VERSITA

Cent. Eur. J. Med. • 5(5) • 2010 • 544-550
DOI: 10.2478/s11536-010-0017-6

Central European Journal of **Medicine**

Serum neopterin as a prognostic indicator in patients with breast carcinoma

Research Article

Belma Kocer[1*], Umit Turan[1], Suleyman Surmeli[1], Buket Altun[1], Sabahat Aksaray[2], Osman Cem Yilmaz[1], Omer Cengiz[1]

[1] *Ankara Numune Training and Research Hospital, 2nd General Surgery Clinics, 06100 Ankara, Turkey*

[2] *Ankara Numune Training and Research Hospital, Department of Microbiology, 06100 Ankara, Turkey*

Received 12 June 2009; Accepted 13 June 2009

Abstract: Neopterin is a useful indicator of the activation state of the cellular immune system, and an elevated level predicts prognosis in different types of tumors. The aim of this study was to evaluate serum neopterin levels if it is viable predictor for prognosis in breast carcinoma patients. Serum neopterin was investigated in 56 breast carcinoma patients, 16 patients with benign breast lesions and 16 healthy women as controls. Neopterin was measured by ELISA (Enzyme-linked immunosorbent assay). The clinicopathological parameters were determined by reviewing both medical charts and pathological records. All patients had been followed-up until September 2009 or death.The mean serum neopterin concentrations were 8.5 ± 5.2 nmol/L in patients with breast carcinoma, 6.5±3.1 nmol/L in patients with benign breast lesion and 8.1 ± 1.9 nmol/L in healthy volunteers ($p>0.05$). Elevated neopterin levels were significantly correlated with age, elevated serum CRP level, advanced stage and presence of the distant metastases ($p<0.05$). Overall survival was significantly shorter in patients with a serum neopterin level >10 nmol/L than patients with neopterin <10 nmol/L level (56 months vs. 76 months, $p=0.011$). Increased preoperative serum neopterin concentrations are associated with a poor prognosis in patients with breast carcinoma.

Keywords: *Neopterin • ELISA • Breast carcinoma • Prognosis*

1. Introduction

Neopterin is a product of human monocytes/macrophages stimulated by IFN-γ which is synthesized by the Th-1 cells. Neopterin plays a role as a sensitive marker of cell-mediated immunity and an indicator of systemic immune activation in patients with malignant disease. In various malignant tumors, autoimmune diseases, allograft rejection and viral infections alter the production of neopterin [1-6]. Frequently, elevated serum and urine neopterin concentrations have positive correlation with the advanced tumor stage and poor prognosis in different tumors [6-9].

Breast cancer is the leading causes of death in women and one in ten women is affected by the disease [10]. The prognosis is related to tumor size, the presence of axillary lymph node metastasis, presence of metastatic disease in breast cancer patients. Most of the histopathological and clinical markers are provide to estimate the prognosis of the breast cancer. There are limited studies that investigate neopterin levels in breast carcinoma, but no study investigates the prediction of serum neopterin levels on prognosis. Murr and colleagues found an elevated neopterin level in the urine of 18% of patients with breast cancer [7]. Yildirim et al. has founded higher serum neopterin level in patients with metastatic breast cancer [11].

The present study aimed to investigate whether serum neopterin level can be used as a significant marker to predict the prognosis in breast carcinoma.

* *E-mail: kocerb@yahoo.com*

2. Material and Methods

The study subjects consisted of 56 breast cancer patients; (age range, 20-81 years; median age, 50.6 years), 16 patients with benign breast lesion and 16 healthy female controls, age- and gender-matched volunteers. Patients with histological confirmed invasive ductal carcinoma of the breast underwent surgery between September 2002 to March 2005 and whose follow-up was completed was included in this study. Women with benign breast lesion like as fibroadenoma, fibrocytic diseases were included this study. The women who have infections, inflammatory or autoimmune diseases were excluded. The informed consent was obtained from all patients to accepted enrollment in the study.

We investigate the relationship between the serum neopterin concentrations and the clinicopathological features, disease free survival and overall survival in breast carcinoma patients.

