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SIGNIFICANCE OF CA 125 AND CEA AS BIOMARKERS IN ASSESSING PROGRESS OF TREATMENT FOR CERVICAL CANCER

Kaiser Jamil*[1], Javeed Ahmad [1], Hajira Fatima [1] and G. Suryanarayana Raju [2]

Genetics Department, Bhagwan Mahavir Medical Research Centre [1], 10-1-1, Mahavir Marg, AC Guards, Hyderabad, Andhra Pradesh, India
Surgical Oncology Division, Nizam's Institute of Medical Sciences [2], Panjagutta, Hyderabad, Andhra Pradesh, India

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Correspondence to Author:

Dr. Kaiser Jamil

Head of Genetics Department, Bhagwan Mahavir Medical Research Centre, 10-1-1, Mahavir Marg, AC Guards, Hyderabad-500004, A.P. India

E-mail: kj.bmmrc@gmail.com

ABSTRACT:

Background: Cervical cancer (CC) is the second leading cause of cancer deaths in women, with more than 80% of these occurring in developing countries that have limited access to screening programs. Therefore there is a need to develop diagnostic and prognostic biomarkers which can be quantifiable and help clinical oncologists at the first interaction with the suspected patients. The aim of this study was to correlate the serum markers with the diagnosis of distant metastasis, disease recurrence, therapy monitoring and prognosis of the cervical cancer.

Methods: The study group consisted of 50 metastatic cervical cancer patients and 50 benign cervical cancer patients in the age group of 20-80 years. Blood samples (5ml) were collected for analysis after Informed consent was obtained from each patient prior to sample collection. The markers (CEA and CA125) were analyzed by ELFA and sandwich ELISA methods respectively and compared to their reference values.

Results and conclusion: Statistical analysis of the results showed that the incidence of the disease showed a direct correlation with the age of the patients. The study also confirmed that elevated serum CA125 and CEA levels warranted further clinical management. It is concluded that CA125 and CEA are useful tumor markers not only for patients in clinical remission following treatment but also as prognostic and diagnostic biomarkers.

INTRODUCTION: Cervical cancer (CC) is the second most common cancer and predominant gynecological cancer in women, causing most cancer related deaths world over [1]. In developing countries cervical cancer is the second leading cause of cancer deaths in women, with more than 80% of these occurring in places where they have limited access to screening programs.

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It has been reported that 12,170 women developed cervical cancer, and about 4,220 women died of cervical cancer in the United States during 2012 (http://www.cancer.org/). And in China, the mortality rate of cervical cancer ranged from 2 to 4 per 105 population in urban areas, 0 to 7 per 105 population in rural areas during the period of 1996 to 2005 [2]. It is a leading cause of cancer mortality, affecting mainly the under developed populations of sub-Saharan Africa, Central and Latin America, and South-Central Asia [3]. In India, cervical cancer kills a woman every seven minutes. In Tamil Nadu, cervical cancer is the second most common cancer affecting women, especially those in rural areas,

whereas in other states the data was not available. Eighty per cent of women in the low socio-economic strata in India are at risk owing to the lack of awareness regarding the disease and the services available to combat the disease, some statistics which were available are listed below:

- 1, 32,082 women are affected by cervical cancer every year in India.
- 74,118 women die of the disease every year in India.
- 4, 93,243 women are affected by cervical cancer every year in the world.
- 2, 73,505 women die of the disease every year in the world.

(According to the National Cancer Registry Programme by Indian Council of Medical Research (ICMR) in the year 2007 supported by WHO)

Remarkably, it generally takes about ten years to arise from precancerous lesion to invasive cervical cancer. For this reason, the effective screening of precursor lesion is of great importance, which makes cervical cancer preventable and curable. Cytological examination and HPV test are the most widely applied screening methods for CC and its precursor (cervical intraepithelial neoplasia, CIN) lesion. Regular Papanicolaou (Pap) tests are an excellent diagnostic tool for detecting not only cancerous, but also precancerous cells, both of which can be treated or removed [4, 5]. Previous observational studies have consistently shown dramatic reductions in the cervical cancer mortality rate after the implementation of population-based screening programs [6, 7].

