Correlation between Magnetic Resonance Spectroscopy and Auditory Brain- stem Response Audiometry in Acute Bilirubin Encephalopathy in Full Term Neonates

Abstract

Background: This research aimed to correlate between MRS and ABR to see whether MRS offers new perspectives on the pathophysiology of hyperbilirubinemia and the early diagnosis of acute bilirubin encephalopathy (ABE) in full-term neonates.

Methods: This observational cohort study was done at Tanta University Hospitals between March 2019 and March 2021. It enrolled 26 newborns with ABE who were diagnosed with BIND-M score (Modified Bilirubin Induced Neurological Dysfunction Score), In addition, 20 healthy, age-matched neonates were enrolled as control subjects. Brain MRS and ABR were done to all enrolled neonates. Ethical committee approval and parent consent were taken prior to the start of the research.

Results: Regarding MRS, a significant negative correlation was detected between NAA/Cr, and NAA/Cho ratio and total plasma bilirubin levels, while only NAA/Cr had a significant positive correlation with the age on admission. On the other hand, Lac/Cr ratio had a significant positive correlation with total plasma bilirubin levels. As regards ABR, there was a significant positive correlation between wave V peak latency, I-III and I-V interpeak intervals, and bilirubin level. There were significant negative correlations between NAA/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals. Significant negative correlations were determined between NAA/Cho and wave V peak latency, I-III, and I-V

interpeak intervals. Finally, correlation between MRS and ABR revealed a significant positive correlation between Glx/Cr and I-V interpeak interval. Also, there were significant positive correlations between Lac/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals.

Conclusion: MRS data especially NAA/Cr and Lac/Cr correlated with wave V and I-V interpeak of ABR.

Keywords: Correlation, Magnetic Resonance Spectroscopy, Auditory Brain- stem Response Audiometry, Acute Bilirubin Encephalopathy, Neonates

Introduction:

Acute bilirubin encephalopathy (ABE) is still an essential etiology of morbidity as well as mortality globally, in particular in low-middle-income nations as it's responsible for about fifteen percent of neonatal mortality. The pathophysiology of such acute life-threatening problem during infancy and its predicted evolution to kernicterus are still incompletely understood [1].

Symptoms of ABE might either ulterior or absent and cases suffering the disease might not show distinct neurological manifestation during the neonatal duration, thus the clinical diagnosis of neonatal bilirubin encephalopathy is totally strenuous [2].

ABR might be an effective method to monitor the auditory brainstem pathway in neonates who are at high risk of neurotoxicity. Diagnosis of the early stages of auditory destruction results from elevated level of bilirubin is crucial at a stage where lasting central impacts might be preventable [3].

Magnetic Resonance Spectroscopy (MRS) is widely applied in clinical research and is determined as an efficient procedure for non-invasive monitoring of brain biochemistry in vivo for animals as well as human. The metabolites of the CNS most adaptable for studying with H^1-MRS are NAA (N Acetyl Aspartate), Cr (total Creatine), and Cho (Choline). furthermore, the ratio of NAA/Cr is considered one of the metabolic markers of the functional condition of neurons and axons in the brain, with a reduced ratio denoting neuronal or axonal loss or dysfunction [4].

1. Patients and Methods:

Study Population and Setting: This prospective cohort study was done at Tanta University Hospitals between Maech 2019 and March 2021. It enrolled 26 newborns with ABE who were diagnosed with BIND-M score (Modified Bilirubin Induced Neurological Dysfunction Score)with the following inclusion criteria; Full term newborns (>37 weeks or more) with total serum bilirubin ≥20 mg/dl. Exclusion criteria included: Newborns with neurological problems influencing BIND-M score (birth asphyxia, neonatal sepsis, metabolic errors), Preterm neonates < 37 Weeks, direct hyperbilirubinemia > 20% of total bilirubin, neonates with total bilirubin less than 20 mg/dl and newborns with problems influencing ABR (family history of childhood hearing loss, congenital infection, chromosomal abnormalities. congenital ear anomalies), In addition, 20 healthy, age-matched neonates were enrolled as a control group.

