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A prospective study to evaluate the efficacy and safety of vitamin E and levocarnitine prophylaxis against doxorubicin-induced cardiotoxicity in adult breast cancer patients

Iman Moustafa[1,2,3], Catherine Connolly[1], Malik Anis[2], Hani Mustafa[2], Frasia Oosthuizen[1] and Michelle Viljoen[4]

Abstract

Background: Doxorubicin induces acute and chronic cardiotoxicity. This study is aimed to evaluate the efficacy and safety of vitamin E and levocarnitine (EL) as cardioprotective agents against acute doxorubicin cardiotoxicity in female adult breast cancer patients.

Methods: A prospective, randomized controlled study was conducted in patients treated with doxorubicin and cyclophosphamide (AC). Patients were randomly assigned to EL plus AC or AC alone for the duration of 4 cycles. Cardiac enzymes (B-type natriuretic peptide, creatine kinase, troponin I (Trop)) and cardiac events were monitored during treatment to evaluate the cardioprotective efficacy of EL.

Results: Seventy-four patients were recruited and received four cycles of chemotherapy. The intervention group ($n = 35$) showed a significant reduction in both the B-type natriuretic peptide and creatine kinase cardiac enzymes compared to the control group ($n = 39$). The median (IQR) change for BNP was 0.80 (0.00–4.00) for IG versus 1.80 (0.40–3.60) for CG groups ($p < 0.001$); creatine kinase was −0.08 (−0.25–0.05) for IG versus 0.20 (0.05–0.50) for CG ($p < 0.001$). The addition of EL decreased the cardiac events by 24.2% ($p = 0.02$). All adverse events were tolerable and manageable.

Conclusion: This study supports the addition of EL as prophylaxis against acute doxorubicin cardiotoxicity and it was also very well tolerated by a majority of the patients. The co-administration of EL at higher doxorubicin (240 mg/m^2) dose should be further investigated.

Keywords

Vitamin E, levocarnitine, prophylaxis, doxorubicin, cardiac toxicity, adult cancer patients

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Introduction

Cancer remains one of the leading causes of death globally.[1] The anthracycline chemotherapeutics – doxorubicin, daunorubicin, epirubicin, idarubicin, and mitoxantrone – are important antitumor agents in the treatment of numerous types of cancer such as breast cancer, ovarian cancer, osteosarcoma and acute myeloid leukemia.[2] While effective, the associated cardiotoxicity remains a limiting factor with the use of doxorubicin.[3,4] The risk of cardiovascular death following chemotherapy was found to be higher than the risk of tumor recurrence in many patients.[5,6]

Associated cardiotoxic effects include cardiac failure, left ventricular dysfunction, pericarditis, myocarditis, atrial fibrillation, ventricular arrhythmias like ventricular tachycardia and/or fibrillation.[7] The mortality rate due to

[1]Department of Pharmacology, School of Health Sciences, University of KwaZulu-Natal, Durban, South Africa
[2]Department of Pharmacy, King Abdulaziz Hospital, Ministry of the National Guard – Health Affairs, Al-Ahsa, Saudi Arabia
[3]King Abdullah International Medical Research Center, Al-Ahsa, Saudi Arabia
[4]Department of Pharmacology, School of Pharmacy, University of the Western Cape, Bellville, South Africa

Corresponding author:
Iman Moustafa, Department of Pharmacy, King Abdulaziz Hospital, Mahassen street, Ministry of the National Guard – Health Affairs, Al-Ahsa, Eastern Region 31982, Saudi Arabia.
Email: emooo74@yahoo.com

congestive heart failure (CHF) related to anthracycline is more than 50%.[8,9] Up to 30% of the patients treated with doxorubicin struggle with CHF.[10]

Doxorubicin has multiple modes of action against cancer cells.[11] The main mechanism of action is the intercalation within deoxyribonucleic acid (DNA) base pairs, causing splintering of DNA strands and the inhibition of DNA and ribonucleic acid (RNA) synthesis. Doxorubicin inhibits the enzyme topoisomerase II (Top II), causing DNA damage and initiation of apoptosis. When doxorubicin is combined with iron, doxorubicin causes free radical-mediated oxidative damage to DNA, further limiting DNA synthesis.[12]

The cardiotoxicity of doxorubicin appears separate from its therapeutic mechanism as cardiomyocytes are generally not replicated and Top II, the primary target of doxorubicin, is not expressed in dormant cells and undetectable in heart tissues.[13] The generation of reactive oxygen species (ROS) is a classical mechanism by which doxorubicin injures the myocardium. There is evidence suggesting that ROS is unlikely to be the primary mechanism of doxorubicin-induced cardiotoxicity.[10] Other proposed mechanisms include impaired mitochondrial function, disruption of Ca^{2+} homeostasis and altered gene and protein expression that triggers cell death.[14]

Doxorubicin induces cardiotoxicity acutely or chronically. The incidence of acute cardiotoxicity is approximately 11%.[15] Acute cardiotoxicity may occur within 2–3 days of its administration.[16] The symptoms are mostly chest pain due to myopericarditis and/or palpitations due to sinus tachycardia, paroxysmal non-sustained supraventricular tachycardia and premature atrial and ventricular beats.[17] Early chronic cardiotoxicity appears between 30 days and one year after doxorubicin treatment was completed, while late chronic cardiotoxicity may appear 6–10 years after doxorubicin treatment was completed.[3] Chronic toxicity manifests as congestive cardiac failure and is due to cumulative dose-related toxicity. The cumulative doxorubicin dose is the most important risk factor associated with chronic cardiotoxicity.[18] The minimum total cumulative dose of doxorubicin that can lead to cardiotoxicity is 250 mg/m^2.[19]

Numerous agents have been investigated as prophylaxis against doxorubicin-induced cardiotoxicity. Dexrazoxane is currently the only FDA-approved medication to prevent and decrease the severity of cardiomyopathy linked to doxorubicin intake of a cumulative doxorubicin dose of 300 mg/m^2.[20] The use of dexrazoxane is, however, limited due to concerns of secondary malignancies.[21] Therefore, keeping the total cumulative doxorubicin dose below a threshold of 450 mg/m^2 is currently the only method that may help to reduce cardiotoxicity.[18] Numerous studies were conducted with antioxidants like vitamin C, vitamin E, selenium, carotenoids, β-carotene, *N*-acetylcysteine, coenzyme Q10, glutathione peroxidase and levocarnitine to determine if these can be used as prophylactic agents against anthracycline-induced cardiotoxicity. The results have however been inconclusive.[17,22] Studies that have explored vitamin E alone or in combination with levocarnitine to mitigate cardiotoxicity of doxorubicin are of low quality and the results are conflicting.[23] Therefore, evidence to support or discard the prophylactic use of vitamin E and levocarnitine in preventing doxorubicin-induced cardiotoxicity remains controversial.

