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Significance Analysis of Different Time Domain Measures Of HRV to Differentiate Normal and On-Music States

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ABSTRACT: The present article establishes the significant difference between normal and on-music HRV data by applying the notion of Two Factor ANOVA. First of all, an attempt has been made to ascertain which of the seven available standard time domain measures of HRV data are significantly different and which are not so by the statistical test single factor ANOVA or its modified forms as and when necessary. It is found that in both of normal/pre-music and on-music states, all the measures show significant difference as a whole; the problem remains to identify exactly the measures, which cause the significant difference. This is done by applying Tukey's multiple comparison test or by Newman-Keul's test on pair wise means as and when required. This shows that there exists exactly one pair of measures, which are similar, but the rest five are significantly different in pre-music/normal state and two pairs of similar measures and the rest three are significantly different in on-music state. On comparing similar measures of normal and on-music states, it is found that there exists exactly one pair of similar measures common to both the states. Finally, the two factor ANOVA is applied, which successfully differentiates the aforesaid two states.

KEYWORDS: HRV data, Bartlett test, modified ANOVA of Brown and Forsythe, Tukey's multiple test, Newman-Keul's test, Two factor ANOVA.

I .INTRODUCTION

During normal sinus rhythm, the heart rate (HR) varies from beat to beat. Heart rate variability (HRV) results from the dynamic interplay between the multiple physiological mechanisms that regulate the instantaneous HR. HRV is a useful signal for understanding the status of the autonomic nervous system (ANS)[1-4]. HRV refers to the variations in the beat intervals or correspondingly in the instantaneous heart rate (HR). The normal variability in HR is due to autonomic neural regulation of the heart and the circulatory system [5]. The balancing action of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), the two branches of the autonomic nervous system (ANS) controls the HR [6-10]. Increased SNS or diminished PNS activity results in cardio-acceleration. Conversely, a low SNS activity or a high PNS activity causes cardio-deceleration[14-18]. The degree of variability in the HR provides information about the functioning of the nervous control on the HR and the heart's ability to respond [11]. So far as the application of statistical methods are concern the following two works may be cited. Andriano L Roque et.al. [19] describes in their article ' The effects of auditory stimulation with music on heart rate variability in healthy women'.Standard statistical methods were used to calculate the means and standard deviations. The normal Gaussian distribution of the data was verified by the Shapiro-Wilk goodness-of-fit test (z value of >1.0). For parametric distributions, the one-way ANOVA was applied for repeated-measures followed by the Bonferroni post-test. For nonparametric distributions, the Friedman test was made and it was followed by Dunn's post-test. The authors compared the geometric indices of HRV between the three moments (Group 1, control condition vs. classical baroque vs. excitatory heavy metal; Group 2, control condition vs. classical baroque vs. excitatory heavy metal vs. white noise). The differences were considered significant when the probability of a Type I error was less than 5% ($p<0.05$). They used the Software Graph Pad Stat Mate version 2.00 for Windows (Graph Pad Software, San Diego, CA, USA).Bianca

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CR de Castro et al. in their article [20] establishes that 'Previous exposure to musical auditory stimulation immediately influences the cardiac autonomic responses to the postural change maneuver in women'. Statistical tools and software's used are the same as in [19]. In the first protocol they compared the indices: RMSSD, pNN50, LF [nu], HF [nu] and LF/HF). They compared the HRV indices between the four moments in the first protocol (seated rest vs. 0–5 min after the volunteers stood up vs. 5–10 min after the volunteers stood up vs. 10–15 min after the volunteers stood up). In relation to the second protocol for parametric distributions, they applied the ANOVA for repeated measures followed by the Bonferroni post-test (SDNN), for non-parametric distributions they used the Friedman test followed by the Dunn's test on (RMSSD, pNN50, LF [nu], HF [nu] and LF/HF). They compared the HRV indices between the five moments in the second protocol (seated rest vs. 10 minutes musical auditory stimulation vs. 0–5 min after the volunteers stood up vs. 5–10 min after the volunteers stood up vs. 10–15 min after the volunteers stood up). Differences were considered significant when the probability of a Type I error was less than 5% ($p < 0.05$).There are many HRV measures that can be defined in the time domain. In this article, we consider only some promising measures [12]. These are mean RR interval (mRR), standard deviation of RR interval (SDRR), mean heart rate (mHR), standard deviation of heart rate (SDHR), root mean square successive difference (RMSSD), Number of pairs of adjacent RR intervals differing by more than 20 ms to all RR intervals (pRR20), and Number of pairs of adjacent RR intervals differing by more than 50 ms to all RR intervals (pRR50) [12]. Obviously it is necessary to check which of them are significantly different; otherwise unnecessarily some measures are included, which are not significantly different and as such do not give any new result. It is also necessary to see whether the measures differ in the HRV data of persons, when they listen to and do not listen to some suitably chosen music. Lastly it is to seen whether it is possible to differentiate between normal and on-music HRV data based on the significantly different measures only. In this article, an attempt has been made to answer all of the above questions by means of some well known statistical tests.

II. MATERIALS AND METHODS

A. ACQUISITION HRV DATA

The ECG signals in digitised form are recorded from different subjects (age between 20 to 30 years) by 'HRV data logger machine. All the signals are recorded at School of Bio-Science and Engineering, Jadavpur University, Kolkata, India under normal room temperature and least noisy environment. Recording is done in two stages. At the first stage ECG is taken at normal condition, when the subjects do not listen to music. In the second stage ECG signals are taken, when subjects do listen to Rabindra Sangeet of some special choice. All signals are taken in ten minutes duration. Finally, recorded signals are processed by MATLABR2010a software using moving window integration of a digital filter and converted into HRV signals.

