

Inflammatory Markers-Serum Level of C-Reactive Protein, Tumor Necrotic Factor- α , and Interleukin-6 as Predictors of Outcome for Peripartum Cardiomyopathy

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Received: 18 September 2012 / Accepted: 3 December 2012 / Published online: 14 August 2013
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Abstract

Aim/Objective Peripartum cardiomyopathy (PPCM) is a disorder of unknown etiology in which symptoms of heart failure occur between the last month of pregnancy and 5 months post-partum. These findings prompted us to carry out a more detailed study aimed at correlating plasma levels of C-reactive protein TNF- α and IL-6 as prognostic value for major clinical in-hospital events and 6-month follow-up in patients with PPCM.

Materials and Methods After ethical clearance, in the present prospective case–control study, a total of 86 subjects were enrolled [patients ($n = 46$) and controls ($n = 40$)]. After checking for the inclusion and exclusion criteria, informed consent was obtained and patients were enrolled. The details of history of pre-eclampsia and mode of delivery were obtained from the patients. The history of onset of symptoms and signs was recorded at the first presentation and at 6 months. Clinical assessment, echocardiography, and blood analysis were done at baseline and after 6 months of standard therapy. All patients received treatment with diuretics and the ACE inhibitor (ramipril), Carvedilol if not contraindicated, and inotropic support inj-Dobutamine. Inflammatory markers (C-reactive protein,

TNF- α , and IL-6) were measured at baseline and at 6 months. Data were analyzed using the SAS version 9.1 statistical program.

Results The characteristics of the study population at first presentation to the cardiac clinic are similar (compared with controls): 0.91 % of the study patients were diagnosed as PPCM patients for the first time and 49 % patients presented within one month after delivery. C-reactive protein (22 vs 08 mg/dl, $p < 0.05$), TNF- α (9.6 vs 3.2 pg/dl, $p < 0.001$), and IL-6 (73.19 ± 34.4 vs 31.52 ± 8.83 pg/dl, $p < 0.005$) were significantly abnormal, and these patients showed significantly higher LV dimensions, LV EDD (61.6 ± 7.1 vs 46 ± 9 mm $p < 0.004$) LV ESD (53.1 ± 7 vs 32 ± 8 , $p < 0.005$), and significantly lower echocardiographic left ventricular ejection fraction (LVEF) (25.9 ± 8.2 vs 55 ± 12 $p < 0.001$) and correlate well with NYHA FC and death. LVEF improved from 25.9 ± 8.2 to 42.9 ± 13.6 % at 6 months ($p < 0.0001$). Patients who completed 6 months of standard care showed a significant reduction of heart rate, LV dimensions, and NYHA FC ($p < 0.001$). However, normalization of LVEF (>50 %) was only observed in 11 (35 %) patients. Seven patients died within 6 months of diagnoses and eight patients were lost to follow-up.

Conclusions Plasma markers of inflammation were significantly elevated in PPCM patients and correlated with increased LV dimensions and lower EF at presentation. Baseline CRP, IL-6, TNF- α , and higher NYHA FC were the only predictors of mortality. These results contribute to inflammation which may contribute to the pathogenesis of PPCM and its complications and predictors of mortality.

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Keywords Peripartum cardiomyopathy · IL-6 · Tumor Necrotic Factor- α · Left ventricular failure · LV ejection fraction

Introduction

Peripartum cardiomyopathy (PPCM) is a disorder of unknown etiology in which symptoms of heart failure occur between the last month of pregnancy and 5 months post-partum. Adherence to this time interval was emphasized to exclude preexisting causes of heart failure that may be exacerbated by pregnancy rather than arising as a result of pregnancy [1–4].

We studied the mechanism of pathogenesis, clinical features, and prognostic markers in this disease. Heart failure is characterized by activation of pro-inflammatory cytokines TNF- α and IL-6 and elevation of C-reactive protein [5, 6]. C-reactive protein is an acute-phase protein which, due to its binding specificity for phosphocholine present in a large variety of molecules, recognizes a range of pathogenic targets including membranes of apoptotic and reactive cells [7]. Cardiac biomarkers, including B-type natriuretic peptide, are elevated in patients presenting with PPCM, although these markers are not unique to PPCM. Elevations of troponin T (TnT) appear to have prognostic significance in this group. The TnT level ≥ 0.04 ng/ml at presentation predicts persistence of systolic dysfunction with a sensitivity of 55 % and specificity of 91 % [8]. Inflammatory cytokines (TNF- α and IL-6) are elevated in women with PPCM compared to pregnancy controls [9, 10]. However, these cytokines are elevated in patients with other cardiomyopathies. As this inflammatory marker is associated with adverse prognosis in patients with idiopathic dilated cardiomyopathy [5, 6, 11], we investigated whether levels of plasma C-reactive protein and IL-6 at baseline could predict clinical outcome in patients with PPCM. In chronic heart failure, low levels of total cholesterol have been found to be a predictor of impaired survival. The lipid profile in patients with PPCM has not been studied to date. In addition, cardiac myocytes have been noted to undergo apoptosis in animal models of PPCM [12, 14] as well in the failing human heart [14]. These findings prompted us to carry out a more detailed study aimed at correlating clinical evaluation with plasma levels of C-reactive protein and IL-6.

