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ASSESSING CARDIAC AND NEUROLOGICAL MATURATION DURING THE INTRA-UTERINE PERIOD

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Abstract

The world's first magnetoencephalography (MEG) system specifically designed for fetal and newborn assessment has been installed at the University of Arkansas for Medical Sciences. This non-invasive system called SARA (Squid Array for Reproductive Assessment) consists of 151 primary superconducting sensors which detect biomagnetic fields from the human body. Since the installation of SARA, significant progress has been made toward the ultimate goal of developing a clinical neurological assessment tool for the developing fetus. Using appropriate analysis techniques, cardiac and brain signals are recorded and studied to gain new understanding of fetal maturation. It is clear from our investigations that a combination of assessment protocols including both fetal heart and brain activity is necessary for the development of a comprehensive new method of fetal neurological testing. We plan to implement such a test protocol for fetuses at high-risk for neurological impairment due to certain maternal risk factors and/or fetal diagnostic findings.

Keywords

fetal magnetoencephalogram; fetal magnetocardiogram; HRV measures

INTRODUCTION

Although there has been a decrease in perinatal morbidity and mortality rates of the fetus and neonate over the past four decades, antenatal hypoxia and/or asphyxia continues to be significant health problem leading to major motor and cognitive disabilities such as cerebral palsy, hearing and visual impairment, and mental retardation. Though for many years both clinicians and the general population have believed that birth trauma and perinatal asphyxia were the primary cause of these handicaps, clinical studies in recent years have disputed this misconception^{1,2,3,4,5,6,7} Most experts in this field have estimated that the incidence of cerebral palsy associated with labor events is around 10%. One major problem arises from the fact that it is currently impossible to determine the timing, the type, the duration, or the severity of the insults that are associated with neurological deficits in the newborn. Terms such as perinatal asphyxia, intrapartum asphyxia, hypoxic-ischemic encephalopathy,

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neonatal neurological dysfunctional syndrome, and fetal-neonatal anemia have all been used to describe the affected newborn. The non-selective use of these terms has produced great confusion in the clinical literature. Because a thorough investigation attempting to identify the cause of neonatal neurological depression is often not attempted or is incomplete, the diagnoses of perinatal asphyxia is many times made by default,⁸.

Electronic fetal heart rate monitoring is commonly used to assess fetal well-being in-utero. While a reassuring fetal heart rate pattern predicts the birth of an infant with a five-minute Apgar score of seven or greater with an accuracy 99%, non-reassuring tests have been associated with a false positive rate of greater than 50%.⁹ Traditionally the neurological maturation of the fetus has been assessed using ultrasound studies^{10,11}. Although it has been possible to observe and evaluate fetal neurological development on a limited basis using ultrasound, direct access to the fetal electro-cortical signals is required for specific testing and analysis. Currently, evidence-based clinical decisions concerning fetal neurological damage as a result of hypoxia or asphyxia cannot be made with conventional techniques.

To address these issues, SARA was developed through a grant provided by the National Institutes of Health/National Institute of Neurological Disorders and Stroke and is currently under operation at the University of Arkansas for Medical Sciences (UAMS).^{12,13} The system has a 151 primary sensor array curved to fit the shape of the maternal abdomen. This instrument is completely non-invasive and detects weak biomagnetic fields associated with the electrophysiological activity in the human body. In order to investigate the cardiac and neurological status of the fetus, we conduct serial fetal magnetocardiography (fMCG) and fetal magnetoencephalography (fMEG) recordings starting at 28 weeks of gestation^{13,14,15,16,17,18,19}. Newborn follow-up studies using the same fMEG protocol²⁰ are performed within two weeks of delivery using a specially designed cradle that can be attached to the SARA system. All studies are performed with the aim of providing improved monitoring techniques for maternal-fetal health and assisting physicians in the management of pregnancy and delivery. Some of the key results obtained through analysis of the signals obtained by SARA from the fetal cardiac and brain systems will be discussed in the next sections.