This study is set out to investigate the potential of vitamin E and levocarnitine used in combination as a strategy to prevent doxorubicin-induced cardiotoxicity in the acute phase of chemotherapy in patients with breast cancer.

Methods and patients

Study design and setting

This study was a prospective, randomized controlled, open-label and single-center study conducted at the Department of Oncology, King Abdul-Aziz Hospital (Eastern Region, Saudi Arabia) from October 2018 to April 2021.

Study population – patient selection and eligibility criteria

The following inclusion and exclusion criteria were applied during this study.

Inclusion criteria:

- Patients with a diagnosis of breast cancer at any stage (I, II, III, IV) attending the Department of Oncology, King Abdul-Aziz hospital;
- Female patients only;
- Aged 18 years or older but younger than 75 years when starting the first cycle of chemotherapy;
- Chemotherapy regimen must include doxorubicin and cyclophosphamide;
- Performance status of 0–2 in the Eastern Cooperative Oncology Group (ECOG) score;
- Oestrogen receptors, progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) can be positive or negative;
- Patients with a left ventricular ejection fraction (LVEF) of >50%.

Exclusion criteria:

- Patient age <18 or >75 years when starting the first cycle of chemotherapy;
- Patients with an LVEF of <50% before enrolment to the study;
- History of ionizing radiation to the chest wall;
- Prior exposure to anthracyclines;
- Pre-existing cardiac disease or risk factors, including:
  - History of myocardial infarction (MI) or active cardiac disease (e.g. CHF or hypertension);
  - Diabetes mellitus (Type I and II);

- Metabolic abnormalities (familial hypercholesterolemia, Gaucher disease, Hunter syndrome, Krabbe disease and maple syrup urine disease).
- Pregnant patients;
- Patients treated with glimepiride, metformin, pioglitazone, probucol, dexrazoxane, PDE5 inhibitor (sildenafil), nitric oxide, superoxide dismutase, endothelin receptor antagonist (bosentan), beta-blockers, amiodarone, angiotensin-converting enzyme inhibitors, calcium channel blockers or digoxin;
- Patients with renal and hepatic insufficiency/impairment;
- Patients with any abnormal baseline bloodwork.

Patient enrolment and baseline assessments

After an initial clinical evaluation and explanation of the study protocol to prospective patients, those consenting to participate underwent a baseline clinical evaluation which included various baseline laboratory tests (white blood cells (WBCs), haemoglobin (Hgb), platelets (Plts), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Bilirubin (Bil), alkaline phosphatase (ALK) and serum creatinine (SrCr).

Cardiac events were assessed with cardiovascular imaging using multigated acquisition (MUGA), which was used to assess the LVEF. Baseline analyses were performed one day before the initial chemotherapy cycle (day 0). Venous blood samples of 3 mL were collected and sent to the hospital laboratory for analyses of the variables listed. The cardioprotective efficacy was measured by monitoring the three serum cardiac biomarker enzymes B-type natriuretic peptide (BNP), creatine kinase (CKMB) and troponin I (Trop) at the baseline.

Subjects were selected according to the eligibility criteria and the performance status (PS) as assessed by the ECOG score. Performance status is an indicator of whether the patient will be able to tolerate chemotherapy and respond to treatment or not. The ECOG has scores from 0 to 5; a score of 0 means the patient is fully active, a score of 1 means the patient is restricted in physical activity but able to carry out light house or office work, a score of 2 means the patient is capable of self-care but unable to carry out any daily work activities, a score of 3 means the patient is capable of limited self-care, a score of 4 means the patient cannot care for him/herself and is totally confined to bed or chair and a score of 5 implies death.[24]

Randomization and intervention

Randomization. Breast cancer patients meeting the inclusion criteria were randomly allocated in a 1:1 ratio to either the intervention group (IG) or the control group (CG) (Figure 1). All recruited subjects received the doxorubicin and cyclophosphamide protocol every 21 days for 4 cycles as part of the standard chemotherapy protocol. Both groups received 60 mg/m^2 and 240 mg/m^2 cumulative doses of doxorubicin. Doxorubicin was administered as an intravenous push over 15 min.

Intervention schedule. The IG received a combination of vitamin E (600 mg three times daily) and levocarnitine (Day 1 = 3000 mg IV; Day 2–21 = 300 mg four times daily). Levocarnitine IV was administered in the hospital before chemotherapy administration. A dosage with vitamin E and oral levocarnitine (Day 2–21) was self-administered by the patient (Table 1).

Levocarnitine was used as intravenous and oral products (Table 1). The commercial product, 1000 mg levocarnitine per ampoule (5 mL) for injection was procured from Help S.A. Greece, while the oral commercial product of levocarnitine was an L-carnitine solution (6000 mg levocarnitine per bottle (300 mg/mL)), purchased from Sterop, Belgium. The commercial product of alpha-tocopherol was vitamin E capsules (45 mg α-tocopherol acetate (45 mg = 100 IU)) bought from Gericare, USA.

An accurate and current accounting of the dispensing and the return of oral vitamin E and levocarnitine (EL) for each subject were maintained on an ongoing basis by a principal investigator. The number of oral vitamin E and levocarnitine dispensed and returned by the subjects were recorded on the investigational drug accountability record. If the chemotherapy cycle is needed to be delayed because of any complication, then the patient continued to receive vitamin E and levocarnitine.

