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Fibroblast growth factor 23 in children with HF: a prospective cohort study

Keywords: Prognosis, fibroblast growth factor 23, children and HF

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Abstract

Background: Elevated fibroblast growth factor 23 (FGF23) levels have been associated with mortality in adults with heart failure (HF), but data on FGF23 levels in pediatric HF are limited. In this prospective cohort study, we aimed to assess prognostic value of fibroblast growth factor 23 in children with HF.

Methods: We prospectively enrolled 40 children with chronic HF and 20 matched healthy controls. For each patient complete diagnostic workup was performed including transthoracic echocardiography with tissue Doppler study to assess cardiac systolic and diastolic functions. Serum FGF23, renal function tests, parathyroid hormone, serum calcium and phosphate were measured for patients & controls. N-terminal Pro-Brain Natriuretic Peptide (NT-proBNP) was measured for patients. Symptom severity was assessed with modified Ross HF classification for children. Patients were followed for 1 year for mortality or hospitalization for HF which were considered as poor prognosis criteria.

Results: Compared to control group, patients with HF had significantly higher FGF23 levels (60.20 ± 11.04; 355.68 ± 97.27 pg/ml respectively, P <0.001). Three patients died and eleven were admitted with HF. These fourteen patients with poor prognosis had significantly higher FGF23 level than those with favorable prognosis (421.86 ± 75.50; 320.04 ± 89.56 pg/ml, respectively P <0.001). FGF23 was positively correlated with NT-pro-BNP and left ventricular end diastolic diameter and negatively with ejection fraction and fractional shortening. Ability of FGF23 to predict poor prognosis in patients was analyzed using receiver operating characteristic curve. The cutoff point was 375pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV=91.7 and AUC=0.878. Multivariate logistic regression analysis revealed that only FGF23 & Ross class III are independent predictors of poor prognosis in HF children.

Conclusion: FGF23 levels are elevated in children with HF and are associated with increasing HF clinical severity. FGF23 has a good prognostic accuracy in detecting HF children with poor prognosis.

Keywords: Prognosis, fibroblast growth factor 23, children and HF

What is known about the subject? FGF23 has been related to adverse cardiovascular events in adult patients with HF. Increased FGF23 levels have been associated with left ventricular (LV)

hypertrophy and impaired LV function. Studies on adult populations had demonstrated that FGF23 could be used to predict clinical outcome in patients with HF.

What this study adds? FGF23 levels were increased significantly with increasing HF clinical severity and in patients with poor prognosis. FGF23 has a potential good prognostic value as a novel biomarker in pediatric HF with cutoff point >375pg/ml predicting children with poor prognosis.

Introduction:

Pediatric HF (HF) is a complex disease process, which can occur secondary to a variety of etiologies, including congenital heart diseases, cardiomyopathy, or acquired conditions as well. It remains a major cause of morbidity and mortality in childhood with significant health & economic burden worldwide (1).

Exploring new cardiac biomarkers for HF helps clinicians identify disease progression, predict the clinical outcomes, provide protective strategies, and select the proper treatment and monitor therapy (2).

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone secreted by osteoblasts and osteocytes in response to increased phosphate levels regulating renal phosphate homeostasis and vitamin D metabolism, by stimulating phosphaturia and inhibiting calcitriol synthesis (3).

Besides its role in mineral metabolism, FGF23 has a direct action on the cardiovascular system and has been recently related to adverse cardiovascular events in adult patients with HF and involved in cardiac remodeling (4-7). Increased FGF23 levels have been associated with left ventricular (LV) hypertrophy and impaired LV function (8-11). It has been linked with alterations in myocyte calcium handling (12) and upregulation of the renin-angiotensin system (13). Interestingly, adult studies showed that FGF23 not only could predict clinical outcomes in patients with acute and stable HF (14, 15) but also was independently associated with all-cause death and HF in a community-living older person (16).

Comparable data about FGF23 in children with HF are limited. Therefore, we aimed to assess the prognostic significance of FGF 23 in children with HF and to determine a cutoff point for predict children with poor prognosis.

Methods

This prospective cohort study was carried out at the Pediatric cardiology unit of Menoufia University Hospital between January 2020 and February 2021. Informed consent was obtained from the guardian of each participant included in the study. All procedures performed during the study were following the ethical standards of Menoufia University Institutional Research Committee.

Patient and public involvement statement: Patients and/or the public were involved in the design, conduct, reporting and dissemination plans of this research. Public healthcare clinics, but not families, were involved in the content and design.

Eligibility criteria

Inclusion criteria: For the case group, we targeted children with HF as defined by the International Society for Heart and Lung Transplantation Practice Guidelines for Management of HF in Children (17) during follow-up clinic visits. The control group included a group of age and sex-matched healthy children who were referred to the cardiology clinic because of innocent murmurs.

Exclusion criteria: diseases affecting FGF 23 as chronic kidney disease and rickets, any congenital anomalies other than congenital heart disease, and age less than 2 months and more than 18 years.

All included patients & controls were subjected to detailed history taking with collecting patient demographics and comprehensive clinical examination. Patients were assessed for the functional status of HF by Modified ROSS classification system of HF in infants and children (18). Patients were followed for 1 year for an endpoint of all-cause mortality or HF hospitalization and patients were classified accordingly into 2 groups: patients admitted to hospital with HF or died (poor prognosis) and patients who survived and did not require admission to hospital with HF (favorable prognosis).

Investigations:

Blood samples were collected then centrifuged, aliquoted, labeled, and stored at −80 °C until batched assays were performed. Because of the well-defined role of FGF23 in renal & bone diseases investigations using standard procedures were performed including complete blood count (CBC) and renal functions, serum calcium, serum phosphate and parathyroid hormone. Estimated glomerular filtration rate (eGFR; in ml/min/1.73 m2) was determined using an established formula of e GFR=k× height (cm)/serum creatinine (mg/dl) (19). Serum intact FGF23 levels were measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit "Elabscience, USA". To correlate FGF23 with other biomarkers of HF, we measured NT-proBNP using ELISA kits in children with HF.

Echocardiography

Transthoracic echocardiography (TTE) imaging with tissue Doppler study was done upon entry into the study using Philips HD 11 XE device (Philips, Bothell, WA), with transducers of 3–8 and 1–3 MHz depending on patient size. An echocardiographic examination was performed by the two authors who are experienced with echocardiography & unaware of the serum test results. M-mode tracing was obtained in the parasternal short-axis view at the level of the papillary muscles of the LV, and LV end-systolic and end-diastolic diameters were measured. LVEF was

measured using the modified Simpson's method, as recommended by the American Society of Echocardiography (19, 20).

Statistical analysis: -

The data collected were tabulated and statistically analyzed using Statistical Package of Social Science (SPSS) software Version 22. The Kolmogorov-Smirnov, Shapiro, and D'agstino tests were used to verify the normality of the distribution of variables. Categorical variables were compared using the chi-square test or Fisher exact test. Student t-test was used to compare two groups for normally distributed quantitative variables, whereas Mann- Whitney U test was used to compare two groups for abnormally distributed quantitative variables. Pearson coefficient was used to correlate between quantitative variables. The receiver operating characteristic (ROC) curve was performed to assess the prognostic performance of FGF23 with respective maximum accuracy points for both sensitivity and specificity (an area of more than 50% gives acceptable performance and an area of about 100% is the best performance for the test). Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. Univariate and multivariate logistic regression analysis was done to detect the most independent factor(s) that predict poor prognosis in children with HF (all-cause mortality and HF hospitalization). All statistically significant parameters in univariate analysis were included in multivariate regression analysis. The significance of the obtained results was judged at the 5% level.

Results:

The study included 60 children divided into two independent groups. The case group included 40 children with HF, with a mean age of 51.0 months (± 61.21), and the control group included 20 matched clinically healthy children with a mean age of 53.70 months (± 68.73).

The clinical characteristics of patients with HF are shown in **Table 1**. The etiology of HF was congenital heart disease in 19 patients, dilated cardiomyopathy in 15 patients, hypertrophic cardiomyopathy in 4 patients, and rheumatic heart diseases in 2 patients (aortic regurgitation and mitral regurgitation) with a mean duration of HF = 19.53 ± 23.49 months. According to the modified Ross score, 14 patients (35%) were in class I and 15 patients (37%) were in class II and 11 patients (27.5%) were in class III. **Table 2** showed a comparison of demographic, clinical data, laboratory, and echocardiographic data between case and control groups. Age, sex, weight, height, BSA, systolic and diastolic blood pressure did not differ significantly between the two groups. Heart rate and respiratory rate were significantly higher in cases. Regarding laboratory data, there

was no significant difference between the two groups as regards CBC parameters, BUN, serum creatinine, serum calcium, serum phosphorus, and eGFR. FGF23 levels were significantly in higher in patients with HF (355.68 ± 97.27 pg/ml) compared to healthy controls (60.20 ± 11.04 pg/ml) ($p < 0.001$). Cases had significantly higher LVEDD, LVESD, and E/e`and lower EF% and FS than controls.

Table 3 showed a comparison of demographic, clinical data, laboratory, and echocardiographic data between patients with a poorer prognosis and the others with a more favorable prognosis. Out of 14 children with poor prognosis, 11 children required admission to hospital with HF and 3 children died during the study. The three children were suffering from dilated cardiomyopathy. One of them had sudden death shortly after complaining from palpitation and chest pain at home. The other two patients were admitted to our intensive care unit, presenting in cardiogenic shock, and treated with intravenous catecholamines, frusemide, inotropes including milirinone and levosimendan. They were intubated and mechanically ventilated with positive pressure ventilation. Their status deteriorates and finally they died. One died on day 6 & the other on day 8 of admission. Unfortunately, ventricular assist devices & heart transplantation are not available for children in our country.

There was no statistically significant difference between the two groups as regards age, sex, duration of HF, heart rate, respiratory rate, blood pressure, weight, length, and BMI. Most patients with poor prognosis (8 patients) were in class III according to the modified Ross classification and 10 patients had dilated cardiomyopathy. Patients with poor prognosis had statistically significant higher troponin I, NT-proBNP, and FGF 23 levels compared to patients with favorable prognosis. Regarding echocardiographic parameters, patients with poor prognosis had statistically significant higher LVEDD, LVESD, and E/e` ratio and lower EF and FS than those with favorable prognosis. Serum levels of FGF 23 were increased with increasing Ross class **(Figure 1)**. The relationship of FGF23 with other variables that indicate severity of HF was determined by calculating the Pearson correlation coefficient. FGF 23 was positively correlated with LVEDD ($r=0.437$. $P<0.001$) and NT- proBNP ($r= 0.977$, $P<0.001$) and negatively correlated with EF ($r= -0.328$, $P=0.039$ and FS ($r= -0.365$, $P=0.021$).

FGF23 was further analyzed using ROC curve (Fig. 2) the best cutoff point to detect poor prognosis was >375pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV= 91.7 and AUC=0.878.

Univariate and multivariate regression analysis for variables that may affect the patient's prognosis are demonstrated in **Table 4.** All statistically significant parameters in univariate analysis were included in multivariate regression analysis model to detect the most independent predictor of poor prognosis which revealed that FGF 23 level (P=0.022) and modified Ross classification (P= 0.046) were the only independent predictors of poor prognosis in children with HF.

Discussion:

The current study showed that children with HF had a significantly higher level of FGF 23 than controls and that FGF23 levels were increased significantly with increasing HF clinical severity and in patients with poor prognosis.

Isakova et al. reported about a 2-fold increase in FGF23 levels in the pediatric HF population compared to healthy controls, and found a significant association between FGF23, the severity of HF, and left ventricular dilatation (21). Studies in the adult population demonstrated that FGF23 was elevated in patients with HF with preserved ejection fraction (22) and with reduced ejection fraction (4). Also, Anderson et al. found increased serum levels of FGF23 in patients with acute decompensated HF (23). In our study elevated FGF-23 cannot be explained by impaired renal function as largely described (7, 24) as our patients had normal renal function and GFR indicating another underlying mechanism. Studies showed that FGF23 is expressed in the heart and that it is significantly enhanced in clinical and experimental settings of cardiac remodeling and HF independent of renal function. Also, FGF23 may promote cardiac injury by endocrine and paracrine/autocrine mechanisms (25, 26).

Regarding heart failure, NYHA classification, biomarkers as NT-pro BNP and left ventricular (LV) systolic function play a major role in risk stratification (27, 28). Our study demonstrated that FGF23 was increased with increasing disease severity assessed by modified Ross classification, NT-proBNP and LV function.

The new finding in our study is that FGF23 shows a statistically significant increase in HF children with poor prognosis (death and HF hospitalization) than those with good prognosis. In multivariate logistic regression model, FGF 23 and Ross class III were found to be an independable predictors of poor outcome (P=0.022). This agreed with the adult studies done by Gruson et al. (4) who found that FGF 23 was the strongest predictor of long-term cardiac death, and Plischke et al. (15) who found that FGF23 level was significantly higher in patients reaching the combined endpoint of cardiac hospitalization or death (poor outcome). Imazu et al. found that FGF23 was

higher in patients with HF hospitalization than in non-hospitalized patients and also found that elevated FGF23 is associated with poor outcomes (death, implantation of LV assist device, and rehospitalization) (29).

