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Increased Plasma Nitrite and von Willebrand Factor Indicates Early Diagnosis of Vascular Diseases in Chemotherapy Treated Cancer Patients

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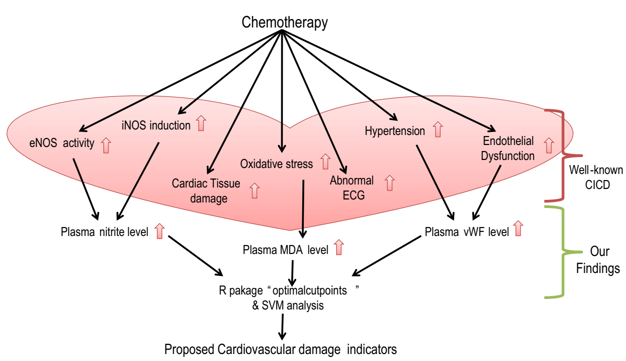
Abstract
Chemotherapy induced cardiotoxicity leads to development of hypertension, conduction abnormalities, and congestive heart failure. However, there is no simple test to detect and assess cardiovascular risk in a chemotherapy treated cancer patient. The aim of the present study on cancer patients treated with ($n = 66$) and without ($n = 66$) chemotherapy is to identify indicators from plasma for vascular injury. The levels of plasma nitrite, asymmetric dimethyl arginine (ADMA), von Willebrand factor (vWF), cardiac troponins, lipid peroxidation (MDA), and lactate dehydrogenase (LDH) were estimated. An R package, namely, Optimal Cutpoints, and a machine learning method—support vector machine (SVM) were applied for identifying the indicators for cardiovascular damage. We observed a significant increase in nitrite ($p < 0.001$) and vWF ($p < 0.001$) level in chemotherapy treated patients compared to untreated cancer patients and healthy controls. An increased MDA and LDH activity from plasma in chemotherapy treated cancer patients was found. The R package analysis and SVM model developed using three indicators, namely, nitrite, vWF, and MDA, can distinguish cancer patients before and after chemotherapy with an accuracy of 87.8% and AUC value of 0.915. Serum collected from chemotherapy treated patients attenuates angiogenesis in chick embryo angiogenesis (CEA) assay and inhibits migration of human endothelial cells. Our work suggests that measurement of nitrite along with traditional endothelial marker vWF could be used as a diagnostic strategy for identifying susceptible patients to develop cardiovascular dysfunctions. The results of the present study offer clues for early diagnosis of subclinical vascular toxicity with minimally invasive procedure.

Graphical Abstract
Schematic representation of chemotherapy induced elevated plasma nitrite level in cancer patients.



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Keywords Chemotherapy · Nitrite · von Willebrand factor · Cardiotoxicity · Vascular disease · Support vector machine (SVM)

Introduction

Chemotherapy induced cardiovascular dysfunction (CICD) includes coronary artery disease, cardiomyopathy, cardiac arrhythmias, and congestive heart failure. The risk of death due to CICD is higher than the actual risk of tumor recurrence [1] with a sevenfold higher mortality rate, 15-fold increase in the rate of heart failure, tenfold higher rates of cardiovascular disease, and ninefold higher rates of stroke than for the general population [2]. Higher incidences of hypertension, dyslipidemia, acute coronary syndromes, and stroke have been reported in long-term cancer survivors [3–6]. In addition to these conditions, endothelial dysfunction and enhanced atherosclerotic processes have also been reported [7].

Lack of complete understanding of the multi-factorial cellular and molecular drivers underlying CICD has led to the difficulty in the identification of the optimal therapeutic approaches for protection against CICD. It is, therefore, necessary to identify the indicator for CICD that makes certain patients more susceptible than others in order to improve the clinical outcome of chemotherapy treatment regimens. Although there exist diagnostic techniques such as serial monitoring of left ventricular ejection fraction using non-invasive cardiac imaging, multiple gated acquisition scans (MUGA) [8], echocardiography [9], and flow-mediated dilation (FMD) [10], their practical use in the clinical cancer setting is almost nil. Alternatively, indicators of cardiovascular dysfunctions could well be used as tools for early diagnosis of cardiovascular damages. von Willebrand factor (vWF) has been proved as a suitable marker of endothelial dysfunction in vascular diseases [11, 12]. Nitric oxide (NO) plays diverse roles in the cardiovascular function and diseases [13–15]. Asymmetric dimethylarginine (ADMA) is a potent endogenous competitive inhibitor of nitric oxide synthase (NOS) which has been shown to predict the occurrence of acute coronary events [16]. Lipid peroxidation is caused by the action of reactive oxygen species (ROS) on cellular lipids and is an indicator of cellular damage in the cardiovascular system [17]. Measurement of LDH activity in serum plays an important role in the diagnosis of acute myocardial infarction [18]. Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are biomarkers used for the determination of the myocardial dysfunction [18]. Indicators of cardiac damage could well be used as tools for early diagnosis of cardiotoxicity before the occurrence of any permanent cardiac damage.

An earlier study by our group demonstrated the dampening of vascular functions by chemotherapy drugs through interference with NO signaling using cell based models [19]. Circulation of endothelial markers could be used for the determination of the risk of developing vascular dysfunctions under chemotherapy as well as to screen for its early detection.

