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Objective Characterization of Autonomic Profile Using Time Series Data of Cardiac Signal in Diabetes Mellitus

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Abstract: *Variation in heart rate reflects the resultant effect of sympathetic and parasympathetic systems in a time-related dynamic fashion and an expression of complex temporal dynamics of cardiac chronotropy. By analysing Heart Rate Variability (HRV) the overall cardiac health and the state of the autonomic nervous system can be assessed. But the parametric expression of HRV variables are made in a single numerical value that inadequately expresses the attributes of heart rate dynamics. The beat-to-beat temporal dynamics remains unexpressed. The individual and 'pure' effect of sympathetic and parasympathetic systems on cardiac autonomic status remains submerged within the overall influence of ANS and cannot be segregated and separately evaluated, without application of blocker, in an invasive experimental setting. There are also contradictory and paradoxical findings of HRV studies in stress and in patients of autonomic neuropathy.*

In the present endeavour, the temporal dynamics of the beat-to-beat variation has been analysed in a simple and innovative descriptive protocol along with standard HRV protocol in normal control volunteers and patients of Diabetes Mellitus. A long-term descriptive study in resting basal state has been conducted to objectively characterize the detailed temporal dynamics of cardiac chronotropy. At the same time, parasympathetic and sympathetic modulatory influence has been segregated to extract the features of each arm of ANS.

Background: *Autonomic nervous system with its sympathetic and parasympathetic componentsis responsible for homeostasis and cardiac chronotropy reflects the combined effect of both these components in a temporal dynamic fashion. But stating the heart rate as a single value per unit time e.g. 70 beats/minute ignores the dynamicity of the temporal property of heart. Rather heart rate variability can give a better picture of the overall autonomic activity of an individual. The different HRV parameters viz. time-domain, frequency domain and non-linear dynamics provide significant and crucial insight into the cardiac chronotropy.*

However, majority of parametric expression of the different HRV variables is done in a single numerical value which is a mere average expression of the different attributes pertaining to the heart rate dynamics. The beat-to-beat temporal variation remains unexpressed.

In the present project we intend to evaluate the temporal dynamics of cardiac chronotropy by a novel descriptive tool. Along with that the conventional HRV evaluation will also be undertaken.

Diabetes Mellitus is one of the most common diseases known to produce autonomic neuropathy. Studies conducted in the last two decades on the outcome of diabetes on HRV have shown convincing results of altered HRV parameters in patients of long-standing Diabetes Mellitus. But certain issues remain yet unsettled. Various articles expressed contradictory findings with respect to various HRV variables. In one study no change in HRV parameters was observed between control group and diabetic subjects in resting state, although autonomic activity was found to be reduced significantly among the diabetics upon application of physiological stress [1]. Similarly, another study conducted among Indian population showed that both sympathetic and parasympathetic components of the ANS are affected in Diabetes with parasympathetic dysfunction predominating over sympathetic dysfunction [2]. But the SEARCH CVD Study pointed towards a parasympathetic loss with sympathetic override [3].

For a sensitive physiological system as the autonomic nervous system, application of even a minimal amount of stress (viz. anxiety among the patients) during the course of experiment can lead to an effectively significant change in the autonomic activity. However, in none of the studies conducted so far has the issue of reducing this amount of stress been clearly stated. Also there is no clear cut statement whether the 'resting state' in these studies were quantified as per the definition of physiological resting state or basal state [4] and that is probably the reason behind the discrepancies in the findings.

In our study we intend to comfort the patient with prior explanation of the procedure and using common techniques like playing music or television to ameliorate this stress and anxiety factor and also strictly adhere to the definition of basal state when it comes to evaluation of the HRV parameters in resting state.

Materials and Methods: *In this institution based descriptive and analytical study**,** a small population of normal healthy volunteers (n = 30), age range between 18 – 33 years, and a group of diabetic patients (n = 23), age range between 18-33 years who met the WHO criteria for Diabetes Mellitus were selected to evaluate the temporal dynamics of the RR interval time series during long term rest. Descriptive work-up was done by continuous digital electrocardiography during rest. Data mining and statistical analysis was carried out using standard statistical and mathematical software tools. Descriptive, correlation and comparative analysis of the results obtained were done between cases and controls along with motion path analysis of the sequence of appearance of scatter points in Poincaré plot for HRV.*

Results: *The study shows alteration in long-term and short-term HRV parameters among patients of Diabetes Mellitus both during basal state. There is obvious dampening of both sympathetic and parasympathetic components of autonomic nervous system in the diabetic subjects that leads to the characteristic pattern alteration in HRV parameters. Our study supports the findings in previous researches conducted on HRV in diabetic patients but provides new insights into understanding the probable mechanism behind neurological manifestations in this disease. Statistically significant differences ($p < 0.05$) were observed between normal controls and diabetic cases with regard to time-domain, frequency-domain and non-linear HRV parameters that suggests presence of autonomic dysfunction from prolonged hyperglycemia.*

Conclusion: *Autonomic profile of the normal subjects and patients of Diabetes Mellitus can be objectively characterized by evaluation of RR interval time series data.*

Key Word: *HRV; diabetes; stress; glutamate*

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I. Introduction

Autonomic nervous system with its sympathetic and parasympathetic components is responsible for homeostasis and cardiac chronotropy reflects the combined effect of both these components in a temporal dynamic fashion. Heart Rate Variability (HRV) is now considered as the gold standard for assessment of cardiac autonomic status [2]. Since 1990s a large number of research publications appeared in different leading journals quantitating the different attributes of autonomic profile in health and various diseases states.