In our study, according to ROC analysis, the best cutoff point to differentiate between patients with poor prognosis and those with favorable prognosis was >375pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV= 91.7 and AUC=0.878. In the study done by Cornelissen et al. (14) FGF 23 measured on day 1 and day 2 after admission of adults with acute HF predicted one-year outcome as accurate as the Seattle HF model. Moreover, a recent study by the same authors (30) found that FGF23 levels outperformed the GRACE score in one-year mortality prediction in adults with acute myocardial infarction and concomitant HF. However, FGF23 had no discriminative ability for survival prediction in patients without acute HF. They demonstrated that LogFGF23 levels above 1.71 predicted death at one year with a sensitivity of 0.75 and a specificity of 0.74 in patients with acute HF resulting from myocardial infarction.

Our study has some limitations. First, it was a single-center study with modest sample size. Second, the diversity of HF etiologies in our patients may offer some limitations to data analysis however, previous studies showed that whatever the etiology of acute or chronic HF, FGF23 levels were elevated. Further studies are needed to exactly understand the pathophysiologic role of FGF23 in pediatric HF.

Conclusion:

Serum levels of FGF 23 were elevated in children with HF and these levels were correlated with Modified Ross staging of HF, NT- pro BNP and echocardiographic assessment of LV function. FGF23 has a potential prognostic value as a novel biomarker in pediatric HF which may help to identify high-risk patients that are more prone to complications and need a closer follow-up and more aggressive treatment.

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Table (1): Clinical characteristics of patients with HF (n=40)

Characteristic	No.
Etiology of HF	
Congenital heart diseases (CHD)	19
Ventricular septal defect (VSD)	5
Patent ductus arteriosus (PDA)	4
Atrial septal defect (ASD)	2
Mitral stenosis+ Mitral regurgitation	2
Fallot tetralogy with mild Pulmonary stenosis	1
Aortic regurgitation (AR) + VSD	1
PDA + VSD	1
Aortic stenosis +AR	1
Mitral stenosis	1
VSD+ASD	1
Dilated cardiomyopathy (DCM)	15
Hypertrophic cardiomyopathy (HOCM)	4
Rheumatic heart diseases (MR and AR)	2
Medications	
Diuretics	36
Angiotensin converting enzyme inhibitors	33
B blockers	17
Lanoxin	5
Anticoagulants	2
Modified Ross class	
Class I	14
Class II	15
Class III	11
Duration of HF (months) Mean ± SD Median (range)	 19.53 ± 23.49 10 (2-96)

Table (2): Comparison of demographic, clinical, laboratory and echocardiographic data between case and control groups

Parameter	Case group (n=40)	Control group (n=20)	p-value
Demographic and clinical parameters			
Age (months) ‡	12.0 (6.50 – 108.0)	12.0 (6.50 –102.0)	0.863
Females † no (%)	21 (52.5)	12 (60	0.582
Heart rate (Beats/minute) §	128.50 ± 22.99	110.30 ± 17.76	**0.001**
Respiratory rate (per minute) ‡	53.50 (40.0 – 60.0)	38.0 (20.0 – 41.0)	**<0.001**
Systolic blood pressure (mmHg) §	95.95 ± 10.40	95.75 ± 10.14	0.944
Diastolic blood pressure (mmHg) §	59.82 ± 6.71	63.10 ± 7.48	0.091
Weight (Kg) ‡	9 (8.75-25.0)	10.50 (7.0-26.50)	0.445
Length or height (cm) ‡	0.77 (0.68-1.24)	0.81 (0.71-1.21)	0.433
Body mass index (kg/m2) §	16.16 ± 2.94	16.98 ± 3.38	0.342
Laboratory parameters			
Hemoglobin (gm/dl) §	10.88 ± 0.92	11.17 ± 0.96	0.252
White cell count (*10^3/ul) §	7.03 ± 1.80	6.80 ± 1.57	0.629
Platelet count(*10^3/ul) §	265.55 ± 67.16	258.0 ± 62.12	0.676
Serum creatinine (mg/dl) §	0.63 ± 0.16	0.67 ± 0.18	0.332
BUN (mg/dl) §	14.18 ± 3.21	13.40 ± 2.91	0.367
eGFR§	77.51 ± 30.80	77.95 ± 39.52	0.536
Serum calcium (mg/dl) §	9.35 ± 0.64	9.30 ± 0.52	0.788
Serum phosphate (mg/dl) §	2.98 ± 0.56	3.0 ± 0.36	0.542
PTH (pg/ml) §	42.85 ± 6.34	40.20 ± 4.73	0.131
Serum FGF23 (pg/ml) §	355.68 ± 97.27	60.20 ± 11.04	**<0.001**
NT-proBNP (pg/ml)	380.2 ± 103.9	--	
Echocardiographic parameters			
IVSd (cm)‡	0.50 (0.40-0.60)	0.50 (0.40-0.60)	0.733
IVSs (cm)‡	0.60 (0.50-0.75)	0.60 (0.50-0.70)	0.350
LVEDD (cm)‡	4.0 (3.0-4.45)	2.80 (2.35-3.85)	**0.001**
LVESD(cm) ‡	2.75 (1.90-3.30)	1.75 (1.55-2.70)	**0.003**
LVPWd (cm) ‡	0.50 (0.40-0.60)	0.50 (0.40-0.70)	0.611
LVPWs (cm) ‡	0.70 (0.60-0.80)	0.65 (0.6-0.85)	0.642
EF%‡	58.95 (44.0-72.0)	67.60(63.5-73.0)	**0.001**
FS‡	29.45 (21.75-36.0)	34.80 (32.0-36.25)	**0.047**
E/A‡	1.33 (1.21 – 1.50)	1.47 (1.22 – 1.56)	0.106
E/e`‡	9.82 (7.93-11.89)	7.33 (6.14-8.81)	**0.005**
TAPSE(cm)‡	1.81 (1.20-2.70)	1.60 (1.05-2.13)	0.295

Data are presented as no, percentage, mean ± standard deviation or as median (IQR).

†χ2 test/Fisher's exact test was used.

‡Mann-Whitney test was used.

§t-test was used

BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion.

Table (3): Comparison of demographic, clinical, laboratory and echocardiographic data between patients with poor and favorable prognosis.

Parameter	Poor Prognosis (n = 14)		Favorable prognosis (n = 26)		P
Sex†	No	Percentage	No	Percentage	
Male	7	50.0	12	46.2	0.816
Female	7	50.0	14	53.8	
Age (months) ‡	67.79 ± 72.57, 24.0 (3.0 – 192.0)		41.96 ± 53.52, 12.0(2.0 – 144.0)		0.130
Mortality n (%)	3 (21.4%)		0 (0%)		
Diagnosis©					
Congenital heart disease	2	14.3	17	65.4	0.002
Dilated cardiomyopathy	10	71.4	5	19.2	
Hypertrophic cardiomyopathy	2	14.3	2	7.7	
Rheumatic heart disease	0	0.0	2	7.7	
Modified ROSS class†					0.003
Class I	1	7.1	13	50.0	
Class II	5	35.7	10	38.5	
Class III	8	57.1	3	11.5	
Duration of HF (Months) ‡	19.07 ± 21.57, 12.0 (2.0 – 60.0)		19.77 ± 24.86, 6.5 (2.0 – 96.0)		0.967
Hear rate (Beats/minute) §	126.07 ± 24.35, 130.0 (90.0 – 170.0)		129.81 ± 22.60, 135.0 (85.0 – 165.0)		0.630
Respiratory rate‡	47.0 ± 15.0, 51.0 (20.0 – 65.0)		49.46 ± 14.09, 55.0 (20.0 – 65.0)		0.664
Systolic blood pressure§	95.36 ± 8.65, 95.0 (85.0 – 110.0)		96.27 ± 11.38, 95.0 (80.0 – 120.0)		0.795
Diastolic blood pressure§	60.0 ± 6.50, 60.0 (50.0 – 70.0)		59.73 ± 6.94, 60.0 (50.0 – 80.0)		0.905
Weight (kg) ‡	20.29 ± 16.66, 12.0 (5.0 – 55.0)		14.73 ± 13.07, 9.0 (4.0 – 45.0)		0.130
Length (m) ‡	1.0 ± 0.36, 0.83 (0.65 – 1.61)		0.88 ± 0.32, 0.74 (0.55 – 1.50)		0.220
Body mass index (Kg/m^2) §	17.08 ± 2.29, 16.69 (11.83 – 21.48)		15.67 ± 3.17, 16.20 (8.65 – 20.66)		0.150
Hmoglobin (gm/dl) §	10.64 ± 1.05, 10.65 (8.50 – 12.50)		10.99 ± 0.83,11.15 (8.50 – 12.50)		0.124
White blood count (*10^3/ul)§	7.19 ± 2.02, 7.10 (4.30 – 12.0)		6.93 ± 1.71, 6.80 (4.30 – 10.50)		0.671
Platelet count (*10^3/ul)§	263.93 ± 75.60, 265.0 (170.0 – 400.0)		266.42 ± 63.74, 255.0 (156.0 – 400.0)		0.913
Serum Creatinine (mg/dl) §	0.59 ± 0.09, 0.60 (0.50 – 0.80)		0.65 ± 0.19, 0.60 (0.40 – 1.0)		0.182
BUN (mg/dl) §	14.14 ± 3.18, 13.50 (10.0 – 20.0)		14.19 ± 3.29, 13.0 (10.0 – 20.0)		0.964
eGFR‡	87.23 ± 34.36, 75.63 (53.57 – 160.0)		72.28 ± 28.01, 65.88 (37.0 – 145.0)		0.190
Calcium (mg/dl) ‡	9.34 ± 0.63, 9.15 (8.50 – 10.20)		9.36 ± 0.66, 9.20 (8.40 – 11.0)		0.989
Phosphate (mg/dl) ‡	3.16 ± 0.88, 2.90 (2.60 – 6.0)		2.88 ± 0.24, 2.85 (2.60 – 3.50)		0.528
Parathyroid hormone	45.79 ± 6.61, 48.0 (34.0 – 55.0)		41.27 ± 5.70, 40.0 (32.0 – 52.0)		0.055
NT-proBNP (pg/ml) §	457.57 ± 82.61, 416.0 (340.0 – 600.0)		338.54 ± 90.19, 350.0 (210.0 – 560.0)		<0.001
FGF23 (pg/ml) §	421.86 ± 75.50, 397.50 (329.0 -560.0)		320.04 ± 89.56, 326.0 (200.0 - 550.0)		<0.001
IVSd (cm) ‡	0.74 ± 0.48, 0.50 (0.40 – 1.90)		0.54 ± 0.25, 0.50 (0.30 – 1.50)		0.123
IVSs (cm) ‡	0.85 ± 0.50 , 0.70 (0.40 – 2.0)		0.68 ± 0.26, 0.60 (0.47 – 1.50)		0.305
LVEDD (cm) ‡	4.41 ± 0.72, 4.20 (3.50 – 5.90)		3.56 ± 0.93, 3.20 (2.30 – 5.90)		0.005
LVESD (cm) ‡	3.10 ± 0.69, 3.0 (2.0 – 4.0)		2.32 ± 0.75, 2.0 (1.40 – 3.90)		0.002
LVPWd (cm) ‡	0.74 ± 0.42, 0.55 (0.50 – 1.70)		0.55 ± 0.24, 0.50 (0.40 – 1.50)		0.023
LVPWs (cm) ‡	0.87 ± 0.42, 0.70 (0.40 – 1.80)		0.73 ± 0.29, 0.65 (0.40 – 1.80)		0.180
EF%§	46.86 ± 18.38, 42.0 (22.0 – 80.0)		65.46 ± 10.88, 68.0 (42.0 – 82.0)		0.003
FS‡	23.43 ± 9.19, 21.0 (11.0 – 40.0)		33.15 ± 6.25, 34.0 (21.0 – 50.0)		0.002
E/A§	1.30 ± 0.48, 1.37 (0.50 – 2.25)		1.34 ± 0.31, 1.35 (0.52 – 1.92)		0.776
E/É‡	11.87 ± 3.87, 11.54 (4.40 – 17.40)		8.71 ± 2.64, 8.78 (3.55 – 13.25)		0.012
TAPSE (cm) ‡	2.07 ± 0.79, 1.90 (0.90 – 3.20)		1.67 ± 0.79, 1.30 (0.80 – 3.20)		0.067

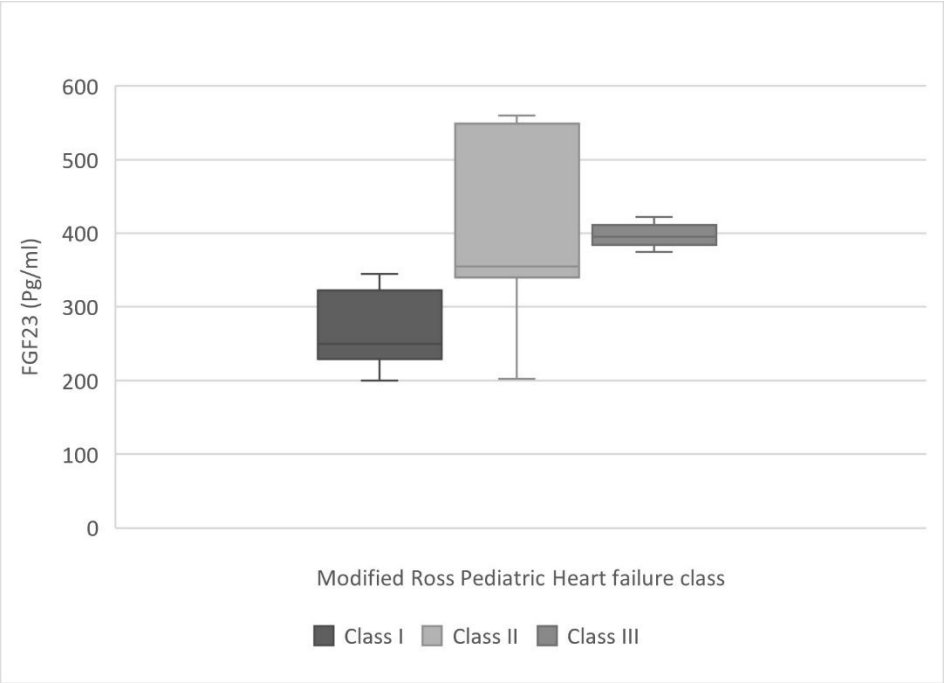
Data are described as number and percentage or as mean ± SD, median (range).

