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Diagnostic and Prognostic Role of Plasma Osteopontin Levels in Children with Pulmonary Hypertension Associated with Congenital Heart Disease at Tanta University Hospitals

Rana Ahmed El-Zayady [a*], Ahmed Hamdy Shabana [b], Hesham Ahmed El-Serogy [c] and Amr Mohamed Zoair [b]

[a] M.B.B.CH, College of Medicine, Tanta University, Egypt.
[b] Department of Pediatrics, College of Medicine, Tanta University, Egypt.
[c] Department of Clinical Pathology, College of Medicine, Tanta University, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Natural progression of CHD often results in the development of pulmonary arterial hypertension (PAH), which is a leading cause of death and disability.
Aim: The goal of this work is to clarify the prognostic and diagnostic usefulness of OPN in children with PAH-CHD, as well as to determine whether or not its level correlates with clinical and echocardiographic data of PAH-CHD.
Methodology: Fifty CHD children and twenty-five healthy children served as a control subject in this research. Plasma OPN levels were measured using ELISA kits after a thorough medical history, physical examination, and series of tests (radiological, laboratory, and diagnostic).
Place and Duration of Study: Patients were chosen from those hospitalised to the Tanta University Hospital Pediatric Cardiology Unit, Pediatric Department, between April 2021 and April 2022.
Results: There was significant increase of plasma OPN levels in PAH- CHD patients as compared to CHD patients without PH as compared to control group. OPN levels was also found to be

**Corresponding author: E-mail: ranaelzayady@gmail.com;*

positively correlated with age, disease severity, mPAP and RV diameter and negatively correlated with RV E/A ratio. OPN can be used as a diagnostic biomarker with sensitivity 92% and specificity 96% and as a prognostic biomarker with sensitivity 86% and specificity 90%.
Conclusion: Plasma levels of OPN were significantly elevated in PAH-CHD children, and these levels were correlated to the severity of PH and echocardiographic parameters. OPN levels were markedly elevated in PAH-CHD patients with bad prognosis.

Keywords: Osteopontin; children; pulmonary hypertension; congenital heart diseases.

1. INTRODUCTION

Approximately 1% of neonates have congenital heart defects (CHD) at birth. Anomalies incorporating the interconnection between the heart chambers, or the great arteries are associated with higher pulmonary blood flow and pressure over time, despite the absence of severe pulmonary stenosis, resulting in gradual remodelling of the pulmonary arteries and increased pulmonary vascular resistance (PVR) [1].

Inadequate treatment of CHD is contributed to pulmonary arterial hypertension (PAH), particularly in the presence of a significant left to right (L-R, systemic-to-pulmonary) shunt that must be reversed. It is possible that chronic revealing of the pulmonary bed to elevated blood flow and pressure may cause vascular rearrangement and dysfunction. A rise in PVR and, in extreme cases, a reversal of the shunt, may result from this [2]. In terms of hemodynamics, PAH is known as a rise in mean pulmonary artery pressure (mPAP) of at least 25 mmHg while left-sided filling pressures remain normal (left ventricular end-diastolic pressure or pulmonary capillary wedge pressure 15 mmHg) [3]. No longer the definition of PAH relies on the systolic pulmonary artery pressure (PAP) or exercise-derived values [4].

PAH linked with CHD (PAH-CHD) in children results in a low survival probability if a timely diagnosis and proper treatment are not administered. Undiagnosed PAH-CHD may result in a variety of consequences and is linked with a very high morbidity rate, including, for example, PAH-CHD. Cyanosis is linked to exercise intolerance and a variety of possible problems, such as hyperviscosity, erythrocytosis, and irregularities of haemostasis, stroke, endocarditis and cerebral abscesses. Cardiac problems include syncope, congestive heart failure, cardiac arrhythmias and sudden death. In Eisenmenger's syndrome patients, this impairment is substantially more severe than in Other PAH -CHD patients. Additionally, chronic hypoxemia linked with Eisenmenger's disease has a variety of complications. Among them include secondary polycythemia, gout, hyperviscosity, joint and long bone discomfort due to hemoptysis, hypertrophic osteoarthropathy, and thrombosis [2]. Biomarkers in the blood, such as N-terminal pro-B-type natriuretic peptide (NT-pro BNP), osteopontin (OPN) and B-type natriuretic peptide (BNP) [5] and NT-pro BNP [6], may aid in the diagnosis, severity, and prognosis of paediatric PAH.