The present study aim at identification of predictive indicators of cardiovascular dysfunctions such as plasma nitrite, ADMA, vWF, cardiac troponins, lipid peroxidation, and lactate dehydrogenase (LDH) levels in cancer patients before and after chemotherapy, and investigate the correlation between these and clinical and laboratory findings of cardiologic functions. In addition, the strength of endothelial activity in patient's serum samples was also tested using in vitro laboratory assays.

Patients and Methods

Study Population

The patients attending the outpatient clinic of the Department of Surgical Oncology, Rajiv Gandhi Government General Hospital, Chennai, India since January 2016 were included. Inclusion criteria were histologically diagnosed cancer patients who were yet to start any type of treatment and who were under neo-adjuvant chemo and radiotherapy. Patients with residual or recurrent cancers post multimodality management and patients with previous history of cardiovascular problems were excluded. The patients (age range 25–70) were categorized into two groups. The first included cancer patients ($n = 66$) who had clinical and histopathological evidence of cancer (carcinoma type) and were receiving neither chemotherapy nor radiotherapy. The second group ($n = 66$) consisted of neo-adjuvant chemotherapy treated cancer patients who mostly received platinum drugs followed by paclitaxel. Tumors were classified according to the UICC criteria [20]. Sixty-six healthy volunteers served as members of the control group. Both the patients and control group had a similar socioeconomic background.

Sample Collection

Blood samples (about 3 ml) were collected in tubes containing sodium EDTA by venipuncture in cancer patients, chemotherapy treated patients, and healthy volunteers. Plasma was separated and stored at − 80 °C till use.

Biochemical Measurements

Determination of Nitric Oxide Metabolites

Since NO is highly reactive and volatile in vivo (half-life of 3–5 s), nitrite (NO_2^-) and nitrate (NO_3^-) in plasma are used as a measure of the production of the NO radical in the blood stream [21]. In the present study, the plasma levels of N_2O_3, i.e., the sum of NO_2^- and NO, were measured using 2,3-diaminonaphthalene (Cat no. D2757 Sigma) for assessment of NO bioavailability [22]. The level of nitrite was expressed in µM.

Measurement of ADMA

Plasma concentrations of asymmetric dimethylarginine (ADMA) were assayed using Human ADMA ELISA kit (Cat no. E1887Hu, Bioassay Technology Laboratory, Shanghai, China) as per the manufacturer's protocol. The level of ADMA was expressed in nM/ml.

TBARS Assay (Lipid Peroxidation)

Plasma malondialdehyde (MDA) concentration was measured as an index for lipid peroxidation as described in Moselhy et al. [23]. Diluted plasma (1:2) was heated in a boiling water bath with the 2-thiobarbituric acid (TBA) reagent (20% TCA, 0.5% w/v TBA, and 2.5N HCL) for 20 min. In order to reduce the interference of iron in peroxidation reaction of unsaturated fatty acid, 0.05 mM EDTA was added to the reaction mixture as iron chelator. After cooling, the reaction mixture was centrifuged at 10,000 RCF for 5 min. The absorbance of the supernatant was measured at 540 nm using a microplate reader (Bio-Rad Model 680). The absolute concentration of MDA in the sample was calculated using the extinction coefficient of MDA (1.56×10^5 M/cm).

LDH Activity Assay

The LDH activity in plasma was determined following the method of Li et al., [24]. LDH transforms NADH (fluorescently active) to NAD (fluorescently inactive) along with the production of lactate from pyruvate. In the enzyme coupled assay, we measured the concentration of NADH fluorometrically (using Varian Cary Eclipse Fluorimeter) at $E_x/E_m = 345/465$ nm, which is proportional to LDH activity.

Determination of Cardiac Troponins

Cardiac troponin T and I were estimated following the method of chemiluminescence immunoassay using cardiac troponin I (Abbott Diagnostics) and cardiac troponin T (Roche Diagnostics) commercial kits. The results were expressed in ng/ml.

Measurement of von Willebrand Factor (vWF)

von Willebrand Factor was assayed in plasma samples by homemade sandwich ELISA [25]. Briefly stated, microplate wells were coated with polyclonal Rabbit Anti-Human von Willebrand Factor antibody (A 0082; Dako) and diluted plasma (1:40) was added to the plate, with appropriate controls. Next, rabbit anti-vWF HRP-conjugated antibody (P 0226; Dako) was added and incubated for 60 min. The absorbance was read at 450 nm.

In vitro Laboratory Assays

Since angiogenesis represents a part of endothelial functions, a chick embryo angiogenesis assay, an indirect measure of the function of endothelium, was adopted to check the angiogenic potential of serum samples collected from cancer patients under chemotherapy. Wound healing assay was also performed to assess the migration property of endothelial cells under the treatment of patients' serum sample.

Chick Embryo Angiogenesis (CEA) Assay

Fertilized eggs were purchased from the Poultry Research Centre, Potheri, Chennai to perform in vivo angiogenesis assay [26]. Eggs were incubated at 37 °C (60% relative humidity) for 4 days, and, on the fifth day, sterile discs (made of Whatman filter paper) soaked in the patients' serum were placed on the chorioallantoic membrane (CAM) for 4 h. Images were captured using a Stereo Microscope (Nade, NSZ-810) at 0, 2, and 4 h of incubation. Then the images were processed by Adobe Photoshop 8.0 and analyzed using AngioQuant v1.33 software [27].