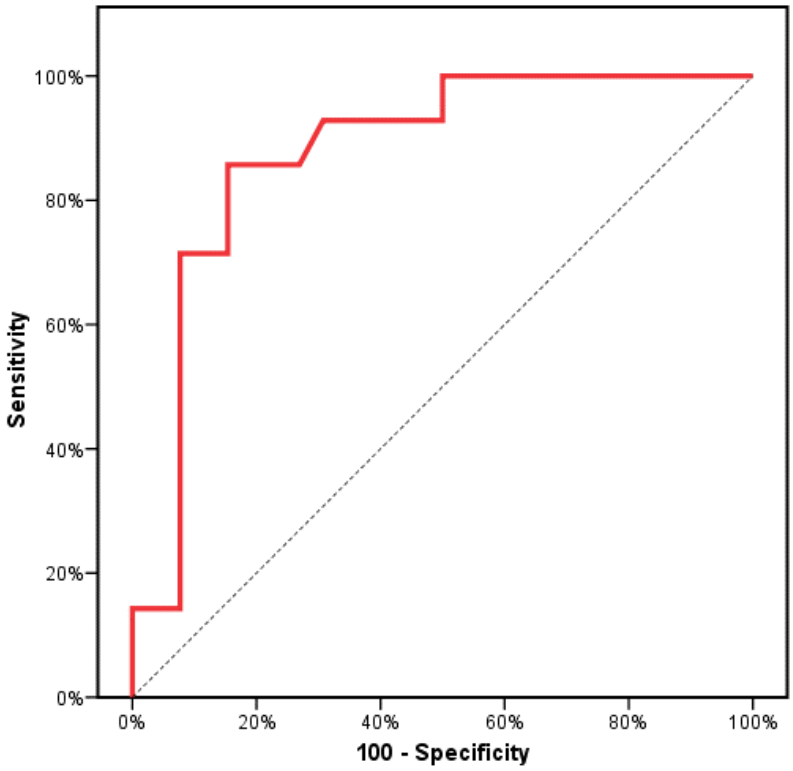
†χ2 test/Fisher's exact test was used.
© Monte Carlo test was used.
‡Mann-Whitney test was used.
§t-test was used
[FE]P: P value of Fisher exact test
BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion

Table (4): Univariate and multivariate Logistic regression analysis for predictors of poor outcome in children with HF

	Univariate		#Multivariate	
	P	OR (95%C.I)	P	OR (95%C.I)
Sex (male)	0.816	1.167(0.318 – 4.284)		
Age (months)	0.205	1.007(0.996 – 1.018)		
Hear rate (Beats/min.)	0.620	0.993(0.965 – 1.022)		
Respiratory rate	0.599	0.988(0.944 – 1.034)		
Systolic blood pressure	0.789	0.991(0.930 – 1.057)		
Diastolic blood pressure	0.902	1.006(0.912 – 1.110)		
ROSS class III	0.021*	13.0(1.478 – 114.358)	0.046*	0.224(0.051–0.972)
Duration of HF	0.928	0.999(0.971 – 1.027)		
Weight (kg)	0.250	1.027(0.982 – 1.074)		
Length (m)	0.270	2.991(0.427 – 20.933)		
Body mass index (Kg/m^2)	0.153	1.201(0.934 – 1.554)		
IVSd (cm)	0.129	4.895(0.631 – 37.938)		
IVSs (cm)	0.182	3.515(0.556 – 22.231)		
LVEDD (cm)	0.013*	3.076(1.265 – 7.482)	0.439	0.631(0.197 – 2.024)
LVESD (cm)	0.007*	3.874(1.446 – 10.376)	0.646	1.688(0.181 – 15.761)
LVPWd (cm)	0.111	6.373(0.655 – 61.975)		
LVPWS (cm)	0.228	3.254(0.479 – 22.113)		
EF%	0.002*	0.919(0.871 – 0.970)	0.612	0.581(0.071–4.740)
FS	0.002*	0.846(0.760 – 0.943)	0.751	1.972(0.030–130.528)
E/A	0.769	0.769(0.133 – 4.446)		
E/É	0.011*	1.382(1.076 – 1.775)	0.410	0.700(0.299–1.635)
TAPSE (cm)	0.129	1.909(0.828 – 4.403)		
Calcium (mg/dl)	0.944	0.964(0.346 – 2.688)		
Phosphate (mg/dl)	0.238	2.943(0.490 – 17.657)		
Parathyroid hormone (pg/ml)	0.037*	1.135(1.008 – 1.277)	0.168	1.191(0.929–1.526)
Troponin	0.051	10.0(0.992 – 100.821)		
NT-proBNP (pg/ml)	0.004*	1.015(1.005 – 1.026)	0.761	1.015(0.924–1.114)
Serum Creatinine (mg/dl)	0.262	0.073(0.001 – 7.027)		
BUN (mg/dl)	0.962	0.995(0.810 – 1.222)		
Hemoglobin (gm/dl)	0.437	0.982(0.938 – 1.028)		
White cell count (*10^3/ul)	0.662	1.085(0.753 – 1.562)		
Platelet count (*10^3/ul)	0.910	0.999(0.990 – 1.009)		
eGFR	0.150	1.016(0.994 – 1.038)		
FGF23 (pg/ml)	0.009*	1.014(1.004 – 1.025)	0.022*	1.026(1.004 – 1.048)

OR: Odd's ratio, C.I: Confidence interval, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate. *: Statistically significant at $p \leq 0.05$





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Fibroblast growth factor 23 in children with or without heart failure: a prospective study

Keywords: Prognosis, fibroblast growth factor 23, children and Heart failure

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Abstract

Background: Elevated fibroblast growth factor 23 (FGF23) levels have been associated with mortality in adults with heart failure (HF), but data on FGF23 levels in pediatric HF are limited. In this prospective cohort study, we aimed to assess prognostic value of fibroblast growth factor 23 in children with HF.

Methods: We prospectively enrolled 40 children with chronic HF and 20 matched healthy controls. For each patient a complete diagnostic workup was performed including transthoracic echocardiography to evaluate cardiac systolic and diastolic functions. Serum FGF23, renal function tests, parathyroid hormone, serum calcium and phosphate were measured for patients & controls. N-terminal Pro-Brain Natriuretic Peptide was measured for patients. Symptom severity was assessed with modified Ross HF classification for children. Following the patients for a year, Patients were followed for one year and clinical worsening events such as death or HF hospitalization were recorded.

Results: Compared to controls, patients with HF had significantly higher FGF23 levels (355.68 ±97.27; 60.20±11.04 pg/ml respectively, P <0.001). Three patients died and eleven were admitted with HF. In comparison to patients without clinical worsening events, these fourteen patients exhibited significantly higher FGF23 levels (320.04 ± 89.56; 421.86 ± 75.50 pg/ml, respectively P <0.001). FGF23 was positively correlated with NT-pro-BNP and left ventricular end diastolic diameter and negatively with ejection fraction and fractional shortening. The ability of FGF23 to predict unfavorable prognosis in patients was analyzed using receiver operating characteristic curve. The optimal cutoff point was 375pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV=91.7 and AUC=0.878. Multivariable regression analysis revealed that only FGF23 & Ross class III are independent predictors of clinical worsening events in HF children.

Conclusion: FGF23 levels were elevated in children with HF and were increased significantly with increasing Ross score class. FGF23 had a good prognostic accuracy in detecting children with clinical worsening events**.**

Keywords: Prognosis, fibroblast growth factor 23, children and heart failure.

What is known about the subject? FGF23 has been related to adverse cardiovascular events in adult patients with HF. Increased FGF23 levels have been associated with left ventricular (LV)

hypertrophy and impaired LV function. Studies on adult populations had demonstrated that FGF23 could be used to predict clinical outcome in patients with HF.

What does this study add? FGF23 levels were increased significantly with increasing HF clinical severity and in patients with clinical worsening events. FGF23 had a potential good prognostic value as a novel biomarker in pediatric HF with cutoff point >375pg/ml predicting children with clinical worsening events.

Introduction:

Pediatric HF (HF) is a complex disease process, which can occur secondary to a variety of etiologies, including congenital heart diseases, cardiomyopathy, or acquired conditions as well. It remains a major cause of morbidity and mortality in childhood with significant health & economic burden worldwide [1].

Exploring new cardiac biomarkers for HF helps clinicians identify disease progression, predict the clinical outcomes, provide protective strategies, and select the proper treatment and monitor therapy [2].

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone secreted by osteoblasts and osteocytes in response to increased phosphate levels regulating renal phosphate homeostasis and vitamin D metabolism, by stimulating phosphaturia and inhibiting calcitriol synthesis [3].

Besides its role in mineral metabolism, FGF23 has a direct action on the cardiovascular system and has been recently related to adverse cardiovascular events in adult patients with HF and involved in cardiac remodeling [4-7]. Increased FGF23 levels have been associated with left ventricular (LV) hypertrophy and impaired LV function [8-11]. It has been linked with alterations in myocyte calcium handling [12] and upregulation of the renin-angiotensin system [13]. Interestingly, adult studies showed that FGF23 not only could predict clinical outcomes in patients with acute and stable HF [14, 15] but also was independently associated with all-cause death and HF in a community-living older person [16].

Comparable data about FGF23 in children with HF are limited. Therefore, we aimed to measure and compare FGF 23 levels of children with HF with those of healthy children, to correlate them with indicators of HF severity and assess the prognostic significance of FGF 23 in children with HF.

Methods

This prospective cohort study was carried out at the Pediatric cardiology unit of Menoufia University Hospital between January 2020 and February 2021. Informed consent was obtained from the guardian of each participant included in the study. All study procedures were carried out in accordance with the ethical standards of Menoufia University Institutional Research Committee.

Patient and public involvement statement: Patients and/or the public were involved in the design, conduct, reporting and dissemination plans of this research. Public healthcare clinics, but not families, were involved in the content and design.

Eligibility criteria

Inclusion criteria: For the case group, we targeted children with HF defined by the International Society for Heart and Lung Transplantation Practice Guidelines for Management of HF in Children [17] as a syndrome resulting from ventricular dysfunction, volume, or pressure overload, alone or in combination. Definition of chronic HF has been changed from the traditional definition of a syndrome that results from inadequate cardiac output to maintain end-organ perfusion to the new definition in adults which incorporates disorders of ventricular filling, that is, diastolic dysfunction or HF with preserved ejection fraction [18].

So, Patients with chronic heart failure who fulfilled one or more of the following criteria were included in the study:

1) Patients with reduced LV ejection fraction (EF%), fractional shortening (FS) and/or tricuspid annular plane systolic excursion (TAPSE).
2) Patients with symptoms and signs of HF[#] and structural cardiac abnormality.
3) Asymptomatic patients with prior symptoms and signs of HF controlled on anti-failure medications with evidence of structural cardiac abnormality.

[#]Symptoms and signs of HF were respiratory distress, dyspnea on exertion, feeding problems, failure to thrive, diaphoresis, hepatomegaly, cool extremities, and poor peripheral perfusion [19].

