Heart rate variability: Linear and non-linear analysis of pre-awake period for normal and intrauterine growth restricted children at 10 yr

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1.0. Introduction:-

Heart rate variability (HRV) is a powerful non-invasive tool reflecting autonomic cardiac function that predicts death and risk of arrhythmic complication in humans [1]. Reduced HRV is known to be associated with increased risk of cardiac mortality in adults [2]. HRV is defined as the variation over time of the interval between consecutive heart beats, and it is predominantly dependent on the extrinsic regulation of the heart rate. . The HRV reflects the ability of the heart to respond to any unpredictable stimuli and can be used to assess the overall cardiac health and the state of the autonomic nervous system (ANS), which is responsible for balancing cardiac activity [3]. Heart rate is controlled by the sympathetic and the parasympathetic branches of the ANS, the parasympathetic (vagal) branch causes the heart to slow down by means of releasing acetylcholine, while the sympathetic nerves cause the heart to beat faster through the release of noradrenalin [4]. The alterations in the ANS function reflected by reduced HRV are known to be associated with increased risk of cardiovascular mortality.

The definition of intrauterine growth restricted (IUGR) children by the world health organisation (WHO) is for birth weight below the 10 percentile for gestational age [5]. IUGR may result in significant fetal morbidity and mortality if not adequately diagnosed and managed. In UK the percentage of low birth weight is around 7% according to the 2007 UK government office for national statistics [6] and in America alone 350,000 children are born weighing less than 2.50 kg, and one third of them, approximately 100,000 have true IUGR and the rest are defined as small for gestational age (SGA) [7]. Low birth weight (LBW) is considered as the marker of fetal growth restriction. A baby having birth weight less than 2.5 kg is low birth weight (world health organization 1993). In our previous study, we have examined alterations of HRV parameters in low birth weight IUGR children (birth weight <2.5 kg) and compared the results with normal and IUGR children having both weight > 2.5 kg by analysing 24 hour RR –interval time series data of 9-10 years old children . We found a significant difference in most of HRV measures in low birth weight IUGR children compared to normal and those IUGR children having birth weight > 2.5kg. In the present

study, linear and non linear HRV measures are used to analyse the 15 min pre-awake period RR-interval time series data of normal and growth restricted children and compared the results 24 hour RR-interval time series data of these groups to find the correlation between them. The 15 min pre-awake period was chosen due to the fact that several studies proved that the occurrence of sudden death (SD) during 24 hrs shows that the peak time for sudden death is from 6 am to mid day. Sudden death occurs to 20 % of people with obstructive upnea, 41% for people without obstructive upnea, and 30% for general population [8]. When studying the circadian variability in the occurrence of sudden death in patient with hypertrophic cardiomyopathy, it's found that SD occurs with increased frequency after awaking during morning hours (usually 6:00am to noon) [9] and the same findings is supported by Thakur , where he found that sudden death occurrence rate is low from midnight to 6:00 am and a 2.4-fold increase between the rate at 6:00 and the rate at noon. [10]. The selection of 15 min pre-awake is to detect any alteration in HRV measures at the beginning of the period when the rate of sudden death is high.

2.0. Materials and Data

In co-operation with parents, researchers and staff at the Leicester Royal Infirmary have compiled biometric data from a demographic of 70 children, both at birth and again at approximately 10 years of age. Data collected includes body mass index, head circumference measurements, medical history and prescriptions, creatinine to cortisol ratio, blood pressure readings, parental smoking and maternal smoking history.

The ECG was recorded over a period of 24 hours, at a sample rate of 128 Hz (every 7.8125 ms). A 24-hour ECG at 10 years old for each child was taken, starting at approximately 3PM. This was provided with parentally noted number of sleep hours. The ECGs of the study population will be analysed according to birth weight of the child (IUGR or not). RR data was extracted using Pam and Tompkins QRS detector from the raw ECG for all children and used for the analysis. The QRS detection was done by filtering the ECG signal through a second order Butterworth band pass filter, with comparison to an adapting threshold. The lower cut-off frequencies for the band pass filter were set to 14Hz, and the upper frequency to 24Hz. The threshold was set to 65% of the previous detection, the QRS was found using the filtered ECG signal, and from the QRS the RR intervals could be extracted. The particular QRS detector that was used had previously been employed in another HRV study done by [11].

The recording was done by a Del Mar Reynolds Medical Limited Lifecard CF ambulatory recorder. Table 1 shows the classification of the RR data available for this study, the first row gives the details of the groups of the children involved in the study, the second row shows the birth weight details and the last is gender related data, which states how many males and females are in each group.