Circulating biomarker in PAH: [7].

The following is a summary of the plasmatic biomarkers presently utilised in PAH:

Indicators of:

Activation of dysfunctional neurohormones (endothelin, natriureticpeptides, copeptin and adrenomedullin).

Myocardial damage (troponins).

Inflammation/oxidative stress (isoprotans, interleukins [IL], C-reactive protein).

Vascular injury / remodelling (growth differentiation factor-15, von Willebrand factor angiopoietin).

End organ failure (sodium, uric acid, creatinine).

OPN a pleiotropic cytokine, was found as one of the top five genes over expressed in the lung explants of patients with group I PAH, in contrast to controls. Its regulation correlates highly with hemodynamic severity [8].

The expression of OPN was first discovered in cancerous epithelial cells, but it is now recognised to be present in other cell types, involving fibroblasts and cardiomyocytes. Tissue remodelling, inflammation, scarring and

metastasis are just a few of the many biological processes in which OPN plays a role. Either as a soluble cytokine or an immobile part of the extracellular matrix, it is thought to mediate signals between the two [6,9].

It was shown that lung OPN is highly expressed with age, boosting the proliferative ability of PA-SMCs in older children relative to younger and promoting pulmonary vascular remodelling. OPN was protected against hypoxia-induced PH and was unaffected by age-dependent pulmonary vascular remodelling. In patients with iPAH or COPD, pulmonary vascular remodelling was linked to high OPN regulation in pulmonary arteries [10,11].

2. THE OBJECTIVES OF THIS WORK WERE

1. To evaluate OPN plasma levels in PAH children related with CHD (PAH-CHD).
2. To evaluate the OPN prognostic and diagnostic value of in these patients and correlation of its levels with echocardiographic and clinical data of PAH-CHD.

3. METHODS

3.1 Study Design: Cross-Sectional Comparative Study

This research included 50 CHD children: Twenty-five children with PAH-CHD, twenty-five CHD children clinically descrbied and by Echo-Doppler, and 25 matched age and sex children as controls.

Study location and population and sampling Technique: Patients were randomly selected between April 2021 and April 2022, from patients hospitalised to the Pediatric Cardiology Unit, Pediatric Department, Tanta University Hospital. While an age-matched control group was recruited from family and neighbors.

The used statical tests were:

1-Chi-square test
2 -Monte Carlo correction
3 - F-test (ANOVA)
4- Kruskal Wallis test
5 - Spearman coefficient
6 -Receiver operating characteristic curve (ROC)
7 - Sensitivity
8 - Specificity
9 - Positive Predictive value (PPV)
10 – Negative Predictive value (NPV)

Patients were classified into two groups:

Group 1: Twenty-five PAH children related to CHD (PAH-CHD).
Group 2: Twenty-five CHD children without PAH.

25 matched age and sex healthy children as controls.

Inclusion criteria: less than 18 years CHD children with or without PAH.

Exclusion criteria: (Lung diseases, heart failure, renal diseases, acute or chronic illness or inflammation, brain tumors, epithelial cell tumors, bone tumors, or any type of cancer).

All children included in the study were subjected to the following:

- Full history taking.
- Thorough clinical examination: including signs of CHD, heart rate, signs of PH, respiratory rate and complete local cardiac assessment.

Grade of PH was assessed as mild (mPAP = 25-40 mm Hg), moderate (mPAP = 41-55 mm Hg) or severe (mPAP >55 mm Hg) [10].

- Investigations:

A. Plain X-ray chest and heart: Cardiothoracic ratio (CTR) was performed for evaluation of cardiomegaly.
B. ECG: Using 3 channel 1000 apparatus.
C. Echocardiographic evaluation using the Vivid 7 ultrasound system (GE Medical System, Horten, Norway) with 7 and 4s MHz multi-frequency transducers.