In the parameters of HRV (Time domain, Frequency domain, non-linear dynamic etc.) the various aspects of the Heart Rate Dynamics are expressed. The important application of heart rate variability analysis is in the diagnosis and prognosis of patients with autonomic neuropathy, especially diabetes mellitus. Heart rate variability gives information about the sympathetic-parasympathetic autonomic balance and thus about the risk for sudden cardiac death in these patients.

Studies have revealed that parasympathetic tone dominates the resting state, while stress leads to prompt withdrawal of vagal tone and subsequent sympathetic activation. Conversely, recovery is characterized by parasympathetic activation followed by sympathetic withdrawal, although clarification of the normal trajectory and autonomic basis of heart rate decay following stress is needed. Abnormalities in autonomic physiology especially increased sympathetic activity, attenuated vagal tone, and delayed heart rate recovery have been associated with increased mortality [5].

Besides basal conditions the changes in sympatho-vagal balance occur due to reduced responsiveness to an excitatory stimulus that characterizes numerous patho-physiological states. Chronic imbalance of the autonomic nervous system is a prevalent and potent risk factor for adverse cardiovascular events, including mortality, an issue which is not widely recognized by clinicians [6].

Zhong *et al* in 2007 evaluated the nonlinear interactions between the sympathetic and parasympathetic nervous systems in the form of frequency and amplitude modulations in human heart rate data [7]. However, in spite of all these, the beat-to-beat temporal dynamics remain unexpressed. Also, many of these multi domain parameters are practically of similar implication, measure the same physiological phenomenon with a different kind of parametric expression.

Diabetes mellitus is one of the most common diseases known to produce autonomic neuropathy. Studies conducted in the last two decades on the outcome of diabetes on HRV have shown convincing results of altered HRV parameters in patients of long-standing diabetes mellitus. Besides macrovascular and microvascular complications, the major cause of death among diabetic patients is cardiovascular mortality. Cardiovascular mortality has been related to the cardiac autonomic neuropathy that is too often associated with Diabetes. Screening for cardiac autonomic neuropathy is, therefore, recommended at the diagnosis of Diabetes

Mellitus, especially for patients with a history of poor glycaemic control, macrovascular/microvascular complications, and increased cardiovascular risk [8].

Effects of hypoglycaemia on cardiac autonomic regulation may contribute to the occurrence of adverse cardiac events. Koivikko *et al* have concluded that hypoglycemia results in the reduction of cardiac vagal outflow in both diabetic and nondiabetic subjects [9]. Altered autonomic regulation may contribute to the occurrence of cardiac events during hypoglycemia. The spectral components of short-term HRV calculated by using the FFT and AR methods were not interchangeable and FFT analysis was preferred in diabetic patients [10]. In frequency domain, the analysis of sympathetic (LF) and parasympathetic (HF) component evidenced an association between the offspring of type 2 diabetic subjects and a sympathetic over activity [11]. A global reduction and alteration of circadian rhythm of autonomic activity are present in offspring of type 2 diabetic patients with and without insulin resistance. Diabetic patients had lower values for time-domain and frequency-domain parameters than controls [12]. Most heart rate variability parameters were lower in diabetic patients with chronic complications than in those without chronic complications. Type 2 diabetic patients with microalbuminuria have diminished heart rate variability in response to deep breathing, change of position and the Valsalva maneuver, but they preserve BP response to postural change [13]. Therefore, microalbuminuria seems to be associated with early diabetic autonomic neuropathy(DAN), but not with advanced DAN. It was concluded that cardiac (parasympathetic) autonomic activity was diminished in diabetic patients before clinical symptoms of neuropathy become evident [14-16].

In the present study, we aim to evaluate and compare the autonomic profile among diabetic patients and normal subjects using RR interval time series data.

II. Materials and Methods

This descriptive analytical study was carried out in the Department of Physiology, Medical College & Hospital, Kolkata in collaboration with Department of Medicine and Department of Endocrinology, Medical College & Hospital, Kolkata from December 2017 to December 2019. A total of 13 healthy volunteers and 23 diabetic patients, both males and females, aged ≥ 18 years were selected for the study.

a. Inclusion/Exclusion criteria of controls:

i. Inclusion criteria: 18-33 years old volunteers who,

1. Have a normal physical examination
2. Have no associated co-morbidities
3. Are not on any medications
4. Have a normal ECG

ii. Exclusion criteria: 18-33 years old volunteers who,

1. Have cardiovascular co-morbidities
2. Have abnormal ECG
3. Are on medications that might hamper the normal autonomic functioning of the body (e.g. antihypertensives, antidiabetics, etc.)
4. Volunteers less than 18 years or more than 33 years old

Inclusion criteria for Case:

Patient of DM according to current WHO diagnostic criteria for Diabetes:

a. Fasting blood glucose≥126mg/dl or 2hrs post-prandial blood glucose≥200mg/dl who are attending Diabetes OPD of Medical College & Hospital, Kolkata

b. Patient of Impaired Glucose Tolerance Test according to current WHO diagnostic criteria for diabetes i.e. Fasting blood glucose<126mg/dl & 2hrs post-prandial blood glucose between 140 and 200mg/dl

c. Patient of Impaired Fasting Glucose according to current WHO diagnostic criteria for diabetes i.e. Fasting blood glucose between 110-125mg/dl and 2hrs post prandial plasma glucose<200mg/dl

Exclusion criteria for Cases:

a. Patient developing life-threatening complication like DKA, coma etc.
b. Patient suffered from neuropathy of etiologies other than DM

b. Sample Size Estimation:

i. Estimation of sample size in controls

(https://www.rnoh.nhs.uk/sites/default/files/sample_size_formula_for_a descriptive_study.pdf)

