AAJMS [Formerly IJMS] May 2021;4(2):22-34;ISSNe 2522-7368
DOI: http://doi.org/10.32441/aajms.4.2.3

Breast Cancer: Role of Hormones Receptors and Tumor Markers

Abdulghani Mohamed Alsamarai, Tikrit University College of Medicine, [TUCOM], Tikrit, Iraq. Email: abdulghani.Mohamed@tu.edu.iq, galsamarrai@yahoo.com; Mobile: +9647701831295,
ORCID: http://orcid.org/0000-0002-7872-6691
Sheylan Salah Abdulla, Erbil Polytechnic University, Health Technical College, Medical Laboratory Technique, Erbil, Iraq.
Email: sheylan.salah@epu.edu.iq; sheylan2000@yahoo.com
Mobile: +9647504244479
ORCID: http://orcid.org/0000-0001-8584-1391
Suzan Khaled Mohammed Saraj, Kirkuk Health Authority, Kirkuk, Iraq. Email: suzansarag@yahoo.com
Mobile:+9647731625525
ORCID: https://orcid.org/0000-0002-5512-716X
Correspondence author: Abdulghani Mohamed Alsamarai, Tikrit University College of Medicine, [TUCOM], Tikrit, Iraq. Email: abdulghani.Mohamed@tu.edu.iq, galsamarrai@yahoo.com; Mobile: +9647701831295,
ORCID: http://orcid.org/0000-0002-7872-6691
Received: 6/1/2021 Accepted: 9/3/2021 Published: 1st May, 2021

1. Introduction

Breast cancer is the common malignant disease in Iraqi community and for the first rank for the new cases incidence, death rate and five years prevalence for the year 2020 [1]. In Iraq, new cases of breast cancer for 2020 are 22.2%, while it is 20.9% for Syria, 20.8% for Jordan, 20.6% for Kuwait, 16.4% for Egypt, 14.2% for Saudi Arabia, 12.9% for Iran, and 10.3% for Turkey [1]. The breast cancer etiology still unclear, however, recent studies implicated many risk factors, such as viral infections and hormones [2-8]. Unfortunately, recent studies indicated a shift toward younger age group in breast cancer development [9].

2. Hormone receptors

The presence or absence of hormone receptors, including the ER and PR, individually or together has been suggested to be prognostic- and predictive factors for breast cancer.

2.1. Estrogen receptor (ER)

The ER is a member of the nuclear hormone family of intracellular receptors which is activated by the hormone 17β-estradiol [10]. The main function of ER is as a DNA-binding transcription factor which regulates gene expression [11]. Estrogen receptor is of two types (α and β) that are coded by separate genes. The β isoform is encoded by the ESR2 gene, while α isoform is encoded by the ESR1. Hormone-activated ERs form dimers [12]. These two forms of ERs are co-expressed in various cell types including thyroid, bone, adrenals and female rat brain [13]. This may lead to the formation of homodimer ER α (αα) or ER β (ββ) or heterodimer ERαβ (αβ) [14]. There is significant overall sequence homology among the two isoforms [15]. ESR1 is encoded on chromosome 6 (6q25.1) and ESR2 is encoded on chromosome 14 (14q). Both ERs are widely expressed in different tissue types, however, there are some differences in their expression patterns [16]. In breast tissue, ERs are expressed by both normal and malignant cells. About 20% of the Terminal Duct Lobular Units (TDLU) in the breast of premenopausal women express the ER, a value that doubles

during the follicular phase. The average extent of expression of ER by the TDLU cells of postmenopausal women is approximately 50% [17].

Expression of ERs increases dramatically in early hyper-proliferative premalignant lesions ERs are over-expressed in around 70% of breast cancer cases, and are referred to as "ER positive" tumors. With an elevated ratio of ER+ to ER- cells in comparison to normal breast tissue. Moreover, higher expression of ER is associated with higher breast cancer survival [18]. ER+ tumors tend to develop in older women (peaking of 70 years at age), whereas ER- tumors tend to develop at an earlier age (peaking at 50). Mortality from premenopausal ER+ breast cancer is higher in women younger than 35 than in older women [19]. Mammary cells proliferation stimulated by binding of estrogen to ER which contributed to increase in cell division and DNA replication and mutation rate increase. This resulted in to cell cycle disruption, DNA repair processes and apoptosis with subsequent tumor formation. Additionally, estrogen metabolism leads to the production of genotoxic by-products that could directly damage DNA, resulting in point mutations [20].

Recent study in Kirkuk, Iraq, shows that ER is significantly higher in women with breast cancer as compared to controls with odd ratio of 32.25 for local cut-off value and 56.6 for international standard cut-off value [5]. In addition, Area Under Curve determined in ROC analysis indicated an association between breast cancer and ER [5]. Other study in Erbil, Iraq shows that ER is significantly higher in women with breast cancer than in controls and there is a positive significant association between breast cancer and ER serum levels as determined by OR but not when AUC determined using ROC analysis [21]. Age influence serum levels of ER in women with breast cancer [21]. Additionally, the frequency of ER positivity was 66.2% in women with breast cancer, while it was 24% in control group and the difference was highly significant [21].

2.2. Progesterone Receptor (PR)

The progesterone receptor (PR) also known as NR3C3 (nuclear receptor subfamily 3, group C, member 3), is an intracellular steroid receptor that binds progesterone. PR is encoded by the PGR gene which lies on chromosome 11 (11q22) [22]. This gene has two main forms, A and B that differ in their molecular weight (A: 94kDa and B: 114kDa) [23]. These two isoforms are transcribed from distinct, estrogen-inducible promoters within a single-copy PR gene; the only difference between them is that the first 164 amino acids of B are absent in A [24].