The control group included a group of age and sex-matched healthy children who were referred to the cardiology clinic for innocent murmurs and found to be free of any cardiac disease.

Exclusion criteria: diseases that affect FGF 23 as chronic kidney disease and rickets, any congenital anomalies other than congenital heart disease, and age less than 2 months and more than 18 years.

All of the included patients & controls were subjected to detailed history taking with collecting patient demographics and comprehensive clinical examination. Patients were assessed for the functional status of HF by Modified ROSS classification system of HF in infants and children [20]. Patients were followed for 1 year for an endpoint of all-cause mortality or HF hospitalization and patients were classified accordingly into 2 groups: patients with clinical worsening events (death or hospitalization with HF) and patients without clinical worsening events.

Investigations:

Blood samples were collected then centrifuged, aliquoted, labeled, and stored at −80 °C until batched assays were performed. Because of the well-defined role of FGF23 in renal & bone diseases investigations using standard procedures were performed including complete blood count (CBC) and renal functions, serum calcium, serum phosphate and parathyroid hormone. Estimated glomerular filtration rate (eGFR; in ml/min/1.73 m2) was determined using an established formula of e GFR=k× height (cm)/serum creatinine (mg/dl) (19). Serum intact FGF23 levels were measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit "Elabscience, USA". As there is no clear FGF23 cut-off value for the pediatric population and the available assays are not standardized. So, we used the most similar assay reference values (Kainos Laboratories, Japan) with normal FGF23 cutoff level in children <71 pg/ml. [21] To correlate FGF23 with other biomarkers of HF, we measured NT-proBNP using ELISA kits in children with HF.

Echocardiography

Upon enrollment, a Philips HD 11 XE equipment (Philips, Bothell, WA) was used for transthoracic echocardiography (TTE) imaging with tissue Doppler study, usng transducers of 3-8 and 1-3 MHz depending on patient size. The two authors (first and last) who have more than 10 years of echocardiography expertise performed the echocardiographic examination and were not aware of the serum test results. To reduce the interobserver variability, both were present during each echo examination to double check the echo results separately. Only agreed-upon results were recorded. M-mode tracing was obtained in the parasternal short-axis view at the level of the papillary muscles of the LV, and LV end-systolic and end-diastolic diameters were measured. LVEF was measured using the modified Simpson's method, as recommended by the American Society of Echocardiography [22, 23]. EF% and FS were considered reduced if < 55% and ≤ 25 respectively [24]. TAPSE was considered reduced if < 10 mm [25].

Statistical analysis: -

The data collected were tabulated and statistically analyzed using Statistical Package of Social Science (SPSS) software Version 22. The Kolmogorov-Smirnov, Shapiro, and D'agstino tests were used to verify the normality of the distribution of variables. Categorical variables were compared using the chi-square test or Fisher exact test. The student t-test was used to compare two groups for normally distributed quantitative variables, whereas Mann- Whitney U test was used to compare two groups for abnormally distributed quantitative variables. Pearson coefficient was

used to correlate between quantitative variables. The receiver operating characteristic (ROC) curve was performed to assess the prognostic performance of FGF23 with respective maximum accuracy points for both sensitivity and specificity (an area of more than 50% gives acceptable performance and an area of about 100% is the best performance for the test). Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. Univariable and multivariable logistic regression analysis was done to detect the most independent factor(s) that predict clinical worsening events in children with HF (all-cause mortality and HF hospitalization). All statistically significant parameters in univariable analysis were included in multivariable regression analysis. The significance of the obtained results was judged at the 5% level.

The sample size was calculated using the following formula:

$$n \geq \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\frac{1}{2}\log_e \frac{1+r}{1-r}} \right)^2 + 3$$

where n = sample size, $Z_{1-\alpha/2}$ =1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail), r = 0.63 (correlation coefficient between FGF23 and left ventricular end-diastolic diameter in a previous study of Isakova et al [26], β = 0.05.

Results:

The study included 60 children divided into two independent groups. The case group included 40 children with HF, with a median age of 12.0 months (IQR= 6.5 – 108), and the control group included 20 matched clinically healthy children with a median age of 12.0 months (IQR= 6.5 – 108).

Table 1 displays the clinical features of patients with HF. Congenital heart disease was the cause of HF in 19 patients, dilated cardiomyopathy in 15, hypertrophic cardiomyopathy in 4, and rheumatic heart disease in 2 patients, with a median HF duration of 10 months (IQR= 6-27).

Fourteen patients (35%) were classified as being in class I, fifteen patients (37%) as being in class II, and eleven patients (27.5%) as being in class III by the modified Ross score.

Table 2 compared the case and control groups' demographic, clinical, laboratory, and echocardiographic data. Age, sex, body mass index (BSA), height, weight, and blood pressure (systolic and diastolic) did not significantly differ between the two groups. Both heart rate and respiratory rate were significantly greater in case group. There was no statistically significant difference between the two groups in terms of laboratory data for the CBC parameters, BUN,

serum creatinine, serum calcium, serum phosphorus, and eGFR. FGF23 levels were significantly higher in HF patients than in healthy controls (355.68 97.27 pg/ml vs. 60.20 11.04 pg/ml; p <0.001). Compared to controls, cases had significantly higher LVEDD, LVESD, and E/e`and lower EF% and FS.

Table 3 showed a comparison of demographic, clinical data, laboratory, and echocardiographic data between patients with and without clinical worsening. Of the 14 children who experienced clinically worsening events, 11 needed hospital admission for HF, and 3 passed away during the study. The three children were suffering from dilated cardiomyopathy. One of them had sudden death shortly after complaining from palpitation and chest pain at home. The other two patients were presented with cardiogenic shock and admitted to our intensive care unit. Their condition worsened until they eventually passed away. One died on the sixth day, and the other on the eighth.

Regarding age, sex, the duration of HF, heart rate, respiratory rate, blood pressure, weight, height, and BMI, there was no statistically significant difference between the two groups. According to modified Ross classification placed the majority of the patients with clinically deteriorating episodes (8 patients) in class III, while 10 patients had dilated cardiomyopathy. When compared to patients with a good prognosis, participants with clinically deteriorating episodes had statistically significantly higher troponin I, NT-proBNP, and FGF 23 levels. Patients with clinically deteriorating episodes showed statistically substantially greater LVEDD, LVESD, and E/e' ratios as well as poorer EF and FS than those with good outcomes in terms of echocardiographic measures.

There was no statistically significant difference between the two groups as regards age, sex, duration of HF, heart rate, respiratory rate, blood pressure, weight, length, and BMI. Most patients with clinical worsening events (8 patients) had Ross class III and 10 patients had dilated cardiomyopathy. When compared to patients with a good prognosis, participants with clinically deteriorating events had significantly higher troponin I, NT-proBNP, and FGF 23 levels. Patients with clinically worsening events showed significantly greater LVEDD, LVESD, and E/e' ratios as well as poorer EF and FS than those with good outcomes in terms of echocardiographic measures. FGF 23 serum levels increased significantly with increasing Ross score class **(Figure 1)**. Pearson correlation coefficient was used to determine the relationship between FGF23 and other variables

that indicate severity of HF. FGF 23 was positively correlated with LVEDD (r=0.437. P<0.001) and NT- proBNP (r= 0.977, P<0.001) and negatively correlated with EF (r= -0.328, P=0.039 and FS (r= -0.365, P=0.021).

Prognostic performance of FGF23 was analyzed using ROC curve (Fig. 2). AUC was equal to 0.878 (95% CI = 0.767 – 0.989; p < 0.001). The optimal cutoff point to detect unfavorable prognosis was >375pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV= 91.7. Univariable and multivariable regression analysis for variables that may influence the patient's prognosis are illustrated in **Table 4.** The FGF 23 level and the modified Ross classification were the only independent predictors of unfavorable outcomes in children with HF (P=0.022 and P=0.046, respectively) after all statistically significant parameters from the univariable analysis were incorporated in a multivariable regression analysis model.

Discussion:

The current study showed that children with HF had a significantly higher level of FGF23 compared to controls and that FGF23 levels were increased significantly with increasing Ross score class and in patients with clinical worsening events.

Isakova et al. compared FGF23 levels of 20 children suffering from HF caused by various etiologies and 17 healthy controls. They reported about a 2-fold increase in FGF23 levels in patients compared to healthy controls and found a significant association between FGF23 levels and both clinical severity of HF (assessed by NYHA/Ross class and NT-pro BNP level) and left ventricular dilatation. Both associations were independent of eGFR [26]. Studies in the adult population demonstrated that FGF23 was elevated in patients who had HF with preserved ejection fraction [27] and with reduced ejection fraction [4]. Additionally, Anderson et al. found elevated serum levels of FGF23 in patients with acute decompensated HF [28]. In our study elevated FGF-23 cannot be explained by impaired renal function as has been largely described [7, 29] indicating another underlying mechanism. Studies revealed that FGF23 is expressed in the heart and that, regardless of renal function, it is markedly increased in clinical and experimental conditions of cardiac remodeling and HF. FGF23 may promote cardiac injury through endocrine, paracrine/autocrine, and other mechanisms [30, 31]

Risk stratification for heart failure is greatly influenced by the NYHA classification, biomarkers including NT-pro BNP, and left ventricular systolic function [32, 33]. Our study demonstrated that FGF23 was increased with increasing disease severity assessed by modified Ross classification, NT-proBNP and ventricular function indices. FGF23 had postulated to have a direct cardiac and vascular toxicity [34, 35] Another possibility is that FGF23 suppresses calcitriol synthesis, which then activates the renin-angiotensin-aldosterone system [36, 37].

Our study's novel conclusion is that FGF23 levels are statistically significantly higher in HF children with clinical worsening events (death and hospitalization) than in HF children without such events. FGF 23 level and Ross class III were found to be independent predictors of unfavorable outcomes in a multivariable logistic regression model. This was in line with adult studies conducted by Gruson et al. [4] who found that FGF 23 was the strongest predictor of long-term cardiac death, and Plischke et al. [15] who showed that FGF23 level was significantly higher in patients reaching the combined endpoint of cardiac hospitalization or death. Imazu et al. reported that FGF23 levels were greater in HF hospital patients compared to non-hospitalized patients and that raised FGF23 is linked to poor outcomes (death, LV assist device implantation, and rehospitalization) [38].

The optimal cutoff value in our study to distinguish patients with clinical worsening events from those without was >375pg/ml, with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV=91.7, and AUC=0.878. In the study by Cornelissen et al., [14] FGF 23 measured on days 1 and 2 after admission for patients with acute HF accurately predicted one-year outcomes as did the Seattle HF model. In addition, a later study by the same authors showed that in patients with acute myocardial infarction and concomitant HF, FGF23 levels outperformed the GRACE score in one-year mortality prediction. FGF23, however, lacked any discriminative capacity for predicting survival in patients without acute HF [39]. They demonstrated that in patients with acute HF caused on by myocardial infarction, LogFGF23 levels of 1.71 accurately predicted death at one year with a sensitivity of 0.75 and a specificity of 0.74.

Our study has some limitations. First, it was a single-center study with small sample size. Second, data analysis may be limited by our patients' diverse HF etiologies. However, previous research has demonstrated that FGF23 levels were elevated in HF patients regardless of the cause. Third, the multivariable model's interpretation may be limited by the small number of our patients.

Thus, multicentric studies involving a larger number of participants may be warranted. Additionally, further research is needed to fully comprehend the precise pathophysiologic function of FGF23 in pediatric HF.

Conclusion:

Children with HF had elevated serum levels of FGF 23, and these levels significantly increased with increasing modified Ross class. FGF 23 levels were positively correlated with NT-proBNP and LVEDD and negatively correlated with EF and FS. FGF23 has a potential prognostic value as a novel biomarker in pediatric HF which may help to identify high-risk patients that are more prone to complications and need a closer follow-up and more aggressive treatment.

