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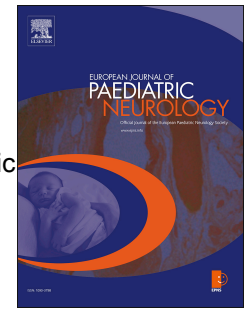

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Abnormal heart rate variability at school age in survivors of neonatal hypoxic-ischemic encephalopathy managed with therapeutic hypothermia

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Running head: Heart rate variability in HIE survivors

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ABSTRACT

Background and Objective. Major deficits in the autonomic nervous system function, detected by measuring heart rate variability (HRV), are reported in neonatal hypoxic-ischemic encephalopathy (HIE)). However, it is unknown if they will recover in the long-term. Because of the possible implications for the neurological outcome, this study aimed to evaluate the HRV at school age, in a cohort of children who survived HIE managed with therapeutic hypothermia.

Methods. A cross-sectional study of HRV in 40 children: 20 HIE survivors and 20 healthy peers. All underwent 5-minutes plethysmography using the PPG Stress Flow device (BioTekna Italy). Absolute and normalized HRV spectral power in the very low frequency (VLF), low frequency (LF), and high frequency (HF) bands and total power were compared between patients and healthy children. The outcome evaluation included neurological, cognitive (WISC-IV), and psychosocial (Parent Stress Index-Short Form-PSI-SF and psychosocial interview) measures.

Results. All mean HRV values were significantly higher in survivors of HIE, compared to healthy peers, with the larger effect size for the HF band (Total Power 8.57$\pm$0.59 vs 7.82$\pm$0.77 ms^2, p .003 ES 0.21; HF 7.82+0.77 vs 8.57+0.59 ms^2, p .001 EF 0.24). None of the children had major health, neurological and psychosocial (PSI-SF/interview) problems. The IQ (WISC-IV) was normal in 17/20 patients, borderline in 2, and <70 in 1.

Conclusions. HRV measures highlight autonomic dysfunction at school age in survivors of neonatal HIE, in the absence of major neurodevelopmental and psychosocial problems. The significance of this finding for children's future life needs further neuropsychiatric investigations and longer follow-up.

Keywords HRV, spectral power, parasympathetic, autonomic nervous system, long term outcome, plethysmography.

MAIN TEXT

1, Introduction

Heart rate variability (HRV) refers to changes over time in beat-to-beat intervals. It is regulated by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS), giving a good measure of its function.[1] ANS plays a major role in the regulation of the body functions such as heart rate, breathing, blood pressure, and temperature contributing to the constancy of internal environment and the adaptation to external perturbations, and it is crucial for homeostasis and body health.[2,3] The ANS regulatory action is controlled by a complex network (the so-called central autonomic network) involving the brainstem, forebrain, and prefrontal cortex.[4,5]

Hypoxic-ischemic encephalopathy (HIE) is a major cause of brain injury in the neonatal period, and brain regions crucial for the central autonomic control such as the prefrontal cortex, hippocampus, and brainstem[4,5] are affected in HIE.[6] ANS involvement, in hypoxic-ischemic brain injury, is further supported by observations during complicated labor[7] and by the occurrence of autonomic symptoms in moderate and severe HIE encephalopathy.[8]

An easy and non-invasive way to measure ANS activity is the analysis of heart rate variability.[1] Analogous to the EEG, power spectral analysis can be used to separate HRV into its component rhythms that operate within different frequency ranges supplying both frequency and amplitude information.[9] The total frequency power informs on the general ANS activation while the three frequency bands measurable in a short term recording (5 minutes), high-frequency (HF) low-frequency (LF), and very low frequency (VLF) represent the parasympathetic and sympathetic modulation of the heart rate.[1]

In health, sympathovagal balance reflects physical and psychological adaptability to a changing environment.[10] By contrast, ANS imbalance has been related, in the adult population, to health threats and disease.[11] In childhood, ANS dysfunction has been implicated in a variety of conditions, including gastrointestinal and connective tissue disorders, diabetes, chronic

fatigue syndrome, and neuropsychiatric disorders.[12,13] In neonatal HIE, abnormalities of HRV have been reported only in the perinatal period and were associated with EEG and MRI severity.[14-17] Moreover neonatal HRV measures were only correlated with the outcome at 2 y of age. [18] Goulding and colleagues showed that HRV tested at 48 hours of life had a positive predictive value of 100% for an abnormal outcome defined as death, cerebral palsy, or a Griffith's Quotient lower than 87.[18]