PR is expressed in reproductive tissue and has important roles in folliculogenesis, ovulation, implantation and pregnancy [25]. Estrogen is necessary to induce the progesterone receptors (PRs) activity. PRs become hyper phosphorylated upon binding of the steroid ligand. PR phosphorylation is complex, occurring in different cellular compartments and perhaps requiring multiple serine kinases [26]. After progesterone binds to the receptor, restructuring with dimerization follows and the complex enters the nucleus and binds to DNA [27]. There, transcription takes place, resulting in formation of messenger RNA that is translated by ribosomes to produce specific proteins [28]. About 65% of ER-positive breast cancers are also PR-positive and about 5% of breast cancers are ER-negative and PR-positive. If cells have receptors for both hormones or receptors for one of the two hormones, the cancer is considered hormone-receptor positive. [29]. some studies suggest that expression of PR is stimulated by atypical and increasing ratio of PR-A to PR-B, which is almost one in normal breast tissue, but varies extensively in malignant cells [30]. Approximately 60% of invasive breast tumors express PR-A or B [23].

Alobaidi et al [5] found that PR is significantly higher in women with breast cancer as compared to controls and AUC and OR confirm the association between breast cancer development and high serum levels of PR. Abdulla [21] in a case control study in Erbil,

Iraq shows that PR is significantly higher in women with breast cancer than in controls and there is a positive significant association between breast cancer and PR serum levels as determined by AUC determined using ROC analysis, but with OR of 1.134 [21]. Age influence serum levels of PR in women with breast cancer [21]. Additionally, the frequency of PR positivity was 29.1% in women with breast cancer, while it was 17.7% in control group and the difference was significant [21]

3. **Tumor markers**

Tumor markers are biochemical signs of tumor existence and consist of cell surface antigens, cytoplasmic proteins , enzymes and hormones [31], produced by tumor cell or other cells of the body in response to cancer or certain benign(noncancerous) conditions. These substances can be found in the blood, urine, tumor tissue, or in the other tissues [32]. The appearance of tumor markers and their concentrations are related to the genesis and growth of malignant tumors in patients. An ideal tumor marker should be highly sensitive, specific, and reliable with high prognostic value, organ specificity and it should correlate with tumor stages. However, none of the tumor markers reported to date has all these characteristics [33]. Inspite of these limitations, for many malignancies, serum tumor markers play an important role in patient management [34]. Tumor markers that correlated with tumor size may be used as tool for diagnosis and monitoring of cancer prognosis. However, they lack specificity and elevation of their levels, although very suggestive, does not always prove the presence or recurrence of cancer and does not predict the number and localization of tumor sites [35]. In breast cancer the most widely used serum markers are carcinoma antigen 15.3 (CA15.3) and carcinoembryonic antigen (CEA). Less widely used markers include CA27.29, tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS) and the shed form of HER-2 [36].The uses of tumor markers in breast cancer, according to a study done by [37] include the following: i) Aiding early diagnosis, ii) Determining prognosis, iii) Predicting response to therapy, iv) Surveillance after primary treatment, v) Monitoring response to therapy in advanced disease.

3.1. Carcinoma antigen 15.3 (CA15.3)

The CA15.3 is also known as Mucin1 (MUC-1) and it is the most widely used marker in breast cancer [38]. The Mucins are large (> 200 K.D) glycoproteins with a high carbohydrate content (50–90% by weight) expressed by a variety of normal and malignant secretary epithelial cells [39]. The CA15.3 is a transmembranous glycoprotein expressed at the apical cell surface of normal glandular epithelium such as breast, ovary, salivary glands, stomach, pancreas, bladder, uterus, small intestine and colon tissue [40]. It is also present on hematopoietic cells like B cells, and resting as well as activated T cells [41].

Many functions have been proposed for CA15.3. The extensive expression of CA15.3 from mid-gestation throughout adulthood in secretary epithelial tissues and the elevated level of expression found in carcinomas and metastatic lesions suggest functions in epithelial morphogenesis and tumor progression [42]. It can function at several levels: firstly, by steric-hindrance by the large glycosylated extracellular domain, secondly, by remodeling the cytoskeletal networks [43], and finally, by down-regulating the activities of other molecules such as catenins , cadherins , or integrins via signal transduction events [44]. Paradoxically, CA15.3 has been proposed to act both as an adhesive and anti-adhesive molecule as the extended conformation may contribute to its anti-adhesive properties, resulting in reduced cell-cell aggregation and decreased adherence to extracellular matrix [45]. There are differences between CA15.3 expression on normal cells and tumors. For example, the monoclonal antibody demonstrates a significant reactivity with CA15.3 on paraffin embedded breast cancer, but minimal reactivity with normal breast cells or benign breast lesions [46]. On normal cells CA15.3 is located on the apical surface and is extensively

glycosylated, however, in tumors, the usual structure of the tissue is disrupted so that CA15.3 may be found on multiple cell surfaces [47].

Furthermore, abnormal glycosylation in the cancer result in less complex and fewer carbohydrate side chains [48]. Therefore, in tumors, there is a great exposure of CA15.3 epitopes on the immune system, compared to normal cells [49]. Although the function of CA15.3 is not clearly established, it may allow for tumor growth through its interactions with adhesion molecules and lymphocytes. It is commonly found in a variety of malignant tumors including breast cancer, lung cancer, ovarian cancer, and endometrial cancer, carcinoma of pancreas, colon and prostate [47].

