this point.

As it was the case for the previously methods, there has been no difference between cell from control group and those from the diabetes patients.

At all diabetics, no matter the type of disease, evolution or it severity, the majority of the smear was occupied by cells with eosinophilic grit unevenly distributed inside the cytoplasm and granules of keratohyalin (well-defined, large and more intensely colored comparing with the grit).

In terms of chromatin, it has been noticed cells with different condensing levels, their aspect ranging from uniform-blue (sometimes less bright) to hyperchromasia or chromatin clusters under the nuclear membrane (especially in the complicated forms of type II DM). Also, numerous binucleated cells along with defined intranuclear inclusions have been observed. Likewise, patients with complicated forms of DM have presented deep nuclear pyknotic cells, some with complete loss of the nucleus, as well as cellular clusters in which there were cells with different dimensions and with a varied nucleocytoplasmic ratio (reactive changes in case of abundant leukocyte inflammation). There was no sign of defined nuclear atypia, squamous dysplasia or malignancy.

The HE staining was a real help in terms of highlighting the leukocyte inflammation. Therefore, patients with complicated DM forms have presented a strong inflammation either associated or not with the proliferation of bacterial flora or with modifications of oral saprophytes.

Conclusions

Oral brush cytology, a standalone method, is a method with reduced value for diagnosing DM, detecting reactive modification induced by the diseases, but without specific elements for a type of DM. However, used with other *screening* methods, it can prove its usability in appreciation of both the presence and severity of the disease.

FINAL CONCLUSIONS

1. It has been found a correlation between heart rate variability parameters (HRV) derived from the photoplethysmographic signal and from those derived from the electrocardiographic signal, each method having advantages and disadvantages, especially when it comes to the comfort of the subject and examiner; a major downfall of using photoplethysmography is its high susceptibility to artefacts.

2. HRV is globally more reduced in patients who suffered from a recent acute myocardial infarct.

3. HRV is globally lower than the hypertensive patients; this modification can be due to the effects of increased arterial tension on sympathovagal balance, on one hand, and the effects of antihypertensive medication, on the other hand.

4. HRV is globally diminished in diabetes patients, aspect which does not correlate with the complexity of cardiac rhythm.

5. Conventional cigarette smoking rapidly drops the HRV parameters, with the values slowly returning to those recorded before smoking.

6. Electronic cigarette smoking results in acute increase of HRV parameters, which lasts as long as the patient smokes, the effects rapidly disappearing.

7. Diabetes mellitus induces unspecific changes of oral squamous cells, discernable on cytological smears collected from this site: the reactive increase of the nucleocytoplasmic ratio, binucleated cells, keratinization modifications (keratohyalin granules, intracytoplasmic keratin with lamellar orientation), cytoplasm dystrophy (lipid vacuoles), chromatin orientation abnormalities, as well as the presence of abundant inflammation and a rich microbial flora.

REFERENCES

Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. *Defining the normal bacterial flora of the oral cavity*, J Clin Microbiol, 2005, 43(11), pp. 5721–5732

Aggarwal S, Tonpay PS, Triskha S, Bansal A. *Prevalence of Autonomic Neuropathy in Diabettes Mellitus*, Curr Neurobiol, 2011, 2(2):101-105

Ahmed MT, Garib BT. *Cytological Features of Oral Cytobrush Smears in Type II Diabetes Mellitus Patients*, Tikrit Journal for Dental Sciences, 2012, 1, 6-12

Ahrné S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE, Molin G. *The normal Lactobacillus flora of healthy human rectal and oral mucosa*, J Appl Microbiol, 1998, 85(1), pp. 88–94

Alberti S, Spadella CT, Francischone TR, Assis GF, Cestari TM, Taveira LA. *Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry*, J Oral Pathol Med, 2003, 32(9), pp. 538–543

Allen J. *Photopletismography and its application in clinical physiological measurement*, Physiol Meas, 2007, pp. 1-39

Al-Maskari AY, Al-Maskari MY, Al-Sudairy S. *Oral manifestations and complications of diabetes mellitus: a review*, Sultan Qaboos Univ Med J, 2011, 11(2), pp. 179–186. Apetrei E. *Cardiologie clinică*, Editura Medicală Callisto, București, 2015, pp. 133-138

