HEART RATE VARIABILITY IN 10 YEAR OLD CHILDREN:

NORMAL AND INTRAUTERINE GROWTH RESTRICTED

A thesis submitted for the degree of

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by

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Declaration of Originality:

A thesis submitted in fulfilment of the requirements for the degree of Doctor of philosophy in the Department of Engineering, University of Leicester, UK.
All work presented in this thesis is Original unless otherwise acknowledged in the text or by references.

Signed:…….. Date:……

Taher Biala

In the Name of Allah, the most Beneficent and the most Merciful

DEDICATION

Dedicated with love to my Parents, wife, sons, my family and friends

ABSTRACT

Heart rate variability (HRV) analysis is a powerful non-invasive tool used to determine the state of the heart and assess the development of the Autonomic Nervous System (ANS). The reduced HRV has been associated with negative outcome of ANS. This work describes the results obtained by HRV analysis of two groups of children, 41 Intrauterine growth retarded (IUGR) and 34 normal for a period of 24 hours. The main objective of this work was to explore the autonomic control in IUGR children by performing HRV analysis and comparing the results with normal children to find differences in HRV at 10 years of age. Barker theory and hypothesis states that the IUGR can be prone to coronary heart diseases or hypertension in their adulthood.

Signal processing was performed on the ECG signal (data) provided which included filtering and detecting the QRS to find the RR segments. In the time domain analysis many parameters were calculated for all 75 children. Several comparisons between IUGR and normal children groups using night time and one hour RR data collected at night using several variables were computed. Frequency domain analysis of RR has been performed by autoregressive model (AR) Fast Fourier Transform (FFT) after re-sampling RR data of all 75 children. Calculation of the frequency components, large frequency, high frequency, and ratio of LF/HF, were obtained using FFT, AR and Lomb periodogram. Statistical analyses were performed to compare between IUGR and normal children. Time and frequency analysis comparison between the two groups of children showed no significant statistical differences, but the analysis has shown significant difference when dividing IUGR into IUGR1 (< 2.5 kg) and IUGR2 (≥ 2.5 kg), and highlighted differences in Respiratory Sinus Arrhythmia (RSA) at night time. QT algorithm was developed to measure HR, QRS, ST and QT. It was found that QT for IUGR children is slightly higher than that of normal children. Using Poincaré plots, significant difference was found between female and male children. Females had low long term variability. The 24 hr and 15 min pre-wake HRV time domain and Frequency domain analysis showed that IUGR children have reduced HRV which is a marker of a negative outcome of the ANS.

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Table of Contents

List of Figures

List of Tables

List of Publications

Journals:

"Heart rate variability: Linear and non-linear analysis of pre-arousal period for normal and intrauterine growth restricted children at 10 yr", Biala, T.A., Schlindwein, F.S., Aziz, W., Wailoo, M., (accepted 2012).

"Heart Rate Variability Analysis of Normal and Intrauterine Growth Restriction Children Using Linear and Non Linear Techniques", Aziz, W., Schlindwein, F.S., Wailoo M., Biala T.A. (accepted 2011);

Conferences:

"Childhood prognosis of heart disease in later life" Biala, T., Schlindwein, F.S., 3rd Festival of Postgraduate Research, Leicester, 26 June 2008.

"Heart rate variability in 10 year olds – normal and intrauterine growth restricted", Taher Biala, Jone Larsen, Cheryl Day, Fernando Schlindwein. Michael Wailoo, 19th Biennial International Eurasip Conference BIOSIGNAL 2008, Brno, Czech Republic, pp. 6-8, June 29 - July 1, 2008.

"Heart rate variability in intrauterine growth retarded infants and normal infants with smoking and non-smoking parents, using time and frequency domain methods", Cripps, V. A., Biala, T., Schlindwein, F.S. and Wailoo, M., 13th International Conference on Biomedical Engineering (ICBME 2008), Singapore, 3rd to 6th December 2008.

"Respiratory Sinus Arrhythmia in 10 year olds – normal and intrauterine growth restricted" Taher Biala, Fernando Schlindwein. Michael Wailoo and Michael Bankart, BIOSIGNALS 2009, Porto, Portugal, 14-17 January 2009.

"Development of QT measurement algorithm for Comparison between Intrauterine growth retarded and Normal children at 10 years old", Taher Biala, Schlindwein, F.S., Michael Wailoo, Medical Physics and Biomedical Engineering World Congress 2009. Munich, Germany, PGBIOMED'09 (12-14 July 2009).

"Assessing cardiovascular status in children", Taher Biala, Schlindwein, F.S., Michael Wailoo, Developmental Physiology Conference sponsored by The Royal College of Pathologists, Leicestershire, 18th – 19th June 2009.

"Heart Rate Variability in 10 Year Olds –Normal and Intrauterine Growth Restricted", Taher Biala, Jone Larsen, Cheryl Day, Fernando S. Schlindwein, Michael Wailoo, Michael Bankart. University of Leicester, The 2nd Academic Symposium of Libyan Students Universities of UK, Bradford; Saturday 20th June 2009.

"Development of QT measurement algorithm for comparison between intrauterine growth retarded and normal children at 10 years old", Taher Biala, Schlindwein, F.S., Michael Wailoo, World Congress on Medical Physics and Biomedical Engineering 2009. Munich, Germany, (7-12 September 2009).

"Heart rate variability using Poincaré plots in 10 year old healthy and intrauterine growth restricted children with reference to maternal smoking habits during pregnancy", Taher Biala, Michael Dodge, Fernando S. Schlindwein, Michael Wailoo, Computing in Cardiology 2010, Belfast, Northern Ireland, UK, September 26-29, 2010.

"Assessing electro cardiac signalling in children", Biala T.A., Wailoo, M, Schlindwein, F.S., Developmental Physiology Conference, Lutterworth, 15-16th June 2011.

Abbreviations

ANS	Autonomic nervous system
AR	Autoregressive model
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CTG	Cardiotocography
CTI	Cardiac time intervals
ECG	Electrocardiogram
FFT	Fast Fourier transform
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
IMD	Index multiple deprivation
IUGR	Intrauterine growth restricted children
LF	Low frequency
NN	Normal to normal interval
OSA	Obstructive sleep apnoea
P	Probability value
PSD	Power spectral density
QRS	QRS in ECG
QT	QT wave in ECG
RMSSD	Root mean square standard deviation
RR	Beat to beat interval
RSA	Respiratory sinus arrhythmia
SDANN	Averaged standard deviation of NN
SDNN	Standard deviation of NN
SGA	Small for gestational age
ST	ST wave in ECG
TINN	Triangular index of NN
VLF	Very low frequency
Z score	standard deviation scores

1 Introduction

This work is a continuation to a research started 2004 at Leicester Royal Infirmary looking at the development of IUGR and normal children (Chakraborty *et al.*, 2007). The previous research investigated the catch up growth of the IUGR compared to the normal children and difference in the blood pressure between the two groups of children. This thesis presents the results of using Heart rate variability measures to assess cardiovascular system development of normal and IUGR children at 10 yrs of age.

1.1 Intrauterine Growth Restriction (IUGR):-

Intrauterine growth restriction (IUGR) is associated with delay in foetal growth at any stage of the pregnancy and is a continuation of conditions which consequently result in the failure of the foetus to reach its expected growth potential (Pollak and Divon, 1992). There are two types of IUGR, namely symmetric and asymmetric. If the growth restriction happens to the foetus in the first or the second trimester, the infant will have symmetrical growth restriction. Asymmetric growth describes the infant with a decrease in growth velocity in the last trimester. IUGR complicates 3-10% of all pregnancies. The markers to monitor IUGR are low birth weight, premature birth and 'small for gestational age', these conditions can lead to adverse prenatal outcomes (Fairley and Leyland, 2006). An infant born less than 37 weeks from first day of menstrual period is preterm and the growth retarded infants are defined by their biometric dimension being less than the 10^{th} percentile for gestational age (Peleng *et al.* 1998), and the abdominal circumference being less than the 2.5^{th} percentile. The suboptimal fetal growth can be called IUGR or sometimes 'small for gestational age' (SGA), IUGR and SGA are two terms which are used interchangeably, but by definition they are different conditions of fetal growth, where SGA is defined by the World Health Organisation as a birth weight below the 10^{th} percentile for gestational age, but IUGR is a pathological condition in which the fetus is unable to grow to its potential growth (Wintour and Owens, 2006). This means that all IUGR are SGA but not all SGA are IUGR. Both are considered as risk factors to future diseases. Figure 1.1 shows growth percentiles for fetal weight versus gestational age.

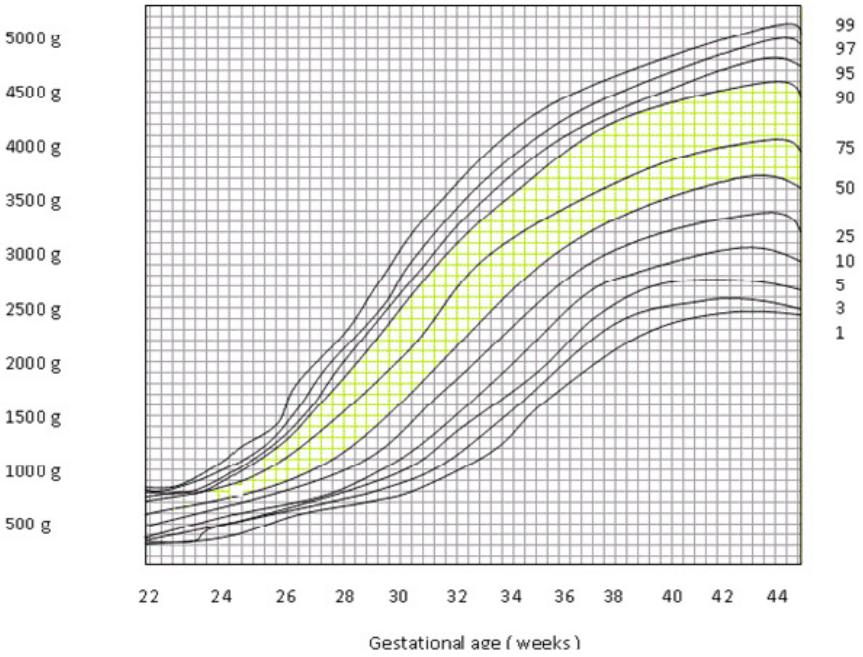

Figure 1.1: Growth Percentile (right y axis) for fetal weight (left y axis) versus gestational age (bottom x axis). Modified from an original by (Peleng D et al. 1998).

Normal intrauterine growth pattern is divided into three stages, the first stage is from 4 to 20 weeks, when rapid cell division and multiplication (hyperplasia) takes place and the embryo grows to a fetus. Next, from 20 to 28 weeks gestation, where hyperplasia decreases and the cells increase in size (hypertrophy). In the last 28 to 40 weeks there is a rapid increase in cell size, accumulation of fat, muscle, and connective tissue. Most of the fetal weight gain (95%) occurs during the last 20 weeks of gestation and because the process of development and weight gain is very delicate, any disturbance during this period can cause the fetus to suffer from restricted growth. Previous work by (Chakraborty *et al.*, 2007) on this data showed that the IUGR gained weight faster after birth than the control children, but at 10 years the IUGR children remained lighter and shorter than the children in the control group, which means that there is 'no catch up' growth.

Most physicians believe that IUGR is caused by a disease process during one or more of the three partitions that maintain and regulate fetal growth (Vandenboshe and Kirchner, 1998). Table 1.1 presents the following conditions associated with IUGR.

The three partitions that maintain and regulate fetal growth are:

- Maternal compartment
- Placenta
- Fetus

Table 1.1: The conditions associated with IUGR (Vandenboshe and Kirchner, 1998).

Intrauterine growth retardation (IUGR) **Conditions associated with IUGR**	
Maternal history	Alcohol use Cocaine use Smoking Malnutrition Use of prescription drugs warfarin (Coumadin, Panwarfarin) and phenytoin (Dilantin) Prior history of IUGR pregnancy Residing at altitude over 5,000 ft (1,500 m)
Medical conditions (of mother)	Chronic hypertension Preeclampsia early in gestation Diabetes mellitus Systemic lupus erythematosus Chronic kidney disease Inflammatory bowel disease Severe lung disease
Infectious diseases	Syphilis Cytomegalovirus Toxoplasmosis Rubella Hepatitis B Herpes simplex virus 1 or 2 HIV-1
Congenital disorders (of fetus)	Trisomy 21 (Down syndrome) Trisomy 18 (Edwards syndrome) Trisomy 13 (Patau syndrome) Turner's syndrome

It is estimated that at least 13.7 million infants are born every year at term with low birth weight (LBW), this represents 11% of new born in developing countries. LBW defined as < 2500g affects 16% of all newborns. In developing countries the IUGR defined as birth weight below the 10^{th} percentile of birth weight for gestational age reference curve represents 23.8% of all infants born every year (30 million). About 75% of these infants are born in Asia, 20% in Africa and 5% in Latin America. This shows that many developing countries exceeded the internationally recommended cut-off levels for triggering public health action, IUGR (>20%) and LBW (> 15%) (de Onis *et al.*, 1998).

A study was done on the mean birth weight of different ethnic groups; the result was that there is significant difference observed between the birth weight of a baby from European

mothers (3357 g), Afro-Caribbean mothers (3173 g) and from mothers from the Indian subcontinent (3096 g). Also there is a significant interethnic difference in length of gestation, parity, maternal height, booking weight, and smoking habit, all of which affect birth weight. The difference in birth weight between the ethnic groups will be greater if the effect of smoking is excluded. This is due to that the smoking factor (mother smoking during pregnancy) may act as a confounder in affecting the fetal growth. When comparing non-smokers, Afro-Caribbean, European with Indian and Pakistanis, an even greater ethnic influence on birth weight was found (Wilcox *et al.*, 1993).

1.2 Barker's Theory (The fetal origin hypothesis)

In the search for disease causation, Barker (Barker D 2003), (Barker D 2004), along with others around the world, came up with a hypothesis to answer the question "is there any association between heart diseases in adulthood and growth in infancy or in childhood". In Hertfordshire, a study was done on 10636 men born between 1911 to 1930 to register all births and the weight at 1 year(Barker, 1989). Hazard ratios for coronary heart diseases decreased when the birth weight increases, and there was a strong trend with the 1 year weight. Another similar study was done on women (Osmond *et al.*, 1993), where the same trend was found with birth weight, but there was no trend with the 1 year weight. Another finding was about type 2 diabetes in men, where it decreased sharply with increasing birth weight (Hales *et al.*, 1991).

The fetal origin hypothesis proposes that coronary heart disease, type 2 diabetes, stroke and hypertension occur in development plasticity, as a result of under nutrition during fetal life and infancy. There seems to be three kinds of process that explain the relationship between under nutrition in fetal life and infancy (Barker, 2004), firstly people with low birth weight have fewer cells in their key organs, such as kidneys, and hypertension can be associated with reduced number of glomeruli, which can lead to increased blood flow through each glomerulus. Studies of the kidneys of people killed in road accidents and being treated for hypertension showed that they had fewer but larger glomeruli. Secondly, the process of hormones setting and metabolism, where an undernourished baby can get the habit of thrifting away from food, especially in conditions of plenty, after birth. Insulin resistance is associated with low birth weight, and the blood glucose concentrations are used to develop the brain but at the expense of glucose transport into the muscles and muscle's growth. The third link between low birth weight and disease in later life is for

people who were thin at birth and had a rapid weight gain after birth and those with low income, who had a higher risk of coronary heart disease in later life.

Weinberg (Weinberg, 2004) and Yu-Kang (Yu-Kang *et al.*, 2005) showed that there is no correlation between birth weight and blood pressure, but both are positively correlated with current weight. A complicating factor is that adjustment in current weight can induce negative correlation between birth weight and blood pressure. These findings raise doubts to the fetal origin hypothesis.

1.3 Heart Rate Variability (HRV)

1.3.1 Historical review and definition of heart rate variability

The first clinical importance of HRV was realised in 1965 by (Hon and Lee, 1965), who noted that fetal stress was preceded by alteration in the interbeat intervals before any changes in the heart beat. After that (Sayers, 1973) reported the existence of physiological rhythms imbedded in the beat to beat heart rate signal. In the 1970s a number of bedside tests were developed for short-term RR analysis to detect autonomic neuropathy in diabetic patients (Ewings *et al.*, 1985). In 1977 a study by (Wolf MM *et al.*, 1978) found that reduced HRV was associated with high risk of post-infarction mortality, and that was caused by the remodelling of the arrhythmia substrate after acute myocardial infarction (Huikuri Heikki and Stein Phyllis, 2012). Power spectral analysis of heart rate fluctuation was introduced by (Akselrod *et al.*, 1981) to evaluate and quantify the beat-to-beat cardiovascular control. In the late 1980s, the clinical importance of using HRV non-invasively gained weight as it proved to be a predictor of mortality following an acute myocardial infarction (Kleiger RE *et al.*, 1987). HRV has the ability to provide more understanding into physiological and pathological conditions and to improve risk stratification (The Task Force, 1996) and with the availability of the new digital, high frequency ECG recording systems and the use of both HRV linear and non-linear techniques, HRV analysis is attracting renewed attention.

The normal clinical features of the electrocardiogram shown in figure 1.2 include wave amplitude and inter wave timings (Clifford *et al.*, 2006), the locations of different waves are marked by letters P, Q, R, S and T. In HRV, the R to R (beat to beat interval) is the raw measurement used to quantify HRV. The baseline variability of RR time series can be

affected by many factors such as age, gender, activity, medication and health. There are two basic ways of looking into the changes, one is associated with the mean beat-to-beat rate (heart rate), and the other is associated with the variance of the sequence of heart beat intervals (Clifford *et al.*, 2006) .

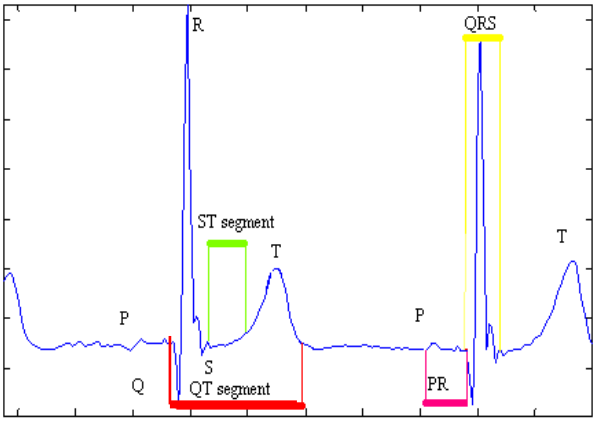

Figure 1.2: A Typical ECG with two heart beats and intervals details.

The cardiac action potential initiated by a group of cells named as sinoatrial node (SA) which is located in the right atrium. This group of cells self depolarise at a regular rate hence it works as an automatic pacemaker. This intrinsic pacemaker is influenced by both parasympathetic and sympathetic branches of the autonomic nervous system. The ECG is a standard clinical tool used by the cardiologists to measure the electrical activity of the heart. The p wave represents depolarisation of the left and right atria. The QRS complex reflects the depolarisation of the ventricles. The T wave is the repolarisation of both ventricular muscles (Boron and Boulpaep, 2003).

1.3.2 Heart rate variability and the autonomic nervous system

On the short scale the timing of successive heartbeats is irregular and these short-term oscillations reflect changes in the balance between the sympathetic and the parasympathetic branches of the autonomic nervous system (ANS). This is called the sympathovagal balance, see figure 1.3.

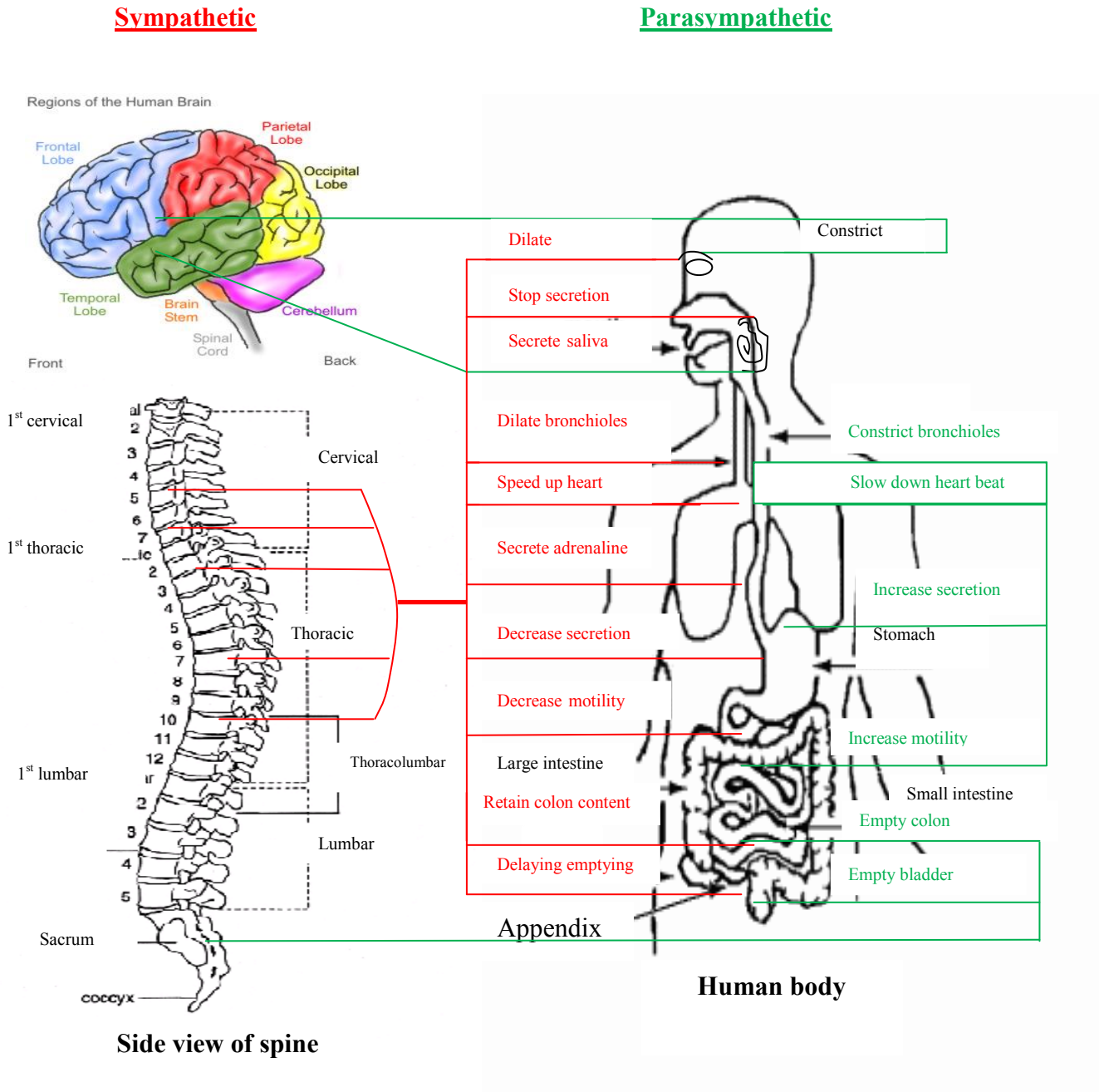

Figure 1.3: Innervation of the major organs by the autonomic nervous system (ANS), Redrawn and modified from (Boron and Boulpaep, 2003).

This irregularity in instantaneous heart rate is well known and is named as heart rate variability (Clifford *et al.*, 2006). In general HRV metrics can be evaluated into either statistical time-based metrics (time domain analysis), or frequency-based metrics that evaluate power, or ratios of power in certain spectral bands. These metrics can be calculated on a short scale (about 5 min) or on a long scale (up to 24 hours).

HRV can be used to aid the assessment of autonomic functions (Boardman, 2003), which beforehand had only been possible using invasive methods. HRV has been used clinically to assess the damage caused to autonomic nervous system in patients suffering from diabetes with neuropathy. It has been shown that HRV could be used in assessing the prognosis after myocardial infarction (Kleiger RE *et al.*, 1987).

1.4 Why measuring HRV for normal and IUGR children at 10 yrs?

The answer to this question is that by measuring HRV one can assess the development of the nervous and cardiovascular systems for normal and IUGR when their age is around 10 years. The following is a literature review of research work done concerning IUGR children. Here a selection of papers and research regarding IUGR in the literature was reviewed to see if any looked at HRV analysis of 10 year-old children and then compared the results and the clinical implications of the results with the outcomes of the research by Barker (Barker, 2004) and others who investigated the fetal origin hypothesis and concluded that, for men, the risk ratio for coronary heart disease fell with increasing birth weight and the trend is very strong with weight at 1 year. Another study on women has confirmed the same findings, but presenting no trend with weight at 1 year. Regarding glucose tolerance or type 2 diabetes, it fell steeply for men and women, with increasing birth weight. At 11 years of age, for boys and girls the same general pattern continues, where the risk of the two disorders fall with increasing birth weight and rise with increasing body mass index. In his preliminary studies Van Leeuwen shows that age-adjusted cardiac time intervals (CTI) are shorter in growth retarded fetuses (Van Leeuwen, 2004).

When looking at the birth weight, postnatal growth and blood pressure at 7 years old, (Anusha *et al.*, 2007)concluded that infants who were small for gestational age (SGA), were not at increased risk of high blood pressure at 7 years of age, but those who crossed their weight percentile during early childhood showed an increased risk. Singal found that

for 6-8 years old children, the promotion of faster weight gain for the IUGR in infancy can increase the blood pressure at later life (6-8 yrs) (Singal *et al.*, 2007). Another study for 8-13 years old children (Franco *et al.*, 2006) concluded that children with history of low birth weight show impaired endothelial function and increased blood pressure uric acid levels. Keijzer-Veen found that at the age of 19 years old the prevalence of hypertension is high in individuals who were born preterm when compared with the general population (Keijzer-Veen *et al.*, 2005), while in others who were born very preterm, no support to the hypothesis that low birth weight is linked with increased BP at young adult age could be found.

Dale Spence *et al*. (2007), when studying the effect of IUGR on quality of life at 50 yrs, found that the two groups (IUGR and Normal), reported similar health quality of life on each of the eight dimensions of the short form health survey (SF-36) and that there are no significant differences between them. SF-36 is used to assess quality of life and general health all over the world. The eight dimensions measured in this form are physical functioning, role limitation due to physical problems, role limitation due to emotional problems, social functioning, mental health, energy/vitality, pain and general health perception (Spence *et al.*, 2007). When looking at the effect of childhood socio-economic condition on coronary heart disease on later life, the result was that this factor will cause a modest persisting influence on risk of coronary heart diseases (CHD) in later life. A paper (Galland C. Barbara *et al.*, 2006) on HRV and cardiac reflexes in small for gestational age SGA infants, suggests reduced autonomic activity and cardiac reflexes in SGA infants, and the findings of sympathetic components of the control of HRV is higher, which might be linked to higher risk of CHD in later life. Detrended time series analysis of the R-R intervals suggests that IUGR fetuses have significantly reduced HRV compared to the other groups of children (Govindan *et al.*, 2006). Other non linear HRV measure known as Sample Entropy approach was used to study obstructive Sleep apnoea syndrome (Al-Hangari and Sahakian, 2007). They proved that normal subjects have significantly more complex HRV pattern than the OSA. Classical Cardiotocography, (CTG), is used to analyse the fetus' heart rate (Signorinin and Magenes, 2003), and to distinguish between normal and pathological fetuses. Myung-Kul Yum *et al.* (2000) found that in IUGR fetuses, the approximate entropy was significantly lower, and the long term fractional scaling was higher than in normal fetuses (Yum *et al.*, 2001). In epidemiological studies,

findings support the use of records with the length of at least 5 min long segments for HRV measures (Schroeder *et al.*, 2004).
The previous literature review on research conducted to date on IUGR infants shows that many studies on IUGR infant were done to find causes and effects on the well known association of IUGR with high risk of mortality, yet there is no study that looks at HRV of IUGR children at 10 yrs.

The novelty of this research is to look at HRV of children at 10 years, IUGR and normal, in an attempt to investigate any relation between HRV measures, RSA, QT syndrome and the fetal origins hypothesis, to try and identify any factor or factors, such as age, gender, maternal smoking, sleeping, or breast feeding, which have an effect on the development of coronary heart disease.

1.5 The organisation of the thesis

This thesis is organised as follows: The first chapter reviewed the important theories and clinical importance of methods used in this work, first IUGR was explained and the importance of understanding IUGR children and associated diseases which can be detected as soon as possible with the hope of preventing any future CHD was highlighted. The second chapter shows the use of time domain analysis described by the Task Force in 1996, in this chapter the results of developed algorithms were discussed and novel results after dividing the IUGR children into IUGR1 (less than 2.5 kg), and IUGR2 (higher than or equal to 2.5 kg) were discussed including the clinical implications of these results. The third chapter looks at the data used in this project and the pre-processing needed to be done on the data in order to eliminate noise and artefacts so HRV measures do not get affected by the classical noise associated with ECG signals such as mains noise and ectopic beats. The modulation of HR by respiratory frequency is also mentioned.

The fourth chapter discusses the algorithms and the novelty associated with results from the FFT, AR and Lomb methods. Significant difference was found between IUGR whose parents smoke at 10 yrs and IUGR with non-smoking parents. RSA was observed by the use of frequency methods. Chapter 5 describes the development of an algorithm using Matlab to measure all important ECG intervals, the main purpose and the novel result in this chapter is measurement of QT interval, which has been associated with long QT syndrome or diseases. Other intervals measured are heart rate, QRS, and ST segment. QRS

detection and wave delineation was used to calculate all the above mentioned intervals. The sixth chapter explains the use of Poincaré plots to compare between IUGR and Normal children, especially when analysing the difference between children whose mothers smoked during pregnancy and whether the child is normal or IUGR. From this study it is found that maternal smoking can have an adverse effect on IUGR children.

The seventh chapter investigates the 15 min pre-awake period using the linear and non-linear measures. The novel and important results on children at 10 yrs showed many significant differences in many HRV measures when comparing between IUGR1, IUGR2 and Normal children. We focus on this period around waking up as it has been identified as a vulnerable and critical period which has been associated with sudden death by many researchers. Chapter 8 discusses the results from all the previous chapters and illustrates the importance and the clinical implications of all results, and outlines the outcome and the conclusions from this thesis. Adding to the conclusions some future work is suggested to improve and to produce additional interesting results in the field.

Appendix (A) shows some details of the data collected which was used in this project and appendix (B) includes two of my publications. Appendix (C) includes the results of the algorithm developed in this thesis.

2 Project Data and Signal Processing

The data used in this project were collected for a research which started when the 10 years old children under study here were in the womb of their mothers. A series of antenatal ultrasound scanning was taken to identify intrauterine growth retarded babies (Chakraborty, 2010). A random sample of normal infants was selected for the purpose of comparison. When those children were 10 years of age, they were subjected to a cardiovascular study, where data such as weights, heights, and parental heights were measured. The 24 hour (Holter) ECG of all IUGR and normal children were recorded using a lifecard CF ambulatory recorder (Delmar-Reynolds Medical Limited, Hertford, UK). Ambulatory electrocardiogram monitors the electrical activity of the heart while the patient performs usual daily activities. Many heart problems occur only during certain activities, such as exercise, eating, emotional stress, or even sleeping. A continuous 24-hour recording is much more likely to detect any abnormal heartbeats that occur during these activities(Yanowitz, 2010).

The most common type of ambulatory monitoring is called Holter monitoring. The recording device of a Holter monitor is worn on a strap at waist or over shoulder. The electrical signals of heart are picked up by two small metal pads (electrodes) attached to chest, and these are connected to the recorder by wires. Holter monitoring provides a continuous 24 to 72 hour record of the electrical signals from heart. After the monitoring period, health professional will compare the timing of activities and symptoms with the recorded heart pattern.

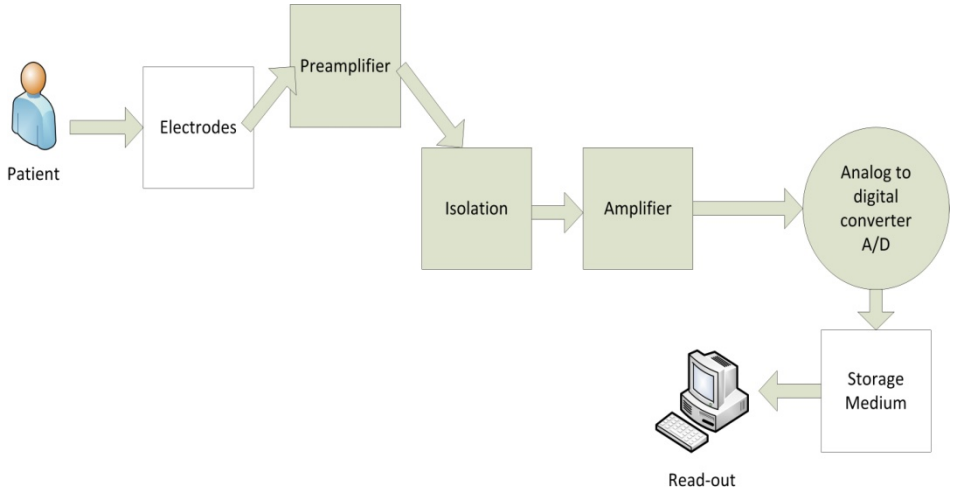

Figure 2.1: Block diagram of the Holter monitor.