Wound Healing Assay

The wound healing method was used for the assessment of the cell migration under the treatment of patients' serum by following the method of Majumdar et al. [28]. Briefly, EA.hy926 cells were cultured and grown till confluence. A scratch injury with a sterile micropipette tip was made on the cell monolayer. Then the cells were incubated at 37 °C with the patients' serum (8%), without FBS for 4 h. Cells incubated in the plain medium (DMEM) and in the growth medium (DMEM containing 8% FBS) were used as the control group. Bright field images (40×) of the wounds were taken using an Olympus IX71 inverted microscope adopted with a DP71 camera. The area of the wound was quantified and analyzed by ImageJ [29].

Statistical Analysis

The detailed statistical analysis performed is described hereunder:

- A non-parametric Kruskal–Wallis rank sum test was performed for the assessment of the effect of different categorical variables on healthy, cancer, and chemotherapy data in R language and environment.
- Further, an R Package, namely, Optimal Cutpoints [30], was used for Selecting Optimal Cutpoints in Diagnostic Tests and for the identification of the indicators among the different groups. The ROC (receiver operating characteristic) curve indicates the diagnostic accuracy of a continuous test to distinguish healthy and diseased populations. The area under the ROC curve (AUC) is widely used as an index to measure the performance of a classifier [31].
- We have developed a unified support vector model based on the machine learning method, Support Vector Machine (SVM), which classifies healthy, cancer, and chemotherapy data based on prior knowledge about ROC curve of individual indicators.

Results

The basic clinical parameters relating to patients from whom plasma nitrite was measured are shown in Table 1. An increased number of individuals were seen with hypertension among chemotherapy treated cancer patients compared to cancer patients without any type of treatment. There was an increased percentage of chemotherapy treated patients with abnormal ECG compared to cancer patients without any type of treatment. The predominant type of carcinoma was oral cancer in the present study group.

A significant increase in nitrite level in chemotherapy treated patients compared to untreated cancer patients and healthy controls (Fig. 1A) was detected. Results obtained from 'Kruskal–Wallis test' showed the presence of a statistically significant difference in nitrite values between three groups ($\chi^2(2) = 58.667$, $p < .001$). A post hoc Dunn test with Bonferroni adjustment of p values supported the significant differences between healthy volunteers versus cancer patients ($p < .001$), cancer patients versus chemotherapy treated cancer patients ($p < .05$), and healthy volunteers versus chemotherapy treated cancer patients ($p < .001$). Among chemotherapy treated patients, concurrent chemoradiotherapy (CCRT) treatment showed a statistically significant increase in nitrite level compared to stand alone chemotherapy (CT) treatment ($p < .05$) (Fig. 1B).

There was a statistically significant decrease in ADMA level among chemotherapy treated cancer patients compared to untreated cancer patients ($p < .05$). However, there was no statistically significant difference between healthy and cancer groups ($p > .05$) (Fig. 2A). The level of MDA in plasma of chemotherapy treated patients was significantly higher ($p < .001$) than plasma MDA content of cancer patients without chemotherapy and the plasma MDA content of healthy volunteer. There was no statistically significant difference in level of MDA in plasma between the healthy volunteer group and cancer patient group without chemotherapy (Fig. 2B). A significantly increased LDH activity in plasma of cancer patients treated with chemotherapy compared to the other groups, healthy volunteer and cancer patients without chemotherapy, was observed, indicating that there is significant cell damage in chemotherapy treated patients (Fig. 2C).

Measurement of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) revealed the presence of an elevated level of cTnI and cTnT in chemotherapy treated cancer patients compared to untreated cancer patients. However, the difference was not statistically significant ($p > .05$ for cTnI and cTnT; Fig. 3A). A significant increase in vWF level in chemotherapy treated patients compared to untreated cancer patients and healthy controls (Fig. 3B) was observed. According to Kruskal–Wallis test, there is a statistically significant difference in vWF values between the three groups ($\chi^2(2) = 82.916$, $p < .001$). p values supported the significant differences between healthy volunteers versus cancer patients ($p < .001$), cancer patients versus chemotherapy treated cancer patients ($p < .05$), and healthy volunteers versus chemotherapy treated cancer patients ($p < .001$). Among chemotherapy treated patients, concurrent chemoradiotherapy (CCRT) treatment showed a statistically significant decrease in vWF level compared to chemotherapy (CT) treatment ($p < .05$) (Fig. 3C).

The results of the chick embryo angiogenesis (CEA) assay revealed that serum collected from chemotherapy treated patients decreased in vivo angiogenesis compared to the serum collected from healthy volunteers and cancer patients. Three angiogenesis parameters such as length, size, and junction of blood vessels upon treatment with serum of three groups are shown in Fig. 4A–C, respectively. We found that there is 12.9% decrease in length ($p < .05$), 9.2% decrease in size ($p < .05$), and 23.9% decrease in junctions ($p < .05$) of vasculature at 4 h using the serum from chemo-treated patients compared to that of the cancer patients. Serum collected from chemotherapy treated patients decreased migration of endothelial cells compared to healthy volunteers and cancer patients as demonstrated by wound healing assay (Fig. 4D). Analyses of results by one-way ANOVA and Turkey post hoc tests indicate that there is 64.5% decrease in the wound healing index for chemo-treated cancer patients compared to cancer patients ($p < .05$). Also, there is a decrease of 66.2% wound healing index for

