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RESEARCH ARTICLE

SERUM NITRIC OXIDE AND PEROXYNITRITE IN BREAST CANCER PATIENTS

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ABSTRACT

Cancer results from deregulation of cellular pathways, leading to rapid multiplication of cells that trigger formation of new blood vessels (angiogenesis), which are required for tumour growth. A marker of angiogenesis thus can help in management of tumour. In this regard, serum nitric oxide was measured in breast cancer patients. Several tumour cell lines found to express enzyme NO synthase. In tissues with increased production of NO, reaction of nitrogen and oxygen (O_2) leads to reactive nitrogen species like dinitrogen trioxide (N_2O_3) and peroxynitrite (ONOO). These reactive nitrogen species will inhibit DNA repair system and also cause oxidative and nitosative stress

Objective/Aim: Aim of this study was to measure and compare serum nitric oxide and peroxynitrite in biopsy proven breast cancer patients and healthy controls

Materials and Methods: This prospective cross-sectional study included 46 newly diagnosed (preoperative) breast cancer female patients (Mean Age =56±12 years). Controls consisted of 46 healthy females were included in the study with no previous disease, alcohol or any drug consumption. Blood samples were collected from all the subjects into empty red capped vacutainer and were analyzed for serum Nitric oxide and peroxynitrite.

Results: Nitric oxide (NO) and Peroxynitrite were significantly increased in breast cancer patients compared with controls.

Conclusions: Stimulation of host defence system against tumor growth results in elevated NO levels in cancer patients. Over expression of Nitric oxide may lead to DNA damage by synthesis of carcinogenic nitrosamines, production of RNS and inhibition of DNA damage repair enzyme mechanism.

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INTRODUCTION

Breast cancer is the second most frequent cancer in females and the fifth most common cause of mortality (Jin, 2009). Cancer results from deregulation of cellular pathways, leading to rapid multiplication of cells that trigger formation of new blood vessels (angiogenesis), which are required for tumour growth (Comoglio, 2002). Tumour angiogenesis is the result of activation of wide variety of signalling systems which include angiogenic factors such as nitric oxide (Reuter, 2010). A marker of angiogenesis thus can help in management of tumour. In this regard, serum nitric oxide was measured in breast cancer patients. Several tumour cell lines found to express enzyme NO synthase (Masuda, 2000).

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In tissues with increased production of NO, reaction of nitrogen and oxygen (O_2) leads to reactive nitrogen species like dinitrogen trioxide (N_2O_3) and peroxynitrite (ONOO) (Masuda, 2000). These reactive nitrogen species will inhibit DNA repair system and also cause oxidative and nitosative stress (Marnett, 2000). Burney *et al*., 1999;Yu *et al*., 2005; Niles *et al.,* 2006), Therefore aim of this study was to measure and compare serum nitric oxide and peroxynitrite in biopsy proven breast cancer patients and healthy controls

MATERIALS AND METHODS

Subjects

Ethical clearance was received from Kasturba hospital Manipal, Manipal University. This prospective cross-sectional study included 46 newly diagnosed (preoperative) breast

cancer female patients (Mean Age =56±12 years) admitted under surgical oncology, Kasturba Medical College Hospital, Manipal, Manipal University between March 2014 and October 2014. All patients were non-smokers non-alcoholic and were not on any long term medications. None of the patients had any other serious medical or surgical illness. Controls consisted of 46 healthy females were included in the study with no previous disease, alcohol or any drug consumption

Blood collection

Blood samples were collected from all the subjects into empty red capped vacutainer and were stored at 4°C. Later serum was separated and stored at – 20°C until use (Cortas, 1990). Serum was used for the measurement of nitric oxide and peroxynitrite.

Measurement of serum nitric oxide: Nitric oxide in serum was determined by Kinetic – cadmium reduction method (Cortas, 1990). Serum nitric oxide by measured by kinetic cadmium reduction test. Nitric oxide production was determined by the evaluation of its oxidation products (nitrites and nitrates), where nitrates were reduced to nitrites with cadmium fillings, the total concentration of nitrites was then measured by using Griess reaction.

Measurement of serum peroxynitrite: Serum peroxynitrite level was measured by modified method of vanuffelen (Al-Nimer, 2010). This is based on peroxynitrite (ONOO-) mediated nitration of phenol resulting in formation of nitrophenol which was detected spectrophotometrically at 412 nm.