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Table (1): Clinical characteristics of patients with HF (n=40)

Characteristic	No.
Etiology of HF	
Congenital heart diseases (CHD)	19
Ventricular septal defect (VSD)	5
Patent ductus arteriosus (PDA)	4
Large ostium secundum atrial septal defect (ASD)	2
Parachute mitral valve causing severe MS	1
Parachute mitral valve causing severe MS & moderate MR	1
Dysplastic mitral valve causing severe MS & MR	1
Fallot tetralogy with mild Pulmonary stenosis	1
Aortic regurgitation (AR) + VSD	1
PDA + VSD	1
Aortic stenosis +AR	1
VSD+ASD	1
Dilated cardiomyopathy (DCM)	15
Hypertrophic cardiomyopathy (HOCM)	4
Rheumatic heart diseases *Severe MR and mild AR* *Moderate MR and AR*	2
Medications	
Diuretics	36
Angiotensin converting enzyme inhibitors	33
B-blockers	17
Lanoxin	5
Anticoagulants	2
Modified Ross class	
Class I	14
Class II	15
Class III	11
Duration of HF (months) Median (IQR)	10 (6-27)

Table (2): Comparison of demographic, clinical, laboratory and echocardiographic data between case and control groups

Parameter	Case group (n=40)	Control group (n=20)	p-value
Demographic and clinical parameters			
Age (months) ‡	12.0 (6.50 – 108.0)	12.0 (6.50 –102.0)	0.863
Females † no (%)	21 (52.5)	12 (60	0.582
Heart rate (Beats/minute) §	128.50 ± 22.99	110.30 ± 17.76	**0.001**
Respiratory rate (per minute) ‡	53.50 (40.0 – 60.0)	38.0 (20.0 – 41.0)	**<0.001**
Systolic blood pressure (mmHg) §	95.95 ± 10.40	95.75 ± 10.14	0.944
Diastolic blood pressure (mmHg) §	59.82 ± 6.71	63.10 ± 7.48	0.091
Weight (Kg) ‡	9 (8.75-25.0)	10.50 (7.0-26.50)	0.445
Length or height (m) ‡	0.77 (0.68-1.24)	0.81 (0.71-1.21)	0.433
Body mass index (kg/m2) §	16.16 ± 2.94	16.98 ± 3.38	0.342
Laboratory parameters			
Hemoglobin (gm/dl) §	10.88 ± 0.92	11.17 ± 0.96	0.252
White cell count (*10^3/ul) §	7.03 ± 1.80	6.80 ± 1.57	0.629
Platelet count(*10^3/ul) §	265.55 ± 67.16	258.0 ± 62.12	0.676
Serum creatinine (mg/dl) §	0.63 ± 0.16	0.67 ± 0.18	0.332
BUN (mg/dl) §	14.18 ± 3.21	13.40 ± 2.91	0.367
eGFR§	77.51 ± 30.80	77.95 ± 39.52	0.536
Serum calcium (mg/dl) §	9.35 ± 0.64	9.30 ± 0.52	0.788
Serum phosphate (mg/dl) §	2.98 ± 0.56	3.0 ± 0.36	0.542
PTH (pg/ml) §	42.85 ± 6.34	40.20 ± 4.73	0.131
Serum FGF23 (pg/ml) §	355.68 ± 97.27	60.20 ± 11.04	**<0.001**
NT-proBNP (pg/ml)	380.2 ± 103.9	--	
Echocardiographic parameters			
IVSd (cm)‡	0.50 (0.40-0.60)	0.50 (0.40-0.60)	0.733
IVSs (cm)‡	0.60 (0.50-0.75)	0.60 (0.50-0.70)	0.350
LVEDD (cm)‡	4.0 (3.0-4.45)	2.80 (2.35-3.85)	**0.001**
LVESD(cm) ‡	2.75 (1.90-3.30)	1.75 (1.55-2.70)	**0.003**
LVPWd (cm) ‡	0.50 (0.40-0.60)	0.50 (0.40-0.70)	0.611
LVPWs (cm) ‡	0.70 (0.60-0.80)	0.65 (0.6-0.85)	0.642
EF%‡	58.95 (44.0-72.0)	67.60(63.5-73.0)	**0.001**
FS‡	29.45 (21.75-36.0)	34.80 (32.0-36.25)	**0.047**
E/A‡	1.33 (1.21 – 1.50)	1.47 (1.22 – 1.56)	0.106
E/e`‡	9.82 (7.93-11.89)	7.33 (6.14-8.81)	**0.005**
TAPSE(cm)‡	1.81 (1.20-2.70)	1.60 (1.05-2.13)	0.295

Data are presented as no, percentage, mean ± standard deviation or as median (IQR).

†χ2 test/Fisher's exact test was used.

‡Mann-Whitney test was used.

§t-test was used

BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion.

Table (3): Comparison of demographic, clinical, laboratory and echocardiographic data between patients with and without clinical worsening outcomes.

Parameter	Patients with outcomes (n = 14)		Patients without outcomes (n = 26)		P
Sex†	No	Percentage	No	Percentage	
Male	7	50.0	12	46.2	0.816
Female	7	50.0	14	53.8	
Age (months) ‡	67.79 ± 72.57, 24.0 (3.0 – 192.0)		41.96 ± 53.52, 12.0(2.0 – 144.0)		0.130
Mortality n (%)	3 (21.4%)		0 (0%)		
Diagnosis©					
Congenital heart disease	2	14.3	17	65.4	0.002
Dilated cardiomyopathy	10	71.4	5	19.2	
Hypertrophic cardiomyopathy	2	14.3	2	7.7	
Rheumatic heart disease	0	0.0	2	U7.7	
Modified ROSS class†					0.003
Class I	1	7.1	13	50.0	
Class II	5	35.7	10	38.5	
Class III	8	57.1	3	11.5	
Duration of HF (Months) ‡	19.07 ± 21.57, 12.0 (2.0 – 60.0)		19.77 ± 24.86, 6.5 (2.0 – 96.0)		0.967
Hear rate (Beats/minute) §	126.07 ± 24.35, 130.0 (90.0 – 170.0)		129.81 ± 22.60, 135.0 (85.0 – 165.0)		0.630
Respiratory rate‡	47.0 ± 15.0, 51.0 (20.0 – 65.0)		49.46 ± 14.09, 55.0 (20.0 – 65.0)		0.664
Systolic blood pressure§	95.36 ± 8.65, 95.0 (85.0 – 110.0)		96.27 ± 11.38, 95.0 (80.0 – 120.0)		0.795
Diastolic blood pressure§	60.0 ± 6.50, 60.0 (50.0 – 70.0)		59.73 ± 6.94, 60.0 (50.0 – 80.0)		0.905
Weight (kg) ‡	20.29 ± 16.66, 12.0 (5.0 – 55.0)		14.73 ± 13.07, 9.0 (4.0 – 45.0)		0.130
Length (m) ‡	1.0 ± 0.36, 0.83 (0.65 – 1.61)		0.88 ± 0.32, 0.74 (0.55 – 1.50)		0.220
Body mass index (Kg/m^2) §	17.08 ± 2.29, 16.69 (11.83 – 21.48)		15.67 ± 3.17, 16.20 (8.65 – 20.66)		0.150
Hmoglobin (gm/dl) §	10.64 ± 1.05, 10.65 (8.50 – 12.50)		10.99 ± 0.83,11.15 (8.50 – 12.50)		0.124
White blood count (*10^3/ul)§	7.19 ± 2.02, 7.10 (4.30 – 12.0)		6.93 ± 1.71, 6.80 (4.30 – 10.50)		0.671
Platelet count (*10^3/ul)§	263.93 ± 75.60, 265.0 (170.0 – 400.0)		266.42 ± 63.74, 255.0 (156.0 – 400.0)		0.913
Serum Creatinine (mg/dl) §	0.59 ± 0.09, 0.60 (0.50 – 0.80)		0.65 ± 0.19, 0.60 (0.40 – 1.0)		0.182
BUN (mg/dl) §	14.14 ± 3.18, 13.50 (10.0 – 20.0)		14.19 ± 3.29, 13.0 (10.0 – 20.0)		0.964
eGFR‡	87.23 ± 34.36, 75.63 (53.57 – 160.0)		72.28 ± 28.01, 65.88 (37.0 – 145.0)		0.190
Calcium (mg/dl) ‡	9.34 ± 0.63, 9.15 (8.50 – 10.20)		9.36 ± 0.66, 9.20 (8.40 – 11.0)		0.989
Phosphate (mg/dl) ‡	3.16 ± 0.88, 2.90 (2.60 – 6.0)		2.88 ± 0.24, 2.85 (2.60 – 3.50)		0.528
Parathyroid hormone	45.79 ± 6.61, 48.0 (34.0 – 55.0)		41.27 ± 5.70, 40.0 (32.0 – 52.0)		0.055
NT-proBNP (pg/ml) §	457.57 ± 82.61, 416.0 (340.0 – 600.0)		338.54 ± 90.19, 350.0 (210.0 – 560.0)		<0.001
FGF23 (pg/ml) §	421.86 ± 75.50, 397.50 (329.0 -560.0)		320.04 ± 89.56, 326.0 (200.0 - 550.0)		<0.001
IVSd (cm) ‡	0.74 ± 0.48, 0.50 (0.40 – 1.90)		0.54 ± 0.25, 0.50 (0.30 – 1.50)		0.123
IVSs (cm) ‡	0.85 ± 0.50 , 0.70 (0.40 – 2.0)		0.68 ± 0.26, 0.60 (0.47 – 1.50)		0.305
LVEDD (cm) ‡	4.41 ± 0.72, 4.20 (3.50 – 5.90)		3.56 ± 0.93, 3.20 (2.30 – 5.90)		0.005
LVESD (cm) ‡	3.10 ± 0.69, 3.0 (2.0 – 4.0)		2.32 ± 0.75, 2.0 (1.40 – 3.90)		0.002
LVPWd (cm) ‡	0.74 ± 0.42, 0.55 (0.50 – 1.70)		0.55 ± 0.24, 0.50 (0.40 – 1.50)		0.023
LVPWs (cm) ‡	0.87 ± 0.42, 0.70 (0.40 – 1.80)		0.73 ± 0.29, 0.65 (0.40 – 1.80)		0.180
EF%§	46.86 ± 18.38, 42.0 (22.0 – 80.0)		65.46 ± 10.88, 68.0 (42.0 – 82.0)		0.003
FS‡	23.43 ± 9.19, 21.0 (11.0 – 40.0)		33.15 ± 6.25, 34.0 (21.0 – 50.0)		0.002
E/A§	1.30 ± 0.48, 1.37 (0.50 – 2.25)		1.34 ± 0.31, 1.35 (0.52 – 1.92)		0.776
E/É‡	11.87 ± 3.87, 11.54 (4.40 – 17.40)		8.71 ± 2.64, 8.78 (3.55 – 13.25)		0.012
TAPSE (cm) ‡	2.07 ± 0.79, 1.90 (0.90 – 3.20)		1.67 ± 0.79, 1.30 (0.80 – 3.20)		0.067

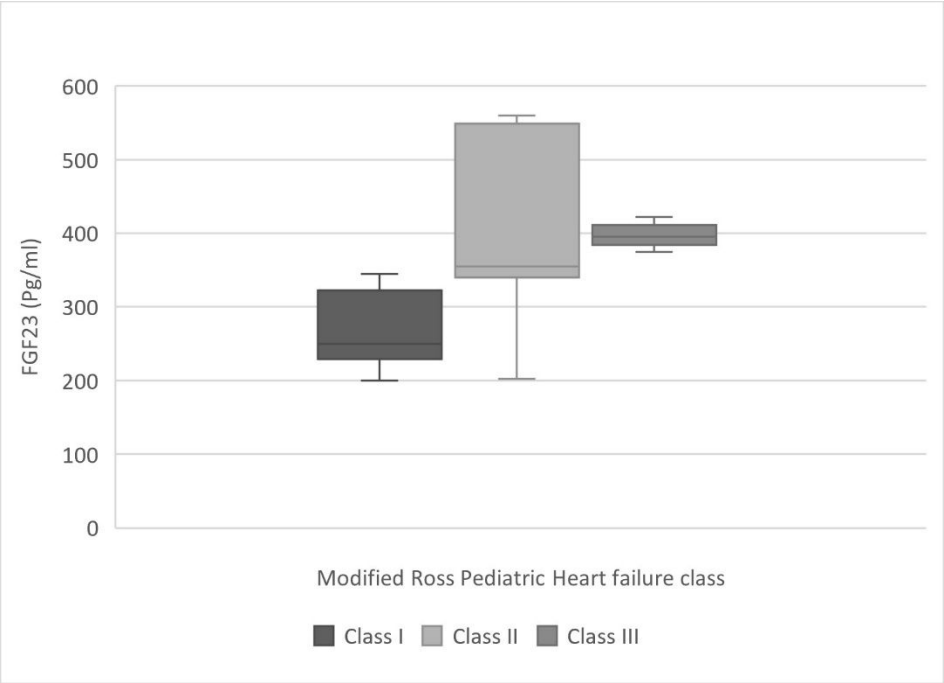
Data are described as number and percentage or as mean ± SD, median (range).