Table 1 The baseline characteristics of patients

S no.	Parameters	Cancer patient (%)	Cancer patient after chemotherapy (%)
1	Age (mean ± SEM)	51.59 ± 1.29	50.65 ± 1.07
2	Gender		
	M	44	42
	F	56	58
3	Menopause attained	45	46
4	Family history of cancer		
	Yes	5	5
	No	95	95
5	BP		
	Normotensive	83	68
	Hypertensive	17	32
6	ECG		
	Normal	86	56
	Abnormal	14	44
7	Pulse rate		
	Normal	83	74
	High	17	26
8	Type of cancer		
	Oral Ca	63	38
	Breast Ca	8	15
	Ovary Ca	8	18
	Cervical Ca	13	15
	Others	8	14
9	Grade of tumor		
	I	74	68
	II	18	24
	III	8	8
10	Type of treatment		
	CT		45
	CCRT		55
11	Type of drug		
	Platinum drug		57
	Paclitaxel		35
	Others		8
12	Chemo cycles given		
	≤ 2 cycles		15
	≤ 4 cycles		40
	> 4 cycles		45
13	Chemotherapy interval (between treatment and sample collection day)		
	≤ 4 weeks of treatment		39
	≤ 8 weeks of treatment		26
	> 8 weeks of treatment		35

chemo-treated cancer patients compared to healthy volunteers ($p < .05$).

The nitrite level displays good 'diagnostic accuracy' or ability to distinguish between cancer patients before and after chemotherapy (AUC = 0.661) and is more pronounced in men (AUC = 0.628 in women versus AUC = 0.719 in men). The cutpoint obtained using the criterion based on the Youden index for cancer patients was 12.42 µM. Thus, nitrite serving as a "good predictive chemotherapy indicator" (Fig. 5A–C) was demonstrated. Similarly, the vWF level also displayed good 'diagnostic accuracy' to distinguish between the cancer patients before and after chemotherapy

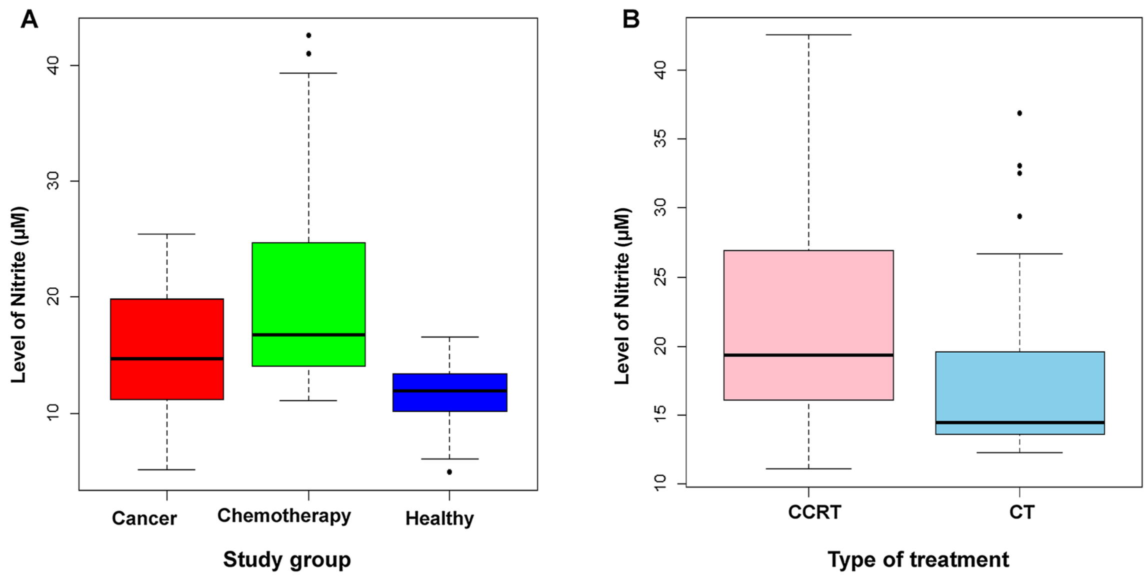

Fig. 1 The study group consists of three groups: healthy volunteers (represented as healthy), cancer patients (represented as cancer), and cancer patients after chemotherapy (represented as chemotherapy). Box-plot figures for nitrite level shown in **A** and **B** ($p < .001$ and $p < .05$, respectively)