The following variables were examined**;** patient's age, histological type, tumor size (≤ 2cm, 2-5cm, ≥ 5cm), lymph node metastasis, the number of lymph node metastases, the stage of tumor**,** histological grade, distant metastasis, local recurrence, hormonal status; estrogen and progesterone receptors, c-erbB-2, vessel or lymphatic invasion of tumor, having neoadjuvant chemotherapy and adjuvant chemotherapy and/or radiotherapy and preoperative serum concentrations of CEA, CA 15-3, IL-6, CRP and neopterin. All patients had been followed up until September 2009 or death. The survival analysis was done. The mean follow-up period was 54 ± 13 months (range: 14–78 months). Six (10.7%) patients were death among this study.

2.1. Blood samples

Peripheral venous blood samples were obtained from breast carcinoma patients, patients with benign breast diseases before surgery to determine the serum concentration of CRP, IL-6 and neopterin. Blood samples were centrifuged at 3,000 rpm for 5 min. The serum was removed and stored at –80°C until biochemical analysis.

2.2. Biochemical determination

The serum concentration of IL-6 was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Bender MedSystems, Vienna, Austria), which was specific for the measurement of natural and recombinant human IL-6 in serum. The detection limit of the assay was 1 pg/ml, and levels >1.4 pg/ml were considered elevated. Neopterin concentrations were measured by ELISA according to the manufacturer's protocol (IBL, Hamburg, Germany). Serum CRP concentrations were measured by the nephelometric method (Dade Behring, Mannheim, Germany). A level lower than 5.0 was considered normal.

2.3. Statistical analysis

Statistical analyses were performed using the SPSS Advanced Statistics 10.0 software package (SPSS Inc., Chicago, IL, USA). The results are expressed as the mean ± SD. Differences between the neopterin positivity within groups were evaluated with the chi-square test. Relationships between clinicopathologic parameters and neopterin positivity were evaluated with the chi-square test, or when the sample size was small, Fisher's exact test. The Kruskal–Wallis and Mann–Whitney U-tests were used to evaluate differences among multiple groups. Nonparametric operating characteristic curves (ROC) were generated to calculate the neopterin cut-off values that affected survival. The probability of disease-free survival (DFS) and overall survival (OVS) were analyzed using the Kaplan–Meier method. The Cox proportional hazards regression model was used for multivariate analysis after univariate analysis to define the relevant prognostic variables. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Serum neopterin and its relationship to clinicopathologic parameters

The clinicopathologic characteristics of the patients with breast carcinoma are shown in Table 1. 46 (83.6%) patients had invasive ductal carcinoma, 5 (9.1%) had mix carcinoma (invasive ductal carcinoma + invasive lobulary carcinoma), 3 (5.5%) patients had invasive lobulary carcinoma and 1 (1.8%) patient had ductal carcinoma insitu. Modify radical mastectomy is performed to 53 (94.6%) patients, 3 (5.4 %) patients had breast conserving surgery.

The mean serum neopterin concentrations were 8.5 ± 5.2 nmol/L in patients with breast carcinoma, 6.5±3.1 nmol/L in patients with benign breast lesion and 8.1 ± 1.9 nmol/L in healthy volunteers. No significant differences were observed among these three group ($p>0.05$).

Serum Neopterin concentration of 10 nmol/L is accepted as the upper normal limit in available literature [12,13]. With this cut-off level, 14 (% 25) patients had elevated preoperative serum neopterin levels. Elevated neopterin levels were significantly correlated with age ($p=0.04$), elevated serum CRP level ($p=0.01$), advanced

Table 1. The relationship between clinicopathologic characteristics and neopterin levels in patients with breast carcinoma (n=56).

Characteristics of the patients		Total n (%)	Elevated neopterin level n (%)	p
Age (years)				
	≤50	29 (51.8)	4 (13.8)	
	>50	27 (48.2)	10 (37.0)	0.04
CA 15-3*				
	Normal	35 (74.5)	8 (22.9)	
	High	12 (25.5)	5 (41.7)	NS
CRP*				
	Normal	20 (47.6)	1 (5)	
	High	22 (52.4)	8 (36.4)	0.01
IL-6*				
	Normal	36 (73.5)	7 (19.4)	
	High	13 (26.5)	5 (38.5)	0.17
TNM stage				
	I	15 (26.8)	1 (6.7)	
	II	31 (55.4)	7 (22.6)	
	III	8 (14.3)	4 (50.0)	
	IV	2 (3.6)	2 (100)	0.01
Grade*				
	1	12 (25)	3 (25)	
	2	26 (54.2)	6 (23.1)	
	3	10 (20.8)	3 (30)	NS
Estrogen Receptor				
	Negative	30 (53.6)	9 (30.0)	
	Positive	26 (46.4)	5 (19.2)	NS
Progesteron Receptor				
	Negative	32 (57.1)	9 (28.1)	
	Positive	24 (42.9)	5 (20.8)	NS
LN metastases				
	Absent	19 (33.6%)	3 (15.8%)	
	Present	37 (66.1%)	11 (29.7%)	NS
Preoperative metastases				
	Absent	54 (96.4%)	12 (22.2%)	
	Present	2 (3.6%)	2 (100%)	p=0.01
Postoperative metastases				
	Absent	47 (83.9%)	10 (21.3%)	
	Present	9 (16.1%)	4 (44.4%)	p=0.14
Exitus				
	Negative	49 (89.1%)	9 (18.4%)	
	Positive	6 (10.9%)	4 (66.7%)	p=0.009

