Diagnostic Value of Serum Vascular Endothelium Factor in Cancer Breast

Laila A. Ahmed[1], Hala M.T. El-Mougy[1], Omayma H.M. Sarhan[1] and Tarek Elbaradey [2]

[1]Department of Medical Biochemistry, Faculty of Medicine(Girls), Al-Azhar University
[2] Oncology Surgery, National Cancer Institute, Cairo University
hala.elmougy@hotmail.com

Abstract: Breast cancer is the most commonly diagnosed cancer among women, and the second leading cause of cancer deaths. The study aimed to determine the usefulness of measuring serum level of VEGF together with CA15-3 in discriminating benign from breast cancer lesion and to find out a relationship between their levels and disease aggressiveness. Ninety female patients were included in this study, 70 with malignant breast cancer (35 with early cancer & 35 with advanced cancer) and 20 cases with benign lesions as control. Serum VEGF and CA15-3 were measured by ELISA. Levels of serum VEGF and CA15-3 were found to be significantly higher in malignant groups than benign group. Regarding malignant cases there was a high significant correlation between early and advanced cases and between serum CA15-3 and serum VEGF. On correlating the level of serum VEGF and CA15-3 with the clinico- pathological data of malignant group, a significant correlation was found with age, size of tumor and metastasis, but no significant correlation with other factors. A significant correlation was found between fixation to chest wall and bilateral primaries with CA15-3 but not with VEGF. Conclusively, it could be suggested that both VEGF and CA15-3 might be measured together as diagnostic and prognostic indicators in breast cancer.
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1. Introduction

Breast cancer is the most common cancer among women in Arab countries; it constitutes 13-35% of all female cancers **(Khalil *et al.*, 2007).**

Angiogenesis is a process characterized by new blood vessels formation from pre-existing vessels that's play an important role in process of tumor growth, invasion, and metastatic dissemination **(Boneberg *et al.*, 2009).** Angiogenesis is mediated by several growth factors , one of them is the vascular endothelial growth factor (VEGF). The VEGF family is composed of VEGF-A (often referred to as VEGF), VEGF-B, VEGF-C, VEGF-D and placental growth factor (**Marianne *et al.*, 2009).**

VEGF is a homodimeric glycoprotein growth factor which acts on vascular endothelial cells via endothelium specific receptor tyrosine kinases to produce a variety of responses including increased proliferation, migration, and vascular permeability partially through stimulation of nitric oxide synthase enzyme in endothelial cells (**Roy *et al.*, 2006).** VEGF over-expression has been detected in many human malignant tumors, including breast carcinomas. VEGF expression is significantly associated with high intra-tumoral microvessel density **(David *et al.*, 2003)**.

Inhibiting tumor angiogenesis is a promising strategy for treatment of cancer and has been successfully transferred from preclinical to clinical application in recent years. Whereas conventional therapeutic approaches as chemotherapy and radiation, are focusing on tumor cells, antiangiogenic therapy is directed against the tumor supplying blood vessels **(Eichhorn *et al.*, 2007)**. Inhibitors of VEGF receptor tyrosine kinases and their downstream effects are attractive anti-angiogenic agents, which could potentially inhibit endothelial cells activation, proliferation, and invasion **(Jain *et al.*, 2009)**.

Cancer antigen;CA15-3 is a glycoprotein encoded by the MUC 1 gene that are heterogeneously expressed on the apical surface of normal epithelial cell types, including those of the breast **(Nicolini *et al.*, 2006).** Elevated CA15-3 is used to anticipate detection of recurrences in patients with breast cancer, and as an additional tool in evaluating therapeutic response of advanced disease. CA15-3 measurement during follow-up has been shown to predict liver and bone metastases **(Keshaviah *et al.*, 2007).**

Aim of study: To determine the usefulness of measuring serum level of VEGF together with CA15-3 in discriminating benign from breast cancer lesion, and to find out a relationship between their levels and disease aggressiveness.

2. Material and methods

The study included 90 female patients with breast masses from the National Cancer Institute in Cairo. They were classified into 3 groups: early breast cancer (35 patients), advanced breast cancer (35 patients), and benign control (20 patients). All groups were subjected to full history, full clinical examination,

histopathological examination of tissue biopsy for estrogen and progesterone receptor determination by immunohistochemistry.

Estimation of serum VEGF and CA15-3 by ELISA technique were done using kits purchased from Ray Biotech. and Nova tech. respectively.

