Original Research Article

Plasma Osteopontin Levels in Children with Pulmonary Hypertension Associated with Congenital Heart Disease

ABSTRACT

Background: Pulmonary arterial hypertension (PAH) is one of the major causes of morbidity and mortality in CHD patients during the natural course of the disease.
Aim: The aim of this work is to evaluate the plasma levels of Osteopontin (OPN) in children with PAH-CHD and to evaluate the diagnostic and prognostic value of OPN in these patients, and correlation of its level with clinical and echocardiographic data of PAH-CHD.
Methodology: This study was conducted on fifty (50) children with congenital heart disease (CHD) and (25) healthy children as a control group were included in this study Medical history , clinical, radiological , laboratory investigations , plasma osteopontin levels were determined using ELISA kits to assay the level OPN in samples.
Place and Duration of Study: Patients were selected from those admitted at Pediatric Cardiology Unit, Pediatric Department, Tanta University Hospital, in the period from April 2021 to April 2022.
Results: Showed that 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 positively correlated with age, disease severity, mPAP and RV Dimeter 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.

Key words: Osteopontin, Children, Pulmonary hypertension, Congenital heart diseases.

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1. INTRODUCTION:

Congenital heart disease (CHD) occurs in approximately 1% of live births. Anomalies involving communications between the cardiac chambers or the great arteries, in the absence of significant pulmonary stenosis, are associated with increased pulmonary blood flow and pressure, leading to progressive remodeling of pulmonary arteries and increased pulmonary vascular resistance (PVR) [1]

Pulmonary arterial hypertension (PAH) is a potential complication of CHD, particularly when a significant left-to-right (L-R, systemic-to-pulmonary) shunt remains uncorrected. Long standing exposure of the pulmonary bed to increased blood flow and pressure can result in vascular remodeling and dysfunction. This may lead to a rise in pulmonary vascular resistance (PVR) and, if severe enough, may cause reversal of the shunt [2]

Pulmonary hypertension is defined hemodynamically as elevation of mean pulmonary artery pressure (mPAP) $\geq$ 25 mmHg with normal left-sided filling pressures (left ventricular end-diastolic pressure or pulmonary capillary wedge pressure $\leq$ 15 mmHg) [3].The definition of pulmonary hypertension is no longer based upon the systolic pulmonary artery pressure (PAP) or exercise derived values [4].

Without early diagnosis and appropriate treatments, PAH associated with CHD (PAH-CHD) in children causes' poor survival rate. Measuring biomarkers in the blood can facilitate pediatric PAH management, as they provide valuable information on disease diagnosis, severity, and prognosis; such as B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-pro BNP) [5] and osteopontin [6]

Osteopontin (OPN), a pleiotropic cytokine, was identified among the top five upregulated genes in the lung explants from patients with group I pulmonary arterial hypertension, compared to normal controls. Its expression correlated strongly to hemodynamic severity [7]

Osteopontin was first found in malignant epithelial cells, but is now recognized to be expressed in various cell types, including cardiomyocytes and fibroblasts. OPN is involved in several biologic processes, such as tissue

remodeling, scarring, inflammation, and metastasis. It can exist as an immobilized component of extracellular matrix or as a soluble cytokine, likely promoting the communication between extracellular matrix and cardiomyocytes [6, 8]

It was found that lung OPN expression increased with age, enhancing the proliferative capacity of PA-SMCs in old children compared with young, and promoting pulmonary vascular remodeling. OPN was not affected by age-dependent pulmonary vascular remodeling and was protected against hypoxia-induced PH. In patients with COPD or iPAH, pulmonary vascular remodeling was associated with marked OPN expression in pulmonary vessels [9]

2. METHODS:

2.1 Sample size: This study was conducted on fifty (50) children with congenital heart disease (CHD) and (25) children of matched age and sex as a control group.

2.2 Study location and population: Patients were selected from those admitted at Pediatric Cardiology Unit, Pediatric Department, Tanta University Hospital, in the period from April 2021 to April 2022.

Patients were classified into two groups:

Group 1: Twenty-five (25) children with pulmonary arterial hypertension associated with congenital heart disease (PAH-CHD).

Group 2: Twenty-five (25) children with CHD with no PAH.

Twenty-five (25) healthy children, of matched age and sex served as a control group.

2.3 Inclusion criteria: Children less than 18 years, with CHD with or without PAH.

2.4 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

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

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 measured for assessment of cardiomegaly.

