International Journal of Research in Medical Sciences
Kadam CY et al. Int J Res Med Sci. 2020 Jan;8(1):189-193
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20195905

Original Research Article

Effect of chemotherapy on serum nitric oxide levels in advanced stage breast cancer patients

Charushila Y. Kadam[1], Subodhini A. Abhang[2]*

[1]Department of Biochemistry, RKDF Medical College, Hospital and Research Centre, Bhopal, Madhya Pradesh, India
[2]Department of Biochemistry, Symbiosis International (Deemed) University and Symbiosis Medical College for Woman, Pune, Maharashtra, India

Received: 22 October 2019
Revised: 28 November 2019
Accepted: 02 December 2019

***Correspondence:**
Dr. Subodhini A. Abhang,
E-mail: subodhini.abhang@gmail.com

ABSTRACT

Background: The role of nitric oxide is still unclear in advanced breast cancer patients undergoing adjuvant chemotherapy. This study was undertaken to investigate the effect of chemotherapy on serum nitric oxide levels in advanced stage breast cancer patients.
Methods: In this observational study, clinically and histopathologically proven sixty female patients with advanced stage breast cancer were included. According to Tumor-Node-Metastasis (TNM) classification, patients were further grouped as stage III and stage IV. Thirty healthy and age-matched female controls were selected for comparison. Blood was collected from healthy controls and from breast cancer patients after surgery prior to chemotherapy and after three weeks of administration of first adjuvant chemotherapy cycle. Serum nitric oxide levels were measured by spectrophotometric method.
Results: Significantly higher concentrations of serum nitric oxide were observed in breast cancer patients before chemotherapy in stage III ($p<0.0001$) and stage IV ($p<0.0001$) of the disease as compare to concentrations in healthy controls. The serum levels of nitric oxide were significantly decreased in stage III as well as stage IV of breast cancer patients after three weeks of receiving first adjuvant chemotherapy cycle as compare to levels before chemotherapy ($p<0.0001$), however serum nitric oxide levels were higher in stage III ($p=0.0036$) and stage IV ($p<0.0001$) of the disease as compare to healthy controls.
Conclusions: Chemotherapy drug administration causes decrease in serum nitric oxide levels in advanced stages of breast cancer patients. Monitoring serum nitric oxide levels could be used to predict patients' response to chemotherapy treatment in breast cancer.

Keywords: Breast cancer, Chemotherapy, Nitric oxide, Oxidative stress

INTRODUCTION

Adjuvant chemotherapy is one of the mainstays of medical intervention for breast cancer after surgical removal of tumor. Adjuvant chemotherapy drugs for breast cancer treatment mostly include anthracyclines, taxanes and alkylating agents.[1] Several experimental studies have shown that anticancer drugs used in the treatment of breast carcinoma kill tumor cells by inducing apoptosis in them and this is done by activating intrinsic or extrinsic pathways of apoptosis.[2] The intrinsic apoptosis signals that are produced following cellular stress due to chemotherapy might be a consequence of oxidative stress by free radicals.[3] Nitric oxide (•NO) is an inorganic free radical gas synthesized from L-arginine by catalytic reaction of NO Synthases (NOSs).[4-6] It plays a variety of regulatory functions in vivo. At low concentrations, •NO acts as a signal transducer in