Among the several factors that contribute to the incidence of CC the most predominant aetiological factor for cervical cancer is persistent infection of certain high-risk types of human papilloma viruses (HR-HPVs),[8] while low risk types are associated with benign cervical lesions and genital warts [9, 10]. HPV has also been detected in a significant proportion of oral, oesophageal, anal, vaginal, vulvar, and penile cancer and in a small percentage of lung, laryngeal, and stomach cancer, as has been shown in some parts of the world [11]. It is also believed that early age of marriage, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking and use of oral contraceptives are also the causes for cervical cancer.

Generally the treatment options for CC are based on the evaluation of the clinical stage of tumor according to the classification of the International Federation of Gynecology and Obstetrics (FIGO). For early-stages (FIGO I-IIA) either surgery or radiotherapy (RT) is employed, whereas for late-stages (FIGO IIBIV) chemotherapy is indicated [12]. Despite the promising results achieved in the last decades with the screening of asymptomatic women by Pap smears and more recently with the advent of vaccines against HPV, CC is still a common disease with about 530,000 new cases and 275,000 deaths per year [13].

The classical management of invasive cervical cancer (ICC) involves evaluating tumor extent which includes tumor size, depth of invasion, microvascular space tumor invasion, spread to regional lymph nodes, and grade of differentiation.

Biomarkers are now being used before diagnosis for screening and risk assessment and during diagnosis especially in cancer cases and they can be used to determine staging, grading, and selection of initial therapy [14, 15]. It is known that a biomarker is either produced by the diseased organ (e.g., tumor) or by the body in response a disease. Hence biomarkers are potentially useful along the whole spectrum of the disease process. Several clinicians have used biomarkers to monitor therapy, select additional therapy, or monitor recurrent diseases [16, 17].

Oncology biomarkers are defined as easily accessible and measurable biologic substances for screening and monitoring of occult tumors [18]. The aim of this investigation was to determine biomarkers for the management of gynecologic cancers which could be used for differential diagnosis between malignant and benign tumor specimens, to monitor and predict responses to treatment, and to detect early stage or occult recurrent diseases. While CA125, CA19-9 and CEA are established biomarkers in patients with gynecologic malignancies [19], the most commonly used serum biomarkers for monitoring disease

progression are CA125 and hCG. Hence we propose to determine these biomarkers in cervical cancer. Also, we propose to determine these biomarkers in CC, and to study the role of serum tumor markers (CA125 and CEA) in assessing the progress of disease which may help in treatment for CC in the south Indian population.

MATERIALS AND METHODS:

Approval of the study: The Institutional Ethics Committee of Bhagwan Mahavir Medical Research Centre (BMMRC) approved the research study.

Study group: The study population included fifty metastatic cervical cancer patients and fifty benign cervical cancer patients in the age group of 20-80 years. Blood samples (5ml) were collected and centrifuged to separate the serum. The serum samples were stored in -20^{o}C till further analysis. Informed consent was obtained from each patient prior to sample collection. The markers (CEA and CA125) were analyzed by ELFA and sandwich ELISA methods respectively. Patient's data were collected from the case files of the Department of Oncology.

Quantification of CA125 and CEA: CA125 and CEA are the tumor associated-antigens present in the serum of the cancer patients, hence can be detected by directing specific antibodies against them. CA125 levels were determined by ELISA Test and CEA levels were determined by ELFA Test.

1. **CA 125:**

ELISA Test: The CA125 ELISA test is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay system utilizes a polyclonal anti-CA125 antibody directed against intact CA125 for solid phase immobilization on the micro titer wells. A monoclonal anti-CA125 antibody conjugated to horseradish peroxides (HRP) is in the antibody-enzyme conjugate solution. The test sample was allowed to react first with the immobilized polyclonal antibody. The wells were washed to remove any unbound antibody. The monoclonal-HRP conjugate reacted with the captured antigen, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies.

