Arch. Bas. App. Med.12 (2024) 77- 81
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

Plasma Levels of Nitric Oxide and Indices of Oxidative Stress in Patients with Breast Cancer and Prostate Cancer

***Jimoh M. A.[1], Rahamon S. K.[2], Yusuff A. O.[2], Onifade A. A.[2]**
[1]*Department of Radiation Oncology, College of Medicine, University of Ibadan.*
[2]*Department of Immunology, College of Medicine, University of Ibadan.*
Accepted: 20 February, 2024.

Abstract
Breast cancer (BCa) and prostate cancer (PCa) are cancers of different organs which share similarities as their development requires gonadal steroids and both are hormone-dependent. Reports have shown that oxidative stress (OS) and alteration in nitric oxide (NO) levels are associated with both BCa and PCa. Due to the dearth of information, plasma levels of NO and OS indices were determined in BCa and PCa patients with a view to identifying the group that is more affected by OS and altered NO levels and thus, could possibly benefit from intensive multi-antioxidant supplementation and/or therapy with NO. Plasma levels of NO, total plasma peroxide (TPP) and total antioxidant potential (TAP) were determined spectrophotometrically, in 88 adults comprising 30 patients with breast cancer, 28 patients with prostate cancer and 30 controls. Thereafter, the degree of OS was determined by calculating the oxidative stress index (OSI). The plasma TPP level and OSI were significantly lower in patients with breast cancer and in patients with prostate cancer compared with the controls. In BCa patients, the plasma TPP level and OSI were significantly higher while the plasma NO level was insignificantly lower compared with patients with PCa. In conclusion, oxidative stress in patients with breast cancer appears to be more severe than in patients with prostate cancer. Therefore, determination of the oxidative stress status of cancer patients may be of clinical importance before the utilization of nitric oxide-based therapies.

Key Words: Antioxidant, Hormone-dependent cancer, Nitric oxide, Oxidative stress, Peroxynitrite

INTRODUCTION

Cancer continues to be a major global cause of death. Each year, over 14 million new cancer cases are diagnosed worldwide and by the year 2030, the incidence of cancer is estimated to double (WHO, 2018).

Each year, more than 2.3 million breast cancer cases are reported, making it the most common malignancy among adults and the most common in women (Harbeck and Gnant, 2017). In 2020, it was estimated to account for about 2,261,419 (11.7%) of the new cancer cases as well as the 4th common cause of global cancer mortality in the same year with an estimated 684,996 (6.9%) cancer deaths (Sung *et al.*, 2021). In Nigeria, the burden of breast cancer is high with an estimate of 28,380 (22.7%) new cases and 14,274 (18.1%) mortality in 2020, making it the most common cancer and the most common cause of cancer mortality in 2020 (IAR, 2020). Globally, prostate cancer is the second leading cancer in men (WHO, 2019). In 2020, an estimated 15,306 (12.3%) new cases and 8,517 (10.8%) mortality from prostate cancer were reported, making prostate cancer the second most common form of cancer and the most common cause of cancer mortality in Nigeria (IAR, 2020). Cancer is a multistage, multistep process involving numerous cellular and molecular events including mutational changes that result in uncontrolled cell proliferation (Klaunig, 2018). A critical component of the events that actively support tumour initiation, promotion and progression is chronic oxidative stress (Klaunig, 2018). Several studies, including epidemiological studies, have shown that a strong relationship exists between oxidative stress and various pathologies including, cancer (Hwang and Bowen, 2007). Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been shown to be pro-tumourigenic as it supports genomic and mitochondrial DNA damage which results in altered signalling pathways, DNA damage and mutation of genes (Klaunig, 2018; Moloney and Cotter, 2018). However, ROS/RNS-associated damages are physiologically mitigated by a series of protective molecules and systems termed the antioxidant defence system which includes some vitamins, minerals, specialized enzymes and other non-enzymatic biomolecules. This system inhibits free radical-induced damage by preventing free radical formation and detoxification of the free radicals (Drozdz-Afelt *et al.*, 2022).

