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Original article

Serum High Mobility Group Box 1 Protein as an Early Marker of Pulmonary Arterial Hypertension in Neonates

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Abstract

Background: Inflammation plays an important role in neonatal hypoxia-induced organ damage. Newborns with perinatal asphyxia often develop persistent pulmonary hypertension of the neonate. This study aimed to early detection of pulmonary arterial hypertension in newborn to improve patient morbidity and mortality by estimated serum high mobility group box 1 protein (HMGB1) by enzyme-linked immunosorbent assay (ELISA).

Methods: This case control study was conducted in Zagazig University Hospital, Pediatric (NICU) and clinical pathology departments, during a period of eight months from March 2020 to January 2021. Following were the three groups into which patients were split: Group 1: fifteen apparently healthy neonates included 11 male and 4 females, group 2: fifteen congenital heart diseases without pulmonary hypertension neonates included 10 male and 5 female and group 3: Fifteen congenital heart diseases with pulmonary hypertension neonates included 10 male and 5 female.

Results: There is a statistically significant difference between the groups that were looked at for HMGB1 expression. When compared pairwise, congenital heart diseases (CHD) with pulmonary hypertension and the other groups show a significant difference (the greatest level was in CHD with pulmonary hypertension) hypertension then CHD without pulmonary hypertension then control group).

Conclusion: HMGB1 levels in newborns with pulmonary hypertension increased.

Keywords: HMGB1, Neonates , pulmonary arterial hypertension.

Introduction

The normal perinatal fetal-to-neonatal circulatory transition is interfered with, leading to pulmonary arterial hypertension. Early-life occurrence of a patent foramen ovale and patent ductus arteriosus causes newborns with increased pulmonary vascular resistance to experience extra pulmonary blood shunting, which causes severe and perhaps unresponsive hypoxemia. persistent pulmonary hypertension of the newborn (PPHN) is a syndrome characterized by severe pulmonary hypertension, which results in hypoxia and blood flow from the right to left extrapulmonary veins. Newborns are susceptible to respiratory distress, acidosis, and refractory hypoxemia when pulmonary perfusion is insufficient [1].

Hypoxemia that responds poorly to extra oxygen is a feature of pulmonary hypertension. Identifying if a hypoxemic newborn has pulmonary arterial hypertension (PAH) is a crucial objective for the initial clinical examination [2].

The term PAH has been used more broadly recently to refer to neonates with a variety of cardiopulmonary disorders, including meconium aspiration, sepsis, pneumonia, respiratory distress syndrome, and congenital heart diseases, all of which share a similar physiology. When pediatric echocardiography is not readily available in a clinical setting, the diagnosis of PAH can frequently be challenging. Oxygen saturation, echo, and chest radiograph are all crucial in the diagnosis and should be taken into consideration on a regular basis when examining the cyanotic newborn [3].

The severity of pulmonary hypertension might vary greatly. A threshold value of 40 mm Hg for Moderate pulmonary hypertension has been described using Doppler estimated pulmonary artery systolic pressure. pulmonary artery pressure surpasses two thirds of systemic blood pressure, severe pulmonary arterial hypertension is anticipated [4].

HMGB1 is a single-chain polypeptide with 215 amino acids. Necrotic cells' passive secretion of this protein and immune cells such macrophages, dendritic cells, and natural killer cells' active secretion during disease states can stimulate both local and systemic inflammatory responses. By encouraging the creation of additional inflammatory cytokines and activating certain immune cells, it can also control the immunological response [5].

Due to its amplifying effect and ability to control other inflammatory cytokines to be released, HMGB1 is a crucial part of the inflammatory network [6].

Tang et al. [7] , According to their findings, HMGB1 serum levels dramatically rise shortly after PAH beginning in babies with the condition. Due to its significant significance in the early stages of PAH, HMGB1 may be a helpful early diagnostic for PAH diagnosis.

We aimed for early detection of pulmonary arterial hypertension in newborn to improve patient morbidity and mortality by estimated serum HMGB1 by enzyme-linked immunosorbent assay (ELISA).

Methods

The pediatric unit of Zagazig University Hospital served as the study's site (NICU) and clinical pathology departments, during a period of eight months from March 2020 to January 2021.

Patients were divided into the following three groups: group (1): apparently healthy neonates included 11 male and 4 females, group (2): congenital heart diseases without pulmonary hypertension neonates included 10 male and 5 female and group (3): congenital heart diseases with pulmonary hypertension neonates included 10 male and 5 female.

