ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study of serum interleukin 18 and nitric oxide levels as prognostic markers in breast cancer patients, and their relation to different treatment modalities.

Mona Kamal Eldeeb[1*], Azhar Mohamed Nomair [1], Hanan Mohamed Nomeir[2], Gehan Abd Elatti Khedr[3], Ramadan Muhammed Rashidy[4]

1. Chemical Pathology Department , Medical Research Institute, Alexandria University, Egypt
2. Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Egypt
3. Clinical Oncology Department, Faculty of Medicine, Alexandria University, Egypt
4. General Surgery Department, Alexandria University Student Hospital, Egypt

Manuscript Info

Manuscript History:

Received: 15 April 2015
Final Accepted: 26 May 2015
Published Online: June 2015

Key words:

IL18 , breast cancer, Nitric oxide

Corresponding Author

Mona Kamal Eldeeb

Abstract

Etiology of breast cancer is mostly hormonal related with other modifying risk factors. The molecular mechanisms linking inflammation and tumorigenesis remained elusive. The role of IL-18 in cancer progression and metastasis remains controversial with insufficient data in the Egyptian population .Thus we aimed to study the rule of IL-18 as a prognostic marker in a group of Egyptian females with breast cancer and its relation to different treatment modalities. Case-control study was carried on 29 cancer breast female patients and comparable 15 controls. Follow up of patients were done at three and six months after completion of their planned treatment, assessments were done including: clinical, radiological examinations (if needed) and laboratory work-up (IL-18, CA 15-3, Nitric oxide) Results: IL18 had significant higher values in patients preoperatively than controls ($p < 0.001$) and no significant differences between the different time intervals. IL18 had negative significant correlation with CA15.3 ($r = -0.433$, $p = 0.019$) at three months post treatment , and negative correlation with NO both in three and six months post treatment ($r = -0.436$, $p = 0.018$ and $r = -0.433$, $p = 0.019$) respectively . IL18 had significant negative correlations with CA 15.3 in relapsed cases ($r = -0.821$, $p = 0.023$). Both NO and CA15.3 had the highest overall performance to predict relapse six months post treatment ($p = 0.039$). Conclusion IL 18 could have a possible role as a prognostic marker in breast cancer patients with a larger scale and a longer period of follow up needed.

INTRODUCTION

Breast cancer represents about 12% of all new cancer cases and 25% of all cancers in women.[(1)] It is the first ranked cancer among Egyptian females (32.0%) followed by liver cancer (13.5%).[(2)] It is a hormone related disease, in addition to other modifying risk factors. [(1)]

Cytokines may control host responses against tumour with increasing evidence that the cytokines contribute to tumour growth, progression and host immune-suppression. [(3)] Molecular mechanisms linking chronic inflammation and tumorigenesis have remained elusive, As some cytokines IL-2, IL-11, transforming growth factor (TGF) beta) stimulate, while others IL-12, IL-18, Interferons (IFNs) inhibit breast cancer proliferation and/ or invasion.[(4)]

Interleukin (IL)-18 was found to enhance the immune defense against tumor cells by activating and inducing the production of IFN-γ. IL-18 also augments the cytotoxic activity of natural killer (NK) and T cells and enhances

their production of other proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α), IL-1β , IL-8, and nitric oxide (NO).[5,6]

Some clinical studies had shown that the level of serum IL-18 may be a prognostic factor in patients with gastric carcinoma, hematological malignancies and metastatic breast cancer . [7]

Nitric oxide (NO) is a potent biological molecule involved in the multi-step process of tumorigenesis, including induction of apoptosis and promotion of angiogenesis.[8] These specific actions of NO in tumor biology are believed to be related to its concentration in tumor tissue and its interactions with other molecules including IL-18, IFN-γ, TNF-α and inducible NO synthase .[5]

Data studying the role of IL18 and NO as a prognostic markers and their correlations with different tumor markers and treatment modalities in Egyptian breast cancer patients is still insufficient , The present work aimed to study the possible rule of IL18 and NO as a prognostic markers and their correlations with different tumor markers and treatment modalities in Egyptian breast cancer in a group of Egyptian females with breast cancer.