Nitric oxide (NO), synthesised by nitric oxide synthase (NOS), is a signalling molecule with well-established pleitropic physiological functions (Soni *et al.*, 2020). Its role in cancer has been reported to be biphasic as it possesses both tumour-promoting and tumouricidal properties (Soni *et al.*, 2020). Its tumour-promoting properties are mediated via angiogenesis, apoptosis, invasion and metastasis. On the other hand, cytostatic and cytotoxic effects on tumour cells are its tumouricidal effects (Korde Choudhari *et al.*, 2013). It is, therefore, not surprising that NO-targeted therapies continue

**Author for Correspondence:* Tel: +2348035628662 E-mail: *jimohmutiualani@yahoo.com*

to gain more interest in breast and prostate cancer management (Soni *et al*., 2020; López-Sánchez *et al*., 2021).

The two most common invasive cancers originating from different organs in men and women are prostate and breast cancers. Although the two cancers are of organs which have different anatomical and physiological functions, they are similar in that their development requires gonadal steroids and the tumours are typically hormone-dependent (Sathyanarayanan *et al*., 2022). Also, oxidative stress and alteration in NO levels are associated with both breast and prostate cancers. However, there is the dearth of information on which of these two hormone-dependent cancers is more affected by oxidative stress and altered NO levels and thus, could possibly benefit from intensive multi-antioxidant supplementation and/or therapy with nitric oxide. This thus serves as the basis for this study.

MATERIALS AND METHODS

Study Participants: A total of 88 adults comprising 30 patients with breast cancer, 28 patients with prostate cancer and 30 apparently healthy individuals, who served as controls, were enrolled into this study. All the study participants with cancer were histologically confirmed.

Ethical Consideration: Before the commencement of the study, ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. Also, informed consent was obtained from each study participant.

Blood Sample Collection: Venous blood (5 ml) was obtained from each study participant and dispensed into K3-EDTA-containing sample bottles. Thereafter, plasma was obtained appropriately, and the samples were kept at -20^0C until analysed.

Laboratory Analyses: Plasma level of nitric oxide was determined spectrophotometrically using Griess-reagent as described by Green *et al*. (1982). Briefly, the method is based on the formation of an azo dye when nitrite (NO_{2-}) reacts with the Griess reagent. Under acidic conditions, NO_2- in the plasma samples reacts with sulfanilamide and N-1-napthylethylenediamine dihydrochloride (NED) to produce a purple-coloured azo compound which strongly absorbs in the visible region. Absorbance of the azo compound that developed was read at 520 nm within 30 minutes of colour development. A standard curve was plotted and the concentration of NO in each sample was extrapolated from the curve.

The plasma level of total plasma peroxide (TPP) was determined spectrophotometrically, using FOX2 reagent as described by Harma *et al*. (2003). Briefly, Aliquots of plasma sample were mixed with FOX2 reagent and incubated for 30 minutes at room temperature. After incubation, the mixture was centrifuged, and the absorbance of the supernatant was measured at 560 nm. TPP level of each sample was then determined as a function of the absorbance difference between the sample and the blank, using H_2O_2 as the standard.

The plasma level of total antioxidant potential (TAP) was also determined spectrophotometrically, using ferric reducing antioxidant power (FRAP) assay as described by Harma *et al*. (2003). Briefly, aliquots of plasma sample were mixed with working FRAP reagent and absorbance (zero minute) value of the mixture was taken immediately. Thereafter, the mixture was incubated at 37°C and absorbance value was taken again after 4 minutes of incubation. Plasma level of TAP was determined as a function of absorbance difference between 4 minutes and 0 minute, using ascorbic acid as standard. The degree of oxidative stress was determined by calculating oxidative stress index (OSI) as the ratio of TPP to TAP multiplied by 100.

Statistical Analysis: Data analysis was done using the SPSS statistical software, version 23.0. Differences in the means of variables were determined using the independent Student's t-test and P-value less than 0.05 was considered as statistically significant. Data were presented as mean ± standard deviation.

