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# Cardiac Myosin Binding Protein C Plasma Level as a Diagnostic and Prognostic Biomarker in Heart Failure in Children

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## ABSTRACT

**Background:** Heart failure is one of the cardiovascular diseases that have high morbidity and mortality especially among children. It accounts for 2% of all pediatric inpatient admission. Cardiac Myosin Binding Protein-C (cMyBP-C) is cardiomyocyte specific sarcomeric protein that regulates sarcomeric structure and its function where it is released in circulation in response to cardiac injury.

**Objective:** To explore the role of cMyBP-C as a biomarker in heart failure in children.

**Patients and Methods:** This prospective cohort study was carried out during the period from October 2017 to October 2018 in Pediatric Cardiology Unit, Zagazig University Hospital. It included 26 selected patients with heart failure diagnosed clinically and by echocardiography.

**Results:** The study revealed that the most common cause pf heart failure in our patients was VSD (30.8%) then DCM (26.9%). 57.7% of studied patients were grade III of ROSS classification while 30.8% were grade IV and 11.5% were grade II. In ECHO findings, mean EF% was 52.3% while mean FS% was 33 % with negative correlation between plasma level of cMyBP-C and EF% and FS%. There was high statistical significant difference in cMyBP-C plasma level at time of admission and after treatment (123.5 ng/ml and 78.8 ng/ml respectively). There was high statistical significant difference in level of cMyBP-C among improved cases before and after treatment. There was no statistical significant difference in level of cMyBP-C before and after treatment among cases with bad prognosis.

**Conclusion:** cMyBP-C could be a prognostic biomarker in children with HF and may be used as a tool to help in HF diagnosis in children as a marker of disease severity.

**Keywords:** Heart failure, cMyBP-C, Children.

## INTRODUCTION

Heart failure was defined by American Heart Association (AHA) as a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood [(1)]. It also can be defined as failure of the heart to supply the blood essential for the metabolic demands of body and it has high morbidity and mortality in children [(2)].

As 87% of new onset case of heart failure reach the diagnosis when the patient reaches the state of sever decompensation [(3)]. It is important to have simple biomarker that can help in early diagnosis and prognosis of adverse outcome to change treatment plan. There are many HF biomarkers that help in assessing the severity of HF and predicting the course of the disease. The release of these biomarkers has different sources; from myocyte injury, ventricular remodeling and reduced coronary reserve [(4)].

cMyBP-C is a thick filament-associated protein and is a specific cardiomyocyte determinant of integrity and function of structure of cardiac sarcomere [(5)]. It regulates cardiac contractility and its phosphorylation by protein kinase contributes to increased cardiac output in response to β adrenergic stimulation. In heart failure, this phosphorylation decreases which leads to contractility dysfunction and the response to β adrenergic stimulation decreases [(6)].

It is confirmed that cardiac stress is associated with decreased level of cMyBP-C phosphorylation, activate cleavage of intact cMyBP-C and release of 40-KDa. 40-KDa is a truncated fragment, which is increased in diseased heart and compete for the normal cMyBP-C binding site to actin and myosin. Thus, interfering with cardiac contractility that causes heart failure. This finding suggests that plasma level of cMyBP-C could be used for early diagnosis of heart failure and predicting its severity [(7)]. This work aimed to explore the role of cMyBP-C as a biomarker in heart failure in children.

## PATIENTS AND METHODS

This prospective cohort study was carried out during the period from October 2017 to October 2018 in Pediatric Cardiology Unit, Zagazig University Hospital. The study includes 26 selected patients with heart failure diagnosed clinically and by echocardiography.

**Ethical approval:**

**The study was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University.**





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A written informed consent was taken from the parents of the participants after full explanation of the aim of the study. They were informed that the participation in the study was voluntarily. The parents were given the opportunity to refuse participation and also were assured that any collected information would be confidential and used for the research purpose only.

***Inclusion criteria***

Infants and children age from 2 month to 5 years old, patient with clinical manifestation of acute heart failure and patient with heart failure due to either acquired or congenital heart diseases.

***Exclusion criteria***

Infants and children < 2 months and > 5 years, patients suffering from any cardiac diseases other than heart failure and patients with heart failure due to causes other than acquired and congenital heart diseases.

**All selected cases were subjected to the following:**

**1-Detailed history taking:** Personal history; complaint, history of present illness e.g. cough, cyanosis, dyspnea…etc. Moreover, past history of diseases, operations, or medication and family history of congenital heart diseases or cardiomyopathy.