Neonatal evaluation: All neonates involved in the study underwent full history commiserating antenatal, perinatal, and postnatal periods. In addition to thorough clinical examination that included BIND-M Score. BIND-M score is used to detect grade of ABE by evaluating mental status, muscle tone, cry pattern and oculomotor or eye movements. Scores of (1–4), (5–6), (7–9) represent mild, moderate and severe ABE respectively [5].

Laboratory investigations: Complete Blood Picture, total serum bilirubin (TSB), direct serum bilirubin, reticulocytic count, Coomb's test, blood groups of the baby and mother. Also, serum albumin and B/A ratio was done on admission.

Finally, ABR and brain MRS were done to all cases on admission.

ABR Protocol: Each neonate was subjected to full otorhinolaryngological examination before undergoing ABR test to exclude any middle ear problems. The test was done in a calm, dark, and electrically shielded room at the Audiology unit of Tanta University Hospital's. The baby should be sleeping usually after feeding. The test was done using SmartEPs (TM, Intelligent Hearing system, Miami, USA). The intensity of the stimulus: initiated at 90 dBnHL decreasing

to 30 dBnHL or to the threshold. Absolute (I, III, and V) Wave Latencies and Interpeak Latencies (I-III, I-V, III-V) were documented and compared between the case group relative to the control group.

MRS Protocol: utilizing a whole-body MR imager and a 1.5-tesla General Electric system (SIG- NATM Explorer, GE Healthcare Systems, Chicago, USA). MRS, Axial FLAIR imaging was utilized for voxels localization. The basal ganglia, which were carefully examined to prevent interference from the skull bone and CSF fluid, were the focus of interest in all infants. The voxel size ranged from 1.5 × 1.5 × 1.5 cm3 to 2 × 2 × 2 cm3. The data were then processed on a GE Advantage workstation, where the magnitude spectra were automatically corrected for baseline and fitted with curves in order to establish the resonance regions of individual metabolites. Long TE was utilized to definitely show the intensity peak of Cho, NAA, and Cr, as well as to determine the ratio of NAA to Cr, NAA to Cho, and Cho to Cr. The primary purpose of short TE was to demonstrate the Lac and Glx peaks.

Statistical data: Data was fed to the computer to be analyzed by IBM SPSS software package version 20.0. *(Armonk, NY: IBM Corp)*. Qualitative data were described by the use of number and percentage. The Shapiro-Wilk test was utilized to verify the normality of distribution. Quantitative data were described via the use of range (minimum & maximum), mean, and SD. The significance of the obtained results was judged at five percent level. Linear Correlation coefficient was used to detect correlation between 2 quantitative variables in the same group.

Results:

Patient characteristics are summarized in Table 1. Gender among the studied group showed male predominance (61.5 %). The median age at admission to the NICU was 52.57 ± 25.52 hours. The median duration of hospitalizationwas 10.69 ± 2.90 days.The medianAdmission TSB was 24.78±1.95 mg/dL. The median B/A ratio on admission was 8.6 ± 0.82 mg/g.

The study exhibited a significant negative correlation between NAA/Cr, and NAA/Cho ratios

and clinical and laboratory data including total serum maximum bilirubin level, B/A ratio, and BIND-M score on admission, while only NAA/Cr had a significant positive correlation with the age on admission. On the other hand, Lac/Cr ratio had a significant positive correlation with maximum total plasma bilirubin level, B/A ratio, and BIND-M score on admission (Table 2)

A significant positive correlation was determined between wave V peak latency, I-III and I-V interpeak intervals, and clinical and laboratory data including maximum bilirubin level, B/A ratio, and BIND-M score on admission. Also, a significant positive correlation was found between wave III and BIND-M score on admission. on the other hand, a significant negative correlationwas revealed between I-V interpeak interval and the age on admission. (Table 3)