RESULTS

Table 1 contains selected characteristics of the patients with cancer. Majority of the patients with breast cancer were at stage 2 of the disease when enrolled into the study. Similarly, majority of the patients with prostate cancer were at stage 2 of the disease when enrolled into the study. Of the 30 patients with breast cancer, 26 (86.7%) had chemotherapy. However, of the 28 patients with prostate cancer, only 7 (25.0%) had brachytherapy as monotherapy, others were on combined therapy (Table 1).

Table 1:
Selected characteristics of the cancer patients

	Breast cancer (n = 30)	Prostate cancer (n = 28)
Age (years)	25 – 73	51 - 78
Stage of cancer		
1	1 (3.3%)	0 (0.0%)
2	21 (70.0%)	25 (89.3%)
3	7 (23.3%)	1 (3.6%)
4	1 (3.3%)	2 (7.1%)
Current Medication		
Chemotherapy	26 (86.7%)	0
Radiation	3 (10.0%)	0
C + R	1 (3.3%)	0
Hormonal	0	0
Brachytherapy	-	7 (25.0%)
B + H	-	7 (25.0%)
R + B	-	7 (25.0%)
C + B	-	1 (3.6%)
C + R + B	-	2 (7.1%)
C + B + H	-	1 (3.6%)
R + B + H	-	1 (3.6%)
C + R + B + H	-	1 (3.6%)
None	-	1 (3.6%)

B = Brachytherapy, C = Chemotherapy, H = Hormonal therapy, R =Radiotherapy

As shown in Table 2, the plasma TPP level and OSI were significantly lower in patients with breast cancer compared with the controls. In contrast, the plasma level of NO was insignificantly higher in patients with breast cancer compared with the controls (Table 2).

Table 2:
Plasma levels of indices of oxidative stress in patients with breast cancer and controls

Parameters	Breast Cancer (n = 30)	Controls (n = 30)	*P*-value
TPP (µmol H_2O_2/L)	32.32 ± 3.71	61.29 ± 7.61	0.002*
TAA (µM Trolox equiv./L)	1454.66 ± 117.67	1747.31 ± 87.49	0.053
OSI (%)	2.73 ± 0.35	3.95 ± 0.48	0.048*
NO (µmole)	48.95 ± 21.67	39.37 ± 17.36	0.131

*Significant at P <0.05, TPP = Total plasma peroxide, TAA = Total antioxidant activity, OSI = Oxidative stress index, NO = Nitric oxide

Patients with prostate cancer had a similar oxidative stress pattern as patients with breast cancer. The plasma TPP level and OSI were significantly lower in patients with prostate cancer compared with the controls. In contrast, plasma NO level was significantly higher in patients with prostate cancer compared with the controls (Table 3).

Table 3:
Plasma levels of indices of oxidative stress in patients with prostate cancer and controls

Parameters	Prostate Cancer (n = 28)	Controls (n = 30)	*P*-value
TPP (µmol H_2O_2/L)	21.30 ± 2.15	61.29 ± 7.61	0.000*
TAA (µM Trolox equiv./L)	1697.73 ± 141.90	1747.31 ± 87.49	0.763
OSI (%)	1.65 ± 0.16	3.95 ± 0.48	0.000*
NO (µmole)	61.93 ± 33.50	39.37 ± 17.36	0.017*

*Significant at P <0.05, TPP = Total plasma peroxide, TAA = Total antioxidant activity, OSI = Oxidative stress index, NO = Nitric oxide.

Comparing patients with cancer, the plasma TPP level and OSI were observed to be significantly higher while the plasma NO level was insignificantly lower in patients with breast cancer compared with patients with prostate cancer (Table 4).