B. ECG: Using 3 channel 1000 apparatus.

C. Echocardiographic assessment: Using Vivid 7 ultrasound machine (GE Medical System, Horten, Norway), with 7 and 4s MHz multi- frequency transducers.

Doppler, two dimensional, and M-mode echocardiography were used for assessment of the following [11]:

1) Type of CHD.
2) Mean pulmonary artery pressure (mPAP).
3) Right ventricular diameter
4) Right ventricular systolic function

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5) Right ventricular diastolic function
6) Using a sandwich enzyme-linked immunosorbent assay test (ELISA).

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Blood sampling:

Two milliliter of random venous blood sample was collected from each subject 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 EDTA vacutainer tube labeled with the patient name. The blood was mixed gently and centrifuged for 20 minutes at 2000-3000 r.p.m for separation of plasma, which was stored at -20℃ till the time of analysis of OPN.

Principle [12]:

- The kit uses a double-antibody ELISA to assay the level OPN in samples.
- OPN was added to monoclonal antibody enzyme well which is precoated with human OPN monoclonal antibody.
- OPN antibodies labeled with biotin and combined with Streptavidin-HRP were added to form immune complex.
- Incubation was carried out and washing again to remove the uncombined enzyme.
- Then chromogen solution A, B was added.
- The color of the liquid changes into blue, and at the effect of acid, the color finally becomes yellow.
- The chroma of color and the concentration of the Human Substance OPN of sample were positively correlated.

3.RESULTS:

Our results showed that the mean age of patients with PAH-CHD was 11.4 ± 4.8 months ,the mean age of patients with CHD without PH was 11.7± 4.6 months, whereas the mean age of the control group was 12.8± 4.3 months, with no statistically significant difference between the three groups as regards the age (P>0.05).

As regards body weight, the mean weight of patients with PAH-CHD was 7.76±1.9 kg, the mean weight of patients with CHD without PH was 8 ±2.2 kg, whereas the mean weight of the control group was 10.2±1.6 kg. The mean weight of PAH--CHD and CHD groups were significantly lower than that of the control group (P<0.001).

As regards sex, the group of PAH-CHD included 10 males and 15 females, the group of CHD patients without PH included 14 males and 11 females, whereas the control group included 13 males and 12 females, with no significant difference between the studied groups (P >0.05) as shown in table (1).

Results also showed that the mean plasma level of OPN in patients with PAH-CHD was 612.002 ± 239.2 nmol/ml, the mean plasma level of OPN in patients with CHD without PH was 228.01 ± 110.3 nmol/ml, whereas the mean plasma level of OPN in the control group was 166.5 ± 82.36 nmol/ml, with significant increase of plasma OPN levels in PAH- CHD patients as compared to CHD patients without PH as compared to control group (P = 0.05) as shown in table (2) .

Our results showed that the mean plasma OPN level in patients with mild PH was 473.57 ± 131.8 nmol/ml, the mean plasma OPN level in patients with moderate PH was 558 ± 45.3 nmol/ml, whereas the mean plasma OPN level in patients with severe PH was 812.64 ± 34.2 nmol/ml, with significant increase of plasma OPN levels according to severity of PH (P<0.001) as shown in table (3).

Results also showed that the mean plasma level of OPN in PAH-CHD patients with bad prognosis (death, CHF or rehospitalization) (780.36 ± 90.25 nmol/ml) was significantly higher than those with good prognosis (550.85 ±70.18 nmol/ml) (P=0.05) as shown in figure (1)

Results also showed that there was no significant correlation between plasma OPN levels and weight, RR, HR, CTR, or RVOT FS% (P>0.05). There was significant positive correlation between plasma OPN levels and RV diameter and mPAP as shown in figure (2 ,3) but there was significant negative correlation between plasma OPN levels and RV E/A ratio as shown in figure (4) (P=0.05).

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Results showed that when using a cutoff value of more than 420 nmol/ml, the sensitivity of OPN as a diagnostic biomarker in PAH- CHD patients was 92%, the specificity was 96%, and area under the ROC curve wasa 0.718as shown in figure (5) and when using a cutoff value of more than 485 nmol/ml, the sensitivity of OPN as a prognostic biomarker in PAH- CHD patients was 86%, the specificity was 90%, and area under the ROC curve was 0.701 as shown in figure (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								
Male	10	40.0	14	56.0	13	52.0	$\chi 2$= 1.77	0.413
Female	15	60.0	11	44.0	12	48.0		
Age (Months)								
Range	2.0 - 48		3.0 - 48		2.0 - 48		F= 0.589	0.558
Mean ± SD.	11.4±4.8		11.7±4.6		12.8±4.3			
Weight (kg)								
Range	2.0 - 13		4.0 -14		4.0 – 15		F= 7.851	0.001*
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 osteopontin (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 Osteopontin (OPN) according to degree of pulmonary hypertension.