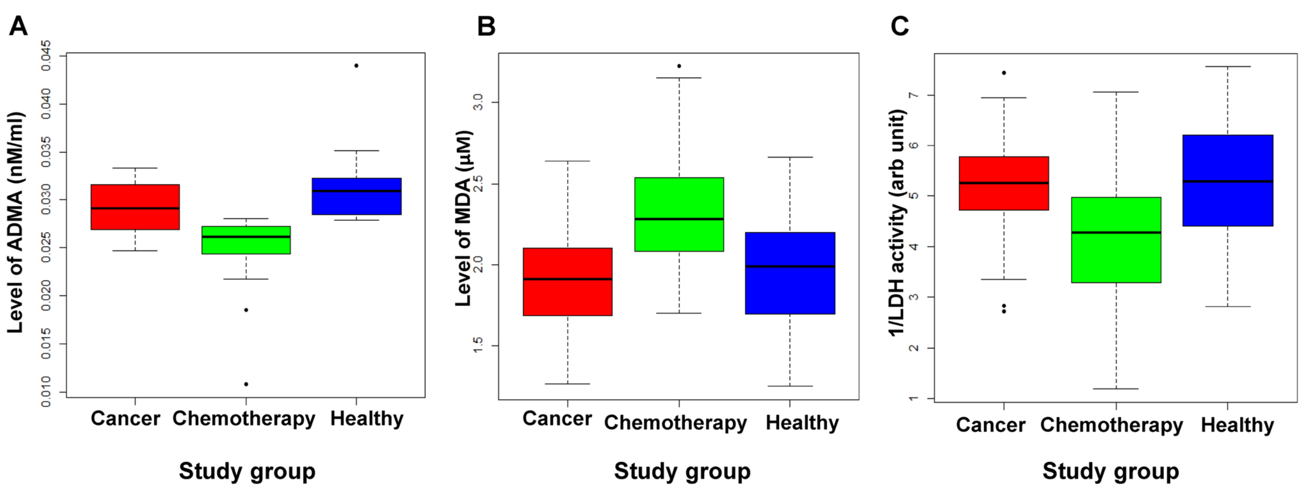

Fig. 2 Box-plot figures have shown in **A** for ADMA level **B** for plasma MDA level; ($p < .001$ for chemotherapy vs. cancer) and **C** 1/LDH (tissue damage) activity in plasma ($p < .001$ for chemotherapy vs. cancer)

(AUC = 0.686; Cutoff = 0.20 arbitrary unit) particularly in women (AUC = 0.725 in women vs. AUC = 0.621 in men) than in men. von Willebrand factor serving as a "good predictive chemotherapy indicator" (Fig. 5D–F) was observed.

We have developed a unified model based on the machine learning method (SVM) which can classify healthy, cancer, and chemotherapy data based on prior knowledge about ROC curve of individual indicators. A SVM model has been built on the basis of three indicators nitrite, MDA, and vWF in the training set to classify cancer patients before and after chemotherapy. A good model was achieved with training set ($N = 99$) with low training error (0.20) and cross validation error (0.23). The performance of the classification model on testing set ($N = 33$) was excellent based on the AUC and confusion matrix table (Table 2) (AUC = 0.915, precision = 82.3% accuracy = 87.8%, sensitivity = 93.3%, specificity = 83.3%) (Fig. 6). We have achieved an excellent classification of cancer patients before and after chemotherapy based on three indicators nitrite, MDA, and vWF.

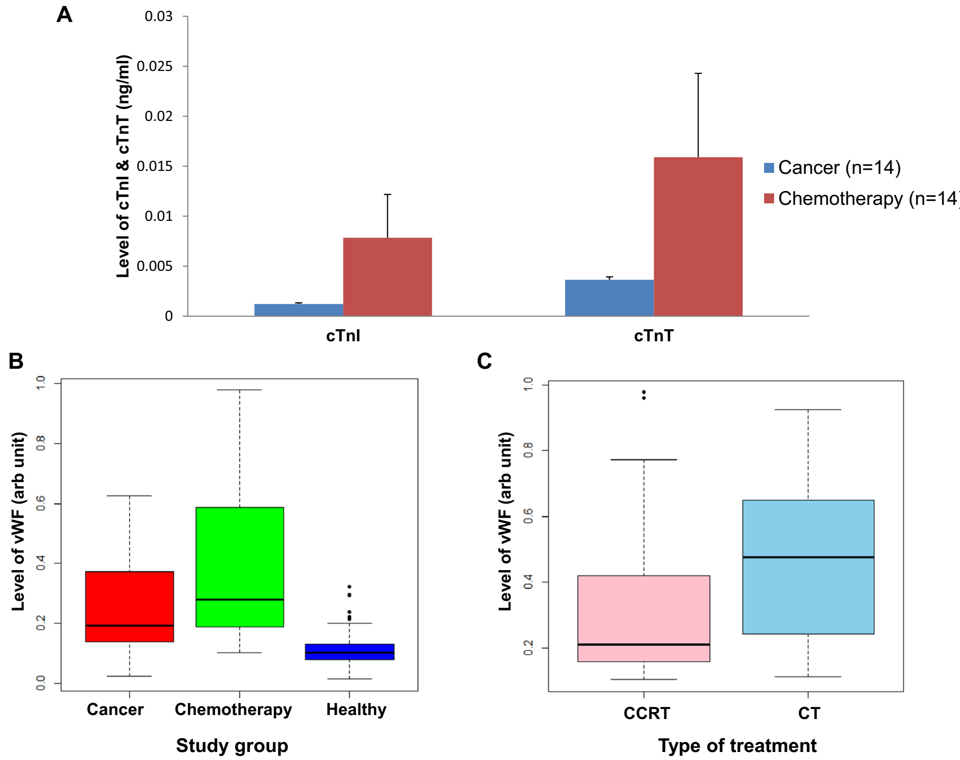

Fig. 3 Bar graph for cTnT and cTnI shown in **A** ($p = .14$ and $p = .156$, respectively). Box-plot figures for vWF shown in **B** and **C** ($p < .001$ and $p < .05$, respectively). (Color figure online)

Discussion

Tracking endothelial dysfunction in cancer patients in real time is a challenging job since it needs specialized equipment such as Flow-Mediated Dilation (FMD). We conceptualized plasma level of NO as a reflection of the endothelial function in cancer patients. However, the major limitation of tracking NO in real time in patients is its gaseous nature and a very short half-life. On the contrary, the metabolites of NO such as nitrite and nitrate can be easily measured due to its extremely stable nature and availability of wide range of techniques to measure them. Our previous work demonstrated that addition of antineoplastic drugs to healthy volunteers' blood sample marginally increased NO levels in plasma and RBC, in contrast to the decreased NO levels observed from treated endothelial cells [19].

