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Estimation of Serum Levels of Pentraxin 3 in Infants with Congenital Heart Disease for Early Detection of Pulmonary Hypertension

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ABSTRACT

Background: Early detection of pulmonary hypertension (PH) in infants with congenital heart disease (CHD) is crucial for timely intervention and management. Serum pentraxin 3 (PTX3) has emerged as a potential biomarker for inflammatory diseases, but its association with PH in the context of CHD has not been thoroughly investigated.

Objective: To estimate serum PTX3 levels in CHD infants for early detection of PH and to correlate its level with the severity of PH.

Methods: This case-control study involved 80 infants, divided into 50 with CHD (further subdivided into (Group IA) 30 with PH and (Group IB) 20 without PH) and 30 healthy controls. Diagnosis of CHD and PH was confirmed via echocardiography, with PH severity graded according to established criteria. Serum PTX3 levels were measured using ELISA.

Results: Infants with CHD and PH (Group IA) exhibited significantly higher serum PTX3 levels (mean ± SD, 8.22 ± 4.38 ng/ml) compared to those with CHD without PH (Group IB) and healthy controls (3.91 ± 1.78 ng/ml, P<0.001). PTX3 levels showed a significant positive correlation with PH severity (r=0.557, P<0.001) and could predict PH incidence with an AUC of 0.832 (95% CI= 0.732 to 0.907), at a cutoff value >4.29 ng/ml with 86.67% sensitivity and 54.00% specificity.

Conclusion: Serum PTX3 levels are significantly elevated in infants with CHD and PH, correlating with PH severity. These findings suggest PTX3 as a promising biomarker for the early detection and severity assessment of PH in infants with CHD.

Keywords: Pentraxin 3, Congenital heart disease, Pulmonary hypertension, Infants, Biomarkers, Early detection.

INTRODUCTION

Congenital heart disease (CHD) is a leading cause of morbidity and mortality among children, making early and accurate identification vital for successful interventions and surgical outcomes [1]. CHD is classified into cyanotic and critical CHD, the latter of which includes right heart obstructive lesions, left heart obstructive lesions, and mixing lesions [2].

Recent reviews indicate a rise in CHD birth prevalence globally, though estimates remain low in less affluent countries due to limited diagnostic technology and missed early-life diagnoses [3].

The occurrence and frequency of pulmonary arterial hypertension (PAH) linked with CHD are 2.2 and 15.6 per million, respectively [4].

PAH, as classified by the 6th World Symposium on Pulmonary Hypertension, is characterized by a mean pulmonary artery pressure exceeding 20 mm Hg. This condition advances over time, causing dysfunction of the right ventricle and ultimately heart failure. It is frequently initiated by defects causing left-to-right shunting or obstructive disease of the left heart, which leads to post-capillary PH [5].

While echocardiography is the preferred screening method for PH, its limited availability necessitates alternative diagnostic approaches. An encouraging approach involves assessing particular biomarkers such as pentraxin 3 (PTX3), a novel biomarker that has proven effective in assessing PAH and differs from C-reactive protein (CRP). PTX3 is abundantly produced in the heart and is synthesized by vascular endothelial cells and macrophages. It has demonstrated specificity in diagnosing PAH, and its clinical applicability extends from adults to neonates and infants [6,7]. Elevated serum PTX3 levels indicate pulmonary artery pressure, making it a useful biomarker for predicting and monitoring the progression of PAH [8].

Hence, this study aimed to estimate serum PTX3 levels in CHD infants for PH early detection and to correlate its level with PH severity.

SUBJECTS AND METHODS

Study design

This case-control study involved 80 infants. The study was performed at Benha University Hospital over a period of 6 months from November 2022 to May 2023.

The subjects were divided into two primary groups: the case group, which included 50 infants diagnosed with CHD, and the control group, comprising 30 healthy infants. Within the case group, further categorization was made: Group IA included 30 infants with CHD and PH, while Group IB consisted of 20 infants with CHD but normal pulmonary pressure. The control group was chosen from healthy infants referred for follow-ups on other diseases, ensuring they were age and sex-matched with the case group.

