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Expression of EPCAM as cancer stem cell marker in breast cancer Iraqi women carcinoma

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ABSTRACT

According to mounting evidence, PC may arise from cancer stem cells (CSCs), which are a distinct population of cancer cells with features similar to those of stem cells. However, the CSCs that spread the cancer cells and tumor that are resistant to medical therapy may be distinct. CSCs have the capacity to naturally resist medical treatment and cause tumor relapse. Breast cancer is made up of a diverse range of tumors with different characteristics, physiologies, and therapeutic approaches. A small glycosyl phosphatidylinositol (GPI)-joined cell surface protein involved in cell adhesion is called EPCAM. It resembles mucin. Enhancing clinical outcomes and preventing the challenge of cancer will be made possible by improving understanding of the processes behind cancer resistance and the development of treatment approaches. Resistance to chemotherapy. Age, grade, tumor size, histological type of tumor, and clinical stage were among the clinicopathological variables that were examined and revealed significant group differences. In this study, sixty patients with breast cancer from Iraqi women were included. They were divided into three groups: Group I, which contained 20 newly diagnosed patients, Group II, which contained 20 relapsed patients who underwent chemotherapy and recovered later, and Group III, which contained 20 patients who displayed resistance or no response to chemotherapy. The percentages of immunohistochemical expression on the basis of cell scoring data of EPCAM positive cells for groups I (42,59%), II (64,68%), and III, with a greater proportion reaching (77,89%) with significant (P 0.05) variations between groups. Additionally, histopathological parameters were examined, revealing various histopathological characteristics in each group of the study, such as high necrosis and severe hemorrhage. The tumor cells also extended to the right from the luminal surface at the upper left to the muscular is propria at the lower right, and there was a high degree of variability in the longitudinal spaces of the tumor mass.

As a last thought, EPCAM marker might be used to target therapy of breast cancer stem cells within the tumor tissue limiting the recurrence of the tumor since they play a part in cancer resistance to chemotherapy and relapsing of the illness.

Key words: EPCAM, IHC, cancer stem cells and breast cancer stem cells.

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INTRODUCTION

Cancer Stem Cells (CSCs) are a unique population of cancer cells featuring stem-cell like characteristics and also, based on growing evidence, they may start the development of PC as is the situation with other neoplasms. CSCs have the ability to inherently resistant against medical treatment and lead to tumor relapse, however, the CSCs that propagate the cancer cells and tumor which are resistant toward medical therapy may be different. Breast cancer is composed of a heterogeneous group of tumors having numerous features, biology, and treatments. The first group "Carcinomas" is cancers that happen from the epithelial part of the breast. The epithelial part is composed of the cells that lining the lobules and terminal ducts; according to normal conditions, these types of epithelial cells are in charge of making milk. The second group "sarcomas" is a type of cancer that starts in tissues like bone or muscle. Bone and soft tissue sarcomas are the main types of sarcoma. Soft tissue sarcomas can develop in soft tissues like fat, muscle, nerves, fibrous tissues, blood vessels, or deep skin tissues. They can be found in any part of the body. [1].

According to the World Health Organization (WHO) [1], cancer is the second leading cause of death globally. Globally, there are more than 18 million instances of cancer, which caused 9.6 million deaths in 2018 and

around 1 in 6 fatalities overall. Additionally, according to the most recent WHO data, breast cancer is the most common malignancy worldwide in 154 out of 185 countries, frequently the leading cause of cancer-related mortality in more than 100 countries, and accounts for the majority of cancers in women, accounting for about 25% of all documented female cancers. In 2018, there were approximately 2.1 million new cases of breast cancer diagnosed.

CSCs are a distinct subpopulation of cancer cells that resemble stem cells. According to mounting evidence, they may also be the precursors of PC, as they are for other neoplasms. When normal tumor cells, which make up the majority of the tumor, divide or undergo self-renewal, cancer stem cells seem to produce daughter cells while still possessing the complete capacity to differentiate and divide as their parent stem cells do. Research had been done on the likelihood of finding specific markers that would identify PC CSCs. This category comprises several specific proteins, including as CD-133, CD-24, CD-44, CXCR-4, EpCAM, ALDH-1, Oct-4, ABCB-1, c-Met, ABCG-2, and nestin [2].

Resistance to chemotherapy and the return of the illness in patients who have relapsed are two key issues in the treatment of breast cancer. Because cancer stem cells have a unique method to withstand chemotherapy and live, researchers determined that this may be caused by the existence of cancer stem cells within the tumor mass.