Subjects with no particular prenatal medications (such aspirin or other non-steroidal anti-inflammatory drugs) and a normal obstetric history for the mother, a gestational age between 34-42 weeks, neonates from first day to 28 days old, no postnatal cyanosis, breathlessness, hypoxemia, or other problems and discharge from the hospital after 3–4 days and In the same ward as the mother, postpartum care is given without specialized medical assistance were included in the study as control group.

Neonates from the first day to 28 days old, congenital heart diseases with-without pulmonary hypertension and more than 34 weeks of gestation were included in the study as case group.

Sepsis or other serious prenatal infections are infections that are serious throughout pregnancy, their mothers had a history of taking unusual medications, such as non-steroidal anti-inflammatory drugs (NSAIDs) or selective serotonin reuptake inhibitors (NSAIDs) during pregnancy and the newborns had severe pathological jaundice, diaphragmatic hernia, severe congenital cardiovascular illness, or aberrant thyroid function were excluded from the study.

All cases had undergone full history taking including prenatal history: history of maternal infection and diseases antibiotic or drug administrated to mother and natal history: sex, weight, gestational age, and invasive procedures like endotracheal intubation and mechanical breathing, complete clinical examination including general reflexes, vital signs, and a comprehensive examination of the nervous, respiratory, cardiovascular, and stomach systems etc. and laboratory investigations including complete blood count, CRP and blood culture.

Measurement of serum HMGB1 by ELISA: HMGB1 was measured using the HMGB1 Direct enzyme linked immunosorbent assay Kit (Shino-Test Corporation, Kanagawa,Japan).

Radiological investigation: Transthoracic echocardiography was performed on each patient after admission. During admission, noninvasively calculate the pulmonary artery pressure. It was performed to detect ductal and atrial shunt if left-to-right, bidirectional or right-to left, to assess cardiac function and to detect structural heart diseases. From the velocity of the tricuspid regurgitant (TR) jet using continuous. Wave Doppler and modified Bernoulli equation:

RVSP or (SPAP) = 4(v) 2 + RA pressure

Where

- RVSP = right ventricular systolic pressure.
- SPAP = systolic pulmonary artery pressure.
- RA pressure = right atrial pressure it was roughly estimated by 10mm/hg

v = velocity of the tricuspid regurgitation jet [8].

The echocardiography was done using vivid 7 machines by general electric using M3 probe. Dimensional echocardiogram and colour flow Doppler were performed to detect ductal and atrial shunt if left-to-right, bidirectional or right-to left, to assess cardiac function and to detect structural heart diseases.

Ethics approval:

Written informed consent was obtained from the participant's relatives. The study was conducted according to the world medical association (Declaration of Helsinki) for studies involving humans. Both the Institutional Review Board and this study's protocol have given their approval [IRB] and the local ethics committee at Zagazig University's Faculty of Medicine.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences). Independent samples Student's t-test, Chi square test, One Way ANOVA test, Kruskal-Wallis H test, Levene's test, a post - Hoc test, Spearman's rank correlation coefficient, receiver operating characteristic (ROC) curve analysis were used.

Results

There was statistically non-significant difference between the studied groups regarding age or gender. There is statistically non-significant difference between the studied groups regarding gestational age, mode of delivery or antenatal history. A larger percentage of the studied neonates within each group had normal antenatal history and delivered by CS mode (Table 1).

Table 2; showed that there was statistically significant difference between the studied groups regarding heart rate and respiratory rate. On LSD comparison, the difference is significant between control group and each of CHD with and without pulmonary hypertension groups.

Table 3; p1 the difference between CHD with pulmonary hypertension and CHD without pulmonary hypertension groups p2 the difference between CHD without pulmonary hypertension and control groups p3 the difference between CHD with pulmonary hypertension and healthy control groups. There is statistically non-significant difference between the studied groups regarding hemoglobin level. All groups had negative CRP. There was statistically significant difference between the studied groups regarding HMGB1. On pairwise comparison, the difference is significant between CHD with pulmonary hypertension and each other group (highest level was in CHD with pulmonary hypertension then CHD without pulmonary hypertension then control group).

Table 4; showed that there was statistically non-significant difference between the studied groups regarding ejection fraction. On comparing groups with congenital heart disease with and without pulmonary hypertension, there is non-significant difference between them regarding type of CHD. However, there is significant difference between them regarding SPAP which was significantly higher among group with pulmonary hypertension.

Table 5; showed that there was statistically significant positive correlation between it and each of estimated SPAP, heart and respiratory

rates. On the other hand, there is non-significant correlation between HMGB-1 and either age, gestational age, Length, weight, head circumference, ejection fraction or hemoglobin.

Table 6; Among factors significantly correlated to HMGB-1 level, linear stepwise regression analysis showed that only SPAP significantly associated with its level (unstandardized beta=1.867, p<0.001).

