MedCrave
Step into the World of Research
Journal of Cancer Prevention & Current Research

Research Article
Open Access
CrossMark

Expression of cytokeratin-18 in serum as a biomarker of apoptosis & necrosis in carcinoma breast

Abstract

Background: Cytokeratin expression is helpful in differentiating between specific breast cancer subtypes and as prognostic indicator.

Objectives: To compare the cytokeratin-18 levels between breast cancer and healthy control. To compare cytokeratin-18 levels before and after neo-adjuvant chemotherapy (NAC) and to correlate cytokeratin-18 levels of pre and post chemotherapy with the percentage response to chemotherapy as evaluated by the RECIST criteria.

Methods: Thirty patients of histologically confirmed invasive breast carcinoma and ten healthy individuals were taken as controls. The estimation of cytokeratin-18 was done at presentation and after two cycles of NAC. The change in level of serum cytokeratin-18 levels following two cycles of chemotherapy has been correlated with the RECIST criteria of response to chemotherapy.

Results: Mean age of the patients was 48.73 years (range 28-80 years). The mean cytokeratin-18 level in carcinoma breast patient pre-treatment was 156.27 IU/L and in control group was 279.30 IU/L. The mean Cytokeratin 18 levels in patients before and after chemotherapy was 156.27 IU/L and 181.77 IU/L respectively ($p<0.05$). The average diameter of tumor before and after chemotherapy was 5.33 and 3.73 cm respectively. On correlation of percentage change of Cytokeratin 18 levels before and after chemotherapy and response to chemotherapy as evaluated by the RECIST criteria the following result was obtained: complete response (n=2): 76% rise of CK-18, partial response (n=21): 24% rise of CK-18, stable disease (n=5): 22% decline of CK-18, progressive disease (n=2), 21% decline of CK-18.

Conclusion: The expression of cytokeratin-18 is a useful biomarker in breast cancer patient. Serum levels of CK-18 can be used to judge the efficacy of neo-adjuvant chemotherapy. Depending on post chemotherapy changes of CK-18 levels, the most appropriate chemotherapy regimen can be selected for individual patients.

Keywords: breast cancer, cytokeratin-18, RECIST criteria, apoptosense-M 30, immunohistochemical assessment, intermediate filaments

Volume 5 Issue 2 - 2016

Agni Gautam Shah,[1] Ram Niwas Meena,[1] Surendra Pratap Mishra,[2] SCU Patne,[3] Shashi Prakash Mishra,[1] Roshni Gavel,[2] Seema Khanna,[1] Rahul Khanna[1]

[1]Department of General Surgery, Institute of Medical Sciences, India
[2]Department of Biochemistry, Institute of Medical Sciences, India
[3]Department of Pathology, Institute of Medical Sciences, India

Correspondence: Rahul Khanna, Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, UP, India,
Email dr_rahul_kanna@rediffmail.com

Received: June 05, 2016 | **Published:** July 14, 2016

Introduction

Keratins are epithelia-specific intermediate filament proteins, which are expressed in a tissue-specific manner.[1] Around 50 keratin genes have been discovered across the species. Immunohistochemical assessment of members of the Cytokeratin (CK) family has been used in the histopathological evaluation of breast carcinoma.

In normal breast tissue, CK5 and CK14 are expressed in myoepithelial cells while CK7, CK8, CK18 and CK19 are expressed in the ductal epithelium. CK20 is not detectable in breast epithelium.[2] During the development of malignancy, the original CK profile of the cell is often retained. As a result, different types of breast cancers can be characterized according to a purported cell of origin within the breast, based on differential CK expression patterns.

In current practice, evaluation of CK profiles is used for the detection of small tumor foci in sentinel lymph nodes or other metastatic sites.[3,4] Cytokeratin expression is also helpful in differentiating between specific breast cancer subtypes,[5] and as a prognostic indicator.[6] There is evidence to suggest that CK-18 is involved in the invasive or growth properties of breast cancer. There is also a relationship between caspase cleaved CK-18 fragments and disease risk of patients with cancer as well as therapeutic response. The measurement of caspase cleaved or total CK-18 levels from epithelial derived tumors could be a simple, non-invasive way to monitor or predict tumor progression, prognosis and response to chemotherapy.