B. DIFFERENT TIME DOMAIN MEASURES OF HRV

The different time domain measures of HRV [12] used in this article are enlisted in the table.1.

mRR	ms	$\dfrac{\sum_{i=1}^{N}(RR_i)}{N}$
SDRR	ms	$sqrt\left(\dfrac{\sum_{i=1}^{N}(RR_i - mRR)^2}{N-1}\right)$
mHR	bpm	$\dfrac{\sum_{i=1}^{N}\left(\dfrac{6000}{RR_i}\right)}{N}$

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SDHR	bpm	$sqrt\left(\frac{\sum_{i=1}^{N}\left(\left(\frac{60000}{RR_i}\right)-mHR\right)^2}{N-1}\right)$
RMSSD	ms	$sqrt\left(mean\left(RR_{i+1}-RR_i\right)^2\right)$
pRR50	%	$\left(\frac{count\left(\lvert RR_1-RR_i\rvert\right)_{>50ms}\times 100}{N-1}\right)$
pRR20	%	$\left(\frac{count\left(\lvert RR_{i+1}-RR_i\rvert\right)_{>20ms}\times 100}{N-1}\right)$

Table.1. List of statistical measures used for the analysis of the HRV data.

C. ANALYSIS OF VARIANCE (ANOVA)

As we deal with equality of population means of more than two samples, so student's t- test is not workable. In fact, consideration of pair of samples at a time, involves increase in type1 error. So we opt for single factor ANOVA.
The basic assumption for applying ANOVA is that the data must be normally distributed (which is usually assumed) and the secondary assumption is that the population variances are same under certain level of confidence, which is normally taken as 0.95. The secondary assumption is always to be verified by Bartlett's test for homogeneity of variances [13].

D. TEST FOR EQUALITY OF VARIANCES (BARTLETT'S TEST)

Null hypothesis H_0: Variances are homogeneous.
Alternative hypothesis H_A: Variances are heterogeneous

Let SS_i be the sum of squares and n_i be the size of the i^{th} sample. Then the pooled variance s_p^2 is given by $s_p^2 = \sum_{i=1}^{k}(SS_i) / \sum_{i=1}^{k} v_i$. The test statistic B is given by

$$B = (\log s_p^2)(\sum_{i=1}^{k} v_i) - \sum_{i=1}^{k} v_i \log s_i^2, v_i = n_i - 1.$$

This formula is calculated with natural logarithm (base e); but very often the biologists prefer the calculations with logarithm (base 10). In that case, we have B=2.30259 $[(\log s_p^2)(\sum_{i=1}^{k} v_i) - \sum_{i=1}^{k} v_i \log s_i^2]$.

The distribution of B is approximated by chi-square distribution with k-1 degrees of freedom. But a more accurate chi-square approximation is obtained by computing a correction factor

$$C = \frac{1}{3(k-1)}\left(\sum_{i=1}^{k}\frac{1}{v_i} - \frac{1}{\sum_{i=1}^{k} v_i}\right).$$

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So the corrected test statistic becomes $B_c = \frac{B}{C}$.

If Bc is less than $\chi^2_{.05,k-1}$, then H_0 holds, otherwise H_A holds.

If H_0 holds for the population variance, then the single factor ANOVA is applied. Otherwise if H_A holds, then one of the following two tests (section E and F) are applied:

E. MULTIPLE WELCH'S TEST [13]

The test statistic is given by

$$F' = \frac{\sum_{i=1}^{k} c_i(\bar{X}_i - \bar{X}_w)^2}{(k-1)[1+\frac{2A(k-2)}{k^2-1}]},\quad c_i = \frac{n_i}{s_i^2}, C = \sum_{i=1}^{k} c_i,\ \bar{X}_w = \frac{\sum_{i=1}^{k} c_i \bar{X}_i}{C}, A = \sum_{i=1}^{k} \frac{(1-c_i/C)^2}{\nu_i}, \nu_i = n_i - 1,$$

where n_i is the size of i^{th} sample. Further F' is associated with degrees of freedom $\nu_1 = k-1, \nu_2 = \frac{k^2-1}{3A}$, where ν_2 is

to be rounded to next lower integer, if it is a fraction.

The distribution of F' is approximated by F distribution with ν_1, ν_2 degrees of freedom.

F. MODIFIED ANOVA OF BROWN AND FORSYTHE [13]

The test statistic is given by $F'' = \frac{\text{Group SS}}{B}$, where Group SS$=\sum SS_i$, $B = \sum_{i=1}^{k}(1-\frac{n_i}{N})s_i^2$.

F'' is associated with degrees of freedom $\nu'_1 = k-1, \nu'_2 = \frac{B^2}{\sum_{k=1}^{n} \frac{[(1-n_i/N)s_i^2]^2}{\nu_i}}$

The distribution of F'' is approximated by F distribution with ν'_1, ν'_2 degrees of freedom.

Again F' and F'' both work well for $n_i \geq 10$. But F' is better, when $4 < n_i < 10$.

If it is proved that there is no significant difference between the population means, then no more investigation is necessary. But if there is a significant difference, then the natural query is to find exactly those population means, which do differ significantly. This is done by Tukey's multi-comparison test.

G. TUKEY'S MULTI-COMPARISON TEST [13]

It consists of the null hypothesis H_0: $\mu_B = \mu_A$ versus the alternate hypothesis H_A: $\mu_B \neq \mu_A$, where the subscripts denote all possible pairs of groups. For k group, $k(k-1)/2$ different pair wise comparisons can be made. The sample means are ranked; pair wise differences between means are determined, and a standard error is computed. The test

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statistic is $q_{\alpha,\nu}$, where α is the level of significance and ν is the degree of freedom. On this basis, it is decided, which means are really different significantly? But sometimes the results become indecisive, viz. $\mu_A = \mu_B$, $\mu_B = \mu_C$. So if $\mu_A \neq \mu_C$, then what happens to μ_B? It escapes classification. In such cases we consider a modified form of Tukey's test known as Newman-Keuls test.

H. NEWMAN-KEULS TEST [13]

The Newman-Keuls test is performed exactly as in Tukey's test, with one expectation that this test uses different critical values for different ranges of means. Thus in Newman-Keuls test the expression of critical value is given by $q_{\alpha,\nu,p}$, where p is the number of means, in the range of means being tested. Due to different choice of p, this test is free from any indecision. The similar classes are always found to be disjoint.

