Diagnostic Performance of Plasma Haptoglobin in Children with Decompensated Ventricular Septal Defect

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Abstract

Background: Recent studies suggest that plasma haptoglobin (HP) is a diagnostic biomarker that may aid in disease assessment and risk stratification of patients with Ventricular septal defect and heart failure (HF). Aim: To investigate the diagnostic accuracy of plasma haptoglobin levels in children with VSD and HF. Methods: This case control study included 36 children diagnosed as Ventricular septal defect with or without heart failure. A control group of 36 healthy children were recruited. Echocardiography and plasma HP levels were assessed among all participants. Results: A statistically significant correlation was found between plasma haptoglobin level and the severity of heart failure. Haptoglobin levels were lower in severe HF compared to the mild HF subgroups. HP had a sensitivity of 94.4%, and a specificity of 85.2%, with an area under curve of 0.92, the optimal haptoglobin cutoff for diagnosing heart failure was ≤7.9 mg/dl (p value ≤0.001). Conclusions: There might be a correlation between the severity of heart failure and haptoglobin levels as the reduction in haptoglobin levels was more pronounced in individuals with severe HF than in those with mild HF. At a sensitivity of 94.4% and a specificity of 85.2%, haptoglobin was an accurate diagnostic tool for identifying decompensated ventricular septal defect cases in our patients.

Keywords: *Plasma Haptoglobin; Ventricular Septal Defect; Heart failure*

1. Introduction

Approximately 20–40% of all congenital heart disorders (CHD) in children include ventricular septal defect (VSD), making it one of the most prevalent CHD in children [1]. If left untreated, this structural cardiac anomaly disrupts normal circulatory hemodynamics leading to increased pulmonary blood flow, volume overload, and ultimately heart failure (HF) [2]. HF in children with VSD imposes significant morbidity and mortality which highlight a critical need for early detection, monitoring, and timely intervention [3]. While echocardiography remains the gold standard for diagnosing VSD, it may not always reflect the systemic inflammatory and oxidative stress responses associated with heart failure [4]. Therefore, identifying reliable biomarkers is essential for improving clinical outcomes.

Because of its anti-inflammatory and antioxidant characteristics, haptoglobin (HP)—an acute-

phase plasma glycoprotein mostly produced by the liver—has become a possible biomarker for cardiovascular disorders [5]. It functions by binding free hemoglobin, preventing oxidative damage and tissue injury [6]. Elevated haptoglobin levels have been reported in various inflammatory and cardiovascular conditions, suggesting its role as a marker for disease severity and prognosis [7]. However, its diagnostic utility in pediatric populations, particularly in children with VSD and heart failure, remains underexplored.

Myocardial dysfunction, fibrosis, and remodeling are all aspects of heart failure that have been linked to oxidative stress [8]. Haptoglobin, through its hemoglobin-binding activity, may counteract oxidative stress and modulate inflammatory pathways, making it a credible candidate for assessing heart failure severity in children with VSD [9].

The current standard biomarkers for heart failure, including B-type natriuretic peptide and N-terminal proBNP have shown utility in adults and children [10]. However, these biomarkers may be influenced by age, renal function, and comorbidities, reducing their specificity in pediatric populations [11]. In contrast, haptoglobin levels may provide additional pathophysiological insights, complementing conventional biomarkers in identifying early heart failure in children with VSD [12].

This study aimed to investigate the diagnostic performance of plasma haptoglobin levels in children with VSD and heart failure and to explore HP correlation with clinical severity of heart failure.

Patients and Methods

This case control study included 36 children diagnosed as Ventricular septal defect with or without heart failure. A control group of 36 healthy children with distributional matching to the patients regarding age and gender were recruited from outpatient clinic. All participants were selected at Pediatric Cardiology Unit of Zagazig University Children's Hospital from June 2020 to June 2024.

Inclusion Criteria: All children admitted to the hospital with VSD were included in this study.

Exclusion Criteria: Patients with VSD in association with other congenital heart diseases, presence of hemolytic anemias, and coexistence of inflammatory diseases affecting other organs or systems were excluded from the study.

