

Genetic Expression of High Mobility Group Box 1 in Neonatal Sepsis

Omima M. Abdel Haie^a, Mona A. Elbeshery^a, Shuzan A. Mohammed^b, Salwa S. Husein^c, Neveen T. Abed^a

^a Department of pediatrics, Benha faculty of medicine, Benha University, Egypt.

^b Department of Medical Biochemistry and Molecular Biology, Benha faculty of medicine, Benha University, Egypt.

^c Department of Pediatrics, Shoubra General Hospital, Egypt.

Correspondence to: Salwa S. Husein, Department of pediatrics, Benha faculty of medicine, Benha University, Egypt.

Email:

salwadr77@gmail.com

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Abstract

Background: High-mobility group box 1 (HMGB1) has important role in a variety of diseases, including neonatal sepsis (NS). The aim of this study is to evaluate the clinical importance of HMGB1 genetic expression in NS demonstrating its diagnostic value and detecting the effect of its genetic expression on the disease outcome. **Patients and Method:** This study was carried out on 100 neonates; 28 neonates with clinical sepsis and had positive blood cultures (confirmed), 22 neonates with clinical sepsis but had negative blood cultures (suspected) and 50 healthy non-infected newborns age and sex matched as controls. **Results:** Expression of HMGB1 in newborns with confirmed NS and those with suspected NS were higher than healthy controls. Also, it was higher in newborns with confirmed NS than those with suspected NS. The sensitivity and specificity of the diagnostic value of HGMB1 were higher than that of C-reactive protein (CRP) and hematological score (H score). Assessment of the HMGB1 for prognosis of the disease was evaluated by univariate analysis ($p < 0.002$) and logistic multivariate regression analysis ($p = < 0.023$). The univariate analysis showed that CRP, H score and HMGB1 are significantly predicting the prognosis of NS, while the multivariate analysis showed that only HMGB1 significantly predict the prognosis of NS. There were significant positive correlations between HMGB1 gene expression and APGAR score at 5 minutes, hemoglobin level, H. score and CRP titer in confirmed N **Conclusion:** HMGB1 is a possible diagnostic and prognostic marker of neonatal sepsis (NS).

Keywords: neonatal sepsis, high mobility group box 1, HMGB1, real-time PCR, gene expression

Introduction:

Neonatal sepsis (NS) is a clinical syndrome of bacteremia in infants < 28 days of life. It manifests by systemic signs of infection, circulatory shock, and multi system organ dysfunction. Early diagnosis and immediate treatment are essential to improve survival⁽¹⁾. Sepsis is life-threatening factor in all ages, especially in the neonatal period. The incidence of sepsis among preterm neonates is 6 times greater than that of full-term infants (approximately 1:1500 in full-term and 1:250 in preterm infants)⁽²⁾. NS accounts for 1.6 million fatalities a year underdeveloped countries⁽³⁾.

The Human high mobility group (HMG) proteins embrace three super-families; HMGB, HMGN, and HMGA. High-mobility group box 1 (HMGB1), the most plentiful and widely-studied HMG protein, recognizes and coordinates the cellular stress response and has a crucial role not only intracellular as a DNA chaperone, chromosome guardian, autophagy sustainer, and protector from apoptotic cell death, but also extracellular as the prototypic damage associated molecular pattern molecule (DAMP). This DAMP, with other factors, has cytokine, chemokine, and growth factor function, regulating the inflammatory and

immune response. These features make HMGB1 a serious molecular target in various human illnesses as infectious diseases, ischemia, immune disorders, neurodegenerative diseases, metabolic disorders, and cancer⁽⁴⁾.

HMGB1 gene is located to chromosome 13⁽⁵⁾. HMGB1 protein is a non-histone DNA-binding protein that has a wide distribution in a series of cells (immune, endothelial or epithelial cells). HMGB1 is included in the regulation of gene replication and transcription. Under external stimuli (infection, trauma, burn, etc.), HMGB1 can be transported from the nucleus to the cytoplasm and then liberated into the blood stream. Through activation of macrophages, neutrophils, and releasing an enormous amount of inflammatory mediators, it plays an important pathological role in various illnesses, as infectious pneumonia, infectious diarrhea, and purulent meningitis⁽⁶⁾. Various biomarkers have been demonstrated for their capability for detection of NS, but none has yet proven adequate sensitivity and specificity⁽⁷⁾.