The correct detection and analysis of the existence of these types of cells within the tumor breast mass might thus aid in the right targeting and elimination of these cells by focusing on their markers. Chemotherapy treatments sometimes result in the tumor returning.

The EPCAM was the main topic of this investigation. A small glycosyl phosphatidylinositol (GPI)-joined cell surface protein involved in cell adhesion is called EPCAM. It resembles mucin. Additionally, EPCAM seems to be linked to matrix adhesion and modulation of cellular shape through stress fibers, processes of nuclear condensation, and also rounding, which shields cancer cells from apoptosis. [3].

In order to aid in cancer targeted therapy, the current study examines the immunohistochemistry expression of EPCAM protein and its function as a cancer stem cell marker in a sample of Iraqi women who have breast cancer.

MATERIALS AND METHODS

Samples collection

The research involved 60 patients, all female, ranging in age from 40 to 65. The samples were gathered in partnership with the Dr. Majed Al-Dari Clinic in Baghdad, Iraq, from the histology section of the Medical City Hospital, Department of Education Laboratories, and Ministry of Health and Environment. Samples from the Raji Al-Hadithy laboratory were cut into sections. Three breast cancer organizations were gathered. 20 samples from breast cancer patients who had just received a diagnosis were included in Group I, 20 samples from patients who had relapsed after receiving chemotherapy, and 20 samples from patients who had shown resistance to or no response to chemotherapy were included in Group II.

Preparation of Avidin-Biotin Complex (ABC) staining system working solution

- PolyExcel H2O2 Peroxide Block: Block for 5 minutes.
- The primary antibody: was created in accordance with the manufacturer's instructions. (Biotechnology PathSitu).
- Only biotinylated Horse Radish Peroxidase (HRP), which is coupled with secondary antibodies, was employed as the AB enzyme reagent.
- PolyExcel Target Binder: The tissue sections were coated with this substance and let to sit at room temperature for 10 minutes.
- PolyExcel PolyHRP: PolyExcel was used to coat the tissue sections. PolyHRP and RT incubation for ten minutes.
- PolyExcel StunnDAB: StunnDAB working solution was applied to

tissue slices, and it was incubated for 5 minutes at room temperature.

- To make the peroxidase substrate, combine 1.6 ml of distilled water with 5 drops of substrate buffer (10x), 1 drop of DAB chromogen (50x), and 1 drop of 50x peroxidase Method.

- Removing the Wax: After the paraffin sections were elaborated, paraffin wax, which is hydrophobic and resistant to aqueous reagents, was infiltrated and enclosed around all of the constituents. The majority of the tissue and cell components is transparent and lacks any natural color. The first step in applying an H&S stain is to completely dissolve the wax with the hydrocarbon solvent xylene.
- Hydration of the Section: Following thorough de-waxing, the slide was moved by changing a series of alcohol to remove the xylene from the tissue, and the tissue was then rinsed in water. Now that the slice is moist, aqueous reagents can easily permeate the tissue and cell components.
- Hematoxylin Nuclear Stain Application: The tissue is now stained using a nuclear stain such as as Harris Hematoxylin, which is a solution of an aluminum salt and an engaged agent (oxidized hematein or hematoxylin). The nuclei and a few other components first take on a reddish-purple hue as a result.
- Finishing the Nuclear Stain by "Bluing": The portion is "blued" by handling a mildly alkaline solution after being washed with tap water. The Hematoxylin takes on a dark blue hue as a result. Section
- Now that the nuclei have been properly stained, they may be washed and examined to determine whether there is an appropriate discrepancy and to determine the degree of background stain.
- Removal of Extra Background Stain (Differentiate): When Harris Hematoxylin is utilized in general, a differential (de-staining) procedure is necessary to remove background staining that isn't specific and to advance disparity. There was a bad acid alcohol used. After this procedure, complete cleaning and bluing are still preferred. "Regressive" stains are those that entail a de-staining or differentiating stage in the staining process.
- Eosin Counterstain Application: Here, the section is stained with an alcoholic solution of eosin. This gives a variety of non-nuclear elements various pink hues.
- Rinse, dehydrate, clear, and mounting (Apply Cover Glass): Following the eosin stain, the slide is moved through a succession of alcohol to remove any water's effects, followed by washing in numerous xylene baths, which "clears" the renders and tissue to a crystal-clear state. Glass cover slip uses a fluffy layer of polystyrene mount ant. If the staining process and all following steps are carried out correctly, the slide will accurately detect all required microscopic elements and remain stable for a number of years.