** All parameters couldn't have been studied in these patients*

stage (p=0.01) and presence of the distant metastases (p=0.01). The patients died during the follow up period had higher incidence of elevated preoperative neopterin levels than the patients still alive (p=0.009). The mean neopterin concentration were increased in advanced age (p=0.03), patients with elevated CRP levels (p=0.007) and CA 15-3 levels elevated (p= 0.03), advanced stage (p=0.04) and with the patients died at follow up period (p=0.01). The mean neopterin level was higher in patients with preoperative metastasis, but it didn't show statistically significance (p=0.09) (Table 2).

Table 2. Association of mean neopterin levels with different clinicopathological parameters.

		Mean neopterin levels (nmol/L)	
Age	≤50 years	7.1 ± 3.9	
	>50 years	10.0 ± 6.0	p=0.03
CRP levels			
	Normal	6.2 ± 2.1	
	Elevated	10.6 ± 6.4	p=0.007
IL-6			
	Normal	7.9 ± 4.7	
	Elevated	9.3 ± 4.1	p=0.12
CA 15-3			
	Normal	7.8 ± 5.0	
	Elevated	11.0 ± 6.3	p=0.03
Stage			
	I	6.3 ± 2.7	
	II	8.1 ± 4.8	
	III	12.8 ± 7.6	
	IV	13.3 ± 0.8	p=0.04
Preoperative Metastasis			
	Absent	8.3 ± 5.2	
	Present	13.3 ± 0.8	p=0.09
Exitus			
	Absent	7.8 ± 4.5	
	Present	13.4 ± 7.9	p=0.01

3.2. Relationship between serum neopterin and DFS and OVS

Of 56 patients, 6 (10.7%) were death among this study. The mean follow-up period was 54 months, OVS was 54+/-13 months (range: 14–78 months) and DFS was 51±16 months (range: 0–78 months). Two (3.6%) patients had distant metastases at diagnosis. The levels of neopterin in metastatic patients were higher than the patients' with no metastatic disease (p=0.09). Nine (16%) patients had postoperative metastases. The patients who were dead at follow-up have significantly higher mean preoperative neopterin levels than those who did not (p= 0.01).

Based on a univariate proportional hazards analysis, advanced size of tumor, advanced age, advanced stage, presence of distant metastasis, absence of estrogen receptor and elevated serum neopterin levels were associated with poor prognosis (p<0.05). OVS was significantly shorter in patients with a serum neopterin level >10 nmol/L than patients with neopterin <10 nmol/L level (56 months vs. 76 months, p=0.01) (Figure 1). In multivariate analysis, only the stage was significant indicators of survival (Table 3).

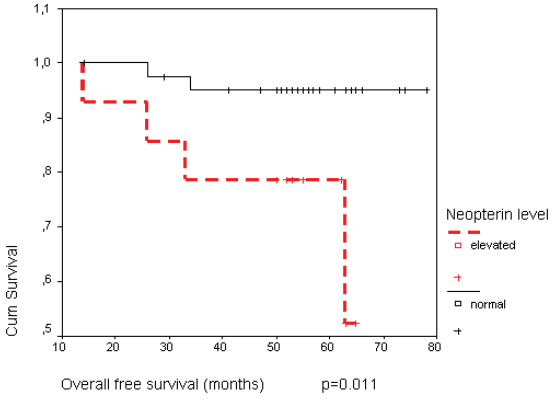
Stage, presence of distant metastases, presence of estrogen receptor and age were factors affecting DFS. Neopterin did not predict DFS in univariant analysis.