Methods

Study setting & design

A case-control study was carried out on 29 female patients with breast cancer their age ranged from 35 to 55years and on a comparable control group of 15 females with age range from 32 to 54 years. Both groups were also comparable regarding menopausal state, parity, age at first birth and lactation. Patients were selected from the Clinical Oncology Department, Faculty of Medicine, Alexandria University and from the Surgery Department of Alexandria University Student Hospital .Written consents were taken from all participants and approval of the Ethical Committee was also obtained.

To all patients surgery was done as a primary treatment modality, either modified radical mastectomy (MRM) or breast conservative surgery (BCS). Different forms of post operative treatments (chemotherapy, radiotherapy and/or hormonal) were given to patients according to clinical and pathological indication for every case. Follow up of patients were done at three and six months after completion of their planned treatment, where assessments were also done including: clinical examination, radiological investigations (if needed) and laboratory work-up (measurement of IL-18, CA 15-3 , Nitric oxide and other routine laboratory investigations). Subjects were excluded if they had obvious inflammatory conditions, infectious, allergic, autoimmune, or other systemic diseases such as diabetes mellitus and hypertension.

History taking and clinical examination

History was taken from all patients with concern on: menopausal status , parity, age at first live birth and presence of mass or nipple discharge.

Clinical examination of patients were done with focus on: tumor size, presence of lymph nodes, metastasis and type of surgery either breast conservative surgery or modified radical mastectomy. In addition to radiological examination to detect presence of tumor and/or metastasis.

Laboratory Investigations

A diagnostic work up for all patients was followed that included; 1) Pathological examination of the specimen to determine tumor size, pathology type (infiltrative ductal carcinoma (IDC), invasive lobular carcinoma(ILC) or others), tumour grade, lymph node status , estrogen receptors (ER),progesterone receptors (PR), HER 2 and Ki67. In addition to routine laboratory investigations done (preoperatively and at 3 and 6 months after operation) included: complete blood picture(CBC)[9], fasting blood glucose level, erthyrocyte sedimentation rate (ESR), renal function tests (urea and creatinine) and liver function tests (alanine and aspartate aminotransferase).[10]

Enzyme-linked immunosorbant assay (ELISA) sandwich technique for measurement of IL-18 (eBioscience high performance assay, Vienna, Austeria)[11] was done as follows: serum was obtained by centrifugation at X 8000g for 15 minute and stored at -20^{o}C until assay. The assay procedure was done according to manufacture instructions and detection limit of the human IL-18 was (9 pg/ml) . CA 15-3 was determined by Chemiluminascence technique using the 1000 Immunoassay System and results were expressed as unit per ml. [12] Nitric oxide (NO) levels were also measured in the deproteinized serum samples using the Griess assay method,[13] which was based on reduction of nitrate to nitrite by nitrate reductase in the presence of β-nicotinamid dinucleotide phosphate (β-NADPH) where the concomitant oxidation of β- NADPH was monitored by the decrease in absorbance at 340 nm and concentrations were deduced from the appropriate standard curve and results were given as micromoles per liter .

Statistical analysis

Statistical analysis was performed using SPSS version 20.0. Normality testing was done through the Kolmogrov–Smirnov test. Normally distributed data was expressed in mean ± SD and F test (ANOVA) was used with repeated measures for comparing between the different periods. Abnormally distributed data was expressed in median (Min.- Max.) Wilcoxon test was used to compare between the different periods. Correlations between

studied parameters were done through the Spearman's rank correlation coefficient. Agreement of the different studied parameters at six month post operation was used and expressed as sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Receiver operating characteristic curve (ROC) was plotted to analyse a recommended cutoff, the area under the ROC curve denotes the diagnostic performance of the tests. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

Results

Table (1) showed the distribution of the studied cases according to different parameters. Where 17 (58.6%) of patients had T2 disease (size and/or extension of the primary tumor) at presentation. As regarding the degree of spread to regional lymph nodes 14 patients (48.3%) had N1(regional lymph node metastasis regional lymph node metastasis) while10 patients (34.5%) had N0 (absent lymph nodes) and only one patient (3.4%) of the 29 cases had distant metastasis. On pathological examination 25 (86.2%) had infiltrating ductal carcinoma (IDC), 26 (89.7%) were ER +ve, 23 (79.3%) were PR +ve and only10 (34.5%) were HER 2 +ve. According to mode of treatment received 18 (62.1%) of patients underwent breast conservative surgery. Patients received combinations of different treatment modalities where 27(93.1%) of patients received chemotherapy, and /or radiotherapy and/ or received hormonal therapy. During follow-up only 7 (24.1%) of patients developed relapse.