Many people have irregular heartbeats from time to time. The importance of irregular heartbeats depends on the type of pattern they produce, how often they occur, how long they last. Because arrhythmias can occur irregularly, it may be difficult to record an arrhythmia while patient is in the doctor's office. A standard ECG monitors only 40 to 50heartbeats during the brief period the patient is attached to the machine. A Holter monitor records about 100,000 heartbeats in 24 hours and is much more likely to detect a problem.(Yanowitz, 2010)

One factor affecting ambulatory ECG measurement is that external conditions can be far from stable and may produce nonstationary changes in a time series, making the assessment of the physiological events more difficult or even impossible, than under stable laboratory conditions. Sapoznikov *et al.* (1994) proposed a method for separating non-periodic (nonstationary) changes from periodic ones.

The blood pressure was recorded using 90217 ULTALIT oscillometric ambulatory blood pressure monitor for 24 h. Cortisol excretion was measured from two samples of urine, one for finding night level, and the other to find the morning surge in Cortisol production, Cortisol is a steroid hormone which is considered as a marker of physiological alterations due to stress stimuli and the level of Cortisol is found to be increased during high stress periods such as students' final exams. (Kirschbaum and Hellhammer, 1994, de Weerth *et al.*, 2003).

The medical state of the children was recorded and any medication used for any illness was written in a spreadsheet along with all other data. Table 2.1a and 2.1b show a summary of the data used in this work and related data. Parents kept a diary of all daily activity of their child such as sleeping and waking up times, see appendix A. The data of table 2.1a and 2.1b shows that both groups of children are of normal gestational age, around 38 to 39 weeks. This means that gestational age during pregnancy is not a differentiating factor in the growth of the children during infancy.

Table 2.1: The data of all Normal and IUGR children used in this work – Continuous variables (Chakraborty S. et al. 2007).

Continuous variables	IUGR N=41 Mean (95% CI)*	Control N=34 Mean (95% CI)	Difference of means (95% CI)	Probability value
Gestation (weeks)	38.98(38.56, 39.40)	39.18(38.86, 39.49)	-0.2(-0.7,0.3)	0.45
Birth weight kg	2.56(2.43,2.69)	3.53(3.36,3.69)	-1.0(-1.2,-0.8)	<0.0001
Birth weight z score	-2.1(-2.3,-1.8)	0.1(-0.1,0.4)	-2.2(-2.6,-1.8)	<0.0001
Placental (a) weight(Grams)	488(448,528)	631(583,679)	-143(-203,-82)	<0.0001
Breast feeding duration(Weeks)	4.2(1.9,6.5)	10.5(7.9,13)	-6.3(-9.7,-2.8)	0.0006
Maternal height (b) (cm)	161.8(159.2,164.4)	161.7(158.9,164.4)	0.2(-3.6,3.9)	0.93
Parental height (b) (cm)	175.O(172.5,177.6)	178.2(176.3,180.1)	-3.2(6.3,0.03)	0.052
Current IMD (c) score	21.2(15.9,26.5)	12.9(9.1,16.8)	8.3(1.6,14.9)	0.02
Current age(years)	9.36(9.12,9.54)	8.96(8.78,9.23)	0.4(0.09,0.7)	0.01
Final weight(kg)	28.32(26.78,30.46)	32.64(30.48,34.80)	-3.8(-6.5,-1.1)	0.007
Final weight z score	-0.4(-0.7,-0.1)	0.6(0.3,1.0)	-1(-1.5,-0.5)	<0.0001
Change in weight(kg)	26.26(24.49,28.03)	29.53(26.95,31.27)	-2.9(-5.6,-0.1)	0.04
Change in weight z score	1.7(1.3,2)	0.5(0.1,1)	1.2(0.6,1.8)	<0.0001
Final height(cm)	131.4(129.3,133.4)	133.8(132,135.5)	-2.4(-5.1,0.3)	0.08
Final height z score	-0.6(-0.9,-0.3)	0.16(-0.1,0.5)	-0.8(-1.2,-0.4)	0.0002
Final BMI	16.63(15.83,17.42)	18.11(17.22,19)	-.1.5(-2.7,-0.3)	0.01
Final BMI z score	-0.1(-0.4,0.3)	0.8(0.4,1.1)	-0.8(-1.3,-0.3)	0.002

Table 2.2: The data of all Normal and IUGR children used in this work – Categorical variables (Chakraborty *et al*. 2007).

Categorical variables	IUGR N=41 Number (%)	Control N=34 Number (%)	Probability value
Gender			0.12
Male	19(46.3)	22(64.7)	
Female	22(53.7)	12(35.3)	
Breast feeding	19(46.3)	25(73.5)	0.02
Maternal smoking in pregnancy	16(39)	9(26.5)	0.25
Current smoking	21(51.2)	12(35.3)	0.17
Significant medical problem	12(29)	4(12)	0.07
Currently on medication	11(27)	4(12)	0.16
Normal development	37(90)	34(100)	0.11
Mainstream schooling	40(97)	34(100)	0.36

NB:

a: Probability values were derived from t tests for continuous variables and from Chi-square tests for categorical variables.

b: Placental weights are available for 35 IUGR and 32 controls.

c: Maternal and paternal heights are available for 39 IUGR.

d: Index of Multiple Deprivation (IMD) score is a measurement of socio-economic deprivation which is inversely related to deprivation.

*: CI means confidence interval.

2.1 Data Reading

The Leicester Royal Infirmary (Chakraborty, S. *et al.* 2007) uses Delmar Reynolds software (DR) for data reading, analysis and saving. The data files for the raw ECG and RR data for all the 75 children (41 IUGR, 34 normal), were stored in binary format files. The Delmar Reynolds manuals were consulted to know how the data file was formatted and then algorithms were self-written in Matlab, so the data could be read in Matlab environment allowing us to carry out further analysis. Figure (2.1), shows the raw ECG extracted by using the Matlab program for reading the data. The beginning of the files contains information about the Delmar Reynolds software used to store the binary data format and then is the ECG data. Figure (2.2) shows an enlarged segment from the raw ECG data.

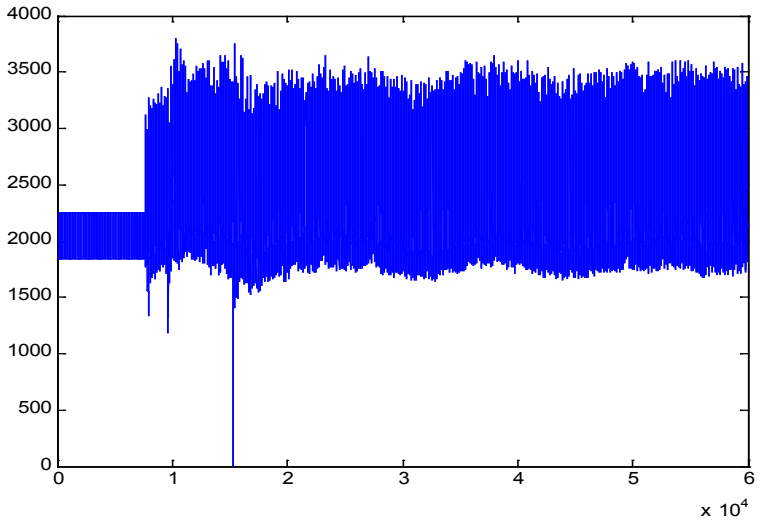

Figure 2.2: Beginning of ECG data file and ECG data were collected for 24 h.

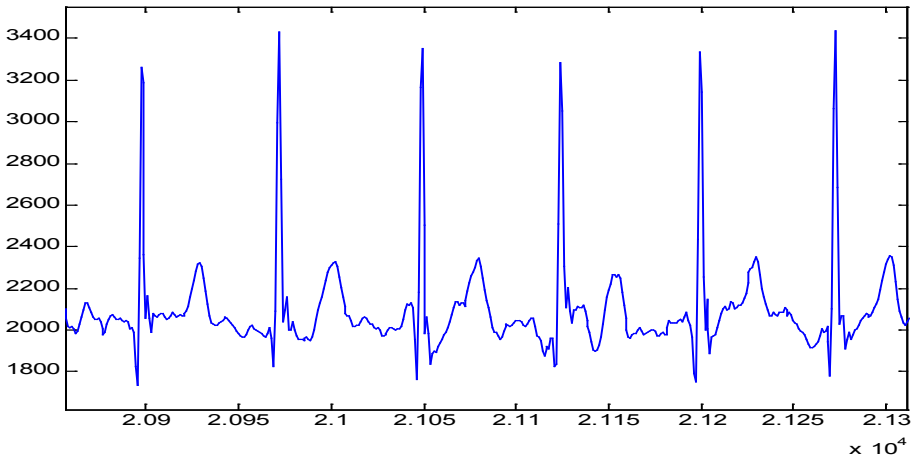

Figure 2.3: A small zoomed segment of the ECG data file in fig. 2.2.

2.2 Sampling Frequency

The Delmar Reynolds software uses a sampling frequency of 128 Hz, which means a sample every 7.8125 ms. This is considered as low sampling frequency by the Task Force of the European Society of Cardiology (1996), who recommended that the sampling frequency should be 250 Hz or above. Davingnon uses 333 Hz as sampling frequency to find the normal ECG standards for infants and children (Davingnon *et al.*, 1979/80). Another study, (Macfarlane *et al.*, 1989), used 500 Hz. In 1990 the American Heart Association recommended 500 Hz; this is needed due to the high frequency contents of the ECG in young children (Dickinson, 2005). A recent study to find the normal limits for the paediatric electrocardiogram by (Rijnbeek *et al.*, 2001), uses 1200 Hz. Using a lower sampling frequency means that time jitter will be produced for estimation of fiducial point of the R-waves, but a frequency as low as 100 Hz may be satisfactory if an algorithm of parabolic (or cubic spline) interpolation is used to refine the R wave fiducial point (The Task Force, 1996).

2.3 Digital Filtering and QRS detection

A classical technique based on a bandpass filter and an adaptive threshold was used for detection of the QRS, (Schlindwein *et al.*, 2000).

A second order Butterworth band pass filter with cut-off frequencies of 14 Hz and 24 Hz followed by rectification and then an adaptive threshold was used for QRS detection (Schlindwein *et al.*, 2006). The value chosen for the adaptive threshold of the detector tends towards 65% of the moving average value of the maximum magnitude of the previous QRS complexes. To reduce the possibility of detecting false positives we have used an absolute refractory period (300 ms). Figure (2.3) shows detected QRS from raw ECG and filtered ECG, fig (2.4), shows an enlarged filtered ECG and the behaviour of the adaptive threshold (Pan and Tompkins, 1985). The delay between the filtered ECG signal and the original ECG is caused by the group delay of the Butterworth band pass filter and it does not affect the measurement of RR intervals, as successive QRS complexes will have the same delay. Forward and reverse filtering (filtfilt.m) can be used to completely eliminate these delays.

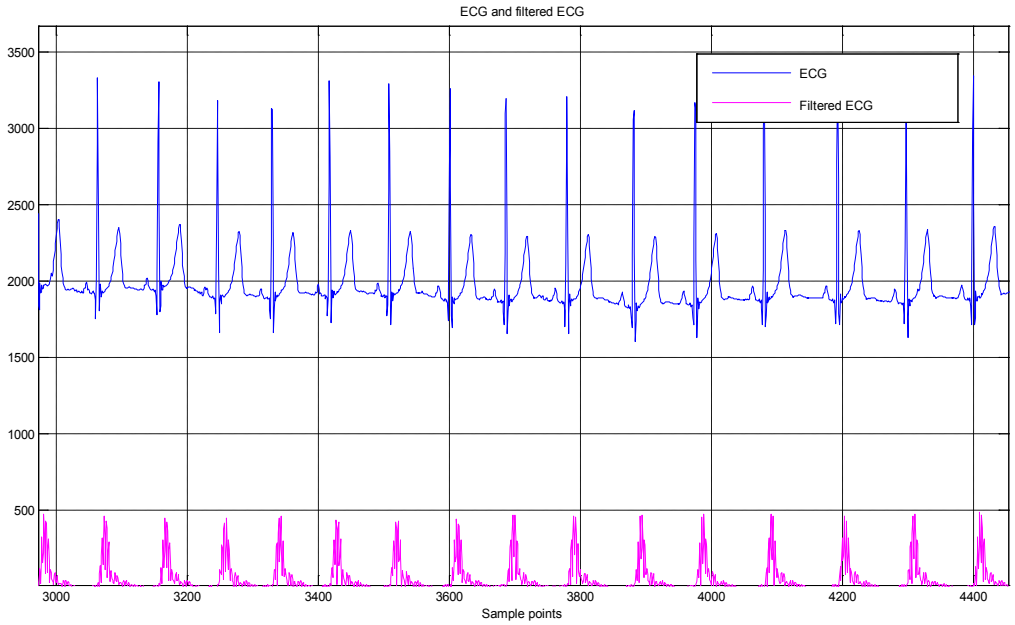

Figure 2.4: Raw ECG and Filtered ECG.

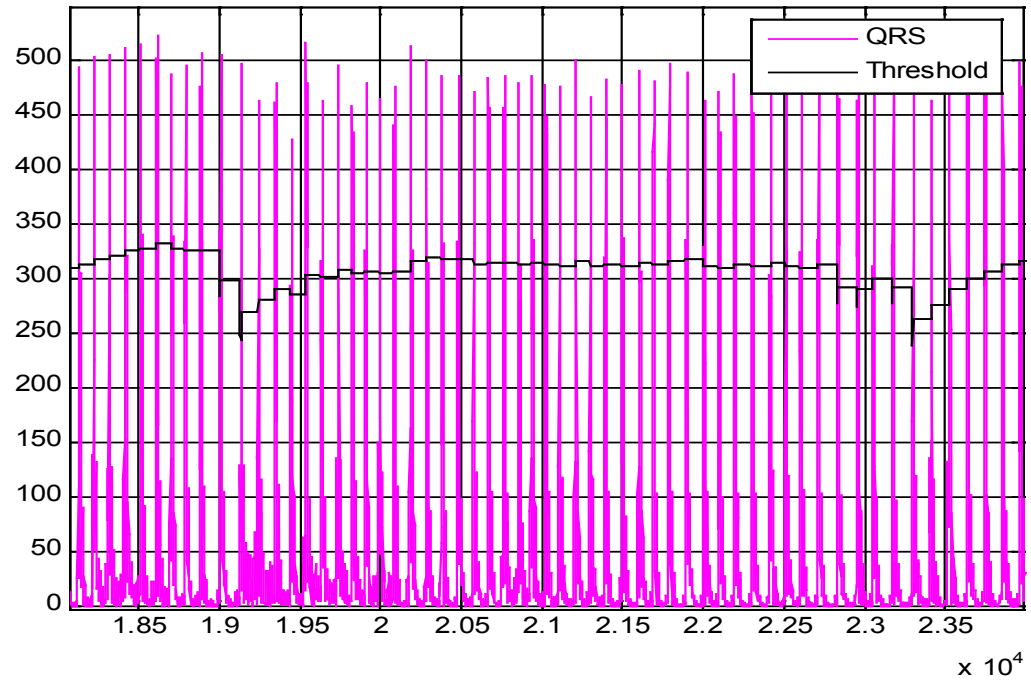

Figure 2.5: Filtered ECG and the behaviour of the adaptive threshold. Notice how the adaptive threshold adjusts itself in a way that all R waves will be detected while the T waves will be rejected.

2.4 **Re-sampling RR data**

The RR series is subjected to a process of interpolation to obtain equally spaced data to be used for standard spectral analysis as both FFT and autoregressive model (AR) must have equally spaced data to perform spectral analysis. The signal has been re-sampled at 4 Hz after a cubic spline data interpolation. This will give us the results in Hertz and allows the spectrum analysis up to 2 Hz. Figure 2.5 shows the detected ECG, filtered ECG, RR data and interpolated RR data.

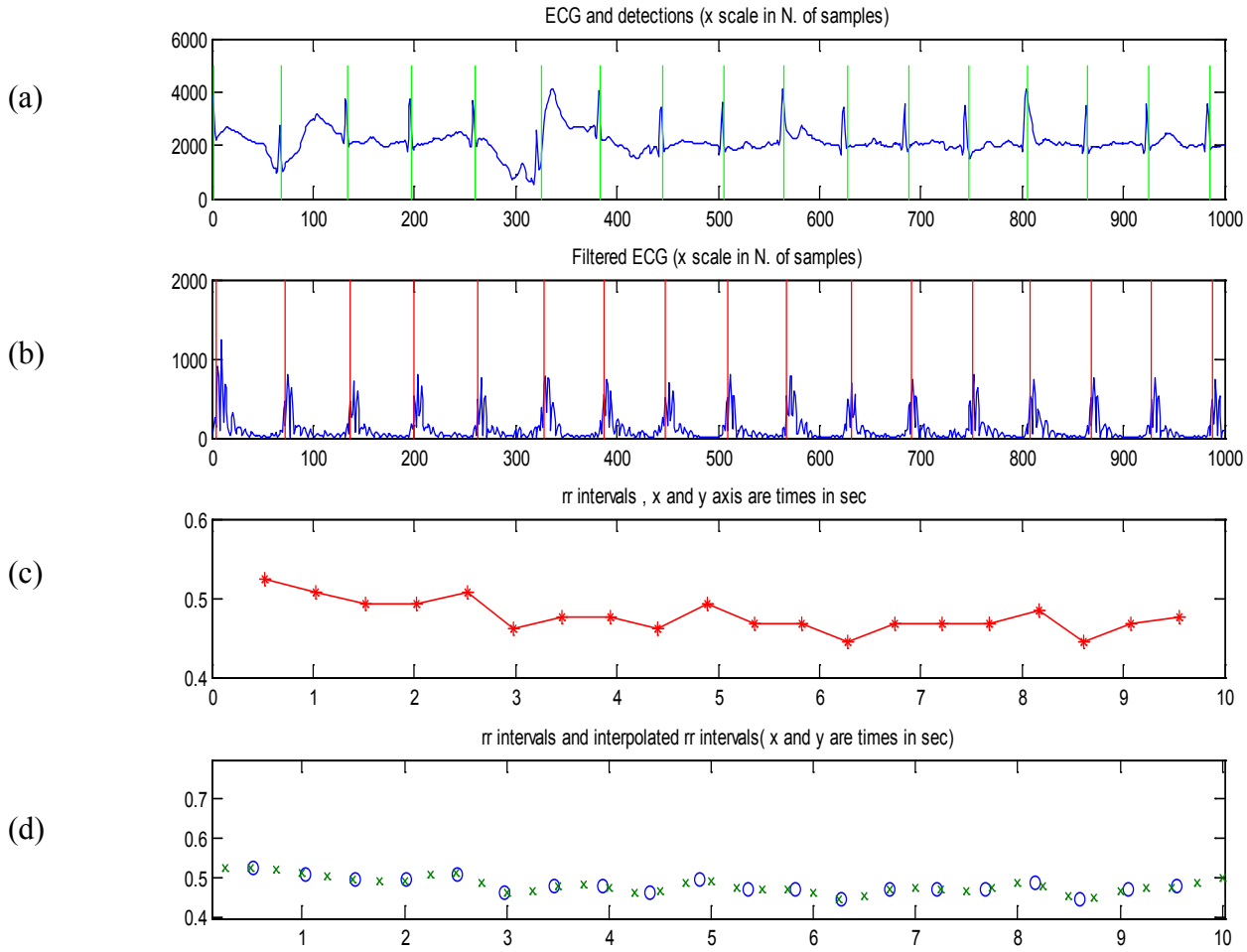

Figure 2.6: (a) The ECG with detected R waves, (b) the filtered ECG and detected R waves, (c) raw RR data series and (d) raw RR data series and the interpolated RR series re-sampled at 4 Hz.

2.5 Artefacts in RR Interval Time Series

In practice, heart rate variability (HRV) analysis is performed using intervals between successive R waves (RR intervals). The RR interval time series used for HRV analysis should contain only RR intervals. Ambulatory ECG recording is exposed to many physiological and technical disturbances. Errors of physiological origin in QRS detection arise from disturbances and extraordinary waveform in the measured ECG signal (Pahlm and Sornmo, 1884, Thomas *et al.*, 1979). Abnormal initiation of the heart beat (ectopic beat) can lead to a variety of morphologies of QRS complexes and cause difficulties in both their classification and their detection. Potential physiological sources of errors also include: abnormally large P or T wave and electromyogram (EMG) similar enough to QRS complexes in amplitude and frequency content to cause spurious detection. The ability of QRS detector to tolerate variations in ECG waveforms depends on the recognition criteria themselves and the pre-processing of the raw ECG data, of which most essential part is filtering (Friesen *et al.*, 1990, Hamilton and Tompkins, 1986).

Like physiological disturbances in the ECG signal, the tolerance of different QRS detection procedures can vary with respect to technical disturbances (Friesen *et al.*, 1990, Hamilton and Tompkins, 1986). The disturbances of technical origin include movement of electrodes relative to heart and skin or other changes in conductivity between the electrodes and skin, which result in baseline shift. Thus RR intervals obtained from ambulatory recording often include abnormal intervals called artefacts, which do not represent the sinus rhythm and differ in length from normal RR intervals. These artifacts lead to spurious transient spikes in the resulting RR interval time series.

2.5.1 Detection and Correction of Artefacts

The decision whether an abnormal beat should be corrected or not usually forms the most difficult step in the artefact removal. Error detection algorithms attempt to differentiate normal RR intervals from abnormal ones. In computerized artefact detection, relatively simple artefact detection criteria supported by additional visual verification are still being used (Tikkanen, 1999). This is because results obtained with simple procedures are comparable with more complex solutions (Tikkanen,

1999, Mulder, 1992). The simple artefact detection criteria described in the literature include absolute upper and lower limits for acceptable interval (300-1500 ms), absolute or relative difference from the previous accepted interval (e.g. 20-40%) from previous RR interval, from mean or from a fitted polynomial representing the baseline (Tikkanen, 1999). (Malik *et al.*, 1989) used four simple artefact detection criteria and found none of them to be significantly better than the others. A single artefact detection criteria always has its particular weaknesses, a combination of criteria should be preferred (Sapoznikov *et al.*, 1994)

The two basic procedures mainly used for removing individual artefacts from an RR interval time series are:

- Total exclusion of abnormal intervals
- Substitution of a better matching value.

The exclusion approach is widely used, suits well for time domain analysis, and can also be used with frequency domain analysis if only a few beats are to be excluded, whereas the substitution approach is used widely with both time domain and frequency domain analysis (Tikkanen, 1999). The substitution can take the form of simply replacing the abnormal value with a local mean or median value, but more sophisticated procedures include linear, non-linear or cubic spline interpolations or more complicated predictive modelling (Lippman *et al.*, 1994).

If the artefacts are of technical origin, then substitution approach can be used with good justification in a physiological sense, whereas if artefacts are due to physiological or mental factor, both approaches can be used with success. (Lippman *et al.*, 1994) showed that the simple deletion method and more complex non-linear predictive interpolation method gave the best results for removing ectopy from 5 minute RR interval time series. In general, the removal of artefacts tends to increase the low frequency component of the spectrum and reduce the standard deviation, but it should be noted that the sum of the intervals after correction does not always equal the sum of the original intervals.

2.6 Discussion

ECG data in this work, stored in a binary format, was subjected to many signal conditioning steps to have very reliable and accurate RR intervals to calculate HRV measurements.

The first was filtering the signal to remove any unwanted noise like the 50 Hz mains. Then the signal was checked to remove any ectopic beats which can, if present, give wrong HRV measures. Faulty lead connection was considered in the algorithm because that might affect the results if any lead was removed by any physical activity or taken of the measurement position during the 24 hr period when reading data (ECG) from the child. Spline interpolation was performed on the signal to produce RR ready for the frequency domain analysis (FFT and AR). The use of refractory period prevents the QRS detector from looking for QRS wave within a certain period of the signal. This will avoid considering a high T wave as a QRS wave, because after detecting one QRS the search for a new QRS will always start after the refractory period finishes(300 ms) in this algorithm.

To do accurate ECG intervals measurements the up sampling of the ECG from 128 Hz to 512 Hz was implemented. This will give us more accurate measurements of ECG interval such as QRS, QT, ST and any other interval measurements.

3 Time Domain Analysis

The simplest way to evaluate the variation in heart rate is using time domain measures (The Task Force, 1996). In continuous ECG recording, each QRS is detected and the R to R or so-called normal to normal (NN) interval (after removing extra-systoles) or the instantaneous heart rate is determined. Figure 3.1 shows a graph of 24 hr RR intervals with high RR at night time and low RR at day time.

A Matlab algorithm (timedomain.m), see the programs CD enclosed with this thesis, was developed to measure the TD HRV measures. Data (subjectNo.dat) were read in by a subprogram (extractRR.m) .Then the following time domain variables were then calculated: mean NN interval, the mean heart rate, the difference between the longest and the shortest NN interval, the difference between night and day heart rate. Appendix C gives a printout summarising the results. The time-domain analysis results summarised in table 3.1 showed no significant differences between the IUGR and the Normal Group (Biala T. *et al.*, 2008). The p values of comparing between all HRV of the time domain variables presented in table 3.1 show no significant difference between the normal children and the IUGR children, all with $p>0.05$.

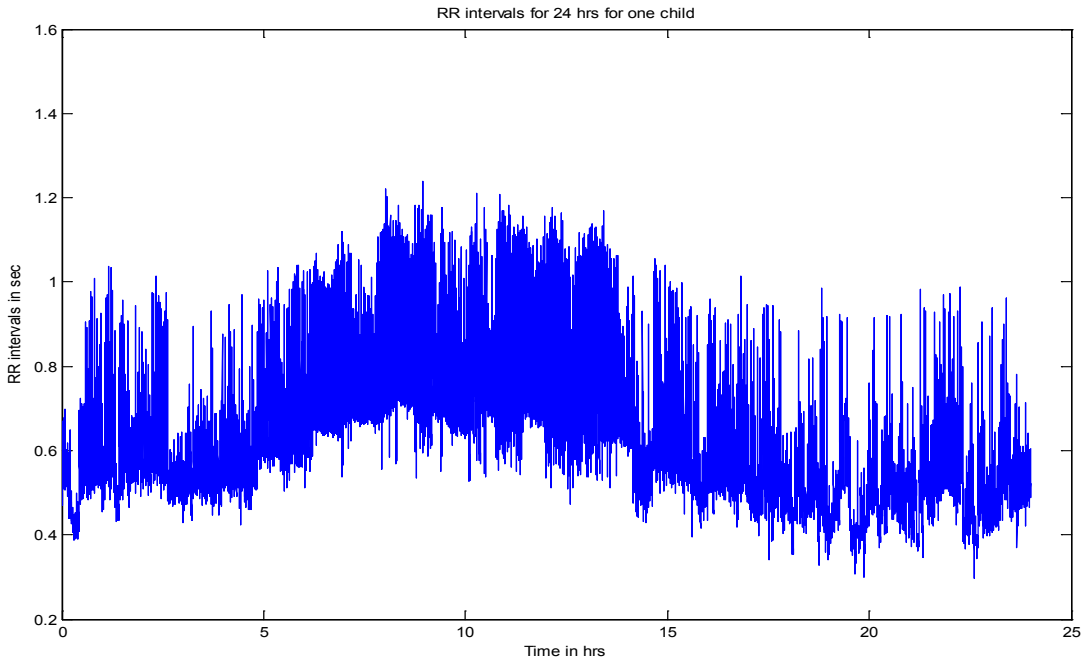

Figure 3.1: The 24 hr RR intervals for 10 yrs child.

Table 3.1: Time domain HRV measures.

Subject	Normal No=34 Mean (SD)	IUGR No=41 Mean (SD)	p value	95% Confidence Interval for Difference[a]	
				Lower Bound	Upper Bound
Mean 24 (s)	0.676 (0.057)	0.685 (0.066)	0.581	-0.04	0.023
Mean day (s)	0.612 (0.052)	0.623 (0.064)	0.494	-0.04	0.019
Mean night (s)	0.822 (0.082)	0.828 (0.084)	0.794	-0.047	0.036
SDNN	0.159 (0.029)	0.150 (0.034)	0.289	-0.008	0.025
SDNN day	0.118 (0.023)	0.111 (0.023)	0.238	-0.005	0.019
SDNN night	0.121 (0.031	0.114 (0.032)	0.381	-0.009	0.023
Mean RR (5 min) (s)	0.676 (0.057	0.684 (0.066)	0.571	-0.04	0.022
Mean day (5 min) (s)	0.596 (0.053)	0.607 (0.065)	0.443	-0.042	0.019
Mean night (5min) (s)	0.811 (0.077)	0.816 (0.079)	0.814	-0.044	0.035
SDANN	0.132 (0.023)	0.124 (0.031)	0.261	-0.006	0.022
SDANN day	0.074 (0.018)	0.068 (0.016)	0.202	-0.003	0.014
SDANN night	0.070 (0.019)	0.065 (0.020)	0.359	-0.005	0.014
Shortest NN int.	0.304 (0.028)	0.315 (0.041)	0.201	-0.03	0.006
Longest NN int.	1.394 (0.131)	1.442 (0.765)	0.740	-0.342	0.244
Range	1.090 (0.130)	1.127 (0.775)	0.804	-0.334	0.259

3.1 The Mean and the Standard Deviation:-

In this work, where we have the ECG records for 24-hour periods, the mean and SD for the averaged segments of 5 min were calculated. Figure (3.2) shows the RR mean and SDANN for a normal child. The night time is well defined and marked between the two brown lines. The mean of RR is high at night, which means a low heart rate. The standard deviation of averaged 5 min, SDANN (ms), is plotted under the RR curve and it shows that there is high variability of the RR at night due to the influence of the parasympathetic and sympathetic branches of the ANS. At day time, when the child is active, the sympathetic branch of the ANS is dominant, RR is short, and so is SDANN. Periods before going to bed and awakening are characterised by a sudden rise in RR, positive slope, and then a decrease, a negative slope, respectively.

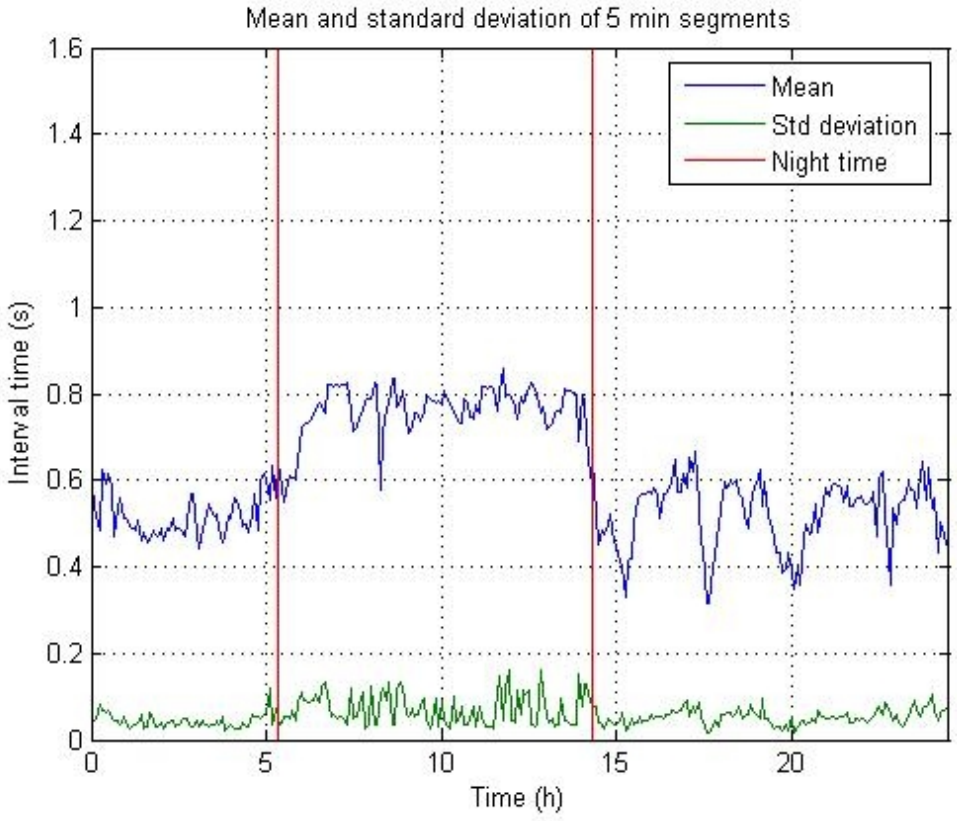

Figure 3.2: A normal child average RR and SDANN over 24 hr.