Methodology and Operational Design:

All patients included in this study underwent a comprehensive evaluation. Full history taking was performed, including personal details, family history focusing on consanguinity and any previous family history of cardiac disease. Perinatal history, including mode of delivery, gestational age, NICU admission, and past hospital admissions, were also documented. We performed a thorough clinical examination which included anthropometric measurements and assessments of pallor, jaundice, cyanosis, facial characteristics, decubitus, and a comprehensive head-to-toe examination. Vital signs were recorded, and a detailed local cardiac examination was conducted. Heart failure was graded according to Modified Ross classification to mild, moderate and severe degrees [4].

Echocardiographic studies were performed using an Epic CVx Release 6 Philips machine with S8-3 or X5-1 MHz probes. Measurements included LV systolic and diastolic dimensions, aortic and LA dimensions, fractional shortening, and ejection fraction of the LV. Cardiac dimensions and LV ejection fraction were measured in alignment with the recommendations of the American Society of Echocardiography (19).

Tests for liver function (SGPT, SGOT, Serum Albumin), C-reactive protein (CRP), and serum electrolytes (Na, K, Ca) were evaluated. Eliza testing was used to determine the hemoglobin levels. Blood samples were taken from 72 participants using sterile syringes in EDTA tubes, centrifuged for 20 minutes at speeds ranging from 2000-3000 RPM. After allowing the samples to clot at room temperature for 10-20 minutes, the centrifuge was turned off. Until analysis, the samples were kept at -20°C. Following the manufacturer's directions, samples were produced and diluted using a human haptoglobin kit. The samples, enzyme-labeled antibodies, and standards were all incubated at 37°C for 60 minutes. After a thorough washing of the plates, chromogen solutions A and B were introduced, and the plates were incubated for 10 minutes. After adding a stop solution, the optical density (OD) was determined. Linear regression analysis was used to construct a standard curve.

Statistical Analysis:

Utilizing SPSS (Statistical Package for the Social Sciences) version 26, data analysis was carried out. Whenever applicable, chi-square tests, Fisher's exact tests, or Monte Carlo testing were used to compare categorical variables, which were presented using absolute frequencies. To ensure that parametric test assumptions were correct, Kolmogorov-Smirnov test was employed. Depending on the distribution of the data, quantitative variables were presented as medians and interquartile ranges or as means and standard deviations. When comparing two groups, normally distributed data was analyzed using independent sample t-tests, whereas non-normally distributed data was analyzed using the Mann-Whitney test. One-way ANOVA was used for normally distributed data in comparisons involving more than two groups, and Tukey HSD was used for post-hoc analysis when p values were <0.05. Spearman rank correlation and Pearson correlation were used for correlation analysis whenever appropriate. In order to find the best cutoff values for certain quantitative parameters used for medical diagnosis, Receiver Operating Characteristic (ROC) curves were utilized. A statistically significant result was defined as a p-value less than 0.05, while a very significant difference was shown by a p-value less than or equal to 0.001.

Results

Table (1) Demographics of research groups:

	VSD group	**Control group**	χ^2	**p**
	N=36 (%)	**N=36 (%)**		
Sex:				
Female	16 (44.4%)	18 (50%)	0.233	0.64
Male	20 (55.6%)	18 (50%)		
	Median (IQR)	**Median (IQR)**	Z	p
Age (month)	12(2.25 – 34.5)	24(8.5 – 48)	-1.899	0.06

IQR : interquartile range, χ^2 : Chi square test, Z : Mann Whitney test

In terms of gender and age, study members did not differ statistically (table 1).

Table (2) Heart failure severity in patients according to Ross classification:

	N=18	**%**
Heart failure		
Mild	5	27.8%
Moderate	8	44.4%
Severe	5	27.8%

Eighteen patients with VSD had heart failure; 44.4% of them had moderate heart failure while

27.8% had severe of heart failure (table 2).