To estimate mean with an adequate level of precision i.e. confidence interval of appropriate narrowness, for an acceptable margin of error **m** which is equal to half the maximum width of the Confidence Interval allowable, we find an estimate of the standard deviation (**SD**) of the mean outcome.

$$n \geq \frac{4X\ (SD)\text{^}2}{m\text{^}2}$$

In the present study, we require a 95% confidence interval for the mean of a continuous variable with a standard deviation of 15 to be no wider than 10 (i.e. $\mathbf{m \leq 5}$).

$$n \geq \frac{4X15\text{^}2}{5\text{^}2}$$
$$n \geq 36$$

Using the formula: In order to estimate the mean of continuous variable (**SD=15**) with 95% confidence interval no wider than 10, 36 participants would be required
However, due to limited duration of the present study we decided to keep the sample size within 30 as per thumb rule principle [17].

ii. Estimation of sample size in cases

As per recent studies, the prevalence of DM in Kolkata is 11.7% [21]. The sample size for the present study is calculated by the help of the formula Z^2pq/l^2, where Z is the 95% confidence limit=1.96, p=prevalence of DM (here it is 11.7), q is the complement of p, l=precision (here 5% absolute precision was assumed).
Thus, the sample size was 147 (as per the Epi info software for sample size calculation) in a finite population i.e. OPD patients of diabetic clinic of MedicalCollege & Hospital, Kolkata.

c. Sampling Design:

i. Sampling design in controls

Thirty subjects were recruited by local advertisements and notification in the institutional campus, and who agreed to participate after oral and written explanation of the study.

The study was performed on a population of healthy volunteers and diabetic patients recruited as per advertisement mentioned above. In order to achieve a reasonable degree of randomization in this institution based study, we followed the following procedure,

a) The study was conducted on the subjects within the age group of 18 – 33 years for controls. This is because the age related development of autonomic inefficiency become significant above that age [18, 19].
b) At first 250 individuals of equal proportion of male and female (125 each) were shortlisted in response to the advertisement.
c) Every individual in each sex group were blindly assigned with a number from 1 to 125 in each group. From a random number generator algorithm 15 study subjects finally recruited from each group constituted requisite randomized study sample size of 30.
d) However, due to repeated malfunctioning of the recording device, loss of data due to sudden and unwarranted hardware crash and lack of compliance of study participants we could finally analyze the ECG data of 13 healthysubjects.

ii. Sampling design in cases

The OPD of diabetic clinic of MedicalCollege & Hospital, Kolkata runs twice a week. As per record of the diabetic clinic, there is on an average 150 attendance per clinic day. Annual attendance of 14,400 (approximately) is assumed. Out of these imaginary patient queue 147~150 was selected for the present study in a two-year period for data collection i.e. ~13 patients per month which means ~3 patients per clinic day. The OPD attendant takes the OPD ticket of patients deposited in a box for a certain time (e.g. for half an hour) and then he collects ticket bunch for making entry into the OPD registers before sending them to the doctors. Thus, there are 5-6 bunches during the OPD hours of the clinic. Starting unbiasedly, three study subjects were selected randomly on each clinic day from one of those bunches and subsequently data were collected from them with their informed consent. On next day, the very next bunch was considered for selecting three study subjects and on next day from next bunch……. & again from the 1st bunch and so on. Thus, a ***schedule samplingprotocol***was followed to avoid selection bias [20, 21].

However, due to repeated malfunctioning of the recording device, loss of data due to sudden and unwarranted hardware crash and lack of compliance of study participants we could finally analyze the ECG data of only 23 patients for long term evaluation. Also female patients later didn't turn up for participating in this study. So all HRV records were obtained only from male patients.

d.Study variables:

1. Clinical variables

2. Experimental variables:

a) Time Domain Parameters
b) Frequency Domain Parameters
c) Non-linear Dynamic Parameters

e. Methods of data collection:

1. History taking
2. Clinical evaluation
3. Electrocardiological evaluation during rest and physiological stress

f. Experimental protocol:

After informed and written consent the descriptive study was done in a quiet room (temperature 22°C), between 8.00 AM and 2.00 PM, after an overnight fasting. The subjects were instructed to abstain from beverages containing caffeine and from alcohol from 20.00 hours the preceding day. Smoking was allowed on the morning of the measurements. During sessions the subjects were not allowed to speak; they were entertained with music to prevent mental stress or falling asleep. Throughout the session, a single lead ECG and a single-lead respiration signal (thoracic impedance) was recorded on an RMS POLYRITE machine (Version 3.0.16). For monitoring purposes, the ECG was also continuously visualized and the arterial blood pressure was intermittently measured at the left upper arm (Nassan Multiparameter Monitor).

For diabetic patients, the experimental protocol remained the same. However, since they were on a fasting state so continuing long term ECG for 6 hours was not feasible. Many of them complained of uneasiness during the course of ECG and therefore, the process had to be aborted after a while. Symptomatic patients whose capillary blood glucose test showed low sugar levels were immediately asked to take their meals and leave for home. So in majority of cases, the long term evaluation could be continued for a maximum of 3-4 hours. This has led to loss of a considerable amount of ECG data from our patients. Software used for data analysis were Kubios HRV Software, MATHEMATICA and MATLAB platform.

g. Pre-processing of data:

Once the ECG recording was taken, it was found that the ECG data was contaminated with noise. This noise was of various types, e.g. white noise, pure line interventions, motion artifacts, baseline shifts and drifts, etc. Standard software algorithms in MATLAB platform [22] were used to filter the ECG data of any such noise and in the process obtain a data with a high S/N ratio.

1) Correction of Baseline shift and drift
2) Correction of the T wave morphology to obtain a clear RR interval
3) Derivation of the RR Interval Tachogram and removal of out lairs.