Statistical Analysis:

Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16. Parametric data was expressed as mean ± SD, and non parametric data was expressed as number and percentage of the total.

3. Results

Statistical analysis of serum levels of VEGF and CA15-3 revealed a high statistically significant difference (*P*=0.0001) between malignant and benign cases (Table 1).

On correlating the levels of serum VEGF and serum CA15-3 with Group I (early breast cancer group) and group Π (advanced breast cancer group) there was statistically high significant correlation (*P* =0.0001) between both groups (Table 2, Figs. 1&2).

Regarding malignant cases there was a high statistically significant correlation between serum CA15-3 and serum VEGF (Fig.3).

Table (3) represents the clinicopathological data of the malignant groups.

On correlating the levels of serum VEGF and CA15-3 with clinico- pathological factors of malignant groups a significant correlation (*P* <0.05) was found with age, size of tumor and metastasis, but no correlation with ER, PR, multiple primers, inflammatory cancer, Peaud` orange, fungation /ulceration, edema, capsular rupture and major intraduct component. A significant correlation (*P* <0.05) was found between fixation to chest wall and bilateral primaries with CA15-3 but not with VEGF (Table 4).

Receiving Operating characteristic Curve (ROC curve) was done to detect the best cut off value which maximizes the discrimination between malignant and benign cases. One hundred twenty was the best cut off value of serum VEGF, the predictivity of serum VEGF was about 85%, the sensitivity was 95.71% and the specificity was 45.00%. For serum CA15-3, 30 was the best cut off value. The predictivity of serum CA15-3 was about 100%, the sensitivity was 52.86% and the specificity was 100% (Table 5).

Positive rate of serum VEGF was 67/70 denoting 95.7% in malignant cases, and 11/20 denoting 55% in benign cases with high statistically significant difference between them (*P* =0.0001). Positive rate of serum CA15-3 was 37/70 denoting 52.8% in malignant cases, and 0/20 denoting 0% in benign cases with high statistically significant difference between them (*P* =0.0001) (Table 6).

Table (1): Serum VEGF and CA15-3 in benign and malignant groups.

		CA15-3(U/l)	VEGF(pg/ml)
Malignant	Mean±SD	103.21±92.41	496.31± 309.09
Benign	Mean±SD	11.85±6.33	173.39±165.64
	P value	0.0001	0.0001

Table (2):Serum VEGF and CA 15-3 in early and advanced breast cancer groups.

		CA15-3(U/l)	VEGF(pg/ml)
Early	Mean±SD	18.81±8.04	286.47±104.74
Advanced	Mean±SD	187.61±50.99	706.14±303.75
	P value	0.0001	0.0001

Table (3): Clinicopathological data of malignant group

Clinicopathological factors		Number	Percent %
Histological types	IDC(intraduct carcinoma)	54	77.14
	Others	16	22.86
Stage	I	3	4.28
	Π	27	38.57
	ΙΙΙ a	5	7.14
	ΙΙΙ Ь	19	27.14
	IV	16	22.86
Grade	I	6	8.57
	Π	51	72.86
	ΙΙΙ	13	18.57
Metastasis	O (negative)	54	77.14
	B (bone)	7	10
	L (lung)	4	5.71
	Ov (ovary)	1	1.43
	V (liver)	4	5.71
ER		52	74.29
PR		48	68.57
Lymph nodes	O(negative)	14	20
	A	28	40
	AC	4	5.71
	AF	13	18.57
	AFCI	1	1.43
	AFS	2	2.86
	AS	1	1.43
	ASI	7	10
multiple primaries	-	14	20
bilateral primaries	-	2	2.86
peaud orange		6	8.57
Fungation/ulceration		5	7.14
fixation to chest wall		11	15.71
Oedema		8	11.43
capsular rupture		30	42.86
Major intraduct component present		2	2.86

Table (4): Relation of serum VEGF, CA15-3 and clinical pathological factors in malignants