**2-Full clinical examination:** General examination. Anthropometric measurements. Cardiac examination: (i) Inspection and palpation for peripheral pulses and the precordium [the presence of a thrill, the point of maximal impulse (PMI), precordial hyperactivity] to determine whether the right ventricle (RV) or left ventricle (LV) is dominant. (ii) Auscultation: heart sounds, murmurs and their characters, and additional sounds as S3 gallop. Manifestations of heart failure like hepatomegaly, edema, ascites …etc. Chest examination: To exclude the patients with chest infections.

**3-Assessment of severity of heart failure using modified ROSS score [8]**

| |
|---|
| **Class I:** Asymptomatic. |
| **Class II:** Mild tachypnea or diaphoresis with feeding in infants. Dyspnea on exertion in older children. |
| **Class III:** Marked tachypnea or diaphoresis with feeding in infants. Prolonged feeding times with growth failure and marked dyspnea on exertion in older children. |
| **Class IV:** Symptoms such as tachypnea, retractions, grunting, or diaphoresis at rest. |

**4-ECG.**

**5-Plain X-ray chest:** For heart size and pulmonary vascular marking.

**6-Echocardiography:**

Transthoracic M-mode and two dimensional echocardiography was performed to all subjects positioned in the left lateral decubitus, using a Vivid 7 dimension machine according to the recommendation of the American Society of Echocardiography [9], to detect the defects of the heart and its functions. Also, to measure the dimensions of the right ventricle end diastolic dimension (RVEDD) and pulmonary artery diameter. Left ventricular end systole (LVES), left ventricular end diastole (LVED) were measured and from which EF, FS of left ventricle were estimated as follows:

**FS (%) =** Dd – Ds /Dd × 100

**EF (%) =** (Dd)3 – (Ds)3/(Dd)3 × 100

Where FS is fractional shortening, Dd is end-diastolic dimension of the LV, and Ds is end-systolic dimension of the LV. This is a reliable and reproducible index of LV function and ejection fraction is related to the change in volume of the LV with cardiac contraction.

**7-Routine laboratory investigation:** Complete blood count, kidney function tests and liver function tests.

**8-Measurment of plasma level of cMyBP-C:** using ELISA tech at time of admission and after treatment.

***Statistical analysis***

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Chi-square ($X^2$) test of significance was used in order to compare proportions between two qualitative parameters.

Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values. Area under curve (AUC) was also calculated, criteria to qualify for AUC were as follows: 0.90 – 1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair; 0.60-0.70 = poor and 0.50-0.6 = fail.

The optimal cutoff point was established at point of maximum accuracy. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following: P-value ≤ 0.05 was considered significant, P-value < 0.001 was considered as highly significant and P-value > 0.05 was considered insignificant.

## RESULTS

**Table (1):** Demographic Characteristics of the studied population

| Characteristics | Studied patients (n=26) |
|---|---|
| ***Age / month:*** Mean ± SD | 15.5 ± 16.9 |
| ***Wight / kg:*** Mean ± SD | 8.38 ± 3.23 |
| ***Height \cm:*** Mean ± SD | 72.19 ±13.03 |
| ***Sex:*** Female No (%)<br>Male No (%) | 13 (50%)<br>13 (50%) |
| ***Consanguinity:*** Yes (%)<br>No (%) | 6 (23.1%)<br>20 (76.9%) |

This table showed that the age of the studied group ranged from 2 months to 5 years with mean of 15.5 months. The mean body weight was 8.38 kg and mean height was 72.19 cm. regarding sex 50% of them were males. Finally, 23.1% had positive consanguinity.

**Table (2):** Clinical ROSS classification among the studied patients

| ROSS classification: | Studied patients (n=26) | |
|---|---|---|
| | No | % |
| **Grade I** | 0 | 0.0 |
| **Grade II** | 3 | 11.5 |
| **Grade III** | 15 | 57.7 |
| **Grade IV** | 8 | 30.8 |

This table showed that more than half of the studied group (57.7%) were of grade III, 30.8% were of grade IV and only 11.5% were of grade II according to ROSS classification.