Monitoring and patient follow-up

The study participants visited the oncology department once every 21 days for 4 cycles of routine standard chemotherapy administration.

Laboratory tests (WBC, Hgb, Plts, AST, ALT, Bil, ALK and SrCr) and serum cardiac biomarkers (BNP, CKMB and Trop) were also tested. All patients were clinically examined for any signs or symptoms of heart failure. Cardiac function was assessed by measurement of the LVEF, utilizing resting MUGA nuclear medicine imaging scans. The LVEF was done as a baseline before the initiation of chemotherapy and within 3 weeks after completing the chemotherapy to measure the cardiac events. Cardiac events were defined as a decline in LVEF from a baseline of ≥10%, a decline in LVEF of at least 20% from the baseline, a decline in LVEF to at least 5% below the baseline, or the development of congestive cardiac failure.[25,26]

Evaluation of the safety of vitamin E and levocarnitine:

At each visit, patients were encouraged to report any adverse effects that occurred throughout the study period. Clinical examinations, including the recording of vital signs as well as full blood counts and biochemistry profiles, were performed one

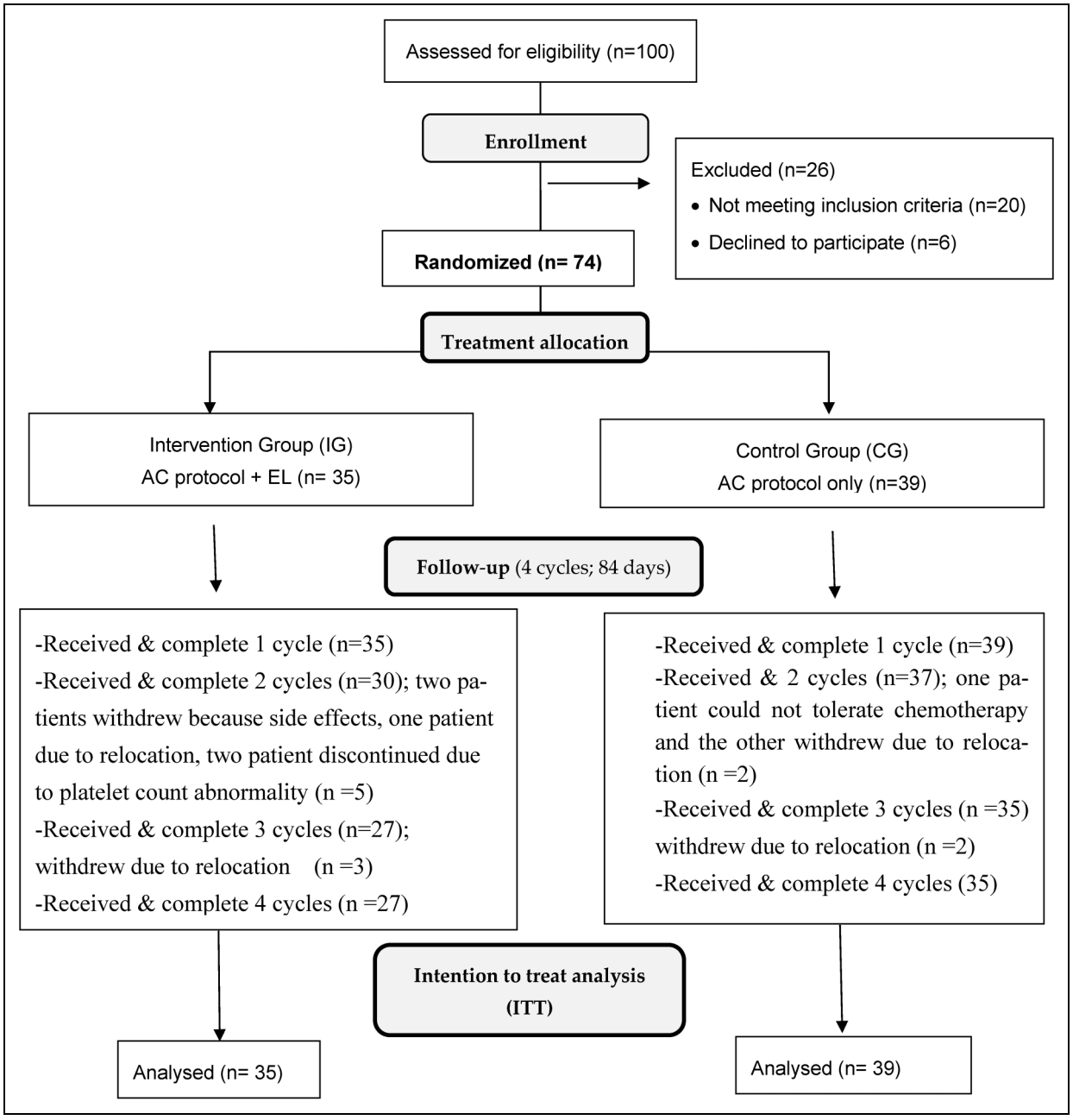

Figure 1. Schematic diagram of study enrolment, randomization and follow-up treatments of study participants. EL, vitamin E and levocarnitine; AC, doxorubicin and cyclophosphamide.

day before chemotherapy for all four treatment cycles. The adverse effects during chemotherapy were carefully recorded and graded. The adverse events were evaluated according to Common Terminology Criteria for Adverse Events (CTCAE).[27]

Statistical analyses

A sample size of 60 patients was determined based on estimating the incidence of doxorubicin-induced subclinical cardiomyopathy to within ±8% (margin of error) and a probability of 95%, assuming an incidence of 11%.[15] The frequency distributions of continuous variables (cardiac enzymes, age and general laboratory testing as Hgb, WBC, Plts, AST, ALT, Bil, ALK and SrCr) were assessed for normality and mean ± standard deviation (SD) or median interquartile range (IQR) were used where appropriate. For the strength of an association between the outcome and exposure variables, bivariate analysis for categorical variables used Chi-square test or Fisher's exact test, where applicable. Continuous variables between the IG (AC + EL) and the CG groups (AC alone) used *t*-tests or Mann–Whitney U-test, where appropriate.