METHODS

Subjects

The studies performed with SARA were approved by the UAMS Human Research Advisory Committee and informed written consent was obtained from all subjects. A total of 433 fetal recordings lasting six minutes were performed on fetuses with gestational ages ranging from 27 to 40 weeks. 317 of these were acquired from 72 fetuses classified as high-risk (HR) due to maternal complications such as pregnancy induced/chronic hypertension, hemoglobinopathies, or smoking²¹ that may compromise normal fetal development. The high-risk group was further subdivided into fetuses of mothers who smoked (SM - Smokers, 55 datasets from 23 mothers), fetuses who were diagnosed with intrauterine growth restriction (IUGR, 36 datasets from 10 fetuses) and all other high-risk fetuses. Of the 72 high-risk fetuses, seven (9 datasets) had poor neonatal outcome. The demographic information of these subjects is given in Table 1. For details of the inclusion criteria for all HR mothers we refer²¹. The remaining 126 recordings were obtained from 37 healthy mothers and fetuses who were classified as low-risk (LR).

General Recording Methods and Analysis

The routine recording sessions range from six to 12 minutes in a continuous mode at a sampling rate of 312.5 Hz and a bandpass of dc to 100 Hz. The position and orientation of

the mother's abdomen, relative to the sensor array, are determined using two localization coils placed at fiduciary points on the mother's right and left side and one on her spine at the level of the umbilicus. These coils do not interfere with the MEG recordings. The fetal head position and distance of head from maternal abdominal surface are determined using a portable ultrasound scanner after the patient sits down in front of the array. A fourth localization coil is then attached to the maternal abdomen at that site to provide additional positional information related to sensor coordinates. The ultrasound exam to evaluate fetal head position and distance from surface is repeated at the end of the study but before the patient moves from the array. The raw SARA recording consists of maternal heart, fetal heart and brain signals. To perform data analysis we cancel the dominant maternal heart vectors by orthogonal projection. This method is first applied to the raw signal in order to remove maternal heart artifact. At this point, the fetal heart data can be analyzed. This process is then reapplied to the residual data set (devoid of maternal heart) to remove fetal heart artifact in order to analyze the brain signals.

RESULTS

Fetal Cardiac System

Beat-to-beat intervals were computed for all the fMCG signals using threshold detection technique. Standard heart rate variability (HRV) measures such as standard deviation of normal to normal intervals (SDNN), root mean square deviation of the successive differences (RMSSD), fraction (p) of the normal intervals greater than the chosen tolerance of x milliseconds from their previous values (pNNx) and the variance of the detrended time series at different time windows s containing s number of beat-to-beat intervals. In order to understand the differential maturation of the high-risk fetuses compared to the low-risk fetuses we divided the data into the following three gestational age (GA) groups, 27-30 wk, 31-35 wk and 36-40 wk. Although we have repeated recordings from the same subjects in multiple gestation ages we treat them all independently based on the following assumptions: dependency of the heart rate on the gestation age and the state of the fetus.

Unlike SDNN which captures the global variability in the beat-to-beat intervals, RMSSD captures the short-term variability (and hence high-frequency component of the HRV). pNNx is another measure which captures the short-term variability and quantifies the fluctuations in the firing of the sinus rhythm. It is computed by calculating the ratio of the number of normal to normal intervals which differ by a chosen tolerance value of x millisecond from their previous value to the total number of intervals. We used three different tolerance values namely 10, 15 and 20 milliseconds and found that the best difference between the groups was observed only with the tolerance of 10 milliseconds²¹. All the three measures are indicators of the alterations in the cardiac dynamics and can be used to quantify the maturation of the fetus based on the cardiac dynamics.

The results obtained for SDNN, RMSSD and pNN10 are shown in Figure 1 in different gestational ages. In SDNN there is a difference between the high-risk risk and low-risk groups in the 27-31 wk and 36-40 wk divisions and no difference in the 31-35 wk division. In RMSSD and pNN10 there is a significant difference between the low-risk and high-risk groups in 31-35 wk and 36-40 wk division as well. In 31-35 wk division low-risk fetuses had lower values of pNN10 and RMSSD compared to high-risk fetuses while in the 36-39 wk division an exactly opposite behavior was observed.²¹ We performed a subgroup analysis by separating the IUGR fetuses and compared their pNN10 measures with low-risk fetuses. We found that in the 31-35 wk division there was a significant difference between IUGR fetuses and low-risk fetuses. Further, in this gestational week division the values of pNN10 for IUGR fetuses were less compared to the low-risk fetuses.²¹ There was no difference between these two groups in the 27-31 wk division. In 36-40 wk division the

comparison was made because of less number of datasets in the IUGR group. We did not find any significant difference between the low-risk fetuses and fetuses of the smokers and fetuses with poor neonatal outcome either.