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

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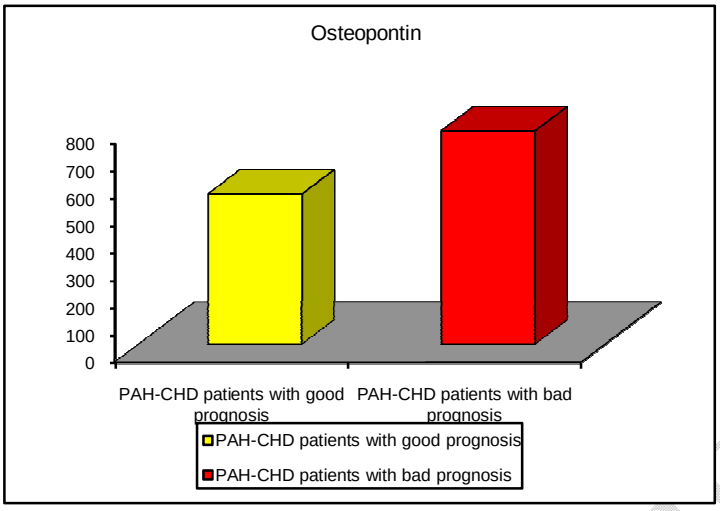

Figure (1):Osteopontin levels in patients with good and bad prognosis in PAH-CHD group.

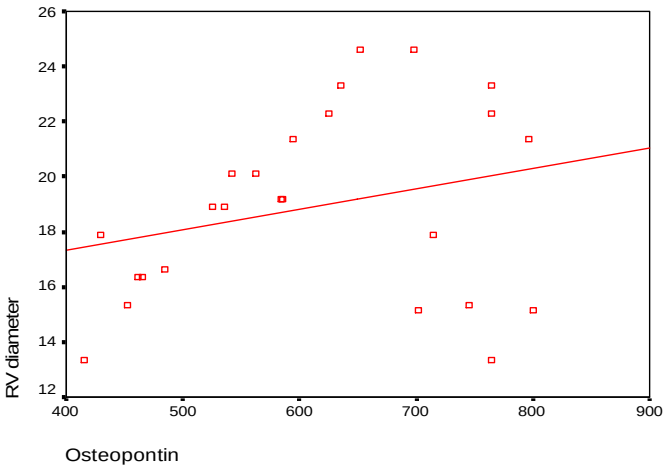

Figure (2): Correlation between plasma OPN levels and RV diameter in PAH-CHD group.

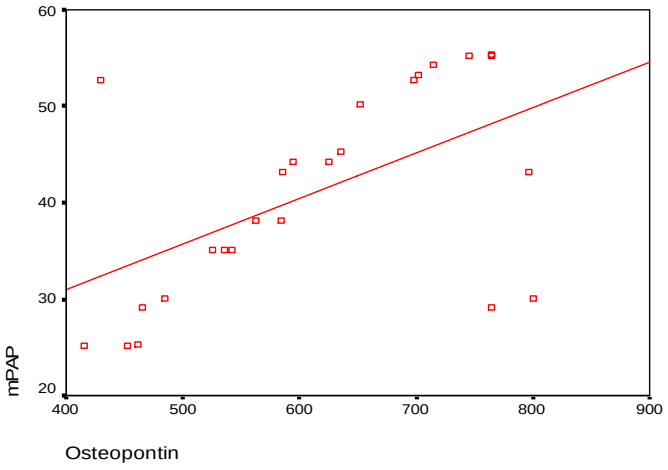

Figure (3) :Correlation between plasma OPN levels and mPAP in PAH-CHD group.

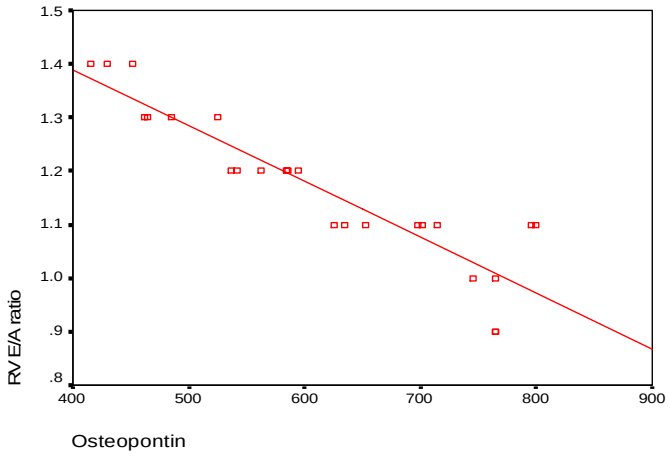

Figure (4): Correlation between plasma OPN levels and RV E/A ratio in PAH- CHD group.