Regarding the comparison between different months according to IL18, CA15.3 and NO (table 2) showed significant higher levels of IL18 in patients preoperatively than controls ($p < 0.001$). No significant differences in IL 18 levels were found between the three different time intervals. CA 15.3 showed significant higher values in preoperative than controls and higher in pre-operation than six months post operation (p value <0.001, 0.013 respectively). While NO showed significant lower levels in control group than pre operation (p value <0.001) and significant higher levels in both three and six months post operation than pre-operation (p value <0.001)

Tables (3) showed that among the patients who received chemotherapy and /or radiotherapy and/ or hormonal therapy (n = 27) there were significant lower values of CA 15.3 in the six months post operation than pre operation (p value =0.027) and significant higher values of NO in three and six months post operation than pre operation were p value was ($p<0.001$). There were no significant difference in IL 18 levels between the three different periods.

Tables (4) and figures (1) showed clinical (sensitivity, specificity , positive and negative predictive values and accuracy) and the overall performance of IL18, CA15.3 and NO to predict relapse in breast cancer cases six months after treatment. Where both NO and CA15.3 showed highest overall performance to predict relapse six months post treatment (p =0.039).

Correlation studies between different studied parameters (table 5) showed that IL18 had negative significant correlation with CA15.3 ($r = -0.433$, $p = 0.019$) at three months post treatment , and negative correlation with NO both in three and six months post treatment ($r = -0.436$, $p = 0.018$ and $r = -0.433$, $p = 0.019$) respectively . In addition CA15.3 had positive significant correlation with NO in six months post treatment ($r = 1.000$, $p < 0.001$)

The studied correlations between IL18 and CA 15.3 in relapsed cases only showed negative significant correlation ($r = -0.821$, $p = 0.023$) as shown in table (6)

Table (1): Distribution of the studied cases according to different parameters

	No. (%)
Tumor size	
T1	9 (31%)
T2	17 (58.6%)
T3	3 (10.3%)
Nodes	
N0	10 (34.5%)
N1	14 (48.3%)
N2	5 (17.2%)
Metastasis	
M0	28 (96.6%)

M1	1 (3.4%)
Type of surgery	
Conservative	18 (62.1%)
MRM	11 (37.9%)
Pathology type	
IDC	25 (86.2%)
ILC	3 (10.3%)
Others	1 (3.4%)
Pathology grade	
G1	2 (6.9%)
G2	23 (79.3%)
G3	4 (13.8%)
ER +ve	26 (89.7%)
PR +ve	23 (79.3%)
HER 2 +ve	10 (34.5%)
Ki 67	
Low	6 (20.7%)
High	4 (13.8%)
Not available	19 (65.5%)
Chemotherapy	27 (93.1%)
Radiotherapy	27 (93.1%)
Hormonal therapy	27 (93.1%)
Follow up	
Non-relapsed	22 (75.9%)
Relapsed	7 (24.1%)

Table (2): Comparison between different months according to IL18, CA15.3 and NO

	Preoperative	**After 3 month**	**After 6 month**	**Controls**
IL18(pg/ml)	163.47 (60.02 – 1187.23)	163.96 (5.47 – 704.38)	188.89 (44.70 – 902.57)	112 (40.05-400.78)
P				<0.001*
P_1		0.496	0.112	
CA 15.3(U/L)	33.50 (0.03- 80.80)	22.50 (8.50 – 80.30)	20.80 (1.60 – 47.30)	12.60(1.56-32.40)
P				<0.001*
P_1		0.567	0.013*	
NO(µmol/L)	290.45 ± 126.28	3962.42 ± 1722.82	54057.59 ± 23503.67	62.5±23.2
P				<0.001*
P_1		<0.001*	<0.001*	