The corrected database was saved and archived in specific drive destination in PC.

h. Analysis of RR tachogram by Kubios HRV software:

The set of RR were analysed using the Kubios HRV software. 30 different parameters were analysed and the data was tabulated in 2 master charts.

Correlation test was performed. A cut off value of ≥0.75 was taken. All correlation values equal to and above 0.75 were noted and tabulated.

Descriptive evaluation for evolution and proportional distribution of pairs of pattern alteration of the RR interval time series data was conducted with each RR interval tachogram. The results and outcome were presented in the form of line diagram and block diagram.

III. Results

I. Demography:

Table 1: Age-Sex Distribution of Controls

Age (years)	Long term rest		Tilt Table experiment		Cold pressor test	
	Male	Female	Male	Female	Male	Female
< 20	0	1	6	1	1	1
20-25	5	0	5	1	0	1
26-30	3	1	4	3	2	1
> 30	3	0	7	3	2	2
Total	11	2	22	8	5	5
Grand Total	13		30		10	

Fig. 1: Age-Sex Distribution of Controls

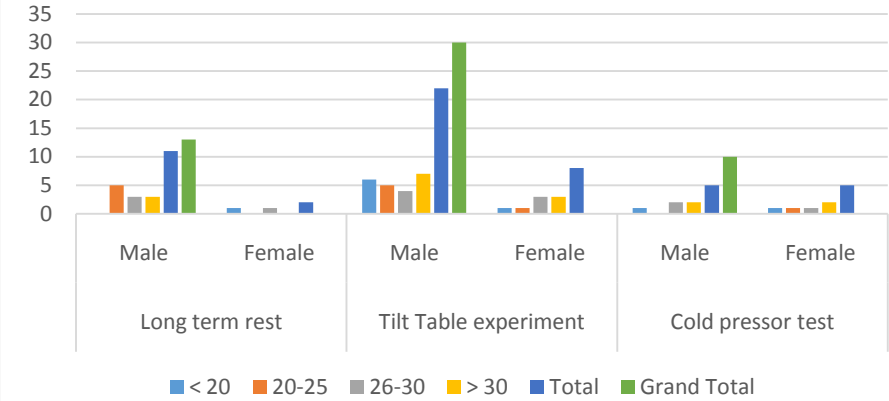
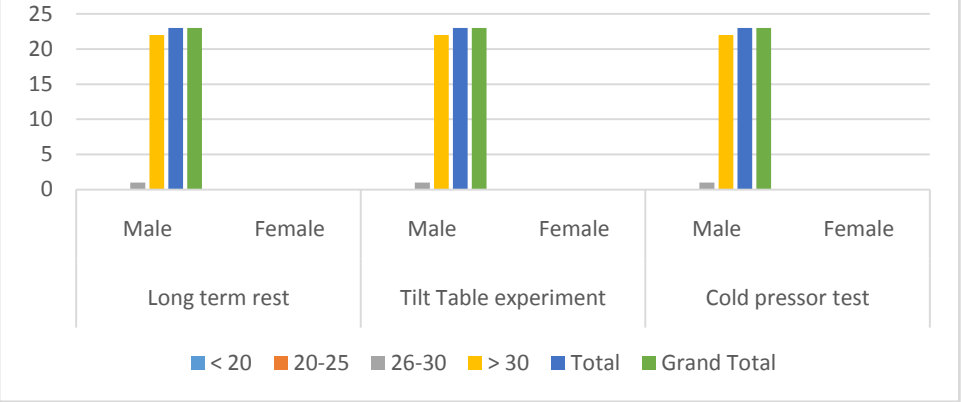


Table 2: Age-Sex Distribution of Patients

Age (years)	Long term rest		Tilt Table experiment		Cold pressor test	
	Male	Female	Male	Female	Male	Female
< 20	0	0	0	0	0	0
20-25	0	0	0	0	0	0
26-30	1	0	1	0	1	0
> 30	22	0	22	0	22	0
Total	23	0	23	0	23	0
Grand Total	23		23		23	

Fig. 2: Age-Sex Distribution of Patients

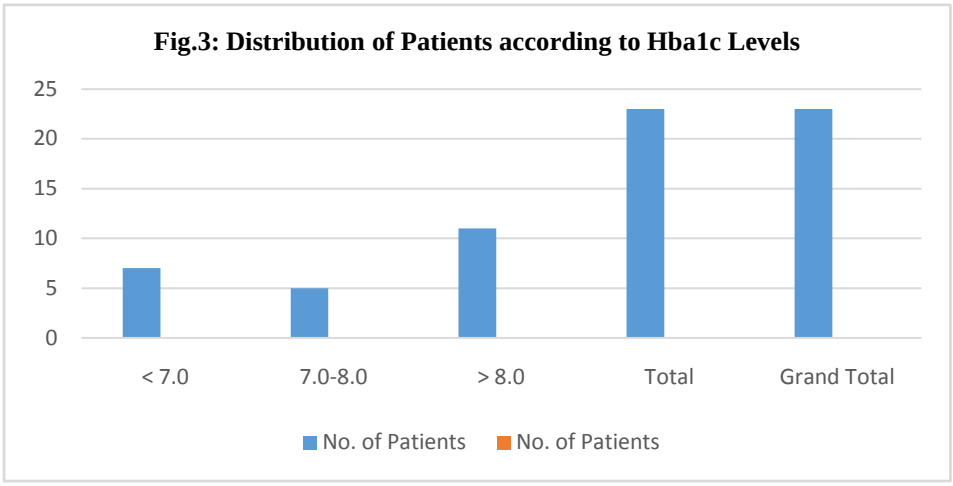


Presence/absence of confounding variables like stress, anxiety, last meal time, environmental temperature, etc. may affect the objective outcome of the present state.