The present study was conducted for the investigation of the possible changes in blood plasma nitrite level along with vWF, lipid peroxidation, and LDH as predictive indicators of CICD. A significant increase in blood plasma nitrite level among chemotherapy treated patients compared to untreated patients was observed. The difference was more pronounced among concurrent chemo-radiotherapy-treated patients (Fig. 1). Similarly, long-term cancer survivors who were treated with cisplatin showed an impaired NO-dependent vasodilation in the brachial artery [32]. A significant increase in the level of nitrite was observed in the group of lung cancer patients, post chemotherapy treatment [33]. High levels of NO production have been associated with several forms of impaired cardiac functions, including dilated cardiomyopathy and congestive heart failure [14]. Guerra et al. [34] noted the positive correlation in the increased plasma nitrite with myocardial damage in doxorubicin-treated rats. Interestingly, the results of the present study showed cancer as a disease itself inducing nitrite generation even before the chemotherapy cycles when compared to the baseline (Fig. 1). Analogous results were found in previous studies [35, 36].

Lacunae in knowledge pertaining to the source of NO and the mechanism that causes an increased nitrite in CICD exist. We measured ADMA, a natural inhibitor of NOS enzyme for partially addressing this issue, and observed a significant decrease among chemotherapy treated cancer patients compared to untreated cancer patients. Such observation suggests a reduced inhibition of NOS, which might be the reason for increased NO production under chemotherapy treatment. However, the sample size for ADMA measurement was small ($n = 14$) to enable derivation of any conclusion from the present study. Other possible sources of increased nitrite in plasma were the release of heme-bound nitrite from RBC [37], tissue damage [38], iNOS induction [39], and release of NO from nitrosylated proteins [40] upon chemotherapy (Graphical Abstract). Lactate dehydrogenase (LDH) is an intracellular energy producer which leaks into the plasma upon cell damage. Burtis et al. [41] showed the determination

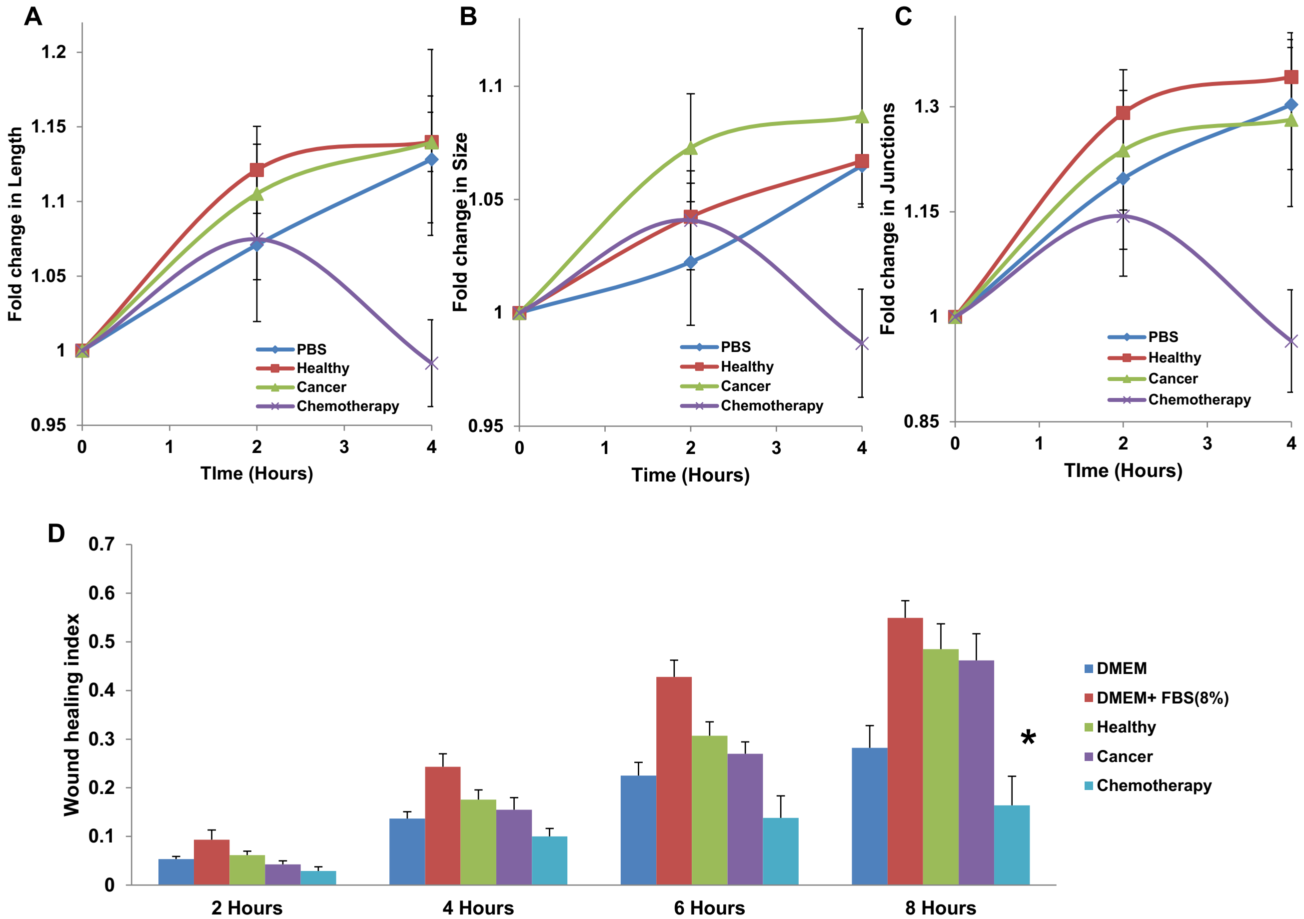

Fig. 4 Line graphs showing angiogenic activity in terms of **A** length of the blood vessel, **B** size of the blood vessel, and **C** and junctions of the blood vessel. **D** Bar graph showing scratch wound assay. *Indicates statistical significance. (Color figure online)

of LDH activity in serum having important clinical significance in the diagnosis of acute myocardial infarction.