The level of significance was set at $p < 0.05$. All statistical analyses were performed using the IBM Statistical Package for the Social Sciences SPSS® software version 20.

Results

Demographic characteristics

Patient enrolment was done from October 2018 to December 2020, and 100 patients were assessed for eligibility (Figure 1), 26 patients did not meet the set inclusion criteria. Seventy-four patients were randomly assigned to the IG group ($n = 35$) or the CG group ($n = 39$). The mean (±SD) age of the selected study population was 52.51 ± 12.54 years for the IG group and 51.46 ± 9.25 years for the CG group ($p = 0.68$), before the initiation of the chemotherapy treatment cycles. No variables were statistically different between the two groups (Table 2).

Table 1. Chemotherapy protocol schedule and prophylaxis interventions.

	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
Groups	*D1*	*Day 2 −21*	*D1*	*Day 2 −21*	*D1*	*Day 2 −21*	*D1*	*Day 2 −21*
CG	AC		AC		AC		AC	
IG	AC		AC		AC		AC	
	L IV	*L PO*	*L IV*	*L PO*	*L IV*	*LPO*	*L IV*	*L PO*
	Vitamin E-α tocopherol (600 mg three times daily for 84 days)							

CG, control group; IG, intervention group; AC, doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2; L IV, 3000 mg levocarnitine IV in 375 mL normal saline 0.9% over 30 min; L PO, 1 mL (300 mg) levocarnitine per mouth four times daily.

Treatments received and biomarker analysis

Cardiac biomarkers. Table 3 presents a comparison of the three cardiac enzymes (BNP, CKMB and troponin I) among the two groups at the baseline and after each of the four chemotherapy cycles (days 21, 42, 63 and 84). There were no statistically significant differences between the two groups at any time point (baseline, days 21, 42, 63 or 84) for the BNP and troponin I enzymes.

However, the median (IQR) of the CKMB value was significantly ($p = 0.03$) higher at the baseline for the IG 0.60 (0.30–0.70) compared to the CG group 0.40 (0.30–0.50). On the other hand, the median (IQR) for CKMB of the IG group was stable and significantly lower compared to the CG on day 21 (IG 0.4 (0.30–0.50) versus CG 0.50 (0.40–0.70), $p = 0.02$), on day 42 (IG 0.4 (0.30–0.60) versus CG 0.2 (0.40–0.90), $p = 0.03$),

Table 2. Patient sociodemographic and clinical characteristics at the baseline.

Characteristic	Intervention ($n = 35$)	Control ($n = 39$)	Test	*p*-Value
AGE (mean ± SD)	52.51 ± 12.54	51.46 ± 9.25	*I*	0.68
Ethnicity				
Arabic	33.00 (94.29%)	38.00 (97.44%)	*FE*	0.60
Not Arabic	2.00 (5.71%)	1.00 (2.56%)		
Performance status				
0	29.00 (82.86%)	33.00 (84.62%)	*FE*	0.51
1	5.00 (14.29%)	3.00 (7.69%)		
2	1.00 (2.86%)	3.00 (7.69%)		
Body surface area (BSA) (m^2)				
≤2	28.00 (80.00%)	33.00 (84.5%)	*FE*	0.6
>2	7.00 (20.00%)	6.00 (15.38%)		
Stages				
I	1.00 (2.86%)	3.00 (7.69%)	*FE*	0.70
II	18.00 (51.43%)	16.00 (41.03%)		
III	10.00 (28.57%)	11.00 (28.21%)		
VI	6.00 (17.14%)	9.00 (23.08%)		
Oestrogen receptor status				
Positive	24.00 (68.57%)	29.00 (74.36%)	χ^2	0.58
Negative	11.00 (31.43%)	10.00 (25.64%)		
Progesterone (PR) receptor status				
Positive	21.00 (60.00%)	28.00 (71.79%)	χ^2	0.28
Negative	14.00 (40.00%)	11.00 (28.21%)		
Human epidermal growth factor receptor 2 status (HER2)				
Positive	10.00 (28.57%)	13.00 (33.33%)	χ^2	0.66
Negative	25.00 (28.57%)	26.00 (66.67%)		

SD; standard deviation; I; independent *t*-test; χ^2; chi-square test; FE; Fisher's exact test.

Table 3. Cardiac enzymes at the baseline and after each cycle.

	Intervention group (IG)			Control group (CG)			
Tests	Median	IQR		Median	IQR		*p*-Value
Baseline							
BNP (pmol/L)	3.40	2.90	4.70	3.30	2.90	5.60	0.62
CKMB (µg/L)	0.60	0.30	0.70	0.40	0.30	0.50	0.03[a]
Trop (pg/mL)	3.20	2.40	6.20	3.90	2.20	7.00	0.50
Cycle 1 (Day 21)							
BNP (pmol/L)	4.30	2.90	6.90	4.50	3.50	7.30	0.30
CKMB (µg/L)	0.40	0.30	0.50	0.50	0.40	0.70	0.02[a]
Trop (pg/mL)	1.90	1.00	2.40	1.90	0.70	5.10	0.35
Cycle 2 (Day 42)							
BNP (pmol/L)	4.20	3.00	6.40	2.90	3.45	7.70	0.19
CKMB (µg/L)	0.40	0.30	0.60	0.20	0.40	0.90	0.03[a]
Trop (pg/mL)	3.70	2.90	5.80	0.80	3.10	9.20	0.06
Cycle 3 (Day 63)							
BNP (pmol/L)	4.90	3.40	6.90	5.90	3.50	7.80	0.12
CKMB (µg/L)	0.40	0.30	0.80	0.60	0.40	0.90	0.03[a]
Trop (pg/mL)	3.30	2.60	7.60	6.60	3.30	12.30	0.06
Cycle 4 (Day 84)							
BNP (pmol/L)	5.25	3.30	6.90	5.70	4.20	9.70	0.35
CKMB (µg/L)	0.40	0.30	0.50	0.60	0.40	0.80	0.01[a]
Trop (pg/mL)	6.30	3.60	9.40	8.05	4.75	13.25	0.15

BNP: B-type natriuretic peptide; Trop: troponin I; CKMB: creatine kinase; IQR: interquartile range; [a]statistically significant at ≤0.05.