The following plot, figure 3.3, shows the mean RR and the SDANN for an IUGR child where it can be noticed the changes over the 24 hr between high RR to low RR, which means the rising and falling of heart rate during the day and the night.

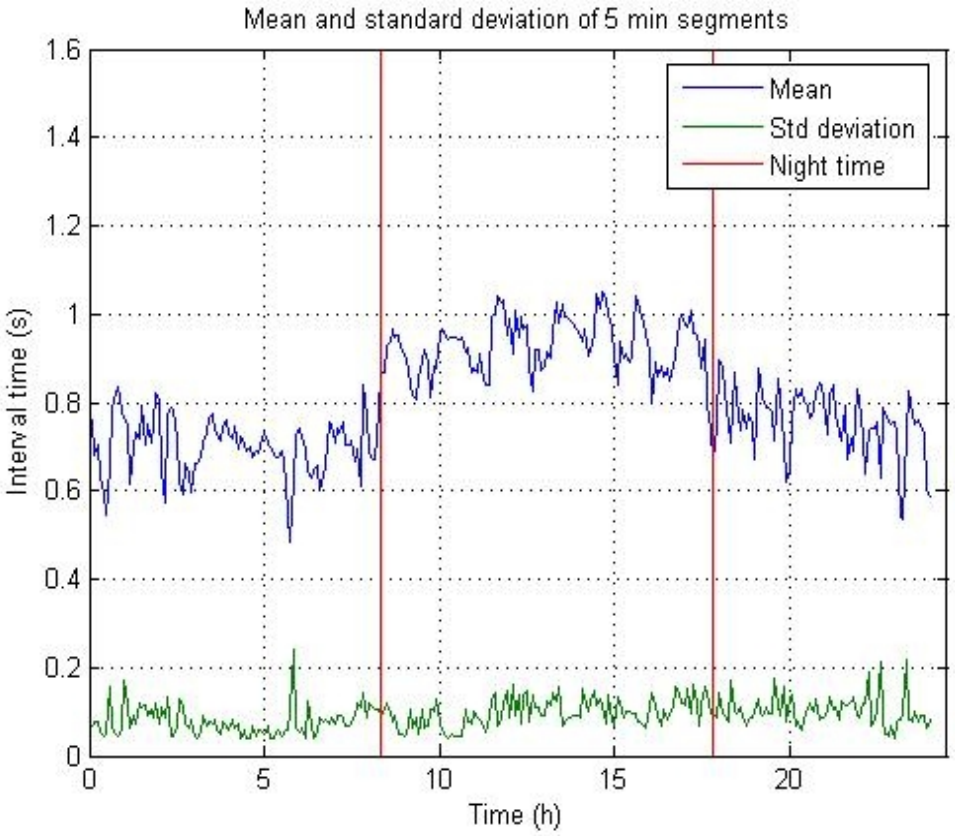

Figure 3.3: An IUGR child average RR and SDANN over 24 hr.

Normal and IUGR RR-mean and SDNN box-whiskers for the two groups were plotted. This is first to visualise and to study the Normal RR mean (figure 3.4) for a 24 hr period, including both day and night and the Normal RR SDNN (figure 3.5) and then to look at the IUGR RRmean and SDNN. The

RR mean during night time shows higher values because the heart rate drops during the night and the parasympathetic mode of the ANS is dominant. The respiratory sinus arrhythmia phenomenon is very clear at night time due to the modulation of the heart rate by the breathing, which is more evident with regular breathing. The mean RR of the IUGR children (figure 3.6) shows the outliers marked in red. These were removed prior to performing HRV analysis. Figure 3.5 shows the SDNN (a very

common measure of the HRV) of the normal children. Notice that it is higher than the SDNN for the IUGR children (figure 3.7). Low value of SDNN is a proven marker for heart diseases.

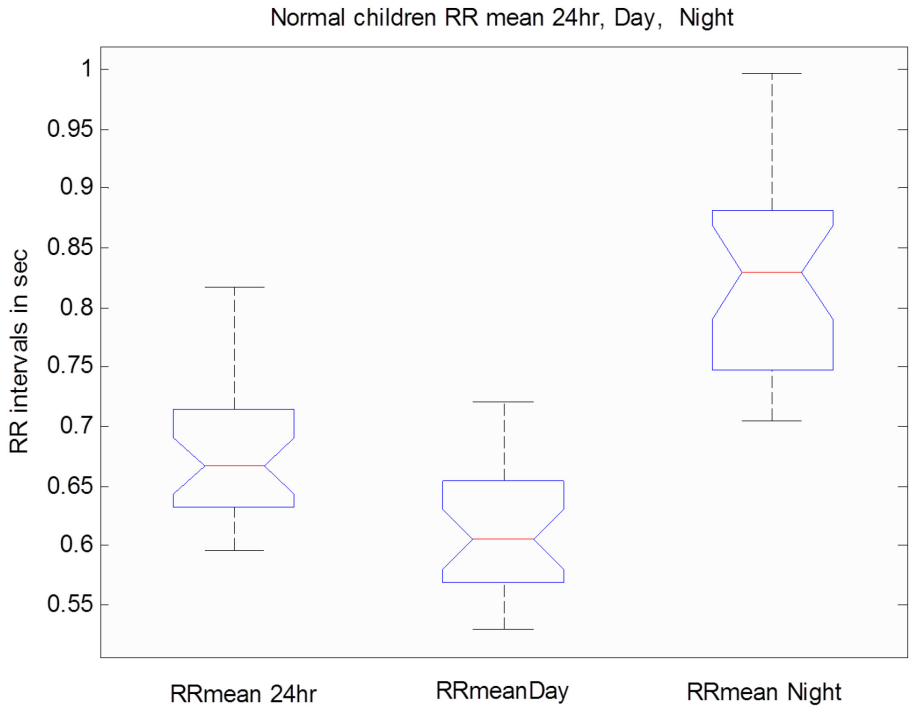

Figure 3.4: Normal Children RR mean for 24 hr, day and night.

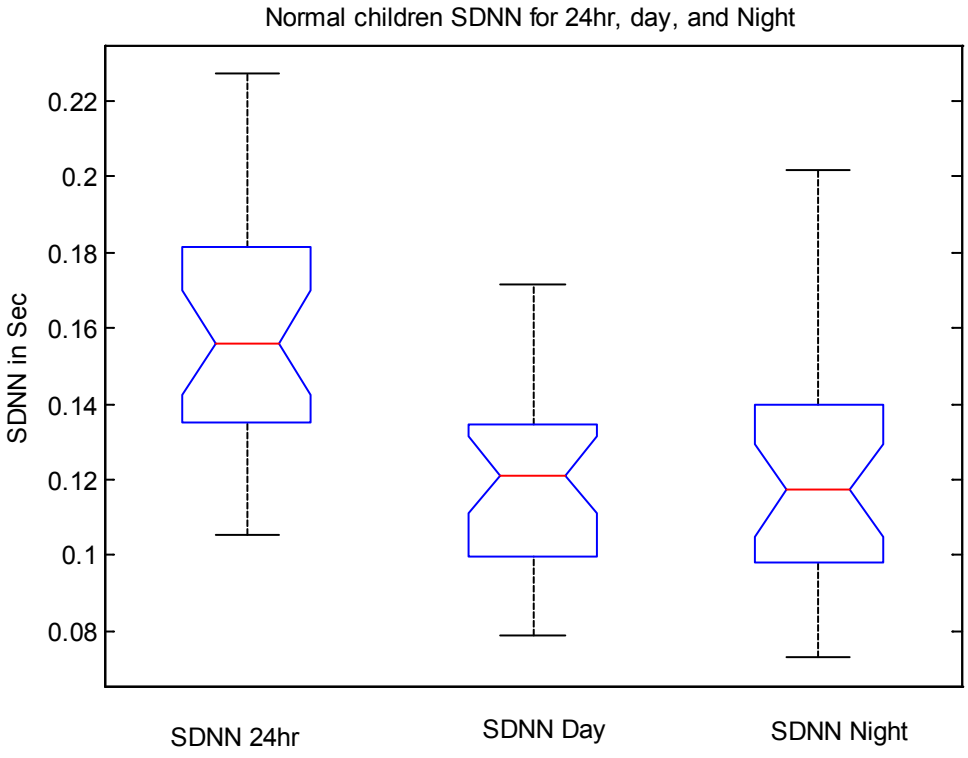

Figure 3.5: Normal children SDNN for 24hr, day and night.

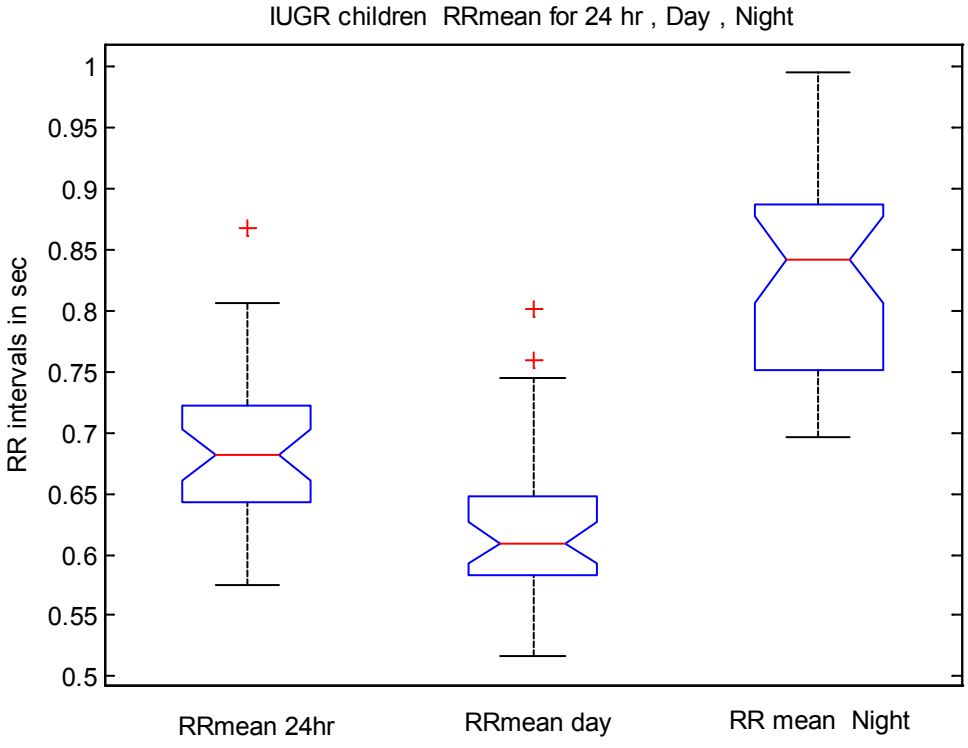

Figure 3.6: IUGR RR mean for 24hr, day and night.

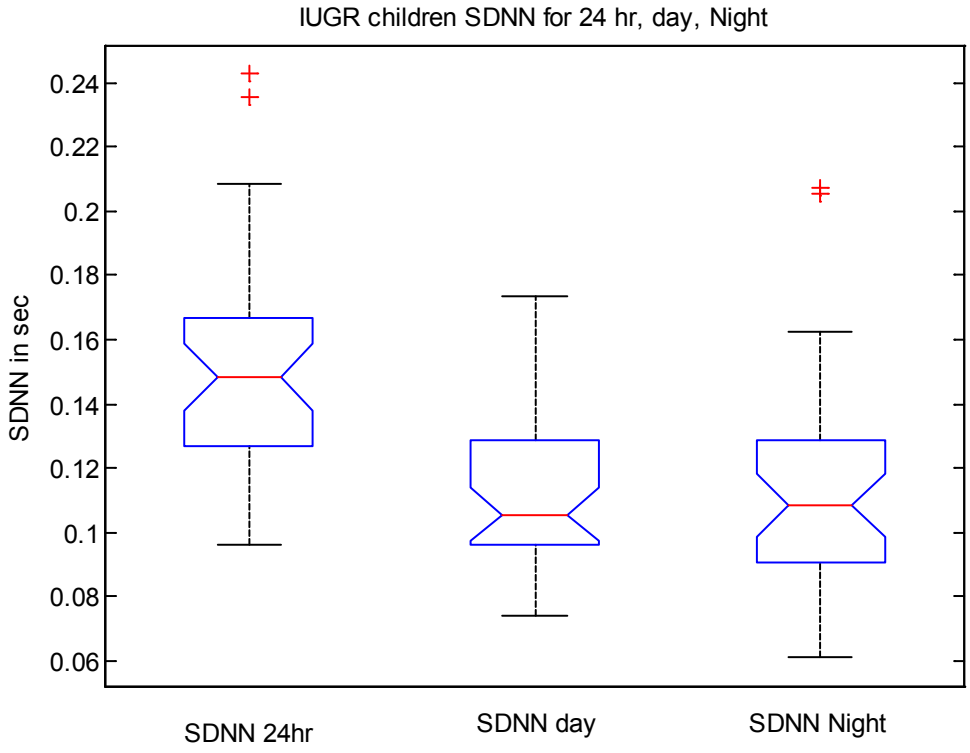

Figure 3.7: SDNN of IUGR children for 24 hr, day and night.

The RR mean results for the normal children show that at night time the parasympathetic branch of ANS is dominant and heart rate slows down, but at day time when the children are very active and playing around, the sympathetic branch is dominant and heart rate is faster. The same applies for the IUGR children, and it was found that there is no significant difference between the mean RR results for Normal and IUGR, in all day, all night and 24 hr periods ($p>0.05$). The high SDNN for 24hr period is due to the consideration of the whole span of tile day and night and it includes the waking up and the sleeping transition which is not considered in the day and night analysis for both IUGR and normal children data. The results of the time domain analysis shows that there is no significant difference between all the HRV measures ($p>0.05$).

3.2 Heart rate plots

The inverse of the R to R (NN) intervals is the instantaneous heart rate. In figure 3.7, at day time the heart rate for this particular child is high, it is in the range of approximately 78 bpm to 160 bpm. At night time when parasympathetic tone is

dominant and the child is at rest, the heart rate tends to be as low as 60 bpm. The heart rate is equal to 1/RR , and the unit is beat/min.

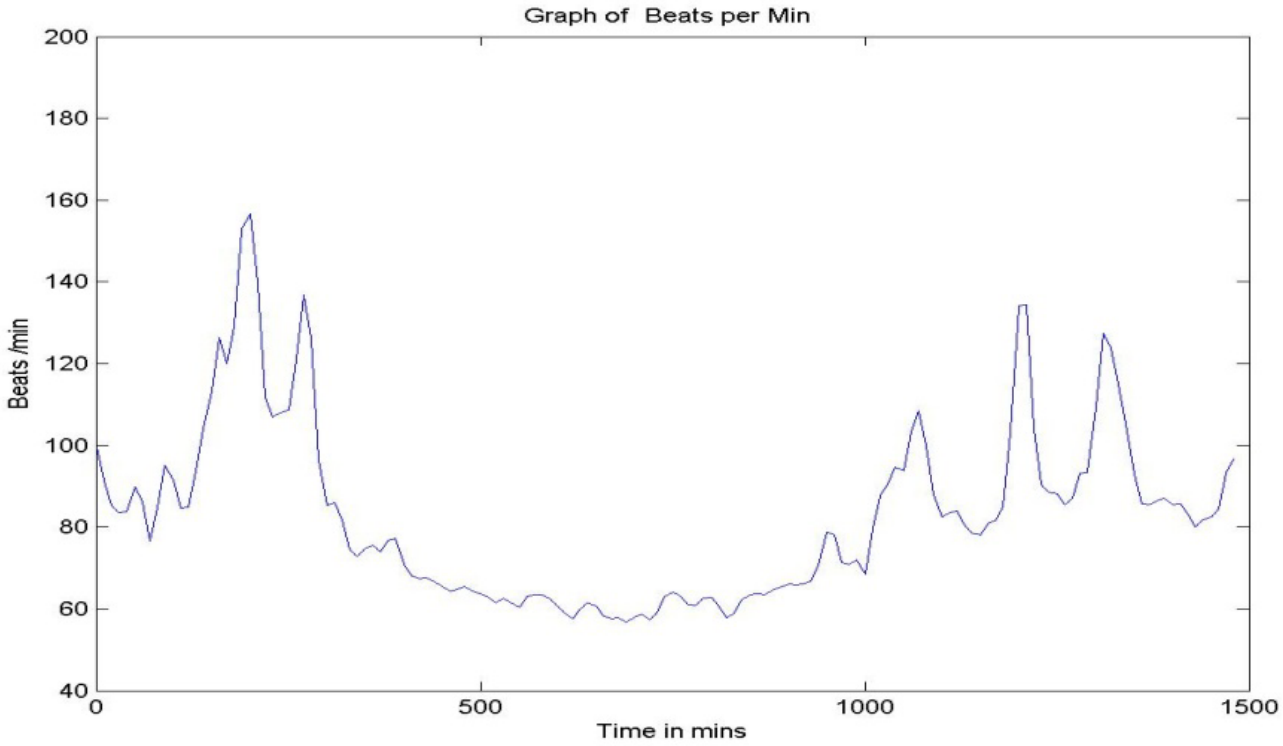

Figure 3.8: A Graph of the heart rate in beats per min for 24hr.

3.3 Sample Density Distribution

Sample density distribution plots (which are the plots of all NN intervals against their number of occurrences) and Poincaré plots (the plots of the present NN against the previous NN) along all the 24 hr are part of the geometrical tools for HRV Poincaré analysis They are used to visualise the distribution of the RR intervals and how they behave in terms of their statistical distribution. The 'incorrect' NN intervals are usually either shorter or longer than the population of 'correct' NN intervals (Malik, 1997). Figure (3.9) shows the sample distribution plot for a child, it has a peak of RR around 0.6 s and another lower peak at 0.8 s. The range of the shortest RR to the longest RR is from 0.3 s to just under 1.3 s.

Triangular index (HRV index) is the value obtained by dividing the area integral of the distribution D by the maximum *Y,* where Y=D(X), and X is the most frequent NN.

$$HRV\ index\ = \frac{(total\ Number\ of\ all\ NN\ intervals)}{Y} \ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

And the interval histogram (TINN) measure is = M-N (3.2)

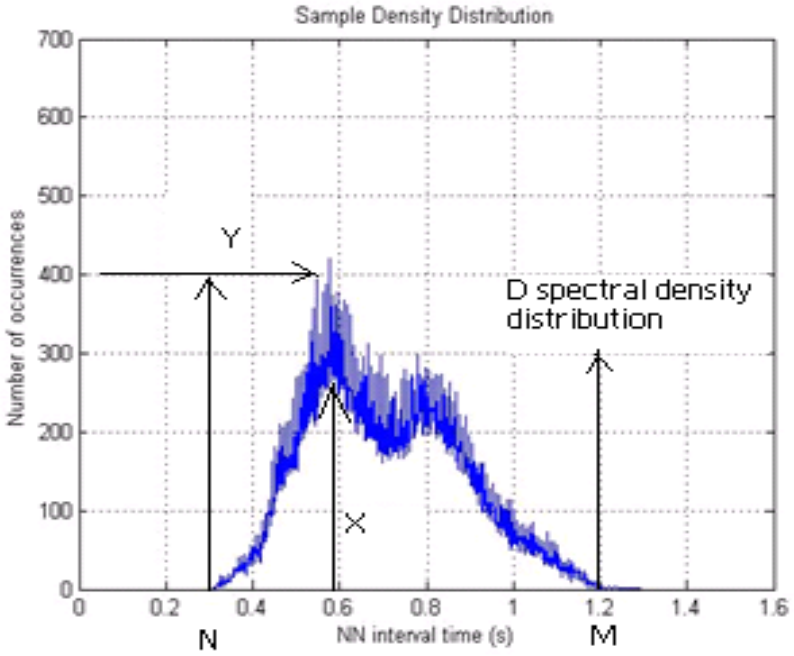

Figure 3.9: The sample density distribution of the RR intervals for a child.

3.4 Statistical results for Night comparison

It is well known that RR data time series is a non-stationary signal and it is unpractical to compare between subjects given the features of RR time series, so a possible way of dealing with such a signal is to segment the time series at an identified length and analyse the segments in isolation (Clifford *et al.*, 2006). It is recommended (by Task Force 1996) to take short segment of 5 or 10 min and I have taken 10 min, then I added more than that to study the trend in the data. Interestingly enough a lot of results from this study do agree with other results in this thesis or confirm results by other researchers.

In this work, one hour of RR was separated from the 24 hour record of RR to look at a period of night when the children having their highest RR and what effect has some known factors (IUGR, smoking, breastfeeding, gender) on the ANS. This was done for the purpose of comparing between different groups of children with specific characteristics, such as IUGR group against Normal, or Normal children with parental smoking during pregnancy against normal non smoking parent,… etc. The selected one hour starts after the maximum value of RR of every child; that means it is at night

time when the parasympathetic tone is at its highest level. A Matlab algorithm enclosed in the CD with this thesis was developed. The program is to find the maximum RR and the following hour for all normal children as one group then to find the same for the IUGR group. This was done to see the effect of gender, breastfeeding and smoking. The results plotted and can be compared graphically.

It is quite clear that at night time the variation in RR is less and hence the comparison between different groups of children will be more adequate if the night time is used as this is not affected by the children's day activities.

3.5 Night Time RR comparison

3.5.1 Night Time 1 hr comparison (IUGR, Normal)

The night time 1 hr RR comparison between IUGR and Normal groups shown in figure 3.10 indicates that they are very similar values of RR. The later results show that there are differences between other groups like males and females, but overall results of all the IUGR and Normal cancel any differences. The trend of the plots for both normal and IUGR starts from high values of RR because the RR data has been chosen arbitrarly to start from the highest value of RR.

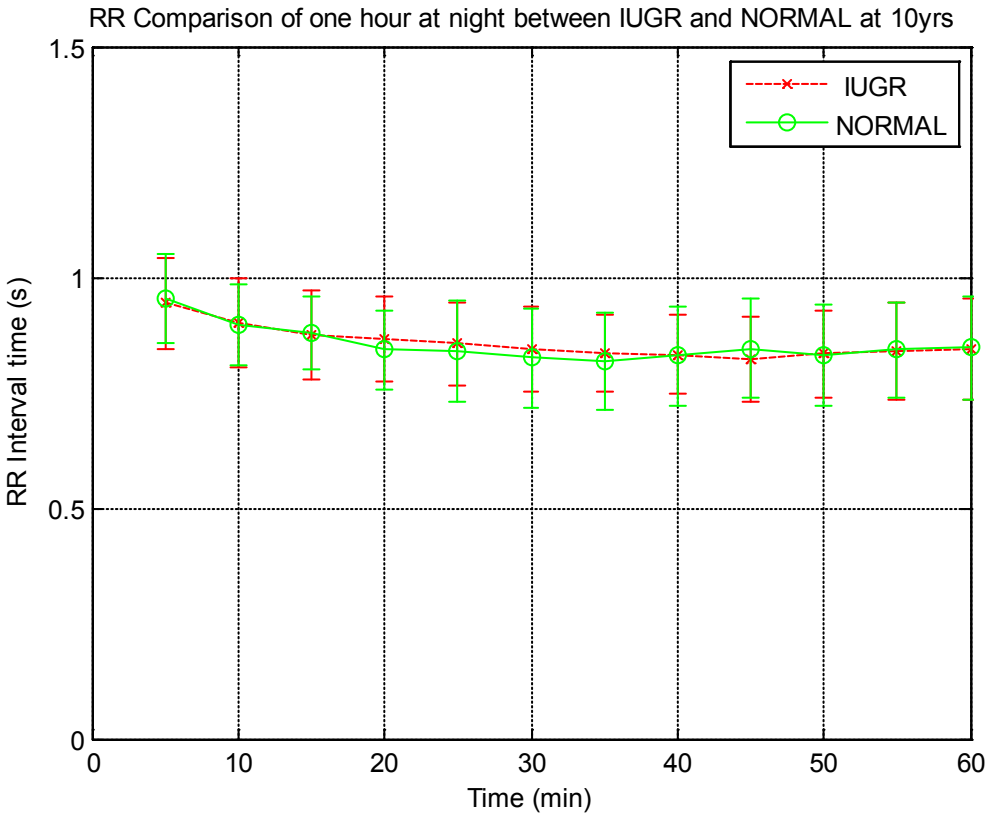

Figure 3.10: The one hour RR comparison at night between IUGR and Normal children.

3.5.2 Night time 1 hr comparison (Male, Female)

The graphs shown in Figures 3.11 and 3.12 allow a comparison between males and females in IUGR and Normal groups; it is obvious that the males have higher RR intervals at night than the females. This agrees with the findings of Rijnbeek *et al.* (2001), whose study confirms that the boys have longer RR intervals, and the girls have a higher heart rate than the boys at the same age. It is well known that the smaller the body size the shorter the RR intervals and the higher the heart rate. This also means infant's heart rate is higher than the adult's.

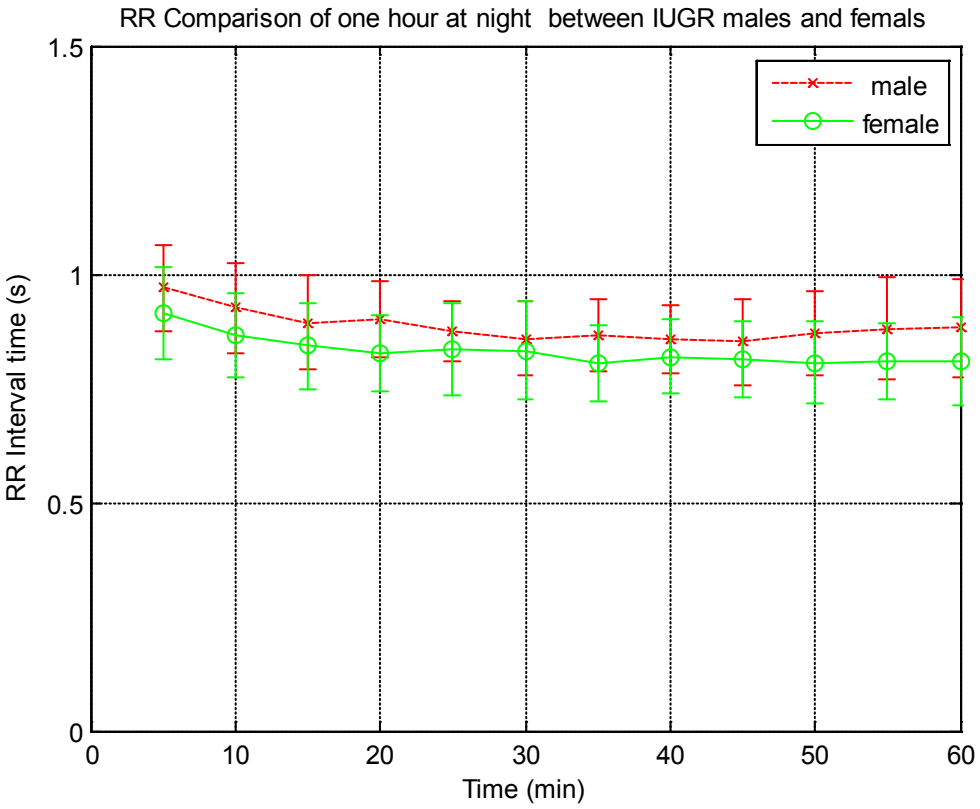

Figure 3.11: Comparing IUGR males and females.

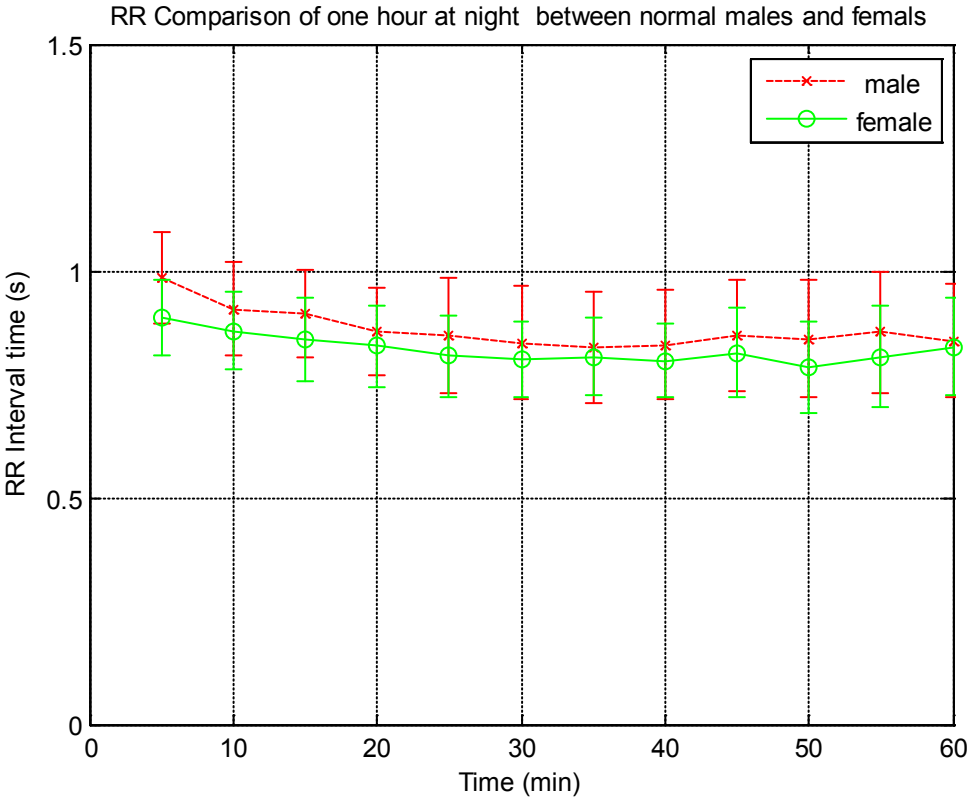

Figure 3.12: Comparing Normal males and females.

3.5.3 Night time 1h comparison (Breast Feeding)

Breast milk has always been encouraged for the infants as the best nutrition in the first months of life. Breastfeeding has been associated with a better cardiovascular risk profile (Ravelli *et al.*, 2000), and better cardiovascular outcomes in later life, such as less ischemic heart disease, (Fall *et al.*, 1992) and lower risk of obesity in adulthood (Dewey, 2003). Research studies on the effect of breast feeding on cardio respiratory risk factors in adult life, show that there was no substantial long-term protective effect of breastfeeding for >1 month on other cardio respiratory risk factors in adult life. Breast-feeding is shown to be linked with a lowering of blood pressure (at 7.5 yrs) in children born at term (Martin *et al.*, 2004). When breastfeeding IUGR children were compared with non-breastfeeding (fig 3.13), the breastfeeding IUGR have longer NN intervals than the non-breastfeeding ones. For the normal children there is no effect of breastfeeding on NN intervals, see fig (3.14).

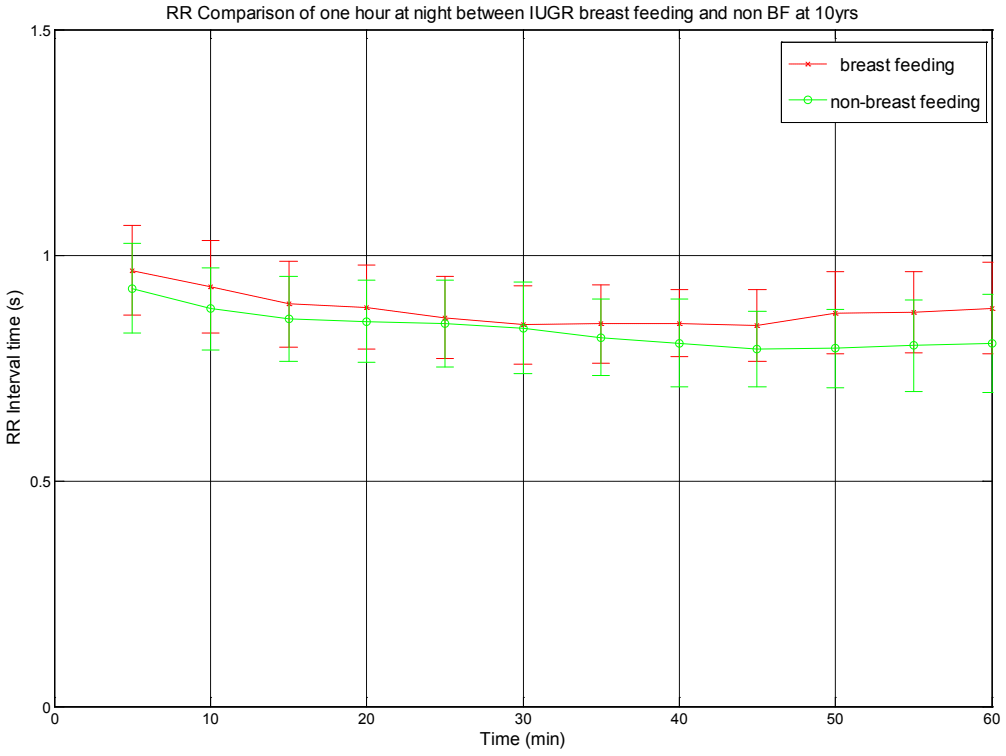

Figure 3.13: IUGR breastfeeding and non-breastfeeding.