In the present study, we compare cytokeratin-18 levels between breast cancer patients and age and gender matched healthy controls and compare the changes in cytokeratin-18 levels before and after neo-adjuvant chemotherapy. We also correlate the percentage change of cytokeratin-18 levels pre and post chemotherapy with the percentage response to chemotherapy as evaluated by the RECIST criteria.

Methods

This prospective study was undertaken on thirty patients with previously untreated, histological confirmed invasive breast carcinoma in the Department of General Surgery with collaboration of Department of Biochemistry and Department of Pathology, Institute of Medical Sciences, Banaras Hindu University between October 2013 and June 2015. The ethics committee of the approved the study protocol. In this study, we excluded pregnant women, patients on

OCPs for last 3 months, receiving HRT and patients with inflammatory breast diseases. Ten normal age matched women free from all diseases were recruited as control subjects.

The estimation of cytokeratin-18 was done by human apoptosense-M 30 (Ap-M30) ELISA kit at presentation and after two cycles of neo-adjuvant chemotherapy (combination of cyclophosphamide, adriamycin and 5FU given at 3 weekly interval). The initial values of CK-18 was correlated with stage of disease, various patient parameters such as parity, menopausal status, use of OCP, HRT, Tumor grade and hormonal receptor status. The change in level of serum cytokeratin-18 following two cycles of chemotherapy has been correlated with the RECIST criteria of response to chemotherapy in the tumor.

The statistical analysis was done using SPSS for Windows version 16.0. Chi-square test was used to compute the statistical association between 2 variables, and the Student's't' test was used to compare means. For paired samples Paired Student's't' test was used. The critical value of 'p' indicating the probability of significant difference was taken as <0.05.

Results

In our study, the mean age of the patients was 48.73 years. Breast cancer was found to be more common among post menopausal women 70% (21/30). There was no history of OCP intake or family history of breast cancer in our patients. All the patients were married with 86.6% patient having two children or more. Most of the patients had infiltrating ductal carcinoma (93.33%) on FNAC. The clinical characteristics of the breast cancer patients included in this study are summarized in (Table 1).

Table 1 Clinical characteristics of the breast cancer cases

Variables		No. (%)
Breast Pain (n=30)	Present	24(80%)
	Absent	6(20%)
Side (n=30)	Right	15(50%)
	Left	15(50%)
Lump (n=30)	Present	100(100%)
	Absent	0(0.0%)
NAC involvement (n=30)	Present	22(73%)
	Absent	8(26.7%)
Nipple retraction (n=30)	Present	19(63.3%)
	Absent	11(36.7%)
Nipple Discharge (n=30)	Present	11(36.7%)
	Absent	19(63.3%)
Skin involvement (n=30)	Present	16(53.3%)
	Absent	14(46.7%)
Ulceration (n=30)	Present	7(23.3%)
	Absent	23(76.7%)
Peau de orange (n=30)	Present	16(53.3%)
	Absent	14(46.7%)
Fixity chest wall (n=30)	Present	2(6.7%)
	Absent	28(93.3%)
Edema arm (n=30)	Present	2(6.7%)
	Absent	28(93.3%)
Lymph node involvement (n=30)	Present	27(90%)
	Absent	3(10%)
Ipsilateral axillary (n=30)	Present	27(90%)
	Absent	3(10%)
Supraclavicular (n=30)	Present	1(3.3%)
	Absent	29(96.7%)
Number of lymph node (n=30)	0	3(10%)
	1	21(70%)
	2	3(10%)
	3	3(10%)
	No nodes	3(10%)
Fixity of Nodes (n=30)	Present	3(10%)
	Absent	24(80%)
T staging (n=30)	T3	13(43.3%)
	T4a	1(3.3%)
	T4b	15(50%)
	T4c	1(3.3%)
N staging (n=30)	N0	3(10%)
	N1	23(76.7%)
	N2a	3(10%)
	N2c	1(3.3%)
M staging (n=30)	M0	100(100.0%)
	M1	0(0.0%)

The mean cytokeratin-18 level in carcinoma breast patient was 156.27±95.015 IU/L and in control group was 279.30±37.883 (p-value <0.001) (Table 2). Two of our patients had stage II and twenty eight had stage III breast cancer. There was minimal difference in CK-18 levels as per the stage distribution (Table 3).