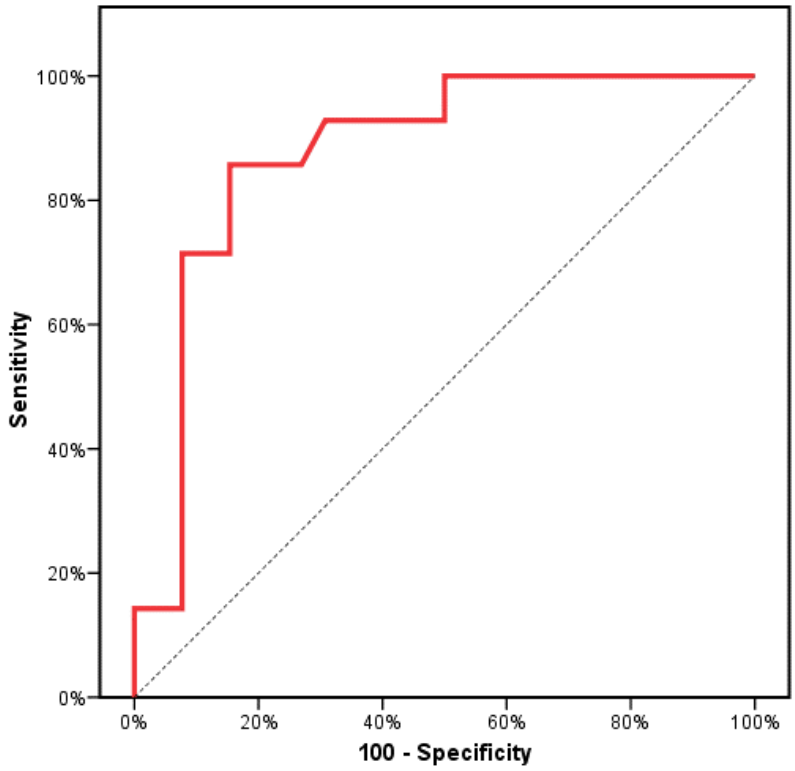
†χ2 test/Fisher's exact test was used.
© Monte Carlo test was used.
‡Mann-Whitney test was used.
§t-test was used
^{FE}P: P value of Fisher exact test
BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion

Table (4): Univariable and multivariable Logistic regression analysis for predictors of poor outcome in children with HF

	Univariable		Multivariable	
	P	OR (95%C.I)	P	OR (95%C.I)
Sex (male)	0.816	1.167(0.318 – 4.284)		
Age (months)	0.205	1.007(0.996 – 1.018)		
Hear rate (Beats/min.)	0.620	0.993(0.965 – 1.022)		
Respiratory rate	0.599	0.988(0.944 – 1.034)		
Systolic blood pressure	0.789	0.991(0.930 – 1.057)		
Diastolic blood pressure	0.902	1.006(0.912 – 1.110)		
ROSS class III	0.021*	13.0(1.478 – 114.358)	0.046*	0.224(0.051–0.972)
Duration of HF	0.928	0.999(0.971 – 1.027)		
Weight (kg)	0.250	1.027(0.982 – 1.074)		
Length (m)	0.270	2.991(0.427 – 20.933)		
Body mass index (Kg/m^2)	0.153	1.201(0.934 – 1.554)		
IVSd (cm)	0.129	4.895(0.631 – 37.938)		
IVSs (cm)	0.182	3.515(0.556 – 22.231)		
LVEDD (cm)	0.013*	3.076(1.265 – 7.482)	0.439	0.631(0.197 – 2.024)
LVESD (cm)	0.007*	3.874(1.446 – 10.376)	0.646	1.688(0.181 – 15.761)
LVPWd (cm)	0.111	6.373(0.655 – 61.975)		
LVPWS (cm)	0.228	3.254(0.479 – 22.113)		
EF%	0.002*	0.919(0.871 – 0.970)	0.612	0.581(0.071–4.740)
FS	0.002*	0.846(0.760 – 0.943)	0.751	1.972(0.030–130.528)
E/A	0.769	0.769(0.133 – 4.446)		
E/É	0.011*	1.382(1.076 – 1.775)	0.410	0.700(0.299–1.635)
TAPSE (cm)	0.129	1.909(0.828 – 4.403)		
Calcium (mg/dl)	0.944	0.964(0.346 – 2.688)		
Phosphate (mg/dl)	0.238	2.943(0.490 – 17.657)		
Parathyroid hormone (pg/ml)	0.037*	1.135(1.008 – 1.277)	0.168	1.191(0.929–1.526)
Troponin	0.051	10.0(0.992 – 100.821)		
NT-proBNP (pg/ml)	0.004*	1.015(1.005 – 1.026)	0.761	1.015(0.924–1.114)
Serum Creatinine (mg/dl)	0.262	0.073(0.001 – 7.027)		
BUN (mg/dl)	0.962	0.995(0.810 – 1.222)		
Hemoglobin (gm/dl)	0.437	0.982(0.938 – 1.028)		
White cell count (*10^3/ul)	0.662	1.085(0.753 – 1.562)		
Platelet count (*10^3/ul)	0.910	0.999(0.990 – 1.009)		
eGFR	0.150	1.016(0.994 – 1.038)		
FGF23 (pg/ml)	0.009*	1.014(1.004 – 1.025)	0.022*	1.026(1.004 – 1.048)

OR: Odd's ratio, C.I: Confidence interval, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate. *: Statistically significant at $p \leq 0.05$





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Fibroblast growth factor 23 in children with or without heart failure: a prospective study

Keywords: fibroblast growth factor 23, children and Heart failure, prognosis

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Abstract

Background: Elevated fibroblast growth factor 23 (FGF23) levels have been associated with mortality in adults with heart failure (HF), but data on FGF23 levels in pediatric HF is limited. In this prospective cohort study, we aimed to assess the prognostic value of fibroblast growth factor 23 in children with chronic HF.

Methods: We prospectively enrolled 40 children with chronic HF and 20 matched healthy controls. For each patient, a complete diagnostic workup was performed, including transthoracic echocardiography to evaluate cardiac systolic and diastolic functions. Serum FGF23, renal function tests, parathyroid hormone, serum calcium and phosphate were measured for patients and controls. N-terminal pro-brain natriuretic peptide was measured in patients. The severity of symptoms was assessed using modified Ross HF classification for children. Patients were followed for one year, and clinical worsening events such as death or HF hospitalization were recorded.

Results: Compared to controls, patients with HF had significantly higher FGF23 levels (355.68 ±97.27; 60.20±11.04 pg/ml respectively, P <0.001). Three patients died and eleven were admitted with HF. In comparison to patients without clinical worsening events, these fourteen patients exhibited significantly higher FGF23 levels (320.04 ± 89.56; 421.86 ± 75.50 pg/ml, respectively; P <0.001). FGF23 was positively correlated with NT-pro-BNP and left ventricular end diastolic diameter and negatively correlated with ejection fraction and fractional shortening. The ability of FGF23 to predict clinical worsening events in patients was analyzed using a receiver operating characteristic curve. The optimal cutoff point was 375 pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV=91.7 and AUC=0.878. Multivariable regression analysis revealed that FGF23 is the only independent predictor of clinical worsening events in children with chronic heart failure.

Conclusion: FGF23 levels were elevated in children with chronic HF, and they increased significantly as Ross score class increased. FGF23 levels increased in patients who experienced clinically worsening events.

What is known already known on this topic? Increased FGF23 levels have been associated with left ventricular (LV) hypertrophy and impaired LV function. Adult studies showed that FGF23 is related to adverse cardiovascular events in patients with HF and that it could be used to predict the clinical outcome of those patients, but data about FGF23 in children with HF is limited.

What this study adds? FGF23 levels increased significantly with increasing HF clinical severity and in patients experiencing clinically worsening events. FGF23 may have a potential prognostic value as a novel biomarker in pediatric HF with a cutoff point >375 pg/ml predicting children with clinical worsening events.

Keywords: fibroblast growth factor 23, children and heart failure, prognosis.

Introduction:

Pediatric HF (HF) is a complex disease process, that can occur secondary to a variety of etiologies, including congenital heart diseases, cardiomyopathy, and acquired conditions as well. It remains a major cause of morbidity and mortality in childhood, with a significant health and economic burden worldwide.[1]

Exploring new cardiac biomarkers for HF helps clinicians identify disease progression, predict clinical outcomes, provide protective strategies, select the proper treatment, and monitor therapy.[2]

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone secreted by osteoblasts and osteocytes in response to increased phosphate levels, regulating renal phosphate homeostasis and vitamin D metabolism, by stimulating phosphaturia and inhibiting calcitriol synthesis. [3]

Besides its role in mineral metabolism, FGF23 has a direct effect on the cardiovascular system. It has been recently linked to adverse cardiovascular events in adult patients with HF and is involved in cardiac remodeling. [4-7] Increased FGF23 levels have been associated with left ventricular (LV) hypertrophy and impaired LV function. [8-11] It has been linked with alterations in myocyte calcium handling [12] and upregulation of the renin-angiotensin system. [13] Interestingly, adult studies showed that FGF23 not only predicted clinical outcomes in patients with acute and stable HF [14,15] but was also independently associated with all-cause death and HF in community-living older persons. [16]

Comparable data about FGF23 in children with HF is limited. Therefore, we aimed to measure and compare FGF23 levels of children with chronic HF with those of healthy children, to correlate them with indicators of HF severity, and to assess the prognostic significance of FGF23 in children with chronic HF.

Methods

This prospective cohort study was carried out at the Pediatric Cardiology Unit of Menoufia University Hospital between January 2020 and February 2021. Informed consent was obtained from the guardian of each participant included in the study. All study procedures were

carried out in accordance with the ethical standards of Menoufia University Institutional Research Committee.

Patient and public involvement statement: Patients and/or the public were involved in the design, conduct, reporting, and dissemination plans of this research. Public healthcare clinics, but not families, were involved in the content and design.

Eligibility criteria

Inclusion criteria: For the case group, we targeted children with chronic HF, defined by the International Society for Heart and Lung Transplantation Practice Guidelines for Management of HF in Children [17] as a syndrome resulting from ventricular dysfunction, volume overload, or pressure overload, alone or in combination. The definition of chronic HF has changed from the traditional definition of a syndrome that results from inadequate cardiac output to maintain end-organ perfusion to the new definition in adults, which incorporates disorders of ventricular filling, that is, diastolic dysfunction or HF with preserved ejection fraction. [18]

So, patients with systolic and diastolic chronic heart failure who fulfilled one or more of the following criteria were included in the study:

- patients with reduced LV ejection fraction (EF%), fractional shortening (FS), and/or tricuspid annular plane systolic excursion (TAPSE).
- symptomatic[#] patients with evidence of structural cardiac abnormality.
- asymptomatic patients with prior symptoms and signs of HF controlled on anti-failure medications with evidence of structural cardiac abnormality.

All patients included in the study did not have their structural cardiac abnormalities corrected.

[#]Symptoms and signs of HF were respiratory distress, dyspnea on exertion, feeding problems, failure to thrive, diaphoresis, hepatomegaly, cool extremities, and poor peripheral perfusion. [19]

The control group included a group of age- and sex-matched healthy children who were referred to the cardiology clinic for innocent murmurs and found to be free of any cardiac disease.

Exclusion criteria: diseases that affect FGF23 such as chronic kidney disease and rickets, any congenital anomalies other than congenital heart disease, and age less than 2 months and more than 18 years.

All the included patients and controls were subjected to detailed history taking, including collecting patient demographics and a comprehensive clinical examination. Patients were assessed for the functional status of HF using the Modified Ross classification system for HF in infants and children.[20] Patients were followed for 1 year for an endpoint of all-cause mortality or HF hospitalization, and patients were classified accordingly into 2 groups: patients with clinical worsening events (death or hospitalization with HF) and patients without clinical worsening events.

Investigations:

Blood samples were collected, then centrifuged, aliquoted, labeled, and stored at −80 °C until batch assays were performed. Samples were withdrawn from patients and controls once they were included in the study according to the previously mentioned inclusion criteria during their visit to the cardiology clinic. Because of the well-defined role of FGF23 in renal and bone diseases, investigations using standard procedures were performed, including a complete blood count (CBC), renal functions, serum calcium, serum phosphate, and parathyroid hormone. Estimated glomerular filtration rate (eGFR; in ml/min/1.73 m2) was determined using an established formula of eGFR=k × height (cm)/serum creatinine (mg/dl) [19]. Serum intact FGF23 levels were measured by an enzyme-linked immunosorbent assay (ELISA) commercial kit from "Elabscience, USA." There is no clear FGF23 cut-off value for the paediatric population, and the available assays are not standardized. So, we used the most similar assay reference values (Kainos Laboratories, Japan) with a normal FGF23 cutoff level in children of less than 71 pg/mL. [21] To correlate FGF23 with other biomarkers of HF, we measured NT-proBNP using ELISA kits in children with HF.

Echocardiography

Upon enrollment, Philips HD 11 XE equipment (Philips, Bothell, WA) was used for transthoracic echocardiography (TTE) imaging with tissue Doppler study, using transducers of 3–8 and 1-3 MHz, depending on patient size. The two authors (first and last), who have more

than 10 years of expertise in echocardiography, performed the echocardiographic examination and were not aware of the serum test results. To reduce the interobserver variability, both were present during each echo examination to double check the echo results separately. Only agreed-upon results were recorded. M-mode tracing was obtained in the parasternal short-axis view at the level of the papillary muscles of the LV, and LV end-systolic and end-diastolic diameters were measured. LVEF was measured using the modified Simpson's method, as recommended by the American Society of Echocardiography. 22, 23 EF% and FS were considered reduced if < 55% and ≤ 25 respectively 24. TAPSE was considered reduced if < 10 mm 25.