P : Stastical significance difference between patients preoperatively and controls

Table (3): Comparison between different months according to IL18, CA15.3 and NO in cases with different postoperative treatment modalities (chemotherapy, radiotherapy and /or hormonal therapy) (n = 27)

	Preoperative	After 3 month	After 6 month
IL18(pg/ml)	164.72 (60.02 - 1187.23)	163.96 (5.47 - 704.38)	188.89 (44.70 - 902.57)
P		0.829	0.212
CA 15.3(U/L)	32.40 (0.03 – 80.80)	22.50 (8.50 – 80.30)	20.80 (1.60 – 47.30)
P		0.755	0.027*
NO(µmol/L)	294.33 ± 129.28	4015.36 ± 1763.67	54779.77 ± 24060.93
P		<0.001*	<0.001*

P: Stastical significance difference between patients preoperatively and three and six months post-treatment

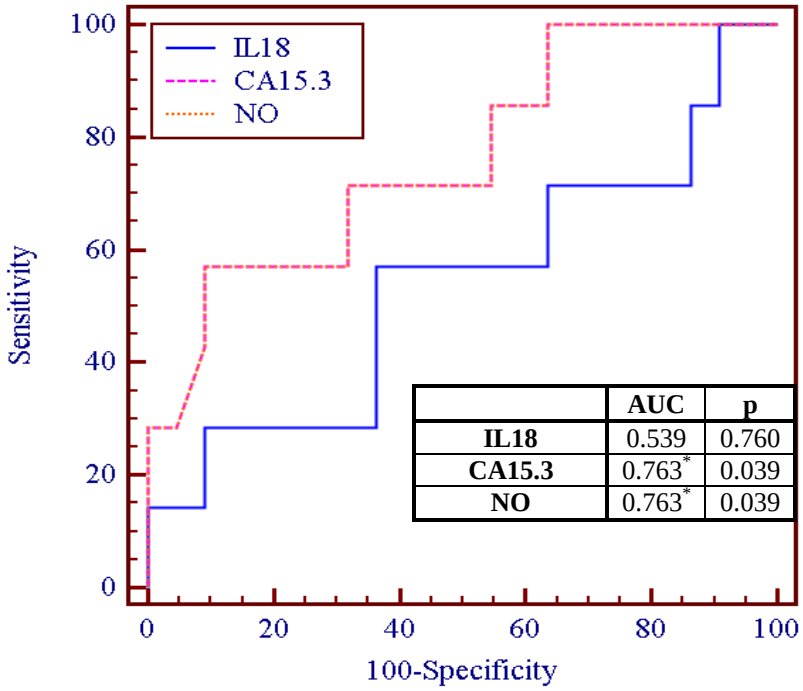

Figure (1):ROC curve for IL18, CA15.3 and NO after 6 month to predict relapse

Table (4): Clinical (sensitivity, specificity , positive and negative predictive values and accuracy) of IL18, CA15.3 and NO after 6 months with relapse in breast cancer cases

	Non-relapsed	Relapsed	Sensitivity	Specificity	PPV	NPV	Accuracy

IL18	**>131.19**	14	4	42.86	63.64	27.27	77.78	58.62
	≤131.19	8	3					
CA15.3	**≤22.2**	15	2	71.43	68.18	41.67	88.24	68.97
	>22.2	7	5					
NO	**≤56623.01**	15	3	57.14	68.18	36.36	83.33	65.52
	>56623.01	7	4					

Table (5): Correlation between IL18, CA15.3 and NO in each period

	Preoperative		**After 3 month**		**After 6 month**	
	r_s	**P**	**r_s**	**p**	**r_s**	**P**
IL18 vs CA 15.3	-0.303	0.110	-0.217	0.259	-0.433*	0.019
IL18 vs NO	-0.249	0.194	-0.436*	0.018	-0.433*	0.019
CA 15.3 vs NO	0.169	0.380	0.186	0.333	1.000*	<0.001

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Table (6): Correlation between IL18 and CA15.3 in different periods in relapsed cases