Methods

Study Design and Patient Enrollment

All patients ($n = 46$) and controls ($n = 40$) gave written informed consent before study entry. The study was conducted at Narayana medical college, Nellore, AP. History of

pre-eclampsia and mode of delivery were obtained from the patient and confirmed by examining the obstetric card carried by each patient. The history of onset of symptoms and signs was recorded at the first presentation of the patients (baseline) and after a follow-up period of 6 months (6 months visit). These were the two time points of the study.

Inclusion criteria were as follows: (i) age ≥ 16 and ≤ 40 , (ii) New York Heart Association functional classes (NYHA FCs) II–IV, (iii) symptoms of congestive heart failure that developed in the last month of pregnancy or during the first 5 months post-partum, (iv) no other identifiable cause for heart failure, (v) left ventricular ejection fraction (LVEF) ≤ 40 % by transthoracic echocardiography, and (vi) sinus rhythm.

Exclusion criteria were as follows: (i) significant organic valvular heart disease, (ii) systolic blood pressure (SBP) ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg, (iii) clinical conditions other than cardiomyopathy that could increase inflammatory markers by screening serum for rheumatoid arthritis and HIV and evidence of sepsis, (iv) treatment with anti-inflammatory drugs, (v) severe anemia (hemoglobin concentration < 9 g/dl), (vi) serum creatinine level more than 2 mg/dl, and (vii) metabolic disorders affecting lipoprotein metabolism, i.e., thyroid disease.

Clinical assessment, echocardiography, and blood analysis were done at baseline and after 6 months of standard therapy. Inflammatory markers were measured at baseline only. All patients received treatment with diuretics and the angiotensin-converting enzyme inhibitor (ramipril) and inotropic support inj-Dobutamine. Patients with an EF ≤ 25 % or LV thrombus received anti-coagulation therapy. Carvedilol was added after resolution of overt heart failure, and the dose was slowly titrated up to a target of 25 mg twice daily as long as SBP was ≥ 100 mmHg or symptoms such as dizziness did not occur. Patients attended the cardiac clinic at least once a month for routine follow-up.

Plasma levels of C-reactive protein were measured as part of the routine investigation by the hospital laboratory using a commercially available enzyme-linked immunosorbent assay (Roche Diagnostics). The assay had a sensitivity of 0.1–10 mg/L and included standards that were run in parallel. All patients had serum IL-6 levels measured by the ELISA method, M/s Immunotech France (IL-6 ELISA KIT-Beckmen). Obtained values were used to calculate plasma levels in patient and control samples.

The manufacturer supplied a reference range for normal values. However, as those values were obtained in samples from western population groups, we collected blood from 20 otherwise healthy age-, race-, sex-, body mass index-, and parity-comparable controls recruited from the local population.

For ROC curve, the best cutoff point of IL-6 (40 pg/ml) was identified to assess the increased risk of cardiovascular

events (sensitivity 83.7 %, specificity 63 %, AUC 0.671, $p = 0.007$). The normal range for IL-6 by this method was 0–39.9 pg/ml in the local population. In the group with <40 pg/ml (Group I), there were 40 patients and the average IL-6 was 31.52 ± 8.83 pg/ml (range: 24–39.8 pg/ml), whereas in the group with >40 pg/ml (group II), there were 46 patients with an average IL-6 of 73.19 ± 34.4 pg/ml (range: 40.1–210 pg/ml) ($p < 0.0001$).

Functional Class, Echocardiography, and Cardiac

Scintigraphy

A physician who was provided the clinical data, but was blinded to the protocol and unaware of the results of the laboratory tests, performed the assignments of each patient to the NYHA FC during baseline and follow-up visits.