This study aimed to evaluate the HMGB1 gene expression levels in NS and correlate it with the clinical and laboratory findings in

NS. We aimed also to detect the effect of disease outcome on HMGB1 expression and whether it can be used for diagnosis and prognosis or not.

Patients and methods

Patients

This comparative case-control study included 28 neonates with confirmed (proven) sepsis and 22 with suspected sepsis and 50 apparently healthy, age and sex matched controls where confirmed sepsis is defined as clinical sepsis with positive blood cultures, and suspected sepsis is defined as clinical sepsis with negative blood cultures. Sepsis neonates were selected from those admitted in neonatal intensive care unit (NICU) of Benha University Hospital in the period from August 2020 to January 2021 while apparently healthy neonates were selected from those seeking medical advice at the outpatient clinic for simple medical conditions.

Ethical consideration

An informed consent was obtained from parents or health care providers of each neonate included in this study. The study was approved by the Ethical Committee of Benha Faculty of Medicine, Benha University (MS21.7.2019).

Inclusion criteria

Preterm and full-term neonates were included in the study if presented with signs and symptoms suggestive of sepsis based on *Töllner* sepsis scoring system ⁽⁸⁾.

Exclusion criteria

Neonates with congenital malformations, chromosomal abnormalities, birth injuries and acute kidney injury presented by oliguria and high creatinine level > 1.5 mg/dl or persistent increase > 0.3 mg/dl for at least 48 hours were excluded.

Methods:

Each neonate included in this study was subjected to full history taking, through clinical examination and laboratory investigations.

Laboratory investigations:

Sampling:

A venous blood sample (3 ml) was taken from each neonate. Blood was collected at the first time of suspicion of sepsis for complete blood count (CBC) and for calculation of H. score according to the study done in 1988 ⁽⁹⁾. The blood sample was divided into 2 parts. One part (2 ml) was put on EDTA for CBC (0.5 ml), culture (1ml) and HMGB1 mRNA quantitation (0.5

ml). The other part (1 ml) was left to clot for half an hour then centrifuged at 3000 rpm to separate serum for C- reactive protein (CRP) titer and serum creatinine measurement. Sera for HMGB1 mRNA quantitation were kept at -80°C till the time of extraction. HMGB1 mRNA quantitation was performed by quantitative real-time PCR (qRT-PCR) in the Molecular Biology and Biotechnology Unit, Faculty of Medicine, Benha University.

Relative quantitation of HMGB1 mRNA by qRT-PCR:

Sampling:

A venous blood sample (about 1 ml) was obtained from each neonate into sterile EDTA-containing vacutainer tube, mixed well and was kept at -80°C till mRNA extraction was performed.

Steps:

1. Total mRNA Extraction:

Total mRNA was extracted via miRNeasy Mini Kit (Qiagen) according to the manufacturer instructions followed by Ultraviolet Spectrophotometric Quantification of RNA by Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). RNA preparations with an optical density (OD)

ratio at 260/280 nm of 1.9-2.3 were accepted (10).

2. Reverse transcription of mRNA into cDNA:

Reverse transcription (RT) was performed in a Veriti™ Thermal Cycler (*Applied Biosystems*), using SenScript™ RH (-) cDNA Synthesis Kit (*Intron, Biotechnology*). The reaction mix included 10 µl of 2X RT reaction mix solution, 1 µl of the enzyme mix solution and 2.5 µl of the template RNA being completed up to 20 µl by DNase/RNase Free Water. This step was performed at 45 °C for 1 hour followed by RTase inactivation at 85 °C for 10 minutes.

3. Relative quantitation of HMGB1 mRNA:

This step was carried out in a *Stepone* real time PCR system (*Applied Biosystem, Singapore*). Singleplex reactions were done using *Hera Sybr Green qPCR kit (Willowfort, UK)*. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the endogenous housekeeping gene. Melting curve analysis was done in each run to ensure specificity. The primers (*Willowfort, UK*) for HMGB1 were FP: 5'-TAACTGAATAGGGGCGTGGTCT-3', RP: 5'-GAAAATGTGCTGGCTGTAGTGG

-3' and the GAPDH primers were FP: 5'-GCACCGTCAAGGCTGAGAAC-3' and RP: 5'- TGGTGAAGACGCCAGTGGA -3' (11). The reaction mix contained 10 µl master mix (2X), 1 µl of each primer, 2 µl cDNA and 6 µl Nuclease free water. The reactions were cycled for 45 cycles (Denaturation at 95°C for 10 sec and Annealing / Extension at 57°C for 30 sec).