physiological processes such as vasodilatation, neurotransmission, platelet aggregation, etc. or at high concentrations it is cytotoxic for tumors and pathogens.[5,6] Moreover, despite of these beneficial effects, accumulating evidence suggests that NOS activity increases in some invasive tumors and chronically elevated levels of •NO are genotoxic that may also play role in pathogenesis of cancer.[5] Genotoxicity of •NO is attributed to its reaction with oxygen and superoxide. •NO influences initiation of cancer by nitrosative deamination, DNA strand breakage or DNA modification by peroxynitrite.[5,6] Some studies suggest that, formation of peroxynitrite induces apoptotic DNA fragmentation and p-53 dependent apoptosis.[6] •NO may also suppress antitumor defense and enhance tumor growth, invasion and metastasis by promoting angiogenesis and blood flow in tumor neovasculature.[6] •NO may promote production of prostaglandins by activating Cyclooxygenase-2 (COX-2). Prostaglandins, induces angiogenesis and suppresses apoptosis through enhancing Bcl-2 protein synthesis.[6,7] Recently, it has been shown that •NO acts as antiapoptotic modulator that suppresses key step in apoptotic signaling pathway by preventing Bcl-2 cleavage and cytochrome c release from mitochondria.[7] The role of •NO in cancer biology is poorly understood. •NO has been demonstrated to be both pro- and anti-apoptotic depending on concentration and cell types.[6,7] Recently, it has been reported that increased iNOS is associated with poor outcome and decreased survival in ER- negative breast cancer.[8] In another study, it has been shown that chemotherapy influences inducible nitric oxide synthase activity in breast cancer cell line.[9] Moreover, it has been reported that aberrant high levels of iNOS/NO are associated with less effectiveness of platinum based neoadjuvant chemotherapy in triple negative breast cancer.[10] However, to date, serum nitric oxide levels in advanced stage breast cancer patients undergoing adjuvant chemotherapy is still unclear. The present study was thus undertaken to evaluate the serum levels of nitric oxide during adjuvant chemotherapy in advanced stage breast cancer patients and to assess its clinical significance.

METHODS

In this observational study, sixty histopathologically proven female breast cancer patients diagnosed with invasive ductal/lobular carcinoma were included. According to Tumor-Node-Metastasis (TNM) classification, patients were further grouped as stage III and stage IV. The patients were of 30-74 years age. Thirty healthy and age matched female volunteers were selected as controls for comparison. The clinicopathological characteristics of the patients are given in Table 1. This study was approved by Institutional Ethical Committee (Ref. No. BJMC/IEC/Pharmac/D1210137-39). The study was carried out from November 2010 to July 2014. The After obtaining prior written consent, blood samples were collected from healthy volunteers as well as post-operative breast cancer patients before chemotherapy and after three weeks of administration of first adjuvant cycle of FEC[5-fluorouracil, epirubicin, cyclophosphamide]/ AC [Adriamycin (doxorubicin), cyclophosphamide] / PC [paclitaxel]. The serum obtained after centrifugation was stored in aliquots at -80ºC until analysis.

Inclusion criteria

- Histopathologically proven female breast cancer patients receiving chemotherapy.

Exclusion criteria

- Care was taken to exclude patients with allergic and infectious diseases, hypertension, diabetes mellitus, other systemic ailments and male breast cancer patients to avoid false positive results.

Table 1: Clinicopathological characteristics of breast cancer patients.

	No. of patients	60
Age	Range	30-74
	Mean	53.43±13.46
Type	Invasive ductal carcinoma	48
	Invasive lobular carcinoma	12
Status	Premenopausal	19
	Postmenopausal	41
TNM stage	Stage III	30
	Stage IV	30
Receptor status	ER+	51
	PR+	47
	Her2+	26
Chemotherapy drugs	FEC	34
	AC	12
	PC	14

Measurement of nitric oxide (•NO)

The measurement of serum nitric oxide (•NO) was done in terms of its metabolites, nitrate and nitrite, by kinetic cadmium reduction spectrophotometric method.[11] Briefly, 0.5 ml of serum was deproteinized with 2 ml of 75 mM/L ZnSO4 and 2.5 ml of 55mM/L of NaOH. To 1ml of deproteinized supernatant obtained after centrifugation, 1ml of glycine-NaOH buffer and 2.5 gm of activated cadmium granules were added. After 90 minutes of incubation at room temperature, 2 ml of distilled water was added to this mixture. Then, 1 ml of sulfanilamide and 1 ml of NED was added to 2 ml of above mixture. The contents were mixed well, covered with silver foil and incubated for 20 minutes. The readings were taken at 540 nm. The detection limits in serum were 2-250 µmol/L. The CVs were 9% and 4.7% for nitrate

concentrations of 31.4 µmol/L and 80.2 µmol/L respectively.[11]

The standard curve was prepared for concentrations 10 µmol/L to 100 µmol/L. The •NO concentration in each sample was determined by extrapolating OD values against standard concentrations using the standard curve. •NO values were expressed as µmol/L.