RESULTS AND DISCUSSION

Clinicopathological study

For the purpose of differentiating and comparing between different clinic pathological variables among the patient group based on the information being followed up in the patient history files in the

Medical City Hospital archives in Baghdad, the paraffin embedded breast cancer samples of each group of the study were collected and examined under the consultation of Histopathological and oncologist consultants. (Table 1) details the findings of the clinical pathological characteristics that were observed. All of the research participants were female, and their mean ages ranged from 40 to 65 with a notable difference across groups. Group I (patients with recently discovered breast cancer) exhibits a greater percentage of (Table 1). details the findings of the clinical pathological characteristics that were observed. All of the research participants were female, and their mean ages ranged from 40 to 65 with a notable difference across groups. Compared to Groups II and III, Group I (patients with newly diagnosed breast cancer) has a larger number of elderly women. [4]. It was shown that a sample of breast cancer-stricken women had substantial differences in their ages and other clinicpathological characteristics as determined by hospital case reports. The two forms of breast cancer that are often distinguished by the estrogen hormone are estrogenic and non-estrogenic. Estrogen According to the Iraqi Institute of Cancer Reports, dependent breast cancer is the most prevalent kind of cancer in the country. Results showed that in the group were the older patients regarding to age than the other groups of the study with a significant variations among groups table (3-1) and (3-2), which indicates that the majority of women reach the menopause at age 50 due to the estrogen secretions levels are quaintly drop and reducing by age. Typically, this is because younger people have a higher number of stem cells in their intestinal mucosa, and older people may use a different aberrant pathway to develop cancer stem cells that have distinct chemotherapeutic drug resistance mechanisms. Most samples from group I, which often do not get chemotherapy recorded a larger tumor mass size than other groups who underwent a full course of chemotherapy treatment without response in group III and with a recurrence of the tumor after some time, so typically either slightly affected and regressed or completely regressed the tumor. The two forms of invasive carcinomas are Invasive Lobular Carcinoma (ILC) and invasive ductal carcinoma (IDS), with varying numbers of instances in each group of the research. (Table 2).

Tab. 1. Distribution of breast cancer patients groups according to age

Age	Group I		Group II		Group III		Total	
	No.	%	No.	%	No.	%	No.	%
40-45	6	0.1	8	1.61	12	0	22	25.3
46-50	4	0.1	2	0	2	0.2	8	6.99
51-55	5	0.1	7	2.33	8	1.3	20	24.4
56-65	5	0.1	3	0.03	4	0.3	12	20.7

Tab.2. Classification of breast cancer patients into stage and grade

Grade	Stages										Total	
	T0		T1		T2		T3		T4			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Low	10	36.4	19	22.4	12	0	10	0	16	0	48	39.8
High	2	0	6	14.5	22	55	7	17.5	4	10	45	51.8
Total	12	16.4	25	38.4	34	30.1	17	7.6	20	7.8	93	100

Histopathological sections study

After the preparation of H&E histological slides, histopathological parameters were measured for each group in the study. (Figure 1). depicts the histopathological analysis of group I, which shows early signs of cancer represented by the orientation of the cancer cells, the condensation of chromatin in the rapidly dividing cells, as well as the signs of hemorrhage and hyperplasia. [5,6].

A B

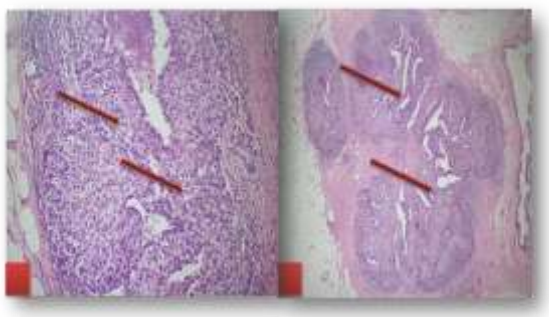
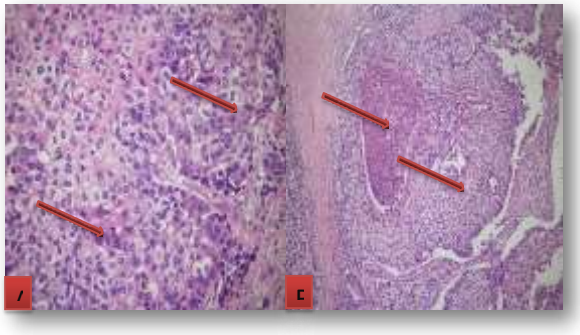