I. CONFIDENCE INTERVALS [13]

Once it is determined, which means are significantly different and which are not so, the next thing is to determine separately the interval of confidence of population means for each significantly different mean and for the pooled mean of similar means. We can calculate confidence intervals of population means for each different population mean X_i and also for the pooled mean of all similar means given by $\overline{X}_p = \frac{\sum n_i \overline{X}_i}{\sum n_i}$, where the summation is over all samples concluded to have come from the same population. The intervals of confidence are given by $\overline{X}_i \pm t_{\alpha(2),\nu}\sqrt{\frac{S^2}{n_i}}$ and $\overline{X}_p \pm t_{\alpha(2),\nu}\sqrt{\frac{s^2}{\sum n_i}}$ respectively.

J. TWO FACTOR ANALYSIS OF VARIANCE [13]

In two-way factorial analysis of variance, we refer to one factor as A and other factor as B. Furthermore, let 'a' represent the number of levels in factor A, 'b' represent the number of levels in factor B, and 'n' the number of replicates. Let the triple subscript X_{ijl} on the variable denotes the value of the replicate l of the combination of level i of factor A and level j of factor B. Each combination of a level of factor A with a level of factor B is called a cell. The cell may be visualized as the "groups" in one factor ANOVA. For the cell formed by combination of level i of factor A and level j of factor B, let $\overline{X}_{ij}$ denote the cell mean. The mean of all bn data in level i of factor A is $\overline{X}_i$, and the mean of all an data of level j of factor B is $\overline{X}_j$. There are total no of N data in the experiment, and the mean of all N data is denoted as $\overline{X}$. We denote Total SS $= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{l=1}^{n}(X_{ijl} - \overline{X})^2$ and Total DF= N-1

Cell SS= $\sum_{i=1}^{a}\sum_{j=1}^{b} n(\overline{X}_{ij} - \overline{X})^2$, Cell DF = ab -1

Within-cells SS = $\sum_{i=1}^{a}\sum_{j=1}^{b}\left[\sum_{l=1}^{n}\left(X_{ijl} - \overline{X}_{ij}\right)^2\right]$, Within-cells DF= (total DF-cells DF) $= ab(n-1)$

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Factor A SS $= bn\sum_{i=1}^{a}\left(\overline{X_{i.}}-\overline{X}\right)^2$. This is associated with Factor A DF$= a-1$

Factor B SS = $an\sum_{j=1}^{b}\left(\overline{X_{.j}}-\overline{X}\right)^2$ .This is associated with Factor B DF $= b-1$

In general the variability among cells is not equal to the variability among levels of factor A plus the variability among levels of factor B. The amount of variability not accounted for is that due to the effect of interaction between factors A and B. This is designed as the $A\times B$ interaction and is readily calculated by difference. It is given by

$A\times B$ Interaction SS = cells SS – factor A SS – factor B SS

This is associated with $A\times B$ interaction DF = (factor A DF) (factor B DF) = $(a-1)(b-1)$

[An interaction between two factors means that the effect of one factor is not independent of the presence of a particular level of the other factor. Therefore interaction among factors is an effect on the variable, which is in addition to the sum of the individual effect of each factor].

Next mean ASS, mean BSS, mean $A\times B$ SS, Mean within cell SS are calculated by dividing each of them by the corresponding degree of freedom DF. These are denoted by MASS, MBSS, $\mathrm{M}(A\times B)$SS and MESS respectively.

We now make the following Hypothesis:

Null Hypothesis $(\mathrm{H}_0)_A$: There is no significant effect of factor A as a whole

Alternate Hypothesis $(\mathrm{H}_A)_A$: There is a significant effect of factor A as a whole

Null Hypothesis $(\mathrm{H}_0)_B$: There is no significance effect of factor B as a whole

Null Hypothesis $(\mathrm{H}_A)_B$: There is a significant effect of factor B as a whole

Null Hypothesis $(\mathrm{H}_0)_{A\times B}$: There is no significant effect of factor A on B

Null Hypothesis $(\mathrm{H}_A)_{A\times B}$: There is a significant effect of factor A on B

Now if $\mathrm{F_A} = \dfrac{\mathrm{MASS}}{\mathrm{MESS}}, \mathrm{F}_B = \dfrac{\mathrm{MBSS}}{\mathrm{MESS}}, \mathrm{F_{A\times B}} = \dfrac{\mathrm{M(A\times B)SS}}{\mathrm{MESS}}$, then

$\mathrm{F_A} < F_{.05(1),a-1,ab(n-1)} \Rightarrow$ Levels of factor A do not differ significantly

$\mathrm{F_A} > F_{.05(1),a-1,ab(n-1)} \Rightarrow$ Levels of factor A differ significantly

$\mathrm{F}_B < F_{.05(1),b-1,ab(n-1)} \Rightarrow$ Levels of factor B do not differ significantly

$\mathrm{F}_B > F_{.05(1),b-1,ab(n-1)} \Rightarrow$ Levels of factor B differ significantly

$\mathrm{F_{A\times B}} < F_{.05(1),(a-1)(b-1),ab(n-1)} \Rightarrow$ Levels of factor A has significant effect on levels of factor B

$\mathrm{F_{A\times B}} > F_{.05(1),(a-1)(b-1),ab(n-1)} \Rightarrow$Levels of factor A has no significant effect on levels of factor B

The following machine formulae may be used for quicker computation.

$$\text{Total SS} = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{l=1}^{n}\overline{X}_{ijl}^{2} - C,\ \mathrm{C} = \frac{\left(\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{l=1}^{n}X_{ijl}\right)^2}{N}$$

$$\text{Cells SS} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\left(\sum_{l=1}^{n}X_{ijl}\right)^2}{n} - C,\ \text{ASS} = \frac{\sum_{i=1}^{a}\left(\sum_{j=1}^{b}\sum_{l=1}^{n}X_{ijl}\right)^2}{n} - C,\ \text{BSS} = \frac{\sum_{i=1}^{b}\left(\sum_{j=1}^{a}\sum_{l=1}^{n}X_{ijl}\right)^2}{n} - C$$

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III RESULTS AND DISCUSSIONS

A. NORMAL HRV DATA

B. HRV DATA UNDER DIFFERENT STATISTICAL MEASURES

The HRV data of the subjects (s1- s16) in normal state is recorded as discussed in section 2.1. The statistical measures as given in section 2.2 are then computed for all the above mentioned 16 subjects. The results are presented in table.2.