Table 2 Comparison of Cytokeratin-18 (M-30 antigen) level between breast cancer patients (pre chemotherapy) and controls

Variables	Cytokeratin pre (Mean±SD)	t-value	p-value
Case (n=30)	156.27±95.015 IU/L	3.963	<0.001
Control (n=10)	279.30±37.883 IU/L		

Table 3 Comparison of pre chemotherapy and post chemo-therapy response with Cytokeratin-18 level as per stage of disease

	Post Chemotherapy (mean±SD)	Pre Chemotherapy (mean±SD)	p-value
All breast cancer patients (n=30)	156.27±95.015	181.77±66.597	0.032
Stage			
II (n=2)	141.00±4.2431	159.00±42.4261	0.077
III (n=28)	57.36±98.374	83.39±68.234	< 0.001
Size of tumor (cm)	5.33±1.749	3.733±1.6595	<0.001

Correlation of pre and post chemotherapy response of tumor (as per RECIST criteria) with cytokeratin-18 level is shown in Table 5. We found that 21 out of 30 (70%) breast cancer patients demonstrated partial response to chemotherapy i.e. reduction in sum of diameters of the target lesion by more than 30%. These patients had a 34% increase in CK-18 levels after the chemotherapy compared to pre-chemotherapy levels (p<0.001). Only 2 patients (out of 30) demonstrated complete response i.e. complete disappearance of tumor on clinical and radiological evaluation and they had 77% rise of CK-18 levels after the chemotherapy. Patients with stable disease (n=5) and progressive disease (n=2) had a post-chemotherapy decline of CK-18 levels by 23% and 22% respectively (Tables 4&5).

Citation: Shah AG, Meena RN, Mishra SP, et al. Expression of cytokeratin-18 in serum as a biomarker of apoptosis & necrosis in carcinoma breast. *J Cancer Prev Curr Res.* 2016;5(2):238–242. DOI: 10.15406/jcpcr.2016.05.00158

Table 4 Tumor response to chemotherapy evaluated as per RECIST criteria

Response	No. (%)
Complete Response	2
Partial Response	21
Stable Disease	5 (16.7%)
Progressive Disease	2
Total	**30 (100.0%)**

Discussion

Every year more than one million new cases of breast cancer are diagnosed worldwide and it is the leading cause of cancer death among women. It is the most commonly occurring neoplasm in women, and the second most common tumor, after lung cancer, in both genders.[7] It has a varied clinical, pathologic, molecular features and treatment modalities. Biomarkers of breast cancer are necessary for prognosis; follow up, prediction of response to chemotherapy and as possible therapeutic targets. Prognostic biomarkers provide information regarding outcome irrespective of therapy, while predictive biomarkers provide information regarding response to therapy.

Cytokeratin are filamentous structures present in cell cytoplasm and nucleus. In eukaryotic cells, the cytoskeleton is composed of three different types of morphologically distinct filamentous structures: microfilaments, intermediate filaments (IF) and microtubules. The integrated cytoskeletal network formed by the three filament systems maintains the mechanical integrity of the cell and participates in several cellular processes such as cell division, motility and cell-cell contact. The IF protein family is the most complex and includes several hundred different members. Based on their characteristics, sequence similarities and expression intermediate filaments are classified. Cytokeratin are type I and II intermediate filaments (acidic and basic proteins respectively). Type III includes desmin, vimentin and glial fibrillary acidic proteins. Type IV is neurofilament proteins. Type V is called nuclear lamins which are exclusive to cell nuclei and types VI include filensin and phakinin.[8]

Table 5 Changes in cytokeratin-18 (M-30 antigen) levels before and after chemotherapy correlated with response to chemotherapy (as per RECISTcriteria)

Response	Pre chemo-therapy	Post chemo-therapy	p-value	Percentage change in CK-18 level
Complete response (n=2)	103.50±36.06	183.00±55.15	0.434	76.81%
Partial response (n=21)	135.62±76.02	181.81±64.24	<0.001	34.05%
Stable disease (n=5)	232.40±117.03	180.0±84.21	0.036	-22.54%
Progressive disease (n=2)	235.50±184.55	184.50±120.91	0.46	-21.65%