4. Data analysis:

Data was analyzed by stepone software version 2.3. HMGB1 expression in healthy controls (HC) was set to 1. The relative quantitation of human **HMGB1** mRNA was normalized against that of human GAPDH. Gene expression fold change in NS was equal to $2^{-\Delta\Delta CT}$, (12). ΔCt was calculated by subtracting the threshold cycle (Ct) value for GAPDH from Ct value for HMGB1. $\Delta\Delta Ct$ was determined by subtracting ΔCt of controls from that of cases. Figure (1)

Statistical analysis:

The collected data were tabulated and analyzed using Statistical Package for Social Sciences Software version 16 software (SPSS Inc, Chicago, Illinois, United States). Categorical data were presented as number

and percentages. Chi square test (X^2) or Fisher's exact test (FET) were used to analyze categorical variables as appropriate. FET was used for small sized groups. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at $P > 0.05$. They were presented as mean \pm standard deviation and range if normally distributed and analyzed by analysis of variance (ANOVA) test for more than 2 independent groups, or presented as median and interquartile range (IQR) if nonparametric and analyzed by Mann Whitney U (MWU) test for 2 independent groups or Kruskal Wallis test (KWT) for more than 2 groups. Significant ANOVA and KW tests were followed by post hoc multiple comparisons using Bonferroni tests to detect the significant pairs. Spearman's correlation coefficient (ρ) was used to assess linear association between 2 quantitative variables. Receiver operator characteristic curve (ROC) curve was used to determine cutoff value of HMGB-1, CRP and H. score with optimum sensitivity and specificity in diagnosis of confirmed sepsis and its prognosis. Statistical significance was accepted at $P < 0.05$. High significance was accepted at $P \leq 0.01$.

Results

This study was carried out on 100 neonates 28 neonates with clinical sepsis and had positive blood cultures, 22 neonates with clinical sepsis but had negative blood cultures and 50 healthy non-infected newborns age and sex matched as controls.

Regarding the demographic data, we found no significant difference between cases and controls as regard to the gestational age, birth weight, age of admission, mode of delivery and the patient sex but there were high significant decreases in the APGAR (appearance, pulse, grimace, activity, and respiration) score in both the confirmed and suspected sepsis compared to controls at 1 minute and only in the confirmed sepsis compared to controls at 5 minutes. One fourth of number of the confirmed NS (7 out of 28 cases) died giving statistical significance compared with either the controls or suspected who showed no deaths (Table 1).

Regarding CBC& CRP, we found that the TLC, H. score, I/T ratio and CRP were highly significantly increased in confirmed sepsis compared to suspected group and controls. On the contrary, Hb and PLT were highly significantly decreased in the

confirmed sepsis than the suspected and controls. (Table 2).

The current study found that HMGB-1 gene expression was highly significantly increased in both confirmed and suspected sepsis compared to controls and highly significantly increased in the confirmed compared to the suspected sepsis (Figure 2-A). The HMGB-1 gene expression also experienced high significant increases in the confirmed and suspected sepsis subgroups (categorized by their onset) versus controls (Figure 2-B). In addition, the HMGB1 gene expression was significantly increased in the dead compared to the living patients (Figure 2-C).

The highest specificity (82%), sensitivity (100%), positive predictive value (PPV) (84.7%), and negative predictive value (NPV) (100%) were found for HMGB1 at cutoff 2.02 fold increases or more in early diagnosis of sepsis with area under the curve (AUC) that accounts for 0.977. The sensitivity of CRP and H. score in detecting sepsis was 94% and 78% while; their specificity was 80% and 56% respectively. The AUC for CRP was 0.95 and while that of H. score was 0.762. (Figure 2-D and Table 3)

The ROC curve analysis of HMGB1 gene expression at cutoff value 4.15 fold increases or more showed that the highest specificity (88.4%), sensitivity (100%), PPV (58.3%), and NPV (100%) and AUC (0.97) were found for HMGB1 for prediction of mortality in neonatal sepsis (prognosis). The sensitivity of CRP and H. score in predicting mortality was 85.7% for both while their specificity was 72.1% and 86% with AUC 0.862 and 0.864, respectively. (Figure 2-E & Table 3)

Univariate logistic regression analysis showed that the CRP, H. score and HMGB1 were high significant predictors for the prognosis of neonatal sepsis, however, after adjustment of the odd ratio in the multivariate analysis only the HMGB1 significantly predicted the prognosis of neonatal sepsis. (Table 4).