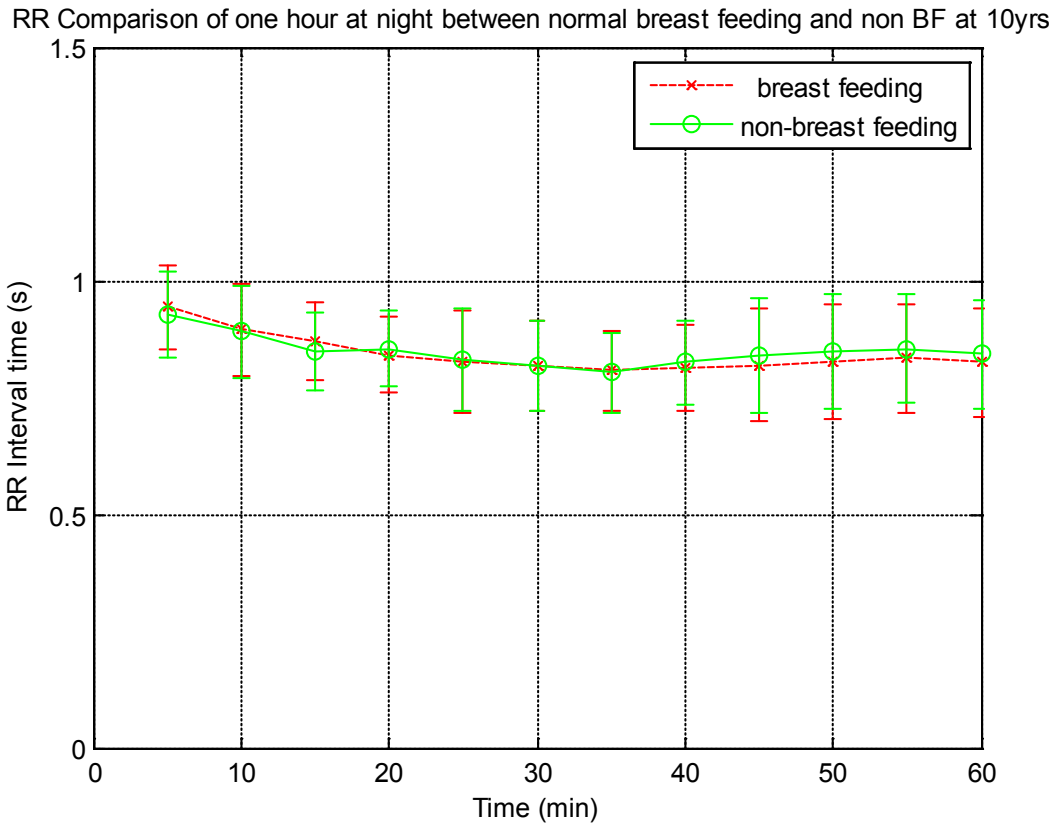

Figure 3.14: Normal breastfeeding and non-breastfeeding.

3.5.4 Night time 1h comparison (Smoking)

The one-hour analysis from this work shows that during pregnancy the IUGR and Normal with non-smoking parents have a lower RR intervals, fig (3.15) and fig (3.16). This implies that smoking has an effect on the ANS. Studies by Blair, Fleming and others, confirm the increased risk of sudden infant death syndrome associated with maternal smoking during pregnancy and evidence of household exposed to tobacco smoke has an independent additive effect (Blair *et al.*, 1996). At 10 yrs old the effect of parental smoking indicates that IUGR have low RR if their parents are Smoking, Figure (3.17). The effect of smoking on Normal children RR at night shown no significant difference, Fig (3.18).

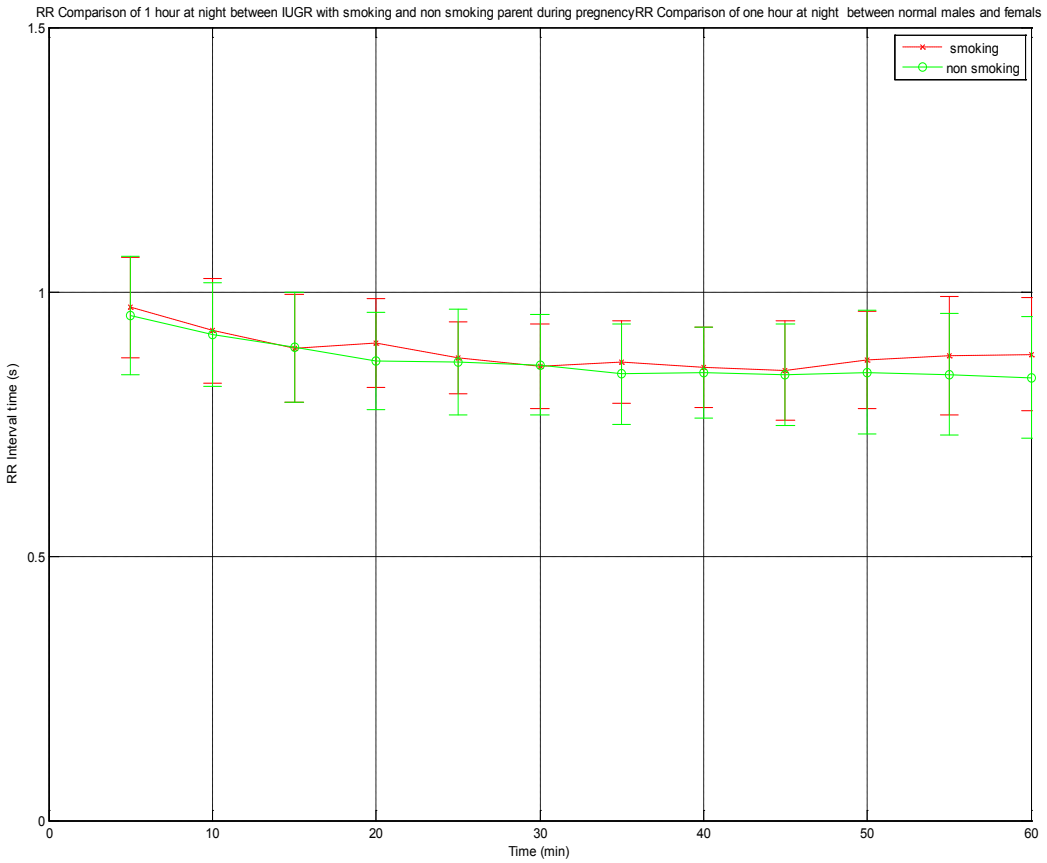

Figure 3.15: Maternal smoking effect on IUGR.

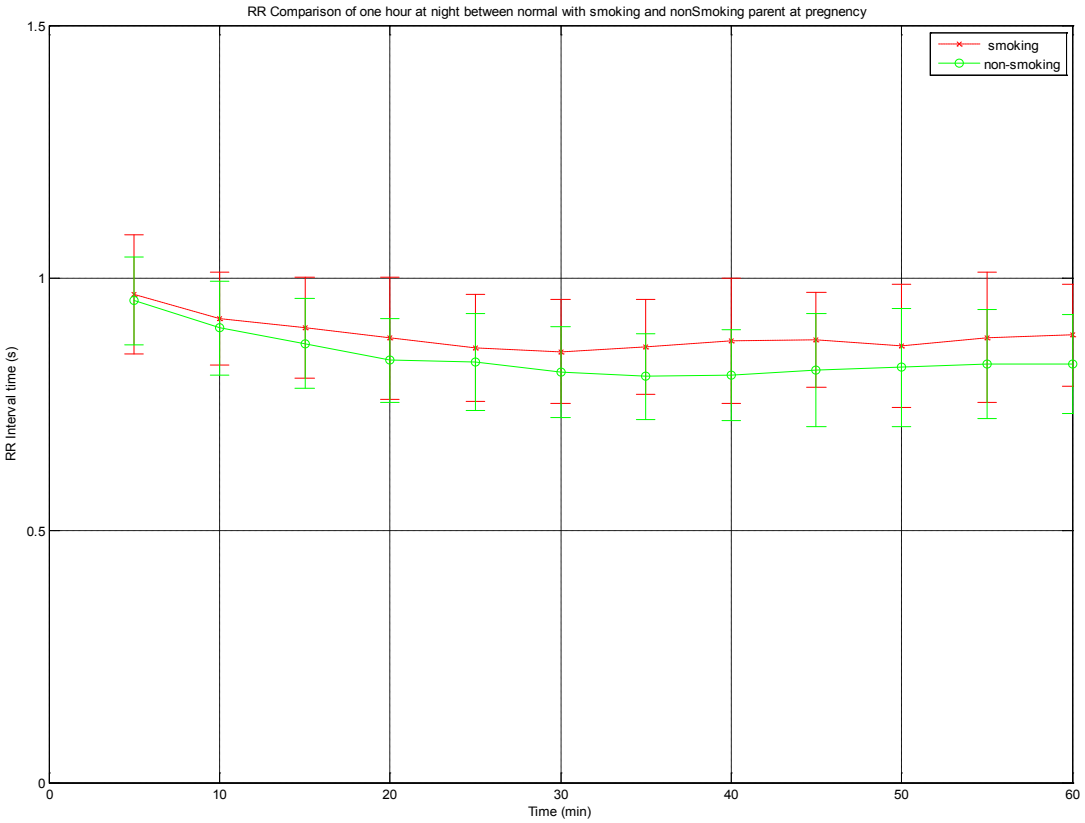

Figure 3.16: Maternal smoking effect on Normal children.

Another study by (Geerts *et al.*, 2008) found no association between maternal exposure to tobacco and diastolic blood pressure, but reported that smoking during pregnancy has a substantial increasing effect on systolic blood pressure in early infancy.

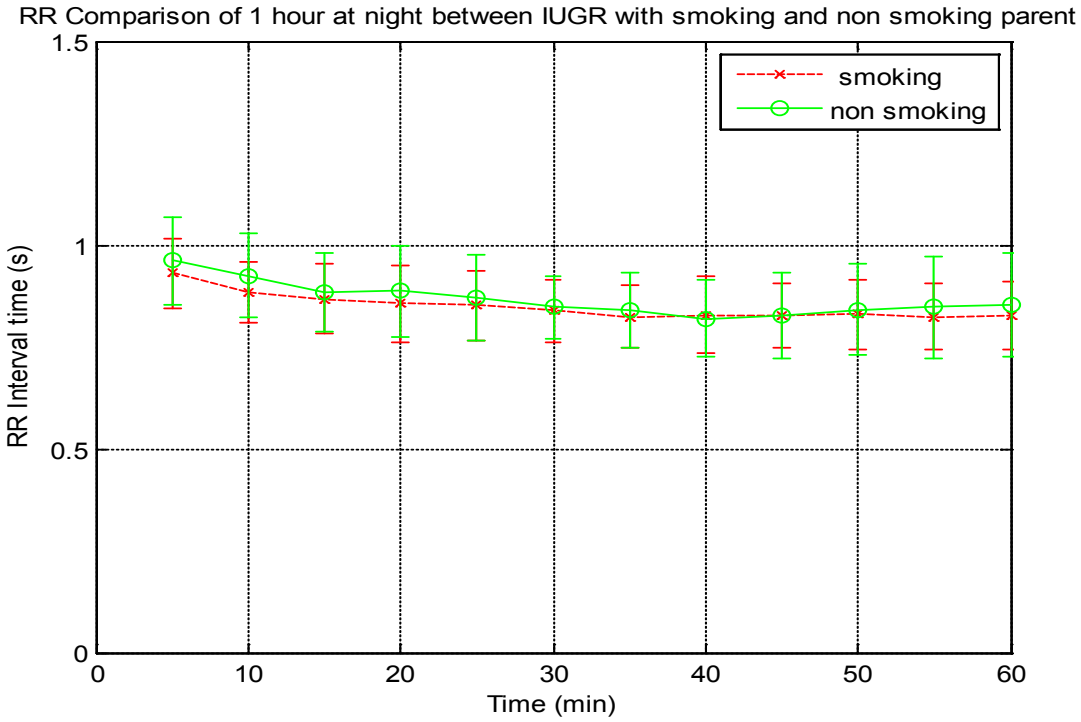

Figure 3.17: Passive smoking effect on IUGR children.

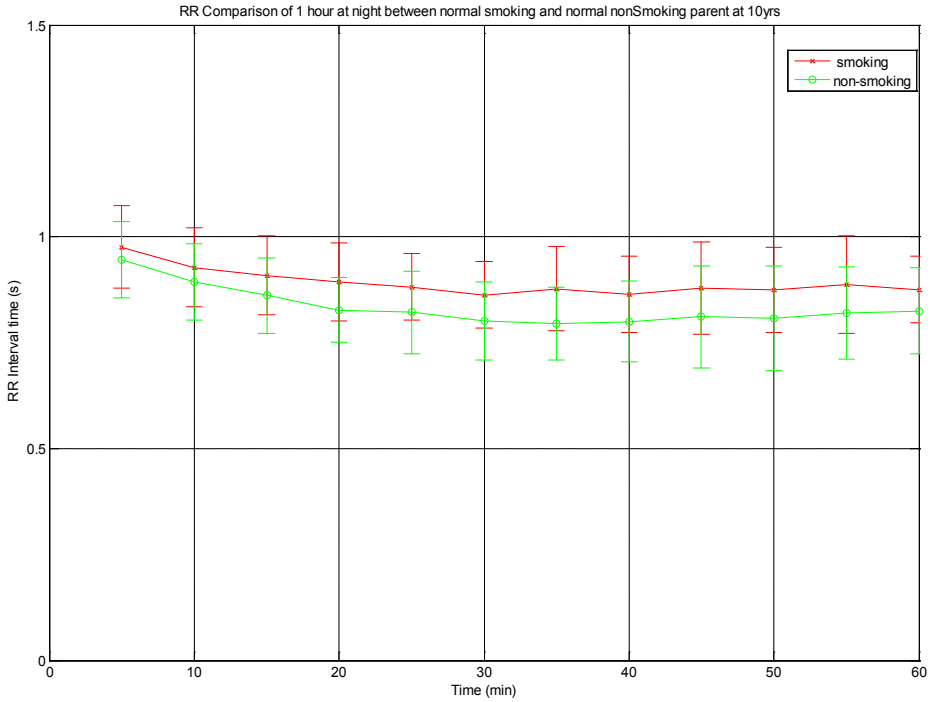

Figure 3.18: Passive smoking effect on Normal children.

3.5.5 Statistical analysis and discussion

All the previous analysis and plots were about one hour comparison of RR intervals at night time and this does not give the whole picture of all the RR night time differences. The nature of non-stationary RR signal can lead to wrong results when doing analysis based on only one hour at night of the IUGR and normal children. Statistical analysis on the previous work (for night time data) was performed by using the Mulifactorial Analysis Of Variance to study the effect of many factors on the RR intervals at all duration of night time and at day time. The Null hypothesis in this analysis is that the outcome RR at night is not affected by any of the factors included in the study. Multifactorial ANOVA is used for analysing the simultaneous effects of two or more independent variables. Table 3.2 shows the listed factors under study using the above method. The SPSS was used to encode the variables to denote the different state of the variable, e.g. breast feeding =1and non breastfeeding = 2. The test of homogeneity of variance was satisfied by having the result of the Levene's test not significant $p>0.05$. The results of running the Multifactorial Analysis of Variance test are shown in table 3.3. During night time the RR intervals are not affected by any of the listed factors (breast feeding, non-breast feeding, smoking and non-smoking during pregnancy, house hold type at the age of 10, males, females, and type of child). The p value was not significant for all factors, i.e. it is >0.05, except for the gender factor where the p value $=0.011$. Males RR intervals at night time have a higher value than females RR intervals (mean difference = 54). The day RR intervals analysis showed that none of the predictors has any effect on the day RR intervals. These results showed no significant difference for all predictors in the study.

When the test was run for dependent variable SDNN at night time, the results shown in table 3.4 gives slightly higher SDNN (HRV) in males, normal, children whose mother did not smoke during pregnancy and children who lives in a non-smoking family at 10 yrs. However, none of the differences reached statistical significance. SDNN is a basic time domain measure for HRV, as explained in chapter 1, and when SDNN is high this means that the subject has high HRV which is a good indication of a healthy subject.

Table 3.2: Factors under analysis to see their effect on RR at night intervals.

Factors		N
Breast-Feed	1.00	44
Non Breast-feed	2.00	31
Non-Smoking in preg.	0.00	50
Smoking in pregnancy	1.00	25
Non-Family Smoking	0	42
Family Smoking	1	33
male	1.00	41
Female	2.00	34
IUGR	1	41
normal	2	34

Table 3.3: The results of Multifactorial Analysis of variance test of RR night as dependent variable.

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Breast-feed	1	842.266	14.965	.469	812.405	872.128
Non Breast-feed	2	825.270	16.801		791.744	858.796
Non- Smoking	0	832.676	14.677		803.388	861.964
Preg.	1	834.860	19.944	.937	795.064	874.657
Smoking in Preg.						
Non- smoking	0	827.209	18.148		790.994	861.964
family	1	840.328	15.933	.622	808.534	874.657
smoking family						
Male	1	860.789	14.314	.011	832.226	889.352
female	2	806.748	15.506		775.806	837.690
IUGR	1	838.639	14.166	.664	810.372	866.907
Normal	2	828.897	16.782		795.410	862.384

Table 3.4: The results of Multifactorial Analysis of Variance test of SDNN night as dependent variable

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Breastfeed	1	91.229	5.801	.659	79.646	102.811
Non Breastfeed	2	95.224	6.536		82.175	108.273
Non-Smoking in Preg	0	93.996	5.674	.886	82.668	105.323
smokingPreg	1	92.457	7.764		76.956	107.958
Non- smoking family	0	93.675	7.041	.930	79.617	107.734
smoking Family	1	92.778	6.178		80.442	105.113
Male	1	98.134	5.563	.223	87.027	109.241
female	2	88.319	6.057		76.225	100.412
IUGR	1	90.642	5.541	.553	79.580	101.704
Normal	2	95.811	6.529		82.775	108.847

4 Frequency Domain Analysis

Spectral analysis of NN interval sequence allows the interpretation of the effects of the sympathetic and parasympathetic influence on the heart rhythm.
Three power spectral analysis methods have been tested on the NN data to find the frequency components of the power spectrum for 10 min segments.

Table 4.1 shows most frequently used frequency domain parameters and their boundaries for short and long term HRV analysis. Short term spectral recordings are characterized by VLF, LF, and HF parameters, while long term recordings include ULF component in addition. The total power (variance) corresponds to the sum of four spectral bands ULF, VLF, LF and HF parameters. The HF power is synchronized with respiratory rhythms, primarily related to the vagal innervations and can be determined by frequency of breathing. The interpretation of LF is controversial, some authors consider LF power as a measure of sympathetic modulation, while others consider it as combination of both sympathetic and parasympathetic activity. The consensus is that LF is sensitive to cardiac sympathetic and parasympathetic nerve activity. The ULF component might reflect circadian and neuroendocrine rhythms and VLF reflects long period rhythms and is affected by temperature regulation and humoral systems (The Task Force, 1996) .

Table 4.1:Frequency domain HRV measures (Redrawn from Task force 1996)

Variable	Units	Description	Frequency Range
		Short term Recordings (5 min)	
Total power	ms^2	Variance of NN intervals 10 min segment	Approx. $\leq$ 0.4 Hz
VLF	ms^2	Power in very low frequency range.	$\leq$ 0.04HZ
LF	ms^2	Power in low frequency range.	0.04-0.15Hz
HF	ms^2	Power in high frequency range.	0.15-0.4 Hz
LF/HF		Ratio of low frequency and high frequency.	
		Long term Recordings (24 hour)	
Total power	ms^2	Variance of all NN intervals	Approx. $\leq$ 0.4 Hz
ULF	ms^2	Power in ultra low frequency range.	$\leq$ 0.003HZ
VLF	ms^2	Power in low frequency range.	0.003-0.04 Hz
LF	ms^2	Power in low frequency range.	0.04-0.15Hz
HF	ms^2	Power in high frequency range	0.15-0.4 Hz

4.1 Fast Fourier Transform

The Fourier model assumes that the RR sequence we observe is the weighted summation of a large number of independent sinusoidal oscillators with different RR intervals (Burr R. *et al.* 1992). In figure 4.1b, a spectrum of 10 min of re-sampled RR can be seen with a high peak frequency around 0.35 Hz.

Periodogram estimation of power spectral density is the most basic nonparametric PSD estimation. The periodogram is the squared magnitude of Fourier Transform of the signal scaled in amplitude by the number of samples.

$$P\left(\frac{k}{NT}\right) = \frac{1}{N}\left|\sum_{n=0}^{N-1} x(nT)\, e^{\frac{-j2\pi nk}{N}}\right|^2 \qquad (4.1)$$

Where $x(n)$ the original signal of length N, and f_s = 1/T is the sampling frequency.

The spectral estimation can be assed according to the issues of spectral leakage, resolution and variance. Spectral leakage is associated with a finite signal samples. The transform of the finite signal samples is treated at a transform of a convolution of infinite number of samples with a rectangular window. At the edges of the truncated signal an unwanted spectral components will be introduced, and this will be higher in case of shorter signal samples. Decreasing this unwanted signal before the performing the Fourier transform the signal may be multiplied by a smooth window function tending to zero on at the edges.
The modified periodogram is defined as follows:

$$P\left(\frac{k}{NT}\right) = \frac{\frac{1}{N}\left|\sum_{n=0}^{N-1} x(nT)\, e^{\frac{-j2\pi nk}{N}}\right|^2}{\sum_{n=0}^{N-1}\left|w(n)\right|^2} \qquad (4.2)$$

Where $w(n)$ is the window function.
The variance can be decreased by computing separate periodograms for *M* signal intervals of length *L* and then averaged.

The averaged periodogram is defined as:

$$P(f) = \frac{1}{M}\sum_{M=o}^{M-1}\left[\frac{1}{Lf_s}\left|\sum_{n=0}^{L-1} x(n+ML)e^{-j2\pi\frac{f}{f_s}n}\right|^2\right] \quad (4.3)$$

Welch periodogram is based on the signal divided into a *M* number of intervals of length *L* overlapping by *D* samples. Each interval is multiplied by a window function $w(n)$. The periodograms are computed for each interval and averaged as shown in the following equation:

$$P(f) = \frac{1}{M}\sum_{M=o}^{M-1}\left[\frac{\left|\sum_{n=0}^{L-1} x(n+MD)w(n)e^{-j2\pi\frac{f}{f_s}n}\right|^2}{f_s\sum_{n=0}^{L-1}|w(n)|^2}\right] \quad (4.4)$$

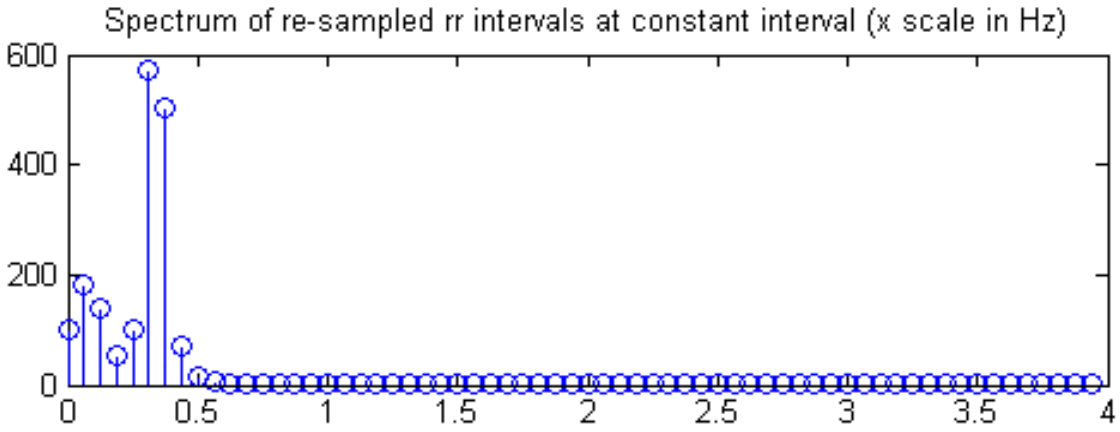

Figure 4.1: FFT of 10 min RR intervals for a child (y scale –PSD(s^2/Hz))

4.1.1 Results and discussion

The frequency domain measures LF and HF of HRV were tested to see if they are affected by other predictors such as IUGR, Normal, smoking during pregnancy and family smoking. The result of the Multifactorial analysis of variance (ANOVA) test is shown in tables 4.1 and 4.2. LF reflects the sympathetic modulation and it increases with stress and tension. LF is shown in table 4.1 to be higher in children who's their mothers smoked during pregnancy and higher in normal children as well as children who were not subjected to smoking in the family when their age is 10 yrs.

Table 4.2: Multifactorial analysis of variance test to study the effect on LF by other predictors

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	1814.373	157.971	.416	1498.883	2129.863
smokingPreg	1	2063.672	228.236		1607.853	2519.490
Non- smoking family	0	2072.157	199.041	.358	1674.645	2469.668
smoking Family	1	1805.888	180.226		1445.952	2165.824
IUGR	1	1873.918	160.832	.578	1552.714	2195.123
Normal	2	2004.126	178.714		1647.210	2361.043

The results of the HF tests in table 4.2 shows that the children who their mother did not smoke during pregnancy and those who were not subjected to family smoking have a higher HF, but the affect of all these predictors on HF are not significant $p>0.05$.

Table 4.2: Multifactorial analysis of Variance test to study the effect on HF by other Predictors

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	2703.890	419.925	.925	1865.241	3542.539
smokingPreg	1	2574.766	606.706		1363.090	3786.441
Non-family smoking	0	2886.545	529.098	.874	1829.864	3943.226
Family. smoking	1	2392.111	479.084		1435.314	3348.908
IUGR	1	2668.599	427.531	.520	1814.760	3522.438
Normal	2	2610.057	475.064		1661.289	3558.826

4.2 Autoregressive model

For short segments of data Fourier-basis spectral estimation has a poor spectral resolution. As sometimes we want to study short segments we also performed the spectral estimation based on the autoregressive model.

The equation of AR process of order p can be written as

$$x_t = n_t + a_1 x_{t-1} + a_2 x_{t-2} + \ldots + a_p x_{t-p} \tag{4.5}$$

Where n_t the white noise driving signal, p is the order of the AR model, and $a_1 \ldots\ldots a_p$ are the parameters of the AR filter.

The AR power spectrum density estimate is given by the following equation (Kay and Marple, 1981) (Boardman *et al.*, 2002):

$$P_{AR}(f) = \frac{\sigma^2 \Delta t}{\left| 1 + \sum_{K=1}^{p} a_k e^{-j 2\pi f k \Delta t} \right|^2} \tag{4.6}$$

Where σ^2 is the variance of the white noise driving function and Δt is the re-sampling interval.

The heart rate variability report produced by the Task force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (The Task Force, 1996) did not select the nonparametric or the parametric spectral method to be more applicable to frequency-domain analysis of HRV. They recommend using both methods to evaluate frequency domain HRV measures because the nonparametric (FFT) has the advantage of algorithmic simplicity and rapidity, while the parametric approach produces smoother spectral components and, if the model order (*p*) is well chosen, it will produce an accurate estimation of PSD even for short time windows.

The AR spectral analysis of 10 min segments for 24 hr will produce a graph as shown in figure 4.2. Figure 4.3 shows the evolution of the AR spectrum of the HRV over 24 hr and figure 4.4 shows the 3D of the AR spectrum evolution of the HRV for the 24

hr. It can be seen that Respiratory Sinus Arrhythmia (RSA) around 0.3 Hz is more apparent at night time, when more regular ventilation is occurring. More details will be given about RSA in a later section. The high peak is due to ultra low frequency ULF (0.0001Hz 0.003Hz) and very low frequency VLF (0.003Hz to 0.04Hz) is due to long-term regulatory mechanisms such as thermoregulatory system, the renin-angiotensin system (which is related to blood pressure and other chemical factors) and other humoral factors (Malik M. and Camm A. J., 1995).

A study by **(**Taylor *et al.*, 1998) showed that VLF fluctuation depends primarily on the parasympathetic branch. Others **(**Serrador *et al.*, 1999) demonstrated that the ULF band is dominated and affected by the physical contributions and that is why HRV in this band tends to increase during exercise.

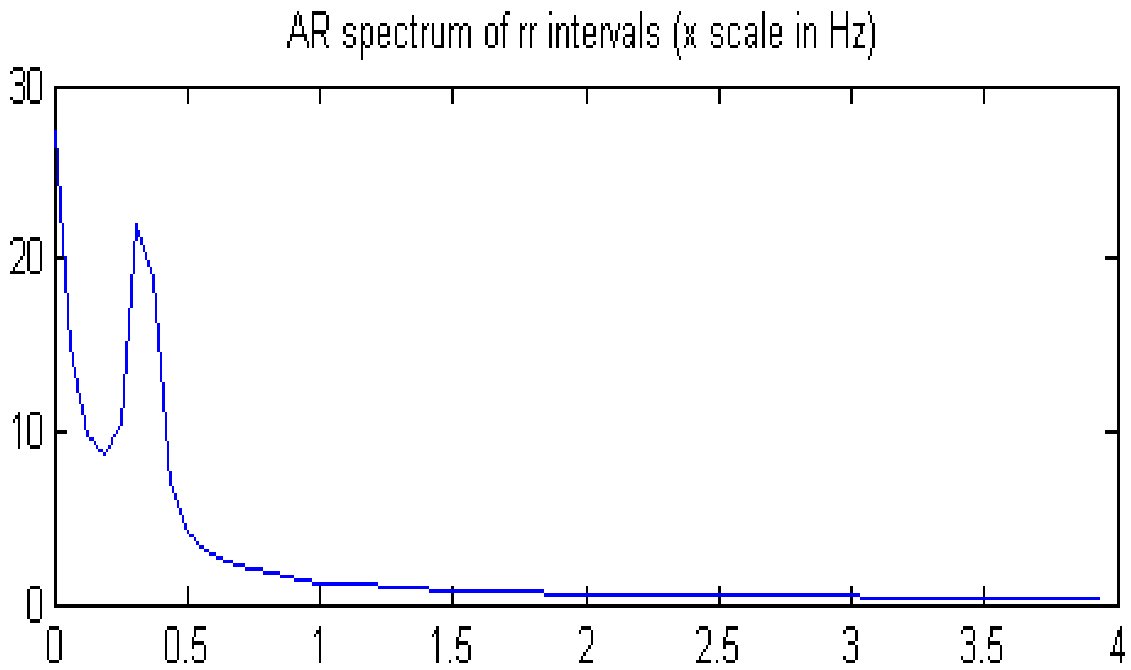

Figure 4.2: Autoregressive spectrum of 10 min (R to R) data after re-sampling (y axis is PSD in S2/Hz).

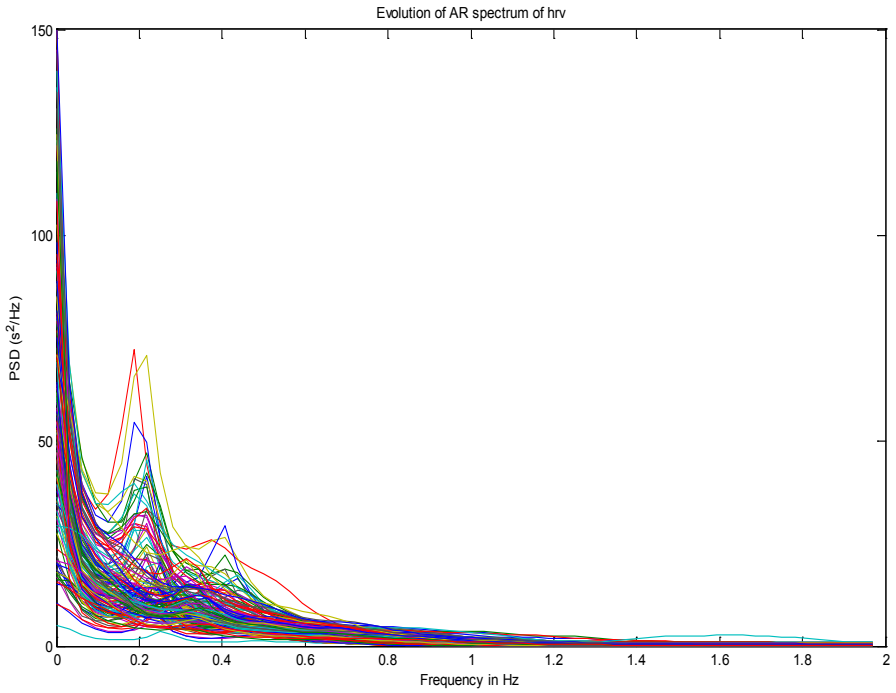

Figure 4.3: Sequence of autoregressive spectra of 10 min (R to R) data after re-sampling.