Table 3. Multivariate proportional hazards models of prognosis in patients with breast carcinoma.

	HR	95% CI	p-value
Stage	4.84	1.581-14.856	p=0.006

HR, hazard ratio

Figure 1. The effect of neopterin levels on overall survival in patients with breast carcinoma.



4. Discussion

Neopterin is synthesized and released from the activated human macrophages by the IFN-γ as part of the human cellular immunity. Elevated serum neopterin concentration has been demonstrated in a wide of diseases which human host-immune system is known to play role such as autoimmune disorders, infections, malignancy and post-transplant rejections.

Increased neopterin levels were found as a prognostic marker for tumor progression and development of metastasis and mortality in various malignant diseases such as gynecological malignancies, adenocarcinoma of the colon and breast cancer [8,9,14,15]. However, no study had been evaluated the prognostic significance of preoperative serum neopterin levels in patients with breast carcinoma. Murr et al found that 18% of the patients with breast cancer had increased urinary neopterin and urinary neopterin levels were highly significant predictor of survival [7]. Yildirim et al. evaluated the serum neopterin levels in breast cancer and they found higher serum neopterin levels in patients with breast cancer than in healthy controls. Serum neopterin levels were similar in the patients with primary breast cancer without metastasis and in controls, but the serum neopterin levels in the patients with metastatic diseases were statistically significantly higher than other groups [11]. It had been demonstrated that urinary neopterin levels had higher in menopausal breast cancer patients than menopausal healthy women [16]. Although Girgin et al had demonstrated that neopterin levels were significantly higher in malignant breast disease than in patients with benign breast diseases [17]; in our study serum neopterin levels has not show any statistically significant difference between the patients, controls and proliferative diseases. However, its level has been correlated with age, presence of metastatic disease, TNM stage, CA 15-3 level and CRP level. The patients having preoperative metastases and the patients died during the follow up period had elevated preoperative neopterin levels.

Neopterin levels were higher in patients who were older than 50 years than in younger patients in our study. Bayram et al have also founded that neopterin levels in menopausal women higher than premenopausal women's [16]. It may have resulted from co-morbid diseases such as atherosclerosis, malnutrition or chronic obstructive pulmonary diseases that led to chronic immune activation [12,18]. Melicharova et al. has demonstrated that presence of two or more comorbid conditions and older age than 70 years resulting in significantly increased urinary neopterin levels in breast carcinoma patients [19].

In the present study serum CRP and IL-6 levels, were also evaluated as the pro-inflammatory parameters in breast cancer. Serum neopterin levels were correlated with these parameters, it is statistically significant with CRP but not IL-6. Neopterin has been shown to correlate with serum concentration of C-reactive protein in renal cell carcinoma [20]. The synthesis of C-reactive protein is induced by IL-6. Administration of recombinant human IL-6 was also shown to increase neopterin concentration in patients with metastatic RCC [21].

In the study by Murr and colleagues [7] urinary neopterin levels did not correlate with tumor size or lymph node status of patients who had a primary malignant tumor of breast. In our study, the neopterin level was independent of tumor burden nor axillaries lymph node status or receptor status correlate with neopterin level.

In many malignancies elevation of neopterin correlates with the stage of the neoplastic process [6,7]. In our study, the number of patients with elevated neopterin levels increased in advanced stage. The mean neopterin levels were also high in patient with metastatic diseases. The causes underlying an increased neopterin biosynthesis have not fully determined.

Neopterin is a marker of immune function but also reflects the prognosis of carcinoma, and increased neopterin concentrations are associated with a poor prognosis. We found that age, CRP levels, distant metastatic disease, TNM stage, tumor size and the serum neopterin levels predict the survival. We may explain the relationship between the survival and the neopterin concentrations by the human host immune response is stronger against to the aggressive tumors. If the immune system is unable to eliminate malignant tumors cells, activation of the immune system may persist and lead to chronic macrophage activation so we detect highly serum neopterin levels in advanced malignant patients.