II. Distribution of patients according to Hba1c level and duration of DM:

Table 3: Distribution of Patients according to Hba1c Levels

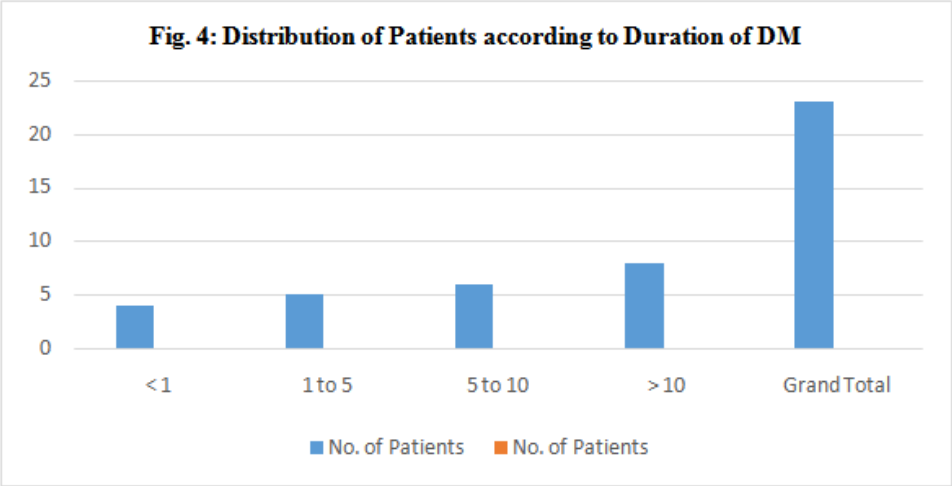
Hba1c (%)	No. of Patients	
	Male	Female
< 7.0	7	0
7.0-8.0	5	0
> 8.0	11	0
Total	23	0
Grand Total	23	



Majority of the patients participating in the study showed poor control of Diabetes during their 1st OPD visit.

Table 4: Distribution of Patients according to Duration of DM

Duration of DM (years)	No. of Patients	
	Male	Female
< 1	4	0
1 to 5	5	0
5 to 10	6	0
> 10	8	0
Grand Total	23	



Majority of the patients participating in the study had long-standing Diabetes.
The values of descriptive statistics for various HRV parameters in both controls and diabetic cases were tabulated and analysed.

All the parameters were found to be more or less normally distributed, with lesser degree of skew or kurtosis.

The value ranges of the individual parameters were all within the normal ranges as per standard literature [23, 24].

These descriptive parameters also validate the normalcy of the recruited study subjects, on whom further evaluation were conducted.

III. Study of the pattern alteration of RR interval time series data and their percentage distribution in long term basal state for controls and patients:

Study of pattern alterations of RR interval time series were conducted in both controls and patients.The RR interval time series data was analyzed in order to extract the features pertaining to the temporal dynamics. Subsequent pairs of RR intervals may be of following types:

i. Increased followed by increased (i-i)
ii. Increased followed by decreased (i-d)
iii. Increased followed by equal (i-e)
iv. Decreased followed increased (d-i)
v. Decreased followed by decreased (d-d)
vi. Decreased followed by equal (d-e)
vii. Equal followed by increased (e-i)
viii. Equal followed by decreased (e-d)
ix. Equal followed by equal (e-e)

We developed appropriate MATLAB code to separate and fractionate each pair of pattern alteration of the RR interval time series data in different designated bins in a progressive manner, till the end.

Bar charts were drawn to represent the relative proportion of each pair of pattern alteration.
To study the distribution of these pairs of pattern alteration of the RR interval time series in the population, the cumulative values of each pattern pairs were normalized to percentage proportions. Subsequently, these normalized percentage values were averaged over the whole population to obtain a signal averaged value.

Table 5: Comparative evaluation of the pattern alteration (i, d, e, ii, dd, ee, id, di, ei, ie, de, ed) of RR interval time series during long term basal state between diabetic patients (n=23) and normal controls (n=13)

	Patient (n = 23)	Control (n = 13)	1-tailed	2-tailed
i	47.81826 ± 2.50751	46.18846 ± 1.731198	0.0279	0.0558
d	48.33435 ± 2.61087	50.661 ± 2.261586	0.9932	0.0136
e	3.832522 ± 2.01824	3.144615 ± 1.91491	0.1626	0.3252
ii	20.63983 ± 3.50119	20.78369 ± 4.36111	0.5432	0.9136
dd	21.14013 ± 3.57961	20.78369 ± 6.09270	0.413	0.826
ee	0.205957 ± 0.22964	0.181462 ± 0.21993	0.377	0.754
id	25.27304 ± 2.79532	24.03692 ± 5.51969	0.194	0.388
di	25.46174 ± 2.95724	23.97308 ± 5.52987	0.1499	0.2998
ei	1.712739 ± 0.83857	1.428154 ± 0.80999	0.164	0.328
ie	1.896087 ± 1.00062	1.365846 ± 0.84291	0.0613	0.1226
de	1.727826 ± 0.87649	1.596923 ± 0.87950	0.336	0.672
ed	1.913478 ± 1.06293	1.532846 ± 0.90372	0.1401	0.2802

Fig. 5: Comparative evaluation of the pattern alteration (i, d, e, ii, dd, ee, id, di, ei, ie, de, ed) of RR interval time series during long term basal state between diabetic patients (n=23) and normal controls (n=13)