Subjects	mRR (ms)	SDRR (ms)	mHR (bpm)	SDHR (bpm)	RMSSD (ms)	pRR20 (%)	pRR50 (%)
s1	605.8	483	153.72	349.69	628.2	821	85.3
s2	883.6	147.8	73.64	57.82	185.5	225	32.7
s3	669.3	178.7	100.9	79.43	219.1	207	24.7
s4	713	659.9	134.62	243.96	752	611	77.5
s5	623.3	240.6	117.31	66.95	289.6	628	69.7
s6	849.8	184.8	79.76	72.69	243.9	298	45.1
s7	648.5	281.9	124.98	125.82	337.5	654	75.4
s8	639.9	158.8	101.9	94.38	214.5	131	14.9
s9	792.7	231.1	103.07	130.91	279.5	283	40.3
s10	842.1	328.1	108.26	145.12	421.5	71	70.3
s11	642.5	267.9	153.58	290.74	363.6	670	76
s12	715.1	512.5	150.34	503	682	859	76.2
s13	749.8	233.8	104.08	108.68	288.1	580	69.3
s14	912.1	144	67.85	28.74	181	218	34.1
s15	796.6	719.6	185.5	564.04	1000.5	652	88.9
s16	722.2	141	87.22	34.24	174.8	217	27.5

Table.2. Different statistical measures for the HRV data in Normal / Pre-music states.

C. BARTLETT'S TEST

To carry out the test for the equality of the population variances we first calculate T_i, CF, $\sum SS_i$, $\sum v_i$, $\sum v_i \log S_i^2$, $\sum \frac{1}{v_i}$. These are presented in table.3.

Subjects	mRR (ms)	SDRR (ms)	mHR (bpm)	SDHR (bpm)	RMSSD (ms)	pRR20 (%)	pRR50 (%)		
T_i	11806.3	4913.5	1846.73	2896.21	6261.3	7125	907.9		
T_i^2	8856712.1	2027847.1	229469.38	933643.07	3346255.5	4196929	60534.37		
CF	8711795	1508905.1	213150.73	524252.02	2450242.4	3172851.6	51517.651		
SS_i	144917.11	518941.93	16318.647	409391.05	896013.17	1024077.4	9016.7194	3018676.1	$\sum SS_i$

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v_i	15	15	15	15	15	15	15	105	$\sum v_i$
S_i^2	9661.1406	34596.129	1087.9098	27292.736	59734.212	68271.829	601.11463		
$\log S_i^2$	3.9850284	4.5390275	3.0365929	4.4360471	4.7762231	4.8342415	2.7789573		
$v_i \log S_i^2$	59.775426	68.085413	45.548894	66.540706	71.643347	72.513623	41.684359	425.79177	$\sum v_i \log S_i^2$
$\frac{1}{v_i}$	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.4666667	$\sum \frac{1}{v_i}$

Table.3. Statistical table comprising $T_i, C.F, \sum SS_i, \sum v_i, \sum v_i \log S_i^2, \sum \frac{1}{v_i}$ in the Normal / Pre-music states.

The pooled variance s_p^2 is calculated as $s_p^2 = \sum_{i=1}^{7}(SS_i) / \sum_{i=1}^{7} v_i =$ 28749.296

$$B = 2.30259\left[(\log s_p^2)(\sum_{i=1}^{k} v_i) - \sum_{i=1}^{k} v_i \log s_i^2\right] = 97.547129\,,\; C = \frac{1}{3(k-1)}\left(\sum_{i=1}^{k}\frac{1}{v_i} - \frac{1}{\sum_{i=1}^{k} v_i}\right) = 3.2857143$$

$$\mathrm{B_c} = (\mathrm{B/C}) = 29.688257 > \chi^2_{.05,k-1} = 24.996\text{. So } \mathrm{H}_A \text{ holds and } \sigma_1^2 \neq \sigma_2^2 = ------ \neq \sigma_7^2.$$

Hence in this case ANOVA cannot be applied. Again as the sample size is greater than 10, so it is better to apply modified ANOVA of Brown and Forsythe as given below.

$$\text{Group SS} = \sum SS_i = 3018676.1;\ \mathrm{B} = \sum_{i=1}^{k}(1-\frac{n_i}{N})s_i^2 = 172495.78.\ \mathrm{F}'' = \frac{\text{GroupSS}}{\mathrm{B}} = 17.49$$

$$v_1' = 15, v_2' = \frac{\mathrm{B}^2}{\sum_{k=1}^{n}\frac{[(1-n_i/N)s_i^2]^2}{v_i}} = 59.175 \approx 59.\ \text{Hence } \mathrm{F}'' = 17.49 > \mathrm{F}''_{(.05,15,59)} = 1.84 \text{ and so } \mathrm{H_A} \text{ holds.}$$

Thus there is a significant difference between the different measures in the normal state.

D.. TUKEY'S MULTI-COMPARISON TEST

We calculate Ranked Sample Means (μ), given by table.4.

1	2	3	4	5	6	7
56.74375	115.4206	181.0131	307.0938	391.3313	445.3124	737.8938

Table.4. Ranked sample means (μ) for different statistical measures for HRV data in normal states.

To test each $\mathrm{H_0}$: $\mu_B = \mu_A$, we calculate S.E= $\sqrt{\frac{\text{MESS}}{16}} = \sqrt{\frac{28749.3}{16}}$ =42.39; A, B = 1, 2, 3,…., 7.