The same physician evaluated all patients. Two-dimensional and M-mode echocardiography with Doppler color flow mapping was performed using an echocardiograph (Philips i 33, WA, USA machine) attached to a 2.5 or 3.5 MHz transducer. All studies were performed and interpreted by the same operator who was unaware of the other parameters investigated. LV dimensions were measured according to the American Society of Echocardiography Guidelines [15]. Measurements of LV dimensions and function were determined on an average of ≥ 3 beats. LVEF and calculations of LV performance were made.

Statistical Analysis

Data were analyzed using the SAS version 9.1 statistical program. Results are expressed as mean \pm SD or median (range). The paired t test was used for the comparison of baseline data with the 6-month data. The McNemar test was used for calculating the differences on the basis of the NYHA FC by grouping the patients into two classes (I, II and III, IV). The multiple logistic regression was performed including predictor variables [NYHA FC, end-diastolic diameter (EDD), end-systolic diameter (ESD), EF, CRP, serum IL-6, and SBP] that had a p value less than 0.15 from the univariate analysis. The resulting model for mortality selected IL-6 and TNF- α and NYHA FC as explanatory variables with a p value less than 0.05 being considered significant.

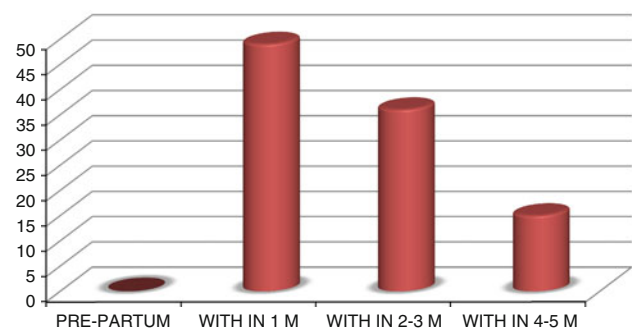
Results

Study Characteristics

The characteristics of the study population at first presentation to the cardiac clinic are similar (compared with

Table 1 Baseline characteristics of study population

	Patients	Control
Age (years)	21 \pm 14.6	23 \pm 15
Gravidity (median)	3 (1–7)	2(1–5)
Type of delivery (%)	82 % vaginal 18 % cesarean	84 % vaginal 16 % cesarean
Body mass index (kg/cm ²)	25.6 \pm 5.1	24.2 \pm 6.2
Blood pressure (mmHg) systolic	108.1 \pm 19	112 \pm 18
Blood pressure (mmHg) diastolic	70.4 \pm 13	68 \pm 15
Heart rate (b.p.m.)	93.5 \pm 18	89 \pm 14



Graph 1 Patients presented with symptoms

controls) as shown in Table 1. Although 91 % of the study patients were diagnosed as PPCM patients for the first time, the remaining 9 (9 %) PPCM patients had been diagnosed at a previous pregnancy. Clinical presentation of PPCM patients within 1 month post-partum was 49 %, within 2–3 month post-partum was 36 %, and within 4–5 month post-partum was 15 % (Graph 1).

Mean plasma levels of hemoglobin and glucose of the PPCM patients were low compared to the control (176 ± 34 vs 189 ± 42 mg/dl). There was an inverse correlation between baseline total cholesterol level and LV EDD and ESD, and therefore a positive correlation to EF ($r_s = 0.36$, $p = 0.006$) was observed.

C-reactive protein (22 vs 08 mg/dl, $p < 0.05$), TNF- α (9.6 vs 3.2 pg/dl, $p < 0.001$), and IL-6 (73.19 ± 34.4 vs 31.52 ± 8.83 pg/dl, $p < 0.005$) were significantly abnormal (Table 2).

Patients showed significantly higher LV dimensions, LV EDD (61.6 ± 7.1 mm vs 46 ± 9 mm $p < 0.004$) and LV ESD (53.1 ± 7 vs 32 ± 8 , $p < 0.005$), and significantly lower echocardiographic LVEF (25.9 ± 8.2 vs 55 ± 12 $p < 0.001$). LV thrombi were detected on echocardiography in 12 % of the patients (Table 3).