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The inclusion criteria were infants under two years old with a diagnosis of CHD confirmed by echocardiographic findings by a pediatric cardiologist.

Exclusion criteria were infants suspected or confirmed to have infections or sepsis (evidenced by elevated CRP >10 mg/dL or +ve blood culture), severe respiratory failure (PaO_2 below 50 mm Hg despite adequate invasive MV), those with disseminated intravascular coagulation, or infective endocarditis.

METHODS

All participants were subjected to history taking (including antenatal, natal, postnatal, previous hospital admissions, medication, and family history), a complete physical examination with a focus on cardiovascular health, and a series of investigations. Routine investigations included CBC and CRP tests, alongside chest X-rays. Specific investigations involved echocardiography to diagnose CHD and PH, estimating systolic pulmonary pressure (SPP) using two-dimensional echo (2D), M-mode, and Doppler, in line with the American Heart Association's guidelines by pediatric cardiologists [9]. PAH was graded as mild (25–40 mmHg), moderate (40–60 mmHg), or severe (over 60 mmHg) according to **Ahmed *et al.*** [10].

To evaluate the plasma levels of PTX3, venous blood samples were collected from each infant immediately after the diagnosis of CHD or PAH through echocardiography and prior to the initiation of any medications or treatments. Plasma was isolated from EDTA-anticoagulated blood samples by centrifugation at 2700 g for 10 minutes and then stored at -80°C until further analysis. PTX3 levels were determined using ELISA kits.

Ethical considerations:

The study was done after being accepted by the Research Ethics Committee, Benha University. All caregivers of the patients provided written informed consents prior to the enrolment of their children. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical analysis was conducted using SPSS v28 (IBM Inc., Armonk, NY, USA). Quantitative variables were expressed as mean and standard deviation (SD) and compared between the two groups using unpaired Student's t-test. Qualitative variables were presented as frequency and percentage (%) and analyzed using the Chi-square test. Pearson or Spearman correlation was employed to assess the degree of correlation between two variables. Receiver operating characteristic (ROC) curve analysis was also performed. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows that patients within case group were then categorized into 2 groups, group IA included 30 infants with congenital heart disease with PH and group IB included 20 infants with congenital heart disease with normal pulmonary pressure.