Table 1:- children groups in 15 min pre awake analysis

	Normal	IUGR-1	IUGR-2	Total IUGR
Recordings	33	20	17	37
Birth Weight (kg)	3.53 ± 0.48	2.29 ± 0.19	2.92 ± 0.36	2.58 ± 0.42
Gender (Male/Female)	21/12	08/12	08/09	16/21

The intrauterine growth Restricted (IUGR) children were divided into two groups depending on their birth weight, so IUGR 1 group is for any IUGR child who has birth weight < 2.5 kg, and IUGR2 group for IUGR children who have birth weight > 2.5 kg [5].

3. Method:-

3.1. Linear HRV measures:-

3.1.1. Time domain analysis:-

Initially time domain analysis was used to compare the results of the 15 min with those of the 24 hr. Two types of measures were used, a) those time domain measures which derived directly from NN intervals or instantaneous heart beat, (b) those which are derived from the differences between consecutive NN. These two types can be derived from small segment of data or all 24 hr data, segments of NN can be used to analyse data when the subject is in different periods of day such as awake, sleep), tilted, rest [12]. The most common time domain measure is the standard deviation of the NN intervals (SDNN), i.e. the square root of variance, where variance is equal to total power of spectrum analysis. SDNN has been standardised into SDANN, the SD of the average NN over 5 min, and SDNN index is over 24hr NN data. Both SDNN and SDANN are time dependent measures. Another common HRV measure is derived from the consecutive differences of NN, RMSSD, which is the measure of high frequency HRV and it reflects the integrity of vagus nerve - mediated by the heart. RMSSD is used as a marker of sudden unexplained death in epilepsy [13]. NN50 is the number of successive NN intervals whose difference is higher than 50 ms, and pNN50, obtained by dividing NN50 by the total number of NN intervals. The last four measures are estimates of high frequency variation. These statistical measures were found to have higher values for conditions such as pre-ventricular contraction (PVC), sick sinus syndrome (SSS) and atrial fibrillation (AF), because of higher variation of RR, and these parameters will have smaller values when RR variation is low, such as in complete heart block (CHB), left bundle branch block (LBBB), and ischemic/dilated cardiomyopathy. NN50 is one member of general NN_x family used to discriminate between normal and different pathological conditions, x value or threshold can be from 50, the standard, to 12 ms [14].

TINN is a geometrical measure of HRV and it is highly insensitive to the quality of NN because the artifacts and ectopic beats are left outside the histogram (triangle). Figure 1

(top), shows the total RR data for a normal male child, one of RR files used in this study. It can be seen very clearly the start of the RR intervals in day time where RR value is smaller than the night RR values. The high RR value during night time is due to the dominance of the parasympathetic branch of the ANS, and for the day time, the sympathetic branch is dominant. Figure 1 (bottom) is an enlargement of the selected 15 min before arousal. The 15 min data are detrended.

Figure 1: 24 hour RR-interval time series data (top) and 15 min pre-awake RR-interval time series data (bottom)

The NN interval histogram is a geometric tool useful for assessing HRV, or the sample density distribution. The measures derived from the histogram are the HRV index = (total number of NN intervals) / Y, where Y is the maximum value. TINN is expressed in ms and these two measures express overall HRV measured over 24 hr and are more influenced by the lower than by the higher frequencies. The major disadvantage is a considerable number of beats is required to construct the geometrical pattern. In figure 2, a histogram of RR interval is shown to the left and a histogram of heart rate is shown to the right, both for a normal male child. Note that the RR histogram is not a normal distribution curve (bell shape).

Figure 2- The histogram of the RR to the left and HR (right) for a normal child

3.2.2. Frequency domain analysis

After frequency domain analysis frequency parameters were identified, these are VLF, LF, HF, and the ratio of LF/HF was calculated for every child, and then analysed to study the behaviour of all the groups. The power spectrum can be calculated by many methods such as FFT or AR. The latter was used in this study. Figure in 2.5 left, is the FFT average spectrum of all data spectrums after window 256 seconds and 50 % overlap to overcome the problem of stationary which characterises the RR signal and any biological signal and reduce the leakage when using FFT. Figure 3 right shows AR-based spectral analysis which has advantages over the FFT that its spectral resolution is better for short data segments and that it produces a smoother spectral estimator. FFT-based spectral estimation is faster in terms of signal processing time.