2. **CEA-ELFA Test:** VIDAS CEA is an automated test for use on the VIDAS instruments, for the quantitative measurement of carcinoembryonic antigen (CEA) in human serum or plasma using the ELFA technique (enzyme linked fluorescent assay). CEA is produced by cells during embryonic and fetal life and production ceases at birth. A very low serum concentration can be detected in healthy individuals. Increased CEA levels can be found in certain cases of cancer such as colorectal, breast and lung cancer and also in non-malignant diseases. Serum CEA levels decrease after treatment and increase in the event of cancer recurrence, residual disease and metastases. The VIDAS CEA test can be used as an additional test for prognosis and therapeutic monitoring of patients with diagnosed malignant carcinomas.

RESULTS:

Correlation of age with the incidence of cervical cancer: Our study included 50 patients with metastatic cervical cancer and 50 benign cervical disease belonging to different age groups. The diagnosis of metastatic and benign cervical cancer was confirmed by the results of CT scan and biopsy. These results were obtained from histopathology department. The serum markers levels of the patients were analyzed by ELISA and ELFA tests. The patients who were tested positive for the markers were categorized according to their age under four age groups as represented in **table 1** below:

TABLE 1: TABLE REPRESENTS THE INCIDENCE OF CERVICAL CANCER IN 4 AGE GROUPS

Sl. No.	Types of the tumor	Age (in years)			
		<14	14-30	30-50	>50
1	Metastatic Cervical cancer	0	13	22	15
2	Benign cervical Cancer	0	15	20	15

1. **Determination of CA 125 in Benign and Malignant samples in cervical cancer patients by ELISA Test:** Reference limit: The cut-off value of CA 125 is <35U/ml for a healthy individual. Table -2 shows the normal values of standard concentrations with different optical densities.

TABLE 2: SHOWING THE STANDARD CONCENTRATIONS AND RESPECTIVE O.D. VALUES

SL. NO.	STD CONC. (U/ml)	OD (450 nm)
A	0.0	0.053
B	15.0	0.11
C	30.0	0.138
D	60	0.217
E	120	0.481

Benign samples of cervical cancer (levels of CA 125): In this study out of 50 benign cases, only 4 samples showed an elevated level of CA 125 (controls) before treatment. In these elevated samples the concentration had reached to 50-60 U/ml as shown in **fig. 1**. Out of 50 metastatic cervical cases 15 cases showed an elevated level of CA 125 before treatment, were concentration reached up to 120U/ml as shown in **fig. 2**.

2. **Determination of CEA in Benign and Malignant samples in cervical cancer patients by ELFA Test:** Reference limit: The cut-off value of CEA for a healthy individual is <5ng/ml. All the 50 benign cases had shown normal levels of CEA as shown in **fig. 4**.

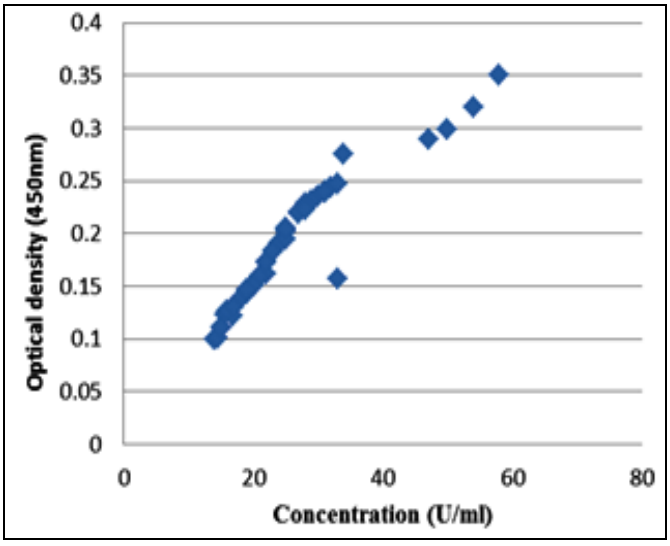

FIGURE 1: SHOWING CA125 LEVELS IN BENIGN SAMPLE OF CERVICAL CANCER (BEFORE TREATMENT)