Parameter	CA15-3	VEGF
	Mean±SD	Mean±SD
PR positive	91.79±85.87	533.32±336.51
PR negative	118.43±99.92	446.96±265.74
	P=0.24635332	*P* =0.234
ER positive	90.77±85.96	520.66±322.23
ER negative	125.59±100.95	452.47±284.94
	P =0.15257528	*P* =0.364
Multiple primaries positive	70.47±76.26	521.68±216.24
Multiple primaries negative	111.39±94.86	394.81±324.85
	P =0.10105931	*P* =0.089
Bilateral primaries positive	14.22±10.02	502.28±65.76
Bilateral primaries negative	105.82±92.46	293.1±311.54
	P =0.000002	*P* =0.045
Inflammatory cancer positive	142.08±81.01	766.28±593.96
Inflammatory cancer negative	100.22±93.11	475.54±273.53
	P =0.32254173	*P* =0.336
Peaud' Orange positive	111.09±102.3	449.03±192.25
Peaud' Orange negative	102.47±92.28	500.74±318.54
	P =0.84898528	*P* =0.573
Fungation/Ulceration positive	134.18±74.87	520.59±251.09
Fungation/Ulceration negative	100.82±93.68	494.44±314.66
	P =0.38966004	*P* =0.834
Fixation to Chest Wall positive	166.97±35.84	634.83±238.87
Fixation to Chest Wall negative	91.32±94.99	470.48±315.44
	P =0.000039	*P* =0.063
Oedema positive	145.44±67.00	714.92±467.58
Oedema negative	97.76 ±94.23	468.10±275.5
	P =0.09996188	*P* =0.184
Capsular Rupture positive	111.21±90.81	513.88±264.89
Capsular Rupture negative	97.21±94.29	483.13±341.23
	P =0.53226449	*P* =0.673
Major Intraduct Component positive	114.05±100.90	289.45±161.93
Major Intraduct Component negative	102.19±92.37	515.70±313.21
	P =0.79112656	*P* =0.163
Metastasis positive	195.66±64.89	669.22±264.65
Metastasis negative	75.8181.13	445.07304.72
	P =0.000001	*P* =0.008
IDC	95.29±92.4	475.49±299.34
Others	129.91 ±90.15	566.56±340.66
	P =0.19184922	*P* =0.345
Age	47.37±12.74	47.37±12.74
	P =0.0001	*P* =0.0001
Size of tumors	3.54±1.94	3.54±1.94
	P =0.0025	*P* =0.0025

A= axillary, F=fixed, S=supraclavicularm, C=contralateral, I=internal mammary

Table(5): Sensitivity and specificity of serum VEGF and CA 15-3 in prediction of breast cancer.

	Cut off value	sensitivity	specificity
VEGF	120 pg /ml	95.71%	45.00%
CA15-3	30 unit /ml	52.86%	100.00%

Table (6): Positivity rate of serum VEGF and CA15-3 in benign and malignant groups.

	Benign No%	Malignant No%	PPV	NPV
VEGF	11/20 55%	67/70 95.7%	85.90	75.00
CA15-3	0/20 0%	37/70 52.8%	100.00	37.74

PPV: Positive predictive value
NPV: negative predictive value

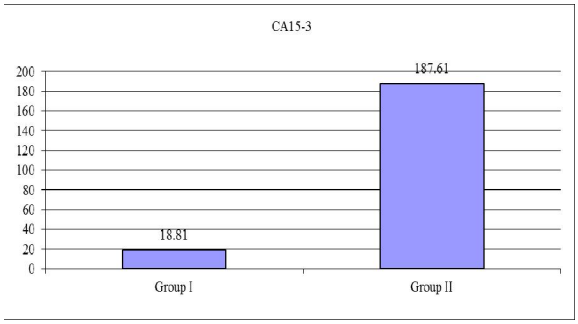

Fig.(1): Mean VEGF(pg /ml) in early cancer (group I) and advanced cancer (group II).

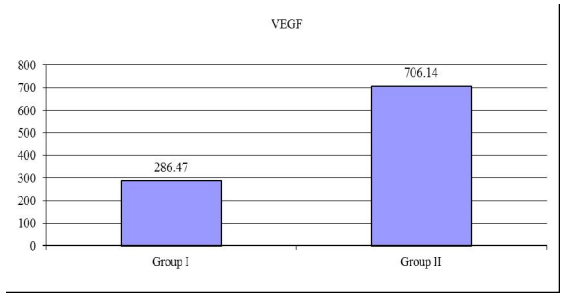

Fig.(2): Mean CA15-3(U/l) and both early and advanced cancer groups.