The present study also found significant positive correlations between HMGB1 gene expression and APGAR score at 5 minutes, Hb, H. score and CRP in confirmed sepsis group. (Figure 3)

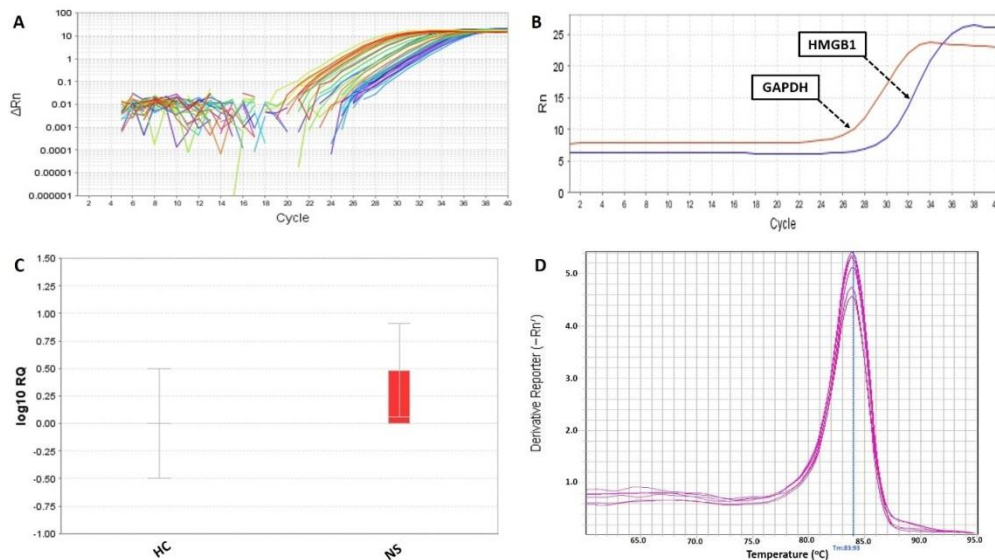


Fig. 1: Photos of HMGB1 relative quantitation by real-time PCR

A: amplification plot of target genes (HMGB1 and GAPDH) in the studied neonates, B: amplification plot of target genes (HMGB1 and GAPDH) in a single sample, C: Gene expression plot of HMGB1 in neonatal sepsis (NS) calibrated to that of healthy controls (HS) and D: Melt curve analysis of HMGB1

HMGB1: High-mobility group box 1, GAPDH: glyceraldehyde-3-phosphate dehydrogenase

Table (1): Demographic and clinical data of the studied neonates.

Variable	Confirmed NS (n. = 28)	Suspected NS (n. = 22)	Controls (n. = 50)	ANOVA or X ²	P	
Mean ± SD (range) or n. (%)						
Gestational age (weeks)	36.7 ± 2.40 (28-40)	36.5 ± 2.64 (27-39)	37.0 ± 1.57 (33-40)	0.34	0.71	
Birth weight (kilograms)	2.89 ± 0.66 (1.25-4.25)	2.65 ± 0.68 (1.05-3.5)	3.01 ± 0.45 (2.15-4.1)	3.07	0.051	
Age of admission (days)	6.67 ± 8.57 (1-27)	6.63 ± 6.93 (1-25)	6.36 ± 6.77 (1-28)	0.021	0.98	
APGAR 1 min	3.87 ± 1.36 ∞ (2-7)	4.60 ± 1.57 ∞ (2-7)	6.5 ± 0.6 (6-8)	23.6	< 0.001 (HS)	
APGAR 5 min	8.3 ± 1.0 ∞ (7-10)	9.0 ± 0.81 (8-10)	9.5 ± 0.51 (9-10)	12.5	< 0.001 (HS)	
MOD						
	NVD	4 (14.3)	8 (36.4)	16 (32.0)	3.77	0.15
	CS	24 (85.7)	14 (63.6)	34 (68.0)		
Sex						
	Male	16 (57.1)	11 (50.0)	28 (56.0)	0.29	0.86
	Female	12 (42.9)	11 (50.0)	22 (44.0)		
Outcome						
	Dead	7 (25) ∞, Δ	0 (0.0)	0 (0.0)	FET	P1 < 0.01 (HS), P2 = 0.01 (S)
	Alive	21 (75)	22 (100)	50 (100)		

NS: neonatal sepsis, APGAR: "appearance, pulse, grimace, activity, and respiration", min: minute, MOD: mode of delivery, NVD: normal vaginal delivery, CS: cesarean section,

ANOVA: analysis of variance, X²: chi square test, FET: Fisher's exact test, ∞: significant versus controls, Δ: significant versus suspected NS, P1: confirmed NS compared with controls, P2: confirmed NS compared with suspected NS

Table (2): Laboratory findings among the studied groups.