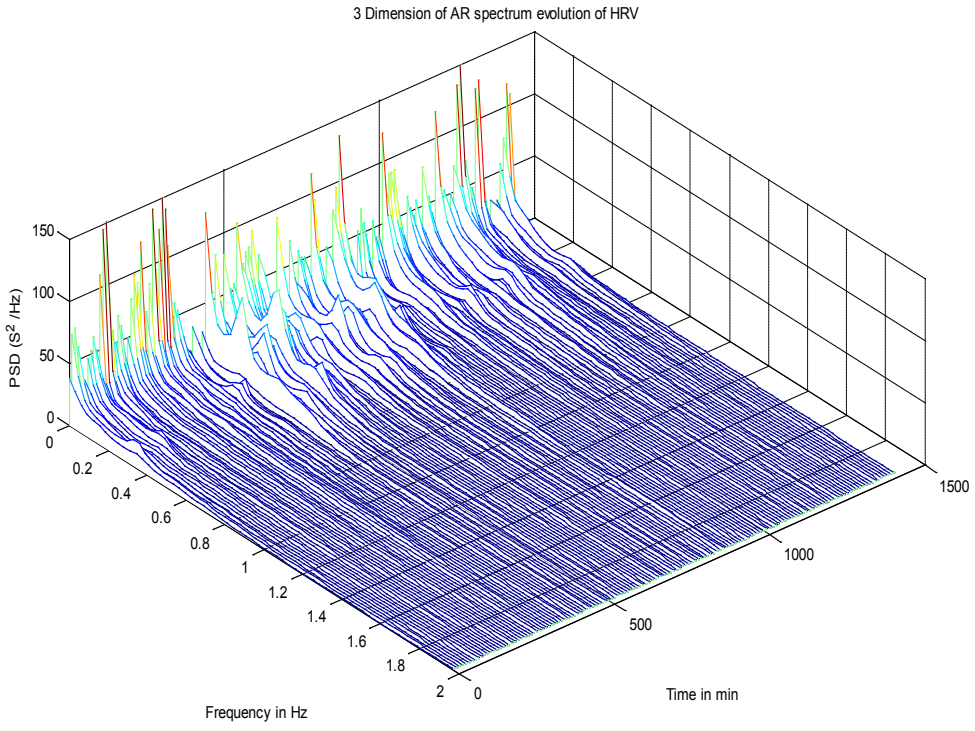

Figure 4.4: 3D sequence of AR evolution of HRV spectra.

4.2.1 Results and discussion

The AR method was implemented using the RSA.m algorithm (programme added to enclosed CD) to find the RSA (area) of the power spectrum between 0.15 Hz and 0.4 Hz, (Biala T. *et al.*, 2009). Data obtained during night time were selected . The starting point was defined using the following line in Matlab:

```
Fprintf ('Where shall we start (in min. Each record = %3.1f min) ?',DataLength_min);
Where=input (' ');
```

The end of the RSA segment analysis can be defined by the following line:

```
Tnum_max=50;          % maximum of 50 segments
Tnum=min (Tnum,Tnum_max);    % limiting max N. segments ...
```

The Trapezoid method in equation (4.7) was used to find the energy (area) of the RSA:

$$A = \frac{(a+b)h}{2} \qquad (4.7)$$

Where (a and b) are the two parallel sides and h is the distance (height) between them.

The result of the algorithm is:

RSA area (energy) = 2.65 u^2, Total area(energy) = 9.05 u^2,percentage= 29.32 percent and the u is arbitrary unit (gain is not calibrated). See Appendix C for results. The t-test showed that there was no significant difference between the two groups for RSA, difference = 0.44, t = 0.32, p=0.7467 (95% CI = -3.1, 2.3). There was homogeneity of variance (Levene's Test, p=0.14) and the data were approximately normally distributed within each group.

Other variables shown in table 4.4 were assessed for significance but none of them significantly predicted (RSA), although IMD (Index of Multiple Deprivation) was borderline (0.06). This means deprived children with high IMD don't have synchronised breathing at night.

Table 4.3: Predictors and their effect on RSA.

Variable	p-value
Sex *	0.32
Breast Feeding (y/n) *	0.50
Parental Smoking (y/n) *	0.99
Household Smoking (y/n) *	0.72
IMD #	0.06
24 hour SBP #	0.59
24 hour DBP #	0.87
BMI #	0.77
Significant Medication *	0.26
Using Medication *	0.36
Birth Weight #	0.45
Length Gestation #	0.50
Weight change from birth #	0.77
Night SBP #	0.79
Night DBP #	0.60
Day SBP #	0.46
Day DBP #	0.64
Cortisol morning #	0.29
Cortisol night-time #	0.66

* = independent t-test, # = correlation

4.3 The Lomb Periodogram

4.3.1 Introduction

Power spectral density (PSD) estimation allows a description of the frequency contents of a time series. The classical methods for estimating the spectrum of a signal or time series are Fourier-based and parametric, of which the AR method is the more popular approach as it involves linear equations, which are computationally easy to solve.

The Fourier and AR methods need a uniformly sampled data series to work, hence they both have the disadvantage of needing re-sampling of the RR data at uniform intervals. This process will alter the frequency content of even a noise-free time series by nonlinear low-pass filtering (Moody, 1993).

The PSD can be adversely affected by the ectopic beats or noise in the time series, because the impulse noise in time series can be transformed to a broad-band "clutter" in frequency domain. The re-sampling can affect more the frequency content of the signal because of the need to put replacements to the noise or to the ectopic beats. Methods such as Lomb periodogram avoid completely the disadvantages associated with re-sampling and interpolation.

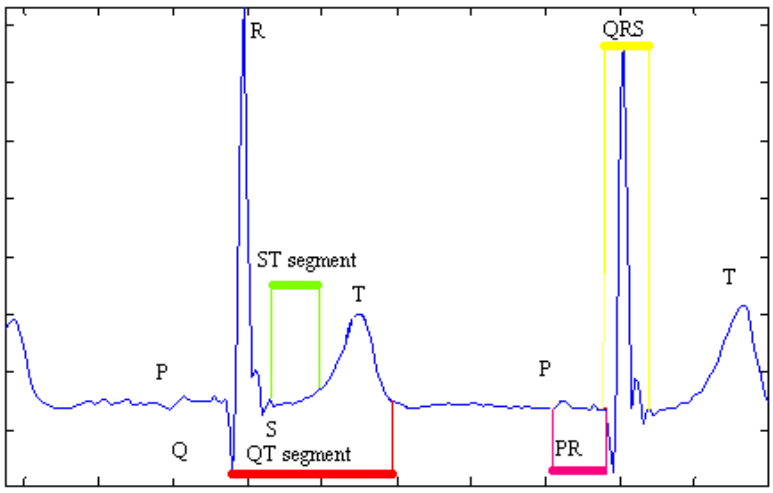

Figure 4.5: ECG recordings of two heart beats.

A study by Shin K. S. 1994 applying Lomb periodogram (LP) (Shin *et al.*, 1994) on various cardiac event series of real ECG, as shown in figure 4.5, showed that LP is very effective in the PSD estimation of HRV, especially in the presence of arrhythmias and dropouts of cardiac events. FFT was used by Clifford G.D. (2005) for HRV analysis of realistic artificial RR interval generator, interpolation and re-sampling. LP was proven to be more appropriate to be used for HRV because of the better provision of PSD estimation .The Lomb-Welch Periodogram method was used by (Thong *et al.*, 2004) to look at the effect of smoking on the HRV analysis. The study showed an increase in LF to HF ratio with little change in mean heart rate. This suggests that nicotine affects both sympathetic and parasympathetic activities.

4.3.2 Method

In this study, the RR data extracted from the recorded ECG over a 24 hr period described in chapter 2 was used, as well as data on family smoking when the children

age is around 10 years. The children in this study were divided into four groups depend on the children being normal or IUGR and family smoking as shown in Table 4.5.

Table 4.4: Grouping employed to children used in this study.

Birth weight of child	Smoking status of parents
IUGR	smokers
IUGR	non-smokers
Normal	smokers
Normal	non-smokers

In this work the Lomb-periodogram method was used to analyse the data in the frequency domain. This is described below.

4.3.2.1 Frequency domain - Lomb periodogram

The FFT requires a uniformly sampled signal, which is not the case in RR data, so the RR data needs to be interpolated and re-sampled at, at least twice the maximum frequency of interest in the original signal to avoid aliasing; more of this was explained in chapter 2. The re-sampling process alters the frequency content of even a noise-free time series by non-linear low-pass filtering (Moody, 1993). The Lomb method, developed by Lomb and later elaborated on by Scargle, is a technique designed to calculate the frequency power of a signal described by unevenly spaced data points (Press *et al.*, 1997), this was implemented in this work to produce Lomb periodogram (Cripps and Biala, 2009). No interpolation of the data series is required. The Lomb normalised periodogram, $P_N(\omega)$, is defined by the following equations as given in (Press W. H. 1992):

The Lomb normalised periodogram equation is

$$P_N(\omega) = \frac{1}{2\sigma^2}\left\{\frac{\left[\sum_j (x_j - \bar{x})\cos(\omega(t_j - \tau))\right]^2}{\sum_j \cos^2(\omega(t_j - \tau))} + \frac{\left[\sum_j (x_j - \bar{x})\sin(\omega(t_j - \tau))\right]^2}{\sum_j \sin^2(\omega(t_j - \tau))}\right\} \quad (4.5)$$

Where τ is defined by the relation

$$\tau = \tan^{-1}\left(\frac{\sum_j \sin(2\omega t_j)}{2\omega \sum_j \cos(2\omega t_j)}\right) \quad (4.6)$$

The average value of the data and the standard deviation are measured by

$$\bar{x} = \frac{1}{N}\sum_{0}^{N-1} x_j \quad , \quad \sigma^2 = \frac{1}{N-1}\sum(x_j - \bar{x})^2 \tag{4.7}$$

where N is the number of data points, τ_i are the data points measured at time t_i, $\omega = 2\pi f$ is the angular frequency and τ is an offset constant that makes $P_N(\omega)$ independent of shifting all the t_i's by any constant. The selection of the offset makes equation (4.5) the solution one will obtain if the harmonic content of a data set, at a given frequency ω, were estimated by a linear least-squares fitting to the model $x(t) = A\cos(\omega t) + B\sin(\omega t)$ (Lomb, 1976). The Lomb periodogram weights the data on a *per point* basis rather than *per time interval* basis (where re-sampling can introduce errors). The implementation of Lomb was done by using the program extractlomb.m. This programme reads the RR data of each child (subject1processeddata.mat) from the respective datas file. Two subfiles (Lomb1.m and Lombplot.m) were used to produce the frequency, time and power for each child and to be save the data in a file called subjectNoLombdata.mat. The Lomb periodogram was plotted using the lomb3D.m algorithm. The LF, HF and LF/HF components were calculated using the Area.m script. All algorithms enumerated above are included in the CD enclosed with the thesis. Their results are collected in Appendix C.

4.3.2.2 Respiratory sinus arrhythmia

In healthy individuals the heart rate varies at the frequency of respiration. This is known as respiratory sinus arrhythmia (RSA). During inspiration, the chest expands causing the intrathoracic pressure to decrease. This increases the amount of venous blood from the body into the right atrium and, via the Bainbridge reflex, causes the heart rate to accelerate. Conversely, during expiration the heart rate decelerates via the baroreceptor reflex (Berne and Levy, 2001) . Recordings of the autonomic nerves show that the sympathetic nerves are activated during inspiration and the parasympathetic during expiration. RSA is most pronounced during the night when we are sleeping and can be seen as an increase in activity around 0.3 Hz, in the HF region. The greater the heart rate variability in an individual, the more pronounced the peak associated with RSA. The identification of RSA was used in this study to identify the start of sleep time and the end of sleep time, or awakening time.

Adding to that, the parents recording of sleep time was used and the mean of RR graphs.

4.3.3 Results

4.3.3.1 FFT

Periodograms for the all children were produced using spline interpolation of the RR intervals, re-sampling at 4 Hz and FFT-based spectral analysis, to allow comparison with the periodograms produced using the Lomb method. The FFT periodogram for NB (A female child) is shown in Figure 4.6. A strong series of peaks centred around 0.25 Hz can clearly be seen, corresponding to RSA activity during sleep, between the hours of 5 and 17 on the plot (the Holters were fitted around 3 pm, which corresponds to time=0 on the plot).

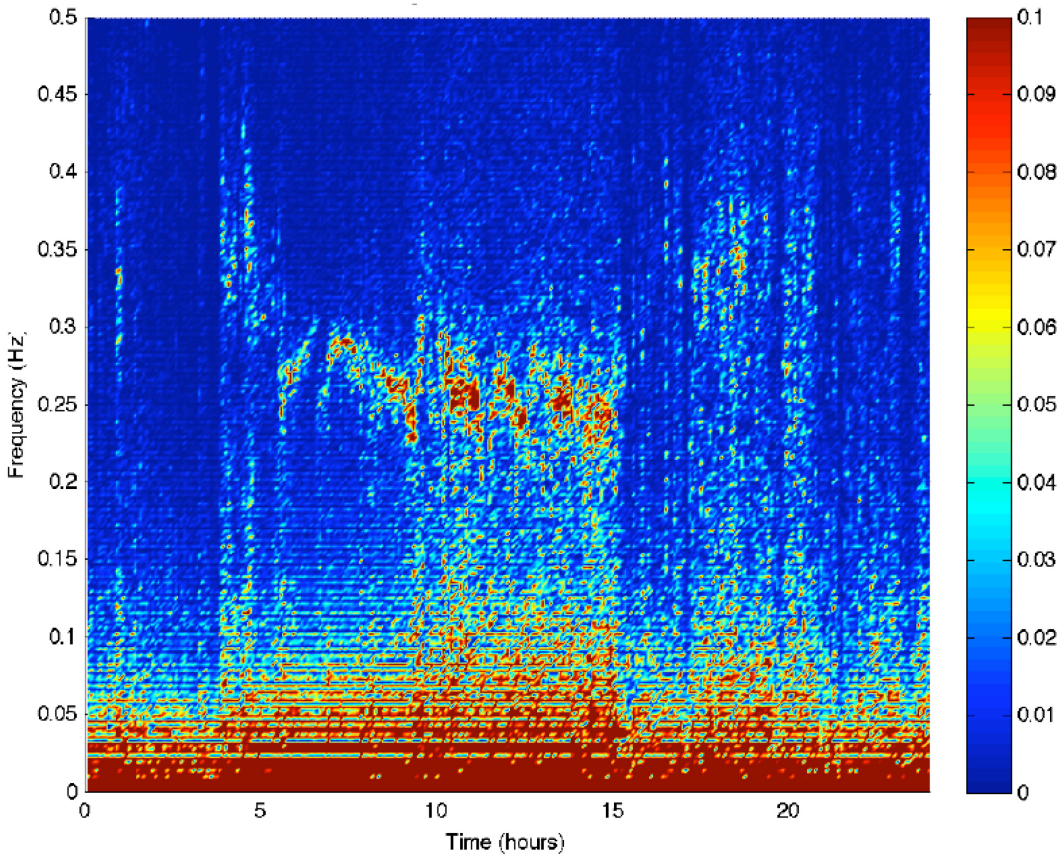

Figure 4.6: FFT periodogram for child NB , an IUGR child at birth whose parents smoked 30 cigarettes a day in total.

4.3.3.2 Lomb periodogram and strength of RSA peaks.

Lomb periodograms were also produced for the same children. Figure 4.7 shows the Lomb periodogram method for child NB.

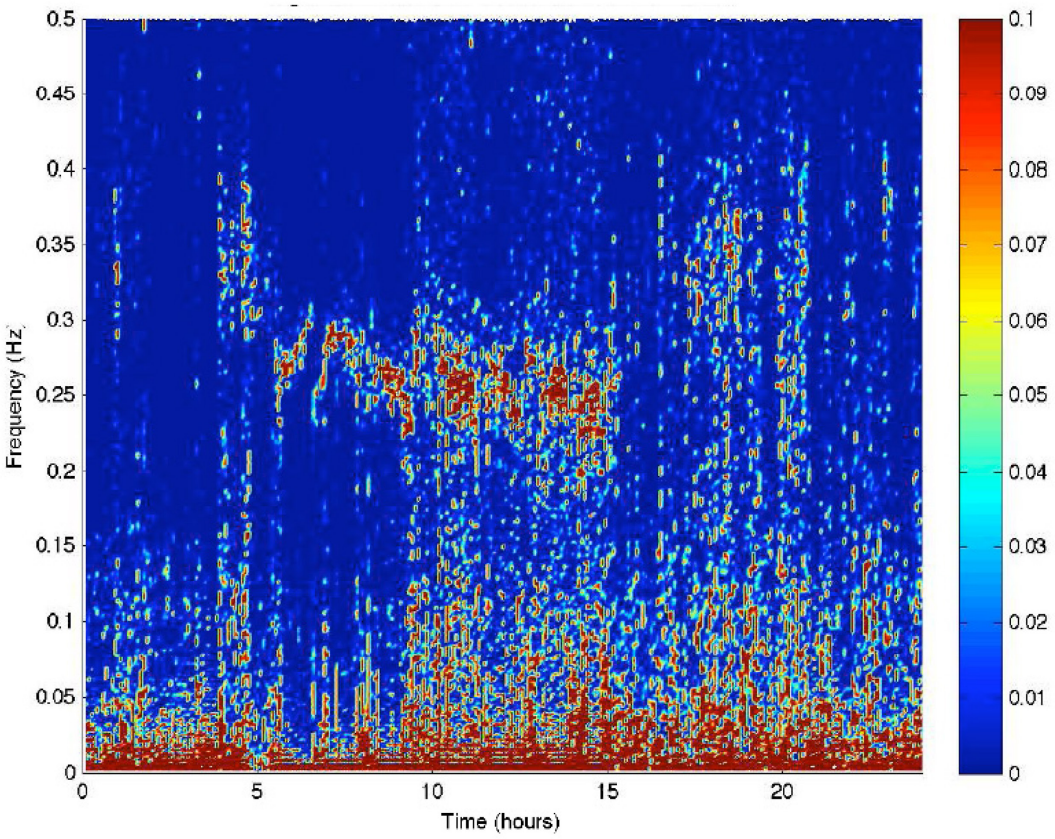

Figure 4.7: Lomb periodogram for child NB, an IUGR child, whose parents smoked 30 cigarettes a day in total.

Strong peaks corresponding to RSA can be seen in both plots (4.6) and (4.7) in the region of 0.2 to 0.3 Hz during the hours that the child was asleep. When comparing visually the FFT and Lomb periodograms (figure 4.6 and figure 4.7), FFT shows less power density at the frequencies of RSA (a future empirical study can be done to quantify the difference between FFT and Lomb), this could be due to the leakage associated with FFT, so that power of the signal spreads to other adjacent frequencies. Alternatively this might have been the effect of the attenuation of high frequencies caused by the Low pass filtering associated with re-sampling and

interpolation. Figure 4.8 shows a Lomb periodogram of an IUGR child whose parents were not smokers.

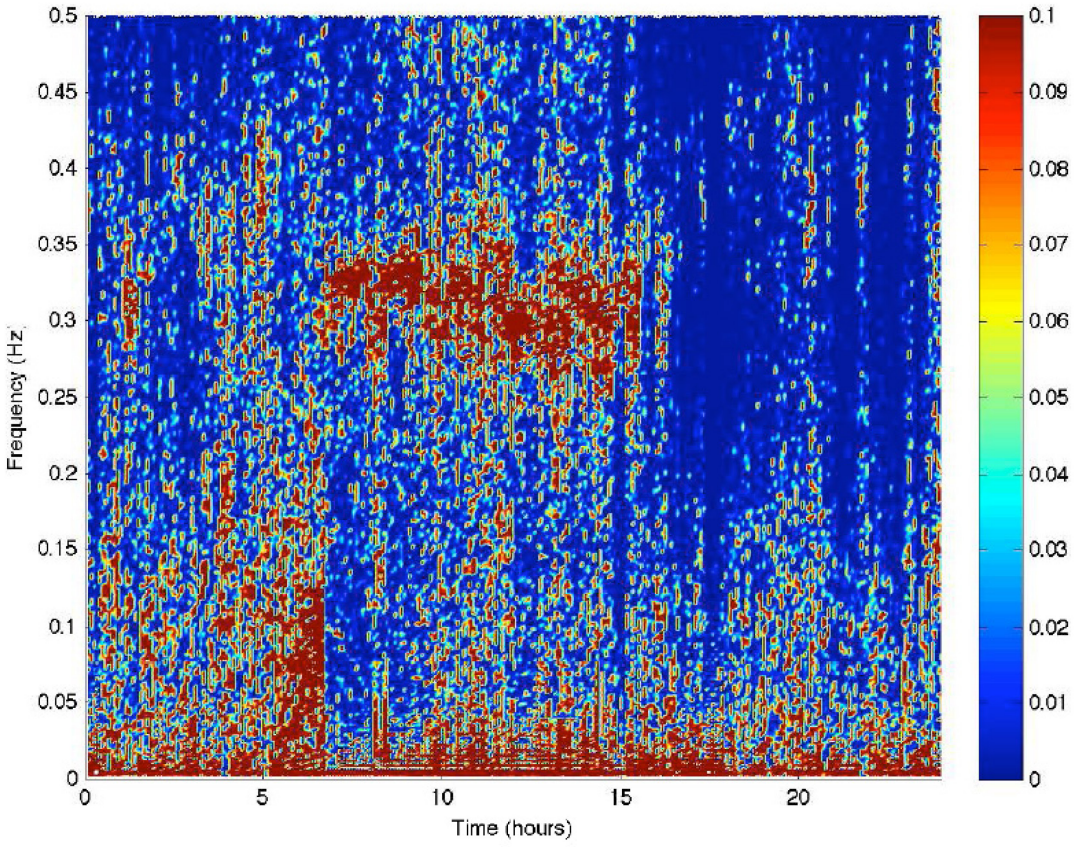

Figure 4.8: Lomb periodogram for an IUGR child whose parents non smokers and the colour scale indicates the strength of the normalised power.

The RSA is related to the efficiency of breathing of the children the higher the HF energy, which is related to the RSA, the better the breathing.

The LF/HF ratio for each child was also calculated, along with the mean ratio and the standard deviation of the group. A Student's t-test was then performed on the pairings, as seen in Table 4.6. The results of comparing the different groups according to mother smoking showed no significant difference between them. This is an observational study and the sample size is small hence the results can give a type 2 errors (i.e. accepting a false Null hypothesis).

Table 4.5: student t-test analysis of LF/HF ratio of comparing between the different groups with regard to smoking during pregnancy

Group 1	Group 2	T – test Prob-asleep	Reject Null Hypothesis	t-test Prob-awake	Reject null hypothesis
IUGR smokers	IUGR non smokers	0.4174	No	0.11	No
Normal smokers	Normal non-smokers	0.1107	No	0.28	No
IUGR smokers	Normal smokers	0.0585	No	0.18	No
IUGR non smokers	Normal non-smokers	0.1135	No	0.79	No

The results of the Multifactorial analysis of Variance test in table 4.7 show that day time activities appears to have produced higher LF/HF ratios for females, , IUGR and non Breast-fed children , but only the effect of gender was significant.

Table 4.6: Multifactorial Analysis Of Variance of LF/HF day as dependent variable.

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	2.641	.306	.610	2.030	3.253
smokingPreg	1	2.332	.443		1.446	3.219
Non-family smoking	0	2.501	.372	.959	1.756	3.246
Family. smoking	1	2.473	.335		1.804	3.142
male	1	2.017	.321	.045	1.375	2.659
female	2	2.957	.333		2.292	3.622
breast feed	1	2.301	.305	.455	1.692	2.910
not breast feed	2	2.673	.372		1.929	3.417
IUGR	1	2.571	.403	.799	1.766	3.376
Normal	2	2.403	.404		1.594	3.211

The results in table 4.8 show that the LF/HF during sleep appears to be lower for male children , those who were breastfed, normal children, and those from families

where there was smoking in the household, however, none of these differences were significant ($p>0.05$).

Table 4.7: Multfactorial Analysis of Variance to study the effect of LF/HF during Asleep.

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	.654	.110	1.000	.434	.875
smokingPreg	1	.654	.160		.335	.974
Non-family smoking	0	.712	.134	.553	.443	.980
Family. smoking	1	.597	.121		.356	.838
male	1	.557	.116	.243	.326	.788
female	2	.752	.120		.512	.991
breast feed	1	.611	.110	.633	.392	.831
not breast feed	2	.697	.134		.429	.965
IUGR	1	.735	.145	.501	.444	1.025
Normal	2	.574	.146		.282	.865

4.3.4 **Conclusions**

In the frequency domain, periodograms were produced using the FFT method and Lomb methods. Comparisons of the spectra produced using the two techniques showed spectral leakage and high frequency attenuation on the FFT periodograms. Therefore, although the Lomb method used in this instance took longer to execute, Lomb periodograms were used for further investigation in the frequency domain.

Analysis of the LF/HF ratios during sleep and awake hours has shown no significant difference between IUGR and normal children.

5 Development of QT measurement algorithm

5.1 Introduction

Previous research on Heart rate variability (HRV) measurements on the data used in this research (Biala *et al.*, 2008) showed that there is no significant difference between the IUGR and Normal children. Respiratory sinus arrhythmia of 10 year old children (Biala *et al.*, 2009) showed no significant difference between the two groups either. When using Lomb frequency domain method (Cripps and Biala, 2009), passive parental smoking on IUGR children caused significant difference compared with the IUGR with non-smoking parents. The previous result was valid only for a selected no of subject, 24 out of 75, but for all subjects there is no effect of passive smoking on IUGR children. The next stage of the research is to measure the different segments and intervals in the ECG of all the children and study the QT intervals. Next is an overview of the ECG features which will give the definition of all waves, segments and intervals associated with ECG.

A typical ECG waveform comprises of an initial P-wave, followed by QRS complex and then a trailing T-wave as shown in figure 5.1. The description of ECG waveforms is as follows:

- **P-Wave** − The low voltage caused by the depolarisation of the atria prior to contraction. The atria contain very little muscle and thus the voltage change is quite small. The normal p-wave measures less than 0.11 second
- **QRS Complex** − The QRS complex is caused by the ventricular depolarisation. The time during which ventricular contraction occurs is referred as the systole. The QRS complex should be less than 0.1 second.
- **T-wave** − T-wave is caused by the ventricular repolarisation. The time between successive ventricular repolarisations is referred to as diastole. Although atrial repolarisation occurs simultaneously, it is not seen due to the low amplitude of the signal generated by this process which overlaps in time with the QRS..

Typical amplitude of P-wave is 0.25 mV, R wave is 1.6 mV, Q wave is 25% of R wave and T wave is 0.1 to 0.5 mV. The accepted normal interval durations are; PR

interval 0.12 to 0.2 second, QT interval 0.35 to 0.44 second, ST interval 0.05 to 0.15 second, P wave duration 0.11second, and QRS duration 0.09 second (Cromwell *et al.*, 1996).

An ECG has many important intervals and segments, such as R to R (used to measure the HRV), QT and ST segments. The long QT syndrome (LQTS) is a familial disease which is caused by stress-mediated life threatening ventricular arrhythmias (Priori S. G. *et al.*, 2001). A rare single gene mutations (the SCN5A gene) in the long QT syndrome have been identified in small numbers of infants dying suddenly and unexpectedly (Blair and Fleming, 2008).

A prolonged QT indicates a myocardium at risk for triggered activity, where the cardiac cells will depolarise rapidly and repeatedly, and this is associated with dangerous Ventricular tachyarrhythmia (Clifford *et al.*, 2006). Changes in the ST segment (depressed, elevated, steeply sloped), indicate various cardiac conditions (Sornmo and Laguna, 2005) .

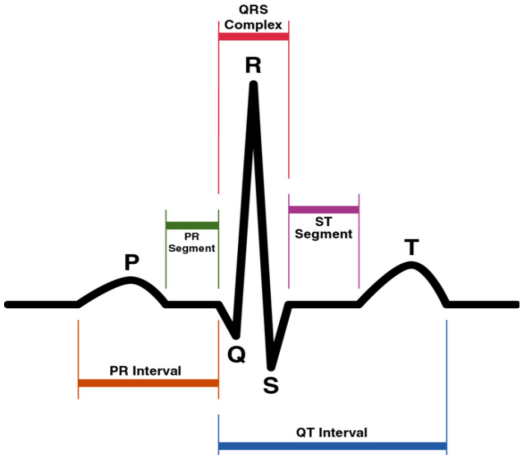

Figure 5.1: Some of the intervals measured in the ECG.

5.2 Aim of work

The main objective of the work described in this chapter is to develop an algorithm to detect QT, other ECG intervals and to find any correlation between QT, ST, QRS, Heart rate (HR) of normal and IUGR children at 10 yrs. The cohort under study is described in chapter 2 as 41 IUGR and 34 as normal. The ECGs of 24 hour for each child were used to find any differences between the two groups.

5.3 Method

The ECG signal was subjected to re-sampling of the ECG from 128 Hz to 512 Hz was implemented (The Task Force, 1996) for jitter reduction. The basis of the QT algorithm developed using MATLAB is, firstly to detect the QRS by the modified Pan and Tompkins technique (Schlindwein *et al.*, 1986) and finding the local maximum value of the ECG, which represents the peak of the R wave, then to window the data before the R peak to find all the details such as: Q (the lowest value before the R peak), start of the Q wave by setting a threshold and comparing the data values with this threshold and when they are equal this point will be marked as the beginning of the Q wave, Secondly, to window the data of the ECG after the R peak, where we can detect, by means of maximum and minimum points of the data, the Q, S and the T waves. The positions of the start of the T wave and the end of the T wave were detected using the same type of threshold comparison used to find the beginning of the Q wave. Figure (5.2) shows the ECG with R, Q, S, and T waves detected, as well as the onset and offset of each of the waves, so that important intervals can be measured. The algorithm was used to find the corrected QT interval, QRS interval, and ST interval by means of detection and delineation of Q, S, and T waves.

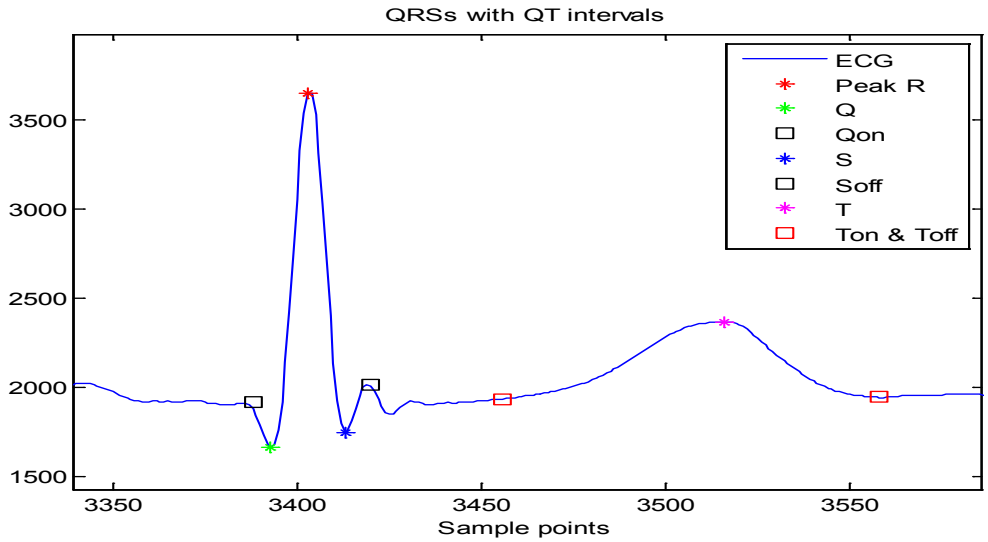

Figure 5.2: Detections and delimitations of ECG waves.

5.4 Results

A self-developed algorithm QTinterval.m was used, (see CD enclosed with this thesis). This algorithm was implemented, to measure the relevant intervals on the children ECG signals. First, ECG data files (e.g. ECG10.mat) are read. Then, the algorithm estimates the locations of all onsets and offsets forthe intervals listed below (prints from the results can be found Appendix C):

Average of QRS = 48.4 samples...or 94.5 ms

STD of QRS = 5.0 samples...or 9.9 ms

Average of QT =181.3 samples...or 354.0 ms

STD of QT = 36.0 samples...or 70.3 ms

Average of ST = 30.9 samples...or 60.3 ms

STD of QT = 36.1 samples...or 70.6 ms

Heart Rate Beat /Min ....102.7 BPM

The corrected value of QT =237.1 samples...or 463.2 ms

Figures (5.3, 5.4, 5.5 and 5.6) show the average values of the HR, ST, corrected QT, and HR. The average values of Normal children were compared with those of the IUGR children. HR is higher in Normal children at day time and at sleep time HR is the same for both groups, see figure (5.3). ST segment in the Normal group is less than that in IUGR as shown in figure (5.4). Figure (5.5), shows IUGR children have a slightly higher corrected average QT. Figure (5.6) shows Normal children have slightly higher RR intervals during awake and asleep. Table (5.1) shows all measured values compared with published Paediatric ECG limits.