Statistical analysis: -

The data collected were tabulated and statistically analyzed using Statistical Package for Social Science (SPSS) Version 22. The Kolmogorov-Smirnov, Shapiro, and D'agstino tests were used to verify the normality of the distribution of variables. The chi-square test or Fisher exact test was used to compare categorical variables. The student t-test was used to compare two groups for normally distributed quantitative variables, whereas the Mann-Whitney U test was used to compare two groups for abnormally distributed quantitative variables. Pearson coefficient was used to correlate between quantitative variables. The receiver operating characteristic (ROC) curve was performed to assess the prognostic performance of FGF23, with respective maximum accuracy points for both sensitivity and specificity (an area of more than 50% gives acceptable performance, and an area of about 100% is the best performance for the test). Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. Univariable and multivariable logistic regression analysis was done to detect the most independent factor(s) that predict clinical worsening events in children with chronic HF (all-cause mortality and HF hospitalization). Because of the small sample size, variance inflation factors (VIF) were calculated for the statistically significant variables in the univariable analysis to measure multicollinearity among the independent variables in the multiple regression model. Only variables with a low VIF (less than 5) were included in the multivariable regression analysis. 26 The significance of the obtained results was judged to be at the 5% level. The sample size was calculated using the following formula: $n \geq \left[\frac{z_{1-\alpha/2} + z_{1-\beta}}{\frac{1}{2}\log_e \frac{1+r}{1-r}}\right]^2 + 3$

where n = sample size, Z1-α/2 =1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail), r = 0.63 (correlation coefficient between FGF23 and left ventricular end-diastolic diameter in a previous study by Isakova et al. 27, β = 0.05. The required sample size to conduct this study was at least 27 patients and 13 controls. We exceeded the sample size (40 patients and 20 controls) to increase the accuracy and significance of the results.

Results:

The study included 60 children divided into two independent groups. The case group included 40 children with chronic HF, with a median age of 12.0 months (IQR = 6.5–108), and the control group included 20 matched clinically healthy children with a median age of 12.0 months (IQR = 6.5–108).

Table 1 displays the clinical features of patients with HF. Congenital heart disease was the cause of HF in 19 patients, dilated cardiomyopathy in 15, hypertrophic cardiomyopathy in 4, and rheumatic heart disease in 2 patients, with a median HF duration of 10 months (IQR = 6-27). According to the modified Ross score, 14 patients (35%) were placed in class I, 15 patients (37%) in class II, and 11 patients (27.5%) in class III.

Table 2 compared the case and control groups' demographic, clinical, laboratory, and echocardiographic data. Age, sex, body mass index (BMI), height, weight, and blood pressure (systolic and diastolic) did not significantly differ between the two groups. Both heart rate and respiratory rate were significantly greater in the case group. There was no statistically significant difference between the two groups in terms of laboratory data for the CBC parameters, BUN, serum creatinine, serum calcium, serum phosphorus, and eGFR. FGF23 levels were significantly higher in HF patients than in healthy controls (355.68 97.27 pg/ml vs. 60.20 11.04 pg/ml; p <0.001). Compared to controls, cases had significantly higher LVEDD, LVESD, and E/e` and lower EF% and FS.

Table 3 showed a comparison of demographic, clinical, laboratory, and echocardiographic data between patients with and without clinical worsening. Of the 14 children who experienced clinically worsening events, 11 needed hospital admission for HF, and three passed away during the study. The three children were suffering from dilated cardiomyopathy. One of them had a sudden death shortly after complaining of palpitation and chest pain at home.

The other two patients were presented with cardiogenic shock and were admitted to our intensive care unit. Their condition worsened until they eventually passed away. One died on the sixth day, and the other on the eighth.

Regarding age, sex, the duration of HF, heart rate, respiratory rate, blood pressure, weight, height, and BMI, there was no statistically significant difference between the two groups. The modified Ross classification placed the majority of patients with clinical worsening events in class III (8 patients). When compared to patients without clinical worsening events, participants with clinical worsening events had statistically significantly higher troponin I, NT-proBNP, and FGF23 levels. Patients with clinical worsening events showed statistically substantially greater LVEDD, LVESD, and E/e' ratios as well as poorer EF and FS than those without clinical worsening events in terms of echocardiographic measures.

FGF23 serum levels increased significantly as Ross score class increased (P <0.001) **[Figure 1].**

FGF 23 was positively correlated with LVEDD (r=0.437. P<0.001) and NT- proBNP (r= 0.977, P<0.001) and negatively correlated with EF (r= -0.328, P=0.039 and FS (r= -0.365, P=0.021).

The prognostic performance of FGF23 was analyzed using a ROC curve **[Figure 2]**. The AUC was 0.878 (95% CI = 0.767-0.989; p 0.001). The optimal cutoff point to predict clinical worsening events was >375 pg/ml with 85.71% sensitivity, 84.62% specificity, PPV=75.0, NPV= 91.7. **Table 4** depicts univariate and multivariable regression analysis for variables that may influence the patient's prognosis. Statistically significant parameters with a low VIF (less than 5) from the univariable analysis were incorporated in a multivariable regression analysis model after the exclusion of NT-proBNP due to multicollinearity between FGF23 and NT-proBNP. The FGF23 level was the only independent predictor of clinical worsening events in children with chronic HF (P=0.027).

Discussion:

The current study showed that children with chronic HF had a significantly higher level of FGF23 compared to controls and that FGF23 levels increased significantly with increasing Ross score class and in patients with clinical worsening events.

Isakova et al. compared the FGF23 levels of 20 children suffering from chronic HF caused by various etiologies and 17 healthy controls. They reported about a 2-fold increase in FGF23 levels in patients compared to healthy controls and found a significant association between FGF23 levels and both the clinical severity of HF (assessed by NYHA/Ross class and NT-pro BNP level) and left ventricular dilatation. Both associations were independent of eGFR. [27] Studies in the adult population demonstrated that FGF23 was elevated in patients who had HF with preserved ejection fraction [28] and with reduced ejection fraction [4.] Additionally, Anderson et al. found elevated serum levels of FGF23 in patients with acute decompensated HF. [29] In our study, elevated FGF-23 could not be explained by impaired renal function, as has been largely described [7, 30], indicating another underlying mechanism. Studies revealed that FGF23 is expressed in the heart and that, regardless of renal function, it is markedly increased in clinical and experimental conditions of cardiac remodeling and HF. FGF23 may promote cardiac injury through endocrine, paracrine/autocrine, and other mechanisms. [31, 32]

Risk stratification for heart failure is greatly influenced by the NYHA classification, biomarkers including NT-proBNP, and left ventricular systolic function. [33, 34] Our study demonstrated that FGF23 was increased with increasing disease severity assessed by modified Ross classification, NT-proBNP and ventricular function indices. FGF23 had been postulated to have direct cardiac and vascular toxicity. [35, 36] Another possibility is that FGF23 suppresses calcitriol synthesis, which then activates the renin-angiotensin-aldosterone system. [37, 38]

Our study's novel conclusion is that FGF23 levels are significantly higher in HF children with clinical worsening events (death and hospitalization) than in HF children without such events. FGF23 level was found to be the only independent predictor of clinical worsening events in a multivariable logistic regression model. This was in line with adult studies conducted by Gruson et al. [4] who found that FGF23 was the strongest predictor of long-term cardiac death, and Plischke et al. [15] who showed that FGF23 level was significantly higher in patients reaching the combined endpoint of cardiac hospitalization or death. Imazu et al. reported that FGF23 levels in

HF hospitalized patients were higher than in non-hospitalized patients, and that elevated FGF23 is associated with poor outcomes (death, LV assist device implantation, and rehospitalization). [39]

The optimal cutoff value in our study to distinguish patients with clinical worsening events from those without was >375 pg/ml, with 85.71% sensitivity, 84.62% specificity, a PPV of 75.0, an NPV of 91.7, and an AUC of 0.878. In the study by Cornelissen et al., [14] FGF23 measured on days 1 and 2 after admission for patients with acute HF accurately predicted one-year outcomes, as did the Seattle HF model. In addition, a later study by the same authors showed that in patients with acute myocardial infarction and concomitant HF, FGF23 levels outperformed the GRACE score in one-year mortality prediction. FGF23, however, lacked any discriminative capacity for predicting survival in patients without acute HF. [40] They demonstrated that in patients with acute HF caused by myocardial infarction, LogFGF23 levels of 1.71 pg/ml accurately predicted death at one year with a sensitivity of 75% and a specificity of 74%.

Our study has some limitations. First, it was a single-center study with a small sample size. Second, data analysis may be limited by the diverse etiologies of heart failure in our patients with predominantly unoperated structural heart diseases. Previous research, however, has shown that FGF23 levels are elevated in HF patients regardless of the cause. Third, the multivariable model's interpretation may be limited by the small number of our patients. Thus, multicentric studies involving a larger number of participants may be warranted. Additionally, further research is needed to fully comprehend the precise pathophysiologic function of FGF23 in pediatric HF.

Conclusion:

Children with chronic HF had elevated serum FGF23 levels, which increased significantly with increasing modified Ross score class. FGF23 may have a potential prognostic value as a novel biomarker in pediatric HF, allowing for identification of high-risk patients who are more prone to complications and need a closer follow-up and more aggressive treatment.

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Table (1): Clinical characteristics of patients with chronic heart failure (n=40)

Characteristic	No.
Etiology of heart failure	
Congenital heart diseases (CHD)	19
Ventricular septal defect (VSD)	5
Patent ductus arteriosus (PDA)	4
Large ostium secundum atrial septal defect (ASD)	2
Parachute mitral valve causing severe MS	1
Parachute mitral valve causing severe MS & moderate MR	1
Dysplastic mitral valve causing severe MS & MR	1
Fallot tetralogy with mild Pulmonary stenosis	1
Suboartic VSD causing severe aortic regurgitation (AR)	1
PDA + VSD	1
Congenital Moderate aortic stenosis + severe AR	1
VSD+ASD	1
Dilated cardiomyopathy (DCM)	15
Hypertrophic cardiomyopathy (HOCM)	4
Rheumatic heart diseases *Severe MR and mild AR* *Moderate MR and AR*	2
Medications	
Diuretics	36
Angiotensin converting enzyme inhibitors	33
B-blockers	17
Digoxin	5
Anticoagulants	2
Modified Ross class	
Class I	14
Class II	15
Class III	11
Duration of heart failure (months); Median (IQR)	10 (6-27)
Duration of follow up till occurrence of endpoint (months); Median (IQR)	12 (10-12)

Table (2): Comparison of demographic, clinical, laboratory and echocardiographic data between case and control groups

Parameter	Case group (n=40)	Control group (n=20)	p-value
Demographic and clinical parameters			
Age (months) ‡	12.0 (6.50 – 108.0)	12.0 (6.50 –102.0)	0.863
Females † no (%)	21 (52.5)	12 (60	0.582
Heart rate (Beats/minute) §	128.50 ± 22.99	110.30 ± 17.76	**0.001**
Respiratory rate (per minute) ‡	53.50 (40.0 – 60.0)	38.0 (20.0 – 41.0)	**<0.001**
Systolic blood pressure (mmHg) §	95.95 ± 10.40	95.75 ± 10.14	0.944
Diastolic blood pressure (mmHg) §	59.82 ± 6.71	63.10 ± 7.48	0.091
Weight (Kg) ‡	9 (8.75-25.0)	10.50 (7.0-26.50)	0.445
Length or height (m) ‡	0.77 (0.68-1.24)	0.81 (0.71-1.21)	0.433
Body mass index (kg/m2) §	16.16 ± 2.94	16.98 ± 3.38	0.342
Laboratory parameters			
Hemoglobin (gm/dl) §	10.88 ± 0.92	11.17 ± 0.96	0.252
White cell count (*10^3/ul) §	7.03 ± 1.80	6.80 ± 1.57	0.629
Platelet count(*10^3/ul) §	265.55 ± 67.16	258.0 ± 62.12	0.676
Serum creatinine (mg/dl) §	0.63 ± 0.16	0.67 ± 0.18	0.332
BUN (mg/dl) §	14.18 ± 3.21	13.40 ± 2.91	0.367
eGFR§	77.51 ± 30.80	77.95 ± 39.52	0.536
Serum calcium (mg/dl) §	9.35 ± 0.64	9.30 ± 0.52	0.788
Serum phosphate (mg/dl) §	2.98 ± 0.56	3.0 ± 0.36	0.542
PTH (pg/ml) §	42.85 ± 6.34	40.20 ± 4.73	0.131
Serum FGF23 (pg/ml) §	355.68 ± 97.27	60.20 ± 11.04	**<0.001**
NT-proBNP (pg/ml)	380.2 ± 103.9	--	
Echocardiographic parameters			
IVSd (cm)‡	0.50 (0.40-0.60)	0.50 (0.40-0.60)	0.733
IVSs (cm)‡	0.60 (0.50-0.75)	0.60 (0.50-0.70)	0.350
LVEDD (cm)‡	4.0 (3.0-4.45)	2.80 (2.35-3.85)	**0.001**
LVESD(cm) ‡	2.75 (1.90-3.30)	1.75 (1.55-2.70)	**0.003**
LVPWd (cm) ‡	0.50 (0.40-0.60)	0.50 (0.40-0.70)	0.611
LVPWs (cm) ‡	0.70 (0.60-0.80)	0.65 (0.6-0.85)	0.642
EF%‡	58.95 (44.0-72.0)	67.60(63.5-73.0)	**0.001**
FS‡	29.45 (21.75-36.0)	34.80 (32.0-36.25)	**0.047**
E/A‡	1.33 (1.21 – 1.50)	1.47 (1.22 – 1.56)	0.106
E/e`‡	9.82 (7.93-11.89)	7.33 (6.14-8.81)	**0.005**
TAPSE(cm)‡	1.81 (1.20-2.70)	1.60 (1.05-2.13)	0.295

Data are presented as no, percentage, mean ± standard deviation or as median (IQR).