For low-risk fetuses there was a positive trend in the pNN10 ($\rho^2 = 0.052$; $P = 0.0114$) and RMSSD ($\rho^2 = 0.048$; $P = 0.012$) as function of gestational age while no such trend was observed for SDNN ($\rho^2 = 0.012$; $P = 0.220$).²¹ Also no trend was observed in any of the HRV measures for the high-risk fetuses.

The values of pNN10 and RMSSD in the 31-35 wk division show that high-risk fetuses demonstrate an increase in maturation rate compared to low-risk fetuses. In the later gestational age, low-risk fetuses tend to mature at normal rates while the high-risk fetuses did not show continuing maturation. This fact combined with the dependency of pNN10 and RMSSD of the low-risk fetuses with gestational age and absence of such a trend for high-risk fetuses clearly indicate that the low-risk fetuses show a progressive maturation while high-risk fetuses show an early maturation. The difference in the pNN10 values between low-risk and IUGR fetuses in 31-35 wk division indicate that the later group lack normal maturation.

In order to quantify the variations in the fetal cardiac beat-to-beat intervals, we performed detrended time series (DTS) analysis. This approach consists of the following three steps: (i) a running average is performed on the beat-to-beat interval time series for a chosen window 'w' (usually in powers of 2) to obtain a trend series, (ii) The trend obtained is then subtracted from the beat-to-beat interval to get the DTS and (iii) standard deviation (SD) is computed for the DTS. The steps (i)-(iii) are repeated for different s values usually ranging from 2 to one fourth of the length of the data and SD is computed for each value of s. Here, we varied s from 2 beats to 256 beats. In Figure 2 beat-to-beat intervals, the trend obtained for a window containing 32 beats and the DTS are shown for a low-risk fetus (Fig 2a-c), high-risk fetus (Fig. 2d-f) and an IUGR fetus (Fig. 2g-i). In general, SD for a large value of s corresponds to the quantifying low-frequency component of the beat-to-beat variability and vice versa. The SD(s) are log-transformed to produce Gaussian-distributed error and then subjected to least-square regression to identify trends with gestation age. The residuals resulting from trend removal are compared between different groups using t-tests with multiple-comparison adjustment via step-down permutation. These results are given in Table 2. Low-risk and high-risk groups show difference only in the low frequency component (at large time scales). Low-risk and high-risk fetuses show significant difference from the IUGR group at high frequency component (at short time scales). This result is in agreement with the pNN10 results obtained for low-risk and IUGR fetuses. Further, low-risk and high-risk fetuses show higher variability compared to high-risk and IUGR fetuses and between the low-risk and high-risk fetuses, low-risk showed higher variability. These results point to fact that in IUGR fetuses the autonomous nervous system is suppressed²².

Fetal Brain System

In order to analyze the fetal brain data, we performed spectral analysis on the residual data that is devoid of maternal and fetal heart signals. The data was filtered in a band width ranging from 0.5-25 Hz using a bandpass filter. We compared the spectral results of the low-risk fetuses with all the high-risk group fetuses. For this purpose power spectra were computed for all the 151 SARA sensors with a frequency resolution of 0.1 Hz. For each sensor, the spectral power was computed in δ (0.5-4 Hz), θ (4-8 Hz), α (8-13 Hz) and β (13-25 Hz) bands and normalized by the total power which is sum of the powers in all the four bands. The power in each band was averaged over all the sensors to get the estimate of the same. The estimated powers in the four bands were compared between different groups using student's *t*-test and a P values less than 0.1 was considered to be statistically

significant. The results are summarized in Table 3. There is a significant difference between the groups at least in one of the spectral bands except of HR and SM where there is no difference between them in any of the spectral bands. A further study in this direction by identifying the continuous and discontinuous brain patterns may be help to understand these differences and will be pursued in the future.