The ability of HRV to recover after neonatal imbalance and/or severe depression is still unknown. However, to study ANS developmental trajectory and outcome could be particularly important, in HIE survivors. In fact, therapeutic hypothermia increased survival, free of major neurological handicaps, but the risk of neurodevelopmental disorders with onset later in life remains high.[19] Azzopardi and colleagues found that,even in the cooled group, a significant proportion of children evaluated at six to seven years of age had intellectual disability, impairment in specific neuropsychological domains, motor impairment, attention deficit hyperactivity disorder and/or sensory deficits.[19]

We choose to focus on the school-age period since the beginning of the learning period, that corresponds to school entry, is an important developmental landmark. At this stage, a latent neurobiological vulnerability may allow neurodevelopmental disorders to emerge, even in the absence of severe brain damage. In fact, at school age, cerebral maturation makes a considerable growth spurt in its complexity, involving neurotransmitters systems[20] and higher-order neuropsychological functions.[21]

The aim of this study was, therefore, to measure the HRV at school age in a cohort of children surviving neonatal HIE managed with therapeutic hypothermia and to compare HRV data between HIE survivors and healthy age-matched children.

2. Materials and methods

2.1 Participants

All term and near-term neonates (≥35 gestational weeks) delivered at the Obstetric Clinic of an Italian University Hospital between January 2010 and December 2013, who underwent therapeutic hypothermia for HIE at the third level neonatal intensive care unit were eligible. In agreement with international guidelines and as previously reported,[22,23] entry criteria for hypothermia at our institution were: age ≥ 35 weeks; (ii) any of the following: arterial umbilical cord or first blood gas analysis (within one postnatal hour) with pH ≤ 7.0, and base excess < 12, or 10-min Apgar score < 5, or need for respiratory support at 10 min of life; (iii) moderate to severe encephalopathy within 6 h of birth scored according to Sarnat & Sarnat criteria.[8]

The inclusion criteria for this study were: age older than five years and reliable performance of the HRV test. The exclusion criteria were: motor phenomena that would have precluded reliable artefact free 5 minutes HRV recording; neurological disorders other than HIE, diagnosed during the follow-up; denied consensus to the study by the parents.

Seven out of 40 eligible patients died in the neonatal period. Of the remaining 33 patients fulfilling the inclusion criteria: 1 was lost to follow up, the parents of 3 refused to participate in the study, 3 were subsequently diagnosed with genetic syndromes, 1 was diagnosed for Paediatric Acute-Onset Neuropsychiatric Syndrome[30] just before the participation to this study and 5 were affected by cerebral palsy. The final patient's cohort consisted of 20 children that constituted the HIE survivors group; their perinatal data are summarised in Table 1.

Twenty healthy children were recruited as the control group and underwent the same protocol as the HIE survivors group.

2.2 Procedure and assessment

Examination of all the subjects was performed by a child neurologist (A.S.) and by a child psychologist (E.C.) and included HRV and neurodevelopmental assessments.

2.2.1 HRV assessment

Testing was performed during daylight hours in a quiet room with a temperature regulated between 20 and 25°C, at least two hours after a food assumption or physical activity.

Data were recorded from photo-plethysmography sensors using software of the PPG Stress Flow device developed by BioTekna (Venice, Italy). HRV parameters were recorded for 5-minutes when the child was sitting on a chair, at rest, not moving with feet flat on the floor. Only artefact-free segments were kept. The amplified signal was low-pass filtered (16 Hz) and digitized at 100 Hz. Collected data were exported for offline processing and analysis. Procedures were in line with the HRV analysis guidelines; measures were analyzed in the frequency domain due to better reliability on short recordings[9]. For each segment, each QRS complex was isolated. The so-called normal-to-normal (NN) intervals (all intervals between adjacent QRS complexes resulting from sinus node depolarization) and the instantaneous heart rate were determined. Segments were analyzed following international standards.[9,25] Total spectral power (Tot Pow: 0.0033–0.40 Hz), reflecting the global ANS activity, was recorded as well as the three main spectral components distinguished in the short-term (5-minutes). The high-frequency (HF) spectral power (0.15–0.40 Hz) representing the parasympathetic activity and corresponding to the heart rate variations related to the respiratory cycle[1,11] the low-frequency (LF) band (0.04–0.14 Hz) representing at rest the baroreflex activity vagally mediated with sympathetic modulation.[26,11] the very low frequency (VLF) component (0.000-0.03) for which experimental evidence suggests intrinsically generated heart activity representing parasympathetic outflow modulated by the efferent sympathetic system.[27,11]