5.4.1 Heart rate analysis:

The heart rate data can show the fact that at day time the heart rate is higher for both IUGR and normal children, but IUGR heart rate is higher than Normal. At night time

the heart rate decreases due to the fact that the parasympathetic branch is dominant at night time, and IUGR children have slightly higher heart rate than Normal children.

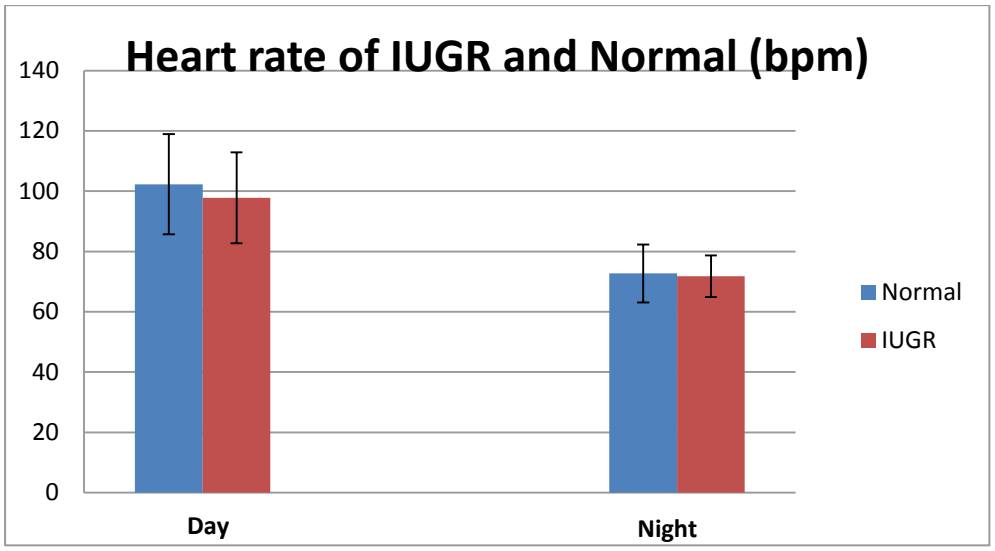

Figure 5.3: Heart rate of IUGR and Normal children.

5.4.2 ST segment analysis:

Due to the slower heart rate at night time, this causes the ST segments and the QT intervals to be longer at night time. The IUGR ST segments are shorter at day time than the normal children, but at night time the ST segments for IUGR children are longer than the ST for normal children.

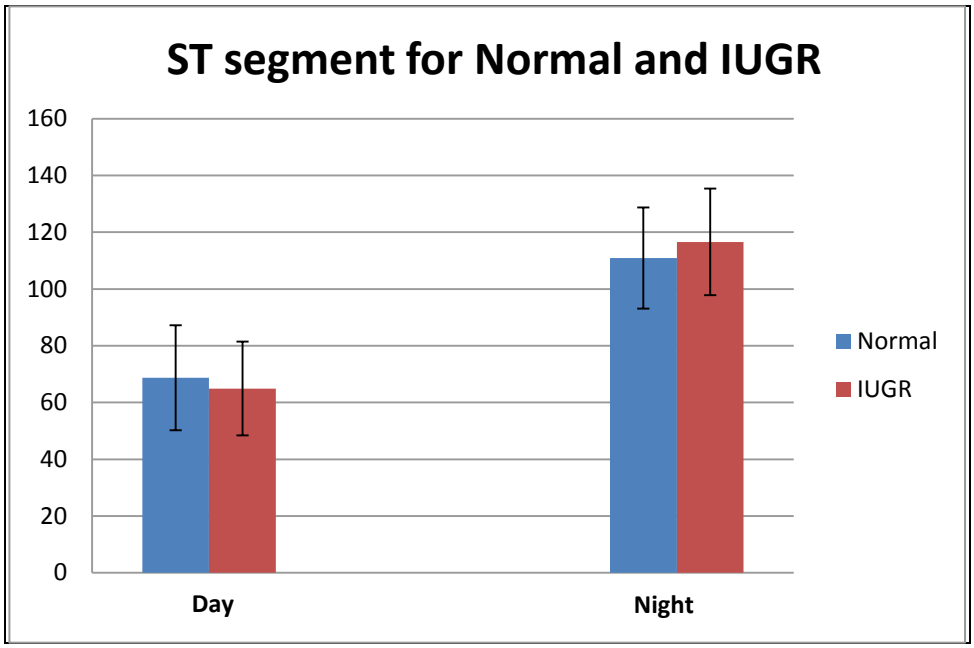

Figure 5.4: ST segment for IUGR and Normal at Day and night time.

5.4.3 QT interval analysis:

QT is a very important feature of the ECG; a long QT can suggest a tendency towards heart diseases. The condition is called QT syndrome where the subject faints and this is associated with mortality (Clifford *al et.* 2006).

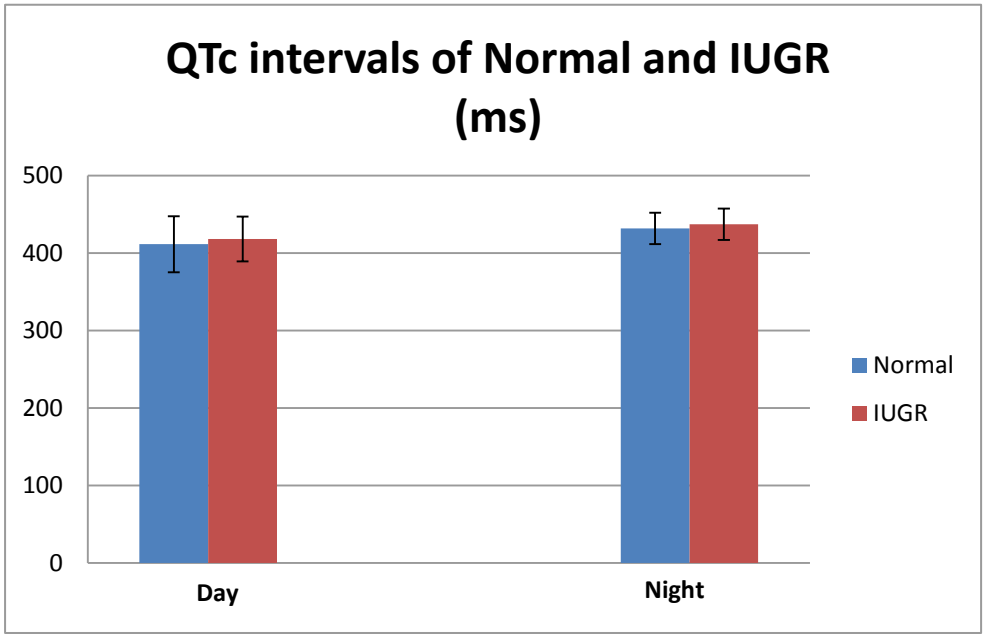

Figure 5.5: QT intervals for IUGR and Normal at day and night.

5.4.4 QRS interval analysis

The QRS intervals are higher at night time and shorter at day time as shown in figure 5.6.

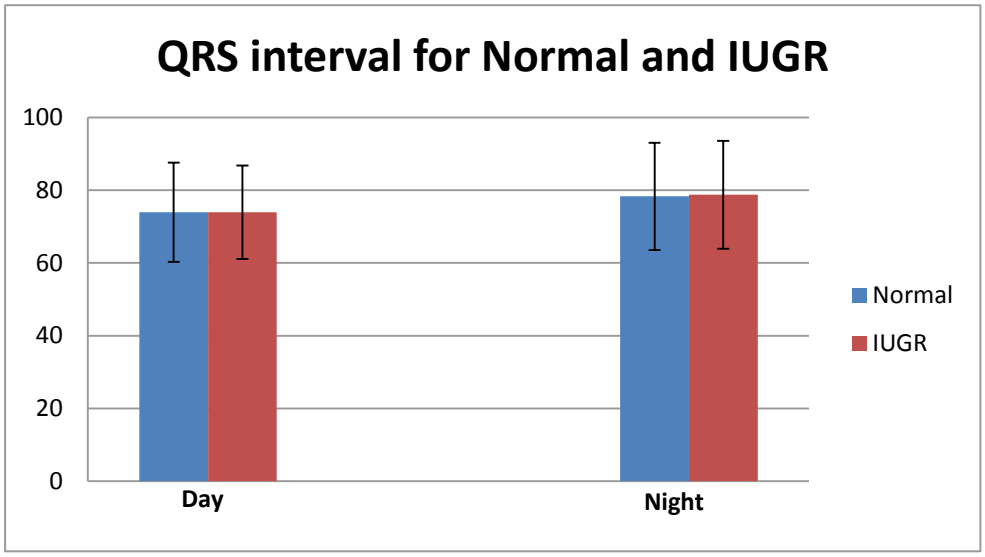

Figure 5.6: QRS intervals for Normal and IUGR children.

Table 5.1: All ECG intervals data for Normal and IUGR children and published Paediatric ECG limits

ECG features / children	Heart rate /day bpm	Heart rate/Night bpm	ST /day (ms)	ST /night (ms)	QTc /day (ms)	QTc/Night (ms)	QRS/Day (ms)	QRS/Night (ms)
Normal	102.31 ±16.60	72.70 ±9.63	68.72 ±18.50	110.94 ±17.83	411.37 ±36.13	431.79 ±20.16	73.96 ±13.65	78.75 ±14.76
IUGR	97.82 ±15.06	71.78 ±6.94	64.91 ±16.52	116.59 ±18.81	418.25 ±28.92	437.22 ±20.17	73.94 ±12.85	78.75 ±14.80
Published Data Paediatric ECG limits*	Boys 78(55,101) Girls 80(58,110)				Boys 411(373,440) Girls 410(365,447)		Boys 85(67, 103) Girls 82(66,99)	

*(Rijnbeek *et al.*, 2001)

Statistical analysis using independent t-tests shows that there is no significant difference between the ECG intervals of the IUGR and the normal children. QTc at

night time comparison gives a p value of 0.064 which is border line non-significant, The mean values indicate that IUGR children might have longer QTc (437.22 ±20.17) than the normal children. Larger samples of IUGR children and normal are needed to confirm this in another study done on the same population's ECG intervals when they become older than 10 yrs.

Table 5.2: Statistical analysis of the ECG intervals results.

ECG intervals	Normal Vs IUGR (*P value)*	95% CI (lower, upper)
HR-DAY	0.195	(-11.4 2.35)
HR-NIGHT	0.623	(-4.58 2.77)
ST-DAY	0.313	(-10.87 3.52)
ST -NIGHT	0.164	(-2.30 13.36)
QT_c-DAY	0.123	(-1.92 15.83)
QT_c-NIGHT	0.064	(-0.420 10.79)
QRS-Day	0.973	(-4.67 4.51)
QRS -Night	0.91	(-4.8 5.43)

5.5 Conclusion

The algorithm tested had shown to measure the correct values of the ECG limits for signals with good SNR. At 10 years of age the measured ECG intervals of all normal and IUGR children was unable to show any deviation from the normal paediatric values (Davingnon *et al.*, 1979/80), (Dickinson, 2005). The algorithm needs to be improved further for general use to cater different changes and shapes associated with paediatric ECG and the ones with higher SNR.

The analysis of the data showed that the IUGR children are relatively more prone to longer QTc intervals. Children with long QT syndrome LQTS are relatively more prone to atrioventricular block, multiform premature ventricular contractions, and torsade de pointes than other children. Patients with QTc of more than 0.60 are at particularly high risk for sudden death (Garson *et al.*, 1993).

6 Heart rate variability using Poincaré plots

6.1 Introduction

Frequency domain measures assume that the R-R interval time series is stationary, or that the variations are harmonic or sinusoidal. In reality, HR fluctuations can be both periodic (e.g., due to respiration) and non-periodic (e.g., due to abrupt changes in the environment or state of the child). Thus HRV may be due to complex, dynamic interactions of biological signals and non-linear techniques may strengthen the analysis of physiological conditions. S maps have revealed complex, non-linear heart patterns in the developing infant [16] and power law slope and Poincaré plots have demonstrated increased risk and adversity in cardiovascular disease patients (Acharya R. U *et al.*, 2006).

Poincaré plot is a quantitative visual tool which can be applied to the analysis of RR interval time series data gathered over relatively short time periods (Kamen *et al*. 1996). In a Poincaré plot each RR interval of a tachogram is plotted as a function of previous RR interval. The geometry of this plot has been shown to distinguish between healthy and diseased subjects in clinical setting. It is very valuable HRV analysis technique due to its ability to display nonlinear aspects of the interval sequence. The Poincaré plot is becoming a popular technique due to its simple visual interpretation and its proven clinical ability as a predictor of disease and cardiac dysfunction (Kamen *et al.*, 1996a).

In this research Poincaré method was used to look at the effect of maternal smoking habit on cardiac function of 10 year-old children (see figure 6.1). These are scatter plots where the current RR is plotted against the previous RR. A graphical presentation of RR can be produced with SD1 as the short term variability and SD2 as the long term variability. The ratio SD1/SD2 represents the randomness in HRV time series, and this ratio has a strong association with mortality in infants with congenital heart defects. The study of 24-hour ECG data of for 27 normal infants (Group I) and 26 infants with congenital heart defects (Group II) after analysis of five-minute segments at birth, showed Group II has reduced SD1 and SD2 and increased SD1/SD2 ($p<0.001$). Post-procedure, greater SD1 and SD2 ($p<0.001$) was seen in

Group II. Poincaré analysis captures congenital differences in autonomic cardiac function and improvements over time (Smith R.L*, et al.* 2009).

The specific aim of this chapter is to report the use of Poincaré plots method to study the effect of maternal smoking habit on the development of the autonomic nervous system of normal and IUGR children.

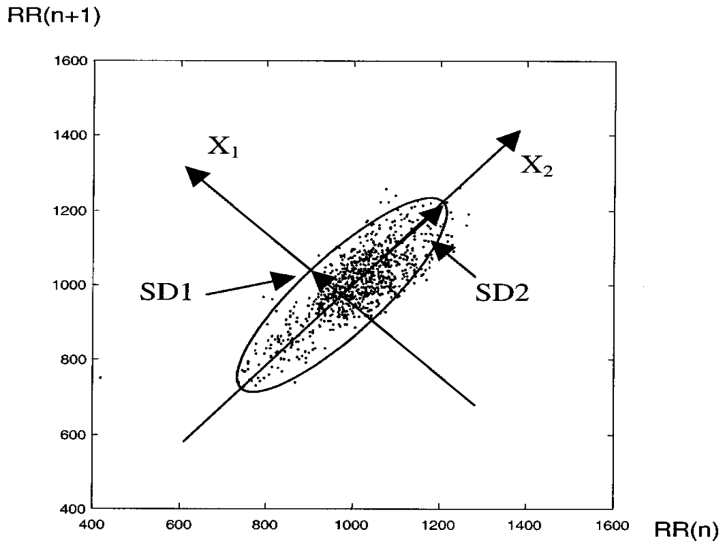

Figure 6.1: Poincaré plot and its measurements (x and y in ms).

6.2 Data

The ECGs of the study population described in chapter 2 at night time were analysed according to maternal smoking status and birth weight of the child (IUGR or not).

The 75 children were sorted into 4 groups, depending on IUGR, Normal and their maternal smoking habit during pregnancy, the classification of the children do change form children who are affected by maternal smoking during pregnancy and those who are influenced by parents who smoke when the children are 10 yrs.

Table 6.1: The breakdown of the population into subsections.

Birth weight of child	Maternal smoking during pregnancy	Number of children
IUGR	Smoker	16
IUGR	Non-Smoker	25
Normal	Smoker	9
Normal	Non-Smoker	25

The emphasis of this part of the work is the study of the development of IUGR children whose mothers smoked during pregnancy.

6.3 Method

6.3.1 Poincaré plots analysis on maternal smoking children

The algorithm for Poincaré analysis (poincareplot.m) uses ellipse fitting, for calculation of SD1 and SD2 values. (See the algorithm in the CD enclosed with this thesis and the 24h results for one child is in Appendix C).Figure 6.1 shows a typical Poincare plot with the ellipse the values of SD1 for short term variability which is caused by RSA and SD2 for long term variability. The new axis oriented with the 45° line of identity are rotated by $\theta = \pi/4$.

$$\begin{bmatrix} x1 \\ x2 \end{bmatrix} = \begin{bmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} RR_n \\ RR_{n+1} \end{bmatrix} \qquad 6.1$$

The dispersion of the points around the x1 axis is measured by the standard deviation SD1and the measure of the cloud along the line of identity is measured by SD2 which is the standard deviation around the x2 axis.

These measures are related to the HRV measures as follow:

$$SD1^2 = Var(x1) = Var(\frac{1}{\sqrt{2}} RR_{\,n} - \frac{1}{\sqrt{2}} RR_{n+1}\ ) \qquad 6.2$$

$$SD1^2 = Var(x1) = \frac{1}{2}Var(RR_n - RR_{n+1}) = \frac{1}{2}SDSD^2 \quad 6.3$$

SD1 is the measure of the poincaré width which is the measure of SD of successive intervals scaled by $1/\sqrt{2}$. SD1 and SD2 can be related by the auto covariance function

$$SD1^2 = \phi_{RR}(0) - \phi_{RR}(1) \quad 6.4$$

$$SD2^2 = \phi_{RR}(0) + \phi_{RR}(1) \quad 6.5$$

Adding 6.4 and 6.5 will give :

$$SD1^2 + SD2^2 = 2SDRR^2 \quad 6.6$$

$$SD2^2 = 2SDRR^2 - \frac{1}{\sqrt{2}}SDRR^2 \quad 6.7$$

The averages of SD1 and SD2 for all children have been tabulated as shown in table 6.2.

Table 6.2: Shows average of SD1, SD2 and SD1/SD2 for all children

Group	Mean SD1	Mean SD2	Mean SD1/SD2
Normal Smoking	70.61	182.87	0.37
Normal Non-smoking	69.04	166.07	0.41
IUGR Smoking	60.33	148.53	0.40
IUGR Non-smoking	69.20	161.64	0.40

6.3.2 Poincaré plots analysis on parental smoking children

The four groups of children (detailed in table 4.1 in chapter 4) with parental habit of smoking where analysed using this non-linear method (Poincaré plot) to look at the effect of smoking habit in the household on all the children. The Poincaré plot uses the current RR interval plotted against the previous. The resulting 'clouds' delimited by the standard deviation in each direction can then be enclosed by an ellipse and measured, giving an indication of the HRV. SD1, the width of the cloud or the standard deviation around the identity axis x1, is a measure of the short-term HRV - more precisely the measure of variability over a single heart beat. SD2, the length of

the cloud along the line of identity or the standard deviation around axis x2, is a measure of long term HRV (Brennan *et al.* 2001).

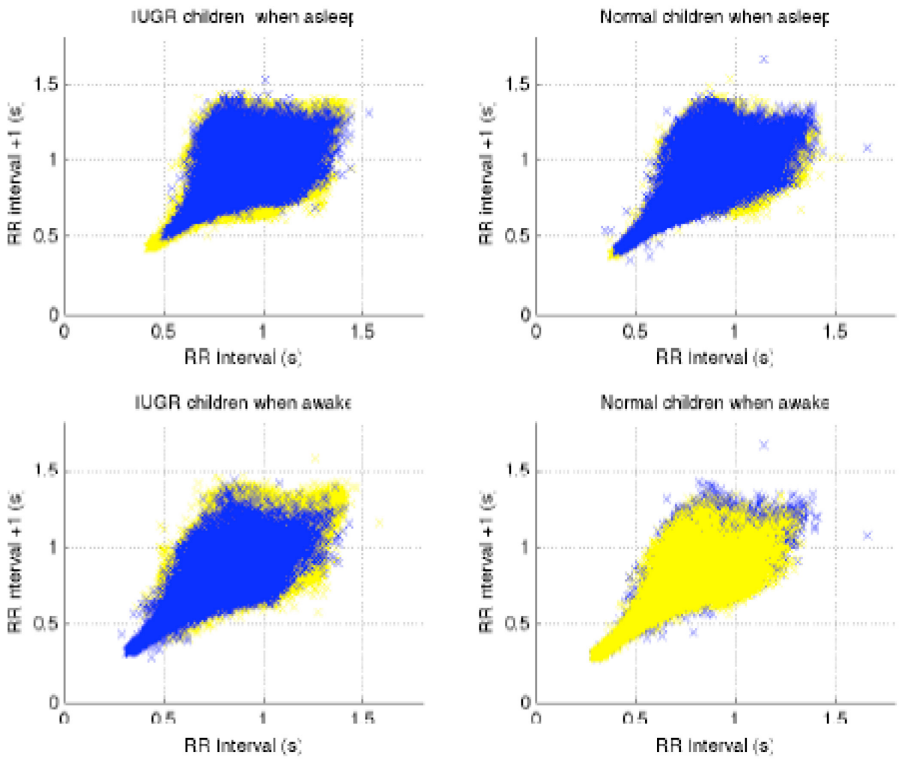

Figure 6.2:Poincaré plots of IUGR and Normal children, the children with smoking parents in blue and the children with non-smoking parents in yellow

Table 6.3: SD1 and SD2 measurements of clouds in the Poincaré plots of figure 6.2

Birth weight	Parents, smoking status	Awake or asleep	SD1(arbitrary units)	SD2(arbitrary units)
IUGR	smokers	awake	0.247	0.505
IUGR	smokers	asleep	0.263	0.453
Normal	smokers	awake	0.224	0.529
Normal	smokers	asleep	0.242	0.495
IUGR	non-smokers	awake	0.259	0.518
IUGR	non-smokers	asleep	0.277	0.505
Normal	non-smokers	awake	0.221	0.484
Normal	non-smokers	asleep	0.271	0.482

The above results show that all children with non smoking family have a higher short term and long term variability during the awake and asleep. The only exception is the normal children with non smoking family during awake period which have lower short and lower long term variability.

6.4 Results

Lomb Periodograms in chapter were used to find the exact start and end of night time for all of the children in this work. The 2D PSD maps are colour coded with low power marked in blue and the highest is in red. Respiratory Sinus Arrhythmia (RSA) is shown in the periodogram in red at frequency of ventilation, around 0.25 Hertz.

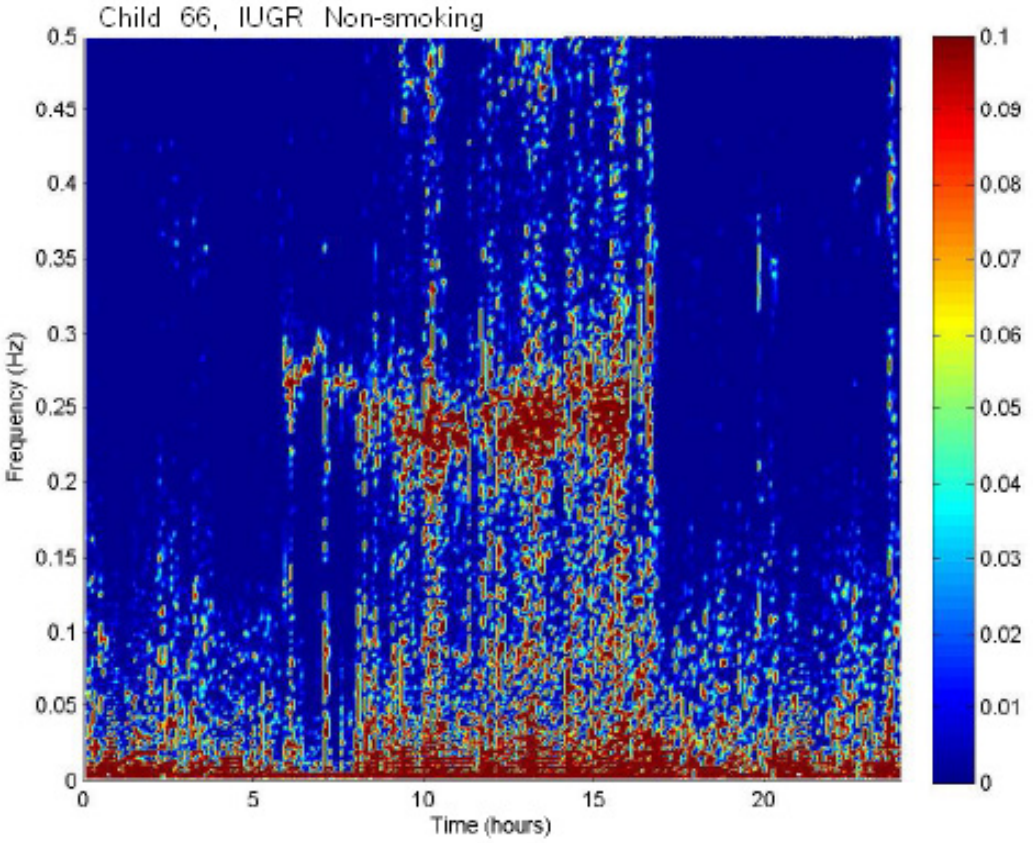

Figure 6.3: Lomb periodogram for an IUGR child with a non-smoking mother during pregnancy, the red colour at 0.25 Hz corresponds to the respiratory sinus arrhythmia (RSA) at night time.

Four Poincaré plots were produced, as seen in Figure 6.4. Measurements and analysis of the values of SD1, SD2 and SD1/SD2 are given in Table 6.5.

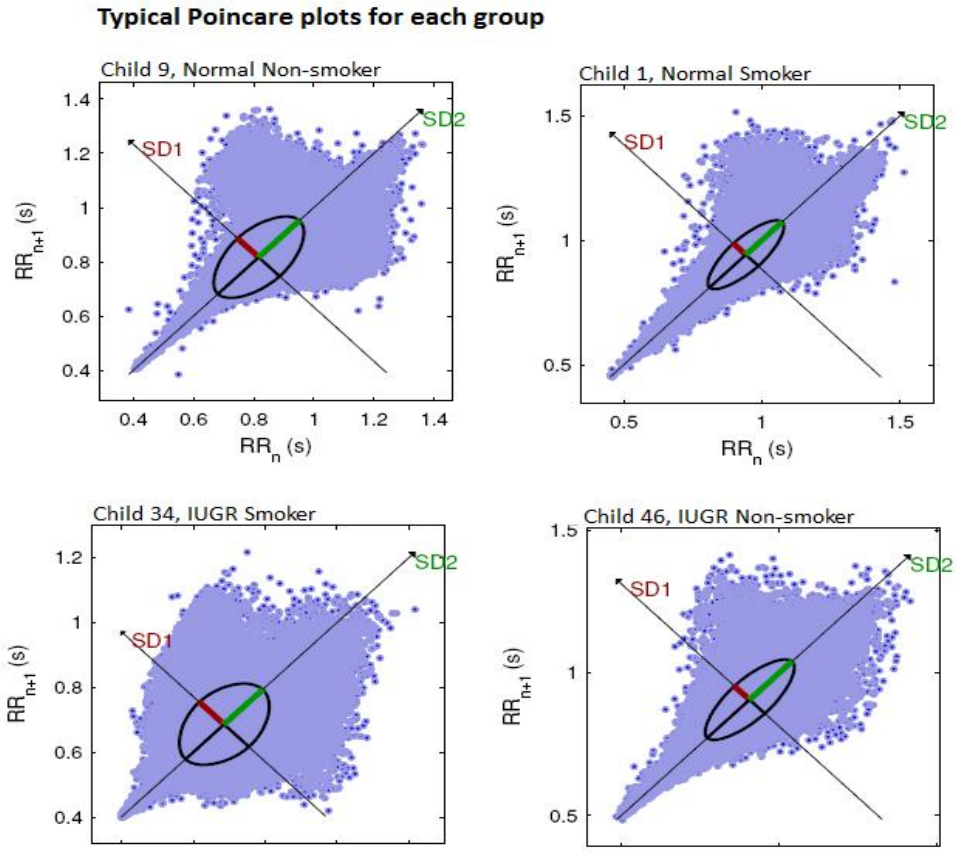

Figure 6.4: Four typical Poincaré plots for two normal and two IUGR children.

From the SD1 and SD2 data for all children a Multifactorial ANOVA test was done to find the p value between all the factors and the dependent variable SD1. Table 6.3 shows the results of the ANOVA test and p values. The results show that there are no significant differences for any of the variables in the statistical model.

The results of the long term variability term (SD2) in table 6.4 show that the males (p=0.04, SD2= 177.646 SE (6.991) have a significantly higher variability measure (SD2) which means that males have a higher long term variability than females.

Table 6.4: Multifactorial ANOVA of dependent SD1

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	69.620	5.922	.549	57.775	81.466
smokingPreg	1	62.598	8.586		45.422	79.773
Non- smoking family	0	67.340	7.214	.812	52.910	81.769
Smoking Family.	1	64.878	6.481		51.915	77.841
IUGR	1	63.250	7.799	.656	47.649	78.850
Normal	2	68.968	7.830		53.306	84.631
breast feed	1	65.289	5.900	.865	53.488	77.090
not breast feed	2	66.929	7.207		52.513	81.345
male	1	71.242	6.215	.252	58.809	83.674
female	2	60.977	6.443		48.089	73.864

Table 6.5: ANOVA test results of SD2 dependent variable and the effect of other factors.

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	158.819	6.660	.537	145.496	172.141
Smoking in Preg	1	166.967	9.657		147.649	186.284
Non- smoking family	0	166.175	8.113	.574	149.945	182.404
smoking Family	1	166.175	7.289		145.031	174.191
male	1	177.646	6.991	.004	163.662	191.629
female	2	148.140	7.246		133.645	162.634
breast feed	1	164.054	6.635	.830	150.781	177.327
not breast feed	2	161.732	8.106		145.517	177.946
IUGR	1	162.892	8.772	1.000	145.346	180.439
Normal	2	162.893	8.807		145.277	180.509

The results of SD1/SD2 in table 6.5 showed that there is no significant difference on the outcome for any of the factors tested. Somewhat lower values of SD1/SD2 were recorded by children whose mothers smoked during pregnancy and by the IUGR children. However, these differences were not significant. Low values of SD1/SD2 are found to be associated with chronic renal failure patients (Claudia *et al.*, 2003).

Table 6.6: ANOVA test results of Dependent variable SD1/SD2 and other factors.

Independent Variable		Mean	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Non-Smoking in Preg	0	.417	.023	.347	.372	.463
smokingPreg	1	.375	.033		.309	.441
Non- smoking family	0	.395	.028	.943	.339	.450
Smoking Family.	1	.397	.025		.347	.447
male	1	.393	.024	.852	.345	.441
female	2	.399	.025		.350	.449
breast feed	1	.392	.023	.834	.347	.438
not breast feed	2	.400	.028		.344	.455
IUGR	1	.383	.030	.611	.323	.444
Normal	2	.409	.030		.348	.469

6.5 Discussion and Conclusion

Poincaré plots were produced for the 75 children observed during the course of this work. The Multifactorial ANOVA test on SD1 by many factors at asleep time in Table 6.3 showed that males, normal, with no smoking mother during pregnancy, no smoking family a higher have short term variability (SD1 is high). Male children proved to have higher term variability (SD2) than females.

The second finding has shown that SD1/SD2 ratio was lower for IUGR children and for those who experience smoking during pregnancy. A decreased SD1/SD2 ratio with an elongated, torpedo-like shape is associated with elevated sympathetic tone, and an increased SD1/SD2 ratio indicates less sympathetic tone (Woo *et al.*, 1992), (Brennan *et al.*, 2001). During anaesthesia, when the autonomic activity lessens this will results in decreased SD1, SD2, and converged Poincaré plots. This condition can be seen in the condition of brain death in which the total autonomic activity is lost (Su *et al.*, 2005).

The width of SD1 can be used to quantify the short-term vagal modulation of the heart rate and the decreased SD1 is shown after the autonomic changes, such as during laryngoscopy or intubation, and therefore it can be used as a sensitive indicator of sympathovagal changes (Kamen *et al.*, 1996b).

The Poincaré analysis of long-term variability, SD2 length reflects the sympathetic modulation (Brennan *et al.*, 2001), suggested that female children have less long term HRV during asleep than their male counterparts, and ($p=0.04$).

7 Linear and non-linear analysis of pre-awake period

7.1 Introduction

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. 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 (Peleng *et al.*, 1998). 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 (http://www.statistics.gov.uk/hub/index.html, 2010) 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) (Vandenboshe and Kirchner, 1998). 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 birth 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 > 2.5 kg. In the present study, linear and non linear HRV measures of 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 midday. In a review of 112 Minnesota residents who died suddenly from cardiac causes between July 1987 and July 2003 it is found that at night time between 6:00 am and 12:00 am Sudden death occurred to 20 % of people with obstructive apnoea, 41% for people without obstructive apnoea, and 30% for general population.(Gami *et al.*, 2005).