In breast cancer lack of sensitivity for early-stage disease combined with a lack of specificity precludes the use of all existing serum markers for early diagnosis of breast cancer [37]. For example , CA15.3 concentrations are increased in 10% of patients with stage I disease , 20% with stage II disease , 40% with stage III disease and 75% with stage IV disease [37]. Increased concentrations of the marker can be found in small proportion of apparently healthy individuals (5%) , in patients with certain benign diseases specially liver diseases and in patients with other types of advanced adenocarcinoma [38, 50].

The available prognostic factors for breast cancer include pathological criteria such as tumor size, tumor grade, and lymph nodes status [51], as well as newer biological factors such as hormone receptors, HER-2, urokinase, plasminogen activator inhibitor [52]. All of these factors require tumor tissue, thus necessitating either biopsy or surgery. Many published studies have addressed the relationship between preoperative concentration of CA15.3 and patient outcome [53]. These studies concluded that high concentrations of the marker (> 35 U/L) at initial presentation predict adverse patient outcome. Indeed, in some studies the prognostic impact of CA15.3 was independent of tumor size and axially node status [54]. Significantly in some reports CA15.3 was found to be prognostic in lymph node negative breast cancer patients, the subgroup in which new prognostic factors are most urgently required [54, 55]. In a study carried out by Molina and Coworkers [56], however, CA15.3 was not prognostic in patients free of axillary nodal metastasis. Although most studies relating to CA15.3 have used preoperative values, concentration during follow-up can also provide prognostic information [37]. Thus, Tampellini and Coworkers [57] reported that patients with CA15.3 values < 30 U/L at the time of first recurrence survived significantly longer than those with higher concentrations. In another report done by Dela and Colleagues [58] found that patients with a CA15.3 lead time of >30 days (i.e. the time between the first abnormal CA15.3 and appearance of clinical manifestation) had a better prognosis than those with a shorter lead time. In that study both the time interval between diagnosis and first abnormal CA15.3 concentration was also of prognostic value. These findings suggest that determination of CA15.3 can provide lead-time prognostic information in patients with breast cancer. Indeed, preoperative concentrations could be combined with existing prognostic factors for selecting patients for adjuvant therapy [37].

In recent years, several reports have shown that serial concentrations of tumor markers increased before radiological or clinical evidence of disease relapse [59]. These studies showed that the mean lead time from marker increase to clinical diagnosis of recurrence varied from 2 to 9 months. The CA15.3 and other mucin related markers may also have a role in predicting response to therapy [60].

The CA 15-3 biomarker is significantly higher in women with breast cancer as compared to controls and a significant positive association between breast cancer and CA15-3 biomarker was demonstrated as determined by odd ratio [4]. Additionally, area under curve determination (AUC=0.99) indicated that CA 15-3 is a predictive biomarker in women with breast cancer [4]. Other study in Erbil, Iraq shows that CA 15-3 is significantly higher in

women with breast cancer than in controls and there is a positive significant association between breast cancer and CA 15-3 serum levels as determined by OR and when AUC determined using ROC analysis [21]. Age not influence serum levels of CA 15-3 in women with breast cancer [21]. Additionally, the frequency of CA 15-3 positivity was 79.1% in women with breast cancer, while it was 57.3% in control group and the difference was highly significant [21]. However, most of the previous studies suggested that CA 15-3 biomarkers is more predictive for monitoring rather than diagnostic marker [61-71].

3.2. Carcinoembryonic antigen (CEA)

The CEA is an oncofetal antigen that was first described by Gold and Freedman [72]. The CEA , a family of glycoproteins, MW~2000 Dalton was first identified in human colon cancer tissue extract [72].The CEA is a glycophosphatidylinositol-linked cell surface glycoprotein of the immunoglobulin gene super family that has been shown to mediate homotypic intercellular adhesion [73]. The normal level of CEA is below 5 ng/ml. However, CEA moderately elevated in 3% of general population and in 19% of smokers who are not with breast cancer [31]. Other non neoplastic conditions associated with elevated CEA levels includes peptic ulcer , inflammatory bowel disease, pancreatitis , hypothyroidism, biliary obstruction and cirrhosis. These false elevations are almost always less than 10 ng/ml and remain stable during serial testing , in contrast to CEA produced by recurrent tumor [74]. Many studies have shown that CEA concentration is known as a marker of malignant transformation and chronic inflammation and it is increased in a variety of cancers e.g. colorectal cancer carcinoma of pancreas, uterine cancer, cancers of the lung, and breast [75].

The CEA was historically considered the standard to which new serum markers are compared [76].The sensitivity increases with advancing tumor stage : CEA values are elevated in approximately 50% of the patients with tumor extension to lymph nodes and 75% of patients with metastasis [74]. The highest values above 100 ng/ml occur with metastasis [77], although poorly differentiated tumors are less likely to produce CEA [74].

Several studies have reported that positive serum CEA levels at the time of primary breast cancer diagnosis may represent a negative prognostic parameter, and correlate with the stage of disease. Several studies have shown that CEA level decrease or increase may reflect the status of disease progression or regression [78]. The literatures also suggest that CEA may be useful in the post surgical follow-up of breast cancer patients for an early diagnosis of recurrence, and for monitoring response to treatment [79]. The availability of the CA15.3 in the last decade has greatly reduced the value of CEA in breast cancer management, and recent studies discourage the routine use of the CEA assay because of its low sensitivity in both early and advanced diseases compared with CA15.3 Nevertheless CEA is still a widely used test for monitoring breast cancer patients [76].