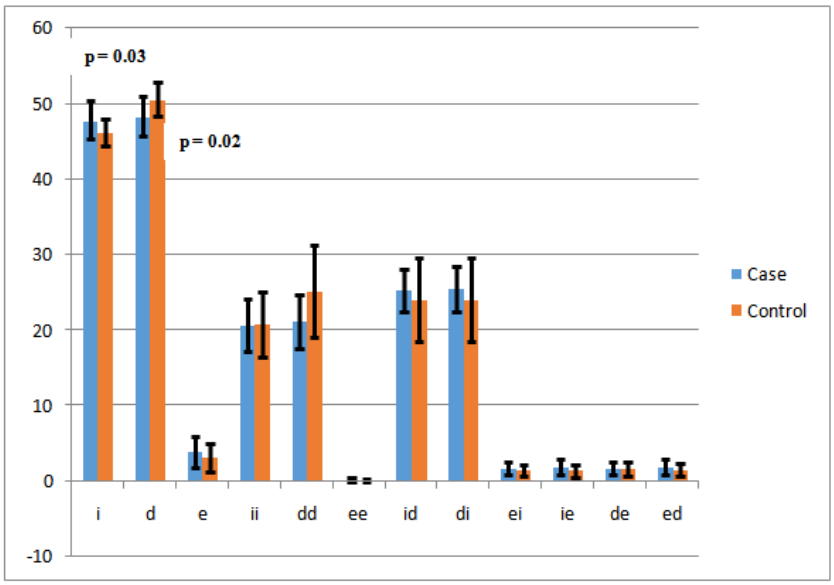


Table 6: Pattern of alteration of Pearson's correlation coefficient (r) in normal controls and diabetic patients during long term rest		
Parameters	**For Controls**	**For Patients**
Mean RR vs RMSSD	0.524	0.143
Mean RR vs pNN50	0.743	0.015
Mean RR vs Total Power	0.596	0.087
SDNN vs RMSSD	0.929	0.803
SDNN vs pNN50	0.841	0.753
SDNN vs HF Power	0.585	0.067
SDNN vs Total Power	0.949	0.964
RMSSD vs pNN50	0.815	0.903
RMSSD vs HF Power	0.753	0.585
RMSSD vs Total Power	0.878	0.719
pNN50 vs LF Power	-0.351	0.065
pNN50 vs HF Power	0.469	0.491
pNN50 vs Total Power	0.77	0.7
LF Power vs HF Power	-0.062	0.393
LF Power vs Total Power	-0.289	-0.106
HF Power vs Total Power	0.501	-0.011

Fig.5 shows that (d) is the most prevalent pattern alteration of the RR interval time series data set in normal controls. Next common preponderance is of (i) variety which is followed by (d-d) pattern, (i-d) pattern, (d-i) pattern and (i-i) pattern. Any match containing 'e' is a rarity. And (e-e) pattern pair is almost absent. However, in patients (d) is the most prevalent pattern alteration of the RR interval time series data set. This is followed in close second by the (i) variety which is followed by (i-d) and (d-i) patterns, (d-d) pattern and (i-i) pattern. Any match containing 'e' is a rarity. And (e-e) pattern pair is almost absent.

Table 5, however, shows that the comparison of the pattern alteration of RR interval time series between controls and patients is statistically significant only for (d) variety with a p value = 0.02 and (i) variety with a p value = 0.03. The remaining varieties are not statistically significant.

IV. Objective Evaluation of Studies of Long Term Rest in Controls and Patients:
The Result of the Heart Rate Variability Evaluation

Obtained values of various HRV parameters were further evaluated. We selected a number of variables among the various HRV parameters. All the time domain parameters and non-linear parameters were utilized. Among the frequency domain parameters, only total power (ms^2), % of total power and LF/HF ratio were found to be important and pertinent physiological variables.

There was a huge number of data which were all apparently Gaussian in nature. Accordingly, we utilized parametric tests e.g. Pearson's r test for correlation and Student's t test (paired) for comparison.

A. Correlation Study:

We conducted correlation study in between each pair of HRV parameters, e.g. Mean RR & SDNN, SDNN & RMSSD, etc.

Out of large numbers of high level correlation parameters (r value ≥ 0.75 and < -0.75), we chose to consider a few HRV parameters for analysis and evaluation for the current study. Most of the parameters of nonlinear domain were kept aside for future activities.

Table 6 summarizes the pattern of alteration of Pearson's correlation coefficient for a few sets of HRV parameters between healthy controls and diabetic subjects during long term resting state.

From Table 6 we can see that SDNN &RMSSD, SDNN &pNN50 and RMSSD & pNN50 are highly correlated in both normal controls and diabetic subjects. RMSSD & HF Power are highly correlated only in normal controls while pNN50 & HF Power are poorly correlated in both the groups.

B. Comparison study of various HRV parameters between normal controls and diabetic patients:

Table 7 represents the comparison among various HRV parameters between normal controls and diabetic patients.