Table 4:
Plasma levels of indices of oxidative stress in patients with cancer

Parameters	Breast Cancer (n = 30)	Prostate Cancer (n = 28)	*P*-value
TPP (µmol H_2O_2/L)	32.32 ± 3.71	21.30 ± 2.15	0.019*
TAA (µM Trolox equiv./L)	1454.66 ± 117.67	1697.73 ± 141.90	0.192
OSI (%)	2.73 ± 0.35	1.65 ± 0.16	0.010*
NO (µmole)	48.95 ± 21.67	61.93 ± 33.50	0.160

*Significant at P <0.05, TPP = Total plasma peroxide, TAA = Total antioxidant activity, OSI = Oxidative stress index, NO = Nitric oxide.

DISCUSSION

Avalanche of reports have established a causal and contributory role of oxidative stress in the initiation, promotion and progression stages of cancer (Klaunig, 2018). This highlights the clinical importance of adequate antioxidants intake in the treatment and prevention of various forms of cancer (Jelic *et al*., 2021).

Oxidative stress has been linked to poor prognosis and disease aggressiveness in patients with breast cancer patients (Scandolara *et al*., 2022). However, available reports on oxidative stress in patients with prostate cancer are conflicting (Drozdz-Afelt *et al*., 2022; Erhabor *et al*., 2022). In this study, TPP and OSI levels, which are indices of oxidative stress, were observed to be significantly lower in patients with breast cancer and patients with prostate cancer compared with the controls. This observation contradicts earlier reports. Nsonwu-Anyanwu *et al*. (2021) and Danesh *et al*. (2022) reported elevated plasma peroxides/oxidants in patients with breast cancer. Similarly, Ahmed Amar *et al*. (2019) reported elevated levels of malondialdehyde, an index of lipid peroxidation in patients with prostate cancer. Although our observations were unexpected, they might be reflective of the nutritional status of the patients with cancer enrolled into this study. Generally, nutritional interventions especially, increased consumption of vegetables and fruits are common supportive clinical approaches to managing cancer (Tan and Norhaizan, 2021). On the other hand, our observations could suggest tumour aggression as the report of Didžiapetrienė *et al*. (Didžiapetrienė *et al*., 2020) showed that the degree of oxidative stress in patients with breast cancer is dependent on the stage of the disease. Patients at advanced stages of cancer are characterised with rapidly proliferating tumour cells which have been shown to be resistant to lipid peroxidation but with concomitant overexpression of antioxidant enzymes (Ma *et al*., 2013). This alteration in the oxidant-antioxidant balance thus, favours the growth of the rapidly dividing cells (Danesh *et al*., 2022).

Improved understanding of the various roles that NO plays in various cancers is contributing to the exploration of its anti-tumourigenic activities (Soni *et al*., 2020). In breast cancer patients, elevated plasma level of NO has been reported (Thomsen *et al*., 1995). Similarly, elevated plasma NO level has been reported in patients with prostate cancer (Nong *et al*., 2011). Our observed elevation in NO level in patients with breast cancer and patients with prostate cancer supports these earlier reports. This observed elevated NO level together with the observed reduction in indices of oxidative stress in patients with cancer compared with the controls demonstrate the classical interplay between reactive oxygen species (ROS) and NO. Reports have shown that alteration in local concentration of either NO or ROS often inversely affect the other, an interaction termed nitroso-redox balance. Therefore, elevation in oxidants level leads to reduction in NO level and vice versa (Mintz *et al*., 2021). At physiological concentration, NO is known to perform a number of antioxidant functions (Mintz *et al*., 2021). This probably explains our observed elevated NO level in patients with cancer as it could be a physiological response to prevent nitrosative stress and aberrant signalling (Hare and Stamler, 2005) which favour tumour promotion and progression.

