Crosstalk between endoplasmic reticulum stress, cytokines and neuroinflammatory biomarkers as a putative panel of biomarkers for neurological outcome prognostication after cardiac arrest and resuscitation

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Post cardiac arrest syndrome (PCAS) yet contributes to unfavorable neurological and cardiac outcome even with successful resuscitation. However, the exact mechanism is yet to be elucidated. **Purpose of the study;** This study investigated the level of expression endoplasmic reticulum (ER) stress downstream biomarkers; Inositol Requiring 1 (IRE1), PKR-like ER kinase (PERK), Activating Transcription Factor 4 and 6 (ATF4 and ATF6), glucose-regulated protein78 (GRP78) and whether **ER stress**-mediated apoptotic caspase-3 is induced in injured brain after resuscitation and their correlation with Inflammatory biomarkers; interleukin (IL)-6, IL-10 and Tumor Necrosis Factor- α (TNF- α) and neuroinflammatory biomarkers; neuron specific enolase (NSE), and S100B as a prognostic value of neurological outcome. **Methods:** Sprague Dawley rats were subjected to 5 min electrically induced CA followed by standard resuscitation. Animals were decapitated at 6, 12, 24h and 48h (n=6 per group) after return of spontaneous circulation (ROSC). Frontal motor and cerebellar cortex were biochemically assessed for inflammatory cytokines and neuroinflammatory biomarkers. Brain tissues were analyzed by real-time RT-PCR and immunoblotting for transcriptional profile and evaluated for histological, ultrastructural and immunohistochemical changes. **Results:** The mRNA levels of PERK, ATF4 and GRP78 were significantly elevated in 6-48 hr after ROSC as well as the associated caspase-3 apoptotic transcription. Quantitative real-time RT-PCR was used to determine the abundance of six representative transcripts in which they were significantly elevated after resuscitation in post-ROSC groups at 6-48 hrs. compared to the control group (*P*-value < 0.05). The markers of ER stress were examined using immunoblotting as well (Figure 1).

Serial IL-6 levels correlated consistently with expression levels of PERK at 12h:($r=0.19$, $p=0.02$) 24h: ($r=0.31$, $p=0.0001$) and 48h: ($r=0.39$, $p<0.0001$). All inflammatory and neuroinflammatory markers except IL-10 correlated with PERK expression level at 48h. Multivariable regression models adjusting for potential confounders confirmed that IL-6 ($\beta=0.3$ (95% CI: 0.03-0.6), $p<0.0001$), TNF- α ($\beta=-0.4$ (95% CI:- 0.5 to 0.2), $p<0.0001$) and NSE ($\beta=-0.4$ (95% CI: - 0.8 to 0.1), $p=0.008$) were associated with PERK expression levels at 48 hrs. post-ROSC. All inflammatory biomarkers were correlated positively with the upregulated levels of ER stress biomarkers.

Table 1: Integrated density (ID) of PERK immunostaining among the studied groups

		Control	12-hrs Post-ROSC	24-hrs Post-ROSC	48-hrs Post-ROSC	ANOVA P value
ID*10 ⁴						
Cerebral Cortex	Mean	64.68	74.61	566.3 ^{ab}	1708.6 ^{abc}	< 0.001*
	±SD	3.23	3.73	28.31	85.4	
Cerebellum	Mean	31.2	28.72	652.2 ^{ab}	1410.4 ^{abc}	< 0.001*
	±SD	1.56	1.43	32.6	70.49	
SD: standard deviation P: Probability *: significance < 0.05						
Test used: One way ANOVA followed by post-hoc tukey						
a: significance relative to Control Group						
b: significance relative to 12-post ROSC Group						
c: significance relative to 24-post ROSC Group						

Table 2: Number of Caspase-3 positive cells / HPF among the studied groups

		Control	12-hrs Post-ROSC	24-hrs Post-ROSC	48-hrs Post-ROSC	Kruskal-Wallis P value
Cerebral Cortex	Median	0.00	0.00	4.00 ^{ab}	10.00 ^{abc}	< 0.001
	IQR	0.00-0.00	0.00-.00	4.00-4.00	7.00-12.00	
Cerebellum	Median	0.00	0.00	4.00 ^{ab}	12.00 ^{abc}	< 0.001
	IQR	0.00-0.00	0.00-1.00	3.00-5.00	10.00-15.00	
IQR: interquartile range P:Probability *: significance < 0.05						
Test used: Kruskal- wallis followed by post-hoc Dunn's test						
a: significance relative to Control Group						
b: significance relative to 12-post ROSC Group						
c: significance relative to 24-post ROSC Group

Figure 1: Brain tissue homogenates were collected, and the expression of markers for ER stress and apoptotic caspase-3 were examined with immunoblotting.

Typical apoptotic nuclei were obviously displayed in frontal cortex and cerebellum 24h after resuscitation and significantly displayed at 48 hrs.post-ROSC. Further, a significant increase in integrated density for PERK immunostaining and number of apoptosis caspase-3 positive cells/HPF were detected (Table1,2). **Conclusions:** ER stress-mediated apoptosis, cytokines and neuroinflammatory biomarkers could serve as a crucial prognostication panel for outcome after cardiac arrest and resuscitation.

Figure 2: PERK expression in the frontal motor cortex of the studied groups; (A) 12-Post ROSC, (B) 24-Post ROSC, (C) 48-Post ROSC. Caspase-3 immunostaining in the frontal motor cortex of the studied groups; (D) 12-Post ROSC, (E) 24-Post ROSC, (F) 48-Post ROSC.