Table 4. Differences in cardiac enzymes at the baseline and the mean of four cycles of chemotherapy treatment in the two respective groups.

	Intervention group (IG)						Control group (CG)					
	Median	IQR		min	max	*p*-Value[a]	Median	IQR		min	max	*p*-Value[a]
BNP (pmol/L)												
Baseline	3.00	2.90	4.70	2.90	7.00	0.012[b]	3.30	2.90	3.90	2.90	58.70	0.006[b]
Cycles 1–4	4.90	3.90	6.90	2.90	10.50		5.50	3.60	7.90	3.10	12.85	
CKMB (µg/L)												
Baseline	0.59	0.30	0.70	0.20	1.20	0.07	0.40	0.30	0.40	0.20	1.00	0.0001[b]
Cycles 1–4	0.40	0.35	0.45	0.30	0.90		0.60	0.40	0.85	0.25	1.79	
Trop (pg/mL)												
Baseline	3.20	2.40	5.70	0.25	17.40	0.14	3.80	1.95	6.90	0.30	80.90	0.007[b]
Cycles 1–4	3.80	2.75	6.05	1.55	15.00		5.53	3.88	8.10	2.65	98.00	

BNP: B-type natriuretic peptide; Trop: troponin I; CKMB: creatine kinase; IQR: interquartile range. [a]Wilcoxon signed-rank test; [b]statistically significant at ≤0.05.

on day 63 (IG 0.4 (0.30–0.80) versus CG 0.60 (0.40–0.90), $p = 0.03$) and on day 84 (IG 0.4 (0.30–0.50) versus CG 0.60 (0.40–0.80), $p = 0.01$).

Table 4 presents the differences in cardiac enzymes at the baseline and the mean of the four chemotherapy cycles in the two respective groups. The median (IQR) levels of BNP increased significantly between the baseline and the mean of the four cycles in both the IG group (baseline; 3 (2.90–4.70) versus mean of 4 cycles; 4.90 (3.90–6.90), $p = 0.012$) and the CG group (baseline 3.3 (2.90–3.90) versus mean of 4 cycles; 5.50 (3.60–7.90), $p = 0.006$).

Both the CKMB and Trop showed no statistically significant difference at the baseline and the mean of four cycles in the IG. However, the median (IQR) of the CKMB (baseline 0.40 (0.30–0.40) versus means of 4 cycles; 0.60 (0.40–0.85), $p = 0.0001$) and Trop (baseline 3.80 (1.95–6.90) versus mean of 4 cycles; 5.53 (3.88–8.10), $p = 0.007$) showed significantly increased levels in CG with a mean of four cycles compared to the baseline values.

Table 5 presents the change in median cardiac enzymes between the two groups over 4 cycles minus the median baseline values. It is evident that there was a statistically significant increase in the median (IQR) for both groups.

Table 5. Differences illustrating the changes in cardiac enzymes between the two groups.

	Median change[a]	IQR		min	max	*p*-Value[b]
BNP (pmol/L)						
Intervention group	0.80	0.00	4.00	−2.40	7.00	<0.001[c]
Control group	1.80	0.40	3.60	−52.80	7.40	
CKMB (µg/L)						
Intervention group	−0.08	−0.25	0.05	−0.4	0.33	<0.001[c]
Control group	0.20	0.05	0.50	−0.35	1.39	
Trop (pg/mL)						
Intervention group	0.70	−0.35	1.25	−10.85	7.75	0.10
Control group	1.78	0.00	4.93	−65.80	80.80	

BNP: B-type natriuretic peptide; Trop: troponin I; CKMB: creatine kinase; IQR: interquartile range; Median change[a]: the median of cycles 1–4 minus median baseline; [b]Wilcoxon–Mann–Whitney test; [c]statistically significant at ≤0.05.

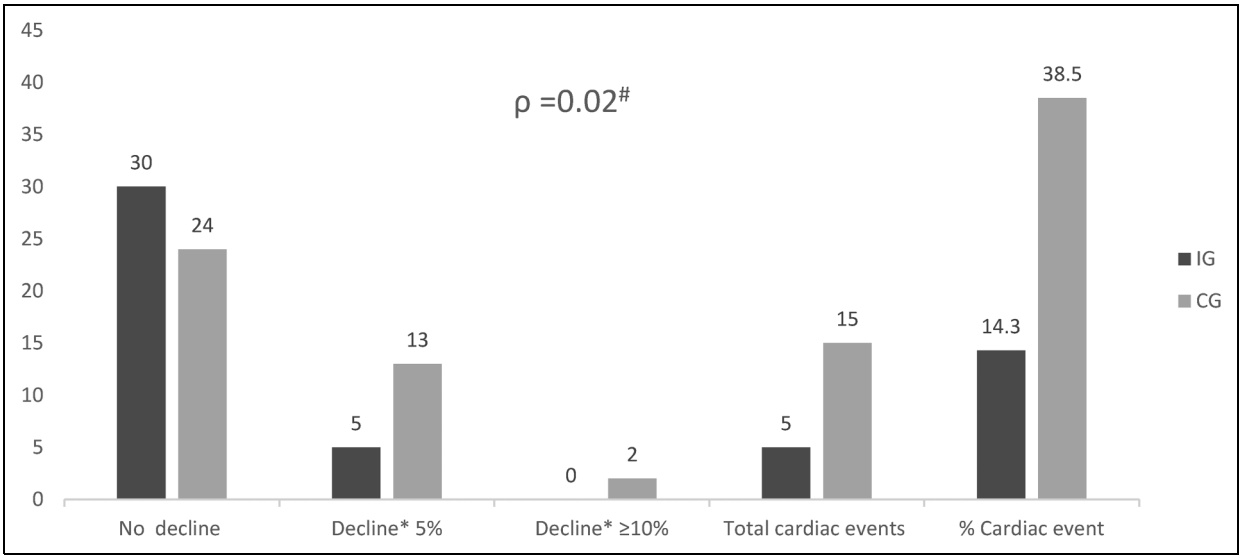

Figure 2. Difference in cardiac events among groups. IG: intervention group; CG: control group, *Left ventricular ejection fraction (LVEF) decline, #Statistically significant ≤0.05.