SUMMARY

In summary, investigation of magnetic fetal brain responses with MEG is becoming an established field of research. Using the SARA system, we have performed many fMEG and fMCG studies in both low-risk and high-risk risk patient populations. In order to improve fetal neurological testing, a combination of comprehensive assessment protocols including fetal heart and brain activity is valuable. We have developed a combination of recording parameters using auditory and visual stimuli and spontaneous brain activity for a multimodal approach to the investigation of fetal health. This protocol can be referred to as SNAP (SARA Neurological Assessment Protocol) and can be used to evaluate the neurological status of the fetus and newborn.

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Figure 1.

Comparison of HRV measures of low-risk and high-risk fetuses in three different gestational age groups (a,d,g) SDNN, (b,e,h) RMSSD and (c,f,i) pNN10. The difference between the groups was assessed using student's t-test is given in the inset and a $p < 0.05$ was considered to be statistically significant.

Figure 2.

Beat to beat intervals (in beats per minute bpm) for (a) low-risk fetus (d) high-risk fetuses and (e) IUGR fetus. (b,e,f) respectively, show the moving average obtained for the data shown in (a,d,e) for a window size of s containing 32 beats. (c,f,i) show the detrended time series DTS which is the original data (shown in the top panel) minus the trend (shown in the middle panel). Note that qualitatively the fluctuations in DTS are high in low-risk fetus and low in IUGR fetus.

TABLE 1

Demographic information of the High-risk mothers

Maternal Age*	Number of Subjects	Race	Number of subjects
18-20	11	White	37
21-35	44	Black	31
36-48	9	Hispanic (Others)	3

* For the remaining seven subjects we don't have this information

The standard deviation (SD(s)) of the Detrended Time Series at different window sizes *s* for the three groups of fetuses are compared for the three groups of fetuses. The raw *p*-values and also the *p*-values adjusted for multiple comparisons using step-down permutations are given. A $p < 0.05$ is considered to be statistically significant.

Table 2

Variable	p-value					
	Low-risk vs High Risk		Low-risk vs IUGR		High-risk vs IUGR	
	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted
SD(2)	0.429	0.532	0.005	0.016	0.081	0.628
SD(4)	0.959	0.959	0.001*	0.003*	0.001*	0.006*
SD(8)	0.822	0.8936	0.010	0.026	0.012	0.035
SD(16)	0.373	0.526	0.121	0.211	0.058	0.098
SD(32)	0.164	0.283	0.491	0.621	0.165	0.199
SD(64)	0.066	0.140	0.704	0.704	0.184	0.199
SD(128)	0.030*	0.082*	0.512	0.621	0.079	0.114
SD(256)	0.0381	0.097	0.275	0.395	0.038	0.081

* statistically significant cases.

Table 3

Comparison of the spectral power of low-risk (LR), high-risk (HR), smokers (SM) and poor outcome (PO) and IUGR fetuses in four different spectral band δ (0.5-4 Hz), θ (4-8 Hz), α (8-13 Hz) and β (13-25 Hz) using t-test. A $p < 0.1$ is considered to be statistically significant.

Group	p-values obtained by testing the null hypothesis that the two groups have the same mean value at the significance level of 0.1			
	δ	θ	α	β
HR – PO	0.094*	0.690*	0.098*	0.082*
SM - PO	0.048*	0.513	0.061*	0.053*
HR - PO	0.536	0.653	0.766	0.536
LR – HR	0.001*	0.001*	0.001*	0.08*
LR-SM	0.001*	0.001*	0.001*	0.02*
LR-PO	0.19	0.001*	0.23	0.001*
IUGR-LR	0.108	0.298	0.122	0.076*
LUGR-HR	0.035*	0.001*	0.04*	0.63
IUGR-PO	0.97	0.003*	0.99	0.002*
IUGR-SM	0.013*	0.001*	0.02*	0.398

* Statistically significant difference.