Association of Breast Cancer with Epstein-Bar Virus and Cytomegalovirus Infection

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Abstract

Cancer is one of the most important health problems of the current era and also a leading cause of death among populations. Breast cancer is the most commonly diagnosed malignancy in women around the world, especially in the Western countries. It accounts for almost one fifth of deaths caused by cancer. In Iraq it has been detected that the number of breast cancer cases are steadily rising since the 1991 war according to the Ministry of health/Iraqi cancer registry of 2014. Breast cancer was most frequent cancer among women and accounts for approximately one-third of the registered female cancers, with sharp increase in incidence of this tumor in young age group. The 2018 cancer registry confirmed the increase in cancer incidence including

breast cancer. In Arab countries including Iraq, breast cancer presented in earlier age than in Western countries. Thus early diagnosis of breast cancer may influence outcomes of treatment. In a recent study in Iraq, age of breast cancer diagnosis is two decades earlier than in Western countries. In conclusion, CMV and EBV infections may play a role in breast cancer development as they have ability for induction of latent infection. This latency phenomenon attribute to continuous exposure of host cells to viral particles in low steady-state and subsequent cell transformation.

Keywords: Breast cancer, Cytomegalovirus, Epstein-Barr virus, CMV IgG, EBV VCA, EBV ENBA-1, Heterophile antibody.

Cancer is one of the most important health problems of the current era and also a leading cause of death among populations. Cancer defined as a type of disorders or disease in which uncontrolled cells division is the main characteristics and spread of these abnormal cells, either by direct growth into adjacent tissues through invasion, or by implantation into distant sites by metastasis (where cancer cells are transported through the bloodstream or lymphatic system) [1]. The uncontrolled cells division attributed to a formation of masses or lump in all cancer types. Breast cancer begins in breast tissue, which is made up of glands for milk production, called lobules, and the ducts that connect lobules to the nipple. The remainder of the breast is made up of fatty, connective, and lymphatic tissue [2].

Breast cancer is the most commonly diagnosed malignancy in women around the world, especially in the Western countries. It accounts for almost one fifth of deaths caused by cancer [3,4]. Every year, one million new cases are reported worldwide, representing 18% of the total number of cancer in women [4,5].

In Iraq it has been detected that the number of breast cancer cases are steadily rising since the 1991 war [6,7] according to the Ministry of health/Iraqi cancer registry of 2014 [8], breast cancer was most frequent cancer among women and accounts for approximately one-third of the registered female cancers, with sharp increase in incidence of this tumor in young age group. The 2018 cancer registry confirmed the increase in cancer incidence including breast cancer [9]. In Arab countries including Iraq, breast cancer presented in earlier age than in Western countries. Thus early diagnosis of breast cancer may influence

outcomes of treatment [10]. In a recent study in Iraq, age of breast cancer diagnosis is two decades earlier than in Western countries [11].

Breast cancer usually affects tissues involved in milk production (Ductal and lobular tissues) [12,13]. It's originated from the terminal ducto-lobular unit of breast tissue. Breast cancer that has not invaded the basement membrane and thus confined within the terminal ducto-lobular units is termed carcinoma in-situ. Mainly, there are two types of in-situ cancers; lobular carcinoma in-situ and ductal carcinoma in-situ [14]. However, others rare forms of breast cancer were reported which include adenoid cystic, papillary and medullary carcinoma [15]. Breast cancer is generally presented with lump in breast, nipple change or discharge and skin contour change [16]. The development of breast cancer involves a progression through series of intermediate processes, starting with ductal hyper proliferation, followed by subsequent evolution to carcinoma in situ, invasive carcinoma, and finally into metastatic disease [17].

As in the case of most of the cancers, staging of breast cancer takes into consideration the size of the tumor (T), the number and location of metastatic lymph nodes (N), and distant organ metastasis (M) [18]. Previous studies have indicated that detection of circulating tumor cells (CTCs) in the peripheral blood can be used in staging and prognosis stratification for breast patients [19,20].

Similar to other human cancer, breast cancer arises from a multifactorial process, but no specific etiological factor has been documented, there for, different breast cancer-associated risk factors have been suggested by epidemiological studies [10, 21, 22] and the genetics of the disease is now provoking speculation regarding possible hereditary influences on breast cancer risks that are related to racial or ethnic ancestry [23].

These risk factors include increasing age; mutation in breast cancer risk genes (including BRCA1, BRACA2 and p53) [10, ,23,24]. Recent study [10] in Iraq indicated a significant association of breast cancer with BRC1 and BRC2 using AUC. Additionally, the mean serum BRC1 was significantly lower in women with breast cancer of $\geq$ 35 years as compared to those with less than 35 years age. Other risk factors include family history of breast cancer, late menopause, dietary habits, endogenous (estrogens and androgens), exogenous hormones (oral contraceptives, hormone replacement therapies), environmental factors (radiation, chemicals and heavy metals) [25] and certain

pathologic findings within breast tissue, including previous breast cancer and various premalignant lesions [26].