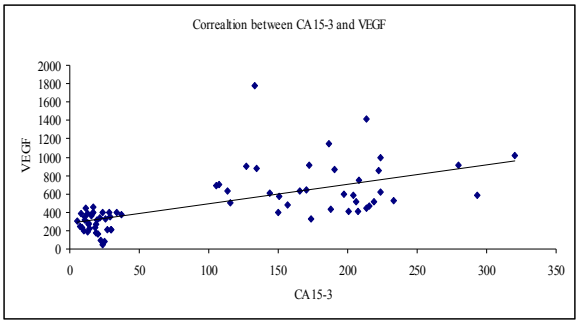

Fig.(3): Correlation between CA15-3(U/l) and VEGF(pg/ml)

Discussion

Breast cancer is the main leading cause of death in women**.** Angiogenesis plays a significant role in cancer by providing increased blood supply to the affected tissues and thus bringing in growth factors, cytokines, and various nutrients for tumor growth. VEGF is the prominent angiogenic agent that is markedly induced in cancer **(Ray *et al.*, 2011).**

In this study, the level of serum VEGF was found to be significantly higher (P<0.05) in malignant groups than in benign group. This finding was in accordance with the result of **Kirwan *et al.*, (2009)** who suggested that VEGF was the major angiogenic factor in breast carcinoma and the most important cytokine involved in tumor spread and disease progression. **Bluff *et al.*, (2009)** suggested that angiogenesis is initiated at the start of hyperplasia, with further increases and morphological evidence of atypia. They found that VEGF was highly increased predicting poor survival and poor clinical response to chemotherapy.**Derya *et al.*(2008)** found that VEGF level was not a diagnosti diagnostic tool for breast cancer but could be a predictor of recurrence risk of early stage breast cancer.

When the mean rank of serum VEGF in malignant groups was correlated to clinicopathological factors, there was no statistically significant correlation (P>0.05) with histological type, capsular rupture, oedema, fungation/ulceration, fixation to chest wall, Peaud` orange, inflammatory cancer and lymph node metastasis.This agreed with results of **Barbro *et al.* (2008)** while **Mohammad *et al.* (2007)** showed that VEGF was associated with lymph node metastasis in breast cancer as VEGF induced lymphangiogenesis was strongly correlated with dissemination to regional lymph nodes.

The presence of Estrogen receptor (ER) and progesterone receptor (PR) is predictive for response to endocrine therapy and improved disease-free survival **(Yi Yan *et al.*, 2009).**

This study showed no significant correlation (P>0.05) between VEGF and both ER and PR. These results agreed with that of **Barbro *et al.* (2008)**. On the other hand **Hyder (2006)** found a significant correlation between VEGF and both ER and PR, explaining it by the effect of estrogen on regulation of VEGF and its receptors in breast cancer as estradiol may tip the scale to favor angiogenesis, increasing bioactive VEGF.

Kataoka *et al.* (2006) explained the relation between VEGF and the poor prognosis in ER-positive patients rather than ER-negative patients by the sensitivity of endothelial cells to VEGF which might be different between ER-positive tumors and ER-negative tumors. In addition, estrogen has been reported to increase VEGF mRNA expression and protein in breast cancer cells and isolated endometrial cells.

A significant correlation was detected between VEGF and age, and size of tumor .This finding was not in accordance with **Xiaowei *et al.* (2008)** but in accordance with **Barbro *et al.* (2008)** who found that high VEGF was not only associated with larger tumors but also with larger metastatic deposits, likely through the growth factor inducing a rich vascular network.

A significant correlation was found between VEGF and metastasis, a result that agreed with **Mohammad *et al.* (2007)** who supposed that early distant spread in patients with higher VEGF levels in their primary tumors as a result of the vessel leakage may be caused by the increased permeability, one of the major effects of VEGF.

There was a significant correlation between VEGF and bilateral primaries, major intraduct component, and grade of the disease. This finding was in accordance with that of **Xiaowei (2008).**

CA15-3 is a glycoprotein that elevates in a proportion of breast cancer patients with distant metastases**.** Its main usefulness is in the surveillance of patients with diagnosed disease and in monitoring the treatment of patients with advanced disease **(Daniele *et al.* 2008).**

Serum level of CA15-3 was found to be significantly higher in malignant group than benign group with high statistically significant difference between them (P=0.0001). This finding is in accordance with **Kim *et al.* (2010)** who said that CA15-3 is known to reflect tumor burden and is associated with poor outcomes.

