



Plasma Levels of Connective Tissue Growth Factor in Children with Congestive Heart Failure

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Authors' contributions

This work was carried out in collaboration among all authors. Author RMW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AHS and HME managed the analyses of the study. Author AMZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: Assessment of plasma level of connective tissue growth factor in congestive heart failure children, assessment of its diagnostic and prognostic role and correlate its level with clinical and echocardiographic assessment of congestive heart failure.

Methods: Connective tissue growth factor level in the plasma was measured in 40 children; 20 of them have congestive heart failure, and 20 are healthy then, correlated with clinical parameters.

Results: The diagnostic and prognostic value of it was evaluated. We compared its levels in both patients and healthy children. We found that connective tissue growth factor level was significantly increased in diseased children. Fractional shortening and ejection fraction correlated negatively with the plasma level of connective tissue growth factor. Heart rate, respiratory rate and calibrated integrated backscatter correlated positively with connective tissue growth factor. Connective tissue growth factor was significantly correlated with the class of heart failure according to Ross classification.

Conclusions: Plasma connective tissue growth factor has a promising diagnostic and prognostic value as a biomarker for congestive heart failure in children with high sensitivity and specificity.

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Keywords: *Connective tissue growth factor; congestive heart failure; children.*

ABBREVIATIONS

| | |
|-------------|--|
| <i>CHF</i> | : <i>Congestive Heart Failure</i> |
| <i>CTGF</i> | : <i>Connective Tissue Growth Factor</i> |
| <i>ECM</i> | : <i>Extra Cellular Membrane</i> |
| <i>ECG</i> | : <i>Electrocardiography</i> |
| <i>LV</i> | : <i>Left Ventricle</i> |
| <i>ECHO</i> | : <i>Echocardiography</i> |
| <i>CBC</i> | : <i>Complete Blood Count</i> |
| <i>CRP</i> | : <i>C - reactive Protein</i> |
| <i>CIB</i> | : <i>Calibrated Integrated Backscatter</i> |
| <i>RR</i> | : <i>Respiratory Rate</i> |
| <i>HR</i> | : <i>Heart Rate</i> |
| <i>CTR</i> | : <i>Cardiothoracic Ratio</i> |
| <i>SBP</i> | : <i>Systolic Blood Pressure</i> |
| <i>DBP</i> | : <i>Diastolic Blood Pressure</i> |
| <i>KG</i> | : <i>Kilogram</i> |
| <i>HF</i> | : <i>Heart Failure</i> |

1. INTRODUCTION

Congestive heart failure (CHF) is defined as a progressive clinical and pathophysiological syndrome caused by an abnormality of cardiac structure or function leading to failure of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolizing tissues, that result in characteristic signs and symptoms including edema, respiratory distress, growth failure and other signs and symptoms of pulmonary and systemic congestion [1].

The progression of heart failure syndrome and left ventricular systolic dysfunction, because of remodeling of the left and right ventricle as a result of the loss of myocytes and maladaptive changes in the surviving myocytes and extracellular matrix, probably occurs in two main ways [2]. One is because of intercurrent cardiac events and the other is as a consequence of the local processes (as the autocrine pathway, molecular adaptations and apoptosis) and systemic processes (as neurohormonal pathways) that are activated as a result of reduced systolic function [3].

Connective tissue growth factor (CTGF) is also called CCN2 which is a cysteine-rich matricellular protein of the CCN family of extracellular matrix-associated heparin-binding protein [4]. It is involved in multiple cellular events such as cell adhesion, cell proliferation, and extracellular matrix (ECM) production [5].

It is highly expressed in myocardial fibroblasts and is responsible for fibrosis, apoptosis and remodeling [6].

CTGF also activates the myofibroblasts and stimulates their deposition and remodeling of ECM (extracellular matrix) proteins. This leads to tissue remodeling and fibrosis. When the tissue remodeling occurs in the vasculature, it can create local hypertension that can induce CTGF expression, thereby setting up a positive feedback loop leading to more tissue remodeling. CTGF also induces the expression of a variety of cytokines such as TGF β and VEGF [7] which induce more expression of CTGF. Thus, there are multiple positive feedback loops involving CTGF expression that can contribute to the progressive nature of fibrosis [7].