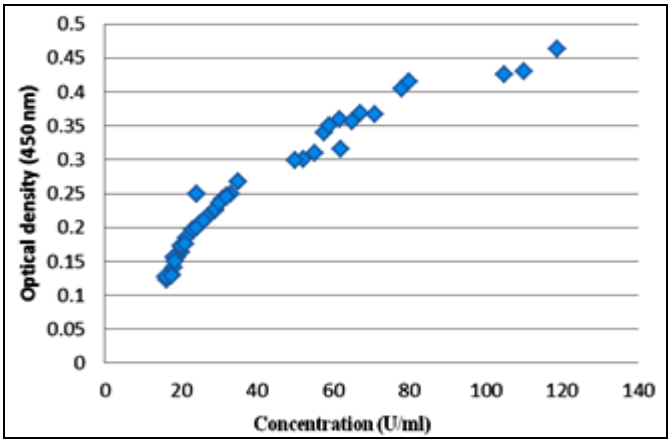

FIGURE 2: SHOWING CA 125 LEVEL IN MALIGNANT SAMPLES OF CERVICAL CANCER (BEFORE TREATMENT)

After the treatment all the 15 cases showed that the level of CA125 had come down to Normal values i.e. <35U/ml as shown in **fig. 3**.

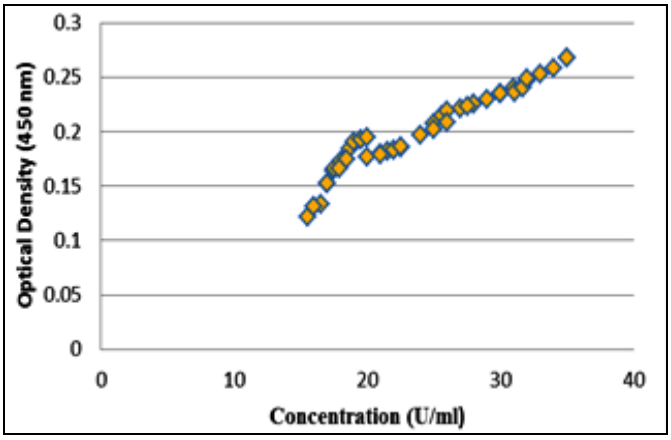

FIGURE 3: SHOWING CA 125 LEVEL IN MALIGNANT SAMPLES OF CERVICAL CANCER (AFTER TREATMENT)

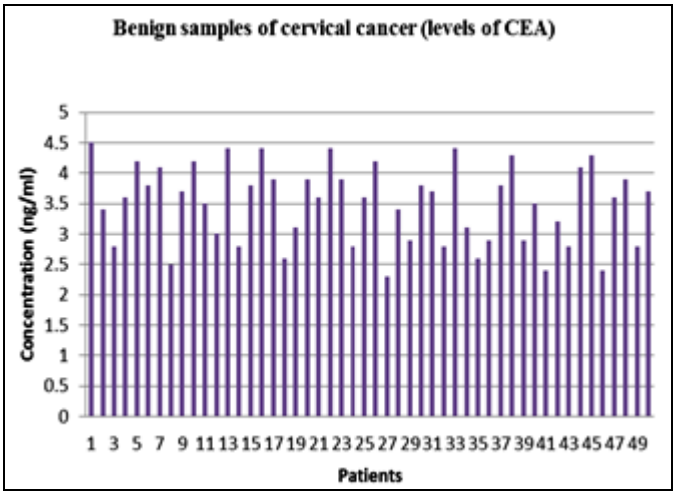

FIGURE 4: *SHOWING BENIGN SAMPLES OF CERVICAL CANCER (LEVELS OF CEA)* CEA LEVELS IN MALIGNANT SAMPLES OF CERVICAL CANCER (BEFORE TREATMENT)

In malignant samples of cervical cancer 40 out of 50 samples shows an elevated level of CEA, whereas standard concentration reached 150 ng/ml. Hence 80% of CC patients had shown an elevated level of CEA as shown in **fig. 5**. But after treatment (chemo-RT) all the 40 cases which showed an increased level of CEA had dropped down to normal levels as shown in **fig. 6**.