Figure 3 shows the power spectrum of FFT (left) and AR (right) for a normal male child

The frequency bands shown in the above figure, are VLF (0 to 0.04Hz), LF (0.04-0.15Hz), and HF (0.15-0.4 Hz) [15]

3.4. Non-Linear HRV measures:-

The non-linear HRV measures used in the analysis include Poincaré plot, detrended fluctuation analysis, approximate entropy, and sample entropy.

Poincaré plot is a way of presenting the nature of RR interval fluctuation; it is a plot of RR as a function of the previous RR. The plot is a visual and a quantitative tool to assess the HRV. The graph gives us two important quantities, SD1 and SD2. SD1 represents the short-term heart rate variability, and SD2 represents the long term variability. SD1/SD2 is the ratio between the two measures, and its value is high in case of PVC, AF, SSS and VF due to high short term variation and low for CHB, Ischemia/dilated cardiomyopathy [16, 17].

Poincaré plot is a graphical representation that shows the correlation between consecutive RR intervals, which means a plot of RR against the previous RR, (RR_n vs. RR_{n-1}) [18]. The shape of the scattered plot is important in the data interpretation and from the plot an elliptic shape is usually fitted around it and its axes, SD1 which describes the short term variability, and SD2, and the long term variability can be obtained.

Figure 4 .- Poincaré plot (left), and DFA plot (right) for a normal male child

The above figures show Poincaré plots and DFA graphs which are used in interpretation of the HRV analysis. SD1 reflects the short term variability of the time series data and SD2 reflects the long term variability. Both were recorded for all IUGR and Normal and same was done with α_1 and α_2 .

Another non-linear parameter is approximate entropy (ApEn), which measures the complexity of the RR signal, with high values meaning high complexity, and low values meaning a more regular signal [19]. Sample entropy is similar to ApEn [20], but it reduces the bias of ApEn and has an agreement with the theory regarding data with probabilistic content, DFA is detrended fluctuation analysis to measure the correlation within the signal. In DFA two parameters are calculated (α_1 , α_2), these are scaling exponents which are the slopes of the regression line relating $\log F(n)$ to $\log n$. $F(n)$ is a function of function of segment length n [21]. α_1 measures the short term fluctuation, and α_2 represents long term fluctuation.

4.0. Results:-

Table 2, shows the average of all HRV parameters with the standard deviation for 15 min pre-awake RR data. Column 1 has a list of all HRV indices, the second column is the HRV measures for all normal children, the 3rd column shows all HRV measures for all IUGR, and the last is the p value of the comparison between the normal and IUGR.

Table 2: HRV measures for normal and IUGR children in 15 min pre-awake

HRV Measure	Normal	IUGR	p-value
RRmean	757.72±76.66	754.19±80.26	0.6224
SDNN	106.672±34.845	99.104±40.123	0.2407
SDANN	48.943±39.364	35.955±26.16	0.1609
HR	82.13±8.89	82.38±8.55	0.7411
RMSSD	88.72±41.46	77.08±44.92	0.1599
NN50	432±166.49	390.83±168.80	0.5481
pNN50	35.89±15.05	32.91±16.44	0.4935
RR triaIndex	531.51±142.71	500.42±142.71	0.2272
TINN	531.51±142.71	500.42±142.71	0.2272
LF	2.842±1.882	2.532±1.896	0.2656
HF	4.336±3.703	3.338±3.739	0.0886
LF/HF	0.924±0.731	1.216±0.859	0.0886
SD1	62.88±29.38	56.42±31.03	0.2588
SD2	151.17±55.57	141.66±54.44	0.4711
ApEn	1.22±0.21	1.26±0.14	0.3550
SampEn	1.14±0.39	1.21±0.29	0.3426
$\alpha 1$	0.89±0.21	0.94±0.24	0.3158
$\alpha 2$	0.91±0.17	0.96±0.16	0.2639

Table 3, give us the HRV measures for 24 RR data, where column 1 lists all the HRV measures and Column 2 and 3 shows the normal children and the IUGR HRV values respectively, and the last column gives us if there is any significant difference between the normal and the IUGR children in the form of p value.