Fig. 1. Histological sections for H&E stained breast tissue of group I (newly diagnosed patients) cancer cells Looks like micro papillary breast carcinoma like to be EMA (inverted polarity)

Ductal carcinoma NST in DDx (Arrows), section shows signs of hemorrhage, hyperplasia and of chromatin condensation inside the cell's nucleus. A) 400X, B) 100X

The return of the tumor mass from the core tissue of the breast mass is seen in group II histological sections (Figure 2). The cancer cells in the tumor are all invasive ductal carcinoma after a full round of treatment. Glycogen-rich invasive cancer without DCIS and intraductal carcinoma



A B

Fig. 2. Histological sections Histological sections for H&E stained breast tissue of group II
(relapsed patients) this is all invasive ductal carcinoma. No DCIS, intraductal and invasive carcinoma with Glycogen rich. A) 400X, B) 100X

(Figure 3). displays the histological sections of group III patients (those with a strong resistance to chemotherapy treatment sessions). The tumor cells extended right from the luminal surface at the upper left towards the muscularis propria right to the lower right, and there is a high notable variability in the spaces among the tumor mass longitudinal spaces. The tumor cells' nuclei are distinguished by what is known as chromatin granularity or clearing, indicating a high proliferative state of the cells [7].

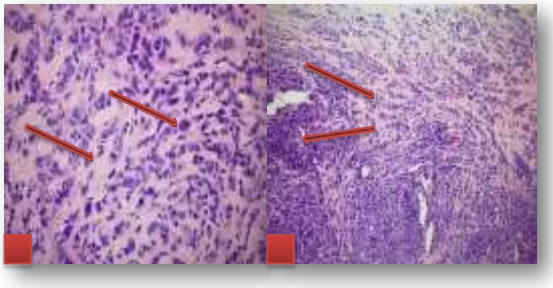
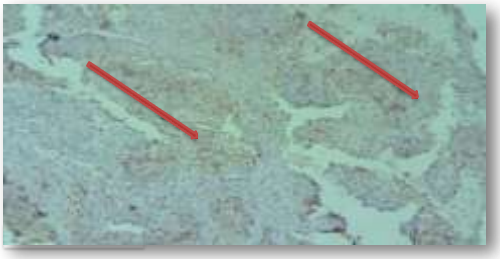


Fig. 3. Histological sections for H&E stained breast tissue of group III (resistant patients), Invasive lobular carcinoma E cadherin to rule out NST carcinoma with lobular feature after a complete course of treatment with no response to the applied chemotherapy, (H&E, X400).

Mesurement of immunohistochemical expression of the selected specific markers within the tumor tissue (EPCAM marker).

In this investigation, the expression of the EPCAM marker was identified, compared for the three patient groups, and then microscopically analyzed on all slides gathered from the three patient groups. This was done using immunohistochemistry staining (IHC). Five fields were used to calculate the percentage of each CD marker on each sectioned slide. While deep or light brown represents the approximate intensity or percentage of marker expression inside the IHC stained tumor mass slides, brown denotes the presence of EPCAM marker in positive tumor cells stained with DAB stain. By counting the positive EPCAM marker cells and comparing their numbers to the overall number of tumor cells in the tumor mass, the proportion of precisely expressed cancer stem cells inside the tumor mass was determined. Five fields were used to calculate the percentage of each CD marker on each sectioned slide. While deep or light brown represents the approximate intensity or percentage of marker expression inside the IHC stained tumor mass slides, brown denotes the presence of EPCAM marker in positive tumor cells stained with DAB stain. By counting the positive EPCAM marker cells and comparing their numbers to the total number of tumor cells in the field, it was possible to analyze and determine the percentage of each marker in all research groups, as well as the percentage of precisely expressed cancer stem cells inside the tumor mass. The approach, which includes dividing the number of positive cells for the designated marker, has been authorized by the American Society for Clinical Pathology

(ASCP). According to the total number of positive and negative cells in five specifically chosen microscopic areas. The outcomes of the EPCAM marker are displayed in (Figures 4 and 5). While the negative EPCAM cells in Group I's tumor mass appear deep blue to violet in color, indicating their reactivity to each measured marker and calculated as cancer stem cells in the tumor mass, the positive EPCAM cells in the tumor mass of Group I appear brownish in color, indicating their positivity to the EPCAM marker.