The result of Tukey's test is summarized in table.5.

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Comparison B vs. A	Difference μ_B-μ_A	SE	q= $\frac{\mu_B - \mu_A}{S.E}$	$q_{0.05,105,k(k=7)}$	Conclusion
7vs1	681.1501	42.39	16.06865	4.24	Reject H_0
7vs2	622.4732	42.39	14.68444	4.24	Reject H_0
7vs3	556.8807	42.39	13.13708	4.24	Reject H_0
7vs4	430.8	42.39	10.16277	4.24	Reject H_0
7vs5	346.5625	42.39	8.175572	4.24	Reject H_0
7vs6	292.5814	42.39	6.902133	4.24	Reject H_0
6vs1	388.5687	42.39	9.166517	4.24	Reject H_0
6vs2	329.8918	42.39	7.782302	4.24	Reject H_0
6vs3	264.2993	42.39	6.234945	4.24	Reject H_0
6vs4	138.2186	42.39	3.260642	4.24	Accept H_0
6vs5	do not test				
5vs1	334.5876	42.39	7.893077	4.24	Reject H_0
5vs2	275.9107	42.39	6.508863	4.24	Reject H_0
5vs3	210.3182	42.39	4.961505	4.24	Reject H_0
5vs 4	do not test				
4vs1	250.3501	42.39	5.905875	4.24	Reject H_0
4vs2	do not test				
4vs3	126.0807	42.39	2.974303	4.24	Accept H_0
3vs1	124.2694	42.39	2.931572	4.24	Accept H_0
3vs2	do not test				
2vs1	do not test				

Table.5. Results of Tukey's Test for different statistical measures for the HRV data in normal states.

It is evident from the table that $\mu_4 = \mu_3$ and $\mu_3 = \mu_1$. But $\mu_4 \neq \mu_1$ and so μ_3 escapes classification. It may come in the first group, as well as in the second group. So, Tukey's Multi-comparison test is not suitable for the purpose. We therefore consider its modification given by Newman-Keuls test. These results are presented in table.6.

Comparison B vs. A	Difference μ_B-μ_A	SE	q= $\frac{\mu_B - \mu_A}{S.E}$	p	$q_{0.05,105,p}$	Conclusion
7vs1	681.1501	42.39	16.06865	7	4.24	Reject H0
7vs2	622.4732	42.39	14.68444	6	4.1	Reject H0
7vs3	556.8807	42.39	13.13708	5	3.92	Reject H0
7vs4	430.8	42.39	10.16277	4	3.69	Reject H0

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7vs5	346.5625	42.39	8.175572	3	3.36	Reject H0
7vs6	292.5814	42.39	6.902133	2	2.8	Reject H0
6vs1	388.5687	42.39	9.166517	6	4.1	Reject H0
6vs2	329.8918	42.39	7.782302	5	3.92	Reject H0
6vs3	264.2993	42.39	6.234945	4	3.69	Reject H0
6vs4	138.2186	42.39	3.260642	3	3.36	Accept H0
6vs5	do not test					
5vs1	334.5876	42.39	7.893077	5	3.92	Reject H0
5vs2	275.9107	42.39	6.508863	4	3.69	Reject H0
5vs3	210.3182	42.39	4.961505	3	3.36	Reject H0
5vs4	do not test					
4vs1	250.3501	42.39	5.905875	4	3.69	Reject H0
4vs2	do not test					
4vs3	126.0807	42.39	2.974303	2	2.8	Reject H0
3vs1	124.2694	42.39	2.931572	2	2.8	Reject H0
3vs2	do not test					
2vs1	do not test					

Table.6. Results of Newman-Keul's Test for different statistical measures for the HRV data in normal states.

From the table 6, we get $\mu_6 = \mu_4$. So the measures corresponding to the mean value pRR20 and SDRR are similar. All other measures are significantly different in the normal state. To verify this argument, we now compute the confidence intervals of the population mean for each of the ranked sample means. These are presented in table.7.

Rank	$\overline{X}_i$	$\overline{X}_i + t_{\alpha(2),v}\sqrt{\frac{S^2}{n_i}}$	$\overline{X}_i - t_{\alpha(2),v}\sqrt{\frac{S^2}{n_i}}$	**Length of the confidence interval of the population mean**
1	56.74375	140.80123	-27.3137	168.115
2	115.4206	199.47808	31.36312	168.115
3	181.0131	265.07058	96.95562	168.115
5	391.3313	475.38878	307.2738	168.115
7	737.8938	821.95128	653.8363	168.115

Table.7. Confidence intervals for significantly different population means.

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Again for the similar measures, pooled sampled mean $\overline{X}_p = \frac{\sum n_i \overline{X}_i}{\sum n_i}, i = 4, 6$ is calculated, which is equal to 376.2031. The confidence interval computed for the similar measures is given by table.8.

Rank	$\overline{X}_p$	$\overline{X}_p + t_{\alpha(2),\nu}\sqrt{\frac{s^2}{\sum n_i}}$	$\overline{X}_p - t_{\alpha(2),\nu}\sqrt{\frac{s^2}{\sum n_i}}$	Length of the confidence interval of the population mean
4,6	376.2031	435.64071	316.7655	118.8752

Table.8. Confidence interval for pooled sample mean.

It is evident from table 7 and 8 that the confidence interval is of minimum length for the common mean $\overline{X}_{4,6}$.

3.2 ON-MUSIC HRV DATA

E. HRV DATA UNDER DIFFERENT STATISTICAL MEASURES

The same seven statistical measures computed for the HRV data of the same 16 subjects (s1- s16), when they listen to some music of special choice is presented in table.9.