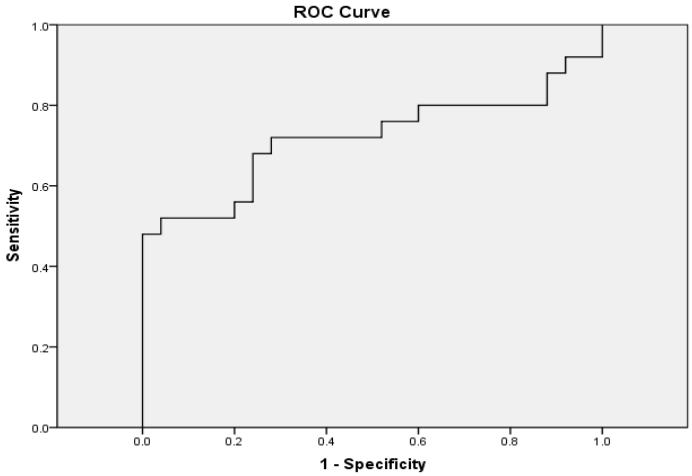

Figure (5):ROC curve for OPN to diagnose PH in CHD patients where the area under the curve (AUC) was 0.718.

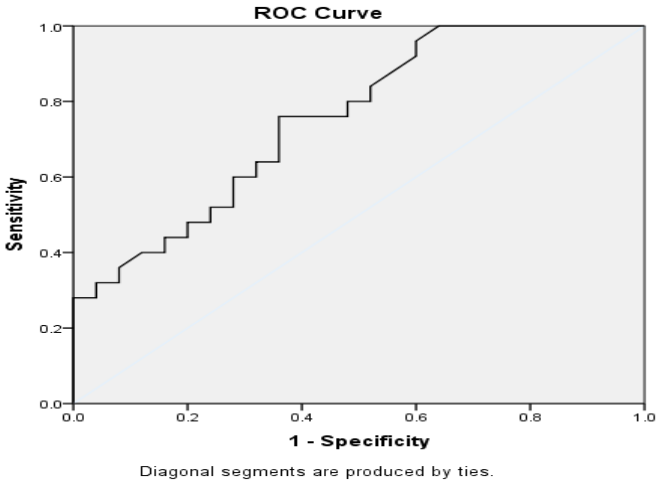

Figure (6):ROC curve for OPN to prognose PH in CHD patients where the area under the curve (AUC) was 0.701.

4. DISCUSSION:

PAH_CHD ia associated with significant decrease in body weight in patients with CHD with and without PH as compared to control group. Agreement with Li *et al*, [13], who reported significant decrease in body weight in CHD patients. Decreased body weight in patients with CHD with or without PH is due to inadequate caloric intake, malabsorption, and increased energy requirements caused by increased metabolism, genetic abnormalities, decreased growth factors and hormonal changes [14]

PAH_CHD patients have significant increase in heart rate and respiratory rate in patients with CHD with or without PH as compared to control group. This was in agreement with Park and Kay *et a*l, [15, 16], who reported that tachycardia and tachypnea in children with CHD, especially severe cases with PH or CHF, occur due to sympathetic activation and catecholamine surge.

It was found that cardiothoracic ratio is increased in CHD either associated with PAH or not, while both groups have higher ratio than control group .This comes in correlation with Pande *et al,* [17] who reported that patients with PAH-CHD have cardiomegaly and increased cardiothoracic ratio.

Echocardiographic results in our study revealed right ventricular dilatation and increased diameter in PAH-CHD group than the other two groups (CHD and control group) [18] who reported chronic pressure overload in patients with PH that causes progressive hypertrophy and dilation of the RV.

The present study also revealed that right ventricular E/A ratio was lower significantly among pulmonary hypertension group than the other two groups. This is explained by the increased PH that decreases the RV diastolic function, which is represented by RV E/A ratio in echocardiographic examination. This also correlates with Milan *et al,* [18].