Table 7; The best cutoff of HMGB-1 in prediction of presence of congenital heart disease without pulmonary hypertension is ≥8.785 ng/mL with area under curve 0.716, sensitivity 73.3%, specificity 66.7%, positive predictive value 68.8%, negative predicative value 71.4% and accuracy 70% (p<0.05).

Table S1; The best cutoff of HMGB-1 in prediction of presence of pulmonary hypertension among patients with CHD is ≥42.895 ng/mL with area under curve 0.996, sensitivity 93.3%, specificity 96.7%, positive predictive value 93.3%, negative predicative value 96.7% and accuracy 95.6% (p<0.05).

Table S2; showed that there was non-significant relation between outcome and HGMB-1 level which was non-significantly higher among those with poor outcome.

Table (1): Comparison between the studied groups regarding baseline data:

Parameters	Groups			F/χ^2	p
	CHD with pulmonary hypertension group	CHD without pulmonary hypertension group	Healthy control group		
	N=15 (%)	N=15 (%)	N=15 (%)		
Age (day): Median Range	7 1 – 27	10 1 – 28	7 1 – 23	1.06	0.365
Gender: Male Female	10 (66.7) 5 (33.3)	10 (66.7) 5 (33.3)	11 (73.3) 4 (26.7)	0.207	>0.999
Gestational age(w) Mean ± SD Range	38.533 ± 1.187 36 – 40	38.933 ± 0.961 38 – 40	38.8 ± 1.207 36 – 40	0.492	0.615
Mode of delivery: Normal CS	5 (33.3) 10 (66.7)	5 (33.3) 10 (66.7)	2 (13.3) 13 (86.7)	2.045	0.526
Antenatal history: NAD Hypertension Diabetes Hypertension and diabetes	12 (80) 1 (6.7) 1 (6.7) 1 (6.7)	12 (80) 0 (0) 0 (0) 3 (20)	13 (86.7) 0 (0) 2 (13.3) 0 (0)	5.504	0.537

Table (2): Comparison between the studied groups regarding vital signs:

Parameters	Groups			F	p
	CHD with pulmonary hypertension group	CHD without Pulmonary hypertension group	Healthy Control group		
	N=15 (%)	N=15 (%)	N=15 (%)		
Heart rate					
Mean ± SD	154.27±26.06	145.2±10.92	122.67±4.43	14.559	<0.001**
Range	118 – 197	124 – 160	114 – 130		
LSD	p_1 0.14	p_2 <0.001**	p_3 <0.001**		
Respiratory rate					
Mean ± SD	60.67 ± 13.5	61 ± 8.91	42.33 ± 6.54	16.865	<0.001**
Range	40 – 84	42 – 74	36 – 55		
LSD	p_1 0.928	p_2 <0.001**	p_3 <0.001**		

F One way ANOVA test
**p≤0.001 is statistically highly significant
*p<0.05 is statistically significant p1 the difference between CHD with pulmonary hypertension and CHD without pulmonary hypertension groups p2 the difference between CHD without pulmonary hypertension and control groups p3 the difference between CHD with pulmonary hypertension and healthy control groups
LSD: least significant difference

Table (3): Comparison between the studied groups regarding laboratory data:

Parameters	Groups			F	p
	CHD with pulmonary hypertension group	CHD without pulmonary hypertension group	Healthy Control group		
	N=15 (%)	N=15 (%)	N=15 (%)		
Hemoglobin (g/dl)					
Mean ± SD	13.79 ± 3.36	12.91 ± 3.78	14.01 ± 2.9	0.454	0.638
Range	9 – 20	9.1 – 22.8	9.6 – 20.1		
WBC ($\times 10^3/mm^3$)					
Mean ± SD	10.3 ± 3.8	10.09 ± 2.3	10.3 ± 4.9	0.438	0.941
Range	9.9	10	9.4		
Platelet count					
Mean ± SD	323.4 ± 70.3	326.2 ± 41.8	226 ± 75.2	0.485	0.538
Range	300	332	193		
CRP:					
Negative	15 (100)	15 (100)	15 (100)		
HMGB1 (ng/mL)					
Median	77.33	9.72	7.66	30.772	<0.001**
Range	40.12 – 147.44	5.93 – 49.99	0.6 – 27.33		
Pairwise comparison	p_1 <0.001**	p_2 0.506	p_3 <0.001**		

F One way ANOVA test, KW Kruskal wallis test
**p≤0.001 is statistically highly significant
*p<0.05 is statistically significant

Table (4): Comparison between the studied groups regarding echocardiographic parameters:

Parameters	Groups			F/t/χ^2	p
	CHD with Pulmonary hypertension group	CHD without pulmonary hypertension group	Healthy Control group		
	N=15 (%)	N=15 (%)	N=15 (%)		
EF (%) Mean ± SD Range	71.87 ± 5.58 60 – 78	76.13 ± 7.21 66 – 87	74.4 ± 4.24 68 – 81	2.05	0.141
Estimated SPAP Mean ± SD Range	61.27 ± 11.11 45 – 78	30.53 ± 4.58 20 – 35		t (9.908)	<0.001**
Type of CHD: ASD ASD+PDA ASD+VSD PDA VSD AV canal PFO+PDA VSD+PFO PDA+ASD+PFO	 3 (20) 0 (0) 4 (20) 0 (0) 6 (40) 1 (6.7) 0 (0) 0 (0) 1 (6.7)	 5 (33.3) 2 (16.7) 1 (6.7) 2 (13.3) 1 (6.7) 0 (0) 2 (13.3) 1 (6.71) 1 (6.7)		13.871	0.085

Chi square test F One way ANOVA t independent sample t test
**p≤0.001 is statistically highly significant

Table (5): Correlation between HMGB1 and the studied parameters:

Parameter	HMGB1	
	R	p
Age (days)	0.034	0.826
Gestational age (weeks)	-0.035	0.820
Weight (kg)	-0.289	0.054
Length (cm)	0.09	0.85
Head circumference (cm)	0.186	0.221
Heart rate (b/min)	0.486	0.001**
Respiratory rate (/min)	0.461	0.001**
Hemoglobin (g/dL)	-0.008	0.957
Estimated SPAP	0.832	<0.001**
Ejection fraction (%)	-0.242	0.109

**p≤0.001 is statistically highly significant
*p<0.05 is statistically significant
r Spearman rank correlation coefficient

Table (6): Linear stepwise regression analysis of factor significantly correlated to serum HMGB level:

	Unstandardized Coefficients		Standardized Coefficients	t	p	95.0% Confidence Interval	
	β	Std. Error	Beta			Lower	Upper
SPAP	1.867	0.238	0.829	7.858	<0.001**	1.38	2.534

Table (7): Area under Curve, Cutoff and validity of HMGB1 in diagnosis of CHD without pulmonary hypertension among the studied patients:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
≥8.785	0.716	73.3%	66.7%	68.8%	71.4%	70%	0.044*

**p≤0.001 is statistically highly significant

Discussion

A disruption in the normal fetal-to-newborn circulatory transition during pregnancy results in pulmonary arterial hypertension. PPHN is a syndrome characterized by severe pulmonary hypertension, which results in hypoxia and blood flow from the right to left extrapulmonary veins. Newborns are susceptible to respiratory distress acidosis, and refractory hypoxemia when pulmonary perfusion is insufficient. PAH is hypoxemia that is poorly responsive to extra oxygen defines this condition. Finding out whether a hypoxemic newborn has PAH is a key objective of the initial clinical examination. [1, 9].

HMGB1 consists of 215 amino acids and is a single-chain polypeptide. This protein's necrotic cells' passive secretion and immune cells such macrophages, dendritic cells, and natural killer cells' active secretion during disease states can stimulate both local and systemic inflammatory responses. By encouraging the creation of additional inflammatory cytokines and activating certain immune cells, it can also control the immunological response [5].

HMGB1 This study demonstrates that infants with PAH have elevated serum levels of HMGB1considerably elevated soon after the commencement of PAH. Because of its amplifying effect and ability to control the production of other inflammatory cytokines, HMGB1 is an essential part of the inflammatory network. Because HMGB1 is crucial in the early stages of PAH, it has the potential to serve as an early marker for the diagnosis of PAH [7].

This is why the study was selected to evaluate the reliability of HMGB1 in pulmonary hypertension diagnosis.

Age, gender, and other factors were statistically non-significantly different between the study groups gestational age, mode of delivery, antenatal history and anthropometric measurement, our results are supported by findings reported by [10, 11].

In contrary D'Angelo et al. [12] reported that the manner of delivery and HMGB1 levels were strongly and directly associated. HMGB1 readings in the spontaneous vaginal group were substantially higher than in the elective or emergency caesarean section group (p=0.004). While Between groups of newborns delivered by caesarean section, both emergency and elective, there was no discernible variation in HMGB1 levels (Group B and C) (p=0.046).

In our investigation, there was a statistically significant difference between the groups that were examined regarding heart rate and respiratory rate. with least significant difference between control group and each of CHD with and without pulmonary hypertension groups and this is in agreement with [13].