Table 7: Comparison among various HRV parameters during long term basal state between normal controls and diabetic patients				
Variable	**Control (n=13)**	**Patient (n=23)**	**% Difference of Mean**	**P value**
	Mean ± SD	**Mean ± SD**		
Mean RR (ms)	765.78 ± 126.14	790.38 ± 116.72	-3.21	0.559
SDNN (ms)	60.25 ± 26.59	43.08 ± 14.6	28.5	0.047
Mean HR (1/min)	80.9 ± 13.26	77.74 ± 11.2	3.91	0.452
STD HR (1/min)	6.48 ± 2.5	4.33 ± 1.54	33.18	0.012
RMSSD (ms)	47.8 ± 27.27	32.73 ± 12.16	31.53	0.078
NN50 (count)	395.46 ± 370.28	233.26 ± 206.12	41.02	0.164
pNN50 (%)	18.52 ± 19.17	9.91 ± 8.88	46.5	0.147
RR Triangular Index	14.98 ± 7.03	11.15 ± 3.54	25.57	0.084
TINN (ms)	331.15 ± 73.97	251.53 ± 75.41	24.04	0.004
VLF Power (%)	50.04 ± 17.45	53.6 ± 12.75	-7.11	0.486
LF Power (%)	28.12 ± 12.69	25.76 ± 7.67	8.39	0.55
HF Power (%)	21.86 ± 12.82	20.55 ± 7.56	5.99	0.741
Total Power (ms2)	3424.69 ± 3212.35	1753.48 ± 1458.08	48.8	0.096
LF/HF	2.01 ± 2.2	1.37 ± 0.45	31.84	0.318
SD1 (ms)	33.81 ± 19.26	23.14 ± 8.6	31.56	0.078
SD2 (ms)	77.79 ± 33.34	56.09 ± 19.52	27.9	0.046
Lmean (beats)	14.24 ± 4.24	13.18 ± 4.76	7.44	0.51
Lmax (beats)	307.69 ± 135.43	277.74 ± 153.43	9.73	0.562
REC (%)	38.26 ± 9.4	36.48 ± 9.53	4.65	0.594
DET (%)	98.47 ± 0.98	98.09 ± 1.17	0.386	0.328
ShanEn	3.41 ± 0.31	3.32 ± 0.35	2.64	0.463
ApEn	1.29 ± 0.21	1.4 ± 0.15	-8.53	0.066
SampEn	1.36 ± 0.33	1.52 ± 0.28	-11.76	0.124

Fig. 6: Comparison of time domain parameters between normal controls and diabetic patients

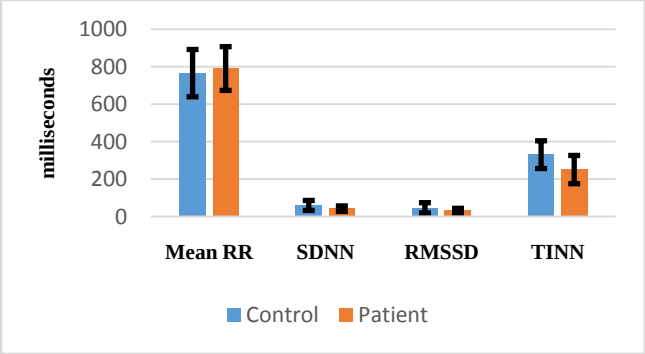


Fig. 7: Comparison of time domain parameters between normal controls and diabetic patients

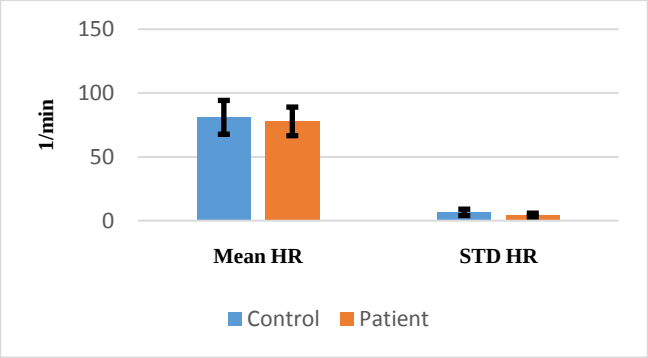
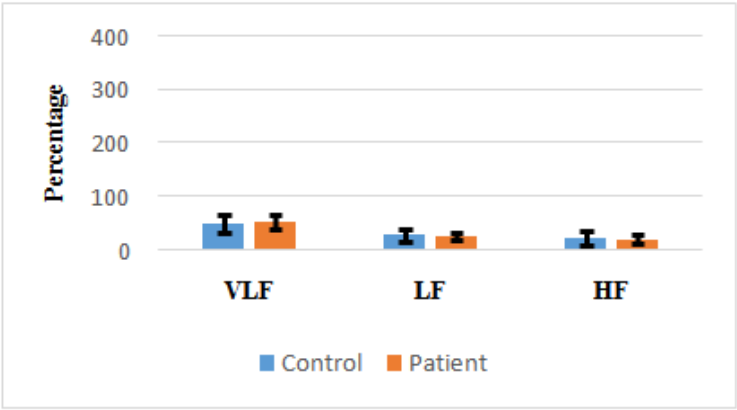


Fig. 8: Comparison of frequency domain parameters between normal controls and diabetic patients



From Table 7 and Fig. 6-8 we can see that there is statistically significant reduction in the values of SDNN, STD HR, TINN and SD2 in the diabetic subjects in comparison to normal controls. Other parameters didn't show any statistically significant change.

IV. Discussion

Physiological variation of autonomic activity, such as respiratory sinus arrhythmia and vasomotor oscillations, cause corresponding fluctuations in heart rate [25, 26]. By RR interval time series analysis, we intend to determine and quantitate the autonomic activity from heart rate variation.

The present study was conducted on a cohort of normal volunteers and patients of Diabetes Mellitus in order to evaluate the autonomic profile of diabetic patients and young adult normal persons in physiological resting state. Conventional HRV evaluation was performed and the results were analyzed by standard statistical tools. Along with that, a descriptive evaluation of the progressive evolution and distribution of the pattern of beat-to-beat alterations of the RR interval time series was conducted by a novel method.

Earlier studies have revealed that parasympathetic tone dominates the resting state, while exercise is associated with prompt withdrawal of vagal tone and subsequent sympathetic activation. Conversely, recovery is characterized by parasympathetic activation followed by sympathetic withdrawal [5]. Stress induced differential activation of two arms of ANS is associated with increased sympathetic and decreased parasympathetic activity and the period of recovery after maximum exercise is characterized by a combination of sympathetic withdrawal and parasympathetic reactivation, which are the two main arms of the ANS [27].