BNP in IG was 0.80 (0–4.00) while in CG it was 1.8 (0.40–3.60), $p < 0.001$ and CKMB in the IG; −0.08 (−0.25–0.05) versus CG; 0.20 (0.05–0.50), $p < 0.001$.

Cardiac events. Five cardiac events were reported in the IG group and 15 cardiac events in the CG group; this is a statistically significant difference ($p = 0.02$). Vitamin E and levocarnitine was thus found to decrease cardiac events by 24.2% (Figure 2).

Safety and mortality

Adverse events. All reported adverse events (Figure 3) recorded in both treatment groups were tolerable and manageable. None of the patients experienced severe or life-threatening adverse events. Abdominal pain and headache were statistically more reported in the IG group albeit categorized as mild and moderate. Early discontinuations of patients in the IG group due to adverse events were due to abdominal pain and gastritis (2.70%). There were no deaths recorded in either group during the study period.

Routine general laboratory testing. Table 6 presents the general laboratory test results comparing the IG and CG groups. Bil and serum creatinine (SrCr) enzyme levels showed significant changes when comparing the two groups. The median of the difference for Bil was higher in the IG group compared to the CG group; IG 1.02 (0.1–3.92) versus CG 0.12 (−0.97–2.68), $p = 0.04$; however, SrCr was lower in the IG group when compared to the CG; IG 0 (−2.75, 3.5) versus CG 5 (0.5, 7), $p = 0.0009$.

Discussion

Few studies have investigated vitamin E and levocarnitine as single agents for prophylaxis against cardiotoxicity; results from these studies were conflicted. No previous studies have

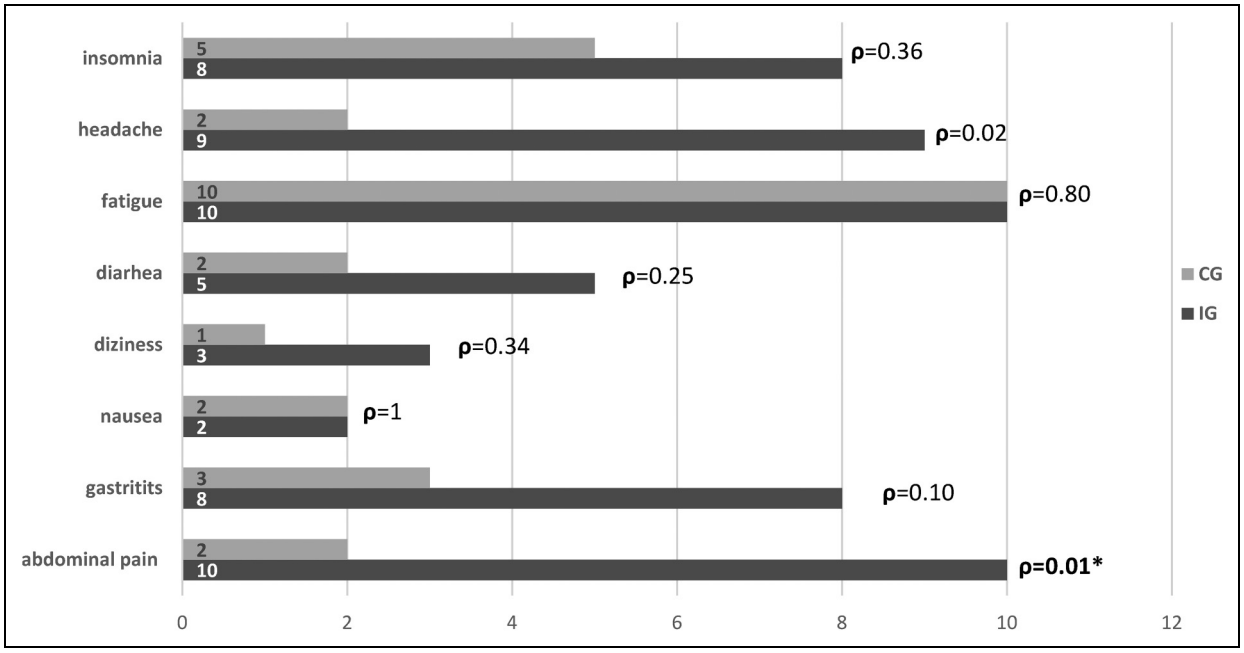

Figure 3. Comparison of reported adverse events during all four cycle treatments for both groups. IG: intervention group; CG: control group. *Statistically significant ≤0.05.

investigated the combination of vitamin E (α-tocopherol form) and levocarnitine for possible cardioprotective properties.[28]

In this study, we investigated the potential of combining vitamin E and levocarnitine to protect against doxorubicin-induced cardiotoxicity in adult breast cancer patients. These patients received a cumulative dose of 240 mg/m^2 doxorubicin in four cycles (60 mg/m^2). Cardiac enzymes were monitored on days 1, 21, 42, 63 and 84.