Table 2 Blood parameters

	Control	Patients	<i>p</i>
Hemoglobin (g/dL)	11.5 ± 2.1	10.5 ± 1.7	
Glucose (mg/dL)	96 ± 18	89 ± 12	
Total cholesterol (mg/dL)	189 ± 42	176 ± 34	
C-reactive protein (mg/L)	08	22 (1–90)	<0.05
TNF α (pg/mL)	3.2	9.6 (0.2–20.0)	<0.001
IL6 (pg/mL)	31.52 ± 8.83	73.19 ± 34.4	<0.005

Table 3 Echocardiographic findings

	Control	Patient	<i>p</i>
Left ventricular EDD (mm)	46 ± 9	61.6 ± 7.1	<0.004
Left ventricular ESD (mm)	32 ± 8	53.1 ± 7	<0.005
Ejection fraction (%)	55 ± 12	25.9 ± 8.2	<0.001
LV thrombus is present	0 %	12 %	<0.005

Follow-Up

At 6 months, only 32 patients' data were available. Seven patients died within the follow-up period of 6 months and eight patients moved to remote areas and were not available for full follow-up assessments. Four patients died despite optimal medical therapy because of progression of heart failure in the hospital and the other three patients experienced sudden death; all the patients died during the first 3 months after enrollment in the longitudinal study.

Women who had a prior history of PPCM had no difference in mortality when compared with the women who had no previous history of PPCM. However, they had intensive monitoring by the cardiologist and obstetrician throughout their pregnancy and post-partum period. Cardiac transplantation or the LV assist device for the population studied was unavailable for the duration of the trial because of economic reasons. During the first months after enrollment, patients received standard therapy for heart failure, which included furosemide [median daily dose 160 mg (80–240)], Ramipril [median daily dose 5 mg (5–20)], and carvedilol [median daily dose 25 mg (6.25–50)]. Carvedilol was titrated up as long as SBP was >100 mmHg or symptoms such as dizziness occurred. Clinical data and NYHA FC were compared at first presentation at the cardiac clinic at baseline (*n* = 46) and after 6 months of standard care (*n* = 32) as detailed in Table 4.

LV Function, Dimension, and Heart Rate

Patients who completed 6 months of standard care showed a significant reduction of heart rate, LV dimensions, and

Table 4 Clinical variables and left ventricular function at baseline and after 6-month follow-up of survivors (*n* = 36)

	Baseline	6 months	<i>p</i> value
Systolic BP (mmHg)	111.1 ± 17.4	116.1 ± 17.6	0.016
HR (b.p.m.)	93.5 ± 18.5	72.6 ± 11.3	0.087
NYHA FC (<i>n</i>)	13	30	<0.001
FCs I & II	23	6	
FCs III & IV			
EDD (mm)	61.6 ± 7.1	55.6 ± 8.9	<0.0001
ESD (mm)	53 ± 7	43 ± 10.3	<0.0001
Echo EF (%)	24.9 ± 8.2	42 ± 13.6	<0.0001

significant improvement in echocardiographic LVEF (*p* < 0.0001, Table 4) and NYHA FC (*p* < 0.001). However, normalization of LVEF (>50 %) was only observed in 11 (35 %) patients.

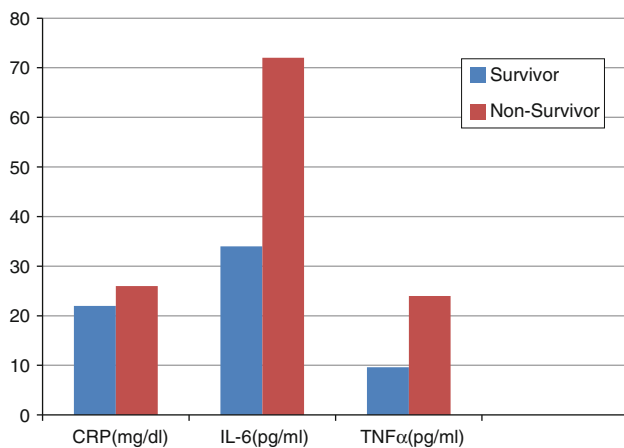
Levels of C-Reactive Protein and Pro-inflammatory Cytokines

The median plasma level of C-reactive protein for the 46 PPCM patients was 10.0 mg/L (range 1–90) with 45 % patients having values of >10 mg/L. Only ten patients had a C-reactive protein level of 3 mg/L. Baseline plasma levels of C-reactive protein correlated positively with LV end-diastolic (*rs* = 0.33, *p* = 0.0026) and end-systolic dimensions (*rs* = 0.35, *p* = 0.0012), whereas the correlation with LVEF (*rs* = 20.27, *p* = 0.015) was inverse (Graph 2).