A significant negative correlation was found between NAA/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals. There were significant negative correlations between NAA/Cho and wave V peak latency, I-III, and I-V interpeak intervals. Also, a significant positive correlation was detected between Glx/Cr and I-V interpeak interval. There were significant positive correlations between Lac/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals. (Table 4, Figures 2&3)

2. **Discussion:**

Bilirubin encephalopathy, one of the preventable causes of handicapping, remains a problem worldwide. The crash cart method to neonates with marked hyperbilirubinemia along with the fast intervention using intensive phototherapy and exchanging blood are the only detected method to avoid the occurrence of bilirubin-induced brain damage[6]. Serum bilirubin level separately is a poor predictor of bilirubin encephalopathy in particular in sick preterm babies. A fast non-invasive indicator of neurological toxicity and imminent neuron injury is needed [7]. This research aimed to correlate between MRS and ABR to see whether MRS offers new perspectives on the pathophysiology of hyperbilirubinemia and the early diagnosis of ABE in

full-term neonates.

Regarding NAA/Cr ratio, we concluded a significant negative correlation between this ratio and maximum total bilirubin level, B/A ratio, and BIND-M score on admission. Conversely, it had a significant positive correlation with the age on admission. (Table 2)

This comes in agreement with Aly and Montaser [8] who demonstrated a significant negative correlation of NAA/Cr ratio with maximum bilirubin level and BIND score. Also, our results come in accordance with Wu et al. [9] who also found a significant negative correlation between NAA/Cr ratios in the basal ganglia and the total plasma bilirubin peak levels.

Similarly, the current study found a significant negative correlation between NAA/Cho ratio, maximum total bilirubin level, B/A ratio, and BIND-M score on admission was found. (Table 2)

On the contrary, we found that Lac/Cr ratio had a significant positive correlation with maximum total blood bilirubin levels, B/A ratio, and BIND-M score on admission. (Table 2)

Regarding correlation of NAA/Cho and Lac/Cr ratios with TSB, B/A ratio, or BIND-M score, we did not find any mention of these notes in variable literatures.

Our results demonstrated a significant positive correlation of wave V peak latency, I-III and I-V interpeak intervals, with maximum bilirubin level, B/A ratio, and BIND-M score on admission. Additionally, a significant positive correlation was found between wave III and BIND-M score on admission. In contrast, there was a significant negative correlation between I-V interpeak interval and the age on admission. (Table 3)

Our data comes in accordance with Jiang et al.[10] who demonstrated that all latencies of waves III and V had significant correlation with the levels of TSB ($r = 0.26\text{-}0.28$, all $P < 0.05$). Moreover, The I-V interval showed correlation with the levels of TSB ($r = 0.24$, $P < 0.05$). Furthermore, other studies concluded a positive correlation between bilirubin level and ABR waves and/or interpeak intervals [11].

Regarding correlation between ABR parameters and MRS metabolic ratios, we found significant negative correlations between NAA/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals. (Table 4, Figures 2&3)

Also, our study found significant negative correlations between NAA/Cho and wave V peak latency, I-III, and I-V interpeak intervals. (Table 4)

Additionally, our results revealed a significant positive correlation between Glx/Cr and I-V interpeak interval. Furthermore, there were significant positive correlations between Lac/Cr and wave III peak latency, wave V peak latency, I-III, and I-V interpeak intervals. (Table 4)

Lower NAA/Cr and NAA/Cho ratio together with elevated Lac/Cr ratio indicated neuronal or axonal loss and dysfunction that coincide with prolonged ABR wave latencies and interpeak intervals which represented damages to various auditory pathway neurons and centers.

It is known that increase in I-III and I-V interpeak latencies represented severely disturbed synapse across the auditory brainstem pathways. Also, disruption of glutamate uptake and the glutamate–glycine cycle results in an increased Glu/Cr ratio in the synaptic gaps which can cause excitotoxic neuronal deaths [12]. This information can explain the correlation between Glx/Cr ratio and I-V interpeak interval.