The dependence on hormones unifies breast cancer and prostate cancer. Recent integrative multi-omic study by Sathyanarayanan *et al*. (2022) provided evidence for common genetic and methylation influences shared by the two cancers. As a signalling messenger, ROS, in a dose-dependent pattern, can activate critical target molecules of gene transcription pathways which are involved in neoplasm growth. These molecules include protein kinase C (PKC), nuclear factor erythroid 2-related factor 2 (Nrf2), mitogen-activated protein

kinases (MAPKs), activator protein-1 (AP-1), Nuclear factor-kappa B (NF-κB) and hypoxia-inducible factor-1α (HIF-1α) (Klaunig, 2018). The observed elevated TPP level and OSI in patients with breast cancer compared with prostate cancer patients indicates that the degree of oxidative stress could be more severe in breast cancer patients than in prostate cancer patients and thus, might benefit from multi-antioxidants supplementation. This observation is further buttressed by slight reduction in TAP level in breast cancer patients. Although our observations appear to favour neoplasm growth, the effect of oxidative stress on cancer is dose dependent. At low ROS concentration or at transient exposure, ROS induces cell proliferation however, cells undergo necrosis or apoptosis when the ROS concentration is high (Klaunig, 2018; Klaunig *et al*., 2011).

The biphasic (pro- or anti-cancer) role of NO in cancer is largely dependent on a number of factors including cell cycle stage, redox status, NO concentration and its distribution. As a signalling molecule, low concentration of NO promotes tumour cell growth and proliferation. At high concentration however, NO generative potent free radicals such as peroxynitrite which inhibits tumour cells growth by damaging the cell membranes (Burke *et al*., 2013; Klaunig, 2018). The observed lower level of NO in breast cancer patients compared with the prostate cancer patients could indicate increased conversion of NO to peroxynitrite in breast cancer patients thereby culminating in the observed elevated oxidative stress in them. This might suggest that there is active cell growth and proliferation in the breast cancer patients necessitating conversion of NO to peroxynitrite to create an anti-tumourigenic environment that will result in cell membrane lysis and inhibition of tumour cells growth.

It could be concluded from this study that oxidative stress in patients with breast cancer appears to be more severe than in patients with prostate cancer. Therefore, determination of oxidative stress status of cancer patients may be of clinical importance before utilization of nitric oxide-based therapies.

REFERENCES

Ahmed Amar, S. A., R. Eryilmaz, H. Demir, S. Aykan, and C. Demir. 2019. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. *Aging Male* 22(3): 198-206.

Burke, A. J., F. J. Sullivan, F. J. Giles, and S. A. Glynn. 2013. The yin and yang of nitric oxide in cancer progression. *Carcinogenesis* 34(3): 503-512.

Danesh, H., N. Ziamajidi, S. A. Mesbah-Namin, N. Nafisi, and R. Abbasalipourkabir. 2022. Association between oxidative stress parameters and hematological indices in breast cancer patients. *Int J Breast Cancer*. 2022:1459410.

Didžiapetrienė, J., B. Kazbarienė, R. Tikuišis, A. Dulskas, D. Dabkevičienė, V. Lukosevičienė, *et al*. 2020. Oxidant/Antioxidant Status of Breast Cancer Patients in Pre- and Post-Operative Periods. *Medicina* (Kaunas) 56(2):70.

Drozdz-Afelt, J. M., B. B. Koim-Puchowska, and P. Kaminski. 2022. Analysis of oxidative stress indicators in Polish patients with prostate cancer. *Environ Sci Pollut Res Int* 29(3): 4632-4640.

Erhabor, O., A. M. Hussaini, A. Abdulwahab-Ahmed, M. Retsky, and T. Erhabor. 2022. Some Oxidative Stress Biomarkers among Patients with Prostate Cancer in Sokoto, North Western Nigeria. *Open Journal of Blood Diseases* 12(3): 60-78.

Green, L. C., D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, and S. R. Tannenbaum. 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 126(1): 131-138.

Harbeck, N., and M. Gnant. 2017. Breast cancer. *Lancet* 389(10074): 1134-1150.

Hare, J. M., and J. S. Stamler. 2005. NO/redox disequilibrium in the failing heart and cardiovascular system. *J Clin Invest* 115(3): 509-517.

Harma, M., M. Harma, and O. Erel. 2003. Increased oxidative stress in patients with hydatidiform mole. *Swiss Med Wkly* 133(41-42): 563-566.

Hwang, E. S., and P. E. Bowen. 2007. DNA damage, a biomarker of carcinogenesis: its measurement and modulation by diet and environment. *Crit Rev Food Sci Nutr* 47(1): 27-50.