The mean serum value of CEA was 4 times higher than that in control and odd ratio and ROC curve analysis indicated a positive association between breast cancer and serum levels of CEA [4]. Thus CEA level may be predictable for early diagnosis, monitoring and recurrence of breast cancer. Other study in Erbil, Iraq shows that CEA is significantly higher in women with breast cancer than in controls and there is a positive significant association between breast cancer and CEA serum levels as determined by OR and when AUC determined using ROC analysis [21]. Age influence serum levels of CEA in women with breast cancer [21]. Additionally, the frequency of CEA positivity was 67.6% in women with breast cancer, while it was 0% in control group and the difference was highly significant [21]

3.3. P53 protein

P53 protein was first identified in 1979 as a transformation-related protein. However, almost 10 years later, researchers discovered the oncogenic properties of p53, which was later called “gain of oncogenic function”. By the early 1990s, p53 became widely recognized as

the first tumor suppressor gene [80]. High rate (>50%) of p53 tumor suppressor gene mutation human cancers, it has attracted the interest of numerous researchers. P53 mutations can lead either to loss or change of p53 binding activity to its downstream targets and may thus induce aberrant cell proliferation, with consequent malignant cellular transformation [80, 81].

Human p53 protein is a nuclear phosphoprotein of MW 53 kDa. The human TP53 gene is located on the short arm of chromosome 17 [82]. P53 protein contains 393 amino acids and is composed of several structural and functional domains [83]. P53 is needed to keep cells under control, so the properly functioning p53 acts as brakes to the cycle of cell growth, DNA replication and division into two new cells [84], and preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress [85]. The capacity of p53 for multiple biological functions can be attributed to its ability to act as a sequence-specific transcription factor, and thus to modulate various cellular processes [80].

The induction of cell-cycle arrest by p53 provides additional time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. However, DNA-repair failure may result in the activation of apoptosis [85], Apoptosis One of the most important roles of p53 is to monitor cellular stress and to induce apoptosis when necessary [85], DNA repair and Inhibition of angiogenesis and metastasis [86].

In Breast Cancer TP53 mutations have been suggested to be an early event in breast carcinomas, while it seems to be a later event in other types of cancer. The frequency and type of mutations vary in different series of breast cancer patients. This may be due to factors such as stage of disease and molecular subtype [87]. P53 mutations are found in 19%-40% of all breast cancers; however certain types of the disease are associated with higher frequencies. For example, in typical medullary carcinomas, p53 mutation occurs in 100% of cases [88].

A potential mechanism for p53 inactivation independent of mutation is alterations identified in both upstream regulatory proteins and downstream p53-induced proteins that may disable or compromise the pathway in breast cancers lacking mutations [89]. Extra nuclear localization of p53 is another mechanism of p53 inactivation independent of mutation. During the cell cycle p53 is differently located in the cell referring that the control of the intracellular localization of p53 is cell cycle regulated [90]. The association between p53 alterations and clinical outcome in breast cancer has been the subject of numerous investigations. The presence of p53 mutations is associated with reduced survival and aggressiveness of breast cancer; therefore it is the most adverse prognostic indicator for both recurrence and death in breast cancer [91]. However, medullary carcinomas are regarded as a prognostically favorable variant. It has been suggested that the diffuse lymphoplasmacytic infiltrate may account for the good prognosis representing a host reaction to tumor cell antigens and the mutated p53 protein may represent one of these antigens [92].

Abdulla in a case-control study in Erbil, Iraq shows that p53 is significantly lower in women with breast cancer than in controls and there is a inverse significant association between breast cancer and p53 serum levels as determined by OR but not when AUC determined using ROC analysis [21]. Age influence serum levels of P53 in women with breast cancer [21]. Additionally, the frequency of p53 positivity was 47.3% in women with breast cancer, while it was 81.2% in control group and the difference was highly significant [21]. Thus p53 may play a potential protective effect in breast cancer.

3.4. Cancer Antigen 27.29

Cancer antigen (CA) 27.29 is a monoclonal antibody to a glycoprotein (MUC1) that is present on the apical surface of normal epithelial cells. CA 27.29 is highly associated with breast cancer; although levels are elevated in several other malignancies [93]. CA 27.29 also

can be found in patients with benign disorders of the breast, liver, and kidney, and in patients with ovarian cysts. However, CA 27.29 levels higher than 100 units per mL are rare in benign conditions [94]. Because of superior sensitivity and specificity, CA 27.29 has supplanted CA 15-3 as the preferred tumor marker in breast cancer. The CA 27.29 level is elevated in approximately one third of women with early-stage breast cancer (Stage I or II) and in two thirds of women with late-stage disease (stage III or IV) [95]. CA 27.29 lacks predictive value in the earliest stages of breast cancer and thus has no role in screening for or diagnosing the malignancy.

Disagreement exists about the ability of CA 27.29 to detect asymptomatic recurrence after curative treatment [96]. In patients at high risk for recurrence of breast cancer (stage II or III) found that CA 27.29 was highly specific and sensitive in detecting preclinical metastasis. The average time from initial elevation of CA 27.29 to onset of symptoms was five months. Because CA 27.29 testing may lead to prompt imaging of probable sites of metastasis, it may be possible to decrease morbidity through earlier institution of therapy [95].