EPCAM

Fig. 4. Immunohistochemical expression of each EPCAM for the group I of newly diagnosed patients showing the positive expressed cancer stem cells with stained with the DABI stain as a brown color (arrows), and the negative cancer cells stained with the counter hematoxylin stain the violet to blue color (arrows), magnification power 400X

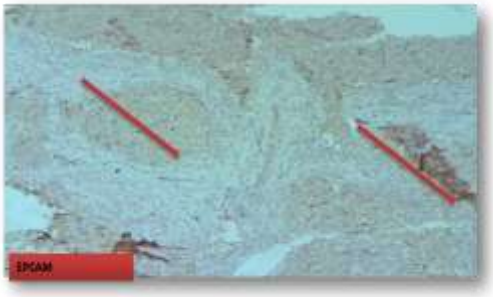


Fig 5. Immunohistochemical expression of each EPCAMfor the group II of Relapsed patients showing the positive moderately high expressed cancer stem cells within the breast tumor mass which stained with the deep DABI stain as a brown color (arrows), and the negative cancer cells stained with the counter hematoxylin stain the violet to blue color (arrows), magnification power 400X

According to the findings, Group III (resistance patients) had the greatest level of expression of the EPCAM marker and had never responded to treatment. Graph (3-6). in (Figure 6).

Based on the acquired data, similar findings were made, showing that the putative stem cell marker EPCAM was substantially related with poorer survival. Particularly, EPCAM could contribute to the development of tumors and the spread of cancer. However, more extensive research required to validate these results. [8,9].

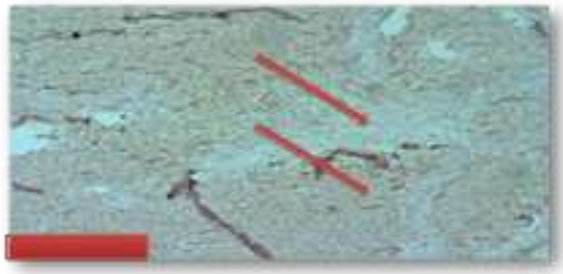


Fig. 6. Immunohistochemical expression of each EPCAM for the group III of Resistance patients showing the positive high expressed cancer stem cells with stained with the deep DABI stain as a brown color (arrows), and the negative cancer cells stained with the counter hematoxylin stain the violet to blue color (arrows), magnification power 400X

Percentage of IHC expression of EPCAM marker upon cell scoring:

(Figure 7). displays the percentages of positive for each CD within each group. Significant group differences are evident in the results. In contrast to the other groups, Group I (newly diagnosed patients) have a weak to moderate expression of EPCAM, whereas Group III exhibits the greatest expression of CD markers. There is a substantial difference between groups, with Group II (relapsed patients) showing a greater expression of CD markers than the initially diagnosed group. After groundbreaking research by that demonstrated the EPCAM marker cells were recognized as cancer stem cells marker for the basal/mesenchymal cell lines MCF 7 and MDA-MB-231, human breast cancer line

[10]. Then who is rich in these cells, endorsed this [11]. Breast cancer stem cells possess many transmembrane proteins that participate in the process of pumping the drugs outside of the cells, as well as these cells proliferate relatively slowly as compared to the surrounding breast cancer cells around the tissue. Breast cancer stem cells can resist chemotherapy causing tumor relapsing down regulation of their DNA repair mechanisms. in many genes responsible for chemotherapeutic resistance and malignant invasion. Later, a wealth of data showed that not all breast malignancies would exhibit this phenotypic trait, highlighting the necessity to identify other breast CSC markers to distinguish these cells from other types of cells in the tumor tissue.

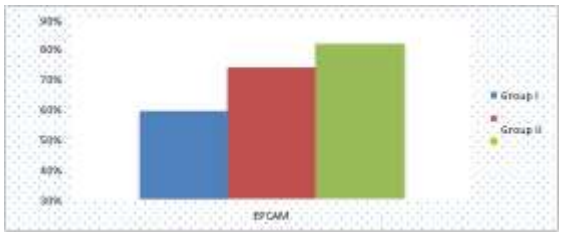


Fig.7. Percentage of immunohistochemical expression upon cell scoring data of EPCAM positive cell for the groups I (newly diagnosed patients), II (relapsed patients) and III (resistant patients) showing significant differences between groups, (data are Mean±SD) Different letters mean significant differences between groups at (P≤ 0.05)