	Preoperative		**After 3 month**		**After 6 month**	
	r_s	**P**	**r_s**	**p**	**r_s**	**P**
IL18 vs CA 15.3	-0.714	0.071	0.250	0.589	-0.821*	0.023

Discussion

Breast cancer is a complex disease; its aetiology is multifactorial, the period of development can span decades and clinical course is highly variable. [7] Cytokines including transforming growth factor-ß (TGF-ß), tumour necrosis factor-· (TNF-·), interleukins IL-1, IL-6, IL-10, IL-12, IL-18 and the interferons all play a key role in controlling the immune response. In addition to their role in chronic inflammation, changes in cytokine levels mediated by the tumour both directly and indirectly, thus they are important parameters that affect the course of disease. [7]

IL-18 is a pleiotropic, pro-inflammatory cytokine that plays a critical role in tumor migration, invasion, and metastasis, with dual effects on tumor development and progression. Also, IL-18 increases the escape immune recognition, increase adherence to the microvascular wall and induce production of angiogenic and tumor growth-stimulating factors via IL-18-dependent mechanism. The role of IL-18 in cancer progression and metastasis remains controversial.[4] Thus we aimed to study the role of IL-18 as a prognostic marker in a group of Egyptian females with breast cancer and its relation to different treatment modalities and tumor markers .

In the present study IL-18 levels were significantly higher in breast cancer patients before operation than controls ($p < 0.001$), this in agree with (Gunel N et al,2002) and (Eissa SA et al, 2005) [5,6] who found significant increase in IL18 level in breast cancer patients than controls which could be attributed to the defense mechanism against tumour growth and metastasis .

IL-18 induces endothelial cell migration and angiogenesis by binding and activating endothelial cells and indirectly by vascular endothelial growth factor. IL-18 mediates inflammatory processes by binding to its specific receptor, IL-18 receptor, and initiating the activation of different signaling cascades leading to changes in target cells gene expression and behavior. Thus IL-18 play an important role as immunomodulatory factors in cancer pathogenesis. [4,14]

The high serum levels of IL-18 in patients before operation at the start of the study in addition to the small percentage of relapse could explain the presence of non significant difference in the IL 18 level between relapsed and non relapsed cases.

The role of IL-18 in immunomodulation and inflammatory process could explain the non significant difference in patients three and six months post treatment than pre-operation .[4,14,15] Chronic inflammation is associated with tumor development and progression. IL-18 plays a central role in inflammation and the immune response, contributing to the pathogenesis and pathophysiology of infectious and inflammatory diseases. [16]

High levels of IL-18 production may play a major role in the growth, invasion and metastasis of renal cancer [16] Higher expression of IL-18 is detected in various cancer cells. [4,17]

IL-18 together with VEGF are markers for transformation of benign cells to metastatic cancer cells. (Jablonska E etal ,2005) [18] reported that concentrations of VEGF and IL-18 in the serum are sensitive tumor markers in this patient group before and after treatment and that high production of VEGF was associated with low production of IL-18. [4] This could also explain the non significant difference in IL-18 levels in patients before and after treatment. Also some anti cancer drugs act by inducing or inhibiting specific cytokines. Alpha-Galactosylceramide (alpha-GalCer) shows antitumor effects by activating natural killer (NK) cells indirectly through stimulation of the secretion of cytokines IL-18.[4]

(Coskun U et al ,2004) [8] found that serum IL-18 levels were decreased by a ratio of 17% after tamoxifen therapy ($P < 0{\cdot}05$). This finding was not unexpected and does not seem to be a desirable effect of tamoxifen therapy, because both tamoxifen and IL-18 have well-known antitumour activities. So it was hypothesized that decreased VEGF may inhibit angiogenesis and increased NO may induce apoptosis with tamoxifen therapy; therefore, serum IL-18 levels can be found to be reduced by a feedback mechanism in IL-18, NO and VEGF activity. Moreover, it can also be considered that the antitumour action of tamoxifen is not mediated by IL-18, and changing levels of IL-18 with tamoxifen therapy may be related to its interactions with other cytokines.