There are more than 20 types of cytokeratins divided into type I and II. Cytokeratin 1-18 are neutral to basic protein components and are classified as type II. Cytokeratin 9-20 are acidic proteins and are called type I. Apoptosis also called as programmed cell death is a physiological process consisting of a well choreographed sequence of events leading to structural and biochemical changes in the cell and eventually apoptosis. Cancer is caused by failure of cells to undergo appropriate apoptotic cell death.[9] Cytokeratin-18 is one of the most common and characteristic member of the large intermediate filament family found in simple or single layer epithelial tissue. Cytokeratin-18 may modulate intracellular signaling and apoptosis via interaction with various proteins.[10] There is evidence to suggest that Cytokeratin-18 is involved in the invasive or growth properties of tumors. There is also a relationship between caspase-cleaved Cytokeratin-18 fragments and disease risk in patients with cancer as well as therapeutic response.

Cytokeratin degradation occurs during apoptosis and caspase cleavage of Cytokeratin leads to formation of apoptotic bodies and amplifies the apoptotic signal. Cellular release of Cytokeratin 18 and 19 fragments into the extracellular space occurs as a result of caspase digestion during intermediate stage of apoptosis and can serve as a marker of apoptosis. The measurement of caspase-cleaved or total CK18 from epithelial- derived tumors could be a simple, noninvasive way to monitor or predict tumor progression,[11] prognosis[12] and response to chemotherapy.[13] The observation regards to patient's demographic data, clinical, pathological and radiological status of carcinoma breast and their correlation with expression of level of cytokeration-18 are documented in Tables 1-5. The tumor size reduction in response to neoadjuvant chemotherapy was determined objectively as per RECIST criteria.

All patients included in this study were female. The age of the patients of carcinoma breast ranged from 28 -80 years with mean age at presentation being 48.73 years. Out of 30 patients, 21 (70%) were postmenopausal. There was no history of OCP intake or family history of breast cancer. All the patients were married with 86.6% patient having two or more children. On clinical staging of disease predominance was seen in stage T4b (50%) followed by T3 (43.3%) and T4a & T4b with 3.3% cases each. Most of the patients (76%) had N1 nodal status.

There was no clinical or radiographic evidence of distant metastasis. Infiltrating ductal carcinoma was found in 93.33% cases on FNAC with 93.3% patients in stage III disease.

In our study the mean cytokeratin-18 level in carcinoma breast patient was 156.27 IU/L and in control group was 279.30 IU/L ($p < 0.001$). This could be attributed to the fact that apoptosis is significantly lesser in breast cancer patients compared to normal people. The basic causes of cancer progression are loss of apoptosis resulting in immortalization of the affected cell population. The mean levels of cytokeratin-18 in patient prior to chemotherapy was 156.27 IU/L & after two cycles of chemotherapy was 181.77 IU/L ($p<0.01$).

Demiray et al.,[14] and Hagg Olofsson et al.,[15] reported significant increases in the levels of caspase-cleaved CK18 after administration of anthracycline therapy. Caspase-cleaved CK18 levels in responders showed significant increases at 24 and 48 h of treatment, while significant increases were not observed in non-responders. Linder Stig et al.,[16] observed increased levels of caspase-cleaved cytokeratin-18 in serum during chemotherapy. These increases were primarily observed in patients who responded to therapy. Interestingly in some responding patients increases in total cytokeratin-18 but not in apoptosis specific caspase-cleaved cytokeratin-18 were observed. Apoptosis may therefore not be the dominating death mode of tumors of these patients.

In our study the diameter of target lesions was measured before and after chemotherapy showing mean size of 5.33cm & 3.73cm respectively. The response as per RECIST criteria of the tumor showed regression in the sum of diameters of target lesions by at least 30%

Citation: Shah AG, Meena RN, Mishra SP, et al. Expression of cytokeratin-18 in serum as a biomarker of apoptosis & necrosis in carcinoma breast. *J Cancer Prev Curr Res.* 2016;5(2):238–242. DOI: 10.15406/jcpcr.2016.05.00158

demonstrating partial response in 70% (21/30) cases whereas stable disease i.e. neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease in the sum of diameters was seen in five cases. In two cases complete response i.e. disappearance of all target lesions in short axis to <10mm was recorded. In two cases the disease was progressive type showing at least a 20% increase in the sum of diameters of target lesion with increase in the sum of at least 5mm.