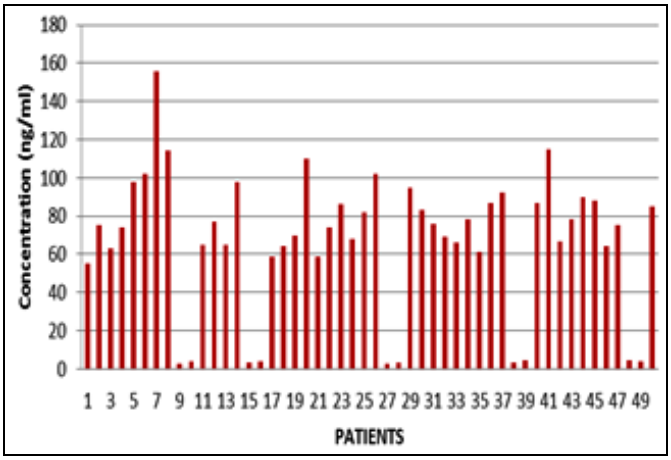

FIG. 5: CEA LEVELS IN MALIGNANT SAMPLES OF CERVICAL CANCER (BEFORE TREATMENT)

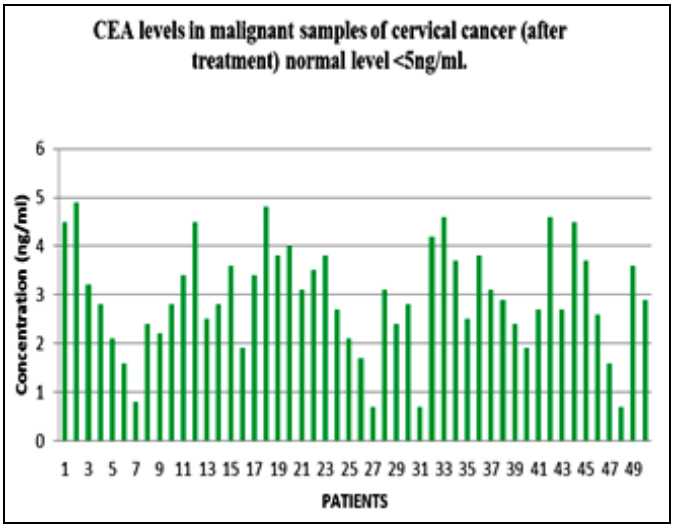

FIG. 6: CEA LEVELS IN MALIGNANT SAMPLES OF CERVICAL CANCER (AFTER TREATMENT)

DISCUSSION: Carcinoembryonic antigen (CEA) is a 200 kDa glycoprotein, isolated first by Gold and Freeman in 1965 [20] using an antibody rose in rabbits by injecting an extract of human colonic carcinoma. Elevated levels were found in patients with colorectal, breast, lung, or pancreatic cancer [21], and also in smokers [8]. The first success in developing a blood test for CEA was in 1965, when the antigen was detected in the blood of some patients with colon cancer.

Blood levels of CEA are also elevated in many other cancers such as those of the thyroid, pancreas, liver, stomach, prostate, ovary, and bladder[8]. Post-operative normalization of serum CEA level has been reported to be a favorable prognostic indicator in lung cancer and the identification of abnormal pre- and post-operative serum CEA levels may be useful in the auxiliary cancer prognosis or postoperative surveillance of colorectal cancer patients [22].

Out of 50 cases in our study in metastatic cervical cancer only 15 cases had shown an elevated level of CA125, but 40 out of 50 cases had shown an elevated level of CEA. These results have shown that CEA is a useful or better biomarker in diagnosing cervical cancer. However, despite a specificity of 90%, in cervical cancer CEA sensitivity as a single marker does not exceed 15% [23, 24]. The combination of CA-19-9, CA125 and CEA improves the sensitivity but on multivariate analysis only CA125 appears as an independent marker [25]. Finally, the addition of CEA does not significantly increase the sensitivity obtained by using SCC alone [26]. Thus to date, SCC still remains the tumor marker of choice in squamous tumors.