Assad S, Ding F, Fu N, Xu YJ. *Correlating Heart Rate Variability with Mental Fatigue*, A Major Qualifying Project Report Submitted to the Faculty of the Worchester Polytechnic Institute, 2012, pp. 10-21

Assadi R. *Conduction System of the Heart*, medscape.com, accesat online pe emedicine.medscape.com la data în decembrie 2016

Aubert AE, Steps B, Beckers F. *Heart Rate Variability in Athletes*, Sports Medicine, 2003, 33(12), 889-919

Balcioğlu AS, Műderrisoğlu H. *Diabetes and cardiac autonomic neuropathy: Clinical manifestations, cardiovascular consequences, diagnosis and treatment*, World J Diabetes, 2015, 6(1), 80-91

Balsamo Gardim C, Alfonso P. de Oliveira B, Fernanda B. Bernardo A, Loch Gomes R, Lopes Pacagnelli F, Lorençoni RM, Vanderlei LC. *Heart Rate Variability in Children with Type 1 Diabetes Mellitus*, Rev Paul de Pediatr, 2014, 32(2), 279-285

Batabyal B, Chakraborty S, Biswas S. *Role of the oral micro flora in human population: A brief review*, J Pharm Pharm Sci, 2012, 3(12), 2138-2148

Billman GE. *The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance*, Front Physiol, 2013, 4, pp. 1-5

Bittencourt M, Busato IM, Albini M, Azavedo-Alanis L, Grégio AM, Soares de Lima AA, Naval Machado MA. *Cytological analysis of epithelial cells in adolescents with type 1 diabetes mellitus*, Arch Oral Dent Res, 2013, 9(2), 185-191

Bortolotto LA, Blacher J, Kondo T, Takazawa K, Safar ME. *Assessment of Vascular Aging and Atherosclerosis in Hypertensive Subjects: Second Derivative Photoplethysmogram Versus Pulse Wave Velocity*, Am J Hypertens, 2000, 13, 165-171

Brennan M, Palaniswami M, Kamen P. *Do Existing Measures of Poincaré Plot geomery Reflect Nonlinear Features of Heart Rate Variability?*, IEEE Trans Biomed Eng, 2011, 48(1), pp. 1342-1347

Brock C, Softeland E, Gunterberg V, Brondum Frokjær, Lelic D, Brock B, Dimcevschi G, Gregersen H, Simren M, Drewes AM. *Diabetic Autonomic Neuropathy Affects Symptom Generation and Brain-Gut Axis*, Diabetes Care, 2013, 36(11), 3698-3705

Brotman DJ, Bash LD, Qayyum R, Crews D, Whitsel EA. *Heart rate variability prediscts ESRD and CKD-related hospitalization*, Clin J Am Soc Nephrol, 2010, 21(9), pp. 15601570

Bušek P, Vaňcova J, Opavsky J, Salinger J, Nevšimalova S. *Spectral Analysis of Heart Rate Variability in Sleep*, Physiol Res, 54, 2005, 369-376

Carel RS. *Cost-Effectiveness Analysis of a Computerized ECG Interpretation System in an Ambulatory Health Care Organization*, J Med Syst, 6(2), pp. 121-130

Chevalier G, Sinatra S. *Emotional Stress, Heart Rate Variability*, Inter Med, 2011, 10(3):1622

ChuDuc H, NguyenPhan K, NguyenViet D. *A Review of Heart Rate Variability and its Applications*, APCBEE Procedia 7, 2013, 80-85

Clifford GD. *Signal Processing Methods for Heart Rate Variability*, Thesis submitted to the University of Oxford, 2002

Comănescu-Neamţu R. *Citologia bucală-jugală în diabet: aspecte morfocitochimice*. IMStomatologie, Timişoara, 1979

Combatalade CD. *Basics of Heart Rate Variability Applied to Psychophysiology*, Thought Technology Ltd., 2010, pp. 5-9

Couceiro R, Carvalho P, Paiva RP, Henriques J, Quintal I et. al. *Assessment of cardiovascular function from multi-Gaussian fitting of a finger photopletismogram*, Physiol Meas, 2015, 36(9), pp. 1801-1825

Cuculici GP, Gheorghiu AW. *Guyton & Hall – Tratat de fiziologie a omului, ediția a 11-a*, Editura Medical Callisto, 2007, pp. 116-119, 123-126, 748-759, 972-976