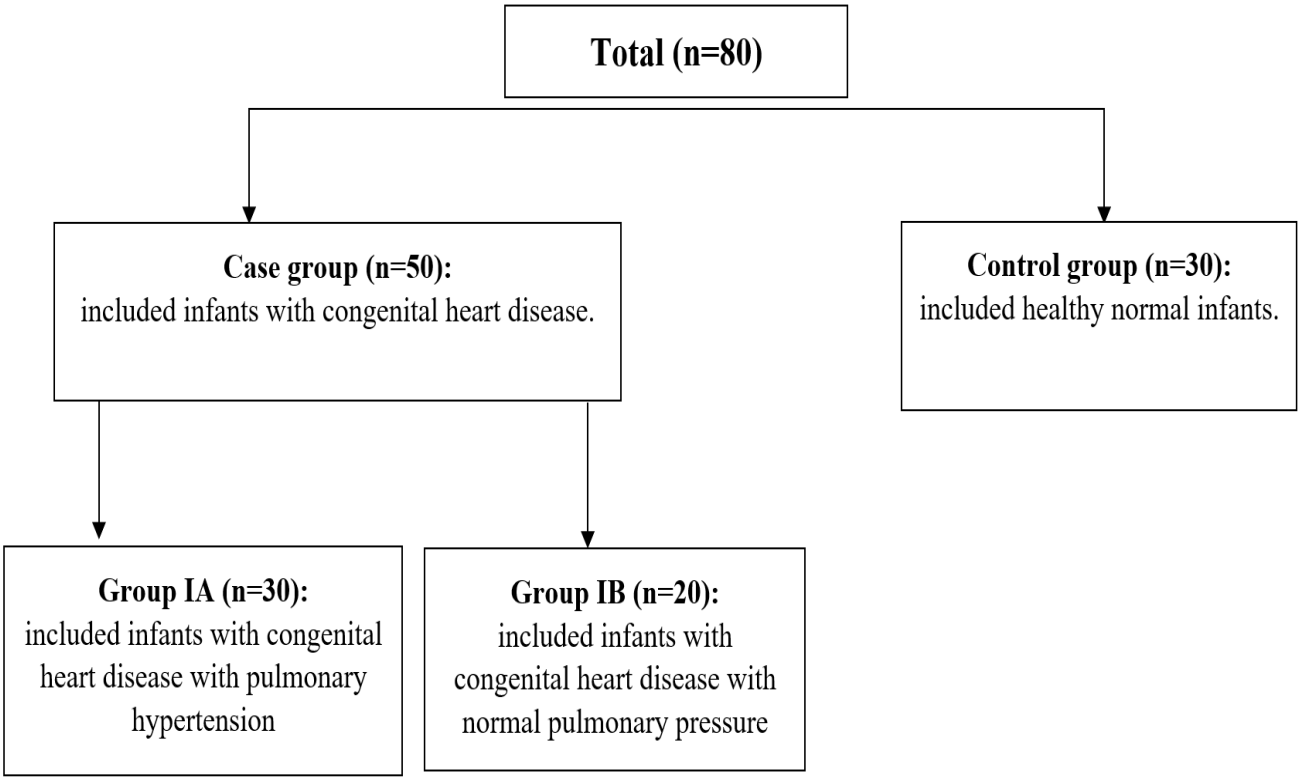

Figure 1: Algorithm illustrating the studied groups.

There were no significant differences in age and sex between the main study and control groups **(Table 1)**.

Table 1: Personal characteristics among studied groups

		Case group (n=50)	Control group (n=30)	P value
Age (months)	**Mean ± SD**	5.6 ± 4.5	7.4 ± 5.1	0.103
	Range	1 - 18	1 - 18	
Sex	**Male**	23 (46%)	14 (46.67%)	0.954
	Female	27 (54%)	16 (53.33%)	

SD: standard deviation.

Echo diagnosis of the study groups (Case group) is illustrated in **Table 2**.

Table 2: Echo diagnosis of the study groups (Case group)

	Case group (n=50)
Isolated large ventricular septal defects	7 (14%)
Large VSD associated with other congenital heart disease	29 (58%)
Complete atrioventricular canal defect	7 (14%)
Complex congenital heart disease	9 (18%)
Isolated patent ductus arteriosus	4 (8%)
PDA associated with other congenital heart disease	8 (16%)
Cyanotic congenital heart disease	5 (10%)
ASD associated with other congenital heart disease	22 (44%)
Isolated ASD	1 (2%)

VSD: ventricular septal defects, PDA: patent ductus arteriosus, ASD: atrial septal defect.

The most common echo diagnosis in both of group IA and IB was VSD associated with other congenital heart disease as shown in **Figure 2**.