1.1 Aim of the Work

The current study aims to evaluate plasma levels of Connective Tissue Growth Factor (CTGF) in patients with CHF, examine its relationship to pathophysiology of heart failure, evaluate the diagnostic and prognostic value of (CTGF) and correlate its level with clinical and echocardiographic assessment of CHF.

2. MATERIALS AND METHODS

Twenty (20) children with CHF were included in the current study. We chose them from those admitted to Pediatric Cardiology Unit, Pediatric Department, Tanta University Hospital, in the time between (August 2017 and Augusts 2018). They were 11 males and 9 females, and their ages ranged from three months to 12 years {infants 2, 1-5 years 10, 5-12 years 8}.

Patients were classified according to Ross Classification System of congestive heart failure in infants and children, into class I, II, III, IV [8].

Twenty (20) healthy children, matched for age and sex, were enrolled as a control group. They were 10 males and 10 females, and their ages ranged from two months to 13 years {infants 1, 1-5 years 7, 5-13years 12}.

Inclusion criteria included infants and children with CHF (diagnosed clinically, and by laboratory and imaging studies) due to heart diseases either congenital or acquired.

Exclusion criteria included patients with renal diseases or failure, hepatic diseases, pulmonary diseases and acute or chronic illness.

All children enrolled in this study were subjected to the following: (1) Complete history taking and

through Complete physical examination: including heart rate (beats/min.), respiratory rate (cycles/min.), temperature (°C), and blood pressure (mm Hg), complete local cardiac examination and manifestations of CHF (symptoms and signs). (2) Plain X-ray chest and heart (postero-anterior view) for cardio-thoracic ratio evaluation for assessment of cardiomegaly. (3) Electrocardiography (ECG): Using 3 channel 1000 apparatus. (4) Doppler & Echocardiography: using a vivid 7 Ultrasound machine (GE medical system, Horten, Norway, with a 3.5 MHz multi-frequency transducer). It was done for evaluation of the following parameters: cardiac causes of CHF, systolic function of left ventricle through LV ejection fraction {EF %} where $EF \% = \frac{(LVEDD)^3 - (LVESD)^3}{(LVEDD)^3} \times 100\%$, LV fractional shortening {FS %} where $FS \% = \frac{(LVEDD) - (LVESD)}{(LVEDD)} \times 100\%$, diastolic function of left ventricle by (pulsed Trans-mitral Doppler) by measuring peak early filling velocity {E wave}, peak late filling velocity {A wave}, E/A ratio and Calibrated integrated backscatter (CIB) measurement of myocardial fibrosis in which calculation of Calibrated Integrated Backscatter measurements of tissue intensity were obtained from sample volumes placed within the pericardium, posterior wall, and anteroseptum (green) in a parasternal long-axis view then, a resultant integrated backscatter curve was derived with standard commercial software (Echopac, General Electric Medical Systems, Milwaukee, Wisconsin), which enables calibrated integrated backscatter to be calculated by subtracting mean pericardial integrated backscatter intensity from mean integrated backscatter intensity of the posterior wall or anteroseptum at end diastole [9]. (5) Routine investigations were done including CBC, CRP, Kidney, and Liver function tests. The results were obtained from patients sheets. (6) Specific estimation of human plasma level of connective tissue growth factor (CTGF) by ELISA Kit supplied by Sun Red, Catalog No.201-12-0147.

2.1 Sampling

Three (3) ml wander blood sample was taken from each patient and control, and put into EDTA vacutainer tube, then centrifugation was done and plasma collected was stored at (-20°C) till the time of assay.

2.2 Statistical Analysis

Collected data were analysed using SPSS version 17 (SPSS Inc., Chocago, IL, USA.

Quantitative data were presented on the form of mean and standard deviation. Qualitative data were presented in the form of *n* (%). Qualitative data were compared using Chi-square test. Mean of the two groups was compared using (*t*) test. Correlation between variable was performed using Spearman correlation coefficient. Receiver operating characteristic curve was used to assess the diagnostic and prognostic value of CTGF to diagnose CHF in children and predict the prognosis of CHF cases at different cutoff points. Significance was judged when *p* < 0.05.