After treatment, all the 15 cases which showed an increased level of CA125 had dropped down to normal. Also all the 40 cases which showed an increased level of CEA had also become normal. This indicates the disease free condition of the patient. Serum levels of CA125 were extensively evaluated in cervical carcinoma. While only 13% to 21% of women with squamous cell carcinoma of the cervix had elevated levels of CA125 [27,

CA125 appeared more sensitive than SCC-Ag for cervical adenocarcinomas. It also serves as an important prognostic factor and an implicit indicator of tumor virulence [23]. CA125 used in combination with CA19-9 can increase the sensitivity to 60%, and up to 70% when combined with both CEA and SCC-Ag [25]. Finally, steady decrease of CA125 levels during treatment for cervical carcinoma correlates with chemosensitivity in 83% of patients [28].

From these data, it is evident that CEA is more significant than CA125 for both diagnosing and monitoring the progress of the treatment in CC.

However, both the biomarkers should be routinely used together in diagnosis to rule out false positives.

CONCLUSION: Tumor markers play a key role in the care of patients with the cervical cancer. Serial determination of the markers such as CEA and CA 125 can be used in aiding diagnosis of distant metastasis, determination prognosis, prospectively predicting response or resistance to specific therapies, surveillance after primary surgery, and monitoring therapy in patients with advanced disease. The tumor marker levels are often measured over a period of time to see if the levels are increasing or decreasing. Usually these "serial measurements" are more meaningful than a single measurement.

Tumor marker levels may be checked at the time of diagnosis; before, during, and after therapy; and then periodically to monitor for recurrence. The levels in the serum of the patients can be analyzed by enzyme linked fluorescent assay (ELFA) and enzyme linked immunosorbent assay (ELISA) respectively. From the results it can be said that the incidence of the disease had shown a direct correlation with the age of the patients. The study also confirmed the validity of serial CEA assay in the diagnosis of metastatic cervical disease and monitoring cervical cancer patients.

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REFERENCES:

1. Garland SM. Can cervical cancer be eradicated by prophylactic HPV vaccination? Challenges to vaccine implementation. Indian J Med Res, 2009; 130: 311-21.
2. Song M, Lin AN. Study on the mortality trend of female cervical cancer from 1996 to 2005 in China. Xiandai Yufang Yixue, 2009; 36:47–50.
3. Ferlay, J., Parkin, D. M., SteliarovaFoucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur. J. Cancer, 2010; 46: 765–781.
4. Abdullah F, Su TT. Enhancement of the cervical cancer screening program in Malaysia: a qualitative study. Asian Pac J Cancer Prev, 2010; 11(5):1359–1366.
5. Ibekwe CM, Hoque ME, Ntuli-Ngcobo B. Perceived benefits of cervical cancer screening among women attending Mahalapye District Hospital, Botswana. Asian Pac J Cancer Prev, 2010; 11(4):1021–1027.
6. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin, 2000; 50(1):7–33.
7. Taylor R, Morrell S, Mamoon H, Wain G, Ross J. Decline in cervical cancer incidence and mortality in New South Wales in relation to control activities (Australia). Cancer Causes Control, 2006; 17(3):299–306.
8. Khan MS, Chaouachi K, Mahmood R. Hookah smoking and cancer: carcinoembryonic antigen (CEA) levels in exclusive/ ever hookah smokers. Harm Reduction J, 2008; 5: 19.
9. Bharadwaj M, Hussain S, Nasare V, Das BC. HPV & HPV vaccination: Issues in developing countries. Indian J Med Res, 2009; 130: 327-33.
10. Kaiser Jamil. Cancer communications for the development of personalized medicine.[Editorial] Journal of Solid Tumors, 2012;Vol. 2, No. 2, 1-3.
11. Shukla S, Bharti AC, Mahata S, Hussain S, Kumar R, Hedau S, et al.,. Infection of human papillomaviruses in cancers of different human organ sites Indian J Med Res, 2009; 130: 222-33.
12. Bidus MA, Elkas JC. Cervical and Vaginal Cancer. International Federation of Gynecology and Obstetrics (FIGO) 2007; 1403–1453 p.
13. Jemal A, Bray F, Center MM, Ferlay J, Ward E. Global cancer statistics. CA Cancer J Clin, 2011 61: 69–90.
14. Rama Mani, Kaiser Jamil and M. Ch Vamsy. Specificity of serum tumor Markers (CA125, CEA, AFP, Beta HCG) in Ovarian Malignancies. Trends in Medical Research, 2007; 2, (3), 128-134.
15. Shaswati Khan and Kaiser Jamil. Linkage disequilibrium analysis determines the association of the haplotypes of MDR1 with IDC breast cancer. International Journal of Genetics and Molecular Biology 2009;Vol. 1 (8), pp. 150-159, © 2009 ISSN2006-9863© Academic Journals .
16. Kaiser Jamil**,** Kalyan Kumar**,** S**.** Hajira Fatima**,** Syed Rabbani**,** Ravi Kumar, Ramesh Perimi et al. Clinical Studies on Hormonal Status in Breast Cancer and its Impact on Quality of Life (QOL). Journal of Cancer Science and Therapy (JCST**)** 2009; 1.2: 83-89 omicsonline.org.
17. Atkinson A J *et al.* NCI-FDA Biomarkers Definitions Working Group; Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin. Pharmaco. Ther, 2001; 69 89–95.
18. P. D. Wagner, M. Verma and S. Srivastava. Challenges for biomarkers in cancer detection. Ann N Y Acad Sci, 2004; 1022, 9-16.
19. R. C. Bast, Jr., T. L. Klug, E. Schaetzl, P. Lavin, J. M. Niloff, T. F. Greber, V. R. Zurawski, *et al.* Monitoring human ovarian carcinoma with a combination of CA 125, CA 19-9, and carcinoembryonic antigen. Am J Obstet Gynecol, 1984; 149(5), 553-9.
20. Gold P, Freeman SO. Demonstration of tumor-specific antigens in human colonic carcinoma by immunological tolerance and absorption techniques. J Exp Med, 1965; 121: 43962.
21. Alaoui-Jamali MA, Xu YX. Proteomic technology for biomarker profiling in cancer: an update. J. Zhejiang Univ Sci B, 1965; 7: 411-20.
22. Wang JY, Lu CY, Chu KS, Ma CJ, Wu DC, Tsai HL, et al. Prognostic significance of pre- and postoperative serum carcinoembryonic antigen levels in patients with colorectal cancer. Eur Surg Res 2007; 39: 245-50.
23. J. M. Duk, H. W. De Bruijn, K. H. Groenier, G. J. Fleuren, J. G. Aalders. Adenocarcinoma of the uterine cervix. Prognostic significance of pretreatment serum CA 125, squamous cell carcinoma antigen, and carcinoembryonic

antigen levels in relation to clinical and histopathologic tumor characteristics. Cancer, 1990; 65(8): 18307.
24. S. N. Bae, S. E. Namkoong, J. K. Jung, C. J. Kim, J. S. Park, J. W. Kim et al. Prognostic significance of pretreatment squamous cell carcinoma antigen and carcinoembryonic antigen in squamous cell carcinoma of the uterine cervix. Gynecol Oncol, 1997; 64(3): 418-24.
25. G. Borras, R. Molina, J. Xercavins, A. Ballesta and J. Iglesias.Tumor antigens CA 19.9, CA 125, and CEA in carcinoma of the uterine cervix. Gynecol Oncol, 1995; 57(2): 205-11.
26. R. Molina, X. Filella, J. M. Auge, E. Bosch, A. Torne, J. Pahisa,et al.CYFRA 21.1 in patients with cervical cancer: comparison with SCC and CEA. Anticancer Res, 2005; 25(3A): 1765-71.
27. P. M. Gocze, H. W. Vahrson D. A. Freeman. Serum levels of squamous cell carcinoma antigen and ovarian carcinoma antigen (CA 125) in patients with benign and malignant diseases of the uterine cervix. Oncology, 1994; 51(5): 430-4.
28. A. Leminen, H. Alftan, U. H. Stenman P. Lehtovirta. Chemotherapy as initial treatment for cervical carcinoma: clinical and tumor marker response. Acta Obstet Gynecol Scand, 1992; 71(4): 293-7.
29. What are the key statistics about Cervical Cancer? http://www.cancer.org/

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