Two dimensional, doppler, and M-mode echocardiography were used to evaluate the following [12]:

1. Type of coronary heart disease.
2. Normalized pulmonary arterial pressure (mPAP).
3. Right ventricular wall diameter
4. Function of the right ventricular systole
5. Function of the right ventricular diastole
6. Employing an enzyme-linked immunosorbent assay sandwich test (ELISA).

Number of investigators: Four investigator had participated in this work.

The 1st: is the main author who suggest the study and revised the research.

The 2nd: Collect cases of research, making tables and write the research.
The 3rd: Did Echo parameters of cases and shared in writing the research.
The 4th: Did the laboratory tests of cases.

Method of collecting data: Each participant was subjected to two millilitres blood drawn from a random vein. by use of disposable sterilized plastic syringes during the first 24 hours of hospitalization. The needle of the syringe was then removed, and each sample was allowed to pass gently along the wall of Patient-specific EDTA vacutainer labeled tube. The blood was gently mixed, centrifuged at 2000-3000 r.p.m. for 20 minutes to separate the plasma, and then frozen at -20°C until the time of OPN analysis.

Availability of diagnostic and prognostic test: OPN is available and easily obtained with a high sensitivity and specificity.

Principle [13]:

- Testing for OPN concentration makes use of a double-antibody elisa in this kit.
- The human OPN monoclonal antibody-coated enzyme well was supplemented with OPN.
- Biotinylated OPN antibodies were mixed with streptavidin-hrp to create an immunological complex.
- A second round of washing and incubation was performed to get rid of any unbound enzyme.
- Chromogen solutions a and b were then added.
- Under the influence of acid, the liquid becomes blue, and then yellow.
- Human substance OPN sample concentration strongly associated with colour chroma.

4. RESULTS

Our data showed no statistically remarkable changes in age (P>0.05) among the three groups studied: patients with PAH-CHD were, on average, 11.4 ± 4.8 months old, while patients with CHD without PH were, on average, 11.7±4.6 months old, and the control group was, on average, 12.8 ± 4.3 months old (P > 0.05).

Patients with PAH-CHD weighed 7.76±1.9 kg on average, whereas those with CHD without PH weighed 8±2.2 kg and those in the control group weighed 10.2±1.6 kg. Mean body weight was considerably lower in the PAH-CHD and CHD groups compared to the control group (P< 0.001).

Table 1 shows that no significant variation was recorded between the sexes among the groups tested (P > 0.05). There were 10 males and 15 females in the PAH-CHD group, 14 men and 11 females in the CHD patients without PH group, and 13 males and 12 females in the control group.

The OPN levels increased considerably in PAH-CHD patients compared to CHD patients without PH contrasted to control group (P = 0.05) Table 2. The mean plasma level of OPN in patients with PAH-CHD was 612.002±239.2 nmol/ml compared to 228,01±110.3 in CHD patients without PAH nmol/ml, whereas the mean plasma level of OPN in the control group was 166.5 ± 82.36 nmol/ml, with significant elevation of plasma OPN levels in PAH- CHD patients as compared to CHD patients without PH as compared to control group (P = 0.05).

Patients with mild PH had a mean plasma OPN level of 473.57± 131.8 nmol/ml, those with moderate PH had a mean of 558± 45.3 nmol/ml, and those with severe PH had a mean of 812.64± 34.2 nmol/ml, as shown in table below. There was a considerable elevation of plasma OPN levels according to severity of PH (P <0.001) Table 3.

Patients with PAH-CHD who had a poor prognosis (death, CHF, or rehospitalization) had a substantially higher mean plasma level of OPN (780.36 ± 90.25 nmol/ml) than those with a favorable prognosis (550.85 ± 70.18 nmol/ml; P=0.05), as shown in Fig. 1.

Weight, RR, HR, CTR, and RVOT FS% were not significantly correlated with plasma OPN levels (P>0.05). (Figs. 2, 3) demonstrate a positive relationship between plasma OPN levels and RV diameter and mPAP (P>0.05), whereas Fig. 4 shows a negative relationship between plasma OPN levels and RV E/A ratio (P=0.05).