The present study also showed a significant elevation of MDA and LDH (Fig. 2). A complex interplay among NO-ROS-NO^2- is implicated under chemotherapy treatment in cancer patients and future studies should explore the dynamics of such molecules. Specifically, a significant increase in both nitrite and vWF levels (Figs. 1, 3) in the present data set was observed, which might predispose the patients to develop chemo-induced thrombosis. This is supported by the fact that vWF is known to increase NO synthesis and bioavailability via eNOS in blood platelets [42]. An increase in cTnI and cTnT (established cardiac biomarkers) was noticed among chemotherapy treated patients in the present study (Fig. 3A). This is in agreement with findings by Cardinale et al. [43] who observed the elevation of cTnI among 204 patients treated with chemotherapy, and cTnI predicted the future left ventricular dysfunction. Similar observations were reported by other studies irrespective of cancer type [44].

The present analysis using Optimal Cut points reveals the display of good ability by "plasma nitrite" to distinguish between cancer patients before and after chemotherapy (Fig. 5). Guler et al. [45] has shown significantly higher nitrite values in children treated with doxorubicin along with abnormal left ventricular ejection fraction and proposed that NO could well be used as a biomarker for doxorubicin toxicity in children. Our results reconfirm the observation by previous studies. Further, the SVM model built on three indicators, nitrite, MDA, and vWF, classified cancer patients before and after chemotherapy with accuracy of 87.8% and AUC value of 0.915 (Fig. 6). We suggest the use of "plasma nitrite" as an "early diagnostic indicator" even in the absence of clinically established cardiac dysfunction among chemotherapy treated cancer patients.

We have extended these in vitro findings to an in vivo setting by incubating chemotherapy treated patients' serum in CEA model. Results show that there is perturbation of endothelial functions (measured in terms of angiogenesis and cell migration; Fig. 4), which suggests clinically