When studying the circadian variability in the occurrence of sudden death in patients with hypertrophic cardiomyopathy, it was found that SD occurs with increased frequency after awaking during morning hours (usually 6:00am to noon) (Maron *et al.*, 1994) and the same findings is supported by Thakur, who found that sudden death occurrence rate is low from midnight to 6:00 am and a 2.4-fold increase occurs between 6:00 am and noon (Thakur *et al.*, 1996). Another research highlighted that the peak time for sudden infant death (SID) occurs just before 6:00AM in the morning (Blair *et al.*, 2006)(Blair and Fleming. 2006). 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.

7.2 Materials and Methods

7.2.1 Data Sets

Table 7.1 shows the details of the RR data available for this study which is described in chapter 2, 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. 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.

Table 7.1: Groups of the children in the this study.

	Normal	**IUGR-1**	**IUGR-2**	**Total IUGR**
Recordings	34	24	17	41
Birth Weight (kg)	3.53 ± 0.48	2.29 ± 0.19	2.92 ± 0.36	2.58 ± 0.42
Gender (Male/Female)	22/12	10/14	9/8	16/21

In figure 7.1 the 15 min RR-interval data extracted from the 24 hr RR data before awakening (bottom).

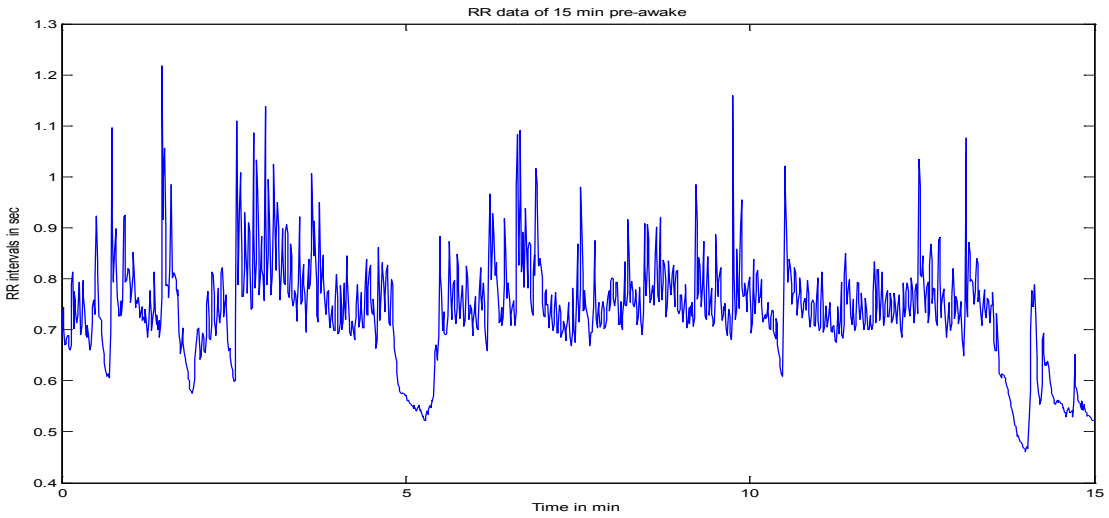

Figure 7.1: One of the 15 min pre-awake RR data under study.

7.3 HRV Analysis Techniques employed

In this study linear (standard time and frequency domain) and nonlinear (Poincaré plot, Approximate Entropy, Sample Entropy and Detrended Fluctuation Analysis) HRV analysis techniques have been used. Heart does not act as a periodic oscillator under physiological conditions (Babloyantz and Destexhe, 1988), (Goldberger and West, 1987) and the classical HRV linear measurements may not detect the nonlinear changes caused by the complex system which effects the RR time series (Karrakchou *et al.*, 1996).

7.3.1 Time Domain HRV measures

The time domain analysis was performed using statistical and geometrical HRV measures (The Task Force, 1996) . The statistical measures included Mean RR, Mean HR, SDNN, SDANN, RMSSD, NN50 and pNN50. SDNN is the standard deviation of normal to normal RR intervals that can be calculated for short periods between 30 s and 5 min duration (short term variability) or calculated for long periods (24 hour) as

a measure of long term variability (Ravenswaaji-Arts *et al*. 1993). SDANN is the standard deviation of average NN intervals calculated over 5 min RR interval time series, RMSSD, NN50, and TINN were explained in the second chapter.

Frequency domain analysis was performed using parametric method autoregressive with model of order p=16. Parametric methods provide smoother spectral components, easy post processing of the spectrum and accurate estimation of power spectral density (PSD) (The Task Force, 1996). The HRV frequency domain measures included low frequency (LF, 0.04-0.15 Hz), high frequency (HF, 0.15-0.4 Hz) and the ratio of LF to HF power (LF/HF) (Marple S.L, 1987) . Prior to the PSD estimation, the RR interval time series was converted into equidistantly sampled time series using cubic spline interpolation as explained in chapter 2 in this thesis.

7.3.2 Nonlinear HRV Measures

The non-linear HRV measures used in the analysis include Poincaré plot, detrended fluctuation analysis (DFA), approximate entropy, and sample entropy (Brennan *et al.*, 2001), (Pincus, 1991), (Richman and Moorman, 2000), (Peng *et al.*, 1995).

Approximate entropy (ApEn), is a complexity measure proposed by Pincus which quantifies the regularity of a time series (Pincus, 1991) . A high value of ApEn indicates randomness and system complexity and smaller values of ApEn indicate regularity of a signal. Sample entropy (SamEn) is the modification of ApEn in which self matches were excluded (Richman and Moorman, 2000) . The parameters used to calculate ApEn and SamEn include length of the vector (m) and tolerance (r). The tolerance is the percentage of the standard deviation of the original time series (for HRV analysis 10% to 25 % of standard deviation).

Detrended fluctuation analysis (DFA) is a scaling analysis technique proposed by Peng and colleagues to detect long range correlations in a non-stationary time series (Peng *et al.*, 1995). The first step in DFA calculation is the integration of the original time series. This integrated time series is divided into boxes of equal length n. This time series is then detrended by subtracting the local trend in each box and root mean square of this integrated and detrended time series, called F(n), is calculated. The fluctuations in the data can be characterised by the scaling exponent (α), which is the slope of the regression line relating log(Fn) to log(n). Typically correlations are

divided into short term, characterized by slope α_1 in the range $4 \leq n \leq 16$, and long term, characterized by scaling exponent α_2 in the range $16 \leq n \leq 64$. The scaling exponent prognostic α_1 was proven to be superior in terms of prognostic ability to detect any coronary heart disease (CHD) than α_2. The physiological signal yields scaling exponent near to 1, indicating fractal like behaviour and altered fractal like behaviour has been reported in patients with cardiovascular disease.

7.3.3 Statistical Analysis

The wilcoxon rank sum test and the independent t-test were used for comparing between two independent groups, depending on the distribution of the data. The Wilcoxon rank sum test is a non-parametric test that is used for data that are not normally distributed and the unpaired sample t-test for data was used to compare between the data that are normally distributed. The difference between the males and females within each group (Normal and IUGR) was calculated and is shown in table 7.2. In table 7.3 the comparison between the IUGR and Normal groups was done in two different time spans (15 mins and 24hr) and results were considered to be statistically significant at $p<0.05$.

Before carrying out multiple comparisons, the ONE WAY ANOVA method was used for the normally distributed data. All variables were tested for normality graphically and numerically. If the outcome variable under test was found to be not normally distributed (Shapiro-Wilk test) or if the homogeneity of variance test (Levene's test) was rejected, the nonparametric two way independent test was used to assess the difference between the groups (Normal, IUGR1 and IUGR2).

The Nonparametric Test (Kruskall-Wallis test) was used for independent samples. If the result of the test was found to be significant, *post hoc* calculations using Mann-Whitney tests were performed to compare the groups. Since multiple comparisons were performed on the same data, a Bonferroni correction was applied to reduce the chance of type I errors. This means that if the Null hypothesis is rejected for $p<0.05$, for multiple comparison this value has to be divided by the number of comparisons.
In other words, for 3 independent variables (normal, IUGR1 and IUGR2) , divide the p-value by 3 (0.05/3 = 0.017) for a significant result. So, to be significant any comparison has to be < 0.017 in the *post hoc* tests.

7.4 Results

The results of this work can be computed using the algorithims developed and explained in previous chapters or using the kubios software (Niskanen *et al.*, 2004). Kubios software has a very good graphical interface which was used to select the 15 min data before awakening and it was used to compute the non-linear measures (ApEn, SamEn and DFA). Table 7.2 displays the sex differences in terms of HRV measures, between Normal and IUGR children. The HRV measures show that the females have significantly lower HRV than the males for some of the outcomes.

Table 7.2: Comparison between males and females HRV measures.

HRV Measures	Normal Children			IUGR Children		
	Male	Female	p-value	Male	Female	p-value
SDNN (ms)	164.52±30.14	143.34±18.87	0.0360	158.84±36.96	148.24±32.98	0.3721
SDANN (ms)	82.39±23.13	69.35±11.27	0.0778	79.92±23.40	74.54±19.18	0.4557
RMSSD (ms)	76.12±32.32	60.18±18.64	0.1289	69.95±32.69	69.30±34.34	0.9544
NN50 (ms)	29502±12899	26169±7303	0.4187	30555±12723	29348±12890	0.7813
pNN50 (%)	24.97±11.51	21.07±8.37	0.3136	25.90±12.48	23.68±11.49	0.5848
LF (ms^2)	2225±1095	1513±449	0.0400	2079±1081	1658±682	0.1642
HF (ms^2)	3086±2474	2036±1380	0.1873	2753±2677	2620±2760	0.8855
LF/HF	0.88±0.35	0.99±0.44	0.4444	1.11±0.58	1.16±0.89	0.8604
SD1 (ms)	53.82±22.86	42.55±13.18	0.1289	54.65±27.16	54.94±29.14	0.9760
SD2 (ms)	225.79±39.52	197.85±26.33	0.0369	213.49±54.89	198.82±51.09	0.4148
ApEn	1.13±0.08	1.14±0.07	0.7209	1.18±0.08	1.15±0.09	0.3318
SamEn	1.05±0.09	1.09±0.08	0.2497	1.13±0.12	1.08±0.13	0.2339

In Table 7.3 the results of linear and nonlinear HRV measures (mean ± standard deviation) for the normal and growth restricted children for 15 min pre-awake period interbeat interval time series and 24 hr data are presented. Although the heart variability of normal children is higher than that of IUGR children, results showed no statistically significant difference between normal and growth retarded children.

Table 7.3: HRV measures for 15 min pre-awake and for 24 hrs RR data.

HRV Measures	15 min Pre-awake Period			24 hour Data		
	Normal	IUGR	p-value	Normal	IUGR	p-value
SDNN	106.672±34.845	99.104±40.123	0.2407	156.82±28.24	152.82±35.21	0.6053
SDANN	48.943±39.364	35.955±26.16	0.1609	77.65±20.46	76.87±21.33	0.8766
RMSSD	88,72±41.46	77.08±44.92	0.1599	70.32±28.86	69.58±33.62	0.9226
NN50	432±166.49	390.83±168.80	0.5481	28290±11179	29870±12830	0.5867
pNN50	35.89±15.05	32.91±16.44	0.4935	23.55±10.51	24.64±11.99	0.6893
RRtriaIndex	22.42±6.99	21.79±7.94	0.5440	40.637± 7.89	54.81±28.28	0.3962
TINN	531.51±142.71	506.59±164.54	0.2379	662.121±46.20	205.16±53.32	0.3507
LF	2.842±1.882	2.532±1.896	0.2656	1966±969	1840±907	0.5742
HF	4.336±3.703	3.338±3.739	0.0886	2704±2177	2678±2724	0.9647
LF/HF	0.924±0.731	1.216±0.859	0.0886	0.92±0.38	1.16±0.78	0.1542
SD1	62.88±29.38	56.42±31,03	0.2588	49.72±20.41	54.81±28.28	0.3962
SD2	151.17±55.57	141.66±54.44	0.4711	215.63±37.42	205.16±53.32	0.3507
ApEn	1.22±0.21	1.26±0.14	0.3550	1.14±0.08	1.16±0.09	0.1908
SamEn	1.14±0.39	1.21±0.29	0.3426	1.07±0.08	1.10±0.13	0.1269
α1	0.89±0.21	0.94±0.24	0.3158	1.06±0.13	1.11±0.12	0.8738
α2	0.91±0.17	0.96±0.16	0.2639	0.93±0.06	0.93±0.07	0.9326

After dividing the IUGR children into two categories; IUGR1 for those with birth weight (BW) < 2.5 kg and the IUGR2 for those with BW > 2.5 kg, HRV values were compared between all three groups of children and the p value was recorded. Table 7.4 shows the 24 hour HRV results. The p value for significant differences between the groups is set to <0.05 and table 7.4 displays the HRV analysis of the 15 min pre-awake.

Table 7.4: HRV measures comparisons for 24 hrs and the p values.

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	0.065	0.002	0.312
SDANN	48.943±39.364	66.66±11.27	88.87±24.28	0.033	0.003	0.091
RMSSD	70.32±28.86	53.72±18.78	86.25±37.93	0.014	0.001	0.073
NN50	28290±11179	25139±10115	35435±13712	1.0	0.026	0.127
pNN50	23.55±10.51	19.83±8.87	30.30±12.93	0.175	0.006	0.026
LF	1966±2214	1418±456	2336±1057	0.017	0.002	0.187
HF	2704±2178	1548±1102	4007±3433	0.012	0.003	0.239
LF/HF	0.92±0.38	1.23±0.66	1.03±0.88	0.163	0.01	0.532
SD1	49.72±20.41	41.56±17.64	70.41±30.85	0.152	0.001	0.006
SD2	215.63±37.42	182.37±39.32	231.97±56.11	0.025	0.003	0.626
ApEn	1.14±0.08	1.17±0.09	1.15±0.08	0.432	1.0	1.0
SamEn	1.07±0.08	1.13±0.13	1.07±0.12	0.07	0.203	1.0

Table 7.5: HRV measures for 15 min comparisons between normal and IUGR with p values.

HRV Parameters	Normal	IUGR1	IUGR2	p-value		
				Normal VsIUGR1	IUGR1VsIUGR2	NormalVsIUGR2
RRmean	757.72±76.66	743.59±75.55	767.45±86.41	0.414	0.348	0.949
SDNN	106.672±34.845	86.38±28.99	122.9±43.01	0.088	0.014	0.758
RMSSD	88,72±41.46	55.75±24.63	103.74±50.73	0.002	0.002	0.306
NN50	432±166.49	333.25±157.13	462.81±158.95	0.089	0.056	1.0
pNN50	35.89±15.05	27.3±14.16	39.93±16.79	0.127	0.044	1.0
RRtriaIndex	22.42±6.99	19.48±6.62	24.11±9.25	0.613	0.224	1.0
TINN	531.51±142.71	451±133.04	562.19±196.04	0.210	0.105	1.0
LF	2.842±1.882	1.791±1.122	3.512±2.289	0.025	0.060	0.522
HF	4.336±3.703	1.876±1.665	1.38±0.95	0.010	0.017	0.639
LF/HF	0.924±0.731	1.38±0.95	0.92±0.57	0.045	0.083	0.932
SD1	62.88±29.38	42.7±17,52	73.57±35.96	0.007	0.004	0.296
SD2	151.17±55.57	123.78±42.12	164.02±61.12	0.267	0.088	1.0
ApEn	1.22±0.21	1.28±0.12	1.25±0.17	0.761	1.0	1.0
SamEn	1.25±0.25	1.14±0.39	1.15±0.33	0.757	1.0	1.0
α1	1.01±0.22	0.89±0.21	0.86±0.24	0.077	0.098	0.790
α2	0.97±0.13	0.91±0.17	0.95±0.19	0.558	1.0	1.0

7.5 Discussion:-

In this study we are looking at the 15 min prior to the awakening. Table 7.1, shows the details of the cohort (75) and the number of children in each of the groups, normal children are 34, and all IUGR are 41 children. The IUGR group is further sub-divided into two groups, IUGR 1 (24 children) and IUGR2 (17 children). In this work comparisons between the normal and IUGR groups were undertaken and, further on, another comparison of results was obtained between the IUGR sub-groups, with regard to their birth weight. In addtion Table 7.2 shows the comparison of the HRV measures between the females and the males for normals and IUGRs. The only significant difference between the males and the females were SDNN, LF and SD2. These were found different when we compared the normal males and females, but with IUGR there is no significant difference between the males and the females with the group. SDNN shows that males have a higher heart rate variability, which is a good marker for a healthy child, and females have a low SDNN. Significantly Low SDNN was found to be associated with the risk of , sudden death (Martin *et al.*, 1986). High LF means that the sympathetic branch in males is significantly more active than in females and SD2 shows that the males have higher long term variability.

Table 7.3 shows the comparison between normal and IUGR children, within the duration of 24 hrs and for 15 min pre-awake. It can be seen that the p values show no significant difference between the two groups in the time domain, frequency domain and using non-linear measures, but one can see that the means of all time domain indices (except for the heart rate) for normal children are higher than IUGR, although the difference is 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 the time domain, it can be seen that ($p>0.05$) when looking at comparison between normal group and IUGR for 15 min pre-awake, but when we analyse SDNN between normal and IUGR it was found that the difference is non-significant (p=NS).

Although it is clear that the longer the data, the higher the SDNN, however 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 it has benn found that RMSSD, NN50, PNN50 are higher in normal than IUGR over 15 min. On the other hand 24 hr RMSSD is higher in normal, while NN50, PNN50 are higher in IUGR; yet emphasising once more that these differences are not statistically significant.

When we analyse table 7.4, it is clear that RMSSD time domain measures show a significant difference between the normal and IUGR1 (birth weight less than 2.5 kg), a low value of RMSSD may be associated with complete heart block (CHB), left bundle branch block (LBBB), and ischemia/dilated cardiomyopathy and this is associated with small RR variation (Acharya *et al.*, 2002), (Acharya *et al.*, 2004). The frequency domain HRV measures that show statistically significant differences are LF and HF. Low HF is a marker of sudden death (Myers *et al.*, 1986), and high LF/HF ratio is associated with CHB and ischemia/dilated cardiomyopathy (Binkley *et al.*, 1991). SD2, which is related to the long term variability, gives $p<0.05$, and SD1/SD2 is high for premature ventricle contraction (PVC), atrial fibrillation (AF), sick sinus syndrome (SSS) and ventricle fibrillation (VF). A low value for the ratio has been associated with CHB (Acharya *et al.*, 2004).

ApEn gives $p>0.05$, but SamEn shows $p<0.05$, when comparing normal and IUGR1 and when comparing IUGR1 and IUGR2. 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 (Tuzcu *et al.*, 2006).

Table 7.5 summarises the results of 15 min pre-awake interval as we compare normal with IUGR1 and IUGR2, as well as IUGR1 against IUGR2. Furthermore, When comparing normal with IUGR1, the RMSSD is significantly different between the two groups ($P=0.002$). The other HRV measures which are SD are HF ($p=0.010$) and SD1 ($p=0.007$). The comparison between IUGR1 and IUGR2 has the following significant difference in the HRV measures: SDNN, RMSSD, HF and SD1 all these have $p<0.017$ correcting for multiple comparison. Regarding normal against IUGR2, $p>0.05$ for all HRV indices. HRV measures show high correlation between the 15 min and the 24 hr data.

7.6 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 term 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, interesting results were found with regard to the development of the autonomic nervous system of the IUGR children. The low value of SDNN for IUGR1 which has been 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, RRtriaIndex and TINN in IUGR1, do represent a low variability in RR and this has been found to be the case in complete heart block (CHB), left bundle branch block (LBBB), and ischemic/dilated cardiomyopathy (Acharya *et al.*, 2002). An important frequency domain result is the high value of the LF/HF ratio (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, might suggest future association with cardiac heart failure (CHF). All these findings suggest the importance of looking after the cardiovascular health of IUGR children from birth, especially when the IUGR children have birth weights 2.5 kg or less.

8 Discussion and conclusion

8.1 The thesis main finding

This study has looked at the Application of HRV analysis to a unique data of 75 normal and IUGR children. The main aim is to find any abnormality in the development of the ANS. The heart pace maker is affected by neurohumoral system driven by stimuli from the brain, human senses and other internal physiological system. Heart rate variability (HRV) signal can be used as a reliable marker of the condition of the heart. The results of this study would be summarised and a conclusion may be drawn in the light of barker theory.

The research started by developing an algorithm that will be able to read paediatric ECG considering the interested frequency for this application which is the HRV analysis. The algorithm used band pass filtering with adaptive threshold, refractory and other routines to improve the signal to noise ratio.

The RR signal was subjected to cubic spline interpolation to 4 Hz for frequency domain analysis using FTT and AR methods. An up sampling to 512 Hz was applied to the original ECG signal of 128 Hz for better detection of ECG intervals.

Time domain analysis showed no significant difference between the normal and the IUGR children at the age of 10 yrs. The time domain analysis showed the influence of the sympathetic branch of the ANS during the day (small RR interval) and the influence of parasympathetic branch (high RR interval) at night. Another study carried out done to look at the one hour analysis during night time. An attempt was made to compare between the IUGR and normal children with the effect of other factors including smoking, gender, and breast feeding. The accuracy of the this analysis was questionable due to the fact that the signal is not stationary and it's very difficult to select the same hour for all the children to obtain an accurate comparison study . Night time, day time or 24 hr comparison proved to be more used in research to find the right HRV measures (Task Force of the European Society of *et al.*, 1996). The one hour study showed some results that confirmed with the consequent accurate results such as the effect of gender on RR data at night time. The effect of many

factors such as gender, IUGR, smoking and breast feeding were chosen to see their influence on RR interval and SDNN at night time. The ANOVA test results showed that such factors no effect don't have any effects on the RR intervals and SDNN at night. For example, smoking during pregnancy increases the sympathetic and reduce vagal activity. HRV decreases with smoking and this is caused by its effect on the ANS.

Low heart rate variability presents a risk factor of sudden death in myocardial infarction patients. A study (Karakaya *et al.*, 2007) on the effect of acute smoking on the ANS showed that mean R-R interval, SDNN, RMSNN, significantly decreased within the first 5-minute period compared with baseline, SDNN increased within the 20- to 30-minute period. The LF/HF ratio significantly decreased within the first 5 minutes after smoking and did not change for the duration of the study. LF and HF increased within the 5 min compared with baseline. The results of this study show that RR at night is not affected by smoking in pregnancy and family smoking (passive smoking) this could be due to the fact that the effect of mother smoking on RR intervals at night diminishes after 10 yrs. The effect of family smoking or passive smoking can be prevented by parents smoking away from the children. Brest feeding showed no significant effect on RR at night but the breast-fed children have higher RR intervals than the RR intervals for the Non breast-fed children. A study (Dahlstrom *et al.*, 2008) showed that LF/HF is correlated with the milk nicotine concentration from a smoking mother. The HRV decreases with increasing milk nicotine. Males have a higher RR at night time (860.789 SE 14.314) than female children (806.748 SE 15.506).

It is well known that HRV is an age and gender related. HRV decreases with the increase in age and the variation is more in female than men (Lavi *et al.*, 2007).

The SDNN measure is lower for children who are IUGR, male and subjected to smoking.

Frequency domain analysis was used to assess the ANS through the frequency components of the power spectrum. FFT was used to evaluate the LF and HF during the 24 hr and it was found the LF is high for children who are subjected to mother smoking during pregnancy. Normal children have a higher LF (sympathetic activities)

during the 24 hr. The HF was found to be high in IUGR children. Low HF was found in subjects with chronic renal failure (CRF) (Acharya *et al*., 2006).

AR method was to used find RSA in children, it is very apparent at night time when breathing is synchronised and the rate of breathing is nearly constant. The results of all children RSA area were compared between the IUGR and the normal children to see if there is any difference occurred. The comparison showed no significant difference between the two groups. Other predictors were tested to see their effect on the RSA and the result was that no predictor had any relation with RSA. IMD which is the measure of poverty was in the border line in predicting the RSA. This means that deprived children have less synchronised breathing at night time. RSA is related with the efficiency of breathing and the efficiency of pulmonary gas exchange is improved by RSA. The larger RSA area, the more efficient is the breathing and consequently the transfer of oxygen to the body. RSA becomes less dominant with age, diabetes and cardiovascular disease.

Lomb peridogram was used due to the advantage of this method when compared with FFT and AR (Moody, 1993). LF/HF at day is affected significantly with gender where females have a higher LF/HF than males. IUGR has a higher sympathetic shown by the high value of LF/HF. Non breast-feeding children have a higher LF/HF. The night time LF/HF was higher for IUGR, non breast feed, and female children.

ECG intervals measurements were calculated by a developed algorithm further more it was found that all the calculated intervals were within the paediatric published limits (Macfarlane *et al.*, 1989, Rijnbeek *et al.*, 2001, Davingnon *et al.*, 1979/80). The intervals were QTc, ST, QTS and heart rate. The other aim of this algorithm is to look at the QTc interval for both groups to see if there is any sign of long QT syndrome in IUGR that might suggest future prognosis of heart disease. QT analysis showed that IUGR group has a higher QT interval than the normal children, and this is an indication that IUGR children could be vulnerable to QT syndrome in the future.

Poincare plot is a non linear technique to measure SD1, SD2 and the ratio SD1/SD2. The calculation was for the night period only to monitor the effect of many factors on theses dependent variables. The result was that there is no factor that affected any of short term variability SD1, the long term variability or the ratio of SD1/SD2. The results showed that males, normal, with no smoking mother during pregnancy, no

smoking family have higher short term variability (SD1is high). Male children proved to have significantly higher term variability (SD2) than females. SD1/SD2 ratio was lower for IUGR children and for those who experience smoking during pregnancy. The ratio is low for slowly varying signals like complete heart block. A decreased SD1/SD2 ratio with an elongated, torpedo-like shape is associated with elevated sympathetic tone, and an increased SD1/SD2 ratio indicates less sympathetic tone (Woo *et al.*, 1992), (Brennan *et al.*, 2001)

The Respiratory Sinus Arrhythmia study of a sample of different children, (IUGR and Normal), did not show statistically significant differences. The energy measurement of RSA for all IUGRs and normal children can be measured, and a similar comparison to RR at night time of RSA is needed for children with variables such as 'smoking', and breast-feeding.

Children with special medication have shown different measurements in comparison to other normal healthy children. 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.

The low value of SDNN for IUGR1 which has been 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, RRtria Index and TINN in IUGR1, do represent low variability in RR and this has been found to be the case in complete heart block (CHB), left bundle branch block (LBBB), and ischemic/dilated cardiomyopathy (Acharya *et al.*, 2002). An important frequency domain result is the high value of the LF/HF ratio (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, might suggest future association with cardiac heart failure (CHF).

IUGR with low birth weight showed a lower HRV than the normal and IUGR with birth weight higher than 2.5Kg. HRV and cardiac reflexes in small for gestational age SGA infants (Galland C. Barbara *et al.*, 2006) , suggests reduced autonomic activity and cardiac reflexes in SGA infants, and the findings of sympathetic components of the control of HRV is higher, which might be linked to higher risk of CHD in later

life. Detrended time series analysis of the R-R intervals suggests that IUGR fetuses have significantly reduced HRV compared to the other groups of children (Govindan *et al.*, 2006). The Sample Entropy showed lower values for IUGR<2.5KG but not significantly different to IUGR2 and Normal children and SamEn was used to study obstructive Sleep apnoea syndrome (Al-Hangari and Sahakian, 2007) and found that normal subjects have significantly more complex HRV pattern than the OSA.

In this work there is a low statistical power due to the small sample size. This means that we can expect some type 2 errors (failing to reject a false null hypothesis).

8.2 Conclusions and future work

The main novelty of this work would be in the application of HRV time domain analysis (TDA) and frequency domain analysis (FDA) to a unique set of data of 75 children (IUGR and Normal), to enhance our understanding of the development of the ANS and improve our ability to identify risk factors, risk period and risk patients. The main findings of the thesis are listed below.

- HRV analysis was applied on IUGR and Normal children at 10 yrs and no significant difference between Normal and all IUGR was found.
- When dividing IUGR into IUGR1 (<2.5 kg) and IUGR2 (≥2.5 kg), there is Significant difference between Normal and IUGR1, IUGR1 and IUGR2.
- Girls have a lower HRV than boys.
- RSA phenomena can be seen very clearly at night time by using FFT, AR, or Lomb periodogram applied on the NN data.
- Lomb method was used to find Frequency domain measures without resampling.
- QT analysis showed that IUGR children have a longer QT interval than the normal children.
- Poincaré plot results seem to indicate more risk on female than male children.
- 24 hr and Pre-awake analysis highlighted that IUGR have lower HRV.
- The work done on the data reading, TDA, and FDA, gives a fairly complete picture of the differences between the two groups, but not a complete one.
- The algorithm used for QRS detection can be reviewed for a better detection and the results of the TDA from the RR files can be compared with the RR found by the QRS detector.
- One of the novelties in this work is finding the difference between Normal and IUGR1, and IUGR1 and IUGR2 in TD, FD and other HRV measures.

The FDA work showed no difference in LF/HF between IUGR and normal groups using the Lomb method. Lomb is superior to standard Fourier-based approaches as it does not require data interpolation and re-sampling. Comparison between AR, FFT and Lomb can be analysed more using the ECG data of this work. The RSA feature is very interesting at night time. The comparison of RSA energy between different

groups of children is useful for the study of the Autonomic Nervous System (ANS), in particular the influence of the parasympathetic branch of the ANS. QT measurements showed that QT interval for IUGR is longer than that for normal children, which might suggest future risk of CHD. Pre-awake study highlighted some significant difference in HRV which might indicate that this period before awake can be critical.
As a continuation to the pre-awakening study, a further study on more periods pre-awake and more periods post-awake to look at the trend of HRV measures would be interesting.

The QT algorithm can be improved to detect a wider variety of shapes of paediatric ECG waves and to deal with ECGs with poorer SNR. The effect of other factors like smoking can be studied to see if ECG is affected by these factors.
A continuation to study the same cohort at a elder age than 10 yrs to analyse the extend of any changes in HRV measures.

The effect of children's ethnicity (African, Asian, ..etc) on their HRV measures can be explored and analysed in comparison with western children. The study of the behaviour of the HRV focussed on children with medical conditions and disabilities such as autism, asthma, epilepsy and other diseases might also show interesting results.

Appendix A

A Sample of Physiological data of the 75 children

category IUGR1	Name	Weight change	Current height(cm)	sex (F=2 or M=1)	current age	Birth weight	current weight
1	HA	28.88	138.00	1.00	9.91	2.82	31.70
1	JB	23.90	122.00	1.00	8.61	2.00	25.90
1	DC	26.20	133.00	1.00	8.88	2.40	28.60
1	NB	31.54	140.00	2.00	9.07	3.46	35.00
1	JC	23.61	127.50	1.00	8.90	3.29	26.90
1	BC	21.88	129.00	1.00	9.11	3.12	25.00
1	PC	22.60	130.00	2.00	8.48	2.40	25.00
1	EM	23.96	130.00	1.00	8.95	3.34	27.30
1	RD	27.22	130.00	2.00	8.82	1.78	29.00
1	EW	24.86	140.00	2.00	10.02	2.54	27.40
1	HD	20.70	127.00	2.00	9.22	2.20	22.90
1	AW	27.54	137.00	2.00	9.49	2.46	30.00
1	EW	24.12	126.50	1.00	9.85	2.28	26.40
1	AFJ	24.30	130.00	1.00	8.98	2.50	26.80
1	LG	21.01	127.00	2.00	9.13	2.50	23.50
1	JH	20.51	122.00	1.00	9.30	2.49	23.00
1	AH	19.54	125.00	2.00	8.66	2.46	22.00
1	GL	25.44	134.00	1.00	9.45	2.46	27.90
1	BL	21.93	131.00	2.00	9.48	2.37	24.30
1	AL	37.53	126.00	2.00	9.76	2.47	40.00
1	LM	22.69	130.00	1.00	9.76	2.21	24.90
1	LP	28.88	133.00	2.00	9.56	3.12	32.00
1	CP	26.90	119.00	2.00	9.55	2.10	29.00
1	JS	20.85	125.00	1.00	9.40	2.55	23.40
1	JS	21.66	128.00	1.00	8.98	2.44	24.10
1	CC	36.65	130.00	2.00	9.11	2.55	39.20
1	AG	33.42	141.00	2.00	10.22	2.38	35.80
1	NH	22.55	128.50	2.00	8.53	2.15	24.70
1	SH	27.02	138.50	2.00	9.57	2.38	29.40
1	GH	30.95	137.00	1.00	10.33	2.05	33.00
1	DM	30.77	138.00	1.00	10.16	3.23	34.00
1	HA	27.74	124.00	2.00	9.13	2.26	30.00
1	AM	15.41	121.00	2.00	8.44	2.70	18.11
1	CP	31.82	139.00	1.00	9.63	2.18	34.00
1	LR	25.65	137.00	2.00	8.58	2.35	28.00
1	CS	25.86	135.00	1.00	9.98	2.44	28.30
1	MS	27.95	135.00	1.00	10.72	3.25	31.20
1	SY	44.34	147.00	2.00	10.16	2.76	47.10
1	AB	22.28	125.00	2.00	8.28	2.72	25.00
1	JB	21.56	126.50	2.00	8.96	3.34	24.90

2	SM	39.00	142.00	2.00	10.29	3.20	42.20
2	TA	33.02	134.50	2.00	8.10	3.18	36.20
2	SA	23.28	131.00	2.00	8.00	3.72	27.00
2	HB	28.78	132.00	2.00	8.22	3.32	32.10
2	SB	32.06	135.00	1.00	8.75	3.64	35.70
2	AB	27.06	136.00	1.00	8.09	4.05	31.10
2	JC	37.82	140.00	1.00	10.90	3.78	41.60
2	BC	37.72	141.00	1.00	9.10	4.08	41.80
2	LG	28.86	131.00	2.00	9.27	2.64	31.50
2	TG	34.24	140.00	1.00	8.75	3.46	37.70
2	HH	22.78	134.00	1.00	9.02	3.22	26.00
2	CL	19.58	130.00	1.00	8.97	3.52	23.10
2	BM	21.44	130.00	1.00	8.89	3.56	25.00
2	ToM	30.40	140.50	1.00	10.43	3.60	34.00
2	TrM	22.26	125.00	1.00	8.34	3.74	26.00
2	RM	27.41	138.50	1.00	9.91	2.49	29.90
2	TYM	28.98	130.00	1.00	9.21	3.52	32.50
2	MO	25.90	133.00	2.00	8.61	4.10	30.00
2	TP	38.32	136.00	1.00	9.49	3.68	42.00
2	JP	45.10	141.00	1.00	8.99	2.80	47.90
2	ER	30.86	136.00	2.00	9.05	2.94	33.80
2	GS	21.90	127.00	1.00	8.74	3.90	25.80
2	CS	28.16	132.00	1.00	8.84	3.84	32.00
2	GS	23.62	129.00	1.00	8.93	2.58	26.20
2	JT	25.20	136.00	2.00	8.81	4.30	29.50
2	CB	30.86	137.50	1.00	8.85	3.64	34.50
2	LW	27.26	129.00	2.00	7.90	3.94	31.20
2	EG	33.16	136.50	2.00	8.62	4.04	37.20
2	RC	33.00	132.00	2.00	8.64	3.20	36.20
2	AS	15.74	120.00	2.00	8.60	3.06	18.80
2	BL	29.32	136.00	1.00	9.84	3.68	33.00
2	LP	28.09	135.00	1.00	8.61	3.91	32.00
2	HN	31.94	131.00	1.00	9.81	4.26	36.20
2	HG	26.60	130.00	1.00	8.12	3.40	30.00

Appendix B

Paper 1 Presented at 19th International conference Biosignal 2008 Brno-Czech Republic June 29 to July 1, 2008

Heart Rate Variability Analysis of Intrauterine
Growth Restricted and Normal Children at 10 yrs.