Alobaidi et al [4] found that mean serum level of CA 27-29 was significantly higher in women with breast cancer as compared to controls. In addition, they reported a significant association between breast cancer development and serum levels of CA 27-29 in both OR and AUC determination. Other study in Erbil, Iraq shows that CA 27-29 is significantly higher in women with breast cancer than in controls and there is a positive significant association between breast cancer and CA 27-29 serum levels as determined by OR and AUC using ROC analysis [21]. Age influence serum levels of CA 27-29 in women with breast cancer [21]. Additionally, the frequency of CA 27-29 positivity was 73% in women with breast cancer, while it was 6.2% in control group and the difference was highly significant [21]

3.5. Other markers.

Determination of breast cancer susceptibility antigen 1 (BRCA1) mean serum value was significantly 5 times higher in breast cancer women than that in controls, while BRCA2 was 3 times higher in patients than in controls [4]. OR and AUC confirmed a significant positive association between breast cancer development and positivity of BRCA1 and BRCA2 [4]. Additionally, the frequency of positivity of BRCA1 and BRCA2 in women with breast cancer were 89% and 88% respectively [4]. Thus both markers may be useful for diagnosis and monitoring of breast cancer cases.

In a case-control study serum mean values of estrogen receptors, progesterone receptors, prolactin, HBA1C, glucose, and calcium were significantly higher in women with breast cancer as compared to matched control [5]. While circulating progesterone, estrogen, vitamin D, Insulin Growth Factor-1, and parathyroid hormone valued were significantly lower in women with breast cancer than in controls.[5]. Odd ratio confirm the association between the evaluated biomarkers and breast cancer. Glucose serum levels were with OR 2.7 and AUC of 0.62, HBA1C OR was 3.21 and AUC of 0.56, IGF-1 OR of 42.35 and AUC of 0.23, calcium OR of 86.55 and AUC of 0.99, PTH OR of 57.6 and AUC of 0.999, prolactin OR of 25.8 and AUC of 0.999 and vitamin D OR of 57.6 and AUC of 0.999. the lower AUC was demonstrated for progesterone and estrogen serum levels. Thus estrogen and progesterone serum levels were with low predictive value in breast cancer, in contrast, ER and PR serum levels were with high predictive value in breast cancer [5].

References

1. WHO. Globocan 2020.
2. Alsamarai AGM, Abdulla SS, Aljumaili ZK. Epstein- Bar Virus and Cytomegalovirus Infection Association with Breast Cancer. Alborg Academy J Med Sci 2021;4(1):8-36.
3.Abdulghani Mohamed Alsamarai, Shilan Salah Abdula, Zakaria Abdula Alkhaiat. Role of Cytomegalovirus and Epstein.Barr Virus in Breast Cancer. World J Pharm Pharma. 2015;4(11):115-142.
4.Alobaidi AHA, Jalaly A, Alsamarai AGM, Sarhan HH. Biomarkers in women with breast cancer: I. CEA, CA 15.3, CA 27-29, BRCA-1, BRCA-2 predictive value. International J Scientific Research Science Engineering Technology 2015; 1(4):442-449.
5.Alobaidi AHA, Jalaly A, Alsamarai AGM. Biomarkers in women with breast cancer: II. Hormones, calcium, vit D, glucose, and IGF predictive value. World J Pharmacy Pharmaceutical Sciences 2015;4 (8):74-100.
6. Alsamarai AM, Alobaidi AHA.Association of human Cytomegalovirus and Epstein-Barr virus with breast cancer. Int J Med Sci [Aalborg Academy J Med Sci] 2018;1(2):1-8.
7. Alsamarai AGM, Abdulla SS, Alobaidi MA. Association of Breast Cancer with Epstein-Bar Virus and Cytomegalovirus Infection. AAJMS 2020;3(2):12-27.
8. Abdulla SS, Alsamarai AGM, Ajumaili ZK. Association of Breast Cancer with Epstein-Bar Virus and Cytomegalovirus Infection: Histological Types and Risk Factors. AAJMS 2020;3(3):20-63.
9. Abdulghani Mohamed Alsamarai , Shilan Salah Abdula. Breast Cancer Frequency Rate Shift toward Younger Age in IRAQ. IJSRSET 2015;5(1): 407-414.
10. Dahlman WK, Cavailles V, Fuqua SA. International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacological reviews 2006; 58 (4):pp773-781.
11. Levin ER. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. Molecular endocrinology 2003; 17 (3): pp309-317.
12. Lee HR, Kim TH, Choi KC. Functions and physiological roles of two types of estrogen receptors, ERalpha and ERbeta, identified by estrogen receptor knockout mouse. Laboratory animal research. 2012; 28(2):71-6.
13. Arts J, Kuiper GG, Janssen JM. Differential expression of estrogen receptors alpha and beta mRNA during differentiation of human osteoblast SV-HFO cells. Endocrinology. 1997; 138 (11): pp5067-5070.
14. Li X, Huang J, Yi P. Single-chain estrogen receptors (ERs) reveal that the ERalpha/beta heterodimer emulates functions of the ERalpha dimer in genomic estrogen signaling pathways. Molecular and cellular biology 2004; 24 (17): pp7681-7694.
15. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. The Journal of biological chemistry 2001; 276 (40): pp36869-36872.
16. Couse JF, Lindzey J, Grandien K. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 1997; 138 (11), pp4613-4621.
17. Allred D, Mohsin S, Fuqua S. Histological and biological evolution of human premalignant breast disease. Endocrine-Related Cancer. 2001;8(1):47-61.
18. Kamby C, Andersen J, Ejlertsen B, Birkler N, Rytter L, Zedeler K, et al. Histological grade and steroid receptor content of primary breast cancer--impact on prognosis and possible modes of action. British journal of cancer. 2005; 58(4):480.