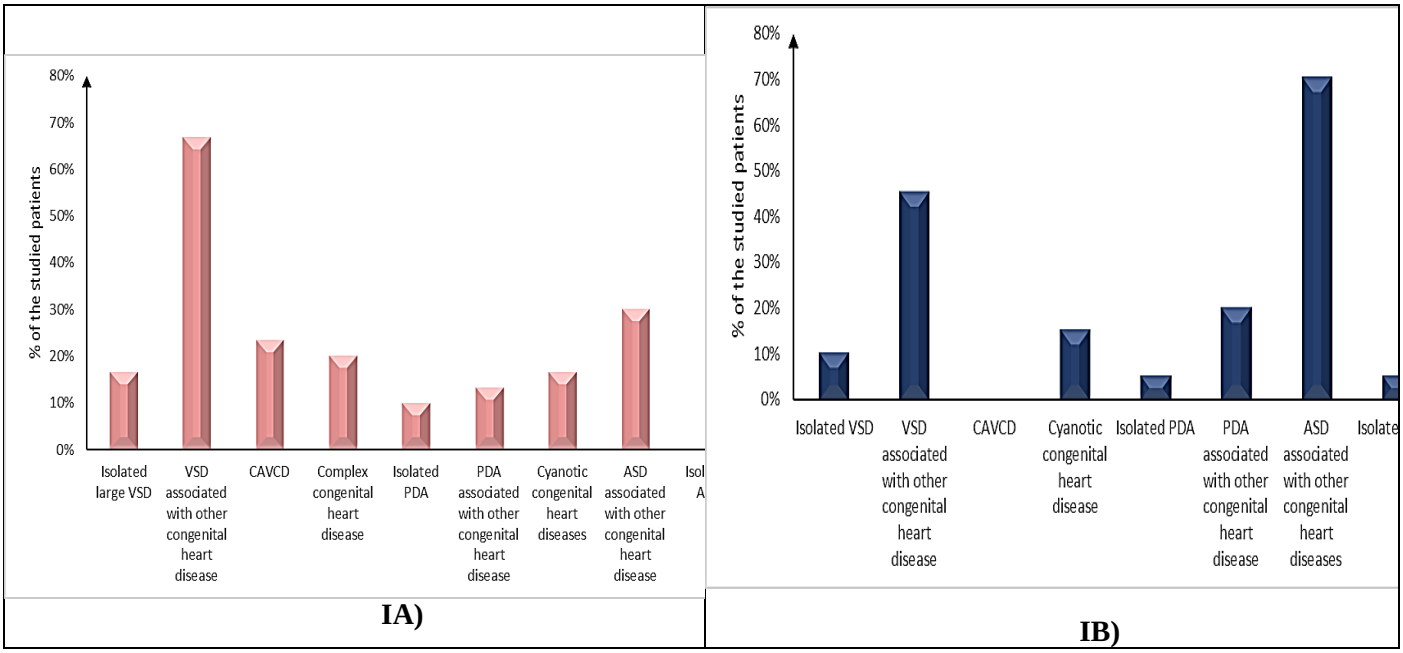

Figure 2: Echo diagnosis among Group IA and Group IB.

The pulmonary artery systolic pressure (PASP) of infants was significantly higher in the study (case group) compared to control group (39.58 ± 15.49 vs. 16.2 ± 5.84 mmHg, respectively, P <0.001). PASP was considerably different among the studied groups (P<0.001). PASP was considerably higher in group IA compared to group IB and control group (P<0.001) and was considerably higher in group IB (but within normal range) compared to control group (P<0.001) **(Figure 3)**.