**Table (3):** Echo-cardiographic findings of the studied patients

| Echo findings | (n=26) Mean ± SD |
|---|---|
| ***LVID d*** | 30.9 ± 13.06 |
| ***LVID s*** | 20.57 ± 12.4 |
| ***FS*** | 33.05 ± 7.98 |
| ***PW*** | 5.5 ± 2.74 |
| ***IVS*** | 6.5 ± 3.17 |
| ***EF (%)*** | 52.3 ± 11.02 |

This table showed that the LVID diameter of the studied group at diastole ranged from 14 to 60 with mean 30.9, while at systole it ranged from 8 to 51 with mean 20.57.

Fractional shortening ranging from10 to 40 with mean 33.05. PW ranged from 0.6 to 11 with mean 5.5. Regarding ejection fraction ranged from 20 to 62% with mean 52.3 %.

**Table (4):** Difference in cMyBP-C level among studied group before and after treatment

| Characteristics | Before (n= 26) | After (n= 26) | Wilcoxon | P |
|---|---|---|---|---|
| ***cMyBP-C(ng\ml):*** (**N=**0.15-32ng/ml) Mean ± SD | 123.5 ± 63.8 | 78.8 ± 50.7 | 5.13 | **0.000 (HS)** |

This table showed that there were high statistical significant differences among studied cases as regards cMyBP-C marker before and after treatment, as it was decreased after treatment.

**Table (5):** Relation between cMyBP-C level and prognosis of cases among studied group

| Prognosis | ***cMyBP-C(ng\ml):*** | | | |
|---|---|---|---|---|
| | **Before treatment** Mean ± SD | **After treatment** Mean ± SD | **Wilcoxon** | **P** |
| **Improved** | 120.3 ± 57.4 | 70.7 ± 30.14 | **3.5** | **0.000 (HS)** |
| **Died** | 189 ± 55.2 | 196.5 ± 57.3 | **1.35** | **0.18 (NS)** |
| **P value** | **0.01 (S)** | **0.000 (HS)** | | |

This table showed that there was a high statistical significant difference in the level of cMyBP-C among improved cases before and after treatment. In addition, the level of cMyBP-C was increased greatly among cases with bad prognosis with no statistical significant difference in the level of cMyBP-C before and after treatment. While, there was statistical significant difference among both improved and those with bad prognosis regarding cMyBP-C level before treatment and high statistical difference after treatment.

**Table (6):** Relation between cMyBP-C level and ROSS classification among studied group

| | ROSS classification | | | KW | P |
|---|---|---|---|---|---|
| | II (n=3) | III (n= 15) | IV (n= 8) | | |
| ***cMyBP-C(ng\ml) before:*** Mean ± SD | 81.3 ± 5.13 | 90.7 ± 41.8 | 198.6 ± 28.2 | **15.95** | **0.000 (HS)** |
| ***cMyBP-C(ng\ml) after:*** Mean ± SD | 61.1 ± 32.5 | 65.7 ± 11.0 | 122.3 ± 65.2 | **10.5** | **0.000 (HS)** |

This table showed a high statistical significant difference among three levels of ROSS classification of the studied patients regarding levels of cMyBP-C before and after treatment, which was higher among patients of grade IV followed by grade III and the least among patients of grade II.

**Table (7):** Correlation between cMYBPC and echo-cardiographic parameters

| Variables | cMYBPC | |
|---|---|---|
| | r | P |
| **LVID d** | 0.643 | 0.000 (HS) |
| **LVID s** | 0.671 | 0.000 (HS) |
| **FS** | -0.456 | 0.02 (S) |
| **PW** | 0.019 | 0.923 (NS) |
| **IVS** | -0.075 | 0.715 (NS) |
| **EF** | -0.624 | 0.001 (S) |

This table showed a high statistically significant positive correlation between cMyBP-C level and LVID diastolic and systolic among studied patients. In addition, there was statistically significant negative correlation with FS and EF.

**Table (8):** Validity data of (ROC) analysis for the cMYBPC levels as a prognostic predictor in studied patients

| Cut-off | Sensitivity | Specificity | PPV | NPV | Accuracy | AUC | P-Value |
|---|---|---|---|---|---|---|---|
| ≥ 63.3 | 81.8% | 75% | 93% | 85% | 91.3% | 0.068 | 0.007 (S) |

**PPV:** Positive Predictive Value **NPV:** Negative Predictive Value **AUC:** Area under curve

This table showed that at cut-off value 63.3, the performance of cMyBP-C as a prognostic predictor for acute heart failure presented with sensitivity of 81.8% and specificity 75% with 0.068 area under ROC curve.