Table 3: HRV parameters of normal Vs IUGR for 24hr

HRV Measures	Normal	IUGR	p-value
SDNN	156.82±28.24	152.82±35.21	0.6053
SDANN	77.65±20.46	76.87±21.33	0.8766
RMSSD	70.32±28.86	69.58±33.62	0.9226
NN50	28290±11179	29870±12830	0.5867
pNN50	23.55±10.51	24.64±11.99	0.6893
LF	1966±969	1840±907	0.5742
HF	2704±2177	2678±2724	0.9647
LF/HF	0.92±0.38	1.16±0.78	0.1542
SD1	49.72±20.41	54.81±28.28	0.3962
SD2	215.63±37.42	205.16±53.32	0.3507
Apen	1.14±0.08	1.16±0.09	0.1908
SamEn	1.07±0.08	1.10±0.13	0.1269

After dividing the IUGR children into two categories; IUGR1 for those whose birth weight (BW) < 2.5 kg and the IUGR2 for those whose BW > 2.5kg, HRV values were compared between all three groups of children and the p value for significant difference was recorded. This table is for the 24 hour RR data. The p value which shows significant difference between the groups is coloured in red, and table 5 is regarding the HRV analysis of the 15 min pre-awake.

Table 4: HRV measures for normal Vs IUGR1, IUGR2 children for 24 hr period

HRV measures	Normal	IUGR1	IUGR2	normalVsIUGR1 (p value)	IUGR1VsIUGR2 (p value)	NormalVsIUGR2 (p value)
SDNN	156.81±28.24	136.99±25.22	171.45±36.78	0.0129	0.0019	0.138
SDANN	48.943±39.364	66.66±11.27	88.87±24.28	0.0322	0.0008	0.091
RMSSD	70.32±28.86	53.72±18.78	86.25±37.93	0.0262	0.001	0.0681
NN50	28290±11179	25139±10115	35435±13712	0.308	0.0128	0.0534
pNN50	23.55±10.51	19.83±8.87	30.30±12.93	0.1916	0.0063	0.0525
LF	1966±2214	1418±456	2336±1057	0.0217	0.0012	0.2207
HF	2704±2178	1548±1102	4007±3433	0.0321	0.0046	0.1078
LF/HF	0.92±0.38	1.23±0.66	1.03±0.88	0.0384	0.4379	0.5602
SD1	49.72±20.41	41.56±17.64	70.41±30.85	0.144	0.0011	0.0066
SD2	215.63±37.42	182.37±39.32	231.97±56.11	0.0209	0.0033	0.2249
Apen	1.14±0.08	1.17±0.09	1.15±0.08	0.4423	0.1198	0.5003
SamEn	1.07±0.08	1.13±0.13	1.07±0.12	0.0209	0.0328	0.8684

Table 5 : HRV parameters for normal, IUGR-1 and IUGR-2 children for 15 min pre-awake

Table 5: HRV measures of Normal and IUGR children for 15 min pre-awake						
HRV	normal	IUGR1	IUGR2	Normal VsIUGR1 (p value)	IUGR1VsIUGR2 (p value)	NormalVsIUGR2 (p-value)
RRmean	757.72±76.66	743.59±75.55	767.45±86.41	0.4142	0.3477	0.949
SDNN	106.672±34.845	86.38±28.99	122.9±43.01	0.0234	0.0071	0.3428
SDANN	48.943±39.364	35.936±21.33	34.167±30.364	0.37162	0.36857	0.10355
HR	82.13±8.89	82.97±8.57	81.64±8.75	0.6530	0.5994	0.9575
RMSSD	88.72±41.46	55.75±24.63	103.74±50.73	0.0023	0.0019	0.3011
NN50	432±166.49	333.25±157.13	462.81±158.95	0.0862	0.0269	0.3593
pNN50	35.89±15.05	27.3±14.16	39.93±16.79	0.0813	0.0466	0.4240
RRtrialIndex	22.42±6.99	19.48±6.62	24.11±9.25	0.1301	0.2203	0.8229
TINN	531.51±142.71	451±133.04	562.19±196.04	0.0425	0.0415	0.8395
LF	2.842±1.882	1.791±1.122	3.512±2.289	0.0475	0.0458	0.49483
HF	4.336±3.703	1.876±1.665	1.38±0.95	0.0026	0.0214	0.66676
LF/HF	0.924±0.731	1.38±0.95	0.92±0.57	0.0475	0.1477	0.9405
SD1	62.88±29.38	42.7±17.52	73.57±35.96	0.0084	0.0044	0.2962
SD2	151.17±55.57	123.78±42.12	164.02±61.12	0.0721	0.0269	0.424
ApEn	1.22±0.21	1.28±0.12	1.25±0.17	0.3041	0.5994	0.6621
SampEn	1.25±0.25	1.14±0.39	1.15±0.33	0.2121	0.2452	0.8229
α_1	1.01±0.22	0.89±0.21	0.86±0.24	0.0781	0.1011	0.7981
α_2	0.97±0.13	0.91±0.17	0.95±0.19	0.2121	0.5774	0.6014

Discussion:-

In this study we are looking at the 15 min period before awakening. Table 1, shows details of the cohort (70) and the number of the other groups, normal children are 33, and all IUGR are 37 children. They are divided into two groups, IUGR 1 (20 children) and IUGR2 (24). In this work comparison between the normal group and IUGR was done, further on, another compared results was obtained between the IUGRs with regard to their birth weight.