Variable	Confirmed NS (n. = 28)	Suspected NS (n. = 22)	Controls (n. = 50)	ANOVA	P
Mean ± SD (range) or n. (%)					
Hemoglobin (mg/dl)	13.4 ± 2.90 ∞, Δ (7.9-21.7)	14.6 ± 3.09 (7.5-22.7)	15.9 ± 1.32 (14-18.7)	10.8	< 0.001 (HS)
TLC (x10 ³ cells)	15.7 ± 6.9 ∞, Δ (6.7-39.2)	10.8 ± 4.8 (4.9-21.8)	11.1 ± 3.01 (6.4-11.6)	9.54	< 0.001 (HS)
PLTs (x10 ³ cells)	194.6 ± 136.1 ∞, Δ (18-704)	264.1 ± 90.8 (73-400)	274.6 ± 75.4 (185-450)	14.1	0.001 (HS)
I/T ratio	0.167 ± 0.07 ∞, Δ (0.08-0.3)	0.117 ± 0.04 (0.07-0.25)	0.109 ± 0.03 (0.07-0.19)	13.5	< 0.001 (HS)
CRP mg/l	57.5 ± 31.5 ∞, Δ (24-145)	33.07 ± 19.2 ∞ (12-100)	3.5 ± 1.0 (1-5)	77.6	< 0.001 (HS)
H. score	2.1 ± 0.87 ∞, Δ (1-3)	0.59 ± 0.66 (0-2)	0.44 ± 0.50 (0-1)	61.1	< 0.001 (HS)

NS: neonatal sepsis, TLC: total leucocyte count, PLTs: platelets, I/T ratio: immature to total neutrophil ratio, CRP: C-reactive protein, H. score: hematological score

ANOVA: analysis of variance, ∞: significant versus controls, Δ: significant versus suspected NS, HS: high significant

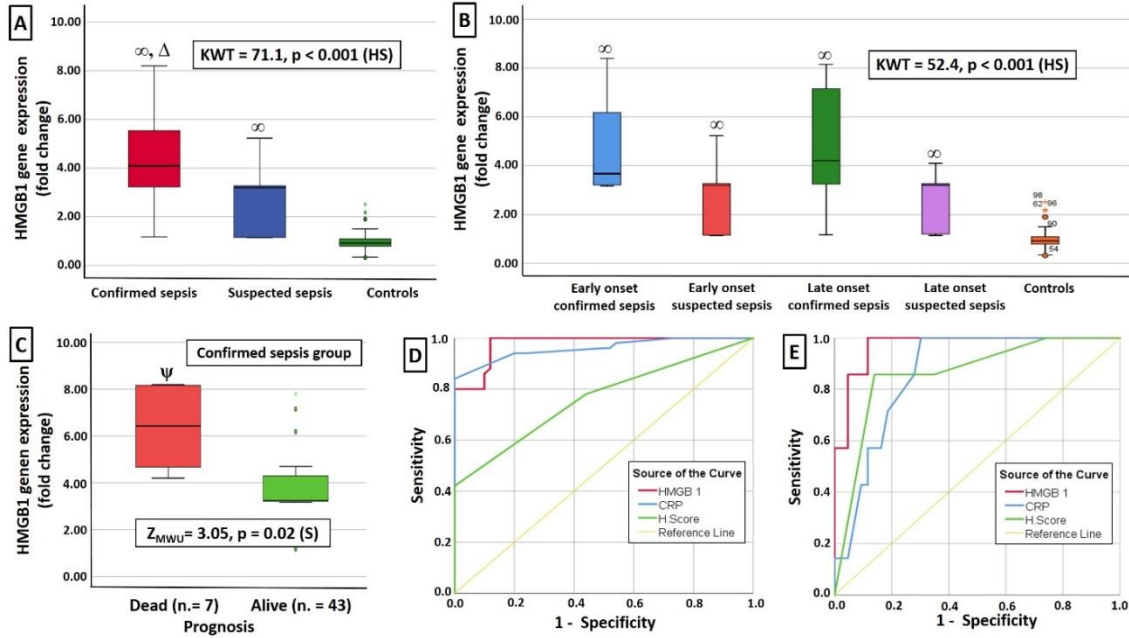


Figure (2): Gene expression of HMGB1 and ROC curve analysis in the studied groups