There are some mechanisms that we can explain how neopterin affects prognosis in carcinoma. Increased neopterin concentration seems to be an indicator of increased oxidative stress in humans [22]. Experiments have shown that neopterin is capable to enhance the oxidative potential of reactive oxygen species (ROS) produced from immune competent cells [22-24]. Reactive oxygen species have been implicated in the initiation and promotion of carcinogenesis, and direct effect on growth factors and other signaling pathways of both antioxidants and oxidants have been demonstrated [25] Therefore, the prognostic value of higher neopterin concentration to predict disease progression and death in malignant diseases could be related to the capacity of neopterin derivatives to induce ROS and oncogene expression [26].

Neopterin and TNF-α stimulate İNOS gene expression and NO production in vascular smooth muscle cell [27,28]. NO produced by macrophages can mediate antibacterial and antitumor function; however, chronic induction of NO and NO synthesis may contribute too many pathologic processes, including inflammation and cancer [29,30]. Nakamura et all demonstrated that nitric oxide induces VEGF-C expression in vitro and *in vivo* [31]. Increased NO generation in cancer cells may contribute to tumor angiogenesis by up-regulating vascular endothelial growth factor and VEGF-induced revascularization may increase the tumor metastatic ability [32]. Therefore, neopterin secreted from the inflammatory cells, may play a critical role in mediating recruited lymphangiogenesis and angiogenesis most likely through the secretion of several cytokines such as NO.

As a conclusion, high levels of the immune activation marker neopterin at the diagnosis of breast carcinoma indicate a poor prognosis. Serum neopterin detection in breast carcinoma is easily done and gives important and valuable prognostic information before surgical intervention is performed.

References

[1] Fuchs D, Weiss G, Wachter H. Neopterin, biochemistry and clinical use as a marker for cellular immune reactions. Int Arch Allergy Immunol 1993; 101: 1–6

[2] Wachter H, Fuchs D, Hausen A, Reibnegger G, Werner ER. Neopterin as marker for activation of cellular immunity: immunologic basis and clinical application. Adv Clin Chem 1989; 27: 81–141

[3] Fuchs D, et al. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today 1988; 9: 150-5

[4] Denz H, Griinewatd K, Thaler J, Huber H, Fuchs D, Hausen A, Reibnegger G, Werner ER, Wachter H. Urinary neopterin as a prognostic marker in haematological neoplasias. Pteridines 1989; 1: 167–170

[5] Melichar B, Solichová D, Melicharová K, Malírová E, Cermanová M, Zadák Z. Urinary neopterin in patients with advanced colorectal carcinoma. Int J Biol Markers 2006; 21: 190–198

[6] Sucher R, Schroecksnadel K, Weiss G, Margreiter R, Fuchs D, Brandacher G. Neopterin, a prognostic marker in human malignancies. Cancer Letters 2009; June 3

[7] Murr C, Bergant A, Widschwndter M, Heim K, Schrocksnadel H, Fuchs D. Neopterin is an independent prognostic variable in females with breast cancer. Clinical Chemistry 1999, 45; 11: 1998-2004

[8] Frick J,Aulitzky W, Fuchs D, Hausen H, Joos H, Reibnegger G, et al. The value of urinary neopterin as an immunological parameter in patients with malignant tumors of the genitourinary tract. Urol Int 1985; 40: 155-9

[9] Melichar B, Solichova D, Svobodova I, Urbanek L, Melicharova K. Urinary Neopterin in Patients with upper Gastrointestinal, Biliary and Pancreatic Carcinomas. Pteridines 2006: 17; 20-24

[10] World Health Organization. Deaths by cause, sex and mortality stratum in WHO regions, estimates for 2002. Available online at: http://www.who.int/whr/2004/annex/topic/en/annex-2-en.pdf. 2009; January 12

[11] Yildirim Y, Gunel N,Coskun U, Pasaoglu H, Aslan S, Cetin A. Serum neopterin levels in patients with breast cancer. Med Oncol 2008; 25: 403-407

[12] Berdowska A and Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. J of Clinical Pharmacy and Therapeutics 2001; 26: 319-329

[13] Honlinger M, Fuchs D, Hausen A, Reibnegger, G, Schonitzer D, Werner ER, et al. [Serum neopterin determination for the additional safeguarding of blood transfusions. Our experiences with 76,587 blood donors]. Dtsch Med Wochenschr 1989;114:172–6

[14] Melichar B, Solichova D, Freedmans RS. Neopterin as an indicator of immune activation and prognosis in patients with gynecological malignancies. Int J Gynecol Cancer 2006; 16: 240-252