3. Conclusion:

MRS data, especially NAA/Cr and Lac/Cr correlated with wave V and I-V interpeak of ABR, therefore MRS could be used in early diagnosis of ABE.

Ethics approval and consent : The current study was approved by the Pediatrics Ethics Committee at Tanta University. We obtained parents' consent before conducting the research. The study was performed based on the Declaration of Helsinki in addition to the principles of good clinical practice.

Approval number (32886/01/19). All parents of the enrolled neonates in the research gave their full and informed permission.

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Table 1: Demographic and clinical data of the studied groups.

	ABE group (n = 26)		Control group (n= 20)		Test of Sig.	p
	No.	**%**	**No.**	**%**		
Gender						
Male	16	61.5	12	60.0	t = 2.065	0.356
Female	10	38.5	8	40.0		
Gestational age (weeks)						
Min.–Max.	37.0 – 40.0		37.0 – 39.0		t=0.397	0.673
Mean ±SD.	37.96 ± 0.66		38.10 ± 1.12			
Weight (kg)						
Min.–Max.	2.80 – 3.60		3.0 – 3.70		t=0.837	0.437
Mean ±SD.	3.07 ± 0.27		3.21 ± 0.19			
Age on admission (hours)						
Min.–Max.	14.0 – 110.0					
Mean ±SD	52.57 ± 25.52					
Duration of hospitalization (days)						
Min.–Max.	5.0 – 14.0					
Mean ±SD	10.69 ± 2.90					
BIND-M score on admission						
Median (IQR)	5 (2– 7)					
Min. – Max.	2.0 – 7.0					
Maximum TB on admission (mg/dl)						
Min. – Max	20.0 – 28.0					
Mean ± SD.	24.78±1.95					
B/A ratio on admission						
Min. – Max.	7.2–10.4					
Mean ± SD.	8.6 ± 0.82					

SD: Standard deviation, p: p value for comparing between the studied groups, *****: Statistically significant at $p \leq 0.05$,
***t**: Student t-test*
B/A ratio: Bilirubin /Albumin ratio

Table 2: Correlation of MRS metabolic ratios with clinical and laboratory data.

	Maximum TB		Age on admission		B/A ratio on admission		BIND-M score on admission	
	R	**P**	**r**	**p**	**r**	**p**	**r_s**	**P**
NAA/Cr	-0.477*	<0.001*	0.381*	0.004*	-0.560*	<0.001*	-0.611*	<0.001*
NAA/Cho	-0.414*	0.002*	0.247	0.066	-0.545*	<0.001*	-0.477*	<0.001*
Cho/Cr	0.016	0.904	-0.044	0.750	0.125	0.360	-0.116	0.394
GLx/Cr	0.111	0.414	-0.106	0.435	0.088	0.518	0.074	0.589
Lac/Cr	0.363*	<0.006*	-0.244	0.070	0.4.4*	<0.001*	0.481*	<0.001*

r: Pearson coefficient, **r_s:** Spearman coefficient
*****: Statistically significant at $p \leq 0.05$

Table 3: Correlation of ABR parameters (wave latencies (ms) and interpeak intervals (ms)) with clinical and laboratory data.

	Maximum TB		Age on admission		B/A ratio on admission		BIND-M score on admission	
	R	**P**	**r**	**p**	**r**	**p**	**r_s**	**p**
Wave I (ms)	-0.153	0.284	0.202	0.154	-0.138	0.335	-0.118	0.409
Wave III (ms)	0.014	0.921	-0.202	0.156	0.054	0.707	0.341*	0.014*
Wave V (ms)	0.391*	0.005*	-0.208	0.144	0.472*	<0.001*	0.447*	0.001*
I-III interpeak (ms)	0.578*	<0.001*	-0.220	0.121	0.472*	<0.001*	0.405*	0.003*
I-V interpeak (ms)	0.390*	0.005*	-0.294*	0.036*	0.372*	0.007*	0.477*	<0.001*
III-V interpeak (ms)	0.136	0.340	-0.261	0.065	0.015	0.915	-0.052	0.717

r: *Pearson coefficient,* ***r_s:*** *Spearman coefficient*

*******: *Statistically significant at $p \leq 0.05$*

Table 4: Correlation between ABR parameters (wave latencies (ms) and interpeak intervals (ms)) and MRS metabolic ratios.