However, in the current study the issue was explored in detail and a fairly complex feature was obtained instead of a simple pattern as stated in the studies aforementioned.

1. Correlation study during long term basal state exhibited a strong correlation between SDNN on one side and RMSSD, NN50, pNN50, RR Triangular Index and Total Power on the other side in normal controls. All these pairs of parameters exhibited a very high correlation having Pearson's correlation coefficient (r) value > 0.84. However, in cases the strength of correlation between the above mentioned pairs of parameters became profoundly weak.

With respect to RMSSD there is a strong correlation with pNN50 in control group which is also maintained in the patient group. From these findings, it can be hypothesized that the time domain parameters do not exhibit any loss of correlation during long term basal state or, in other words, exhibits a similar correlation pattern between controls and cases.

2. Comparative evaluation of the pattern of alteration (i, d, e, ii, dd, ee, id, di, ei, ie, de, ed) of RR interval time series during long term basal state were conducted and represented in Fig. 5 and Table 5. This form of representation utilizes a simple arithmetic tool in the MATHEMATICA platform and is a novel approach. This entails us to study the beat-to-beat temporal variability of cardiac chronotropy. Fig. 5 clearly exhibits a typical pattern of distribution of relative proportion of various types of pattern alteration as stated above in normal controls. In diabetic population although the said visual pattern is somehow maintained but there is a significant rise in percentage proportion of i (increase) pattern of RR interval time series. Similarly, there is a significant decrease in percentage proportion of d (decrease) pattern of RR interval time series in the diabetic patients compared to healthy controls.

3. Comparative evaluation of various HRV parameters between two groups of controls and patients exhibits interesting information in long term basal state. The value of Mean RR is higher among the diabetics and that of Mean HR is lower among the diabetics in comparison to normal subjects (Table 7**).** This suggests a

poorer sympathetic tone than parasympathetic tone in the diabetic group in comparison to normal controls. Thus we may say that sympathetic activity among diabetics is impaired during long term rest**.** However, neither Mean RR nor Mean HR is statistically significant between the two groups.

Variability parameters like SDNN, STD HR and TINN show statistically significant reductions in cases as compared to the normal controls. The order of decrease is in the range of 24-33% (Table 7). The reduction in STD HR among diabetics is suggestive of a reduction in variability in heart rate in the corresponding group which goes well with previous studies on HRV [2, 10, 12, 16]. SDNN is a measure of overall variation and also the extent of sympathetic activity. It is significantly reduced in the diabetic subjects, thereby suggesting an impairment of overall variation in the autonomic activity and also the sympathetic arm of ANS in the diseased population. The same applies for TINN. However, it is noteworthy that no significant difference is observed between cases and controls with respect to NN50, pNN50, LF Power and HF Power while Total Power records a fall to the extent of 48.8% in diabetic patients. But this fall does not reach the level of significance ($p = 0.09$). RMSSD, NN50 and pNN50 are measures of short-term variability and also indices of parasympathetic activity. Even if their values failed to reach the level of statistical significance between cases and controls, yet they show apparent reduction among the diabetics in comparison to normal subjects. This suggests an impairment in parasympathetic activity among patients with Diabetes Mellitus besides an impaired sympathetic tone as mentioned above. This finding also fits well with previous studies on HRV in diabetics [14].

None of the frequency domain parameters are statistically differentiable between the two groups although apparent reduction in the HF and LF bands is noted among the diabetics (Table 7). Reduction of HF band indicates an impaired parasympathetic tone that is already mentioned above. LF band, although conventionally believed to be an index of sympathetic activity, is presently considered to be related to baroreflex function. So its reduction probably indicates an impairment in modulating cardiac autonomic outflows in supine state by baroreflexes among diabetic patients [28]. Among the non-linear parameters only SD2 shows statistically significant ($p = 0.04$) reduction (by 27.9%) in the diabetic patients indicating an impairment in long-term variability of heart rate in the corresponding group during basal state. SD1, an index of short-term variability, shows an apparent reduction in the diabetics but fails to reach the level of statistical significance. Detrended fluctuations also fail to show any statistically significant change between the two groups.

From the findings stated above we may hypothesize that there is a reduction of the long-term controlling parameters of heart rate variability among diabetic patients sparing the short-term variability parameters (only apparent change, not statistically significant). Both the arms of autonomic nervous system seem to be affected by hyperglycemia with a greater impairment in sympathetic activity that is reflected as lower heart rate during basal state among the diseased population as compared to normal controls.

Studies of brain signaling systems in Type 2 Diabetes Mellitus in recent times have provided greater insight into understanding the pathogenesis of diabetes and metabolic syndrome [29]. Study by Valastro et al has suggested upregulation of glutamate receptors in the brain to be the possible underlying mechanism for neurological manifestations in diabetes [30].Studies of long-term exposure to hyperglycemia in streptozotocin-induced diabetic rats have also shown abnormalities in brain glutamate receptors [31, 32].Glutamate receptors have also been detected in the hindbrain where they play an important role in reflexive response to vagal activation [33].The possible malfunctioning of GABAnergic neuron, which is another important component of the baroreflex pathway, in long-term diabetes [34] also remains another speculation.Sowe may hypothesize that it is the chemical dysregulation in the central neuronal circuit that is the reason behind autonomic dysfunction in diabetic patients manifesting as hypo-activity of both sympathetic and parasympathetic components of ANS.

V. Conclusion

The characteristic observations among diabetic patients may be attributed to the altered population and/or functioning of specific subtypes of NMDA receptors with or without involvement of GABAnergic neurons in the central cardiovascular reflex regulatory neuronal circuitry.

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