†χ2 test/Fisher's exact test was used.

‡Mann-Whitney test was used.

§t-test was used

BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion.

Table (3): Comparison of demographic, clinical, laboratory and echocardiographic data between patients with and without clinical worsening events.

Parameter	Patients with events (n = 14)		Patients without events (n = 26)		P
Sex†	No	Percentage	No	Percentage	
Male	7	50.0	12	46.2	0.816
Female	7	50.0	14	53.8	
Age (months) ‡	67.79 ± 72.57, 24.0 (3.0 – 192.0)		41.96 ± 53.52, 12.0(2.0 – 144.0)		0.130
Mortality n (%)	3 (21.4%)		0 (0%)		
Diagnosis©					
Congenital heart disease	2	14.3	17	65.4	0.002
Dilated cardiomyopathy	10	71.4	5	19.2	
Hypertrophic cardiomyopathy	2	14.3	2	7.7	
Rheumatic heart disease	0	0.0	2	U7.7	
Modified ROSS class†					0.003
Class I	1	7.1	13	50.0	
Class II	5	35.7	10	38.5	
Class III	8	57.1	3	11.5	
Duration of HF (Months) ‡	19.07 ± 21.57, 12.0 (2.0 – 60.0)		19.77 ± 24.86, 6.5 (2.0 – 96.0)		0.967
Hear rate (Beats/minute) §	126.07 ± 24.35, 130.0 (90.0 – 170.0)		129.81 ± 22.60, 135.0 (85.0 – 165.0)		0.630
Respiratory rate‡	47.0 ± 15.0, 51.0 (20.0 – 65.0)		49.46 ± 14.09, 55.0 (20.0 – 65.0)		0.664
Systolic blood pressure§	95.36 ± 8.65, 95.0 (85.0 – 110.0)		96.27 ± 11.38, 95.0 (80.0 – 120.0)		0.795
Diastolic blood pressure§	60.0 ± 6.50, 60.0 (50.0 – 70.0)		59.73 ± 6.94, 60.0 (50.0 – 80.0)		0.905
Weight (kg) ‡	20.29 ± 16.66, 12.0 (5.0 – 55.0)		14.73 ± 13.07, 9.0 (4.0 – 45.0)		0.130
Length (m) ‡	1.0 ± 0.36, 0.83 (0.65 – 1.61)		0.88 ± 0.32, 0.74 (0.55 – 1.50)		0.220
Body mass index (Kg/m²) §	17.08 ± 2.29, 16.69 (11.83 – 21.48)		15.67 ± 3.17, 16.20 (8.65 – 20.66)		0.150
Hmoglobin (gm/dl) §	10.64 ± 1.05, 10.65 (8.50 – 12.50)		10.99 ± 0.83,11.15 (8.50 – 12.50)		0.124
White blood count (*10³/ul)§	7.19 ± 2.02, 7.10 (4.30 – 12.0)		6.93 ± 1.71, 6.80 (4.30 – 10.50)		0.671
Platelet count (*10³/ul)§	263.93 ± 75.60, 265.0 (170.0 – 400.0)		266.42 ± 63.74, 255.0 (156.0 – 400.0)		0.913
Serum Creatinine (mg/dl) §	0.59 ± 0.09, 0.60 (0.50 – 0.80)		0.65 ± 0.19, 0.60 (0.40 – 1.0)		0.182
BUN (mg/dl) §	14.14 ± 3.18, 13.50 (10.0 – 20.0)		14.19 ± 3.29, 13.0 (10.0 – 20.0)		0.964
eGFR‡	87.23 ± 34.36, 75.63 (53.57 – 160.0)		72.28 ± 28.01, 65.88 (37.0 – 145.0)		0.190
Calcium (mg/dl) ‡	9.34 ± 0.63, 9.15 (8.50 – 10.20)		9.36 ± 0.66, 9.20 (8.40 – 11.0)		0.989
Phosphate (mg/dl) ‡	3.16 ± 0.88, 2.90 (2.60 – 6.0)		2.88 ± 0.24, 2.85 (2.60 – 3.50)		0.528
Parathyroid hormone	45.79 ± 6.61, 48.0 (34.0 – 55.0)		41.27 ± 5.70, 40.0 (32.0 – 52.0)		0.055
NT-proBNP (pg/ml) §	457.57 ± 82.61, 416.0 (340.0 – 600.0)		338.54 ± 90.19, 350.0 (210.0 – 560.0)		<0.001
FGF23 (pg/ml) §	421.86 ± 75.50, 397.50 (329.0 -560.0)		320.04 ± 89.56, 326.0 (200.0 - 550.0)		<0.001
IVSd (cm) ‡	0.74 ± 0.48, 0.50 (0.40 – 1.90)		0.54 ± 0.25, 0.50 (0.30 – 1.50)		0.123
IVSs (cm) ‡	0.85 ± 0.50 , 0.70 (0.40 – 2.0)		0.68 ± 0.26, 0.60 (0.47 – 1.50)		0.305
LVEDD (cm) ‡	4.41 ± 0.72, 4.20 (3.50 – 5.90)		3.56 ± 0.93, 3.20 (2.30 – 5.90)		0.005
LVESD (cm) ‡	3.10 ± 0.69, 3.0 (2.0 – 4.0)		2.32 ± 0.75, 2.0 (1.40 – 3.90)		0.002
LVPWd (cm) ‡	0.74 ± 0.42, 0.55 (0.50 – 1.70)		0.55 ± 0.24, 0.50 (0.40 – 1.50)		0.023
LVPWs (cm) ‡	0.87 ± 0.42, 0.70 (0.40 – 1.80)		0.73 ± 0.29, 0.65 (0.40 – 1.80)		0.180
EF%§	46.86 ± 18.38, 42.0 (22.0 – 80.0)		65.46 ± 10.88, 68.0 (42.0 – 82.0)		0.003
FS‡	23.43 ± 9.19, 21.0 (11.0 – 40.0)		33.15 ± 6.25, 34.0 (21.0 – 50.0)		0.002
E/A§	1.30 ± 0.48, 1.37 (0.50 – 2.25)		1.34 ± 0.31, 1.35 (0.52 – 1.92)		0.776
E/É‡	11.87 ± 3.87, 11.54 (4.40 – 17.40)		8.71 ± 2.64, 8.78 (3.55 – 13.25)		0.012
TAPSE (cm) ‡	2.07 ± 0.79, 1.90 (0.90 – 3.20)		1.67 ± 0.79, 1.30 (0.80 – 3.20)		0.067

Data are described as number and percentage or as mean ± SD, median (range).

†χ2 test/Fisher's exact test was used.
© Monte Carlo test was used.
‡Mann-Whitney test was used.
§t-test was used
[FE]P: P value of Fisher exact test
BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion

Table (4): Univariable and multivariable Logistic regression analysis for predictors of clinical worsening events in children with chronic heart failure

	Univariable		Multivariable	
	P	OR (95%C.I)	P	OR (95%C.I)
Sex (male)	0.816	1.167(0.318 – 4.284)		
Age (months)	0.205	1.007(0.996 – 1.018)		
Hear rate (Beats/min.)	0.620	0.993(0.965 – 1.022)		
Respiratory rate	0.599	0.988(0.944 – 1.034)		
Systolic blood pressure	0.789	0.991(0.930 – 1.057)		
Diastolic blood pressure	0.902	1.006(0.912 – 1.110)		
ROSS class III	0.021*	13.0(1.478 – 114.358)	0.084	4.850(0.809 – 29.081)
Duration of HF	0.928	0.999(0.971 – 1.027)		
Weight (kg)	0.250	1.027(0.982 – 1.074)		
Length (m)	0.270	2.991(0.427 – 20.933)		
Body mass index (Kg/m^2)	0.153	1.201(0.934 – 1.554)		
IVSd (cm)	0.129	4.895(0.631 – 37.938)		
IVSs (cm)	0.182	3.515(0.556 – 22.231)		
LVEDD (cm)	0.013*	3.076(1.265 – 7.482)		
LVESD (cm)	0.007*	3.874(1.446 – 10.376)		
LVPWd (cm)	0.111	6.373(0.655 – 61.975)		
LVPWS (cm)	0.228	3.254(0.479 – 22.113)		
EF%	0.002*	0.919(0.871 – 0.970)		
FS	0.002*	0.846(0.760 – 0.943)		
E/A	0.769	0.769(0.133 – 4.446)		
E/É	0.011*	1.382(1.076 – 1.775)	0.736	1.022(0.899 – 1.163)
TAPSE (cm)	0.129	1.909(0.828 – 4.403)		
Calcium (mg/dl)	0.944	0.964(0.346 – 2.688)		
Phosphate (mg/dl)	0.238	2.943(0.490 – 17.657)		
Parathyroid hormone (pg/ml)	0.037*	1.135(1.008 – 1.277)	0.320	1.073(0.934 – 1.233)
Troponin	0.051	10.0(0.992 – 100.821)		
NT-proBNP (pg/ml)	0.004*	1.015(1.005 – 1.026)		
Serum Creatinine (mg/dl)	0.262	0.073(0.001 – 7.027)		
BUN (mg/dl)	0.962	0.995(0.810 – 1.222)		
Hemoglobin (gm/dl)	0.437	0.982(0.938 – 1.028)		
White cell count (*10^3/ul)	0.662	1.085(0.753 – 1.562)		
Platelet count (*10^3/ul)	0.910	0.999(0.990 – 1.009)		
eGFR	0.150	1.016(0.994 – 1.038)		
FGF23 (pg/ml)	0.009*	1.014(1.004 – 1.025)	0.027*	1.011(1.001 – 1.021)

OR: Odds ratio , C.I: Confidence interval, IVSd: Interventricular septum thickness in diastole, IVSs: Interventricular septum thickness in systole, LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, LVPWs: left ventricular posterior wall thickness in systole, EF%: Ejection fraction, FS: fraction shortening, TAPSE: Tricuspid annular plane systolic excursion, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate. *: Statistically significant at $p \leq 0.05$.

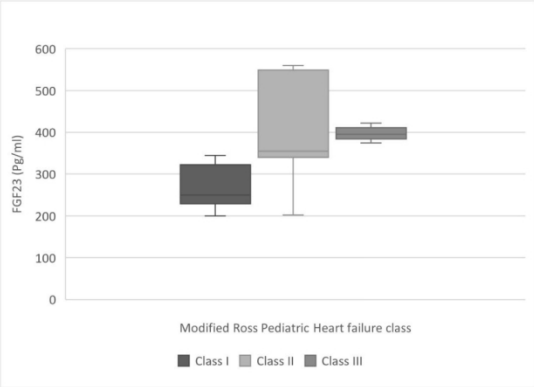

Figure 1. Boxplots showing comparison between FGF23 levels among different Ross Classes (Pf < 0.001). Pairwise comparison between each 2 classes was done using Post Hoc Test (Tukey): P value for comparing class I and II was <0.01, P value for comparing class I and III was <0.01, and P value for comparing class II and III was 0.048.
Pf for ANOVA test.

143x186mm (300 x 300 DPI)

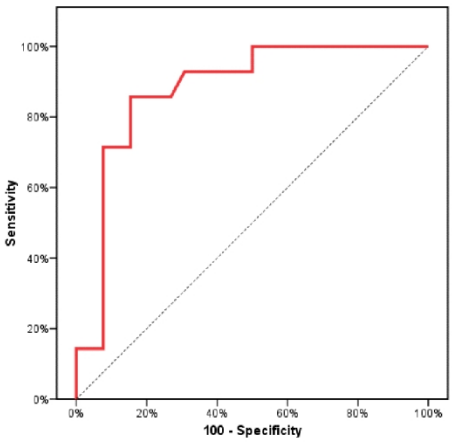

Figure (2): Receiver operating characteristic curve for the ability of FGF23 (pg/ml) to predict clinical worsening events in children with heart failure. The area under the curve was 0. 878 (95% CI= 0.767 – 0.989; p < 0.001).

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