[12] NF-kappa demonstrated that the basal-like breast cancer subtype is controlled non-cell autonomously by cancer stem cell populations. Nat Community 4, 2299 In addition, NSCs from the Breast Cancer Surveillance Consortium (BCSCs) display Oct4, a pluripotency marker, which enables these cells to develop and form diverse tumors. However, the phenomenon has also been linked to breast cancer. A few human basal epithelial cells, for instance, naturally dedifferentiate into stem cells. Malignant tissue has a substantially higher incidence of this type of event. It is also known that the presence of Interleukin 6 (IL-6) can cause non-tumorigenic cancer cells to develop CSCs. This represents a dynamic balance between CSCs and differentiated cells. In order to increase BCSCs, chemotherapy and radiation have been used, demonstrating how resistant they are of these cells in proportion to the tumor's size. The stimulation of pathways that promote self-renewal, including Hedgehog, Notch, and Wnt, is another source of this. For instance, Notch stimulates the expression of Surviving, which downregulates a number of cell cycle check points and prevents apoptosis brought on by medicines or radiation. Another important factor that controls cell survival and the cell cycle is the cycling D1, which is a target of the Wnt and Notch pathways. Additionally, BCSC differentiation and self-renewal as well as tumorigenicity are often dependent on cycling D1. BCSCs survival, metastasis, decreased survival, and relapse survival are all directly correlated with cancer stage. The BCSCs' resistance to conventional treatments necessitates the creation of medications that directly target these cells. these types Therapies can target BCSCs' cell surface markers, influence key signaling pathways, or monitor drug resistance mechanisms inside these cells. [13]. Breast cancer recurrence is a significant clinical manifestation and the main reason for breast cancer fatalities. Following adjuvant therapy, around 10% of individuals with breast cancer will experience an isolated breast cancer recurrence. Many scientists have tried to identify some kind of pattern in the recurrence of breast cancer. This has required studying a variety of breast cancer subtypes, including those that are identified by the presence of certain receptors, such as the HER2/ErbB2 receptor (HER2), the progesterone receptor, and the estrogen receptor, or by their absence, which are referred to as triple negative breast cancers. Its sides are itTherapies can target BCSCs' cell surface markers, influence key signaling pathways, or monitor drug resistance mechanisms inside these cells. [13]. When compared to ER-positive breast cancers, it has been discovered that ER-negative breast cancers had a greater chance of recurrence over the first five years following diagnosis.

Following this, the risk of recurrence in ER-positive breast cancers typically increases during the following 10 years, and 15 years after diagnosis, the risk appears to level off for both subtypes. It is frequently believed that ER-negative/PR negative but HER2-positive tumors have a greater probability of recurrence than ERpositive/PRpositive/HER2negative cancers in cases of ductal carcinoma in situ. In addition to the basic breast categorization there are other sub classifications of breast tumors as well, including basal, HER2-enriched, luminal A, and luminal B. The selection of an appropriate therapy is made challenging by the existence of several subtypes, which frequently overlap but are typically so distinct. As a result, it has been suggested that patients get therapy that is specifically tailored to their needs. [14,15]. Typically, bladder malignancies, breast cancers, kidney cancers, prostate cancers, ovarian cancers, non-small cell carcinomas, as well as other human cancers, have high levels of EPCAM expression cancers. It participates in cell adhesion and metastasis. This suggests that EPCAM may have a significant role in the diagnosis and prognosis of tumors. From a functional standpoint, it can be considered a different ligand for P-selecting, where their interaction makes it easier for tumor cells to go via the bloodstream during metastasis. It increases the tumor cells' adherence and growth to collagen, fibronectin, and lamina. The importance of EPCAM as a novel CSCs marker and a prognostic factor is increased by its metastatic linkages. [16].

CONCLUSION

1. Breast cancer stem cells could play essential roles in tumor resistance leading to possible recurrence of the disease after chemotherapy courses and recovery.

2. Ep-CAM protein could be targeted as a major surface marker for breast cancer stem cells within the breast tumor mass.

3. Immunohistochemistry analysis is one of the methods used efficiently to diagnose and immunophentyping cancer stem cells in breast mass clinical samples.

4. According to clinicopathological study significant differences were showed among groups of patients the study in relation to age, tumor size, grade and type of tumor with a noticeable higher ratio in resistance group.

5. IHC staining analysis showing significant differences in the percentage of expression of EP-CAM among group showing a higher percentage of expression in the resistance group as compared among other groups.

6. EP-CAM marker was highly expressed as more specific marker for breast cancer stem cells in each group.

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