2-A: HMGB1 gene expression in the studied groups, 2-B: HMGB1 gene expression in the studied groups categorized by the onset of sepsis, 2-C: HMGB-1 gene expression in the confirmed neonatal sepsis according to their prognosis (died versus alive cases), 2-D: ROC curve analysis for the performance of HMGB1, CRP and H score in early diagnosis of neonatal sepsis. 2-E: ROC curve analysis for the performance of HMGB1, CRP and H score in prognosis of neonatal sepsis.

HMGB1: high-mobility group box 1, CRP: c-reactive protein, H. score: hematological score

KWT: Kruskal Wallis test, Z_{MWU} : z value of Mann Whitney U test, ∞ : significant versus controls, Δ : significant versus suspected NS, HS: high significant

Table (3): Validity of HMGB1, H. score and CRP for early diagnosis of neonatal sepsis and for prediction of its prognosis (mortality)

Marker	Sens	Spec	PPV	NPV	AUC	95% CI	P
For early diagnosis of sepsis							
HMGB1 ≥ 2.02	100%	82%	84.7%	100%	0.977	0.956-0.999	< 0.001 (HS)
CRP ≥ 22 mg/l	94%	80%	82.5%	93%	0.950	0.921-0.997	< 0.001 (HS)
H. score ≥ 2	78%	56%	63.9%	71.8%	0.762	0.67-0.86	< 0.001 (HS)
For prediction of prognosis (mortality)							
HMGB1 ≥ 4.15	100%	88.4%	58.3%	100%	0.970	0.92-1.00	< 0.001 (HS)
CRP ≥ 44 mg/l	85.7%	72.1%	33.3%	96.9%	0.862	0.76-0.97	0.002 (S)
H. score ≥ 3	85.7%	86%	50%	97.4%	0.864	0.72-1.00	0.002 (S)

Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve, CI: confidence interval, high-mobility group box 1 (HMGB1), CRP: c-reactive protein, H. score: hematological score, S: significant, HS: high significant

Table (4): Univariate and multivariate logistic regression analysis for the predictors of prognosis in neonatal sepsis

Variables	Univariate logistic regression			Multivariate logistic regression		
	B	Crude OR (95% CI)	P	B	Adjusted OR (95% CI)	P
CRP	-0.037	0.96 (0.94-0.99)	0.007 (HS)	0.12	1.01 (0.97-1.06)	0.617
H. score	-1.810	0.16 (0.04-0.64)	0.009 (HS)	-0.007	0.99 (0.14-6.86)	0.994
HMGB1	-1.285	0.28 (0.13-0.61)	0.002 (HS)	-1.472	0.23 (0.07-0.82)	0.023 (S)

OR: odd ratio, CI: confidence interval, CRP: C - reactive protein, H. score: hematological score, HMGB1: high-mobility group box 1, S: significant, HS: high significant