Subjects	mRR (ms)	SDRR (ms)	mHR (bpm)	SDHR (bpm)	RMSSD (ms)	pRR20 (%)	pRR50 (%)
s1	635.4	185.8	126.17	199.96	232.2	331	34.8
s2	731.8	126	85.7	47.14	154.2	101	12.2
s3	654.1	240.2	122.4	269.19	285.3	528	61
s4	812	153.1	101.63	569.41	192.1	256	36.7
s5	623.9	215.4	125.66	201.92	266.5	480	52.9
s6	787.4	243.4	114.87	181.82	289.3	342	47.6
s7	645.2	241.4	125.86	140.99	302.8	617	70.4
s8	584.2	234.3	128.09	277.11	319.3	176	18.2
s9	774	261.8	114.34	301.52	308.8	420	57.5
s10	760.8	614.6	170.05	278.36	830	683	91.8
s11	639.8	156.1	120.99	511.1	207.3	122	13.8
s12	642.5	267.9	153.58	290.74	363.6	670	76
s13	675.3	246.9	116.1	226.93	313.5	404	48.2
s14	792.8	675.2	160.53	245.47	907	620	86.8
s15	772.7	675.1	266.94	984.36	818.4	404	55.2
s16	673.4	404.3	160.16	429.19	518.2	616	73.3

Table.9. Different statistical measures for the HRV data of the subjects in On-music states.

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F. BARTLETT'S TEST

To test the equality of the population variances, T_i, CF, $\sum SS_i$, $\sum v_i$, $\sum v_i \log S_i^2$, $\sum \frac{1}{v_i}$ are calculated, which are presented in table.10.

Subjects	mRR (ms)	SDRR (ms)	mHR (bpm)	SDHR (bpm)	RMSSD (ms)	pRR20 (%)	pRR50 (%)		
T_i	11205.3	4941.5	2193.07	5155.21	6308.5	6770	836.4		
T_i^2	7928416	2028828	326180.4	2388468	3363027	3420072	52756.68		
CF	7847422	1526151	300597.3	1661012	2487323	2864556	43722.81		
SS_i	80993.81	502676.2	25583.18	727456.6	875703.5	555515.8	9033.87	2776963	$\sum SS_i$
v_i	15	15	15	15	15	15	15	105	$\sum v_i$
S_i^2	5399.588	33511.75	1705.545	48497.11	58380.23	37034.38	602.258		
$\log S_i^2$	3.732361	4.525197	3.231863	4.685716	4.766266	4.568605	2.779783		
$v_i \log S_i^2$	59.775426	68.085413	45.548894	66.540706	71.643347	72.513623	41.684359	425.79177	$\sum v_i \log S_i^2$
$\frac{1}{v_i}$	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.0666667	0.4666667	$\sum \frac{1}{v_i}$

Table.10. Statistical table comprising $T_i, C.F, \sum SS_i, \sum v_i, \sum v_i \log S_i^2, \sum \frac{1}{v_i}$ in the On-music states.

The pooled variance s_p^2 is calculated as $s_p^2 = \sum_{i=1}^{k} (SS_i) / \sum_{i=1}^{k} v_i = 26447.27$

$$B = 2.30259\left[(\log s_p^2)(\sum_{i=1}^{k} v_i) - \sum_{i=1}^{k} v_i \log s_i^2\right] = 92.118,\; C = \frac{1}{3(k-1)}\left(\sum_{i=1}^{k} \frac{1}{v_i} - \frac{1}{\sum_{i=1}^{k} v_i}\right) = 3.2857143.$$

$$\therefore B_c = (B/C) = 28.034 > \chi^2_{.05,k-1} = 24.996. \text{ Hence } \sigma_1^2 \neq \sigma_2^2 = \dots\dots\dots \neq \sigma_7^2.$$

Hence in this case also, ANOVA cannot be applied. Again the sample size is greater than 10. So we apply modified ANOVA of Brown and Forsythe.

$$\text{Group SS} = \sum SS_i = 2776963;\; B = \sum_{i=1}^{k} (1 - \frac{n_i}{N}) s_i^2 = 158683.6.\; F'' = \frac{\text{Group SS}}{B} = 17.5$$

$$v_1' = 15, v_2' = \frac{B^2}{\sum_{k=1}^{n} \frac{[(1-n_i/N)s_i^2]^2}{v_i}} = 62.03538 \approx 62. \quad \therefore F''=17.5 > F_{(.05,15,62)} = 1.84.$$

So there is a significant difference between different measures of HRV in on-music state.

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G. TUKEY'S MULTIPLE COMPARISON TEST

The calculated Ranked Sample Means (μ) are given by table.11.

1	2	3	4	5	6	7
52.275	137.0669	308.8438	322.2006	394.2813	423.125	700.3313

Table.11. Ranked sample means (μ) for different statistical measures for the HRV data in On-music states.

To test each H_0:μ_B= μ_A, we find S.E= $\sqrt{\frac{\text{MESS}}{16}} = \sqrt{\frac{26447.26}{16}}$ =40.65654.

The results are summarized in table.12.

Comparison B vs. A	**Difference μ_B-μ_A**	**SE**	$q = \dfrac{\mu_B - \mu_A}{\text{S.E}}$	$q_{0.05,105,7}$	**Conclusion**
7vs1	648.0563	40.65654	15.93978	4.24	Reject H0
7vs2	563.2644	40.65654	13.85421	4.24	Reject H0
7vs3	391.4875	40.65654	9.629139	4.24	Reject H0
7vs4	378.1307	40.65654	9.300611	4.24	Reject H0
7vs5	306.051	40.65654	7.527718	4.24	Reject H0
7vs6	277.2063	40.65654	6.818245	4.24	Reject H0
6vs1	370.85	40.65654	9.121534	4.24	Reject H0
6vs2	286.0581	40.65654	7.035968	4.24	Reject H0
6vs3	114.2812	40.65654	2.810893	4.24	Accept
6vs4	Do not test				
6vs5	Do not test				
5vs1	342.0053	39.5424	40.65654	8.412061	Reject H0
5vs2	257.2134	39.5424	40.65654	6.326495	Reject H0
5vs3	Do not test				
5vs4	Do not test				
4vs1	269.9256	40.65654	6.639168	4.24	Reject H0
4vs2	185.1337	40.65654	4.553602	4.24	Reject H0
4vs3	Do not test				
3vs1	256.5688	40.65654	6.31064	4.24	Reject H0
3vs2	171.7769	40.65654	4.225074	4.24	Reject H0
2vs1	84.7919	40.65654	2.085566	4.24	Accept

Table.12. Results of Tukey's Multi-comparison Test for different HRV data in On-music states.