Statistical analysis

The data for biochemical analysis was expressed as Mean±SD. The statistical significance of the results among different groups was analyzed by using unpaired and paired student's t test. Values of p less than 0.05 were considered statistically significant. Statistical analysis was done using MedCalc for windows version 12.7.0.0 and SPSS version 17.

RESULTS

Table 2 shows the mean serum levels of nitric oxide in healthy controls and breast cancer patients before and after first adjuvant chemotherapy cycle. Significantly higher concentrations of serum nitric oxide were observed in post-operative breast cancer patients before chemotherapy in stage III ($p<0.0001$) and stage IV ($p<0.0001$) of the disease as compare to concentrations in healthy controls. A significant decrease in the concentrations of serum nitric oxide was observed in stage III ($p<0.0001$) and stage IV ($p<0.0001$) breast cancer patients after three weeks of receiving first adjuvant chemotherapy cycle as compare to concentrations before chemotherapy. However, we observed a significantly higher levels of serum nitric oxide in stage III ($p=0.0036$) and stage IV ($p<0.0001$) of the disease as compare to concentrations in healthy controls (Table 2).

Table 2: Depict the serum levels of nitric oxide in healthy controls and in advanced stage breast cancer patients before and after chemotherapy.

Subjects	TNM stage	No. of cases	Nitric oxide (µmol/L)
Healthy controls		30	33.53±5.30
Breast cancer patients			
Before chemotherapy	Stage III	30	55.03±9.24[a]
After chemotherapy	Stage III	30	38.50±7.22[b,c]
Before chemotherapy	Stage IV	30	67.40±10.27[a,c]
After chemotherapy	Stage IV	30	52.46±10.25[a,d]

The data was expressed as Mean±SD.
a $p<0.0001$, significantly different when compared to healthy controls.
b $p=0.0036$, significantly different when compared to healthy controls.
c $p<0.0001$, significantly different when compared to stage III breast cancer patients before chemotherapy.
d $p<0.0001$, significantly different when compared to stage IV breast cancer patients before chemotherapy.