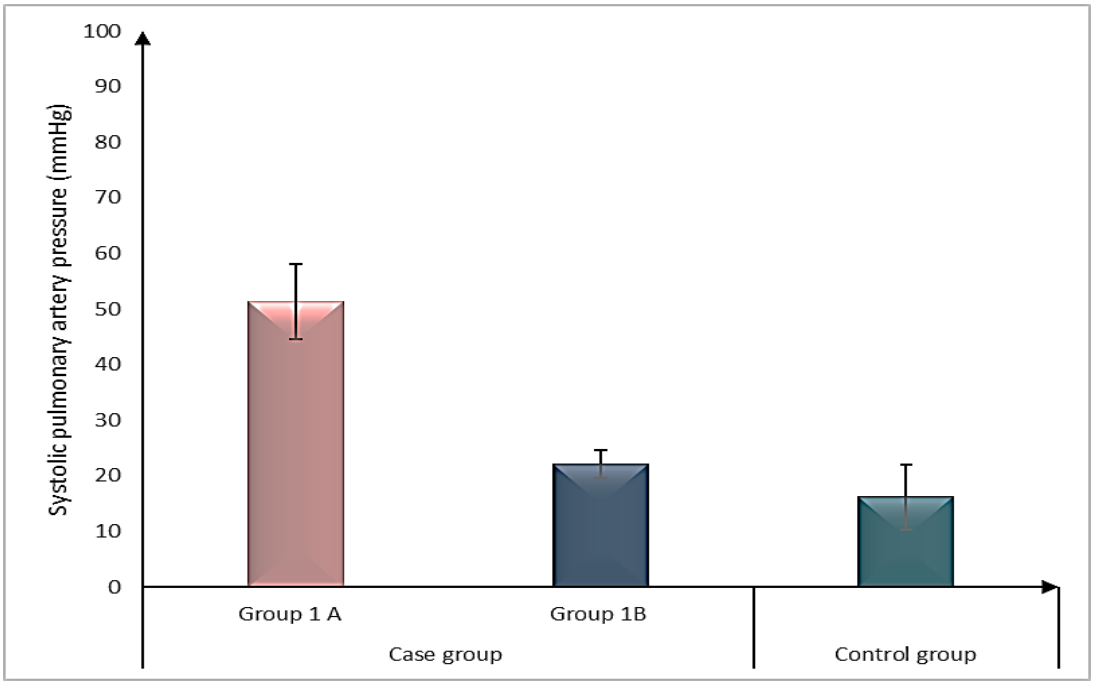

Figure 3: Comparison of systolic pulmonary artery pressure of the two studied groups and control group.

The mean serum PTX3 level was considerably higher in the study group compared to control group (6.89 ± 3.91 vs. 3.91 ± 1.78, respectively, P<0.001). Serum PTX3 was considerably different among the studied groups (P<0.001). Serum PTX3 was considerably higher in group IA compared to group IB and control group (P=0.002, <0.001 respectively) and was insignificantly different between group IB and controls **(Table 3)**.

Table 3: Comparison of the mean serum pentraxin 3 level of the two studied groups and control group

		Case group (n=50)		Control group (n=30)	P value
		Group IA (n=30)	Group IB (n=20)		
Serum pentraxin 3 (ng/ml)	**Mean ± SD**	8.22 ± 2.08	4.88± 1.06	3.91 ± 0.78	**<0.001***
P value		**P1**	**0.002***	**<0.001***	
		P2		0.065	

SD: standard deviation, *: statistically significant, P1: p value compared to Group IA, P2: p value compared to Group IB.

Serum PTX3 demonstrated a significant positive correlation with severity of PH. There were insignificant correlations between serum PTX3 and both age and sex **(Table 4)**.

Table 4: Statistical correlation between mean serum pentraxin 3 level and different parameters

	Serum pentraxin 3 (ng/ml)	
	r	**P**
Age (months)	-0.043	0.703
Sex	0.097	0.394
Severity of pulmonary hypertension	0.557	**<0.001***

r: correlation coefficient, *: statistically significant

The level of serum PTX3 can significantly predict the incidence of pulmonary artery hypertension with AUC of 0.832 (95% CI= 0.732 to 0.907), and at cutoff value >4.29 ng/ml with 86.67% sensitivity, 54.00 % specificity, 53.1% PPV and 87.1% NPV **(Figure 4)**.