As shown in Fig. 5, the results indicated that the sensitivity of OPN at cutoff 420 nmol/ml as a diagnostic biomarker in PAH- CHD patients was 92%, the specificity was 96%, and the area under the ROC curve was a 0.718; and the sensitivity of OPN as a prognostic biomarker in PAH- CHD patients was 86%, the specificity was 90%, and the area under the ROC curve was a 0.701 when using a cutoff value of more than 485 nmol/ml Fig. 6.

Table 1. Demographic data in the studied groups

	PAH-CHD (n = 25)		CHD (n =25)		Controls (n = 25)		Test of Sig.	p
	No	%	No	%	No	%		
Sex							$\chi 2=$	0.413
Male	10	40.0	14	56.0	13	52.0	1.77	
Female	15	60.0	11	44.0	12	48.0		
Age (Months)							F=	0.558
Range	2.0 – 48		3.0 - 48		2.0 – 48		0.589	
Mean ± SD.	11.4±4.8		11.7±4.6		12.8±4.3			
Weight (kg)							F=	0.001*
Range	2.0 – 13		4.0 -14		4.0 – 15		7.851	
Mean ± SD.	7.76±1.9		8 ±2.2		10.2±1.6			
Sig bet Groups	P1=0.119		P2=0.001*		P3=0.01*			

Table 2. Plasma OPN levels in the studied groups

Osteopontin (nmol/ml)	PAH-CHD (n = 25)	CHD (n =25)	Controls (n = 25)	H	p
Range	400 – 850	200 – 250	142.5– 190	3.8	.026*
Mean ±SD	612.002 ± 239.2	228.01 ± 110.3	166.5 ± 82.36		
Sig. bet. groups	P1=0.033*, P2<0.04*, P3<0.034*				

Table 3. Plasma levels of OPN according to degree of pulmonary hypertension

Osteopontin (nmol/ml)	Grades of PH			H	p
	Mild (n = 15)	Moderate (n =6)	Severe (n = 4)		
Mean ± SD	473.57 ± 131.8	558 ± 45.3	812.64 ± 34.2	0.564	<.001*
Post –Hoc analysis	Mild vs moderate	Mild vs severe	Moderate vs severe		
	P1=0.001*	P2=0.001*	P3=0.001*		