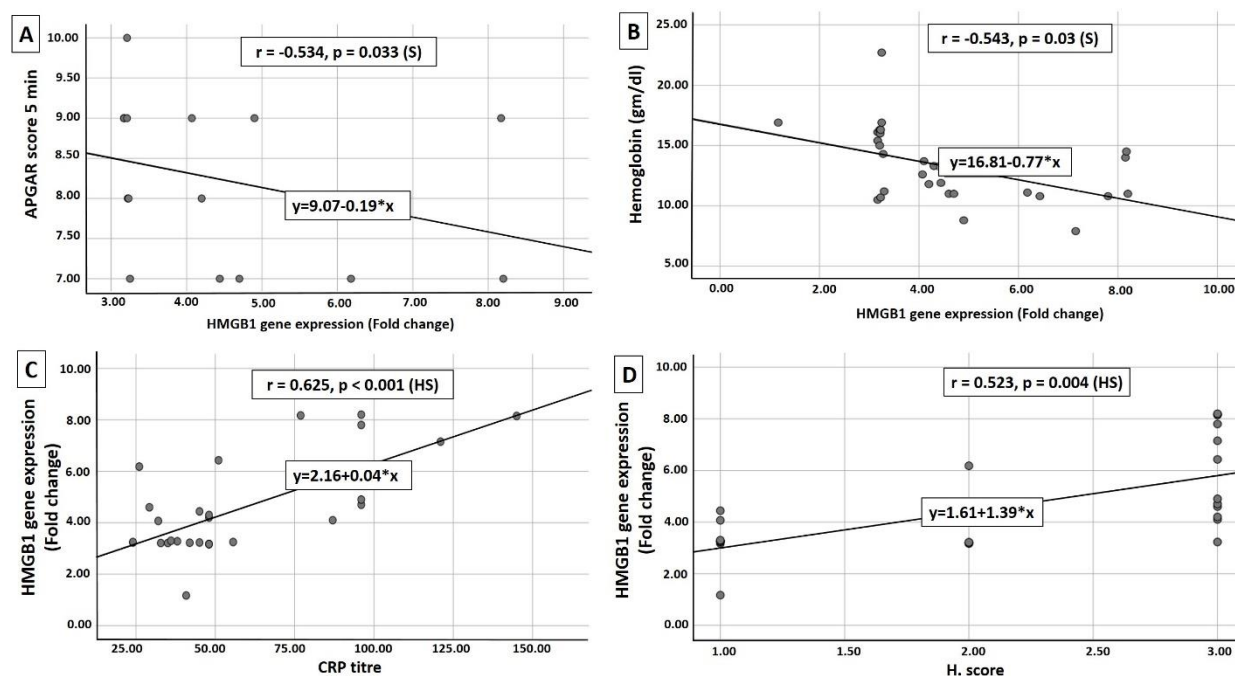


Fig. (3): Correlations between HMGB1 gene expression and the studied variables in the confirmed neonatal sepsis group

APGAR: appearance, pulse, grimace, activity and respiration, HMGB1: high-mobility group box 1, CRP: C- reactive protein, H. score: hematological score

Discussion:

Neonatal sepsis (NS) is a clinical syndrome with hemodynamic alterations and other systemic clinical manifestations caused by pathogenic microorganisms (bacteria,

viruses, or fungi) in a normally sterile fluid (blood or cerebrospinal fluid) in the first month of life. It is a leading cause of neurocognitive sequelae and mortality of newborn (13).

In our study, we found no significant difference between studied groups concerning demographic data. Regarding APGAR score at 1 and 5 minutes, the current study found a high significant decreased Apgar score in patients versus controls which agreed with ⁽¹⁴⁾. In 2020, it was found that premature birth and APGAR scores of < 7 at 5 minutes were found to be associated with overall NS and with early-onset NS ⁽¹⁵⁾.

The current study shows significant increases in TLC, H. score and I/T ratio but significant decreases in HB and PLT in confirmed NS compared to the suspected NS and controls that comes in agreement of a study done 2018⁽²⁾ who reported that H. score reached ≥ 2 in about 50% of cases.

The present study found that CRP levels were significantly higher in NS (confirmed & suspected) compared to controls. In agreement with our finding, some authors found a mean CRP level of 22.1 mg/dl at admission ⁽¹⁶⁾.

Our findings concerning the HMGB-1 gene expression agreed with that study done 2019 ⁽¹⁷⁾ which found that the relative expression of HMGB1 mRNA in peripheral blood mononuclear cells of NS was significantly

higher than that of the local infection and control groups. It has been confirmed that systemic HMGB1 levels are markedly increased in sepsis ⁽¹⁸⁾ and the concentration of circulating HMGB1 is positively correlated with the severity of inflammation ⁽¹⁹⁾. In addition, it was found that HMGB1 expression level was significantly elevated in necrotizing enterocolitis (NEC), when compared to healthy controls and it was significantly increased in stage II NEC (confirmed) than stage I NEC (suspected) ⁽²⁰⁾.

HMGB1 is involved in the pathological progression of sepsis as well. It was significantly higher in some survivors than others ⁽²¹⁾. HMGB1 plays a serious role in the pathogenesis of NS by stimulating TLR4/NF- κ B signaling pathway and enhancing the release of inflammatory mediators as IL-8. HMGB1 blockers as glycyrrhizin can prohibit activation of TLR4/NF- κ B signaling pathway and the release of inflammatory mediators ⁽¹⁷⁾.

Within 24 hours after the onset of sepsis, HMGB1 levels were increased in the liver, small intestine, and other tissues, demonstrating that HMGB1 expression was linked to endotoxin-mediated organ function impairment ⁽²²⁾. HMGB1 is implicated in

various inflammatory diseases, including sepsis, as it is significantly enhanced, and is positively correlated with the severity of disease ⁽¹⁹⁾.