Tables 2 and 3 compare between Normal and IUGR children, for 24hrs and for 15 min pre-awake, it can be seen that the p values show no significant difference between the two groups, this is in time domain, frequency domain and non-linear measures, but one can see that the means of all time domain indices (except for heart rate) for normal are higher than IUGR although not significantly different. The means of all non-linear measures are higher for IUGR than for normal but again this difference is not statistically significant ($p>0.05$). When the IUGR group was divided into IUGR1, IUGR2, and then compared with the normal group, very interesting results were found in all HRV measures, which include time domain, frequency domain, and non-linear indices.

Regarding time domain, the RR from table 2, it can be seen that ($p>0.05$) when looking at normal group and IUGR for only 15 min pre awake, but when we analyse SDNN between normal and IUGR it was found that ($p=NS$) Although it is clear that the longer the data, the higher the SDNN SDANN of the groups are almost equal because we are looking at the short term variability over averaged 5 min segments. When the differences between RR

intervals were analysed (RMSSD, NN50, PNN50) , we can see that all three are higher in normal than IUGR in 15 min , but in 24 hr , RMSSD is higher in normal , and NN50, PNN50 are higher in IUGR, but again these differences are not statistically significant.

When we analyse table 4, it is clear that the time domain measures show a significant difference between the normal and IUGR1 (birth weight less than 2.5 kg), where SDNN and SDANN have the lowest values, and it is confirmed that low SDNN is associated with IUGR1 group and this has been said to be a marker of sudden death [22], and low value of RMSSD maybe associated with complete heart block (CHB), left bundle branch block (LBBB), and ischemia/dilated cardiomyopathy and this is because of small RR variation [23,24]. The other measures that show statistically significant differences are LF, HF and LF/HF. Low HF is a marker of sudden death [25], and high ratio LF/HF is associated with CHB and ischemia/dilated cardiomyopathy [26]. SD2 which is related to the long term variability, gives ($p < 0.05$).

ApEn gives $p > 0.05$, but SamEn shows $p < 0.05$, when comparing normal and IUGR1 and when comparing IUGR1 and IUGR2. As we know that SamEn is a measure of complexity of the biological signal and here SamEn is lower in IUGR1 and higher in the other two groups. Normally AF patients have lower SamEn [27].

Table 5 summarises the results of 15 min pre-awake as we compare normal with IUGR1 and IUGR2, as well as IUGR1 against IUGR2. The HRV measures show high correlation between the 15 min and the 24 hr data. The p value is < 0.05 for SDNN, RMSSD, NN50, pNN50, LF/HF in normal against IUGR1, and IUGR2 against IUGR1. Regarding normal against IUGR2, the ($p > 0.05$) for all HRV indices.

Conclusions

The analysis of the results shows a very high correlation between the short term recording of ECG (15 min pre-awake data) and the long ECG recordings (24 h). When comparing normal children with IUGR children we find no significant differences, this is due to the fact that many of IUGR are in fact normal children or their development of the autonomic nervous system (ANS) is as good as the normal counterparts. After dividing the IUGR children into IUGR1 and IUGR2, we find very interesting results with regard to the development of the autonomic nervous system of the IUGR children. The low value of SDNN for IUGR1 which is found in medical research associated with sudden death can be an important marker when looking after IUGR1 children. The low values of RMSSD, NN50, pNN50, RRtrianindex and TINN in IUGR1, do represent low variability in RR and this found to be the case in complete heart block (CHB), left bundle branch block (LBBB), and ischemic/dilated cardiomyopathy. An important frequency domain result is the high value of the ratio LF/HF (which is the measure or an index of autonomic balance or the balance between the sympathetic and

parasympathetic branches of ANS) for 15 min pre-awake period in IUGR1. This high value of LF/HF in IUGR1, suggests future association with cardiac heart failure (CHF).

All these findings do suggest the importance of looking after the cardiovascular health of IUGR children from birth, especially the IUGR children whose birth weight is less than 2.5 kg.

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