International Agency for Research. 2020. Nigeria. Global Cancer Observatory. Retrieved from https://gco.iarc.fr/today/data/factsheets/populations/566-nigeria-fact-sheets.pdf.

Jelic, M. D., A. D. Mandic, S. M. Maricic, and B. U. Srdjenovic. 2021. Oxidative stress and its role in cancer. *J Cancer Res Ther* 17(1): 22-28.

Klaunig, J. E. 2018. Oxidative Stress and Cancer. *Curr Pharm Des* 24(40): 4771-4778.

Klaunig, J. E., Z. Wang, X. Pu, and S. Zhou. 2011. Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicol Appl Pharmacol* 254(2): 86-99.

Korde Choudhari, S., M. Chaudhary, S. Bagde, A. R. Gadbail, and V. Joshi. 2013. Nitric oxide and cancer: a review. *World J Surg Oncol* 11: 1-11.

López-Sánchez, L. M., R. Mena, S. Guil-Luna, A. Mantrana, J. Peñarando, M. Toledano-Fonseca, *et al*. 2021. Nitric oxide-targeted therapy inhibits stemness and increases the efficacy of tamoxifen in estrogen receptor-positive breast cancer cells. *Lab Invest* 101(3): 292-303.

Ma, Y., L. Zhang, S. Rong, H. Qu, Y. Zhang, D. Chang, *et al*. 2013. Relation between gastric cancer and protein oxidation, DNA damage, and lipid peroxidation. *Oxid Med Cell Longev* 2013: 543760.

Mintz, J., A. Vedenko, O. Rosete, K. Shah, G. Goldstein, J. M. Hare, *et al*. 2021. Current advances of nitric oxide in cancer and anticancer therapeutics. *Vaccines* 9(2): 94.

Moloney, J. N., and T. G. Cotter. 2018. ROS signalling in the biology of cancer. *Semin Cell Dev Biol* 80: 50-64.

Nong, S., Y. Zhang, C. He, B. Cheng, S. Zhou, W. Li, *et al*. 2011. Clinical value of serum interleukin-18 and nitric oxide activities in patients with prostate cancer. Chin. *-Ger. J. Clin. Oncol* 10: 711-715.

Nsonwu-Anyanwu, A. C., A. Usoro, E. B. Etuk, R. C. Chukwuanukwu, and O. U. CA. 2021. Evaluation of biomarkers of oxidative stress and female reproductive hormones in post menopausal women with breast cancer in Southern Nigeria. *Niger J Clin Pract* 24(2): 168-176.

Sathyanarayanan, A., H. M. Tanha, D. Mehta, and D. R. Nyholt. 2022. Integrative multi-omic analysis identifies genetically influenced DNA methylation biomarkers for breast and prostate cancers. *Commun Biol* 5(1): 594.

Scandolara, T. B., J. C. da Silva, F. M. Alves, J. Malanowski, S. T. de Oliveira, V. T. Maito, *et al*. 2022. Clinical implications of lipid peroxides levels in plasma and tumor tissue in breast cancer patients. Prostaglandins Other Lipid Mediat 161: 106639.

Soni, Y., K. Softness, H. Arora, and R. Ramasamy. 2020. The Yin Yang Role of Nitric Oxide in Prostate Cancer. *Am J Mens Health* 14(1): 1557988320903191.

Sung, H., J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, *et al*. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71(3): 209-249.

Tan, B. L., and M. E. Norhaizan. 2021. Oxidative Stress, Diet and Prostate Cancer. *World J Mens Health* 39(2): 195-207.

Thomsen, L. L., D. W. Miles, L. Happerfield, L. G. Bobrow, R. G. Knowles, and S. Moncada. 1995. Nitric oxide synthase activity in human breast cancer. *Br J Cancer* 72(1): 41-44.

World Health Organization. 2018. Cancer Fact sheet N°297".

World Health Organization. 2019. Cancer Retrieved from https://www.who.int/health-topics/cancer#tab=tab_1..