Regarding the studied groups according to clinical examination (cyanosis, grunting and history of ventilation) statistically, there was no difference between the groups, which is consistent with [14].

HMGB1 was discovered to play a role as a late regulator of lethal systemic inflammation in the delayed endotoxin lethality and systemic inflammation in animal models 10 years ago [15].

So, during our study we excluded all patients with sepsis and included all CRP negative patients.

According to complete blood count and CRP level, the examined group and the control group were not statistically different, which was consistent with [10].

In our investigation, there was no appreciable difference between the investigated groups in terms of ejection fraction and type of congenital heart diseases.

However, there is significant difference between them regarding estimated SPAP which was significantly higher among group with pulmonary hypertension with significant

difference (<0.001) and this is in agreement with [14].

HMGB1 is a crucial part of the inflammatory network because it can control the release of other inflammatory cytokines and has an amplifying influence upon secretion [6].

Tang et al. [7] reported that serum levels of HMGB1 in newborns with pulmonary hypertension are significantly increased early after pulmonary hypertension onset.

HMGB1 contributes significantly to the onset of pulmonary hypertension, indicating that it may be a helpful early marker for the identification of pulmonary hypertension [7].

In our study there was difference in HMGB1 between the examined groups that is statistically significant. When compared pairwise, there is a considerable difference between CHD with pulmonary hypertension and each other group (highest level was in CHD with pulmonary hypertension then CHD without pulmonary hypertension then control group).

Our results supported by Huang et al. [14] who reported that The level of HMGB1 was significantly higher in CHD patients with PAH than in CHD patients without PAH and healthy controls (P <0.01, P < 0.001), Fan et al. [10] who said that Patients with CHD had considerably higher levels of HMGB1, which was thought to be a potential indicator of severity and Luo et al. [16] who reported that Serum HMGB1 levels were significantly increased in patients with pulmonary hypertension compared with the control group (P<0.001).

In our result There was a strong link between each and it of SPAP, heart and respiratory rates and this is in agreement with Huang et al. [14] and Luo et al. [16]who reported that The serum HMGB1 levels were significantly positive correlation with estimated systolic pulmonary arterial pressure (eSPAP) linear stepwise regression analysis showed that only estimated SPAP significantly associated with HMGB1 level In our investigation, the relationship between outcome and significance was insignificant and HGMB-1 level and this is against study of Fan et al. [10] who found that HMGB1 levels were significantly increased in patients with CHD who died compared with those patients who survived. in our study there was significant difference between groups according to levels of HMGB1 with The best cutoff of HMGB-1 in prediction of presence of congenital heart disease without pulmonary hypertension is ≥8.785 ng/mL with area under curve 0.716, sensitivity 73.3%, specificity 66.7%, positive predictive value 68.8%, negative predicative value 71.4% and accuracy 70% (p<0.05) and with The best cutoff of HMGB-1 in prediction of presence of pulmonary hypertension is ≥42.895 ng/mL with area under curve 0.996, sensitivity 93.3%, specificity 96.7%, positive predictive value 93.3%, negative predicative value 96.7% and accuracy 95.6% (p<0.05).

Our study was supported by Huang et al. [14] who noted that HMGB1 was considerably greater in patients with CHD who also had pulmonary hypertension compared to healthy controls and patients without pulmonary hypertension (P <0.01,P < 0.001), HMGB1 level is strongly indicated in CHD patients with severe pulmonary hypertension with an AUC of 0.919 (P < 0.001). In order to maximize the combined specificity and sensitivity, the ideal HMGB1 threshold was 13.62 ng/mL. Sensitivity, specificity, were 78.2% and 91.3%, respectively.

Conclusion

According to our research, newborns with pulmonary hypertension had higher HMGB1 levels. In the examination of pulmonary hypertension in conjunction with echocardiography or as an early diagnostic tool upon delivery when echocardiography is not immediately available for confirmation of elevated pulmonary pressure, serum HMGB1 may be taken into consideration.

Declaration of interest

The authors report no conflicts of interest. The authors along are responsible for the content and writing of the paper.

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Table (S1): Area under Curve, Cutoff and validity of HMGB1 in diagnosis of CHD with pulmonary hypertension among the studied patients:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
≥42.895	0.996	93.3%	96.7%	93.3%	96.7%	95.6%	<0.001**

****p≤0.001 is statistically highly significant**

Table (S2): Relation between Outcome and HMGB-1 among the studied participants:

HGMB-1	Outcome		Test	
	Poor	Good	Z	p
Median	51.61	13.09	-1.836	0.066
Range	9.51 – 119.04	0.6 – 147.44		

Z Mann Whitney test