From the above table it is found that $\mu_1 = \mu_2$ and $\mu_6 = \mu_3$ i.e., mean value of corresponding measure MHR and pRR50; pRR20 and SDRR are similar. All other measures are significantly different. For verifying this argument,

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the confidence intervals for the population mean of significantly different measures and of similar measures in on-music state are computed. These are given by table.13 and table.14 respectively.

Rank	$\overline{X}_i$	$\overline{X}_i + t_{\alpha(2),v}\sqrt{\frac{S^2}{n_i}}$	$\overline{X}_i - t_{\alpha(2),v}\sqrt{\frac{S^2}{n_i}}$	Length of the confidence interval of the population mean
4	322.2006	402.8225	241.5787	161.24384
5	394.2803	474.9022	313.6584	161.24384
7	700.3313	780.9532	619.7094	161.24384

Table.13.Confidence interval for population mean of significant different measures in on-music states.

Rank	$\overline{X}_p$	$\overline{X}_p + t_{\alpha(2),v}\sqrt{\frac{s^2}{\sum n_i}}$	$\overline{X}_p - t_{\alpha(2),v}\sqrt{\frac{s^2}{\sum n_i}}$	Length of the confidence interval of the population mean
6,3	365.9844	422.9927	308.9761	114.0166
2,1	94.67095	151.6793	33.6626	114.0166

Table.14. Confidence interval for population mean of similar measures of On-music states.

From table 13 and 14, it is found that lengths of the intervals of confidence of the common population mean $\overline{X}_{6,3}$ and $\overline{X}_{1,2}$ are same and they give the minimum length. In normal state the measures pRR20 and SDRR are similar and the rest are all significantly different. In on-music state there are two sets of similar measures. One is MHR and pRR50, and the other one is pRR20 and SDRR. The rest are significantly different. Finally it may be concluded that the common similar measures in normal and on-music state are pRR20 and SDRR only. The length of the confidence intervals of the corresponding population means also supports the above conclusions. We call these two as similar measures and consider SDRR from them as the similar measure for further analysis. The rest measures are taken as dissimilar measures.

H. TWO FACTORS ANOVA

To establish that the pre-music/normal and the on-music states differ significantly with respect to the aforesaid one similar and five dissimilar measures and also to cross verify the significant difference between the similar and dissimilar time-domain measures [already established in section 3.2], two factors ANOVA is performed. In this case the factors are denoted by A and B respectively, where the factor A has two components viz. similar measure and dissimilar measures and the factor B has two components viz. pre-music states, on-music states. The test is done at 0.95

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confidence level. The similar and dissimilar measures and the related calculations for pre-music / normal states and on-music states are given by table.15 and table.16 respectively.

		Similar Measure	Dissimilar Measures					
	Subjects	SDRR	mRR	mHR	SDHR	RMSSD	pRR50	
	s1	483	605.8	153.72	349.69	628.2	85.3	
	s2	147.8	883.6	73.64	57.82	185.5	32.7	
	s3	178.7	669.3	100.9	79.43	219.1	24.7	
	s4	659.9	713	134.62	243.96	752	77.5	
	s5	240.6	623.3	117.31	66.95	289.6	69.7	
	s6	184.8	849.8	79.76	72.69	243.9	45.1	
	s7	281.9	648.5	124.98	125.82	337.5	75.4	
	s8	158.8	639.9	101.9	94.38	214.5	14.9	
	s9	231.1	792.7	103.07	130.91	279.5	40.3	
	s10	328.1	842.1	108.26	145.12	421.5	70.3	
	s11	267.9	642.5	153.58	290.74	363.6	76	
	s12	512.5	715.1	150.34	503	682	76.2	
	s13	233.8	749.8	104.08	108.68	288.1	69.3	
	s14	144	912.1	67.85	28.74	181	34.1	
	s15	719.6	796.6	185.5	564.04	1000.5	88.9	
Pre-music	s16	141	722.2	87.22	34.24	174.8	27.5	
		T1	T3	T4	T5	T6	T7	T
		4913.5	11806.3	1846.73	2896.21	6261.3	907.9	28631.94
		S1	S3	S4	S5	S6	S7	S
		2027847	8856712	229469.4	933643.1	3346256	60534.37	15454462

Table.15. Normal / Pre-music HRV data under Similar/Dissimilar measures.

		Similar Measures	Dissimilar Measures				
	Subjects	SDRR	mHR	pRR50	mRR	SDHR	RMSSD
	s1	185.8	126.17	34.8	635.4	199.96	232.2
	s2	126	85.7	12.2	731.8	47.14	154.2
	s3	240.2	122.4	61	654.1	269.19	285.3
	s4	153.1	101.63	36.7	812	569.41	192.1
	s5	215.4	125.66	52.9	623.9	201.92	266.5
	s6	243.4	114.87	47.6	787.4	181.82	289.3
	s7	241.4	125.86	70.4	645.2	140.99	302.8
On-music	s8	234.3	128.09	18.2	584.2	277.11	319.3

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	s9	261.8	114.34	57.5	774	301.52	308.8	
	s10	614.6	170.05	91.8	760.8	278.36	830	
	s11	156.1	120.99	13.8	639.8	511.1	207.3	
	s12	267.9	153.58	76	642.5	290.74	363.6	
	s13	246.9	116.1	48.2	675.3	226.93	313.5	
	s14	675.2	160.53	86.8	792.8	245.47	907	
	s15	675.1	266.94	55.2	772.7	984.36	818.4	
	s16	404.3	160.16	73.3	673.4	429.19	518.2	
		T1'	T3'	T4'	T5'	T6'	T7'	T′
		4941.5	2193.07	836.4	11205.3	5155.21	6308.5	30639.98
		S1'	S3'	S4'	S5'	S6'	S7'	S′
		2028827.63	326180.4307	52756.68	7928415.57	2388468.486	3363026.79	16087675.59

Table.16. On-music HRV data under Similar/Dissimilar measures.