The study showed a high significant correlation between serum CA15-3 and serum VEGF in malignant cases. This finding was in accordance with **Iovino *et al.* (2008)**, but was not with **Quaranta *et al.* (2007)**.

In malignant group, serum CA15-3 showed no significant correlation with major intraduct component ,capsular rupture ,oedema ,fungation /ulceration ,ER ,PR ,Peaud` orange ,multiple primaries and grade. This finding was not in accordance with **Chourin *et al.* (2009)** who found a significant correlation between CA15-3 and tumor size, grade, age at time of diagnosis and lymph node invasion which were considered poor prognostic factors. Lymph node invasion was the only prognostic factor for disease-free survival. The presence or absence of hormone receptors did not have any prognostic value.

On the other hand, a significant correlation was evidenced with fixation to chest wall (*P*=0.00003) and metastasis (*P*=0.000001). This finding is in accordance with **Keshaviah *et al.* (2007)**. Patients with high concentrations of preoperative levels of CA15-3 have a significantly worse prognosis than those with low concentrations probably due to a larger burden of occult disease and bilateral primaries.

The sensitivity of serum CA15-3 was 52.86% and specificity was 100.00%. This finding was in accordance with **Chourin *et al.* (2009)** who found that serum CA 15-3 concentration >30 U/l is associated with a poor prognosis supposing cancer metastases.

The low sensitivity of CA15-3 in this study was in accordance with **Duffy (2006)** who found that the main disadvantage of CA15-3 for breast cancer is a lack of sensitivity for low-volume disease. Consequently, the marker is of no value in either screening or diagnosing early breast cancer. Although of little use for early diagnosis, however, CA 15-3 may be the first independent circulating prognostic marker described for breast cancer.

It is good to measure both serum VEGF (high sensitivity and low specificity) and serum CA15-3(low sensitivity and high specificity) to have high sensitivity and high specificity method for screening and diagnosis.

Corresponding author
Hala M.T. El-Mougy
Department of Medical Biochemistry, Faculty of Medicine(Girls), Al-Azhar University
hala.elmougy@hotmail.com

References

1. Barbro K, Linderholm,, Gruvbreger-Saal S, Ferno M, Bendahl B and Malmstrom P (2008): Vascular endothelial growth factor is a strong predictor of early distant recurrences in a prospective study of premenopausal women with lymph-node negative breast cancer. J Vasc Surg., 41:699–707.
2. Bluff JE, Menakuru SR, Cross SS, Higham SE, Balasubramanian SP, Brown NJ, Reed MW and Staton CA (2009):Biomolecular markers of breast cancer. Journal of Molecular Research, 86(10):937-950.
3. Boneberg EM, Legler DF, Hoefer MM, Öhlschlegel C, Steininger H, Füzesi LBeer GMDupont-Lampert VOtto FSenn HJFürstenbergerG (2009): Angiogenesis and lymphangiogenesis are downregulated in primary breast cancer. British Journal of Cancer, 102(4): 605-614.
4. Chourin S, Georgescu D, Gray C, Guillemet C, Loeb A, Veyret C and Basuyau JP (2009): Value of CA 15-3 determination in the initial management of breast cancer patients. Oxford Journals, 20(5): 962-964.
5. Daniele A, Divella R, Tardo SD, Caringella ME, Abbate I, Coviello M, Trerotoli P, Schittulli F and Quaranta M (2008): Breast cancer: Assessing the CA 15.3 clinical usefulness before and after surgery. Int. Surg. Path. J., 56(8):67-75.
6. David C, Tabbara S, Marley E, Talley L, Frost A (2003): "Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention". Biochim. Biophys. Acta ., 1766 (1): 63–78.
7. Derya D, Camlica H, Soydinc H, Derin D and Yasasever V (2008): Serum levels of angiogenic factors in early breast cancer remain close to normal .The Breast J., 34(8): 123–129.
8. Duffy MJ (2006): Serum Tumor Markers in Breast Cancer: Are They of Clinical Value?. Clinical Chemistry J., 52(3) 345–351 .
9. Eichhorn ME, Kleespies A, Angele MK ,Jauch KW and Bruns CJ (2007): Angiogenesis in cancer: molecular mechanisms, clinical impact. Nat Rev Cancer, 8(7):579-591.
10. Hyder SM (2006): Sex-steroid regulation of vascular endothelial growth factor in breast cancer. Endocrine Related Cancer J., 13 (3) 667 - 687.
11. Iovino F, Ferraraccio F, Orditura M, Antoniol G, Morgillo F and Cascone T (2008): Serum vascular endothelial growth factor (VEGF) levels correlate with tumor VEGF and p53 overexpression in endocrine positive primary breast cancer. Oncology and Radiology Journals, 26(3): 250-255.
12. Jain RK, Duda DG and Willett CG (2009): Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol.,6: 327–338.
13. Kataoka N, Cai Q, Wen W, Shu XO, Jin F, Gao YT and Zheng W (2006): Population-Based Case-Control Study of *VEGF* Gene Polymorphisms and Breast Cancer Risk among Chinese Women. Cancer Epidemiol Biomarkers Pre., *15:*1148
14. Keshaviah A, Dellapasqua S, Rotmensz N, Lindtner J, Crivellari D, Collins J, Colleoni M, Thürlimann B, Mendiola C, Aebi S, Price SK, , Simoncini E, Castiglione M, Gelber RD, Coates AS and Goldhirsch A(2007):CA15-3 and alkaline phosphatase as predictors for breast cancer recurrence: a combined analysis of seven International Breast Cancer Study Group trials. Oxford Journals, 18(4): 701-708.
15. Khalil MK, Hussien G and Emam K (2007): Breast cancer statistics in Arab countries. Br. J., 23(2):12-22.
16. Kirwan C, Byrne GJ, Kumar S and Garry McDowell (2009): Platelet release of vascular endothelial growth factor in patients undergoing chemotherapy for breast cancer. Journal of Angiogenesis Research, 8(7):112-121.
17. Kim MJ, Park BW, Lim JB, Kim HS, Kwak JY, Kim SJ, Park SH, Sohn YM, Moon HJ and EK (2010): Axillary Lymph Node Metastasis: CA-15-3 and Carcinoembryonic Antigen