Table (3) Demographic data VSD subgroups:

	VSD without heart failure group	**VSD with heart failure group**	χ^2	**p**
	N=18 (%)	**N=18 (%)**		
Sex: Female Male	 6 (33.3%) 12 (66.7%)	 10 (55.6%) 8 (44.4%)	1.8	0.18
	Median (IQR)	**Median (IQR)**	Z	p
Age (month)	12(1.33 – 34.5)	12(3.38 – 27.75)	-0.65	0.516

IQR: interquartile range, χ^2: Chi square test, Z: Mann Whitney test

When comparing the age and gender distributions of VSD with and without heart failure, no statistically significant differences were found (table 3).

Table (4): Clinical measurements in VSD subgroups:

	VSD without heart failure group	**VSD with heart failure group**	**t**	**P**
	Mean ± SD	**Mean ± SD**		
Respiratory rate(bpm)	39.33 ± 9.29	46.67 ± 7.67	-2.583	0.014*
Heart rate (Bpm)	112.22 ± 23.47	133.89 ± 23.04	-2.795	0.008*
Gestational age (week)	37.44 ± 1.1	37.94 ± 1.11	-1.36	0.183
Height (cm)	71.28 ± 17.57	77.17 ± 17.65	-1.003	0.323
Systolic blood pressure (mmHg)	88.11 ± 10.44	102.11 ± 11.34	-3.854	<0.001**
Diastolic blood pressure (mmHg)	**46.11** ± 9.17	**53.22** ± 11.21	**-1.936**	**0.06**
	Median (IQR)	**Median (IQR)**	**Z**	**P**
Weight (kg)	8(3.65 – 11)	8.75(5.5 – 11)	-0.746	0.456
Body surface Area (m^2)	0.37(0.24 – 0.53)	0.43(0.31 – 0.52)	-1.124	0.261

IQR: interquartile range, * :$p<0.05$ is statistically significant ** :$p\leq0.001$ is statistically highly significant, independent sample t test, Z: Mann Whitney test.

When comparing the VSD subgroups, we found that heart rate, systolic blood pressure and respiratory rate were significantly higher in patients with HF. Factors such as gestational age, height, diastolic blood pressure, weight, and body surface area were not significantly different among the two subgroups (table 4).

Table (5) Laboratory data in VSD with versus without heart failure:

	VSD without heart failure group	**VSD with heart failure group**	**t**	**p**
	Mean ± SD	**Mean ± SD**		
Hemoglobin (g/dl)	10.71 ± 1.69	10.4 ± 0.35	0.52	0.609
WBCS ($10^3/mm^3$)	6.51 ± 2.28	8.24 ± 1.96	-2.408	0.022*
Albumin (g/dl)	3.39 ± 0.4	3.49 ± 0.42	-0.822	0.417
SGPT (U/L)	43.0 ± 1.06	42.78 ± 1.77	0.461	0.648
Sodium (mEq/L)	134.71 ± 4.7	136.67 ± 2.83	-1.537	0.133
Potassium (mg/dl)	3.55 ± 0.64	3.93 ± 0.83	-1.584	0.123
Calcium (mg/dl)	7.25 ± 1.12	9.14 ± 0.89	-5.695	<0.001**
Haptoglobin (mg/dl)	8.07 ± 1.96	6.133 ± 1.58	3.464	0.001**
	Median (IQR)	**Median (IQR)**	**Z**	**p**
Platelet ($10^3/mm^3$)	150(147.5 – 193.75)	295(295 – 300)	-4.828	<0.001**
SGOT (U/L)	45(45 – 81.25)	90(85 – 100)	-4.772	<0.001**
CRP (mg/dL)	0(0 – 1.25)	1(0.5 – 2)	-2.417	0.016*

CRP:C Reactive protein, EF : Ejection fraction ,FS : Fractional shortening ,IQR: interquartile

range, *: p<0.05 is statistically significant, **: p≤0.001 is statistically highly significant, SGOT : serum glutamic-oxaloacetic transaminase ,SGPT : Serum glutamic pyruvic transaminase, t: independent sample t test , Z :Mann Whitney test

Compared to compensated VSD group, the VSD group experiencing heart failure had substantially increased white blood cell (WBC), platelet, SGOT, calcium, and C-reactive protein levels. Hemoglobin, albumin, SGPT, sodium, and potassium levels were not significantly different. Plasma haptoglobin was significantly lower among VSD with heart failure group (table 5).