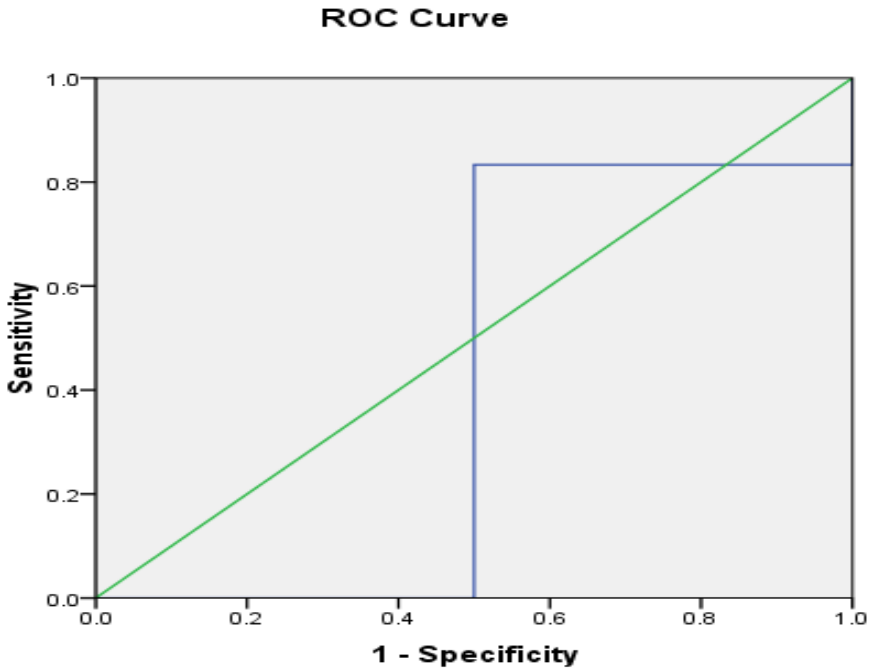


**Figure (1):** ROC for the cMyBP-C levels as a prognostic predictor in studied patients

## DISCUSSION

In current study, mean age was 15.5 month (2month -5years) which agrees with **Nandi, Rossano (10)** who reported that most type of congenital heart diseases are diagnosed during infancy and the greatest incidence of cardiomyopathy was observed during infancy. Thus, are accounting for high percentage of heart failure admission among children.

Our study showed that 23.1% of the studied patients had positive consanguinity. **Ul-Hag *et al.* [11]** study of risk factors predisposing to congenital heart diseases showed that 48.8% of studied cases had positive consanguinity proving that there is high association between consanguinity and congenital heart diseases. This difference in percentage can be explained by different patient population as our study included patients with heart failure due to congenital heart diseases or cardiomyopathy.

The result of the present study showed that more than half of our patients were under weight and height with mean body weight of 8.38 kg and mean height of 72.19 cm (values were compered to age and sex normal centile). The result of our study goes in agreement with the results of **Kotby *et al.* [12]** and **Mohammed *et al.* [13]** who explained that to be due to nutritional defects and feeding difficulties in patients with heart failure. In addition, they reported a significant correction between malnutrition and heart failure and attributed it for several possible explanations including low caloric intake, feeding difficulties, chronic hypoxemia increased metabolic demands and malabsorption.

We classified our patient in this study according to modified ROSS classification at the time of admission where 11.5% were class II, 57.7% were class III and 30.8% were class IV (none of our cases were grade I at time of admission).

Echocardiography data in our study revealed that mean ejection fraction of our patients was 52.3%, which is low. Our results go in agreement with **Jayaprasad *et al.* [14]** who reported that LV dysfunction in children is currently defined by an ejection fraction less than 55%. We found in our study that mean fractional shortening (FS%) was 33% which is in agreement with **Mohammed *et al.* [13]** whose FS% in children with heart failure in their study ranged from 35% to 45% with mean FS% of 33.93%. In addition, both decrease in EF% and FS% in our study are in agreement with **Kusumoto *et al.* [15]** who reported decreased EF% and FS% in heart failure.