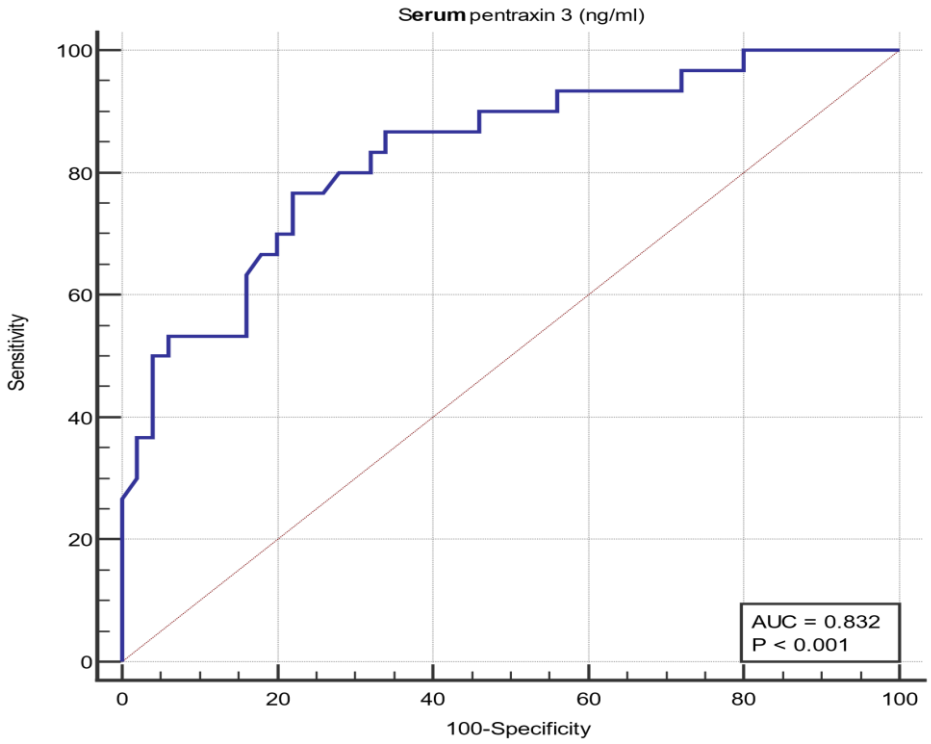

Figure 4: ROC curve analysis of the mean serum pentraxin 3 level for prediction of pulmonary artery hypertension.

In group IA (infant with CHD with PH), serum PTX3 was insignificantly different between cyanotic and acyanotic CHD **(Table 5)**.

Table 5: Statistical comparison between cyanotic and acyanotic congenital heart disease regarding the mean serum pentraxin 3 level in Group IA.

		Group IA (n=30)		P value
		Cyanotic (n=5)	Acyanotic (n=25)	
Serum pentraxin 3 (ng/ml)	**Mean ± SD**	6.01 ± 1.14	8.66 ± 2.16	0.223

SD: standard deviation.

In group IB (infant with congenital heart disease with normal pulmonary pressure), Serum PTX3 was insignificantly different between cyanotic and acyanotic congenital heart disease **(Table 6)**.

Table 6: Statistical comparison between cyanotic and acyanotic congenital heart disease regarding the mean serum pentraxin 3 level in Group IB.

		Group IB (n=20)		P value
		Cyanotic (n=5)	Acyanotic (n=25)	
Serum pentraxin 3 (ng/ml)	**Mean ± SD**	6.4 ± 1.07	4.61 ± 1.41	0.106

SD: standard deviation.

DISCUSSION

Congenital heart disease (CHD) is the most common birth defect, posing challenges due to its variability and severity, necessitating urgent care in a quarter of cases [11]. Its association with PH highlights the need for detailed research and effective management. Despite echocardiography being the standard for PH detection, the potential of biomarkers like PTX3 is being explored for early diagnosis in infants with CHD, aiming to improve outcomes by assessing the severity of PH more accurately [7].

Therefore, this study aimed to estimate serum levels of pentraxin three in infants with congenital heart disease for early detection of PH and to correlate its level with severity of PH.

In our study, we utilized echocardiography to diagnose systolic PAP based on the peak velocity of the tricuspid regurgitation (TR) jet, which directly indicates right ventricular pressure and, by assumption, PAP when combined with an assumed right atrial pressure of 5 mmHg [10]. Regarding the type of CHD and its diagnosis via echocardiography, our findings align with those of **Karakurt *et al.,*** who conducted a cross-sectional study on serum PTX3 and CRP levels in children with severe PAH, primarily from left-to-right shunt defects, assessed by cardiac catheterization and undergoing treatment with specific pulmonary vasodilators. Their control group included 39 age and gender-matched healthy children [12]. Similarly, **Pascall *et al.*** noted that conditions like atrioventricular septal defects or truncus arteriosus often lead to early PAH development [13].