Table (6) Cardiac dimensions and LV systolic function in VSD subgroups:

	VSD without heart failure group	**VSD with heart failure group**	**Z**	**p**
	Median (IQR)	**Median (IQR)**		
MPA (mm)	13(10.75 – 16.25)	15(12.75 – 17.25)	-1.67	0.095
LPA (mm)	7(5 – 11)	7.5(5 – 10.5)	-0.895	0.371
RPA (mm)	7(5.75 – 10.25)	7.25(6 – 8)	-0.352	0.725
LA (mm)	21(15.75 – 30.25)	21(16 – 24)	-0.206	0.837
AO (mm)	13(11.75 – 20.5)	15(11.75 – 18.25)	-0.191	0.849
LVED (mm)	25.5(22 – 42)	26.5(24.25 – 32)	-0.286	0.775
LVES (mm)	16(13.75 – 28.5)	16.5(15 – 18.5)	-0.287	0.774
	Mean ± SD	**Mean ± SD**	t	p
EF (%)	69.06± 8.2	74.06 ± 7.59	-1.899	0.066
FS (%)	38.39 ± 5.54	43.17 ± 5.37	-2.626	0.013*

AO : Aortic Root dimension ,EF : Ejection Fraction ,FS : Fractional Shortening, IQR: interquartile range, LA: Left Atrium dimension, LPA: Left Pulmonary Artery dimension, LVED : Left Ventricular End Diastolic dimension ,LVES: Left Ventricular End Systolic dimension, MPA : Main Pulmonary Artery dimension ,RPA: Right Pulmonary Artery dimension, t :independent sample t test , Z :Mann Whitney test

When comparing patients with and those without heart failure, there were no statistically significant differences in diameters of main pulmonary artery, right pulmonary artery, left pulmonary artery, left atrium, aortic, left ventricular at end diastole, left ventricular at end systole, or ejection fraction. FS was higher in patients with VSD and HF but both subgroups had preserved LV systolic function (table 6).

Table (7) Plasma haptoglobin level among patients with VSD and heart failure:

	Mild HF	**Moderate HF**	**Severe HF**	F	p
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Haptoglobin(mg/dL)	7.08 ± 1.75	6.39 ± 0.86	4.78 ± 1.64	3.759	0.04*
Turkey HSD	P_1 0.66	P_2 0.13	P_3 0.04*		

F :One way ANAOVA test, *p<0.05 is statistically significant, p1 difference between mild and moderate HF group, p2 difference between moderate and severe HF group ,p3 difference between mild and severe HF group

A statistically significant correlation was found between plasma haptoglobin level and the severity of heart failure (p value=0.04). Post hoc analysis revealed that haptoglobin levels were lower in the severe HF compared to the mild HF subgroups (P_3 value=0.04, table 7).

Table (8) Performance of haptoglobin in diagnosis of heart failure:

Cutoff	AUC	95% CI	Sensitivity	Specificity	p
≤7.9mg/dl	0.92	0.854 – 0.986	94.4%	85.2%	<0.001**

The best cutoff of haptoglobin in diagnosis heart failure was ≤7.9 mg/dl with an area under curve of 0.92, sensitivity was 94.4%, and specificity was 85.2% (table 8, figure 1).