Heart Rate Variability in 10 Year Olds –Normal and Intrauterine Growth Restricted

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Abstract. The objective of this work is to verify any correlation between HRV of children at 10 yrs and the Barker Theory and hypothesis which states that IUGR children are prone to coronary disease and hypertension in adulthood. Time domain analysis was done on the raw ECG after filtering and QRS detection. The mean, SDANN, Poincaré plots and the sample density distribution of RR were obtained for all 75 children. It was found that the lowest p-value is 0.29, when comparing time domain measures between the two groups. This means, there is no significant difference between the IUGR and normal children at 10 years. Frequency domain analysis of RR by autoregressive model (AR), Fast Fourier Transform (FFT), and Lomb periodogram was performed for 10 min segments of all 75 children to find frequency components (LF, HF, and LF/HF). IUGR and normal comparison showed that the lowest p-value is 0.48, and for other variables (smoking, body mass index, sex, gestational period) the lowest p-value is 0.27. Frequency domain results support time domain findings that there is no significant difference between IUGR and normal children at 10 years.

1 Introduction

HRV is a powerful non-invasive tool used by physicians to determine the state of the heart and assess the development of the autonomic nervous system (ANS). This work describes the results obtained by HRV analysis of two groups of children, 41 IUGR and 34 normal for a period of 24 h, to find any correlation between HRV of children at 10 years and the Barker Theory and hypothesis which states that IUGR children are prone to coronary disease and hypertension in their adulthood [1]. The HRV analysis is calculated from a raw data of the ECG for all children. Figure (1), shows an ECG and its details [2].

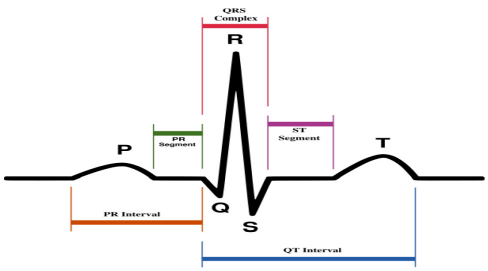

Figure (1.2): A typical ECG waveform.

2 Method

The Task Force [3] specifies the standards used in any HRV studies, where time domain and frequency domain analysis can be used.

2.1 Time domain analysis

(TDA) was done on the raw ECG after filtering it [4], to remove the mains noise and detecting the QRS to find the RR segments, Fig 2.1(a), shows the RR (NN) for 24 hours period. In the time domain analysis the following were calculated for all 75 children, the mean over 5 min intervals for the day and for the night, the SDANN also for day and night. Fig 2.1(b), shows a plot of the mean and SDANN for one child. The Poincaré plot of NN vs. NN-1 and the sample density distribution of RR for all children were plotted. Fig2. 1(c) shows the sample density distribution of RR for one child, and Fig 2.1(d), shows a Poincaré plot of NN-1 vs. NN.

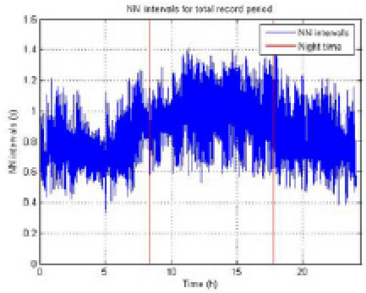

Fig.2.1 (a) NN intervals for 24 hours

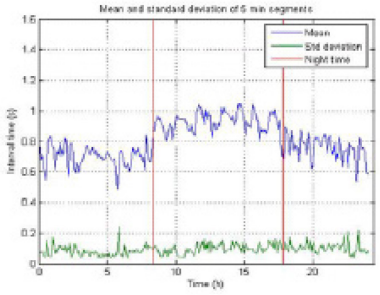

Fig.2.1 (b) Mean and SDANN

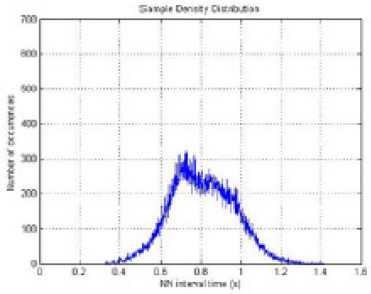

Fig.2.1(c) Sample Density Distribution

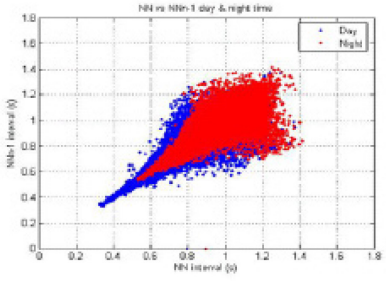

Fig.2.1 (d) Poincaré plot, NN-1 vs. NN

2.2 Frequency domain analysis

Frequency domain analysis (FDA) of RR has been determined by three methods, Fast Fourier Transform (FFT) and autoregressive model (AR), for 10 min segments after re-sampling, and Lomb periodogram, without re-sampling. The Task Force [3] defines low frequencies as between 0.04 Hz and 0.15 Hz and high frequencies as between 0.15 Hz to 0.4 Hz. To measure the low frequency activity and high frequency activity, the results of the Lomb analysis in the power spectra density (PSD) were integrated between the low frequency boundaries and high frequency boundaries for each 10 minute segment in a day. Fig (3) shows the autoregressive spectrum of the HRV for a child with Autism.

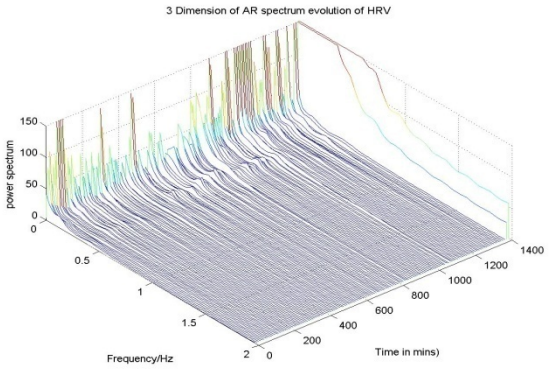

Fig (3), The AR spectrum of a child with Autism

3 Results

For the IUGR, the mean over 24 h, for the day and for the night are, (0.68, 0.61, 0.82), and for control (0.67, 0.59, 0.81). The SDANN over 24 h, day and night for IUGR are (0.12, 0.07, 0.07), and for control, (0.13, 0.07, 0.07).

When performing the t-test for the time domain data to find the p-value, the following results was obtained:-

Table (3.1). Results for Time Domain Analysis (R to R), over 5 minute intervals

Variable	IUGR (ms)	Normal (ms)	Diff (ms)	p-value
Mean 24hr	0.68 (0.07)	0.67 (0.06)	0.0116	0.4641
Mean Day	0.61 (0.07)	0.59 (0.06)	0.0143	0.3531
Mean Night	0.82 (0.08)	0.81 (0.08)	0.0067	0.7396
SDANN 24 hr	0.12 (0.03)	0.13 (0.02)	0.008	0.2881
SDANN Day	0.07 (0.02)	0.07 (0.02)	0.004	0.3629
SDANN Night	0.07 (0.02)	0.07 (0.02)	0.005	0.3379

The power of the frequency components (LF, HF and LF/HF), was calculated from the Lomb periodogram spectral analysis.

When carrying a t-test on the IUGR and normal for low frequency, high frequency and L/H for 10 min segment we get the results recorded in table (3.2). If p value is higher than 0.05, there is no significant difference between the 2 results.

IUGR v Normal

	IUGR Mean *	Normal Mean *	p-value
Night H	0.63	0.60	0.5254
Night L	0.65	0.62	0.5282
Night LH	1.23	1.22	0.8730
Day H	0.66	0.63	0.4782
Day L	0.73	0.74	0.8469
Day LH ratio	1.44	1.44	0.9925

Table (3.2), shows the p- value for significant difference between the variables.

- The calculations of H and L shown in table (3) in arbitrary units squared (gain is not calibrated).

Table(3.3).lists the results of a t-test on IUGR and normal data to find the p-values for candidate predictors such as , the use of medication, significant medication, smoking at pregnancy, body mass index, index of deprivation, and gestational period.

P-VALUES FOR CANDIDATE PREDICTORS

	use med	sig med	sex	cigs p	cigs h	bmi	imd	gest
Night H	0.97	0.55	0.49	0.85	0.69	0.67	0.17	0.38
Night L	0.37	0.19	0.98	0.88	0.86	0.66	0.47	0.39
Night LH	0.69	0.79	0.18	0.76	0.36	0.78	0.45	0.72
Day H	0.25	0.37	0.027*	0.55	0.71	0.19	0.56	0.08*
Day L	0.51	0.49	0.77	0.82	0.64	0.81	0.95	0.09*
Day LH r	0.31	0.46	0.003	0.12	0.98	0.46	0.40	0.56

Table (3.3) - Results of t-test to find p-value for candidate predictors.
* These values are related to t-test type 1 error of 5%.

4 Discussion

In the TDA, changes in the state of the autonomic nervous system (ANS) can be seen very clearly. In the day time sympathetic rhythm is indicated by higher heart rate (low R to R). IUGR=0.61 ms, and for normal =0.59 ms. This is due to the various activities done by the children like running and playing. In the night time the parasympathetic rhythm takes over and low heart rate (high R to R) is dominant. The IUGR mean of RR=0.82 ms, and for normal children=0.81 ms. When carrying the t-test the lowest p-value =0.2881. FDA shows the frequency components of the power spectrum which is associated with known physiological mechanisms, VLF is caused by thermoregulation and humoral factors, LF by baroreflex-related HRV, and HF arises from the respiratory sinus arrhythmia (RSA). The lowest p-value is 0.4782, which means that FDA shows no significant difference between IUGR and normal

groups. Candidate predictors' results showed that none of them has a significant effect on the H, L and L/H ratio .

5 Conclusions

Time domain analysis didn't show any differences between IUGR and normal children at 10 years old. Frequency domain analysis showed the same results, that there is no significant difference between the two groups. Other candidate predictors (body mass index, medication, smoking, gestational period, deprivation) were investigated to see their effect on the HRV, but there was no significant effect to these variables on HRV either.
The main conclusion would be: the heart rate variability in children who were previously growth restricted is no different from that of normal children at age 10 years, which means that at 10 yrs there is no signs of coronary heart diseases or hypertension. This might lead to the conclusion that the development of heart diseases, for IUGR is in a later stage of life, if Barker theory is founded. The results of this study support evidence that IUGR and normal children have no difference at 10 yrs.
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Paper 2 Paper Presented at Biosignal 2009 14th-17th Jan 2009 Porto-Portugal

Respiratory Sinus Arrhythmia: 10 Year olds - Normal and Intrauterine Growth Restricted.

RESPIRATORY SINUS ARRHYTHMIA IN 10 YEAR OLDS - NORMAL AND INTRAUTERINE GROWTH RESTRICTED

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Keywords: Respiratory sinus arrhythmia, Frequency domain analysis, Autoregressive model, hypertension, Barker theory.

Abstract
Frequency domain analysis of RR has been determined by three methods, autoregressive model (AR), Fast Fourier Transform (FFT) and Lomb periodogram for 10 min segments. The first two methods were done after re-sampling and the third method without re-sampling RR series of all 75 children. AR was used in this work, and RSA was identified at night time during sleep. The area of the RSA was calculated for every 10 min interval and compared to the overall area of the 10 min segment, then the average RSA of all segments was calculated, as well as the overall percentage of the RSA energy to the total area for the whole period of sleeping. This was done firstly for a sample of Normal and IUGR 10 year olds. Secondly for all the children under study, an independent t-test concluded that there is no significant difference between the IUGR and Normal (p=0.7467),.

1 Introduction

HRV is a powerful non-invasive tool used by physicians to determine the state of the heart and assess the development of the ANS. This work describes the results obtained by HRV analysis of two groups of children, 41 IUGR and 34 controls for a period of 24 h .The main objective of the work is to find any correlation between HRV of children at 10 years and the Barker Theory and hypothesis, which states that IUGR children are prone to coronary disease and hypertension in their adulthood [1]. The RR interval normally oscillates periodically, shortening with inspiration and lengthening with expiration. This is known as Respiratory sinus arrhythmia, and it's due partly to the Bainbridge reflex via the expansion and contraction of the lungs and the cardiac filling volume caused by variations of intrathoracic pressure [2]. During inspiration, the pressure within the thorax decreases increasing blood influx into the right atrium resulting in a reflex that increases the heart rate (i.e., shortens the RR intervals). During expiration, the reverse of this process results in a slowing of the heart rate.

2 Methods

The Task force [3] specifies the standards used in HRV studies, where time domain and frequency domain analysis can be used to study heart rate variability.

The RR signal is subjected to a process of interpolation to obtain an equally spaced data to be used for spectral analysis. FFT and AR must have an equally spaced data to perform spectral analysis. The signal has been re-sampled at 4 Hz after a cubic spline data interpolation. This will give us the results in Hertz and allows the spectrum analysis up to 2 Hz. The AR spectral method (equation 1),(because of better resolution than FFT when dealing with low sampling Frequency), have been tested on the RR data to find the frequency components of the power spectrum for 10 min segments .The RSA frequency range (HF) is defined to be from 0.15 to 0.40 Hz ,[2].

2.1 Figures and tables:-

The AR spectral analysis of 10 segments produces a graph as shown in Fig (2.1). Graphs (a, b) are for Normal children, and (c, d), for IUGR children.

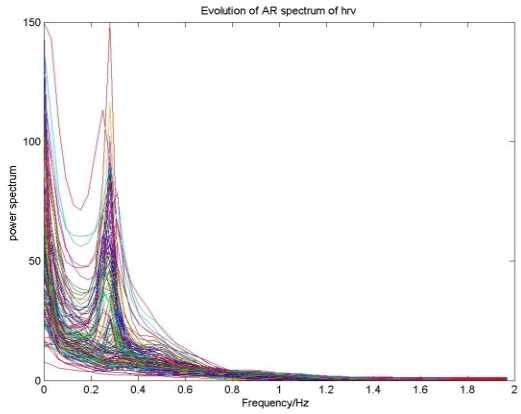

Fig (2.1)a. AR spectrum of Normal child with RSA.

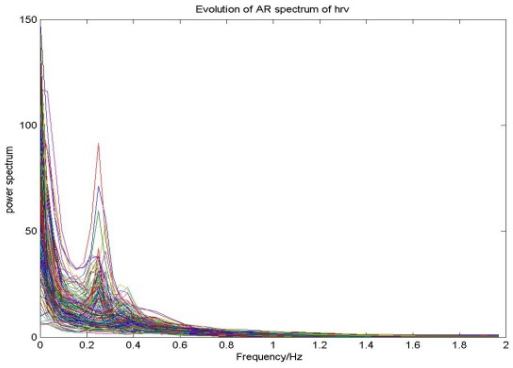

Fig (2.1)b. AR spectrum of Normal child with less RSA.

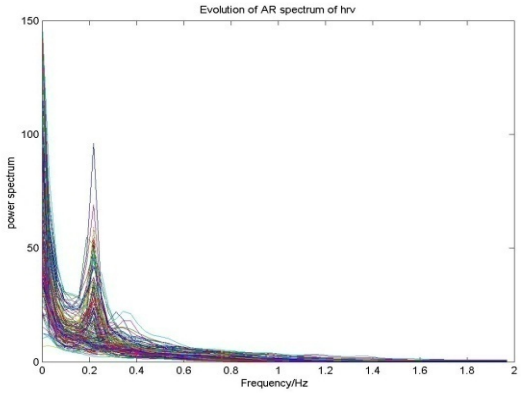

Fig (2.1) c, AR power spectrum of IUGR child with RSA.

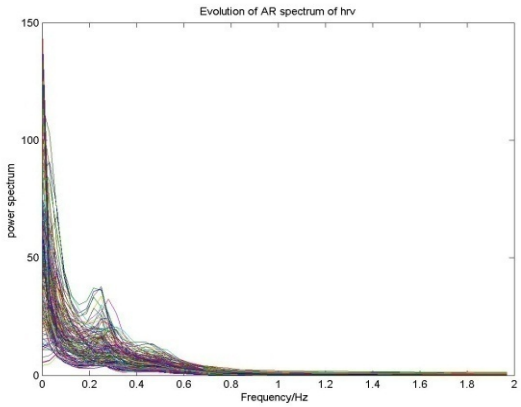

Fig (2.1) d. AR power spectrum of IUGR child with less RSA.

The application of an algorithm to find the energy of the RSA (area), by using Trapezoidal method (equation 2), produced the following table (2.1):-

Table (2.1), The RSA Energy, Total, and % of RSA to Total for IUGR and Normal children.

No	child	RSA (energy) u^2	Total (energy) u^2	% RSA/Total
1	IUGR	4.5936	10.3187	45.6287
2	IUGR	2.3244	5.8750	39.8064
3	IUGR	2.5484	5.5834	45.9105
4	IUGR	5.2631	10.6212	50.0502
5	IUGR	8.5370	18.5431	46.6481
6	Normal	6.9783	12.6834	54.7855
7	Normal	2.6899	6.7861	41.3924
8	Normal	3.6029	9.0093	40.0707
9	Normal	5.0364	11.0507	45.2321
10	Normal	3.7838	8.8850	43.0941

2.2 Equations

The equation of AR process of order p can be written as:-

$$x_t = n_t + a_1 x_{t-1} + a_2 x_{t-2} + ... + a_p x_{t-p}$$

Where n_t is the white noise driving signal p is the order of the AR model, and ($a_1 ..... a_p$) are the parameters of the filter.

The AR power spectrum density estimate is given by the following equation [4]:-

$$P_{AR}(f) = \frac{\sigma^2 \Delta t}{\left| 1 + \sum_{K=1}^{p} a_K e^{-j 2\pi f k \Delta t} \right|^2} \quad \text{.....(1)}$$

Where σ^2 is the variance of the white noise driving function and Δt is the re-sampling interval.

The Trapezoid method in equation (2) was used to find the energy (area) of the RSA:

$$A = \frac{(a+b)h}{2} \quad \text{..... (2)}$$

Where (a and b) are the two parallel sides and h is the distance (height) between them.

Statistically, an independent t-test was used for equality of means between IUGR and Normal in terms of RSA, and to verify the Null hypothesis which is , there is no difference in the RT score (Average of all ratio of RSA energy to total energy within 10 min segment for night time .).

Table (2.2), and (2.3), shows the results of the independent t-test :-

Table (2.2) IUGR and Normal Independent t-test results

Group	n	Mean	SD
IUGR	37	46.83	6.10
Normal	30	47.27	4.65

The t-test showed that there was no significant difference between the two groups for RT score, difference = 0.44, t = 0.32, p=0.7467 (95% CI = -3.1, 2.3). There was homogeneity of variance (Levene's Test, p=0.14) and the data was approximately normally distributed within each group.

Other variables were assessed for significance but none of them significantly predicted (RT) score, although IMD (Index of Multiple Deprivation) was borderline (0.06).

Variable	p-value
Sex *	0.32
Breast Feeding (y/n) *	0.50
Parental Smoking (y/n) *	0.99
Household Smoking (y/n) *	0.72
IMD #	0.06
24 hour SBP #	0.59
24 hour DBP #	0.87
BMI #	0.77
Significant Medication *	0.26
Using Medication *	0.36
Birth Weight #	0.45
Length Gestation #	0.50
Weight change from birth #	0.77
Night SBP #	0.79
Night DBP #	0.60
Day SBP #	0.46
Day DBP #	0.64
Cortisol morning #	0.29
Cortisol night-time #	0.66

* = independent t-test, # = correlation

Discussion

The results obtained are both qualitative and quantitative. The graphs in Fig (2.1) a and b, show the evolution of the AR spectrum of the HRV for a Normal child. It can be seen that the RSA energy in child (a) is higher than the RSA energy for Normal child shown in Fig (2.1) b. The frequency at which RSA occurs in (a) is at 0.3 Hz, but for child (b), RSA occurs at 0.25 Hz. The RSA for IUGR children can be seen in figures (2.1) c and d. Child (d), has RSA at Frequency of 0.2 Hz, and Child (c), at approximately 0.25Hz. The calculations of RSA energy from IUGR and Normal children are shown in table (2.1) in arbitrary units squared (gain is not calibrated). The data presented in the table shows that the lowest energy of RSA is 39.8 u^2, and the highest is 50 u^2 for IUGR. Normal children has the lowest energy of RSA is 40 u^2, and the highest is 54.78 u^2.
The result of the t-test shows no significant difference between the two groups for the RT score, but when looking at other variables, IMD was found to be(p=0.06). Which means that the children who have high index of multiple deprivation (IMD) are very correlated with RT score, and consequently this means that the deprived children don't have a synchronized breathing pattern at night time.

4 Conclusions

This work identifies the RSA in two ways, the first is graphically, and the second, in terms of the RSA Energy (area). RSA is an interesting phenomenon which represents the parasympathetic branch of the Autonomic nervous system. RSA occurs at frequency between 0.15 Hz and 0.4 Hz at night time and at this frequency (HF), RR intervals tend to be longer than at day time due to the parasympathetic tone, and consequently heart rate will be slower. At night ventilation is more regular; hence it is easier to identify the peak corresponding to RSA.

RSA cannot be used to distinguish between the IUGR and normal children, because there is no significant difference between the two groups. Other variables, such as parental smoking and household smoking cannot predict any differences in RSA.

Children who have high index of multiple deprivation (IMD) are very correlated with RT score, and consequently this means that the deprived children don't have a synchronized breathing pattern at night time.

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Appendix C

1-Timedomain.m algorithm results:

Mean over 24 h: 0.6149

Mean day time: 0.5540

Mean night time: 0.7504

SDNN: 0.1371

SDNN day: 0.0838

SDNN night: 0.1131

Mean of all 5 min segments: 0.6140

Mean of day time (5 min): 0.5406

Mean of night time (5 min): 0.7340

SDANN: 0.1146

SDANN day: 0.0505

SDANN night: 0.0622

RMSSD day time: 45.8380

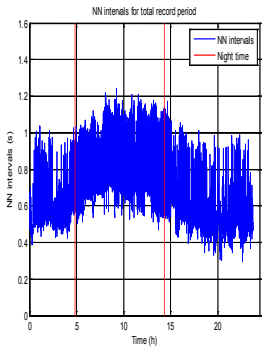
NN50 day time: 8407

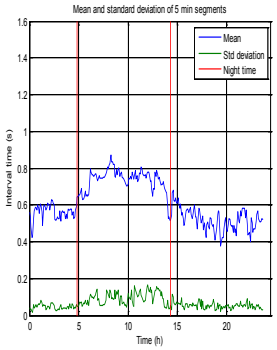
pNN50 day time: 0.0899

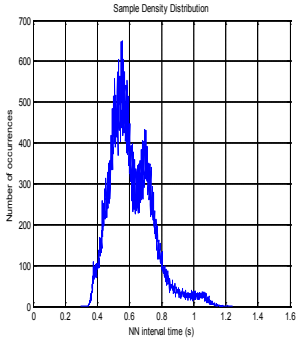
Shortest NN interval: 0.2960

Longest NN interval: 1.2400

Range: 0.9440



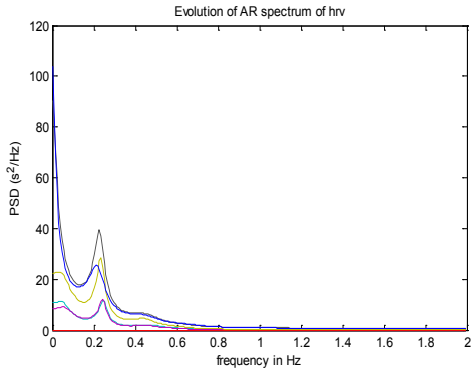




2-RSA.m algorithm results printout:

Opening file:

File opened sucsesfully.
There are 143 segments
bandpass from f1=14Hz to f2=24Hz
Where shall we start (in min. Each record = 10.0 min) ? 450
starting at segment 46. t = 450.0 min.
number of qrs=1357, number of rr=1356
450.0 min, rr_avg = 113.2 samples ... or 884.0+/-63.1ms
RSA area= 1.20 u^2, Total area = 2.86 u^2,percentage= 41.79 percent
number of qrs=1326, number of rr=1325
460.0 min, rr_avg = 115.9 samples ... or 905.4+/-59.6ms
RSA area= 1.20 u^2, Total area = 2.66 u^2,percentage= 45.15 percent
number of qrs=1316, number of rr=1315
470.0 min, rr_avg = 116.8 samples ... or 912.3+/-66.0ms
RSA area= 2.84 u^2, Total area = 6.47 u^2,percentage= 43.85 percent
number of qrs=1360, number of rr=1359
480.0 min, rr_avg = 113.0 samples ... or 882.5+/-90.0ms
RSA area= 4.50 u^2, Total area = 11.71 u^2,percentage= 38.43 percent
number of qrs=1447, number of rr=1446
490.0 min, rr_avg = 106.2 samples ... or 829.3+/-80.0ms
RSA area= 3.65 u^2, Total area = 10.50 u^2,percentage= 34.79 percent
AveRSA = 2.6764 AveTotalArea = 6.8377 AvePercentage = 40.8015



3-Lomb periodogram algorithm results:

Name = subject2

LFsleep = 0.0421

HFsleep = 0.1484

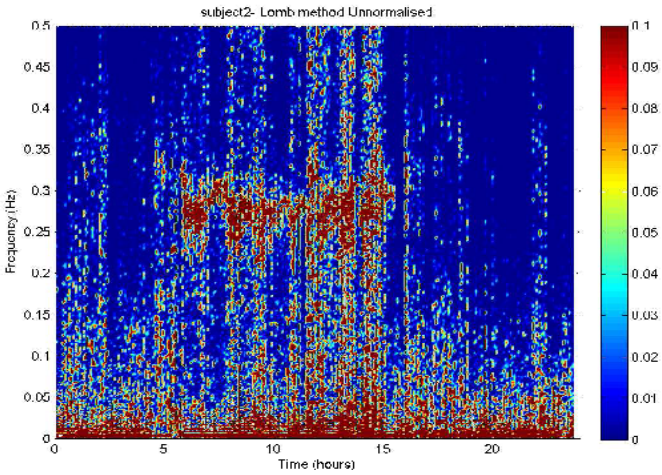
LFHF = 0.2839

LFHFsleep = 0.2267

LFawake = 0.0196

HFawake = 0.0454

LFHFAwake = 2.1487



4-QT.m algorithm results:

Average of QRS = 48.4 samples...or 94.5 ms

STD of QRS = 5.0 samples...or 9.9 ms

Average of QT =181.3 samples...or 354.0 ms

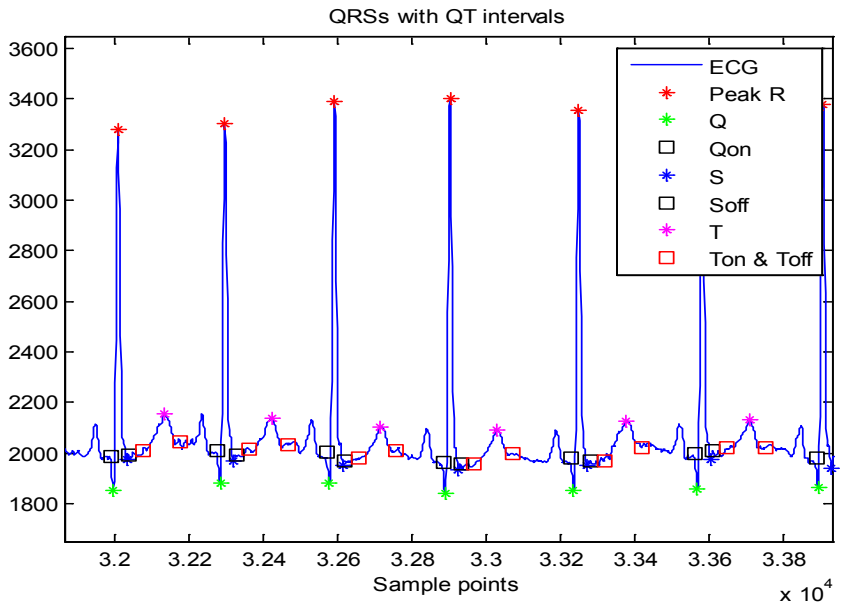
STD of QT = 36.0 samples...or 70.3 ms

Average of ST = 30.9 samples...or 60.3 ms

STD of QT = 36.1 samples...or 70.6 ms

Heart Rate Beat /Min ....102.7 BPM

The corrected value of QT =237.1 samples...or 463.2 ms



5-Poincareplot.m algorithim results:

Opening file: File opened successfully.

File header: Research Tools output from Reynolds Medical Pathfinder

Extracting RR data from source file, please wait.

End of source file.

File closed successfully.

Preparing RR interval plot.

Separating RR intervals into day & night time.

Preparing Scatter plots.

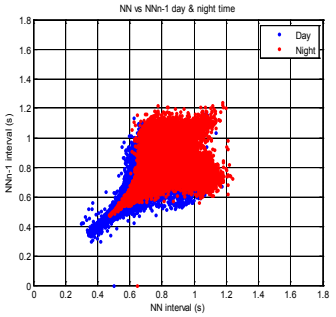
SD1day = 0.0324

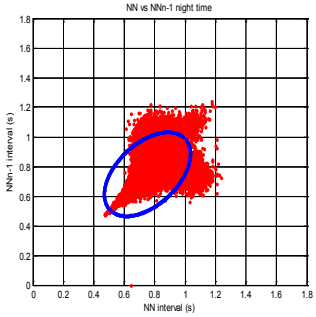
SD2day = 0.1255

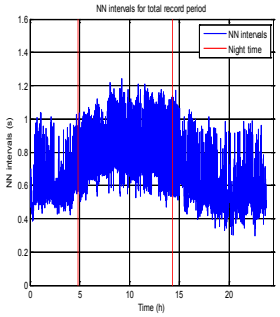
SD1night = 0.0813

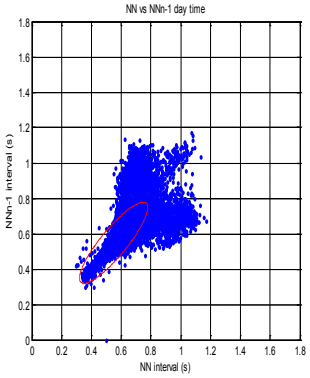
SD2night = 0.1378

End of program.









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