We compared the responses to chemotherapy as measured by RECIST criteria with changes in serum cytokeratin-18 levels. It was observed that in the group of partial response (21/30) there was significant rise in the level of cytokeratin-18 level after chemotherapy (mean=181.81) compared to pre chemotherapy level (mean=135.62). In stable disease group (5/30) also there was significant variation in the form of 23% decline of CK-18 levels. But in complete response group (2/30) and progressive disease group (2/30) the variation was not significant due to less number of cases.

In patients exhibiting Complete Response to chemotherapy (n=2), there was a 76.81% rise in CK-18 levels following chemotherapy as compared to pre chemotherapy levels. This figure was 34.05% in Partial responders (n=21). Patient with stable disease (n=5) and Progressive disease (n=2) had a decline in CK-18 by 22.54% and 21.65% respectively after the chemotherapy. The results show that there is a significant increase in serum cytokeratin-18 levels in patients with a good response to chemotherapy (Cyclophosphamide/ Adriamycin/5-fluorouracil) in the breast cancer patients.

When apoptosis is induced Cytokeratin-18 is cleaved from aspartate amino acids localized at position 238 and 396. Monoclonal antibody M30 recognizes the neo-epitome of Cytokeratin-18 formed after cleavage by the caspases. This newly formed neo-epitope can be regarded as a selective biomarker of apoptosis. It has been reported that the M-30 antigen assay which detects this neo-epitope reflects apoptosis accurately. Therefore M-30 antigen has been used as a marker for pharmacodynamic studies in cancer. Ulukaya Engin et al.,[17] measured the M-30 antigen levels before and after chemotherapy in breast cancer patients. They found that M-30 antigen significantly increases following chemotherapy. The degree of apoptosis is reflected by the M-30 antigen level in serum and may indicate better outcome in patients receiving adjuvant therapy. The M-30 antibody detects a caspase-cleaved products, CK 18- Asp396 (also called M30- antigen) of the cytoskeletal protein cytokeratin-18.

Measurements of cytokeratin-18 release may be particularly useful in clinical situations. Many patients with advanced disease do not respond to therapy with radiologically measurable decreases in tumor mass, but rather with a more vague state of 'stable disease'. This category is likely to be quite heterogeneous, where some but not other patients benefit from chemotherapy.[18] Response to chemotherapy in such patients could probably be better assessed by serial measurement of CK-18 levels during the chemotherapy protocols. Cytokeratin-18 can also be used for the detection of small tumor foci in sentinel lymph nodes or other metastatic sites.[3,4] Cytokeratin expression is also helpful in differentiating between specific breast cancer subtypes,[5] and as prognostic indicator.[19]

Woelfle Ute et al.,[20] reported that loss of Cytokeratin-18 expression might be critical to breast tumor progression. They speculated that Cytokeratin-18 may play an active role or may reflect more upstream processes. Olofsson et al.,[15] postulated that induction of necrotic cell death may be the pathway for clinical efficacy of anthracycline based therapy for breast cancer with defective apoptosis pathway. They suggested that Cytokeratin-18 biomarker may be a useful tool for early prediction of response to chemotherapy in breast cancer. Similar results have been obtained in our study and we found that elevation of serum Cytokeratin-18 levels was highest in patients showing good response to chemotherapy. Patients who had a complete response (n=2) had a 76.81% and partial response (n=21) had a 34.05% rise of Cytokeratin-18 levels post chemotherapy compared to pre chemotherapy levels. Patients with stable and progressive demonstrated a decline of Cytokeratin-18 levels after chemotherapy.

The inference of these findings is that patients with rise of circulating serum Cytokeratin-18 (M-30) levels following chemotherapy are responding to their chemotherapy protocol. Patients with stable or decreasing Cytokeratin-18 (M-30) levels are probably not responding to the chemotherapy and need to be put on alternative or second line chemotherapy protocol. Thus Cytokeratin-18 (M-30) circulating levels in serum may be a useful predictor of response to chemotherapy.