DISCUSSION

In the present study, we evaluated chemotherapy induced changes in serum levels of nitric oxide in advanced stage breast cancer patients and assessed its clinical significance. Numerous reports have demonstrated that the drugs of many classes of antineoplastic agents are known to induce apoptosis that occurs by various mechanisms.[2] Nitric oxide is a short-lived highly reactive free radical either involved in induction or inhibition of apoptosis.[6,7] In this study, we found significantly higher levels of nitric oxide in stage III as well as stage IV post-operative breast cancer patients before chemotherapy as compare to levels in healthy controls ($p<0.0001$). Some investigators have reported elevated levels of nitric oxide at operable stage in serum samples of patients with breast cancer.[12,13] Kilic S et al, reported decreased serum nitric oxide at the early post-operative period but re-increased NO levels in the long term in patients with bladder cancer [A] It is possible that, post-operative higher values of •NO might be due to secretion of •NO by non-immune cell types or by tumor infiltrating inflammatory cells in response to residual disease.[12,14] After three weeks of administration of first adjuvant chemotherapy cycle, we found a significant decrease in the levels of nitric oxide in stage III as well as stage IV of the disease as compare to levels before chemotherapy ($p<0.0001$). However, serum nitric oxide levels were found to be higher after first chemotherapy cycle in stage III ($p=0.0036$) and stage IV ($p<0.0001$) breast cancer patients as compare to levels in healthy controls. The decreased levels of nitric oxide after chemotherapy in breast cancer were reported previously by Abdel-Salem OME et al.[15] However, in contrast to this finding, increased level of nitric oxide after chemotherapy has also been reported.[16] The nitric oxide is synthesized from L-arginine by using iNOS enzyme (one of the isoform of NOS). Sim et al, have shown that as compare to pre-chemotherapy, inducible nitric oxide synthase (iNOS) expression was decreased significantly in lung cancer patients after chemotherapy.[17] Moreover, Garner AP et al, have shown that the capacity of NOS to produce •NO is diminished by one of the cytotoxic drug doxorubicin, with eNOS being particularly susceptible to this doxorubicin mediated inhibition.[18] This reduction in •NO may be intensified by the production of superoxide formed by redox cycling because superoxide is able to quench •NO extremely efficiently to form peroxynitrite. The resulting decreased levels of NO raise the possibility of doxorubicin induced cardiovascular toxicity because NO is involved in regulation of vascular tone and mediates myocardial contractile response. In addition to this, Garner AP et al, and Vasquez-Vivar J, et al, observed that at the flavoprotein domains, all three isoforms of NOS are capable of catalyzing the one electron reduction of doxorubicin with the subsequent production of superoxide ($O2•^-$) and a decrease in •NO.[18,19] Furthermore, doxorubicin directly inhibits NOS activity, which could produce significant alterations in vascular tone in tumors.[18,20] In addition to this, it has been reported that 5-fluorouracil suppresses nitric oxide

synthase by different mechanisms involving inactivation of IkB kinase and through inactivating NF-kB which is critical for expression of iNOS.[21] Cyclophosphamide and paclitaxel has been shown to reduce the generation of •NO due to reduced iNOS expression.[22,23] The observed decrease in •NO after administration of these chemotherapy drugs in this study correlate well with their findings.[18-23]