Recent study indicated that 43.2% of women with breast cancer were of moderate socio-economic status, 68.9% were unemployed house wives, 85.1% from urban area, 64.9% were married, 36.5% were with primary school education level, 67.6% with no family history of breast cancer, 44.6% starting menarche at 13 years old, 8.1% with menopause, 48.6% no history of pregnancy, 16.2% with 6-11 child, 21.6% nulliparous, and 28.4% with history of abortion [11].

Several reproductive risk factors including age at menarche, age at marriage, age at first childbirth, null parity, breast feeding and age at menopause are major risk factors which are mainly responsible for the variation in breast cancer incidence seen in different regions of the world [21,27].

Age plays a crucial role in breast cancer risk, with increasing age the incidence of breast cancer also increases [9]. However, studies in Arab countries indicating a shift in breast cancer incidence toward younger age groups [11]. In a study in Iraq, the breast cancer incidence was 20.3% in women with age of ≤ 20 years, 32.4% in those with age of 21-30 years, 27% in women with age of 31-40 years, 18.9% in women with age of 41-50 years and 1.4% in those with age of 50 years and above. Unfortunately, 52.7% of cases are in the age ≤ 30 years and 79.7% of cases are in the age of ≤ 40 years. [11].

The age specific incidence of breast cancer increases abruptly with age until menopause [28]. Alwan reported a trend for breast carcinoma to affect younger age group [29]. In Iraqi population there is a significant influence of age on CEA, CA 27-29, P 53, ER and PR. However, the age not influenced the serum values of CA 15-3.

Unfortunately, most cancers do not produce any symptoms until the tumors are either too large to be removed surgically or cancerous cells have already spread to tissues, i.e, metastasis has taken place [30]. Cancer detection in its early stage is with vital importance as may influence the treatment outcomes and prognosis. Thus health education programs for women training for self examination of the breast are very important tool for early diagnosis of the tumor. However, simultaneous determination of CEA, CA 27-29 and CA 15-3 may be with predictive value for early diagnosis of breast cancer [10].

Tumor markers are used for population screening, diagnosis, staging and prognosis of breast cancer. They can also predict the

response to therapy, monitor treatment, detect the presence of occult metastasis and monitor the course of the disease [31]. Tumor Markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. A tumor marker produced by the tumor and, when present in significant amounts, indicates the presence of a cancer. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum [32].

Several serum tumor markers (STM) have been proposed to indicate the presence and future behavior of breast cancer (BC), such as carcinoma antigen15.3(CA15.3), carcinoma antigen (CA27-29) and carcinoembryonic antigen (CEA) are the most widely investigated tumor markers in women with breast cancer [33,34]. Other biomarkers emerged for prognosis and prediction of breast cancer [35] these include estrogen receptor, progesterone receptor, p53 and others [36,37]. Recent study in Kirkuk, Iraq, indicated that serum mean value of CA 15-3, CA 27-29, and CEA were significantly higher in women with breast cancer than in controls [10]. Odd ratio and relative risk confirm the association between serum increase of the three markers and breast cancer [10]. Area under curve (AUC) of ROC indicated the high sensitivity of their determination in breast cancer. Additionally, serum mean value of prolactin, progesterone receptor, estrogen receptor, glucose, HbA1C and calcium were significantly higher in women with breast cancer than in controls [38]. However, circulating estrogen, progesterone, IGF-1, parathyroid hormone, and vitamin D mean values were significantly higher in controls as compared to that in women with breast cancer [38].

Viral factors are the most important class of the infectious agents associated with human cancers [39]. It was estimated that 17-20 % of worldwide incidence of cancers attributable to a viral etiology [40]. Recently, viral infection implicated to play a role in breast cancer development [41]. Among viruses Herpesviridae family has been implicated as a cause of breast cancer [42]. Human cytomegalovirus [HCMV] and Epstein –Barr virus [EBV] could potentially involved in breast cancer [43-47]. HCMV women infection in Arab countries including Iraq was with a range of 77.8% to 95.7% [48-51].

In vitro studies suggest oncogenic transforming potential of HCMV [52-54]. In addition, HCMV antigens and DNA were detected in breast cancer tissue and / or elevation of serum HCMV antibodies in

women that was precede the development of breast cancer or higher in women with breast cancer than in controls [46, 55-58].