19. Pollan M. Epidemiology of breast cancer in young women. Breast Cancer Res Treat. 2010; 123 Suppl 1:3-6.
20. Deroo BJ, Korach KS. Estrogen receptors and human disease. The Journal of clinical investigation 2006; 116 (3), pp561-570.
21. Abdulla SS. Risk Assessment for Epestin-Barr and Cytomegalo Viruses Infection in Women with Breast Cancer. Ph D Thesis, Tikrit University College of Science, Tikrit, Iraq, 2016.
22. Leonhardt SA, Boonyaratanakornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids. 2003; 68(10):761-70.
23. Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Research. 2002; 4(5):197-201.
24. Giangrande PH, McDonnell DP. The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. Recent progress in hormone research. 1999; 54:pp291-313.
25. Gadkar-Sable S, Shah C, Rosario G. Progesterone receptors: various forms and functions in reproductive tissues. Frontiers in bioscience : a journal and virtual library. 2005; 10: pp2118-2130.
26. Takimoto GS, Horwitz KB. Progesterone receptor phosphorylation complexities in defining a functional role. Trends in endocrinology and metabolism: TEM 1993; 4 (1): pp1-7.
27. Ismail PM, Amato P, Soyal SM, DeMayo FJ, Conneely OM, O'Malley BW, et al. Progesterone involvement in breast development and tumorigenesis—as revealed by progesterone receptor ―knockout and ―knockin mouse models. Steroids. 2003; 68(10):779-87.
28. Li X, O'Malley BW. Unfolding the action of progesterone receptors. The Journal of biological chemistry 2003; 278 (41): pp39261-39264
29. Ma ZQ, Liu Z, Ngan ES, Tsai SY. Cdc25B functions as a novel coactivator for the steroid receptors. Molecular and cellular biology 2001; 21 (23): pp8056- 8067.
30. Mote P, Bartow S, Tran N, Clarke C. Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. Breast cancer research and treatment 2002; 72(2):163-72.
31. Mohammad H, Talaized AH, Assar S, et al. Evaluation of carcinoembryonic antigen CEA and CA15.3 tumor markers in patients operated for breast cancer. Pak. J. Med. Sc 2007; 23(1) : 115-118.
32. Bigbee W, Herberman RB. Tumer markers and immunodiagnosis. In: Bast RC Jr., Kufe DW, Pollock RE editors. Cancer Medicine. 6th ed.2003; Hamilton, Ontario, Canada: BC Decker Inc.
33. Malati T. Tumor Markers: An overview. Ind. J. Clin. Biochem 2007; 22(2) : 17 –31.
34. Parker C. Active surveillance: towards a new paradigm in the management of early breast cancer. Lancet Oncol. J. 2004; 5: 101-106.
35. Chiu-Shong L , Yeh-You S , Chang-Chien L , et al. Clinical Impact of [18F] FDG-PET in patient with suspected Recurrent Breast Cancer based on a symptomatically elevated Tumor Marker Serum level : a preliminary report. Jap. J. Clin. Oncol 2002; 32 (7) : 244-247.
36. Nicolini A, Carpi A. Post operative follow-up of breast cancer patients : overview of progress in the use of tumor markers. J.Tumor Biol 2000; 21 : 235-248.
37. Michael J. Serum tumor markers in breast cancer: are they of clinical value? Clin. J. Chem 2006; 52 : 345-351.