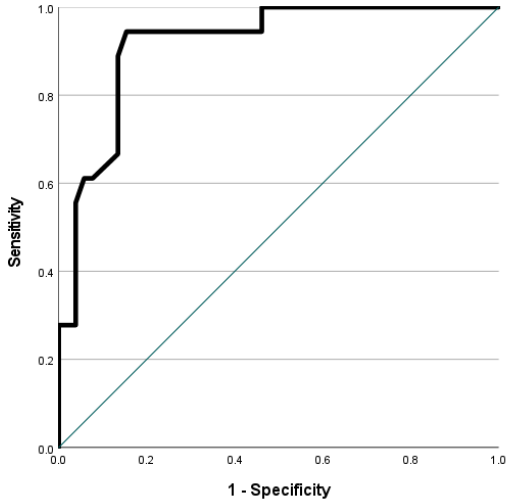

Figure (1) Performance of haptoglobin in diagnosis of heart failure

Discussion

Nearly 1% of live births are affected by heart defects, which are the most frequent birth abnormalities and the main cause of infant mortality. Congenital heart problems occur in 9.1 for every 1000 live births. The most prevalent type of congenital cardiovascular diseases is VSD [13].

After different types of tissue damage, innate immune system-which includes acute phase proteins- is triggered. Its activation causes immunological reactions that play a significant role in the pathophysiology of cardiovascular disease [14]. According to Kushner et al., when inflammation occurs, the plasma concentrations of acute phase proteins (APPs) rise or fall. Numerous cytokines are released into the bloodstream by local inflammatory cells in response to injury [15].

Haptoglobin is one of the proteins that are involved in the acute phase inflammatory response, according to Powanda et al. Additional to its antioxidant and antibacterial properties, it modulates numerous components of the acute phase response and functions as an antimicrobial agent [16].

In our patients with VSD and heart failure, the observed significant decrease in haptoglobin levels compared to those with VSD but no heart failure (6.133 ± 1.58 vs. 8.07 ± 1.96 mg/dL, P = 0.001, table 5) suggests increased hemolysis. This hemolysis may result from turbulent blood flow through the septal defect, leading to mechanical stress and destruction of red blood cells. Additionally, heart failure can exacerbate hemolysis due to associated systemic inflammation and altered hemodynamics. Monitoring haptoglobin levels in these patients could serve as a marker for hemolysis and help assess the progression of heart failure.

In contrast to our results, Karim et al. reported that patients with congestive heart failure had higher plasma haptoglobin levels [17]. The discrepancy between our findings and those of Karim et al., might be explained by differences in patient populations, underlying pathophysiological mechanisms, or the stage and etiology of heart failure.

Our findings highlight the potential diagnostic value of plasma haptoglobin levels in patients with VSD and heart failure. A haptoglobin cutoff value of ≤7.9 mg/dL demonstrated strong diagnostic performance, with an area under the curve of 0.92, indicating excellent discrimination between patients with and without heart failure. At this cutoff, haptoglobin sensitivity was 94.4% and its specificity was 85.2%, (p value<0.001, table 8, figure 1), suggesting that haptoglobin could be a reliable marker for identifying heart failure in VSD patients.

Furthermore, the statistically significant relationship between haptoglobin levels and the severity of heart failure reinforces its clinical relevance in our patients (table 7). The observed trend of lower haptoglobin levels correlating with increased severity of CHF suggests that hemolysis and the associated reduction in haptoglobin might play a key role in the pathophysiology of severe heart failure in VSD patients. These findings could support the integration of haptoglobin level assessment into clinical practice for better risk stratification and management of heart failure in VSD cases. Karim et al. studied Hp phenotypic distribution. They confirmed our results by showing a statistically significant correlation between plasma haptoglobin levels and the severity of heart failure. A larger proportion of the Hp2-2 phenotype was seen in CHF patients compared to healthy controls [17].

Lu et al. found that haptoglobin had a discriminatory effect on cardiovascular-related survival based on the area under the curve (AUC). They reported a greater number of non-survivors in the group with HP levels below 177.1 ng/ml compared to individuals with higher Hp values [18].

Conclusion

There might be a correlation between the severity of heart failure and haptoglobin levels, since the decrease in haptoglobin levels was more pronounced in individuals with severe HF than in those with mild HF. At a sensitivity of 94.4% and a specificity of 85.2%, haptoglobin proved to be a highly accurate diagnostic tool for identifying decompensated ventricular septal defect cases in our patients. Our results supported the integration of haptoglobin level assessment into clinical practice for better risk stratification of heart failure in VSD cases. Further studies are needed to confirm our findings.

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