In conclusion, the expression of cytokeratin-18 as a biomarker in breast cancer patient strongly suggest that caspase-cleaved CK18 can be used to determine the efficiency of cytotoxic drug treatment in patient serum samples. The ability to compare the efficiencies of different treatment modalities would be very useful during development of new anticancer drugs.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Moll R, Franke WW, Schiller DL, et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell*. 1982;31(1):11–24.
2. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. *Histopathology*. 2002;40(5):403–439.
3. Alvarenga CA, Paravidino PI, Alvarenga M, et al. Expression of CK19 in invasive breast carcinomas of special histological types: implications for the use of one-step nucleic acid amplification. *J Clin Pathol*. 2011;64(6):493–497.
4. Effenberger KE, Borgen E, Eulenburg CZ, et al. Detection and clinical relevance of early disseminated breast cancer cells depend on their cytokeratin expression pattern. *Breast Cancer Res Treat*. 2011;125(3):729–738.
5. Reisenbichler ES, Balmer NN, Adams AL, et al. Luminal cytokeratin expression profiles of breast papillomas and papillary carcinomas and the utility of a cytokeratin 5/p63/ cytokeratin 8/18 antibody cocktail in their distinction. *Mod Pathol*. 2011;24(2):185–193.
6. Mehta R, Jain RK, Sneige N, et al. Expression of high-molecular-weight cytokeratin (34betaE12) is an independent predictor of disease-free survival in patients with triple-negative tumors of the breast. *J Clin Pathol*. 2010;63(8):744–747.
7. Bray F, McCarron P, Maxwell Parkin D. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res*. 2004;6(6):229–239.
8. Barak V, Goike H, Panaretakis KW, et al. Clinical utility of cytokeratins as tumor markers. *Clin Biochem*. 2004;37(7):529–540.

9. Petak I, Houghton JA. Shared pathways: death receptors and cytotoxic drugs in cancer therapy. *Pathol Oncol Res*. 2001;7(2):95–106.
10. Weng YR, Cui Y, Fang YJ. Biological Functions of Cytokeratin 18 in Cancer. *Mol Cancer Res*. 2012;10(4):485–493.
11. Koelink PJ, Lamers CB, Hommes DW, et al. Circulating cell death products predict clinical outcome of colorectal cancer patients. *BMC Cancer*. 2009;9:88.
12. De Petris L, Branden E, Herrmann R, et al. Diagnostic and prognostic role of plasma levels of two forms of cytokeratin 18 in patients with non-small-cell lung cancer. *Eur J Cancer*. 2011;47(1):131–137.
13. Brandt D, Volkmann X, Anstatt M, et al. Serum biomarkers of cell death for monitoring therapy response of gastrointestinal carcinomas. *Eur J Cancer*. 2010;46(8):1464–1473.
14. Demiray M, Ulukaya EE, Arslan M, et al. Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. *Cancer Invest*. 2006;24(7):669–676.
15. Hagg Olofsson M, Ueno T, Pan Y, et al. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. *Clin Cancer Res*. 2007;13(11):3198–3206.
16. Stig Linder, Aleksandra Mandic Havelka, Takayuki Ueno, et al. Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker. *Cancer Lett*. 2004;214(1):1–9.
17. Engin Ulukaya, Karaagac E, Ari F, et al. Chemotherapy increases caspase-cleaved cytokeratin 18 in the serum of breast cancer patients. *Radiol Oncol*. 2011;45(2):116–122.
18. I Popov, S Jelic, D Radosavljevic, et al. The role of stable disease in objective response assessment and its impact on survival in advanced colorectal cancer: is 'stable disease' a homogenous response category?. *Neoplasma*. 1999;46(2):132–139.
19. Cheng CW, Yu JC, Wang HW, et al. The clinical implications of MMP-11 and CK-20 expression in human breast cancer. *Clin Chim Acta*. 2010;411(3-4):234–241.
20. Woelfle U, Sauter G, Santjer S, et al. Down-Regulated Expression of Cytokeratin 18 Promotes Progression of Human Breast Cancer. *Clin Cancer Res*. 2004;10(8):2670–2674.

Citation: Shah AG, Meena RN, Mishra SP, et al. Expression of cytokeratin-18 in serum as a biomarker of apoptosis & necrosis in carcinoma breast. *J Cancer Prev Curr Res.* 2016;5(2):238–242. DOI: 10.15406/jcpcr.2016.05.00158