Concentrations in Fine-Needle Aspirates for Preoperative Diagnosis in Patients with Breast Cancer. Radiology, 254: 691-697.
18. Marianne OS, Florence TH, Gabhann FM, and Aleksander S (2009): The Presence of VEGF Receptors on the Luminal Surface of Endothelial Cells Affects VEGF Distribution and VEGF Signaling. PLoS Computational Biology, 8(5):43-70.
19. Mohammad H, Shooshtary S, Talaizadeh AH, Assar S, Arnineh BK, Nateghi J and Rahdar M (2007): Evaluation of carcinoemberionic antigen CEA and CA15.3 tumor markers in patients operated for breast cancer. Pak J Med Sci., 23(1): 115-118.
20. Nicolini A, Tartarelli G, Carpi A, Metelli M, Ferrari P, Anselmi L, Conte M and Miccoli P (2006): Intensive post-operative follow-up of breast cancer patients with tumour markers: CEA, TPA or CA15.3 vs MCA and MCA-CA15.3 vs CEA-TPA-CA15.3 panel in the early detection of distant metastases. BMC Cancer, 6(1):1186-1471.
21. Quaranta A, Johnson K and Tamela S (2007): Tumor markers in cancer breast. Clin Onc J., 67(5):34-54.
22. Ray A, Dhar S and Ray BK (2011): Control of VEGF Expression in Triple-Negative Breast Carcinoma Cells by Suppression of *SAF-1* Transcription Factor Activity. Mol Cancer Res., 9: 1030.
23. Roy H, Bhardwaji S and Herrituala S (2006): Biopsy of vuscular endothelial growth factor. FEBS letters J., 580(9):2879-2887.
24. Vagu V, Anand H, Andrew R and Weilbo V (2007): Recent patents on anticancer drug discovery. Clin. Cancer R., 2(1): 95-71.
25. Xiaowei YD, Yumei GU, Yun Ding WS, Mao J, Tan J, Zhao H, Han X and Sun Y (2008): Telomere Instability in a Human Tumor Cell Line Expressing NBS1 with Mutations at Sites Phosphorylated by ATM .Mol. Bio. J., 34:657-669.
26. Yi Yan G, Skliris P, Penner C, Chooniedass-Kothari S, Cooper C, Nugent Z, Blanchard A, Watson PH, Myal Y, Murphy LC and Leygue E (2009): Correlation of ER, PR, HER-2/neu, p53, and VEGF with Clinical Characteristics and Prognosis in Chinese W omen with Invasive Breast Cancer. The Breast Journal, 14(3): 308–310.

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