[15] Hausen A, Bichlker A, Fuchs D, Hetzel H, Reibnegger G, Wachter H. Neopterin, a biochemical indicator of cellular immune reaction, in the detection and control of patients with neoplastic diseases. Cancer Detect Prev 1985; 8: 121-8

[16] Bayram M, Bayram O, Boyunaga H, Guler Ozer. A research on the level of urine neopterin to see if it may provide a vital clue for a provisional diagnosis of breast cancer in menopausal women. Maturitas 2009; 48: 432-437

[17] Girgin G, Sahin T, Fuchs D, Kasuya H, Yuksek O, Tekin E, Baydar T. Immune system modulation in patients with malignant and benign breast disorders: tryptophan degradation and serum neopterin. Int J Biol Markers 2009; 11: none (Epub ahead of print)

[18] Hoffmann G, Wirleitner B, Fichs D. Potential role of immune system activation-associated production

of neopterin derivatives in humans. Inflamm Res 2003; 52: 313–321

[19] Melicharova K, Kalabova H, Kromova L, Urbanek L, Solichova D, Melichar B. Effect of comorbidity on urinary in patients with breast carcinoma. Eur J Cancer Care (Engl) 2009;13 (Epub ahead of print)

[20] Yoshida N, Ikemoto S, Narita K et al. Serum neopterin levels in renal cell carcinoma. Pteridines 2000; 11: 133-136

[21] Schuler M,Peschel C, Schneller F et al. Immunomodulatory and hematopoietic effects of recombinant human interleukin-6 in patients with advanced renal cell cancer. J. Interferon Cytokine Res 1996; 16: 903-910

[22] Murr C, Fuith LC, Widner B, Wirleitner B, Baier-Bitterlich G, Fuchs D. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? Anticancer Res. 1999;19(3A):1721–8

[23] Weiss G, Fuchs D, Hausen A, Reibnegger G, Werner ER, Werner-Felmayer G, Semenitz E, Dierich MP, Wachter H. Neopterin modulates toxicity mediated by reactive oxygen and chloride species. FEBS Lett. 1993; 19: 89–92

[24] Widner B, Baier-Bitterlich G, Wede I, Wirleitner B, Fuchs D. Neopterin derivatives modulate the nitration of tyrosine by peroxynitrite. Biochem Biophys Res Commun. 1998 Jul 20;248(2): 341–6

[25] Weinberg F, Chandel NS. Reactive oxygen species-dependent signalling regulates cancer. Cellular and Molecular Life Science 2009; Dec;66(23):3663-73. Epub 2009 Jul 24. Review

[26] Murr C, Berchtold J, Norer B, Waldhart E, Wachter H, Fuchs D. Neopterin as a prognostic parameter in patients with squamous-cell carcinomas of the oral cavity. Int J Cancer 1998; 79: 476–480

[27] Djordjevic VB, Stojanovic I, Cosic V, Zvezdanovic L, Deljanin-Ilic M, Dimic S, Kundalic B, Cvetkovic T, Jevtovic- Stoimenov T. Serum neopterin, nitric oxide, inducible nitric oxide synthase and tumor necrosis factor-α levels in patients with ischemic heart disease. Clin Chem Lab Med 2008; 46: 1149-1155

[28] Schobersberger W, Hoffman G, Grote J, Wachter H, Fuchs D. Induction of inducible nitric oxide synthase expression by neopterin in vascular smooth musclecells. FEBS Lett 1995; 377: 461-4

[29] Moncada S, Higgs A. The L-arginine –nitric oxide pathway. N Engl J Med 1993; 329: 2002-12

[30] Knowles RG, Moncada S. Nitric oxide sythases in mammals. Biochem J 1994; 298-58

[31] Nakamura Y, Yasuoka H, Tsujimoto M, Yoshidome K, Nakahara M, Nakao K, Nakamura M, Kakudo K. Nitric oxide in breast cancer: Induction of vascular entothelial growth factor-C and correlation with metastasis and poor prognosis. Clin Cancer Res 2006; 12: 1201-1207

[32] Ambs S, Merriam WG, Ogunfusika MO, Bennett WP, Ishibe N, Hussain SP, Tzeng EE, Geller DA, Billiar TR, Harris CC. p53 and vascular endothelial growth factor regulate tumor growth on NOS2-expression human carcinoma cells. Nat Med 1998; 4: 1371-6