Baseline plasma levels of C-reactive protein, TNF-α, and IL-6 were elevated in patients with PPCM when compared with age-, sex-, body mass index-, and parity-comparable controls (TNF-α 9.6 vs 3.2 pg/ml, IL-6 73.19 ± 34.4 vs 31.52 ± 8.83 pg/ml, C-reactive protein 20.8 ± 13.2 vs 3.1 ± 0.9 mg/L, *p*, 0.01). Plasma C-reactive protein, TNF-α, and IL-6 levels also correlated directly with NYHA FC-IV, complications, and deaths.

Predictors of Mortality

In the population studied, mortality remained high (15 %). Significant differences in the baseline data between the deceased patients and survivors were seen in NYHA FC and values of SBP, end-diastolic and end-systolic dimensions, LVEF, plasma levels of CRP, IL-6, and TNF-α (Table 5). Logistic regression analysis of NYHA FC, SBP, EDD, ESD, EF, CRP, IL6, and TNF-α revealed that only the baseline plasma levels of IL-6, TNF-α, and NYHA FC (OR = 2.67, CI 95 %, 1.04–6.83) were independent predictors of death (Table 5).



Graph 2 Baseline plasma inflammatory markers of deceased patients versus survivors. Only differences in baseline plasma levels of IL-6 and TNF- α were significant, $p < 0.002$

Table 5 Baseline characteristics of deceased versus surviving patients

	Deceased	Survivors	p value
Age (years)	22 \pm 1.2	28 \pm 6	0.24
No. of children (n), median (range)	2(1–4)	3(1–7)	0.14
Onset of symptoms after delivery (months), median (range)	1(1–5)	2(1–5)	0.6
NYHA FC	3.2 \pm 0.8	2.0 \pm 0.6	0.04
EDD (mm)	64 \pm 5	60 \pm 6	0.03
ESD (mm)	57.6 \pm 7	53.2 \pm 8	0.03
Echo EF (%)	20 \pm 4	26 \pm 8	0.04
C-reactive protein (mg/L), median (range)	26	22	0.10
TNF (pg/mL)	9.6	24	<0.001
IL-6 (pg/mL)	34	72	<0.001

Discussion

This study documented the clinical profile of 46 PPCM patients recruited prospectively and examined the role of inflammatory markers at time of diagnosis, in-hospital events, and clinical outcome after 6 months of standard clinical care.

Recovery of systolic function occurs in roughly half of affected women and usually occurs within 6 months of symptom onset [16]. A rapid recovery of EF is often seen in patients after initial diagnosis and diuresis [17]. EF > 45 % at 2 months after diagnosis predicts full functional recovery in 75 % of women with this result [18].

Of these, 15 % of patients died and only 23 % of the studied population normalized their LVEF after 6 months

of therapy. Patients who died had lower NYHA FC, LVEF, larger LV dimensions, and higher CRP, IL-6, and TNF- α at diagnosis compared with those who survived, whereas age, parity, or onset of symptoms did not appear to play a role. None of the patients with PPCM presented with symptoms during the pre-partum period. This is in contrast with studies performed by others [3, 19] and more in keeping with a study from Haiti [4] documenting that 96 % of patients with PPCM developed heart failure in the post-partum period. There was no evidence of chronic disease or cardiac cachexia, which could account for a low lipid profile being a marker of severe chronic disease. We found an association of low total cholesterol levels with larger LV dimensions and lower EF.

Levels of low plasma cholesterol correlated positively with the levels of the inflammatory marker C-reactive protein, IL-6, and TNF- α . Plasma sampled from almost half of the population studied had significantly raised levels of C-reactive protein, IL-6, and TNF- α reflecting possibly the presence of a low-grade chronic inflammatory process due to the release of endotoxin or endotoxin-like substances and subsequent release of pro-inflammatory cytokines [20]. As C-reactive protein, IL-6, and TNF- α measurements are relatively inexpensive, they could provide us with a readily available tool to identify patients with an ongoing inflammatory process and high-risk complications.

This is supported by other previous research in PPCM patients presenting with subsequent pregnancy where we observed an exaggerated post-partum pro-inflammatory cytokine surge, possibly playing a role in the development of PPCM [21]. Plasma levels of CRP, IL-6, and TNF α as markers of potential cardiac inflammation were significantly higher in PPCM patients when compared with healthy controls and are predictors of mortality.

Conclusions

Plasma markers of inflammation were significantly elevated in PPCM patients and correlated with increased LV dimensions and lower EF at presentation. Baseline CRP, IL-6, TNF- α , and higher NYHA FC were the only predictors of mortality. These results contribute to inflammation which may contribute to the pathogenesis of PPCM and its complications [7, 8, 22].

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