Cox et al [46] found that increase in serum CMV IgG levels precede the development of breast cancer in some women. In addition, Alibek et al [42] suggested that the most relevant non-self antigen in breast cancer patients was HCMV as demonstrated using IgG allotypes analysis which are associated with certain tumor antigens. Certain allotype presence with HCMV seropositivity may act synergistically to lead to development of breast cancer [59]. Other studies not detected CMV in breast cancer tissue using RT-PCR [44,60]. Study performed in Erbil, Iraq, indicated that 77.7% of women with breast cancer were seropositive to CMV IgG and OR and AUC ROC confirmed an association between CMV infection and breast cancer [43]. In addition, HCMV DNA was detected in 20% of breast tumor specimens using Real-Time PCR [43]. Other studies reported a very wide range (8.3%-97%) for the detection of CMV DNA in breast cancer tissue [47, 55,61-63]. In contrast, other studies not detected CMV particles in breast cancer biopsies [44,57,60].

HCMV may induce breast cancer through multiple mechanisms, which include, escape of tumor cells from exposure to immune system through HCMV ability to induce immunosupression [64, 65]. In addition, affection of cell cycle regulation, inhibition of apoptosis, activation of angiogenesis and cause increased mutation rate through HCMV gene products [64]. Furthermore, virus encoded interleukins [IL] suggested to play a role such as HCMV expression of viral analogue of human IL-10 [66]. HCMV infection induce immunosupression and this attribute to development of abnormal cells, either directly by initiation of transformation or indirectly through co-infections with other microbes [57,67,68]

The predominant source of HCMV transmission in human is the breast milk [55], and in women seropositive HCMV, more than 90% of their breast milk were positive for HCMV [69, 70]. Although HCMV detected in normal breast tissue [71], the prevalence of HCMV antigens was relatively higher in neoplastic epithelium of patients with breast cancer [47]. Taher et. al. [58] suggest that the role of HCMV in the pathogenesis of breast cancer is unclear. In Iraq, the detection rate of CMV IgG antibody was 100% [72] and CMV DNA in 23.7% of breast cancer tissue [61]. Alsamarai et al [43] found that mean serum of CMV IgG was significantly higher in women with breast cancer (1.4384

IU/ml) as compared to controls (1.125 IU/ml) and 77.7% frequency of positivity in cancer patients.CMV infection may has an association with the development of breast cancer as the virus induce latent infection which mean a continuous exposure of the host cell to the effect of virus.

Richardson et al [56] conclude that CMV is a risk factor for breast cancer as they found a high CMV IgG antibody in breast cancer group as compared to controls. Richardson et al [57] reported in their study and meta-analysis that 96% of serum samples were positive for EBV IgG and 70% positive for CMV IgG, EBV and CMV were detected in 34% and 0% in breast tumor respectively. On the basis of their study findings and the meta-analysis suggest that limitation of molecular analysis may lead to variability in different study's findings, "hit and run" as HCMV and Epstein –Barr virus [EBV] may influence the inconsistent results, one or both viruses has a role at a later stage in breast cancer development, multiple viral infection may increases breast cancer risk, or neither virus has a role [57].

EBV in 1995 was detected in 21% of breast cancer samples [73] and was followed by a series of studies that detected EBV infection in women breast cancer [73-88]. Mazouni et al [89] detected EBV DNA in 33.2% of breast cancer tissue in France, while Aguayo et al [90] detected EBV in 6.5% of breast cancer in Chile. Huo et al [91] in a meta-analysis found that 29.32% of women with breast carcinoma were infected with EBV, with more prevalence in Asia (35.25%) and lowest in USA (18.27%) and conclude that EBV infection is associated with breast carcinoma. Zekri et al [92] found that EBV was detected in 45% and 28% of Egyptian and Iraqi women respectively who are with breast cancer. One study in Iraq reported a detection rate of EBV of 40% in breast cancer tissue [93]. Although, the reported studies concerning the association between EBV infection and breast carcinoma statistically varied widely, two studies reported that EBV seropositivity and IgG mean serum value were not significantly different between breast cancer and control group [46,56]. However, the role of EBV in development of breast cancer should not be excluded.

Our study [43] indicated that EBV VCA IgG mean serum level was significantly higher in women with breast cancer (1.2672 IU/ml) as compared to controls (0.759 IU/ml). In addition, EBV EBNA-1 IgG mean serum value was significantly higher in women with breast cancer (1.492 IU/ml) than in controls (1.131 IU/ml). Also, the

frequency of positivity to EBV VCA IgG and EBC EBNA-1 IgG were significantly lower in controls (2.1%, 0% respectively) than in women with breast cancer (73.6%, 85.1% respectively). All women with breast cancer were positive for heterophile antibodies. Role of EBV in the development of breast cancer may differ geographically and tumor grade, size and histology were significantly correlated with EBV EBNA-1 antibody [94]. Other study found that EBV EBNA-1 antibody was significantly lower in women with benign breast tumor than in those with breast cancer [95]. Study in China suggests a significant association between EBV VCA IgA antibody and breast cancer [96].

In conclusion, CMV and EBV infections may play a role in breast cancer development as they have ability for induction of latent infection. This latency phenomenon attribute to continuous exposure of host cells to viral particles in low steady-state and subsequent cell transformation.

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