In this study, serum PTX3 levels were significantly higher in the study group (6.89 ± 3.91 ng/ml) compared to controls (3.91 ± 1.78 ng/ml, P<0.001), with group IA (8.22 ± 4.38 ng/ml) showing significantly higher levels than both group IB (4.88 ± 1.76 ng/ml, P=0.002) and controls (P<0.001), while group IB and controls did not differ significantly. Supporting these findings, **Wakeel *et al.*** identified PTX3 as a novel predictor for neonatal PH, with significantly higher levels in the PH group versus controls and CHD groups [8]. Similarly, **Farhadi *et al.*** and **Karakurt *et al.*** reported higher PTX3 levels in PH groups compared to CHD without PH and healthy controls, underscoring PTX3's potential as a marker for PH in neonates [9, 12].

Our study found a significant positive correlation between serum PTX3 levels and the severity of PH (r=0.557, P<0.001), but no significant correlation with age or sex. Similarly, **Tamura *et al.*** [14] demonstrated that PTX3 levels were notably higher in PAH patients compared to healthy controls and provided a good balance of sensitivity (74%) and specificity (84%) for PAH detection. Additionally, in patients with connective tissue disease-PAH, PTX3 levels were significantly elevated compared to those without PAH. **Wakeel *et al.*** also found a positive correlation between PTX3 and pulmonary artery pressure, highlighting PTX3 as a primary predictor of PAH [8]. These findings support the role of PTX3 in vascular diseases and inflammation, consistent with its involvement in the pathological processes of PAH, including angiogenesis and vascular remodeling [15].

Carrizzo *et al.* [16] discovered that PTX3 impairs endothelial function in mice by affecting the P-selectin/matrix metalloprotease1 pathway, leading to endothelial cell damage, nitric oxide signaling disruption, and increased blood pressure. This mechanism was observed to be more prevalent in hypertensive patients, suggesting PTX3's potential as a biomarker for hypertension and a target for treatments aimed at mitigating endothelial dysfunction and cardiovascular disease. Moreover, in PAH, PTX3 is

considered a more precise and sensitive biomarker than the traditionally used brain natriuretic peptide, marking a significant advance in diagnostic approaches [17].

Our study found that serum PTX3 levels have a high diagnostic accuracy in predicting PAH, with an AUC of 0.832 (95% CI= 0.732 to 0.907, P<0.001). At a cutoff value of >4.29 ng/ml, it achieved 86.67% sensitivity and 54.00% specificity, along with 53.1% PPV and 87.1% NPV. These results highlight the potential of serum PTX3 as a reliable biomarker for PAH detection. Similarly, **Farhadi *et al.*** reported a comparable diagnostic efficacy with an AUC of 0.683 and a plasma PTX3 cutoff of >2.47 ng/mL, yielding 90.5% sensitivity and 33.3% specificity, underscoring the biomarker's value in clinical settings [9].

The limitations of our study include a relatively small sample size, potentially affecting the generalizability of our findings. Although mean serum PTX3 levels have demonstrated potential as a biomarker for PAH, it's important to acknowledge that elevated levels might also indicate other inflammatory conditions. Furthermore, unaddressed external factors, such as genetic predisposition and environmental influences, could account for the variations observed in the mean serum PTX3 levels, introducing additional complexity to our analysis.

CONCLUSION

Serum PTX3 levels are significantly elevated in infants with CHD and PH, correlating with PH severity. These findings suggest PTX3 as a promising biomarker for the early detection and severity assessment of PH in infants with CHD. Further research is needed to integrate PTX3 measurement into clinical practice for managing CHD-associated PH.

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- **Conflict of Interest:** Nil.

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