CONCLUSION

This finding suggest that chemotherapeutic drug administration causes significant decrease in serum nitric oxide levels in advanced stages of breast cancer patients compare to levels before chemotherapy. The evaluation of serum nitric oxide may provide useful information in predicting and monitoring of patients' response to chemotherapy treatment in advanced stage breast cancer. However, these findings are preliminary. Further large-scale prospective validation studies on monitoring serum nitric oxide levels after chemotherapy in predicting patients' response to treatment in breast cancer is warranted.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Sparano J, Wang M, Martino S, Jones V, Perez EA, Saphner T, et al. Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med. 2008; 358(16):1663-71.
2. Elmore S. Apoptosis: a review of programmed cell death. Toxicologic pathology. 2007;35(4):495-516.
3. Lowe SW. Lin, a W. Apoptosis in cancer. Carcinogenesis. 2000;21(3):485-95.
4. Panis C, Herrera AC, Victorino VJ, Campos FC, Freitas LF, De Rossi T, et al. Oxidative stress and hematological profiles of advanced breast cancer patients subjected to paclitaxel or doxorubicin chemotherapy. Breast Cancer Res Treat. 2012;133(1):89-97.
5. Mates JM, Sanchez-Jimenez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol. 2000;32(2):157-70.
6. Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. Circulat Res. 1999;84(3):253-6.
7. Kim YM, Kim TH, Seol DW, Talanian RV, Billiar TR. Nitric Oxide Suppression of Apoptosis Occurs in Association with an Inhibition of Bcl-2 Cleavage and Cytochrome Release. J Biol Chem. 1998;273(47):31437-41.
8. Glynn SA, Boersma BJ, Dorsey TH, Yi M, Yfantis HG, Ridnour LA, et al. Increased NOS2 predicts poor survival in estrogen receptor-negative breast cancer patients. J Clin Invest. 2010;120(11):3843-54.
9. Öktem G, Bilir A, Selvi N, Yurtseven ME, Vatansever S, Ates U, et al. Chemotherapy influences inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) activity on 3D breast cancer cell line. Oncology Res Featuring Preclin Clin Cancer Therap. 2006;16(4):195-203.
10. Jin Z, Wang W, Jiang N, Zhang L, Li Y, Xu X, et al. Clinical implications of iNOS levels in triple-negative breast cancer responding to neoadjuvant chemotherapy. PloS One. 2015;10(7):0130286.
11. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem. 1990 Aug 1;36(8):1440-3.
12. Konukoglu D, Turhan MS, Celik V, Turna H. Relation of serum vascular endothelial growth factor as an angiogenesis biomarker with nitric oxide and urokinase-type plasminogen activator in breast cancer patients. Ind J Med Res. 2007;125(6):747-51.
13. Günel N, Coskun U, Sancak B, Hasdemir O, Sare M, Bayram O, et al. Prognostic value of serum IL-18 and nitric oxide activity in breast cancer patients at operable stage. Am J Clin Oncol. 2003;26(4):416-21.
14. Kilic S, Bayraktar N, Beytur A, Ergin H, Bayraktar M, EĞRI M. Can the levels of nitric oxide in the urine, serum and tumor tissue be putative markers for bladder cancer?. Int J Urol. 2006;13(8):1079-85.
15. Abdel-Salam OM, Youness ER, Hafez HF. The antioxidant status of the plasma in patients with breast cancer undergoing chemotherapy. Open J Mol Integrat Physiol. 2011;1(03):29.
16. Amin KA, Mohamed BM, El-Wakil MA, Ibrahem SO. Impact of breast cancer and combination chemotherapy on oxidative stress, hepatic and cardiac markers. J Breast Cancer. 2012;15(3):306-12.
17. Sim SH, Ahn YO, Yoon J, Kim TM, Lee SH, Kim DW, et al. Influence of chemotherapy on nitric oxide synthase, indole-amine-2, 3-dioxygenase and CD124 expression in granulocytes and monocytes of non-small cell lung cancer. Cancer Sci. 2012;103(2):155-60.
18. Garner AP, Paine MJ, Rodriguez-Crespo I, Chinje EC, De Montellano PO, Stratford IJ, et al. Nitric oxide synthases catalyze the activation of redox cycling and bioreductive anticancer agents. Cancer Res. 1999;59(8):1929-34.
19. Vásquez-Vivar J, Martasek P, Hogg N, Masters BS, Pritchard KA, Kalyanaraman B. Endothelial nitric oxide synthase-dependent superoxide generation from adriamycin. Biochem. 1997;36(38):11293-7.
20. Luo D, Vincent SR. Inhibition of nitric oxide synthase by antineoplastic anthracyclines. Biochem Pharmacol. 1994;47(11):2111-2.
21. Jung ID, Yang SY, Park CG, Lee KB, Kim JS, Lee SY, et al. 5-Fluorouracil inhibits nitric oxide production through the inactivation of IκB kinase in stomach cancer cells. Biochem Pharmacol. 2002;64(10):1439-45.

22. Loeffler M, Krüger JA, Reisfeld RA. Immunostimulatory effects of low-dose cyclophosphamide are controlled by inducible nitric oxide synthase. Cancer Res. 2005;65(12):5027-30.
23. Miljkovic D, Cvetkovic I, Sajic M, Vuckovic O, Harhaji L, Markovic M, et al. 5-Aza-2′-deoxycytidine and paclitaxel inhibit inducible nitric oxide synthase activation in fibrosarcoma cells. Europ J Pharmacol. 2004;485(1-3):81-8.

Cite this article as: Kadam CY, Abhang SA. Effect of chemotherapy on serum nitric oxide levels in advanced stage breast cancer patients. Int J Res Med Sci 2020;8:189-93.