Measurements of LF and HF power components were made in absolute values of power (milliseconds squared) and in normalized units, which represent the relative value of each power

component in proportion to the total power minus the VLF component. The normalization minimizes the effect of changes in total power on the values of LF and HF components.[9]

2.2.2 Neurodevelopmental assessment

The assessment comprised neurological, cognitive, and psychosocial evaluations.

The neurological evaluation included an anamnestic interview on major health problems allowing hospitalization and on psychomotor development, the occurrence of epilepsy, sensory and motor deficits, and a standard neurological examination.

The general cognitive performance was assessed using the Wechsler Intelligence Scale for Children IV (WISC-IV), standardized for the Italian population.[28]

We included information on psychosocial factors because they could have an independent effect on HRV.[11] They were evaluated by asking about the following events in an in-depth anamnestic interview conducted with the parents: very low socio-economic status (SES), low income, mild traumatic events (such as relocation, economic problems), severe events (parental death, neglect, invalidating pathologies or substance abuse), conflictual relationship between parents, a clinically relevant score to the Parent Stress Index-Short Form (PSI-SF).

PSI-SF is a standardized tool, which yields scores of parenting stress across four domains: parenting distress, difficult child, parent-child dysfunctional interaction, and total stress.[29] The total stress scores, obtained by the sum of the scores of the three subscales, can be interpreted as a stress index related to the parenting role. Responses higher than the 85th percentile (1 standard deviation above the average) are interpreted as “clinically significant” for high levels of family stress.

2.3 Statistical analysis

Results of the WISC-IV were converted to quotients having mean 100 and standard deviation 15. Impairment was defined as a quotient lying two standard deviations below the mean (< 70), the borderline between 70 and 85 quotients.

Normal distribution was assessed by Shapiro-Wilk and Kolmogorov-Smirnov tests, P-P plot, and z-scores distribution. ANS parameters (VLF, LF, HF, and total power-normalized HF, HF%) were compared using the non-parametric Mann-Whitney test. Effect sizes (ES) were expressed as eta-squared.

Statistical significance was set at $p < 0.05$. SPSS Statistics 25.0 (IBM Corp, Armonk, NY) was used for the analysis.

2.4 Ethics Statement

The ethics committee of the University Hospital approved the study (Number 67575 RF-2009-1511075). Parents gave informed consent.

3. Results

The study cohort consisted of 40 children, 20 HIE survivors patients (mean age 6.3 years, range 5.9 - 7.3, 12 males), and 20 healthy children (mean age 6.5 years, range 5.8 – 7, 11 males).

3.1 HRV assessment

As reported in Table 2, mean values in total power, VLF, LF, and HF bands were significantly higher in the HIE survivors, compared to healthy peers, with the larger effect size for the HF band: Total Power 8.57 ± 0.59 vs 7.82 ± 0.77 ms^2, p .003 ES 0.21; VLF power 6.96 ± 0.54 vs 6.44 ± 0.62 ms^2, p.012 ES 0.15; LF power 7.38 ± 0.63 vs 6.77 ± 0.82 ms^2, p.017 ES 0.14; HF 7.82+0.77 vs. 8.57+0.59 ms^2, p .001 EF 0.24; HF% 59.5 ± 11.3 vs 48.4 ± 11.4, p.001 ES 0.24).

3.2 Neurodevelopmental assessment

Neurological examination and IQ were normal in all children in the control group; no psychosocial risk factors were detected by Parent Stress Index-Short Form testing and by the ad hoc anamnestic interview on psychosocial adversities; no major previous health problems were detected from the anamnestic interview.

None of the 20 children in the HIE survivors group had major general medical adversities, visual and auditory impairments or epilepsy. All patients had normal neurological examinations.