Our study showed a significant decrease in the levels of CKMB and BNP in the IG group versus the CG group, while Trop showed only a slight decrease in the IG versus the CG that was not significant (Table 5). While there are no recommendations to specifically monitor cardiac enzyme biomarkers for doxorubicin-induced cardiotoxicity, CKMB, BNP and Trop are the most established cardiac biomarkers for acute cardiotoxicity, MI and heart failure.[29] Moreover, there is no standard recommendation when to monitor the cardiac enzymes after chemotherapy administration.[29]

Some studies found BNP to be a sensitive test and useful for monitoring early anthracycline cardiotoxicity,[30–32] while some studies failed to find any association between BNP and cardiotoxicity.[33–35] A recent study indicated that cancer may possibly increase the BNP levels through inflammation induced by the cancer itself, especially if it has metastasized.[36] In our study, the median change of BNP showed a significant increase in the CG group compared to the IG group (1.80 vs. 0.80 pmol/L) (Table 5); however, this difference was not evident at every cycle (Table 3). This study showed that the BNP levels were lowered by the combination of vitamin E and levocarnitine (EL), which indicated that this combination has a cardioprotective effect.

CKMB is a biomarker that has not been studied adequately to determine its validity in the monitoring of cardiac injury induced by doxorubicin. In this study, the CKMB levels showed significant elevation after every cycle of chemotherapy in the CG versus IG groups (measured after 21 days of chemotherapy). In a study by De Souza et al., it was found that CKMB may be detected for a longer period after a doxorubicin cumulative dose (240 mg/m^2).[37] In the current study, CKMB was determined every 21 days and showed significant decreases in the IG compared to the CG, which indicated that the combination of vitamin E and levocarnitine has a cardioprotective effect.

Troponin is considered the gold standard test to evaluate cardiac injury as it is sensitive and easily available[31]; however, Trop has a limited time for detection[30] and has low specificity as it may increase with conditions such as rhabdomyolysis, renal failure, chronic poor vascular health, hypertensive crisis and sepsis.[38,39] In this study, the combination of vitamin E and levocarnitine (EL) decreased the troponin levels in the IG compared to the CG group; however, it was not significant (Table 5).

Validation of cardiac enzymes as early novel biomarkers for MI was evaluated by Bassan and co-workers and they found diagnostic accuracy of BNP, CKMB and Trop for sensitivity (%) were 70.8, 45.8 and 50.7, for specificity (%) at 68.9, 98.4 and 98.8, positive predictive values (%) at 22.7, 78.6 and 85.7 and negative predictive values (%) at 94.8, 93.4 and 93.3, respectively.[31] It is therefore recommended

Table 6. General laboratory test results comparing the intervention and control groups.

	Intervention group			Control group			
	Median	IQR		Median	IQR		*p*-Value
White blood count (WBC) X 10^9/L							
Median (IQR) at the baseline	7.10	5.63	9.00	6.08	5.16	8.07	0.20
Median (IQR) of cycles 1–4	5.58	3.18	6.81	3.82	3.48	6.18	0.19
Median (IQR) of difference[a]	2.07	0.29	4.28	2.62	0.54	3.51	0.58
Haemoglobin (Hgb) g/L							
Median (IQR) at the baseline	130.00	108.00	133.00	111.00	99.00	128.00	0.01[b]
Median (IQR) of cycles 1–4	113.50	104.75	122.50	102.5	93.75	110.00	0.001[b]
Median (IQR) of difference[a]	8.50	3.00	12.50	5.00	3.25	10.333	0.29
Platelets (Plts) × 10^3/mclL							
Median (IQR) at the baseline	264.00	233.00	379.00	317.00	264.00	370.00	0.16
Median (IQR) of 4 cycles	307.50	257.50	386.50	296	251.75	401.25	0.82
Median (IQR) of difference[a]	−22.50	−61.00	27.00	−9.50	−89.00	60.50	0.53
Aspartate aminotransferase (AST) U/L							
Median (IQR) at the baseline	19.00	11.00	23.00	22.00	15.00	27.00	0.05[b]
Median (IQR) of cycles 1–4	15.25	14.25	19.50	19.50	15.00	23.5	0.006[b]
Median (IQR) of difference[a]	1.00	−0.25	7.25	1.50	−1.50	6.25	0.61
Alanine aminotransferase (ALT) U/L							
Median (IQR) at the baseline	18.00	11.00	26.00	15.00	12.00	27.00	0.29
Median (IQR) of cycles 1–4	20.25	13.5	26.75	17.00	15.00	36.50	0.24
Median (IQR) of difference[a]	−1.00	−6.75	4.50	−1.00	−6.00	3.25	0.93
Bilirubin (Bil) µmol/L							
Median (IQR) at the baseline	6.00	4.60	7.60	5.50	5.20	7.90	0.92
Median (IQR) of cycles 1–4	4.28	3.55	4.68	6.00	4.20	7.57	0.002[b]
Median (IQR) of difference[a]	1.02	0.10	3.92	0.12	−0.97	2.68	0.04[b]
Alkaline phosphatase (ALK) U/L							
Median (IQR) at the baseline	73.00	62.00	84.00	85.00	65.00	105.00	0.007[b]
Median (IQR) of cycles 1–4	73.00	66.75	83	85.75	62.75	111.50	0.02[b]
Median (IQR) of difference[a]	−1.50	−14.00	8.00	−1.50	−13.50	18.50	0.60
Serum creatinine (SrCr) µmol/L							
Median (IQR) at the baseline	63.00	58.00	65.00	62.00	57.00	64.00	0.14
Median (IQR) of cycles 1–4	62.25	59.75	66.00	54.00	49.75	60.00	<0.001[b]
Median (IQR) of difference[a]	0.00	−2.75	3.50	5.00	0.50	7.00	0.0009[b]

WBCs: white blood cells, Hgb: haemoglobin, Plat: platelet count, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALK: alkaline phosphatase, SrCr: Serum creatinine, IQR: interquartile range, [a]change from the baseline and mean or median of cycles 1–4 with standard deviation or IQR, [b]statistically significant at ≤0.05.

that these three enzymes be further validated for the diagnostic accuracy of doxorubicin induced cardiotoxicity but also to consider the cost effectiveness and the precise timing of the effective monitoring of these enzymes.[30]

Cardiac events such as the decline of LVEF can be used as an indicator to predict the cardiotoxicity. This study found that cardiac events decreased significantly (24%) in the IG compared to the CG group. This outcome might be ascribed to the fact that levocarnitine is effective in reversing the oxidative stress arising from iron overload, improving the activity of carnitine acetyl transferase, and improving energy metabolism in mitochondria.[40] Levocarnitine is the active form of carnitine. A relationship exists between the concentration of carnitine and lipid peroxidase levels in myocardial dysfunction.[9] Carnitine supports the transfer of long-chain fatty acids addicted to the mitochondria helping the removal of the toxins outside the cells.[41,42] The supplementation of levocarnitine augments the antioxidant enzymes, develops the lipid profile and diminishes the oxidative stress.[43] Levocarnitine decreases the free radical-prompted lipid peroxidation and improves the mitochondrial antioxidant system.[44] This mechanism possibly underlies the protective role of levocarnitine against doxorubicin cardiotoxicity seen in this study.