The similar measure for pre-music and on-music states and the dissimilar measures for pre-music / normal state and on-music states are summarized in table.17 and table.18a, 18b respectively.

Similar measure in Normal / Pre-music states		Similar measure in On-music states	
SDRR		SDRR	
483		185.8	
147.8		126	
178.7		240.2	
659.9		153.1	
240.6		215.4	
184.8		243.4	
281.9		241.4	
158.8		234.3	
231.1		261.8	
328.1		614.6	
267.9		156.1	
512.5		267.9	
233.8		246.9	
144		675.2	
719.6		675.1	
141		404.3	
T1	TS1	T1'	TS2
4913.5	4913.5	4941.5	4941.5

Table.17. Similar measure in Normal / Pre-music and On-music states.

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Normal / Pre-music states					
Dissimilar Measures					
mRR	**mHR**	**SDHR**	**RMSSD**	**pRR50**	
605.8	153.72	349.69	628.2	85.3	
883.6	73.64	57.82	185.5	32.7	
669.3	100.9	79.43	219.1	24.7	
713	134.62	243.96	752	77.5	
623.3	117.31	66.95	289.6	69.7	
849.8	79.76	72.69	243.9	45.1	
648.5	124.98	125.82	337.5	75.4	
639.9	101.9	94.38	214.5	14.9	
792.7	103.07	130.91	279.5	40.3	
842.1	108.26	145.12	421.5	70.3	
642.5	153.58	290.74	363.6	76	
715.1	150.34	503	682	76.2	
749.8	104.08	108.68	288.1	69.3	
912.1	67.85	28.74	181	34.1	
796.6	185.5	564.04	1000.5	88.9	
722.2	87.22	34.24	174.8	27.5	
T3	T4	T5	T6	T7	TDS1
11806.3	1846.73	2896.21	6261.3	907.9	23718.44
S3	S4	S5	S6	S7	
8856712.09	229469.3783	933643.0697	3346255.53	60534.37	

(a)

On-music States				
Dissimilar Measures				
mHR	**pRR50**	**mRR**	**SDHR**	**RMSSD**
126.17	34.8	635.4	199.96	232.2
85.7	12.2	731.8	47.14	154.2
122.4	61	654.1	269.19	285.3
101.63	36.7	812	569.41	192.1
125.66	52.9	623.9	201.92	266.5
114.87	47.6	787.4	181.82	289.3
125.86	70.4	645.2	140.99	302.8
128.09	18.2	584.2	277.11	319.3

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114.34	57.5	774	301.52	308.8	
170.05	91.8	760.8	278.36	830	
120.99	13.8	639.8	511.1	207.3	
153.58	76	642.5	290.74	363.6	
116.1	48.2	675.3	226.93	313.5	
160.53	86.8	792.8	245.47	907	
266.94	55.2	772.7	984.36	818.4	
160.16	73.3	673.4	429.19	518.2	
T3'	T4'	T5'	T6'	T7'	TDS2
2193.07	836.4	11205.3	5155.21	6308.5	25698.48
S3'	S4'	S5'	S6'	S7'	
326180.4307	52756.68	7928415.57	2388468.486	3363026.79	

(b)

Table.18. Dissimilar measures in (a) Normal / Pre-music states, (b) On-music states.

Now,

TS=Total Similar = 9855, Total Dissimilar = 49416.92, Cell Sum of Square = 1120979.411,
Within Cell Sum of Square = 14737405.45,
Factor A SS= TS2/64+TDS2/ (160-CF) =3.7272E+16, the level of Factor A is a = 6, DF = 5.
Factor B SS= (T2+T12) / (112-CF) =1.38698E+16, the level of Factor B is b= 2, DF =1.
The above calculations are summarized in table. 19.

	SS	DF	MSS
Factor A	3.73E+16	5	7.4544E+15
Factor B	1.39E+16	1	1.387E+16
Error	14737405	220	66988.2066

Table.19. Summary of the results obtained in two factors ANOVA.

Note that the factor $A \times B$ is not considered here as it is not relevant in this context.

$$\therefore F_A = \frac{7.4544E+15}{66988.2066} = 111279292729.747 > F_{(0.05,5,220)} = 4.39.$$

So there is a significant difference between similar/dissimilar measures.

$$\text{Again, } F_B = \frac{1.387E+16}{66988.2066} = 207047839723.815 > F_{(0.05,1,220)} = 3.89.$$

So there is a significant difference between pre-music/normal and on-music states.

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IV CONCLUSIONS

In the present article, the effect of music on HRV has been studied through statistical analysis of different time domain measures [12] available for HRV. For this purpose, the analysis of normal/pre-music and on-music data is taken up sequentially. Firstly, the homogeneity of population variances is tested by Bartlett hypothesis test [13], which fails in both cases. So instead of ANOVA, modified ANOVA of Brown and Forsythe [13] is applied, which shows that all the measures are significantly different. Thus to find exactly the measures, which cause the significant difference, Tukey's multiple comparison test [13] is applied for on-music data and Newman-Keul's test, a modified form of Tukey's test is applied for normal/pre-music data by which one similar measure and five dissimilar measures are identified. With these similar and dissimilar measures two factor ANOVA [13] is performed, which shows that there is a significant effect of music, in the sense that the measured values of HRV data in the normal/pre-music and on-music states do differ significantly. The main goal of this present study is to highlight the application of different statistical tests on bio-medical signals like HRV and at the same time to identify the relevant time domain measures in order to distinguish the pre-music/normal and on-music states.

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