The current study revealed that the ROC curve analysis for HMGB1 gene expression gave 0.977 for AUC, 82% specificity, 100% sensitivity at cutoff 2.02 fold increases or more for diagnosis of sepsis. It was reported that at a 50.65 pg/ml cut-off value for HMGB1 protein measured by ELISA, the AUC was 0.852; the sensitivity was 95.3 %, and the specificity 71.2 % for diagnosis of neonatal NEC ⁽²⁰⁾.

Clinical and preclinical studies reported that HMGB1 is as an interface of sepsis pathogenesis and mortality, so HMGB1 acts as an incoming target for passive immunity in sepsis. Administration of anti-HMGB1 polyclonal antibodies to a murine model of severe sepsis changes the manner of cytokine signaling to a more convenient profile, ameliorates survival, and safeguards against the post-septic immunosuppression ⁽²³⁾. Some researchers ⁽²⁴⁾ demonstrated that HMGB1 is associated with the severity and mortality of sepsis. They explained their results by the promotion of HMGB1 lactylation and acetylation via p300/CBP- and Hippo/YAP-mediated suppression of

deacetylase, respectively. The lactylated/acetylated HMGB1 is liberated from macrophages by exosomal secretion to enhance the endothelial permeability in polymicrobial sepsis.

The present study found that CRP levels were significantly higher in the NS (confirmed & suspected) than controls with cut off at 22 mg/l also, with higher sensitivity (94%) and moderate specificity (80%). Diagnostic accuracy of CRP is largely variable and debate even with high cutoff values. In a study done in 2015⁽²⁵⁾, it was reported that CRP has a sensitivity of 76.92% and a specificity of 53.49% in diagnosis of acute NS with PPV of 80% and NPV of 48.94%. Over all the diagnostic accuracy of CRP in diagnosis of NS was 70.07%. They concluded that CRP estimation has a role in diagnosis of NS but it is not specific enough to depend on as the only indicator. In 2016, it was ^{found} that at cutoff 50 mg/l, CRP sensitivity was sepsis (84.3%), specificity (46.1%), with AUC of 0.683 and CI = 0.529–0.836 ⁽²⁶⁾. Its diagnostic accuracy did not increase even when combined with other parameters of sepsis. However, **Póvoa et al. (1998)**²⁷ found that CRP has a 98.5% sensitivity and 75% specificity. These variations might be due to the accuracy of diagnostic kits, various

etiology of NS, patient-related factors, and individual responses to sepsis or genetic variation ⁽²⁶⁾. Also, *Khair et al. (2012)*²⁸ found that H. score and CRP were useful tests to diagnose NS.

ROC curve was done to determine the role of HMGB1 in predicting the prognosis (mortality) in patients and revealed that there is an increase in expression of HMGB1 in died patients than alive ones with sensitivity (100%), specificity (88.4%) in prediction of mortality. This finding came in agreement with other researchers ⁽²⁰⁾ who found that HMGB1 was a risk factor for mortality in NEC.

In our study, univariate analysis showed that CRP, H. Score and HMGB1 highly significantly predicted the prognosis of NS. This came in agreement with ^(29 & 30) who found that CRP was an important prognostic factor for NS. It was concluded that HMGB1 was a diagnostic and prognostic biomarker for sepsis ⁽²⁰⁾.

Multivariate analysis showed that only the HMGB1 significantly predicted the prognosis of NS. It was shown that the presence of late HMGB1 peak was significantly related to excess mortality (21.9%) than patients with early peak (9.6%)

⁽³¹⁾. HMGB1 was associated with morbidity and mortality within the septic shock patients linked to the more severe illness ⁽³²⁾.

Our study shows that there was a significant positive correlation between HMGB1 and APGAR score at 5 minutes, Hb, H. score and CRP in confirmed NS. In agreement with our results, it was found a high significant positive correlation between HMGB1 and CRP titer ($r = 0.986$) ⁽²⁰⁾. On the contrary, some researchers found no correlation between HMGB1 and CRP ⁽³³⁾.

Conclusion

Expression of HMGB1 is higher in confirmed neonatal sepsis than suspected cases and both are higher than controls. HMGB1 expression is a reliable biomarker for diagnosis and prognosis of sepsis in neonates, this inflammatory biomarker is more superior to CRP and H. score.

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