One of the 20 children in the HIE survivors group had cognitive impairment (IQ < 70). Another 2/20 children exhibited a borderline cognitive profile (70 > IQ < 85). The IQ of the remaining patients was in the range of normality (mean IQ 101 standard deviation 16).

No severe traumatic events (parental death, neglect, invalidating pathologies, or substance abuse) occurred in the lives of the patients; it was only in the case of one child that we found some mild psychosocial risk factors.

4. Discussion

This study found significant differences in HRV measures between school-aged children with a neurodevelopmental risk because of neonatal HIE and age-matched healthy peers. The total power and the power of all HRV frequency bands (VLF, LV and HF), was significantly higher in the HIE survivors compared to healthy children, with the largest effect size for the HF band.

This is the first study that examined HRV a long time after the acute phase of neonatal HIE. Because of the emerging understanding of the association between HRV abnormalities and neuropsychiatric disorders occurring later in childhood or even in adult life,[30-33] our results may have clinical significance since HRV could be a marker of neurobiological vulnerability.

Neonatal research demonstrated changes in the spectral HRV values during the acute neonatal phase of HIE. In most of the studies, changes consisted of depressed HRV power dependent on the severity of brain damage and the neurological short term outcome.[14-18,34] ANS hyperactivity with an HRV profile suggesting stress-related involvement of the ANS sympathetic branch was reported in only one neonatal study. [35] Barbeau and colleagues studied a cohort of neonates affected by mild HIE and mild brain injury on MRI. [35]

In our study, conducted in children at school age, the HRV total power and the power of all frequency bands were increased, consistent with ANS hyperactivation. We did not record data on

the ANS function of our patients during the neonatal period; however, all but one patient suffered from moderate neonatal HIE. Moreover, at school-age, they were substantially free of major neurodevelopmental problems. Possible causes of abnormal HRV such as major health problems and psychosocial risk factors in the post-neonatal life were not observed in our cohort.

Thus, we can presume that the central autonomic nervous system of our patients could have been vulnerable to HIE, stress, and pain occurring during intensive care or exposure to low temperature for hypothermic treatment, in the acute neonatal phase. Subsequently, in the course of post-neonatal ANS development, HRV could have undergone a dysregulated rebound allowing to the finding, at the time of school age, of increased total and VLF, LF and HF bands power.

These results are in line with studies reporting ANS over-activation in the long-term follow-up of children with previous history of preterm birth; the authors interpreted their finding as a compensatory mechanism of the early life excessive stress system activation, even in otherwise healthy subjects.[36-38]

A further important result of our study is the finding of larger effect size for the HF band. This component is thought to reflect parasympathetic activity, and it is called the “respiratory band” because it corresponds to the heart rate variations related to the respiratory cycle.[1,11] Interestingly, a sympathovagal imbalance consisting of a relative increase in parasympathetic compared to sympathetic function was reported also following preterm birth.[39,40]

Our data on HRV profile at school age in HIE survivors confirm, in another type of perinatal adversity, the hypothesis of parasympathetic rebound as the long-term outcome of perinatal sympathetic stress system activation.

Autonomic modulation is programmed during the intrauterine and early postnatal period.[41,42] Evidence from other fields of neonatal medicine suggests a relation between biological early life adversities such as length of gestation or birth weight and programming of the reactivity to stress.[43] Thus, ANS and the other human stress system - the HPA axis – seem to provide the mechanisms linking biological early life adversities with post-neonatal ANS hyperactivity and stress-related

diseases later in life.[43] It could have been interesting to study comprehensively the stress system in our cohort, but unfortunately our study protocol did not include the evaluation of salivary cortisol levels, precluding information on the HPA axis.

The ability to adaptively respond to environmental stressors may be impaired in children without clinical evidence. It may manifest subsequently in later childhood or even in adulthood as difficult resilience to increasing stressors and life challenges.[11] Cognitive disorders also can emerge later in life when school or social demand becomes higher, even if early development is apparently normal. Our finding that, at school age, HIE survivors were free of major neurodevelopmental problems could, therefore, be explained by being too young to manifest an abnormal outcome. A further explanation could be that to highlight subtle psychological or higher cerebral function abnormalities would require tests on neuropsychological functions and the psychopathological profile more sophisticated than those reported in the present study.