Our study reported that there was a significant increase in the plasma level of cMyBP-C in children with heart failure at time of admission and before receiving any anti failure treatment (123.5 ng/ml) as compared to children after treatment (78.8ng/ml). This is in agreement with **Jeong *et al.* [16]** who reported increased plasma level of cMyBP-C in patients with heart failure with or without preserved ejection fraction as compared to healthy control group in their study. In addition, in agreement with **El Amrousy *et al.* [17]** as they mentioned that there was a significant increase in plasma levels of cMyBP-C in children with HF at time of admission as compared to patients after treatment and to control group. There is a limited data about the plasma level of cMyBP-C in children. It was mentioned by **El Amrousy *et al.* [17]** in their study that the plasma level of cMyBP-C in control group ranged between 8-54 ng/dl with mean of 24.4 ± 9.83 ng/ml. This is low compared to the values of plasma level of cMyBP-C in our study for children with heart failure at time of admission (123.5 ± 63.8 mg/ml) and for children after treatment (78.8 ± 50.7ng/ml). Result of our study go in agreement with explanation of **Sadayappan *et al.* [18]** for the high level of plasma cMyBP-C that could occur due to different mechanisms e.g. proteolysis and dephosphorylation in absence of necrosis and apoptosis.

Our study showed that there was high statistical significant association between cMyBP-C plasma level and heart failure grades before and after treatment assessed by ROSS classification of heart failure. It showed that mean cMyBP-C plasma level was higher among patients of grade IV followed by grade III and least among patients of grade II. This is in agreement with **El Amrousy *et al.* [17]** study who reported that the plasma level of cMyBP-C in patients with heart failure were significantly higher in stage IV than that in stage III and stage II.

Our study showed that there was high statistical difference in the plasma level of cMyBP-C among improved cases before and after treatment. While, it showed that the level of cMyBP-C was increased greatly among cases with bad prognosis with no statistical difference in the level of cMyBP-C before and after treatment. The plasma level of cMyBP-C of dead patients were significantly higher than the recovered patient before and after treatment. This is in agreement with the study of **Anand *et al.* [19]** who demonstrated an increased mortality risk with rising cMyBP-C concentration in patients suffering from aortic stenosis.

Our study showed that the patients with higher plasma level of cMyBP-C at time of admission were associated with bad prognosis (7.7%) and even associated with persistent increase in the plasma level of cMyBP-C after treatment indicating persistence of cardiac injury. In the contrary, the patients with lower plasma level of cMyBP-C at the time of admission were associated with good prognosis denoting less cardiac injury and good response to treatment.

In our study, there was negative correlation between cMyBP-C and ejection fraction of studied patients (p= 001). In addition, there was negative

correlation between cMyBP-C plasma level and FS% which agrees with **El Amrousy *et al.* [17]**.

In the present study, ROC curve analysis was performed to evaluate plasma levels of cMyBP-C as a prognostic predictor in patients with acute heart failure. The cutoff point value for cMyBP-C was 63.3, with sensitivity of 81.8%, specificity of 75% with 0.068 area under ROC curve and accuracy of 91.3%. This made cMyBP-c helpful in prediction of adverse outcome and identification of high-risk patients. However, in **El Amrousy *et al.* [17]** study, ROC curve analysis was carried out to identify the cutoff point of cMyBP-C plasma level for heart failure diagnosis, which was 45ng/ml with sensitivity 100%, specificity 96% and accuracy 99.9%.

Our study also showed significant increase in the plasma level of cMyBP-C in mildest HF (ROSS stage II) (81.3ng/ml). This made cMyBP-C could be helpful as a biomarker for early diagnosis of heart failure in children. In **Razzaque *et al.* [7]** study, it was confirmed that cardiac stress could decrease level of cMyBP-C phosphorylation, trigger cleavage of intact cMyBP-C and release of 40-kDa truncated fragment. 40-kDa is increased in diseased heart and compete for normal cMyBP-C binding site to actin and myosin. Thus, altering cardiac contractility causing heart failure. This can suggest that plasma level of cMyBP-C could be used for diagnosis of heart failure and assess its severity.

## CONCLUSION

cMyBP-C could be a prognostic biomarker in children with HF and may be used as a tool to help in HF diagnosis in children as a marker of disease severity (positive correlation between ROSS classification and plasma level of cMyBP-C was proved) and staging in patients with acute heart failure due to congenital heart diseases or cardiomyopathy.

## RECOMMENDATIONS

We recommend cMyBP-C as a prognostic biomarker during treatment of HF in children. cMyBP-C should be measured on admission and before receiving any anti-failure treatment.

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