D'Souza MS, Markou A. *Neuronal Mechanisms Underlying Development of Nicotine Dependence: Implications for Novel Smoking-Cessation Treatments*, Addict Sci Clin Pract, 2011, 6(1), pp. 4-16

Drăgan-Lungulescu M. *Citodiagnostic cu metoda de colorare rapidă APT-Drăgan*, Editura de Vest, Timișoara, 2004, pp. 15-19

Elgendi M, Fletcher R, Norton I, Brearley M, Abbott D, Lovell NH, Schuurmans D. *On Time Domain Analysis of Photoplethysmogram Signals for Monitoring Heat Stress*, Sensors, 2015, 15, pp. 24716-24734

Elgendi M, Jonkman M, DeBoer F. *Heart Rate Variability and the Accelerated Plethysmogram signals measured at rest*, Communications in Computer and Information Science, 2011, 127, pp. 266-277

Elgendi M. *Standard Terminologies for Photoplethysmogram Signals*, Curr Cardiol Rev, 2012, 8, pp. 215-219

Emmerling H. *Systemic diseases with oral signs*, DHV6138/Fall, 2009

Fowler MJ. *Microvascular and macrovascular complications of diabetes*, Clin Diabetes, 2008, 26(2), pp. 77-82

Garg V. *Noninsulin pharmacological management of type 1 diabetes mellitus*, Indian J Endocr Metab, 2011, 15(Suppl 1), pp. 5-11

Ginghină C. *Mic tratat de cardiologie*, Editura Academiei Române, București, 2010, pp. 2628

Gondim RM, Quintanella Farah B, da Franca Bandeira Ferreira Santos C, Mendes Ritti-Dias R. *Are smoking and passive smoking related to heart rate variability in male adolescents ?*, Einstein (Sao Paolo), 2015, 13(1): 27-33

Gurenlian JR. *The Role of Dental Plaque Biofilm in Oral Health*, Int J Dent Hyg, 2007, 81(5), pp. 1-11

Guțiu IA. *Tulburări de conducere ale inimii – fiziopatologie, clinică, tratament*, Editura Tehnică, București, 1994, pp. 101-102, 19-27

Haslbeck M, Luft D, Neundörfer B, Stracke H, Ziegler D. *Diagnosis, Treatment and Followup of Diabetic Neuropathy, 1st Edition*, 2004

Henry BL, Minassian A, Paulus MP, Geyer MA, Perry W. *Heart rate variability in bipolar mania and schizophrenia*, Journal of Psychiatric Research, 2009 (article in press)

Hongo RH, Goldschlager N. *Status of Computerized Electrocardiography*, Cardiol Clin, 24, 2006: 491-504

Jaiswal M, Urbina EM, Wadwa RP, Talton JW, D'Agostino RB, Hamman RF, Fingerlin TE, Daniels S, Marcovina SM, Dolan LM, Dabelea D. *Reduced Heart Rate Variability Among Youth With Type 1 Diabetes*, Cardiovasc Metab Risk, 2013, 36, 1, pp. 157-162

Jajarm HH, Mohtasham N, Moshaverinia M, Rangiani A. *Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method*, Journal of Oral Sciences, 2008, 5(3), pp. 335-340

Jenkins GW, Kemnitz CP, Tortora GJ. *Anatomy and Physiology – From Science to Life*, John Wiley & Sons, Inc., 2007, pp. 709-712

Karakaya O, Barutcu I, Kaya D, Metin Esen A, Saglam M, Melek M, Onrat E, Turkmen M, Esen OB, Kaymaz C. *Acute Effect of Cigarette Smoking on Heart Rate Variability*, Angiology, 2007, 58(5), pp. 620-624

Karim N, Hasan JA, Ali SS. *Heart Rate Variability – A Review*, J Basic Appl Sci, 7(1), 2011, 71-77

Kazanowska K, Hałoń Radwan-Oczko M. *The role and application of exfoliative cytology in the diagnosis of oral mucosa pathology – contemporary knowledge with review of the literature*, Adv Clin Exp Med, 2014, 23(2), pp. 299–305 Kory Calomfirescu Ș, Moș AM. *Complicațiile neurologice în diabet zaharat. Sindroame neurologie paraneoplazice*, Librăriile Crica, 1996, 12-16

Kumar V, Abbas A, Aster J. *Robbins Patologie: Bazele morfologice și fiziopatologice ale bolilor, Ed. A 9-a*, Editura Medicală Callisto, 2014, pp. 730-748