3. RESULTS

Our study involved 20 patients with CHF, involving 9 patient with CHD, 11 of them have cardiomyopathy. (Table 1) demonstrated that there was no significant differences in age, sex in patient group and control group, whereas weight was significantly decreased in patient group as compared to healthy group.

Also, our study show that there was significant increase in HR, respiratory rate, and systolic blood pressure in the heart failure group as compared to healthy group, whereas there was no significant differences between the two studied groups as regards temperature and diastolic blood pressure as shown in (Table 2).

Our study show also, that there were significant increase in cardio-thoracic ratio (CTR) in patients group with a range between (50%-70%) and mean of (60.9 ± 6.31%) as compared to control group and significant increase in calibrated integrated backscatter (CIB) parameter of echocardiography in diseased group as compared to healthy group with a range between (-6)-(-20) and mean of (-12.35) ± 4.756). Also, ejection fraction and fractional shortening decreased significantly in patient group as compared to healthy group with a range between (20-70%) and mean (47.05 ± 16.551%) and range between (14-40%) and mean of (23.5 ± 9.189%) for both parameters, respectively. Table 3.

CTGF levels were significantly increased in our patients group as compared to healthy group with range between (280-1790 pg/ml) and mean of (814.55 ± 355.63 pg./ml) Table 4 and with regard to modified Ross classification, CTGF correlated strongly with the degree of HF and it is significantly increased in relation to Ross classification of CHF Table 5.

Figure 1 show that according to Ross classification, our patients are classified to class I (0%), class II (40%), class III (35%), and class IV (25%).

Through receiver operating curve analysis, it show that cutoff point is more than 100.2 pg/ml and show high specificity, sensitivity, positive predictive value, negative predictive value and accuracy (100%) for each of them (Table 6) (Fig. 2).

4. DISCUSSION

The current study evaluated plasma CTGF level in patients with CHF and correlated it with clinical parameters to evaluate its diagnostic and prognostic values in such children. We found that CTGF level was significantly elevated in patients with CHF. More specifically, CTGF levels increased with the class of heart failure according to Ross classification of congestive heart failure. The increased CTGF correlated negatively with LV systolic function (EF% and FS%) measured by echocardiography. Our present study demonstrated that by using a cutoff point of >100.2 pg/ml, plasma connective tissue growth factor was valid as a diagnostic biomarker of myocardial fibrosis in congestive

heart failure with high sensitivity (100%), specificity (100%), positive and negative predictive values and accuracy (100%). Koitabashi N et al. [10] concluded that CTGF might induce changes in extracellular matrix (ECM) structure via interaction with matrix metalloproteinase.

"CTGF, a profibrotic growth factor, is considered a key molecule in the control of ECM synthesis and may serve as a diagnostic marker and therapeutic target for cardiac fibrosis" [11].

The study demonstrated that there was significant increase of CTGF in the plasma in children with CHF as compared to healthy group, and its level increased with the degree of HF according to Ross classification of CHF. This was in agreement with Koitabashi N et al. [12], who stated that plasma of children with advanced heart failure has high concentration of CTGF that correlated positively with the degree of HF. Our study showed that CTGF positively correlated with CIB which is an indicator of fibrosis, this was in agreement with Miyazaki O et al. [6] which stated that CTGF is highly expressed in myocardial fibroblasts and is responsible for fibrosis, apoptosis and remodeling.