Careful preclinical studies are needed to determine the proper application of IL-18 in cancer therapy as the molecular mechanisms linking inflammation and tumorigenesis have remained elusive, where some cytokines IL-2, IL-11, transforming growth factor (TGF) beta) stimulate tumorigenesis , while others IL-12, IL-18, Interferons (IFNs) inhibit breast cancer proliferation and/ or invasion. [4]

Only one patient in our study had metastasis with no significant increase in IL-18 level than the non-metastatic patients. This could be due to the fact that other studies had larger number of patients with metastasis in compare with other groups, also IL-18 could be associated with different disease processes, such as inflammation and infection so this could affect the precision of the results. [15]

The prognostic value of initially high CA 15-3 level in patients with invasive breast cancer has been widely demonstrated. And the routine use of CA 15-3 in the preoperative assessment of primary breast cancer is strongly recommended . (Alsaeed EF etal , 1995 S. Chourin etal, 2009 and Elbeling FG etal, 2002) [12,19,20] suggested that elevated preoperative CA15.3 levels are associated with advanced TNM staging, histological grade, and basal and HER2/neu-receptor status, and thus a high risk of recurrence locally or at distant sites.

IL 18 was negatively correlated with CA 15.3 six months post treatment together with the significant lower levels of CA15.3 six months post treatment than pre-operation ,this may add to the probable prognostic role of IL-18 in cancer breast patients.

NO appears to have both promoting and inhibiting effects on tumorigenesis. It causes DNA damage and promotes angiogenesis as a potential endogenous carcinogen. Conversely, it suppresses tumor growth and metastasis by inducing apoptosis. [5]

In our study the elevated NO levels in three and six months post treatment may be a result of increased NO synthetase II activity which is stimulated by the host defense mechanism against tumor growth and the non significant low level of IL 18 which may be due to tight regulatory mechanism between NO and IL-18 activity. [5] Which agrees with our result as NO had significant negative correlation with IL-18 both three and six months post treatment. In addition to (Coskun U *et al* , 2004) [8] reported that increased NO production with tamoxifen therapy has an important role in terms of antiproliferative and apoptotic tumor suppressive effects .However , (Kimelberg *et al*, 2000) [21]reported that tamoxifen is a potent inhibitor of NO synthase activity and (Chen *et al*,1999) [22] reported that tamoxifen antagonized oestradiol-stimulated NO release in endothelial cells.

Thus as a result, the effect of tamoxifen on NO expression in carcinogenesis seems to be complex and sometimes contradictory. where in another study Serum nitrate + nitrite levels were found to be elevated by a ratio of 9% after tamoxifen therapy, but this was not statistically significant ($P > 0{\cdot}05$). In addition, some specific actions of NO in tumour biology are considered to be related to its interactions with other molecules and IL-18 and IFN-g [23,24]

In the present work, the overall performance of the three studied parameter to predict relapse after six months of treatment was shown by drawing receiver operating curve (ROC) curve , where the area under the curve (AUC) for NO and CA 15.3 was 0.763, p =0.039 and for IL-18 was 0.539, p=0.760 . This indicates that NO was a better test in predicting relapse in cancer breast patients , thus it could be used as a prognostic marker.

Conclusion:

1. IL 18 could have a possible role as a prognostic marker in breast cancer patients.
2. More studies are needed on a larger number of breast cancer patients with longer period of follow-up to detect the effect breast cancer treatment on IL 18 levels.
3. NO could be used as a prognostic marker in breast cancer patients.

References:

1) Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C et al (2012): Cancer Incidence and Mortality Worldwide 2012: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]

2) Ibrahim AS, Khaled HM, Mikhail NN, Baraka H and Kamel H (2014): Cancer incidence in Egypt: results of the national population-based cancer registry program. Journal of Cancer Epidemiology 2014. Epub 2014 Sep 21.

3) SraboviC N, Mujagic Z, Mujanović-Mustedanagic J, Muminovic Z, Čičkušić E (2011): Interleukin 18 expression in the primary breast cancer tumour tissue. Med Glas Ljek komore Zenicko-doboj kantona . 8:109-115.

4) Kuppala MB, Syed SB, Bandaru S, Varre S, Akka J, Mundulru HP (2012): Immunotherapeutic Approach for Better Management of Cancer - Role of IL-18. Asian Pacific J Cancer Prev .13 : 5353-5361.