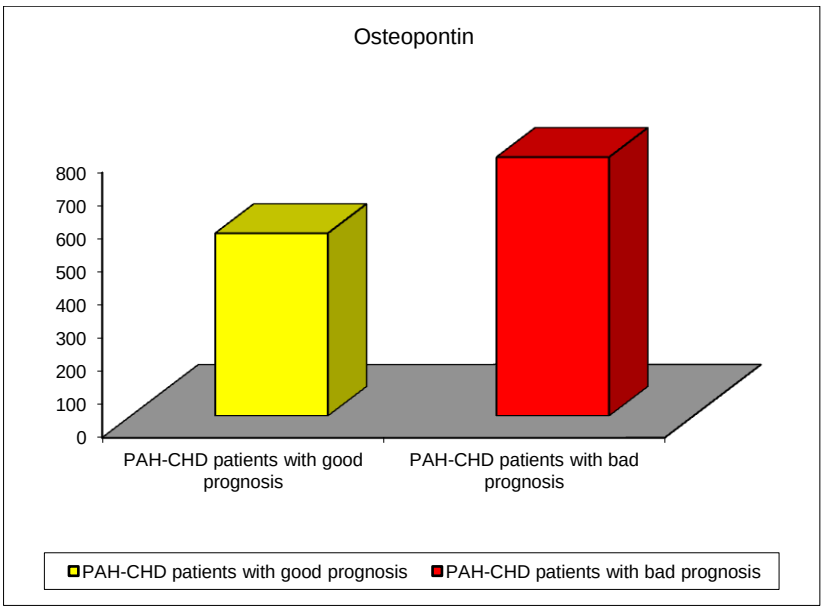

Fig. 1. OPN levels in PAH-CHD patients with good and bad prognosis

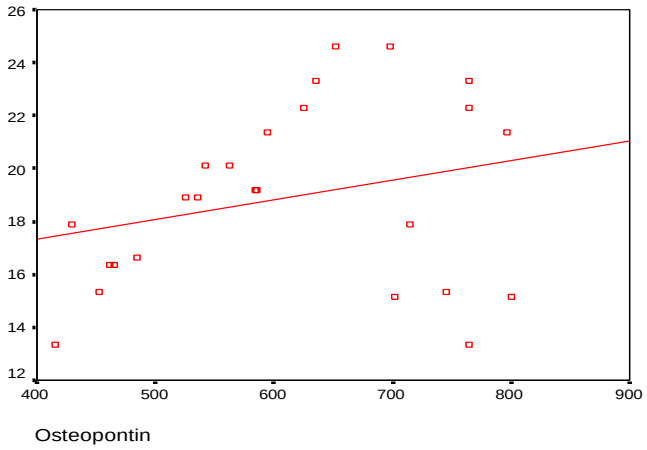

Fig. 2. Correlation between OPN plasma levels and RV diameter in PAH-CHD cases

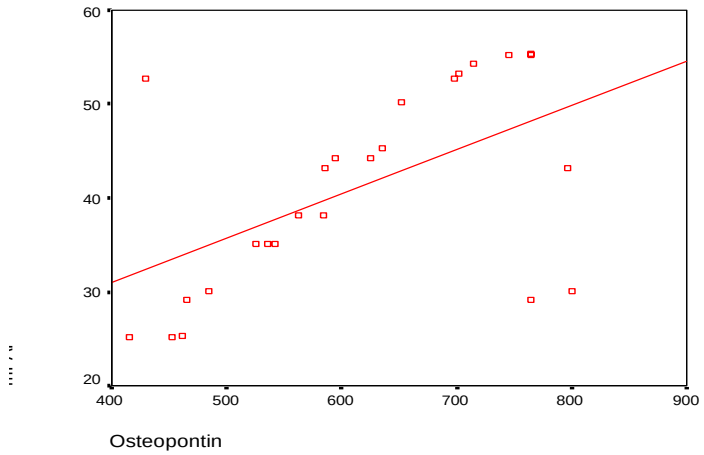

Fig. 3. Correlation between OPN plasma levels and mPAP in PAH-CHD cases

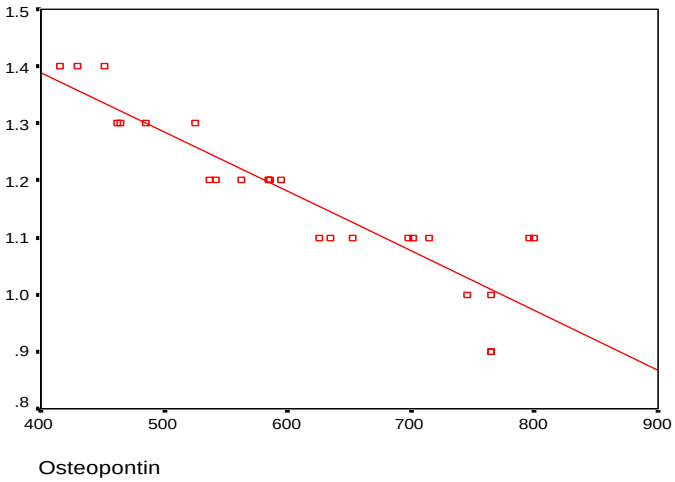

Fig. 4. Correlation between OPN levels in PAH- CHD cases and RV E/A ratio

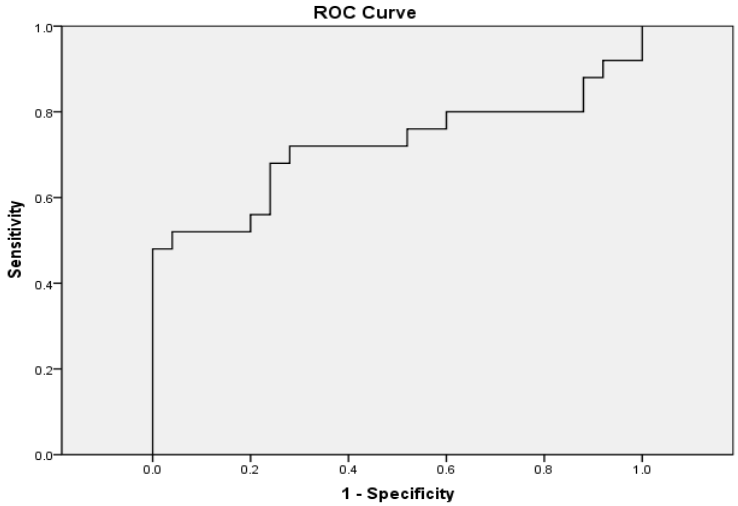

Fig. 5. ROC curve for OPN to diagnose PH in CHD patients where AUC was 0.718

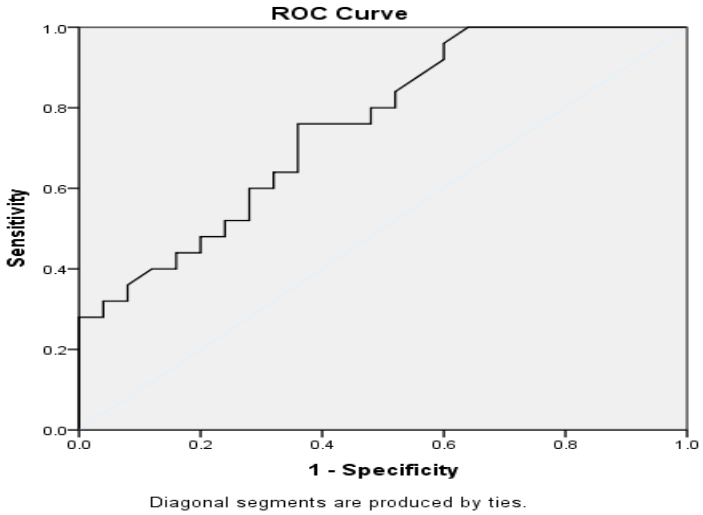

Fig. 6. ROC curve for OPN to prognose PH in CHD patients where AUC was 0.701

5. DISCUSSION

PAH -CHD is related with a considerable reduction in body weight in CHD patients with and without PH, versus the control group. In accordance with li et al. [14], who found considerable weight loss in CHD patients due to insufficient caloric intake, malabsorption, and higher energy needs as a result of increased metabolism, hormonal changes, diminished growth factors and genetic abnormalities contribute to lower body weight in CHD patients with or without PH [15].

In comparison to the control group, PAH-CHD patients had significantly increased heart rate and respiratory rate. Comparable to our results, Park and Kay et al. [16,17] found that tachycardia and tachypnea in CHD children, particularly in severe instances of PH or CHF, are caused by catecholamine surge and sympathetic activation.