In our study, we found that plasma levels of osteopontin (OPN) were significantly elevated in PAH-CHD children as compared to CHD patients without PH as compared to control group. This is in agreement with Saker *et al,* [19] who reported that plasma OPN levels were significantly higher in PAH-CHD patients. Also, our results come in aggretment with Nerukar *et al,* [20]

OPN is diagnostic and prognostic marker in pulmonary hypertension, but there are also other markers that help in diagnosis of pulmonary hypertension such as growth differentiation factor-15, natriuretic peptides, endothelin, and angiopoietin and von Willebrand factor **[**21]

The mechanism of the role of osteopontin in development of pulmonary hypertension is still unclear, but it may be explained by regulation of proliferation, migration, intracellular signaling, and differentiation state of the cells [19]. Interestingly, in hypoxic PAH, increased OPN was mainly localized in pulmonary adventitial fibroblasts but not endothelial or medial cells [22]

Although the mechanisms involved in these responses remain obscure, the possibility that they are coordinated through interactions with surrounding extracellular matrix (ECM) proteins is one possible mechanism .The ECM is a dynamic structure continually changing in response to pathophysiological stimuli. ECM provides signals to adjacent cells via cell surface receptors such as integrins, which ultimately regulate proliferation, migration, intracellular signaling, and differentiation state of the cells [23]

ECM include basic structural proteins such as collagen and elastin, specialized proteins such as fibronectin and proteoglycans, and matricellular proteins including osteopontin and periostin [24]

Recent studies indicated that OPN is an essential contributor to the activated phenotype of plasma artery smooth muscle cells (highly proliferative, migratory) and pulmonary adventitial fibroblasts in hypoxic PAH [19]

Transition from a contractile to a synthetic phenotype appears to be an early, key event during the development of intimal thickening after arterial wall injury. In vitro evidence showed that the mRNA expression of OPN significantly increased when rabbit SMC entered the phenotypic proliferative phase [25]

OPN could be induced in response to pressure or volume overload in systemic arteries. Interestingly, OPN is seldom expressed in normal systemic arteries but is abundant in injured systemic arteries [26]

Furthermore, the increased OPN was mainly detected in muscularized pulmonary arteries in a pattern compatible with endothelial and medial localization. These differences may be attributed to the discrepancy of initial impetuses in the 2 PAH subsets; that is; in hypoxic PAH, alveolar hypoxia may first provoke pulmonary adventitia, whereas in shunted PAH increased intraluminal flow and pressure mainly affect the pulmonary endothelial cells and medial smooth muscle cells [27]).

The current study also showed that OPN levels correlated to the degree of pulmonary hypertension with high levels in severe PH, as it depends on the hemodynamic changes .Also, OPN increased significantly in patients

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with bad prognosis as compared to patients with good prognosis. This is in accordance with Farber and Loscalzo [28].

Moreover, the present study showed that there was significant positive correlation between OPN levels and mPAP and RV diameter. This comes in agreement with Marco *et al*, [29], who found that OPN has significant positive correlation with mPAP ; suggesting a pathological role that is directly linked to the increased pressure in pulmonary vasculature.

The current study also showed that plasma OPN levels have significant negative correlation with RV E/A ratio (diastolic dysfunction) ,that comes in agreement with Marco *et al,* [29], who found that OPN has significant correlation with RV E/A ratio in patients with PAH-CHD .

OPN correlates very well with hemodynamics, while pathway analysis supported a relevant role of OPN in enhancing and promoting vascular remodeling, and in particular PA-SMC proliferation, with interesting similarities to the uncontrolled growth seen in cancer cells. These results set a useful framework to further investigate OPN as a marker of disease severity and possible therapeutic target in PAH [29] .

The current study showed that OPN can be used as a promising predictive biomarker for predicting bad prognosis in PAH-CHD patients. This is in accordance with earlier studies which mentioned that circulating OPN predicts survival in patients with IPAH [30]. Moreover, Rosenberg *et al,* [6] reported that patients with PH and right ventricular dysfunction had significant higher OPN levels compared to PH patients with preserved right ventricular function.

The present study showed that OPN could be used as a diagnostic biomarker with sensitivity 92% and specificity 96%, that comes in agreement with Rosenberg *et al,[*6], who reported that sensitivity was 84% and specificity 65% and can be used as prognostic factor with sensitivity 86% and specificity 90% .

5. 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 of its assessment. Plasma levels of OPN were markedly elevated in PAH-CHD patients with bad prognosis. OPN could be used as a cardiac biomarker in PAH-CHD children, with good diagnostic and prognostic value and high sensitivity and specificity.

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.

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.

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