5) Günel, N., Coşkun, U., Sancak, B., Günel, U., Hasdemir, O. and Bozkurt, Ş(2002): Clinical importance of serum interleukin-18 and nitric oxide activities in breast carcinoma patients. Cancer. 95: 663–667.

6) Eissa SA, Zaki SA, El-Maghraby SM, Kadry DY (2005): Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. J Egypt Natl Canc Inst. 17:51-55.

7) Rao VS, Dyer CE, Jameel JK, Drew PJ, Greenman J. (2006): Potential prognostic and therapeutic roles for cytokines in breast cancer (Review). Oncol Rep. 15(1):179-185.

8) Coskun U, Gunel N, Sancak B, Onuk E, Bayram M, Cihan A.(2004): Effect of tamoxifen on serum IL-18, vascular endothelial growth factor and nitric oxide activities in breast carcinoma patients. Clin Exp Immunol. 137(3):546-551.

9) Buttarello, M; Plebani, M.(2008): Automated blood cell counts: state of the art. American journal of clinical pathology .130 : 104–116

10) Burtis CA, Ashwood ER and Bruns DE.(2008): Tietz fundamentals of clinical chemistry. 6th Ed. Saunders Elseviers, pp :363-372.

11) Liu W, Tang Q, Jiang H, Ding X , Liu Y, Zhu R, etal .(2009): Promoter Polymorphism of Interleukin-18 in Angiographically Proven Coronary Artery Disease. Angiology. 60:180-85.

12) Teitz NW, editor.(1995): Clinical guide to laboratory tests .3rd ed. Philadelphia :W.B. Saunders , pp:106.

13) Moshage H, Kok B, Huizenga JR, Jansen PL.(1995) Nitrite and nitrate determination in plasma: a critical evaluation. Clin Chem. 41:892–896.

14) Volin MV, Koch AE.(2011): IL-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis. J Interferon Cytokine Res. 31: 745-751

15) Fakieh RA, Taha S and Bakhiet M.(2012): The role of IL-18, CCL5 and their receptors as diagnostic markers in breast cancer. GERF Bulletin of Biosciences. 3(2):1-8

16) Sáenz-López P, Carretero R, Vazquez F, Martin J, Sánchez E, Tallada M et al .(2010): Impact of interleukin-18 polymorphisms-607 and -137 on clinical characteristics of renal cell carcinoma patients. Hum Immunol . 71;309-313.

17) Park S, Cheon S, Cho D.(2007) : The dual effects of interleukin-18 in tumor progression. Cell Mol Immunol.4: 329-335.

18) Jablonska E, Puzewska W, Grabowska Z, Jablonski J, Talarek L . (2005): VEGF, IL-18 and NO production by neutrophils and their serum levels in patients with oral cavity cancer. Cytokine .30: 93-99.

19) S. Chourin , D. Georgescu , C. Gray , C. Guillemet , A. Loeb , C. Veyret et al .(2009):Value of CA 15-3 determination in the initial management of breast cancer patients. Ann Oncol . 20: 962-964.

20) Ebeling FG , Stieber P, Untch M, Nagel D, Konecny GE, Schmitt U.M et al .(2002) : Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. B. J. Cancer . 86: 1217–1222

21) Kimelberg HK, Feustel PJ, Jin Y , Paquette J, Boulos A, Keller RW et al .(2000): Acute treatment with tamoxifen reduces ischemic damage following middle cerebral artery occlusion. Neuroreport. 11:2675–2679.

22) Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mandelsohn MH, Shaul PW. (1999): Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest . 103:401–406.
23) Chikano S, Sawada K, Shimoyama T, Kashiwamura SI, Sugihara A, Sekikawa K et al.(2000): IL-18 and IL-12 induce intestinal inflammation and fatty liver in mice in an IFN-gamma dependent manner. Gut . 47:779–786.
24) Brennan P, Zaki G, Spedding A, Langdon J.(2001): Type II nitric oxide synthase expression corralates with lymph node status in oral squamous cell carcinoma. J Oral Pathol Med. 30:129–134.