Cardiothoracic ratio was shown to be elevated in CHD whether related with PAH or not, both groups have a greater ratio than the control group. This is consistent with Pande et al. [18] who concluded that PAH-CHD patients had an elevated cardiothoracic ratio and cardiomegaly.

In our research, the PAH-CHD group had a larger right ventricular dilation and diameter than the other two groups (CHD and control group). Milan et al. [19] showed that chronic pressure

overload in individuals with PH promotes progressive RV hypertrophy and dilation.

The current investigation also demonstrated that the E/A ratio of the right ventricle was considerably lower in the PH group compared to the other two groups. This is due to the elevated PH, which lowers RV diastolic function as measured by the RV E/A ratio on echocardiography. Also consistent with Milan et al. [19].

In our investigation, OPN levels were considerably higher in PAH-CHD children than in CHD patients without PH or in the control group. This is consistent with the findings of Saker et al. [20], who found substantially elevated plasma OPN levels in PAH-CHD patients. Also, our results agree with those of Nerukar et al. [21].

OPN is a prognostic and diagnostic biomarker in PH, although there are additional diagnostic markers, such as growth differentiation factor-15, angiopoietin, natriuretic peptides, von Willebrand factor and endothelin as well [7].

Mechanisms by which OPN contributes to the onset of PH are not fully understood, but may include its effects on cell proliferation, intracellular signalling, migration and differentiation [20]. In hypoxic PAH, increased OPN was shown to be mostly concentrated in pulmonary adventitial fibroblasts, but not in endothelial or medial cells [22].