	NAA/Cr		NAA/Cho		Cho/Cr		Glx/Cr		Lac/Cr	
	r_s	P	R	p	r_s	P	r	p	r_s	p
Wave III (ms)	-0.622*	<0.001*	-0.164	0.249	0.114	0.424	0.190	0.181	0.539*	<0.001*
Wave V (ms)	-0.652*	<0.001*	-0.571*	<0.001*	0.061	0.672	0.038	0.792	0.732*	<0.001*
I-III interpeak(ms)	-0.489*	<0.001*	-0.425*	0.002*	0.225	0.113	0.086	0.548	0.460*	<0.001*
I-V interpeak(ms)	-0.665*	<0.001*	-0.427*	0.002*	0.091	0.525	0.318*	0.023*	0.652*	<0.001*
III-V interpeak(ms)	-0.154	0.282	-0.180	0.247	-0.047	0.743	-0.023	0.874	0.268	0.057

***r:** Pearson coefficient**, r_s:** Spearman coefficient*

****:** Statistically significant at $p \leq 0.05$*

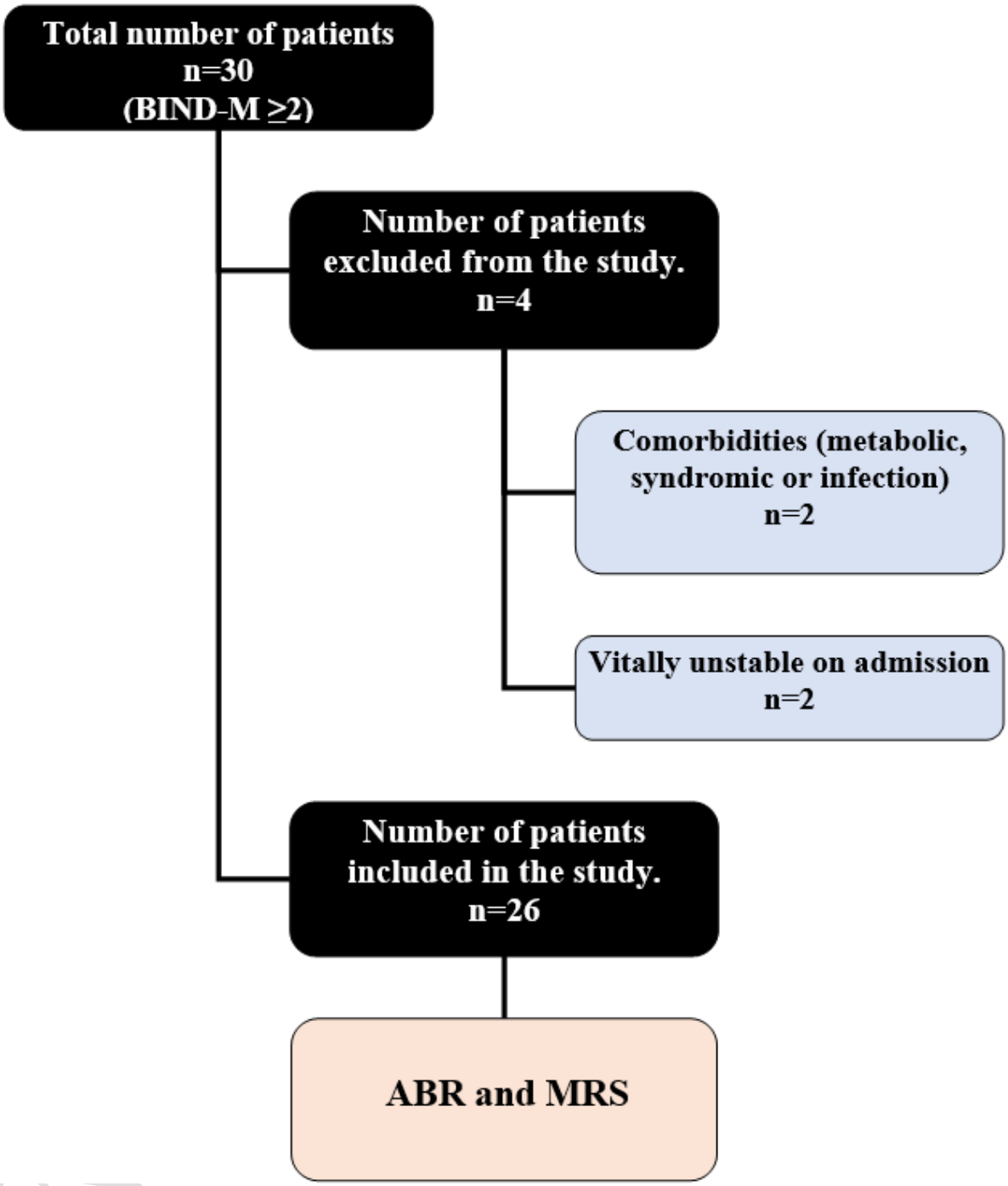

Figure 1: Study population

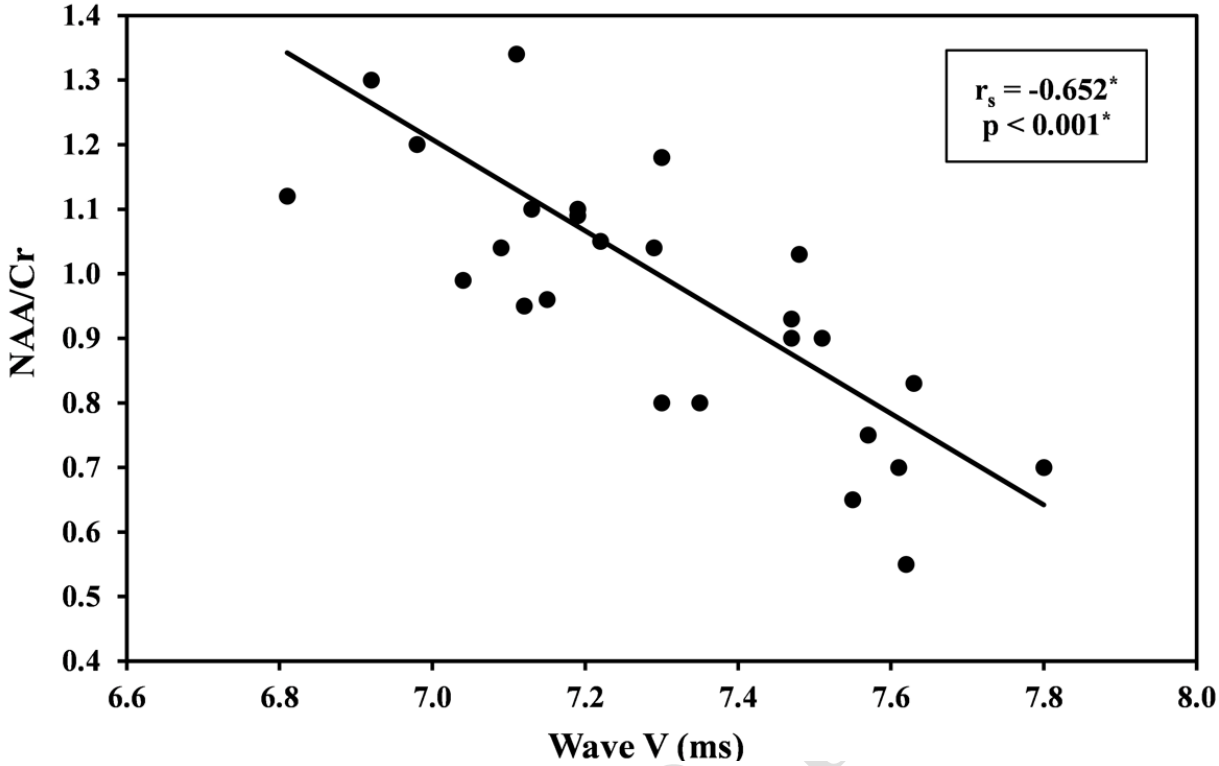

Figure 2: Significant negative correlations between NAA/Cr and wave V peak latency