38. Duffy MJ. CA15.3 and related mucins as circulating markers in breast cancer .Ann. Clin. Biochem 1999; 36:579-586.
39. Devine PL, McKenzie IF. Mucins : Structure , function , and associations with malignancy. J. Bioassays 1992 ;14 : 619.
40. Zotter S, Hangeman PC, Lossnitzer A, et al. Tissue and tumor distribution of human polymorphic epithelial mucin. J. Cancer 2004; 11: 55-101.
41. Chang JF, Zhao HL, Phillips J, Greenburg G. The epithelial mucin , MUC-1 , is expressed on resting T lymphocyte and can function as a negative regulator of T-cells activation. J. Cell. Immunol 2000; 201:83.
42. Gendler SJ, Spicer AP. Epithelial mucin genes. J. Annu. Rev. physiol 2007; 57 : 607.
43. Yamamoto M, Bharti A , Li Y, Kufe D. Interaction of the DF3/MUC1 breast carcinoma-associated antigen and beta-catenin in cell adhesion. J. Biol. Chem 1997; 272 : 12492.
44. Kondo K, Kohno N, Yokiyama A, Hiwada K. Decreased MUC1 expression induces E-cadherin-mediated cell adhesion of cancer cell lines. J. Cancer Res 1998; 58 : 2014.
45. Wesseling J, Vander Valk SW, Hi Heens J. A mechanism for inhibition of E-cadherin-mediated cell-cell adhesion by the membrane-associated mucin episialin/MUC1.J. Mol. Cell. Boil. 1996; 7 : 565.
46. Gendler S, Taylor-Papamitriou J, Duhing T, et al .A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made of tandem repeats. J. boil. Chem 2000; 263:12820-12823.
47. Barratt-Boyes S. Making the most of mucin : a novel target for tumor immunotherapy. J. Cancer immunol. Immunother 1996; 43 : 142-151.
48. Lioyd KO, Burchell J , Kudryashor V, et al. Comparison of O-linked carbohydrate chains in MUC1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. J. Biol. Chem 1996; 271 : 33325-33334.
49. Burchell J, Taylor-Papadimitriou J, Boshell M, et al. A short sequence , within the amino acid tandem repeat of a cancer-associated mucin, contains immunodominant epitopes. Int. J. Cancer 2002; 44 : 691-696.
50. Cheung K, Graves CRL, Robertson JFR. Tumor marker measurement in the diagnosis and monitoring of breast cancer. J. Cancer Treat. Rev 2000; 26 :91-102.
51. Elston CW, Ellis IO, Pinder SE. Pathological prognostic factors in breast cancer. J. Crit. Rev. Oncol. Haematol 1999; 31 : 209-223.
52. Isaacs C, Strearns V, Hayes DF. New prognostic factors for breast cancer. J. Semin. Oncol 2001; 28 : 53-67
53. Ebeling FF, Schmitt UM, Untch M, et al. Tumor markers CEA and CA15.3 as prognostic factor in breast cancer unvariate and multivariate analysis. J. Anticancer Res 1999; 19 : 2545-2550.
54. Kumpulainen EJ, Keskikuru R, Johansson RT. Serum tumor marker CA15.3 and stage are the two most important predicators of survival in primary breast cancer. J. Breast Cancer Res. Treat 2002; 76 : 95-102.
55. Duffy MJ, Duggan C, Keane R, Hill ADK, McDermorr E, Crown J, et al. High preoperative CA15.3 concentrations predict adverse outcome in node-negative and node-positive breast cancer : study of 600 patients with histrologically confirmed breast cancer; 2004.
56. Molina R, Filella X, Alicarte J, et al. Prospective evaluation of CEA and Ca15.3 in patients with locoregional breast cancer. J. Anti cancer Res 2003; 23 : 1035-1042.

57. Tampellini M, Berutti A, Gerbino A, et al. Relationship between CA15.3 serum levels and disease extend in predicting overall survival of breast cancer patients with newly diagnosed metastatic disease. Br. J. Cancer 1997; 75 : 698-702.
58. Dela Lande B, Hacene K, Floiras JL, et al. Prognostic value of CA15.3 kinetics for metastatic breast cancer. Int. J. Biol. Markers 2002; 17:231-250
59. Nicolini A, Ferrari P, Sagripanti A and Carpi A. The role of tumor markers in predicting skeletal metastatic in breast cancer patients with equivocal bone scintigraphy. Br. J. Cancer 1999; 79 : 1443-1447.
60. Ren J, Agata N, Chen D, et al. Human MUC1 carcinoma associated protein confers resistance to genotoxic anticancer agents. J. Cancer Cells 2004; 5 : 163-175.
61. Horobin JM, Browning MCK, McFarlane NP, Smith G, Preece PE, Wood RA, et al. Potential use of tumour marker CA 15-3 in the staging and prognosis of patients with breast cancer. J R Coll Surg Edinb 1991;36:219–21.
62. Duffy MJ, Duggan C, Keane R, Hill ADK, McDermorr E, Crown J, et al. High preoperative CA 15-3 concentrations predict adverse outcome in node-negative and node-positive breast cancer: study of 600 patients with histologically confirmed breast cancer. ClinChem 2004;50:559–63.
63. Jager W. Disseminated breast cancer: does early treatment prolong survival without symptoms? [Abstract]. Breast 1995;4: 65.
64. Nicolini A, Anselmi L, Michelassi C, Carpi A. Prolonged survival by „early" salvage treatment of breast cancer patients: a retrospective 6-year study. Br J Cancer 1997;76:1106–11.
65. Nicolini A, Carpi A, Michelassi C, Spinelli C, Conte M, Miccoli P, et al. "Tumor marker guided" salvage treatment prolongs survival of breast cancer patients: final report of a 7-year study. BiomedPharmacother 2003;57:452–9.
66. Kovner F, Merimsky O, Hareuveni M, Wigler N, Chaitchik S. Treatment of disease-negative but mucin-like carcinoma-associated antigen-positive breast cancer patients with tamoxifen: preliminary results of a prospective controlled randomized trial. Cancer ChemotherPharmacol 1994;35:80–3.
67. Hayward JL, Carbone PP, Heuson J-C, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. Eur J Cancer 1977;13:89–94.
68. Kurebayashi J, Nishimura R, Tanaka K, Kohno N, Kurosumi M, Moriya T, et al. Significance of serum tumor markers in monitoring advanced breast cancer patients treated with systemic therapy: a prospective study. Breast Cancer 2004;11:389–95.
69. Kumar R, Kumar AN, Srivastava A. Breast cancer tumor markers. J Solid Tumor 2012;2:43-46.
70. Ebeling FG, Stieber P, Utch M, Nagel D, Konecny GE, Schmit UM , Fateh-Mghadam A and Seidel D. Serum CEA and CA15.3 as prognostic factors in primary breast cancer , Br J Cancer 2002 ; 22: 86 (8): 1217-1222.
71. Verring A, Clouth A, Ziolkowski Pand Oremek M. Clinical Usefulness of Cancer Markers in Primary Breast Cancer , ISRN Pathology; Volume 2011 (2011), Article ID 817618, 4 Pages .
72. Gold P, Freedman S. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J. Exp. Med 1965; 121 : 439.