Another limit of this study is that our results cannot be generalized to survivors of neonatal HIE with the more severe motor outcome since children with cerebral palsy could not be recruited for HRV testing due to our strict methodological constraints. Moreover, the outcome of this study focussed on neurodevelopmental sequelae and we did not evaluate other possible outcomes known to be related to ANS dysfunction in adults, such as increased BMI/obesity, hypertension and metabolic syndrome.[44,45]

5. Conclusions

In conclusion, this study adds new information on the long term outcome of ANS function (as measured by HRV) in a specific type of perinatal adversity that is neonatal HIE. In fact, we demonstrated, for the first time, significant differences in ANS function between school-aged patients with a history of neonatal HIE managed with hypothermia and age-matched healthy children. These consisted of significantly increased power of all HRV bands suggesting ANS over activation and parasympathetic predominance. These findings are particularly noteworthy since

ANS changes occurred in patients without major motor, sensory, and general cognitive abnormalities, having excluded those with severe motor impairments. The HRV hyperactivity might represent a persistent abnormality of ANS and other functionally linked brain systems. The significance of our finding for children's future life needs further neuropsychiatric investigations and longer follow-up.

Conflicts of Interest: Dario Boschiero is the scientific director of BIOTEKNA Biomedical Technologies. A.S., L.V., M.B., E.C. declare no conflict of interest.

Contributors' statement

Agnese Suppiej conceptualized and designed the study, coordinated and supervised data collection, carried out the neurological evaluations, wrote the final version of the manuscript, reviewed and revised the manuscript.
Luca Vedovelli participated in data collection, performed the statistical analysis of the data, conceptualized and drafted tables and figures and reviewed and revised the manuscript.
Dario Boschiero contributed to the original idea of the study and reviewed the manuscript.
Moreno Bolzon participated in the conceptualization of the study and the perinatal data collection and reviewed the final version of the manuscript.
Elisa Cainelli conceptualized and designed the study, performed HRV and psychological testing, collected and analyzed the data, wrote the first draft, reviewed and revised the manuscript.

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Tables

Table 1

Clinical characteristics of the HIE survivor's cohort.

	Neonates (n=20)
Male	11 (55%)
Birth weight, g	3687 ± 481
Gestational age, weeks	39.6 ± 1.1
pH < 7.0	13/20 (65%)
Base deficit >12	16/20 (80%)
Severe encephalopathy (Sarnat 3)	1/20 (5%)
10 min. Apgar score < 5	5/20 (25%)
Abnormal brain MRI	6/20 (30%)

Table 2

ANS parameters in controls and patients. Results are reported as mean, standard deviation and P-values of Mann-Whitney test. Effect size is calculated as eta-squared.

ANS parameters	**Controls (n=20)**	**Patients (n=20)**	**p-values**	**Effect Size**
VLF power ms^2	6.44±0.62	6.96±0.54	**.012**	**Large,** 0.15
LF power ms^2	6.77±0.82	7.38±0.63	**.017**	**Large,** 0.14
HF power ms^2	6.71±1.08	7.75±0.85	**.001**	**Very Large,** 0.25
HF%	48.4±11.4	59.5±11.3	**.001**	**Very Large,** 0.24
Total Power ms^2	7.82±0.77	8.57±0.59	**.003**	**Large,** 0.21

Highlights:

- Heart rate variability is a reliable measure of autonomic function in children.
- Plethysmography recording of heart rate variability in school-age hypoxic-ischemic encephalopathy survivors
- Frequency analysis of heart rate variability highlighted autonomic over activation in patients compared to healthy peers.
- In the absence of major neurological disability, autonomic involvement may represent a marker of neurobiological vulnerability.

SEZIONE DI PEDIATRIA

Direttore Prof Giuseppe Maggiore

Ferrara, 28 March 2020

To the Editor-in-Chief of Journal of Paediatric Neurology Professor Sameer Zuberi

With regard to the original article “Abnormal heart rate variability at school age in survivors of neonatal hypoxic-ischemic encephalopathy managed with therapeutic hypotermia”, authored by Agnese Suppiej, Luca Vedovelli, Dario Boschiero, Moreno Bolzon, Elisa Cainelli submitted to the Journal of Paediatric Neurology, we state that no honorarium, grant, or other form of payment was given to anyone to produce the manuscript.

All authors report no conflicts of interest.

Kind regards

Agnese Suppiej on behalf of all co-authors

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