Lakhtakia R. *The history of diabetes mellitus*, Sultan Qaboos Univ Med J, 2013, 13(3), pp. 268370

Llewelin JG. *The Diabetic Neuropathies: Types, Diagnosis and Management*, J Neurol Neurosurg Psychiatry*, 2003*, 74(Suppl II), pp. 15-19

Loscalzo J. *Harrison – Medicină cardiovasculară*, Editura All, 2013, pp. 86-91

Loss R, Sandrin R, França BH, de Azevedo-Alanis LR, Gregio AM, Machado MA, Lima AA. *Cytological analysis of the epithelial cells in patients with oral candidiasis*, Mycoses, 2011, 54(4), pp. 130-135 Lungulescu Drăgan M. *Citodiagnostic cu metoda de colorare rapidă APT-Drăgan*, Editura de Vest, 2004, 15-20, 97-116

Majumdar S, Bhagat Singh și colab. A. *Normal Microbial Flora of Oral Cavity*, J Adv Med Dent Sci Res, 2014, 2(4), pp. 62-66

Majumdar S, Singh și colab. AB. *Normal microbial flora of the oral cavity*, J Adv Med Dent Sci Res, 2014, 2(4), pp. 62–66

Makivić B, Djordević Nikić M, Willis MS. *Heart Rate Variability (HRV) as a Tool for Diagnostic and Monitoring Performance in Sport and Physical Activities*, J Exerc Physiol Online, 2013, 3, 103-131

Malik M. *Heart rate variability – Standards of measurement, physiological interpretation, and clinical use*, Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, Eur Heart J, 17, 1996, 354-381

Marsh P. *Role of the Oral Microflora in Health*, Microb Ecol Health Dis, 2000, 12(5), pp. 130137

McCraty R. *Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health Risk*, Glob Adv Health Med, 2015, 4(1), 46-61

Medeiros, JM. *Development of a Heart Rate Variability Analysis Tool*, A Thesis submitted for obtaining the degree of Masters in Biomedical Engineering, Department of Physics, Faculty of Sciences and Technology, University of Coimbra, 2010, pp. 24-25

Meurman JH, Siikala E, Richardson M, Rautemaa R. *Non-Candida albicans Candida yeasts of the oral cavity*, Communicating Current Research and Educational Topics and Trends in Applied Microbiology (A. Méndez-Vilas – Ed.), 2007, pp. 719-731

Middlekauff HR, Park J, Moheimani RS. *Adverse Effects of Cigarette and Noncigarette Smoke Exposure on the Autonomic Nervous System. Mechanisms and Implications for Cardiovascular Risk*, J Am Coll Cardiol, 2014, 64(16), 1740-1750

Mirescu ȘC, Harden SW. *Photopletismography as a Potential Alternative to Electrocardiography for Recording Heart Rate Intervals Used in Variability Analysis*, J Med L, Volume 5 (Special Issue), 2012, pp. 123-128

Mirescu ȘC, Păiș R, Stănoiu BP, diNatale L, Șovrea AS. *The value of exfoliative cytology in the diagnostic of oral mucosa changes in diabetes mellitus*, Rom J Morphol Embryol, 2016, 57(4), 1313-1322

Mirescu ȘC, Petrescu M, Petrescu F, Mirescu NC, David L. *Challenges in implementing heart rate variability testing in a family medicine practice: strengths, pitfalls and caveats*, Studia Biologia UBB, LIX(2), 2014, pp.105-113

Mirescu ȘC. *Computer sound card used as analog-to-digital converter in a teaching physiology laboratory*, Studia Biologia UBB, LX(2), 2015, pp. 85-88

Neamţu E. *Aplicarea şi evaluarea coloraţiei rapide APT-Drăgan în studiul citologiei bucale*. IM-Stomatologie, Timişoara, 1974

Oates PJ. *The polyol pathway and diabetic peripheral neuropathy.* In: Tomlinson DR, editor. *Neurobiology of Diabetic NeuropathyNeurobiology of Diabetic Neuropathy*, vol. 50, London; UK, Academic Press, 2002, pp. 325-392

Ognean I, Dojană N, Roșioru C. *Fiziologia animalelor, Vol I*, Presa Universitară Clujană, ClujNapoca, 2000, pp. 217-218