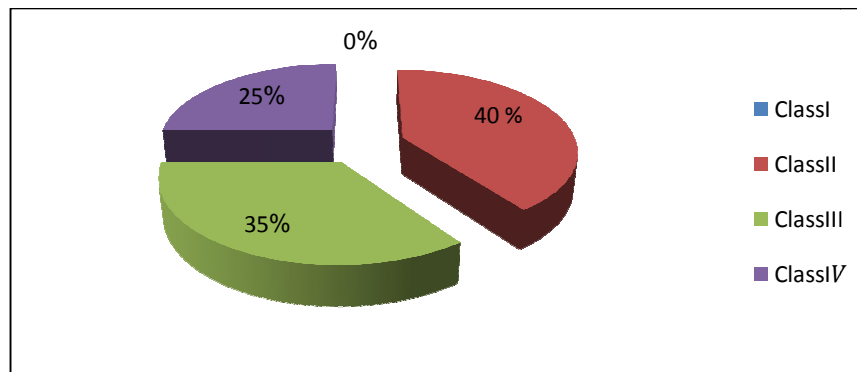


Fig. 1. Classification of the patient group by Ross classification system of CHF

Table 1. Demographic data of the studied groups

| | | Groups | | | | Test | |
|--------------|-----------|---------------------|----------|---------------|----------|------------------|---------|
| | | CHF patients (n=20) | | Control(n=20) | | t/χ ² | P-Value |
| Age (months) | Range | 3-144 | | 2-156 | | -0.820 | 0.417 |
| | Mean ± SD | 58.2±41.75 | | 68.55±39.38 | | | |
| Weight (kg) | Range | 5-38 | | 5-43 | | -2.030 | 0.039* |
| | Mean ± SD | 13.6±8.60 | | 20.5±8.91 | | | |
| | | N | % | N | % | | |
| Sex | Male | 11 | 55% | 10 | 50% | -0.164 | 0.870 |
| | Female | 9 | 45% | 10 | 50% | | |

KG= kilo gram, CHF= congestive heart failure

Table 2. Vital signs of the studied groups

| | | Groups | | Test | |
|-----------------------------|---------------|---------------------|--------------------|-------------|---------|
| | | CHF patients (n=20) | Control (n=20) | t/ χ^2 | P-value |
| HR (Beat/min.) | Range | 115-175 | 80-140 | 9.035 | <0.000* |
| | Mean \pm SD | 141.65 \pm 15.007 | 92.25 \pm 18.511 | | |
| RR (Cycle/min.) | Range | 24-68 | 16-40 | 6.688 | <0.000* |
| | Mean \pm SD | 43.4 \pm 12.1 | 22.4 \pm 6.374 | | |
| Temperature ($^{\circ}$ C) | Range | 36.9-38.5 | 36.6-37.5 | 1.073 | 0.289 |
| | Mean \pm SD | 37.35 \pm 0.460 | 37.08 \pm 0.268 | | |
| SBP (mm Hg) | Range | 70-120 | 60-115 | -2.494 | 0.017* |
| | Mean \pm SD | 94.05 \pm 10.91 | 102.25 \pm 9.283 | | |
| DBP (mm Hg) | Range | 50-70 | 45-80 | -0.966 | 0.340 |
| | Mean \pm SD | 64.35 \pm 7.001 | 66.75 \pm 8.257 | | |

RR= respiratory rate, HR= heart rate; SBP= systolic blood pressure, DSP= diastolic blood pressure

Table 3. Echocardiographic, chest X-ray parameters of both studied groups

| Items | | Groups | | T-test | |
|--------|---------------|----------------------|---------------------|--------|---------|
| | | Patients (n=20) | Control (n=20) | t | P-value |
| CTR% | Range | 50-70 | 45-52 | 8.030 | <0.000* |
| | Mean \pm SD | 60.9 \pm 6.315 | 48.55 \pm 2.246 | | |
| EF (%) | Range | 20-70 | 60-80 | -5.457 | <0.000* |
| | Mean \pm SD | 47.05 \pm 16.551 | 69 \pm 5.779 | | |
| FS (%) | Range | 14-40 | 28-43 | -5.058 | 0.000* |
| | Mean \pm SD | 23.5 \pm 9.189 | 35.35 \pm 4.452 | | |
| CIB | Range | (-6)-(-20) | (-20)-(-34) | 11.656 | <0.000* |
| | Mean \pm SD | (-12.35) \pm 4.756 | (-28.9) \pm 3.766 | | |

CTR= cardio-thoracic ratio; EF= ejection fraction; FS= fractional shortening; CIB= calibrated integrated backscatter