73. Benchimol, Fuks SA, Jotly S, Beauchemin N, Shirota K, Stanners CD. Carcinoembryonic antigen , a human tumor marker , functions as an intercellular adhesion molecule. J Cell. Biol 2005; 327-334.
74. Perkin G, Evand S, Slater ED, Richard JG. Serum tumor markers. J Fam Physicians 2003;68(6):1075-82.
75. Nazli O, Bozdag AD, Tansug T, Kir R, Kaymak E. The diagnostic importance of CEA and CA 19-9 for the early diagnosis of pancreatic carcinoma. Hepatogastroenterology 2000;47(36):1750-2.
76. Guadagni F, Ferroni P, Carlini S, Mariotti S, Spila A, Aloe S, D'Alessandro R, Carone MD, Cicchetti A, Ricciotti A, Venturo I, Perri P, Di Filippo F, Cognetti F, Botti C, Roselli M. A re-evaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study. Clin Cancer Res. 2001 Aug;7(8):2357-62.
77. Ballesta AM, Molina R, Filella X, Jo J, Giménez N. Carcinoembryonic antigen in staging and follow-up of patients with solid tumors. Tumour Biol. 1995;16(1):32-41.
78. Dnistrian AM, Schwartz MK, Greenberg EJ, Smith CA, Schwartz DC. Evaluation of CA M26, CA M29, CA 15-3 and CEA as circulating tumor markers in breast cancer patients. Tumour Biol. 1991;12(2):82-90.
79. Robertson JF, Jaeger W, Syzmendera JJ, Selby C, Coleman R, Howell A, Winstanley J, Jonssen PE, Bombardieri E, Sainsbury JR, Gronberg H, Kumpulainen E, Blamey RW. The objective measurement of remission and progression in metastatic breast cancer by use of serum tumour markers. European Group for Serum Tumour Markers in Breast Cancer. Eur J Cancer. 1999 Jan;35(1):47-53.
80. Bai Land Zhu WG. p53: Structure, Function and Therapeutic Applications. J. of Ca. Molecules 2006; 2(4): 141-153
81. Gasco M, Yulug IG, Crook T. TP53 mutations in familial breast cancer: functional aspects. Hum Mutat. 2003; 21: 301-306.
82. Mills AA. p53: link to the past, bridge to the future. Genes Dev 2005; 19: 2091-2099.
83. Taha I. Hewala, Nadia A. Abd El-Monaim, Medhat Anwar and Samia A. Ebied. "The Clinical Significance of Serum Soluble Fas and p53 Protein in Breast Cancer Patients: Comparison with Serum CA 15-3". Pathology & Oncology Research 2012; 18: (4) 841-848.
84. Balogh GA, Mailo DA, Corte MM, Roncoroni P, Nardi H, Vincent E, Martinez D, Cafasso ME, Frizza A, Ponce G, Vincent E, Barutta E, Lizarraga P, Lizarraga G, Monti C, Paolillo E, Vincent R, Quatroquio R, Grimi C, Maturi H, Aimale M, Spinsanti C, Montero H, Santiago J, Shulman L, Rivadulla M, Machiavelli M, Salum G, Cuevas MA, Picolini J, Gentili A, Gentili R, Mordoh J. Mutant p53 protein in serum could be used as a molecular marker in human breast cancer. Int J Oncol. 2006 Apr;28(4):995-1002..
85. Haupt S, Berger M, Goldberg Z, Haupt Y. "Apoptosis the p53 Network", Journal of Cell Science 2003; 116: 4077-4085.
86. Rahko E. Evaluation of tumor suppressor gene p53, oncogene c-erb-2 and matrixmetalloproteinase-9 as prognostic and predictive factors in breast carcinoma. 2007.
87. Due EU, Phuong VU, Froyland CJ, Meggerud A, Warenberg F, Zhou W, et al. TP53 mutation pattern in breast cancer progression. P53 Marathon Meeting, 3rd International Workshop on Mutant P53, 2007, P. 43.
88. Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. Breast Cancer Res. 2002;4(2):70-6.

89. Lacroix M, Toillon RA, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer. 2006 Jun;13(2):293-325.
90. Lasky T, Silbergeld E. p53 mutations associated with breast, colorectal, Liver, lung and ovarian cancers. Environ Health Perspect 1996; 104: 1324–31.
91. Bergjvist J. HER2 and P53 in human cancer studies of methods and prognostic value. Stockholm 2006.
92. Bqrresen-Dale A. TP53 and Breast Cancer. Human mutation 2003; 21:292-300.
93. Molina R, Barak V, van Dalen A. Tumer markers in breast cancer-European Group on Tumer Markers recommendations. Tumour Biol 2005; 26: 281- 293.
94. Gion M, Mione R, Leon AE, Dittadi R. Comparison of the diagnostic Accuracy of CA27.29 and CA15.3 in primary breast cancer. Clin Chem 1999; 45:630-7.
95. Chan DW, Beveridge RA, Muss H, Fritsche HA, Hortobagyi G, Theriault R, et al. Use of Truquant BR radioimmunoassay for early detection of breast cancer recurrence in patients with stage II and stage III disease. J Clin Oncol 1997; 15:2322-8.
96. Hou MF, Tasi LY, Tasi SM. Evaluation of serum CA27.29, CA15-3 and CEA in patient with breast cancer. Kaohsiung. J. Med Sci 1999; 15:520-258.