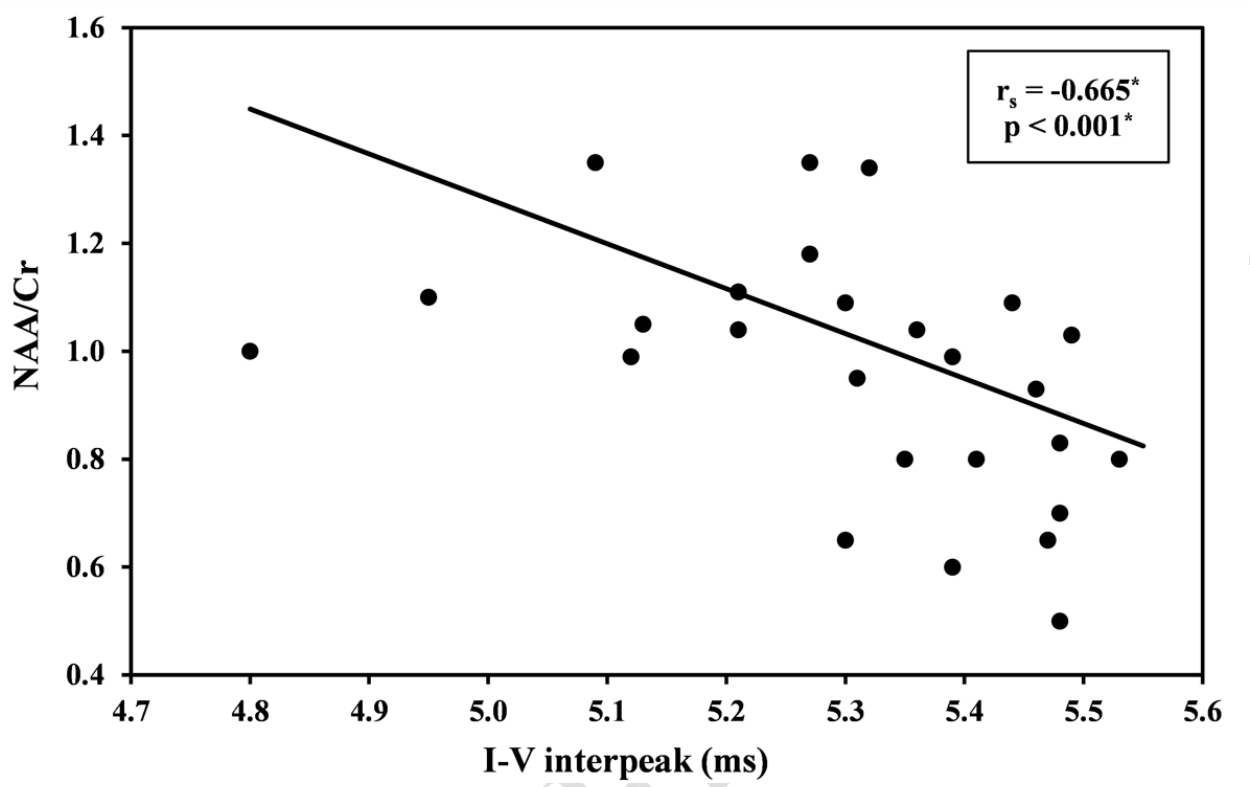

Figure 3: Significant negative correlations between NAA/Cr and I-V interpeak interval.