RESULTS

Table 1. Comparison of serum NO and Peroxynitrite levels in controls and breast cancer patients

Characteristics	Controls (N=46)	Breast cancer patients (N=46)	*p Value
NO (µmoles/L) (Mean ± SD)	132.52± 6.7	166.978± 10.70	*P= 0.001
Peroxynitrite (µmoles/L) (Mean ± SD)	7.73 ± 2.65	16.304± 4.32	*P= 0.001

Student T-test*

Table 2. Comparision of serum NO and peroxynitrite in different stages of breast cancer

Variables	Stage2 (n = 12)	Stage3(n = 21)	Stage4 (n= 9)	P VALUE
Serum NO(µmoles/L) (Mean ± SD)	140.45± 7.1	145.90± 6.70	164.11±5.3	0.05^c (significant)
Serum Peroxynitrite (µmoles/L) (Mean ± SD)	11.00 ± 6.97	14.52 ± 5.91	15.22 ± 3.19	

*Mann-Whitney U-test,
Bonferroni correction for alpha error is used for multiple pairwise comparisons. NO=Nitric oxide
c- P value vs Stage 4 Vs Stage 2 – significant

Statistical Analysis

For descriptive statistics, the frequency and percentage were calculated for qualitative variables, the mean values ± standard deviation (SD) were used for quantitative variables. For comparison between the two groups Student's t-test was used. For correlation, Pearson correlation was used. The Fisher's exact test was used to assess the association among the clinical–pathological features. All p-values were two-sided and a p-value of less than 0.05 was considered to indicate a statistically significant difference.

DISCUSSION

The role of nitric oxide synthase (NOS) in tumor biology has not been defined clearly. Depending on location and concentration it is said to have both tumoricidal, tumor promoting effect (Korde *et al*., 2012; Choudhari *et al*., 2013). In our study, breast cancer patients showed significant increases in serum NO levels when compared with control subjects (P<0.001). However, there was no significant difference between metastatic and non-metastatic cancer which is in accordance with with Günel *et al* and Konukoglu *et al*. Previous studies showed that regardless of tumor grade, there will be macrophage infiltration in invasive ductal carcinoma (Miles *et al*., 1994) and (Hibbs *et al*., 1990). Studies showing increased NO synthesis in intra-tumoral macrophages infiltration indicate its role in immunity in malignancy. Tumour associated macrophages has principally increased tumor necrosis factor which is a cytokine (Miles *et al*., 1994), is a potent inducer of NO synthase (Hibbs *et al*., 1990), may regulate the production of NO in the tumour-infiltrating macrophage population and its interaction with endothelial cells of the tumor vasculature. The NO thus generated by tumours may contribute to increased angiogenesis (Andrade *et al*., 1992; Wood *et al*., 1993) (Weidner *et al*., 1992; Jenkins *et al*., 1995).This may induce DNA damage and help in cancer progression. This study also showed that that the mean level of serum peroxynitrite was significantly higher in breast cancer patients than that of controls (Table 1) and also among cancer patients, it was significantly elevated in stage 4 as compared to stage 2 implicating (Table 2) its prognostic role. Many studies have concluded that peroxynitrite can damage biomolecules by several other mechanisms including lipid peroxidation, protein oxidation and DNA damage. The latter mechanism involves the induction of several transcription factors leading to cytokine-induced chronic inflammation. Peroxynitrite may cause epigenetic perturbations leading to exaggerated nuclear factor kappa-B mediated inflammatory gene expression leading to carcinogenesis. It may also modulate tumor progression by metabolic activation of Has(heterocyclic amines) The N-acetyl transferase enzymes NAT1 and NAT2 catalyse O-acetylation of the N hydroxylated derivatives of HA, (Bell *et al*., 1993).the human mammary ductal epithelial cells may have the ability to produce HA derivatives because of NAT1 (Sadrieh *et al*., 1996). Animal studies have shown that HAs are the reason for tumors at multiple histologic sites (Snyderwine, 1994). Suggested that the presence of eNOS in breast apocrine metaplastic cells offibrocystic disease in the human may turn to metaplastic epithelium which may lead to cancer.

Conclusion

Stimulation of host defense system against tumor growth results in elevated NO levels in cancer patients. Over

expression of Nitricoxidemay lead to DNA damage by synthesis of carcinogenic nitrosamines, production of RNS and inhibition of DNA damage repair enzyme mechanism. Thus, the biological effects of the NO• is a complex process and also depends on the internal and external environment of the target cells as well as the concentration of NO• generated. Thus both NO and peroxynitrite may have a role in the management of breast cancer

Limitation

This study did not compare the results between histological subtypes due to limited number of samples. Also the markers assessed in this study cannot used in diagnostic purposes due their nonspecific nature.

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Conflicts of interest:

There are no conflicts of interest.

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