These reactions may be coordinated by interactions with surrounding extracellular matrix (ECM) proteins, although the processes involved are still poorly understood. In response to various pathophysiological stimuli, the ECM dynamic structure is constantly evolving. Cell surface receptors, such as integrins, receive signals from the ECM, which in turn regulates cell migration, proliferation, intracellular signalling, and differentiation [23].

Basic structural proteins like elastin and collagen, specialized proteins like proteoglycans, fibronectin and matricellular proteins like OPN and periostin are all part of the ECM [24].

Recently, it was reported that the active phenotype (very proliferative, migratory) of plasma artery smooth muscle cells and pulmonary adventitial fibroblasts in hypoxic PAH has been linked to OPN [20].

At an early stage in the progression of intimal thickening after artery wall damage, the transition from a contractile to a synthetic phenotype seems to be a critical event. Evidence from in vitro studies demonstrated that when rabbit SMC reached the phenotypic proliferative phase [25], mRNA expression of OPN dramatically.

OPN might be triggered by pressure or volume overload in the systemic arteries. Interestingly, OPN is rarely produced in healthy systemic arteries but is very prevalent in damaged systemic arteries [26].

Increased OPN was also found to be endothelium and medially localized in the pulmonary arteries that had undergone muscularization. The hypoxic and shunted forms of PAH may differ because they have different etiologies; in hypoxic PAH, alveolar hypoxia may first provoke pulmonary adventitia, while in shunted PAH, increasing intraluminal flow and pressure primarily affect medial smooth muscle cells and pulmonary endothelial cells [27].

High levels of OPN were found in patients with severe PH, since its production is dependent on hemodynamic alterations, as was also shown in the present investigation. Moreover, compared to individuals with a positive prognosis, those with a poor prognosis showed a substantial rise in OPN. This agrees with the findings of Farber and Loscalzo [28].

OPN levels were also shown to positively correlate with both mPAP and RV diameter in the current investigation. This is consistent with the findings of Marco et al. [29], who discovered a positive association between OPN and mPAP, indicating a pathogenic function that is directly related to the elevated pressure in the pulmonary vasculature.

To back up the findings of Marco et al. [29], who reported that OPN had a substantial link with RV E/A ratio in patients with PAH-CHD, the present investigation demonstrated a negative correlation between plasma OPN levels and RV E/A ratio (diastolic dysfunction).

Pathway analysis showed a meaningful function of OPN in improving and promoting vascular remodelling, and in particular PA-SMC proliferation, which has fascinating parallels to the uncontrolled expansion found in cancer cells. These findings provide a solid foundation for future research into OPN as a measure of disease severity and potential treatment target in PAH [29].

Recent research has shown that OPN is a good predictive biomarker for predicting poor prognosis in PAH-CHD patients. Consistent with other research, we find that circulating OPN predicts survival in patients with IPAH [30]. Patients with PH and right ventricular dysfunction exhibited significantly greater OPN levels compared to PH patients with maintained right ventricular function, as described by Rosenberg et al. [6].

This study confirmed previous findings that OPN can be used as a diagnostic biomarker with sensitivity of 92% and specificity of 96% in agreement with Rosenberg et al. [6], who reported sensitivity of 84% and specificity of 65%, and as a prognostic factor with sensitivity of 86% and specificity of 90%.

6. CONCLUSION

Significantly increased plasma OPN levels were found in children with PAH-CHD, and these levels corresponded with the severity of PH and echocardiographic parameters of its evaluation. Patients with PAH-CHD who had a poor prognosis had significantly higher plasma levels of OPN. OPN has the potential to serve as a cardiac biomarker in children with PAH-CHD, with excellent sensitivity and specificity for diagnosis and monitoring of the disease's progression.

ETHICAL APPROVAL AND CONSENT

Approval for this research was gotten from the Ethics and Research Committee of Tanta University. Before being enrolled into the study, informed written consent was obtained from each participant for the case report to be published.

SIGNIFICANCE OF STUDY

This study found that patients with PAH-CHD may have many Complications which may be related to severity of disease and may affect their life. Early detection and follow up will help to reduce disease severity and affectation of heart. OPN can be used as a diagnostic marker for PAH-CHD. It also give an idea about the severity of the disease. It can be used as a prognostic marker.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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