Olinici, CD, Renata V, Doinița C, Gheban D. *Biologia celulară și anatomia patologică*, Editura Floarea Albastră, Zalău, 1999, pp. 195-212

Olokoba AB, Obateru OA, Olokoba LB. *Type 2 diabetes mellitus: a review of current trends*, Oman Med J, 2012, 27(4), pp. 269–273

Orlov S, Bril V, Orszag A, Perkins BA. *Heart Rate Variability and Sensorimotor Polyneuropathy in Type 1 Diabetes*, Diabetes Care, 2012, 35(4), 809-816

Pala A, Chowbey R, Sonvanschi N, Patel D, Shah A, Venkatesch C. *A review of oral manifestations of systemic diseases*, Int J Oral Health med Res, 2016, 2(6), pp. 131-132

Pandit S, Gonsalves M, Karkera B, Jasphin S. *Diabetes: Risk Factor for Oral Cancer ? – A Review*, Int J Adv Health Sci, 2015, 1(10), 25-28

Parahitiyawa NB, Scully C, Leung WK, Yam WC, Jin LJ, Samaranayake LP. *Exploring the oral bacterial flora: current status and future directions*, Oral Dis, 2010, 16(2), pp. 136–145

Paritala SA. *Effects of Physical and Mental Tasks on Heart Rate Variability*, A Thesis Submitted to Louisiana State University, 2009

Park SH, Lee L, Shearston JA, Weitzman M. *Patterns of electronic cigarette use and level of psychological distress*, PloS One, 2017, 12(3), PMID 28278239

Percival RS, Challacombe SJ, Marsh PD. *Age-related microbiological changes in the salivary and plaque microflora of healthy adults*, J Med Microbiol, 1991, 35(1), 5-11

Piero MN, Nzaro GM, Njagi JM. *Diabetes mellitus – a devastating metabolic disorder*, Asian J Biomed Pharm Sci, 2014, 40(4), pp. 1-7

Pilt K, Meigas K, Temitski K, Viigimaa M. *Second derivative analysis of forehead photopletismographic signal in healthy volunteers and diabetes patients*, IFMBE Proceedings, 2013, 410-413

Pinducciu G, Micheletti L, Piras V, Songini C, Serra C, Pompei R, Pintus L. *Periodontal disease, oral microbial flora and salivary antibacterial factors in diabetes mellitus type 1 patients*, Eur J Epidemiol, 1996, 12(6), pp. 631–636

Pradhapan P, Tarvainen MP, Nieminen T, Lehtinen R, Nikus K, Lehtimaki T, Kahonen M, Viik J. *Effect of heart rate correction on pre- andpost-exercise heart rate variability to predict risk of mortality – an experimental study on the FINCAVAS cohort*, Front Physiol, 2014, 5, pp. 1-10

Preston AJ, Gosney MA, Martin MV. *Oral Flora of Elderly Patients following Acute Medical Admission*, Gerontology, 1999

Rivera C, Nuñez-de-Mendoza C. *Exfoliative cytology of oral epithelial cells from patients with type 2 diabetes: cytomorphometric analysis*, Int J Clin Exp Med, 2013, 6(8), pp. 667676

Schipke JD, Arnold G, Pelzer M. *Effect of respiration on shiort-term heart rate variability*, J Clin Basic Cardiol, 1999, 2(1), 92-95

Seifi S, Feizi F, Moazzezi Z, Mehdizadeh M, Zamani B. *Evaluation of oral mucosal epithelium in diabetic male patients by exfoliative cytology method*, J Diabetes Metab Disord, 2014, 13(77), 1-7

Shareef BT, Ang KT, Naik VR. *Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients*, Med Oral Patol Oral Cir Bucal, 2008, 13(11), pp. 693-696

Sharma M, Tiwari SC, Singh K, Kishor K. *Occurence of Bacterial Flora Infections of Diabetic and Non-Diabetic Patients*, Life Sci Med Res, 2011, pp. 1-6

Shilitoe E, Weinstock R, Kim T, Simon H, Planer J, Noonan S, Cooney R. *The oral microflora in obesity and type-2 diabetes*, J Oral Microbiol, 2012, 4

Singh și colab. JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. *Reduced Heart Rate Variability and New-Onset Hypertension. Insights Into Pathogenesis of Hypertension: The Framingham Heart Study*, Hypertension, 1998, 32, 2, pp. 293-297