Table 4. CTGF levels for both studied groups

| | | Groups | | T-test | |
|--------------|---------------|---------------------|--------------------|--------|---------|
| | | Patients n=20 | Control n=20 | t | P-value |
| CTGF (pg/ml) | Range | 280-1790 | 57.6-100.2 | 9.164 | <0.000* |
| | Mean \pm SD | 814.55 \pm 355.63 | 74.215 \pm 14.00 | | |

CTGF= Connective tissue growth factor

Table 5. Mean plasma levels of CTGF in relation to Ross classification of CHF of the patient group

| Class | Ross | | CTGF (pg/ml) | |
|-------|---------|-----------|--------------|--------|
| | N | Range | Mean | SD |
| II | 8 | 280-678 | 526.12 | 152.22 |
| III | 7 | 627-1050 | 783.71 | 153.82 |
| IV | 5 | 1160-1790 | 1319.2 | 264.46 |
| ANOVA | F | | 28.319 | |
| | P-value | | <0.000* | |

Table 6. Cutoff, sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive value (NPV) and accuracy of plasma level of CTGF as a diagnostic predictor of myocardial fibrosis in CHF

| ROC curve between patient and control | | | | | |
|---------------------------------------|-------|-------|------|------|----------|
| Cutoff | Sens. | Spec. | PPV | NPV | Accuracy |
| >100.2 pg/ml | 100% | 100% | 100% | 100% | 100% |

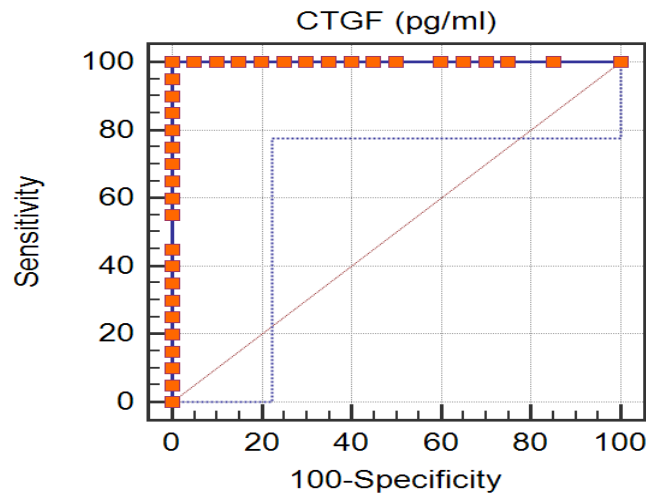


Fig. 2. Cutoff, sensitivity, specificity, Positive Predictive value (PPV), Negative Predictive Value (NPV) and accuracy of plasma level of CTGF as a diagnostic predictor of myocardial fibrosis in CHF

As regards to relation between CTGF and LV systolic function (EF% and FS%), the current study reported that CTGF was significantly negatively correlated with EF% and FS% measured by echocardiography. This was in agreement with Li G et al. [13].

There are some limitations to the current study. First, the sample size wasn't large enough and from one center. Second, we didn't measure the tissue CTGF expression in patients with CHF directly. Third, we did not determine the source of CTGF production although we measured plasma CTGF concentration in this study, because CTGF is included in many fibrotic diseases other than CHF.

In conclusion, this study demonstrated that plasma levels of CTGF were markedly elevated in children with CHF; with significant elevation according to Ross class of CHF, suggesting that myocardial fibrosis and ongoing myocardial damage are related to pathophysiology of heart failure.

Plasma levels of CTGF strongly correlated with clinical and echocardiographic assessment of LV performance of those patients with CHF, and its levels significantly increased in children with adverse outcomes, suggesting its value as a useful diagnostic and prognostic predictor (with high sensitivity and specificity).

Plasma connective tissue growth factor has a promising diagnostic and prognostic value as a biomarker for congestive heart failure in children.

5. CONCLUSION

Plasma connective tissue growth factor has a promising diagnostic and prognostic value as a biomarker for congestive heart failure in children with high sensitivity and specificity.

6. FUNDING TO THE STUDY

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONSENT AND ETHICAL APPROVAL

Written informed consent was obtained from all subjects of the study or their parents or guardians. All information was taken in secret and we used secret codes for each patient. The study was approved by the Ethics Committee of Faculty of Medicine, Tanta University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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