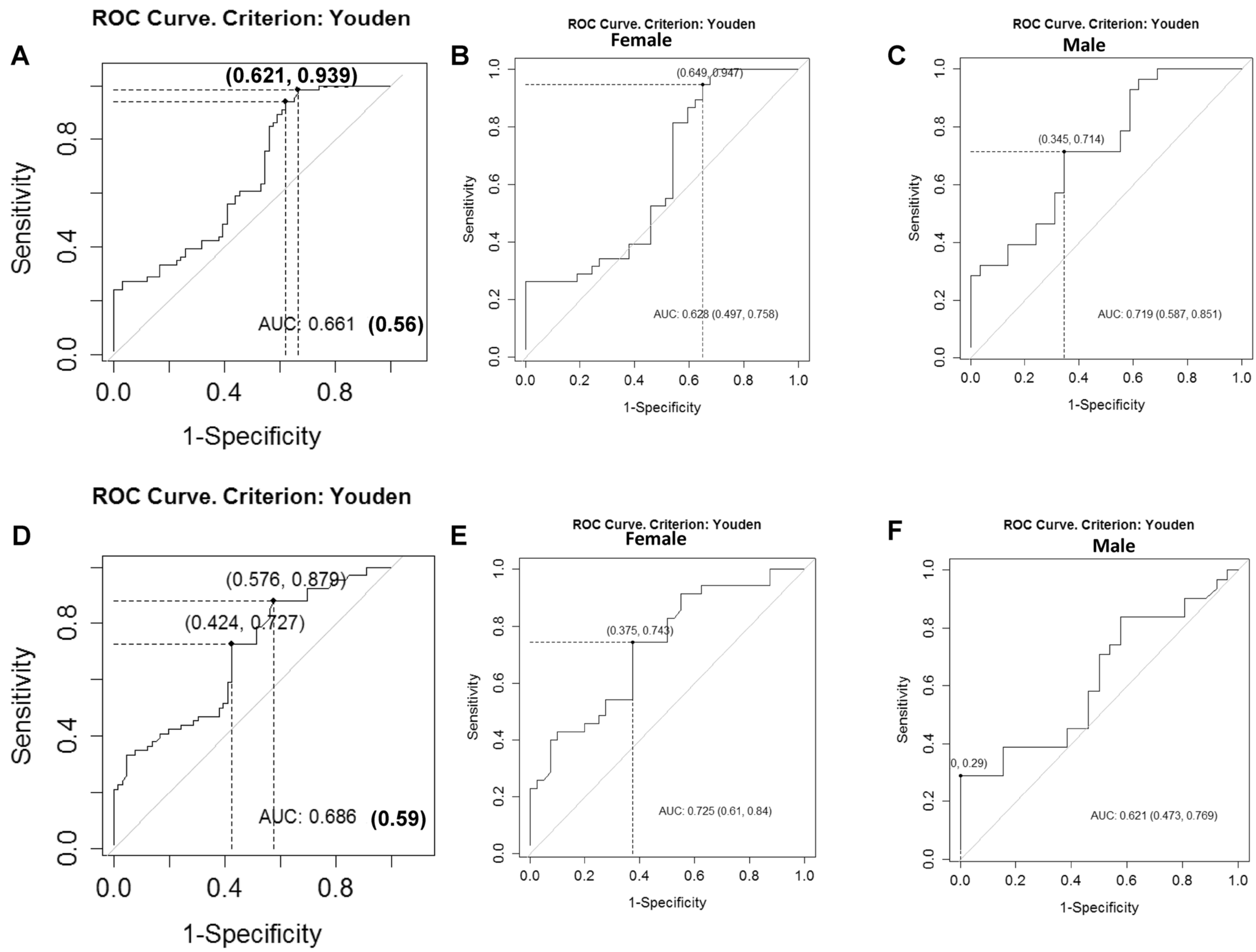

Fig. 5 Analysis of nitrite & vWF level as a biomarker for chemotherapy. An R package namely Optimal Cutpoints was used for selecting Optimal Cutpoints in Diagnostic Tests. The nitrite (**A–C**) & vWF (**D–F**) level displays good 'diagnostic accuracy' to distinguish between cancer patients before and after chemotherapy independent of gender

Table 2 Confusion matrix of SVM classification of cancer patients before and after chemotherapy with definitions and values of metrics

$n=33$	Predicted cancer	Predicted chemotherapy	Total
Actual cancer	TN = 15	FP = 3	18
Actual chemotherapy	FN = 1	TP = 14	15
Total	16	17	

Sensitivity/recall/TPR = TP/actual chemotherapy = 14/15 = 0.933

Accuracy = TP + TN/total = 29/33 = 0.878

Specificity = TN/actual cancer = 15/18 = 0.833

Precision = TP/predicted chemotherapy = 14/17 = 0.823

tolerable doses of chemotherapy indulged in anti-endothelial activities. Although there is no such similar study, Merchan et al. [46] used a similar model and reported that paclitaxel has antiangiogenic property due to its preferential accumulation in endothelial cells.

Conclusion

Early diagnosis of cardiovascular dysfunctions caused by chemotherapy assumes great significance in the identification of patients at risk. In this study, we have clearly showed an increase in the plasma nitrite content along with vWF, MDA, and troponins in cancer patients (Figs. 1, 2, 3) by chemotherapy followed by endothelial dysfunctions (Fig. 4). Hence, nitrite as an indicator along with traditional endothelial marker vWF is proposed as a diagnostic strategy for identifying susceptible patients under chemotherapy. Results obtained from the present study may offer means for a real-time modification of chemotherapy to limit cardiotoxicity. Further, the measurement of endothelial activity in serum

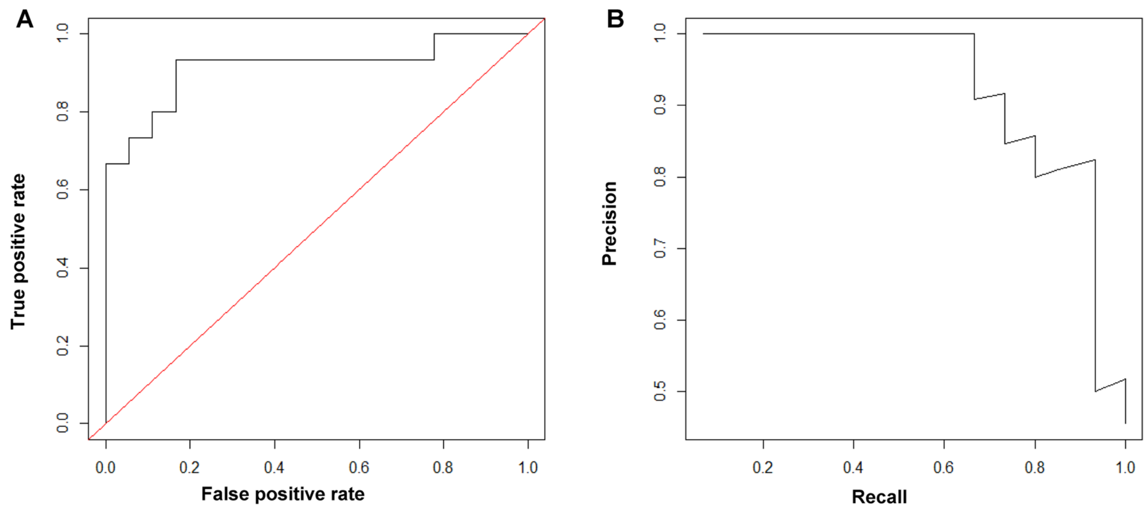

Fig. 6 The SVM classification of cancer patients before and after chemotherapy was based on nitrite, MDA, and vWF. **A** The ROC curve of classification models showing comparison of TPR with TNR. **B** Precision–recall curve of the classification model

serves as a potentially informative indicator for monitoring the chemotherapy associated vascular dysfunctions. However, a study of a large number of patients will be necessary to validate serum as a useful CICD monitoring tool.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical Approval All procedures carried out in the present study are in accordance with the 1964 Helsinki declaration. The study was also approved by the Institutional Ethics Committee, Rajiv Gandhi Government General Hospital (Ref no. 1482), and Institutional Biosafety & Ethical Committee of AU-KBC Research Centre, Chennai, India.

Informed Consent Patients were recruited after obtaining individual written informed consent.

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