Vitamin E is a fat-soluble vitamin that is naturally occurring as tocopherols (αT, βT, γT, δT).[45] α-tocopherol is the most active formula of the tocopherols.[45] Vitamin E has antioxidant activity that may scavenge oxidative stress and enhance the cellular antioxidative capability to diminish the oxidative damage.[46] Within cell membranes, vitamin E has antioxidant activity that prevents the proliferated oxidation of unsaturated fatty acids.[47] Contrary results from a previous trial that investigated single agent α-tocopherol, may be explained by possible inadequate delivery of α-tocopherol to subcellular mitochondrial membranes. Our investigation

was designed to assess whether enrichment of cardiac membranes with α-tocopherol is sufficient to protect against doxorubicin-induced mitochondrial cardiac toxicity as L-carnitine may provoke the antioxidant activity of α-tocopherol. Zou et al. found that levocarnitine augments the lymphatic absorption of α-tocopherol that may facilitate the delivery of α-tocopherol to subcellular mitochondrial membranes.[48] Furthermore, in a study by Augustyniak et al., levocarnitine doubled the serum α tocopherol concentration.[49] Vitamin E has also been found to compensate for the consumed α tocopherol that is consumed during lipid peroxidation,[50] thus preserving cardiac function.[51]

Some studies supported the safety and tolerability of vitamin E and[52–54] we found the safety profile of the combination of vitamin E and levocarnitine tolerable. None of the patients experienced severe or life-threatening adverse events with the addition of these agents. Two patients discontinued in the IG due to abdominal pain probably associated with vitamin E and levocarnitine. No deaths were reported in either group during the 4-cycle treatment period of the current study.

Doxorubicin has toxic effects on kidney function.[55,56] Serum creatinine is not the gold standard marker for renal function although it is widely used in clinical practice.[57] Serum creatinine may be affected by many factors such as weight, gym exercise, liver disease, fluid overload and pregnancy poor nutritional status.[58] Previous studies have reported that vitamin E had protective effects on the kidney as well as slowing progressive kidney diseases.[59–61] Levocarnitine also showed a protective effect on renal function in a preclinical study with doxorubicin-induced nephrotoxicity and certain clinical trials recommended it as a supportive medication in haemodialysis patients.[62–65] However, a study by Sun et al. concluded no relation between the serum creatinine level and levocarnitine.[66] In the current study, the serum creatinine levels were significantly decreased in the IG compared to the CG group (Table 6).

In a review by Adikwu and Nelson, they concluded that the hepatoprotective effect of vitamin E in humans needs more evaluation due to discrepancies in the reports.[67] However, Gokcimen et al. and Gross concluded that vitamin E has a protective effect on doxorubicin hepatotoxicity.[68,69] Levocarnitine has also shown a protective effect against hepatotoxicity induced by doxorubicin.[70–72] It is evident from this study that there was a significant increase in the serum Bil levels in the IG compared to the CG group (Table 6); however, no differences were found in the two groups for the liver enzymes (AST, ALT and ALK). A study by Sotirakopoulos et al. concluded that levocarnitine contributed to an increase in the haematocrit (Hct) and Hgb levels[73] and vitamin E enhanced the haemoglobin levels.[74,75] In this study, the haemoglobin levels significantly increased in the IG group compared to CG group in the mean of four cycles (Table 6). The combination of vitamin E and levocarnitine improved the haemoglobin levels and needs further investigation in a larger group.

Limitations

There are several limitations to this study. First, this was a small-sized single center study where only female patients were recruited as the female gender is a considered risk factor that increases the cardiotoxicity induced by doxorubicin.[76] Second, only acute cardiac toxicity was assessed and chronic cardiomyopathy may develop months to years after the termination of treatment in some patients. Third, using a small total cumulative dose (240 mg/m^2) of doxorubicin may have led to a limited elevation of cardiac enzymes and limited cardiotoxicity appearance which become more apparent at doses above 240 mg/m^2.

Strengths

Previous studies used antioxidants as prophylaxis against cardiac toxicity caused by doxorubicin, mostly *in vitro* with few known *in vivo* studies; this study addressed this gap.

These previous studies used only one cardiac protective agent while this study was a combination of vitamin E and levocarnitine. Using cardiac biomarkers BNP, CKMB and troponin I is highly sensitive and convenient to measure routinely. This is the only study that used cardiac events as well as cardiac enzymes as indicators for monitoring and evaluating cardiotoxicity. This is the only study of this nature conducted in an Arab population.

Conclusions

The combination of vitamin E and levocarnitine as prophylaxis can substantially enhance the supportive care of patients with cancer who receive highly cardiotoxic, doxorubicin-based, chemotherapy. The combination of vitamin E and levocarnitine was safe and tolerable in the patients treated with doxorubicin. We encourage the co-administration of vitamin E and levocarnitine with higher doxorubicin (240 mg/m^2) to be further investigated.

Author Contributions

IM, FO and MV, concepted, designed the study and provisioned the study materials and patients; HM and MA,collected and assembled of data; CC, performed the analysis and interpreted the results; FO and MV, supervisioned the study, IM,drafted the manuscript; FO and MV, reviewed and edited the manuscript. All authors; final approval of manuscript: all authors. All authors have read and agreed to the published version of the manuscript.

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ORCID iD

Iman Moustafa https://orcid.org/0000-0002-9522-7800

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