Solomon D, Nayar R (eds). *The Bethesda System for reporting cervical cytology: definitions, criteria, and explanatory notes*, 2nd edition, Springer, 2004

Soule EK, Maloney SF, Guy MC, Eissenberg T, Fagan P. *User Identified Positive Outcome Expectancies of Electronic Cigarette Use: A Concept Mapping Study*, Psychol Addict Behav, 2017, published online, PMID 28277706

Stys A, Stys T. *Current Clinical Applications of Heart Rate Variability*, Clin Cardiol, 1998, 21, pp. 719-724

Taelman J, Vandeput S, Spaepen A, Van Huffel S. *Influence of Mental Stress on Heart Rate and Heart Rate Variability*, ECIFMBE 2008, IFMBE Proceedings 22, 2008, pp. 13661369

Takahashi N. *Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases*, Elsevier Congress Series 1284, 2005, pp. 103112

Takazawa K. *Second derivative photoplethysmogram*, Vasa, 2015, 44(1), 3-4

Tozoğlu Ű, Bilge OM. *Exfoliative cytology of type 1 diabetic patients*, Eur J Gen Med, 2010, 7(3), pp. 264-268

Tsai JF, Cho W, Jou SH, Lin CM. *Heart rate variability and meditation with breath suspension*, Biomed Res, 2014, 25(1), pp. 6-10

Țibre V. *Corelații clinico-electrofiziologice în complicațiile neurologice periferice după tratamentele oncologice*, Teză de Doctorat, Universitatea de Medicină și Farmacie *Iuliu Hațieganu* Cluj-Napoca, 2007

Vineet RV. *Flora of the oral cavity*, Smashword Edition License Notes, 2015

Vinik AI, Maser RE, Mitchell BD, Freeman R. *Diabetic Autonomic Neuropathy*, Diabetes Care, 2003, 26(5), pp. 1553-1579

Von Borell E, Langbein J, Després G, Hansen S, Letterier C et. al. *Heart rate variability as a measure of autonomic reguation of cardiac activity for assessing stres and welfare in farm animals – A review*, Physiol Behav, 92, 2007, pp. 293-316

Vučović-Rebrina S, Barada A, Smirčić-Duvnjak L. *Diabetic Autonomic Neuropathy*, Handbook of Clinical Neurology, 2013, 117, 279-294

Wardlaw GM, Hampl JS. *Perspectives in Nutrition, 7th Ed.*, 2007, 175-179

Weippert M, Behrens M, Rieger A, Behrens K. *Sample Entropy and Traditional Measures of Heart Rate Dynamics Reveals Different Modes of Cardiovascular Control During Low Intensity Exercise*, Entropy, 2014, 16, 5698-5711

Williams DW, Walker R, Lewis MA, Allison RT, Potts AJ. *Adherence of Candida albicans on oral epithelial cells differentiated by Papanicolaou staining*, J Clin Pathol, 1999, 52(7), pp. 529–531

Zdrenghea D. *Disritmiile cardiace, Vol. I*, Editura Dacia, Cluj-Napoca, 1990, pp. 14-17

Zimmerman RL. *Molecular diagnostics in the cytology laboratory: slowly making its way there,* Diagn Histopathol, 2008, 14(12), pp. 609–613

***American Diabetes Association. *Diagnosis and classification of diabetes mellitus*, Diabetes Care, 2013, 36(Suppl 1), pp. 67-74

***World Health Organisation. *Prevalence of Tobacco Smoking*, 2015, accesat online în februarie 2017

*** European Society of Cardiology. *Atrial Fibrillation 2016 (Management of). ESC Clinical Practice guidelines*, Eur Heart J, 2016, 37(38), pp. 2893-2962

***European Society of Cardiology. *Guidelines for the diagnosis and management of syncope (version 2009)*, Eur Hearth J, 2009, 30, pp. 2631-2671

***European Society of Cardiology. *ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC)*, Eur Heart J, 2012, 33(20, pp. 2569-2619

***GroupHealth. *Type I Diabetes – Treatment Guideline*, 2015, pp. 3-7

****Heart Rate Variability Analysis System – Clinical Information*, Medicore, 2010, p. 9

***World Health Organisation. *Global Report on Diabetes*, 2016, pp. 20-21

***World Health Organisation. *Use of Glycated Haemoglobin (AbA1c) in the Diagnosis of Diabetes Mellitus*, 2011, pp. 4-7