

## LEVELS OF CATHEPSIN-D AND NESTIN IN BREAST CANCER PATIENTS

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### Abstract

**Background:** Breast cancer is the second most frequently cause of cancer deaths among women, therefore, new screening modalities could provide an extraordinary progress in understanding the mechanism of disease. In last decade, the role of Cathepsin-D (Cath-D) and Nestin which recently received attention as tumor markers, are remain unclear.

**Aim:** Measurement the levels of Cath-D and Nestin in breast cancer patients (BCPs) and estimating an association of each marker to stage of disease, early and advanced, and to age of study patients.

**Materials and methods:** An overall 48 adult women who attended to the Kirkuk Oncology Center (Kirkuk province, Iraq) and diagnosed clinically as BCPs; 24 at early stage and 24 at advanced stage, were selected to the present study that carried out from December (2022) to June (2023). The obtained sera were tested by specific ELISA kits.

**Results:** The quantitative findings of ELISA showed that the values of Cath-D and Nestin in BCPs were  $283.997 \pm 18.12$  pg/ml and  $66.258 \pm 2.559$  pg/ml, respectively. Moreover, significant higher values of Cath-D and Nestin were detected in advanced stage ( $395.683 \pm 14.737$  pg/ml and  $78.581$  pg/ml, respectively) when compared to those of early stage ( $172.31 \pm 6.33$  pg/ml and  $53.934 \pm 2.36$  pg/ml, respectively). According to age of study patients, insignificant variation ( $P \leq 0.0$ ) was seen in values of  $\leq 40$  and  $\geq 41$  years old for both Cath-D ( $305.319 \pm 31.55$  pg/m and  $270.027 \pm 21.81$  pg/ml, respectively) and Nestin ( $67.357 \pm 2.81$  and  $64.532 \pm 3.85$  pg/ml, respectively).

**Conclusion:** This represents the first Iraqi study determined Cath-D and Nestin in BCPs. This study demonstrates that these markers were provided additional information about the level of cancer activity in patients at early and advanced stages of disease.

**Keywords:** Tumor marker, Early and advanced stages, ELISA, Age, Kirkuk Oncology Center, Iraq

## INTRODUCTION

Breast cancer is one of the most common cancers in women worldwide, which diagnosed yearly in 1.5 million women, worldwide (Sun et al., 2017). The name of refers to a disease of mammary glands, but it has also a metastatic phenomenon that enable to transfers for distinct organ such as lungs, liver, bones and brain (Del Piccolo et al., 2021; Bisoyi, 2022). The disease develops due to specific gene(s) as a family history, alteration in hormonal levels, sedentary life-style, high dietary intakes and contaminated environments (Shiovitz and Korde, 2015; Golubnitschaja et al., 2016; Nihad, 2018). However, the spontaneous forming of cancer cells occurs by increasing of entropy; evolution and aging of cells; overproduction of free radicals; mechanical cell injury; exposure to chemicals, infectious organisms and radiations; and radiation (He et al., 2021; Ouyang et al., 2022); with the improper working of immune system, or the cells produced at greater amounts for the immune system to eliminate (Sharma et al., 2010; Onkar et al., 2023).

Based on their sites, there are several types of breast cancers that are basically divided into invasive and non-invasive. The most frequent types include ductal and lobular carcinoma in situ and infiltrating ductal carcinoma; while, the less frequent types include medullary, mutinous and tubular carcinomas (Tsutsumi, 2012; Sannachi et al., 2015; De Brot et al., 2017; Natal et al., 2019). However, the classic symptoms of disease include the presence of lump in breast or armpit, which undergoes cyclical changes in size and skin condition, in addition to unusual breast pain or discomfort, persistent tenderness of the breast, scaly or pitted skin on nipple, pain in nipple, nipple discharge and an enlargement in the underarm lymph nodes (Ikhuoria and Bach, 2018; Milosevic et al., 2018; Thota et al., 2020). In metastatic cases, weakness, neurological pain, headaches, weight loss, anorexia, shallow respiration, and bone pain might be involved (Seller and Symons, 2011; Kabalak and Yılmaz, 2023). Based on these symptoms, breast cancer is categorized into in situ carcinoma (0), localized and regional invasive cancer (I, IIA, IIB, IIIA, IIIB, and IIIC), and metastatic (IV), (Trayes and Cokenakes, 2021).

At present, the clinical examination of breast cancer is supported by a number of diagnostic methods such as ultrasounds, imaging, pathological biopsies, and measurement of tumor markers in sera (Alwan et al., 2012; Hao et al., 2020). Tumor markers are typically proteins that are produces by cancer cells, and many of which, can be found in the blood (Rajguru et al., 2020). Tumor markers, as one of the most widely performed identification indexes in clinical laboratories, are characterized by its low cost, easy acquisition of test specimens convenient clinical development, and, and is (Luo et al., 2023). In Iraq, almost studies target the correlation of breast cancer to blood groups (Zaki et al., 2013), chromosomal aberrations (Ahmed and Hidayat, 2020), enzymes (Nsaif et al., 2018), gene expression (Mahmoud, 2014; Rasheed, 2023), hormones (Khalaf et al., 2022) and pathology (Alwan et al., 2017) with limited available data concerned to immune and tumor markers (Zghair et al., 2016; Ibrahim et al., 2021 a, b; Hussain et al., 2022; Khadem et al., 2022). Hence, the current study aims, for first time in Iraq, to measurement the levels of serum Cath-D and Nestin in BCPs using the enzyme-linked immunosorbent assay (ELISA), and estimating the association of each marker to stage of disease, early and advanced, and to age of study patients.

## Materials and methods

### *Ethical approval*

The current study was conducted following the license of the Scientific Committee of the Department of Pathology, College of Medicine, University of Kirkuk (Kirkuk, Iraq).

### *Samples*

An overall 48 adult women who attended to the Kirkuk Oncology Center (Kirkuk province, Iraq) and diagnosed clinically with breast cancer; 24 at early stage and 24 at advanced stage, were selected to the present study that carried out from December (2022) to June (2023). After she agreement, venous blood was inspirited, centrifuged, the sera were saved frozen until be tested by ELISAs.

### *Serology*

According to the manufacturer instructions (SunLong Biotech, China) of the Human Cath-D (Cat. No: SL2372Hu) and Human Nestin (Cat. No: SL2925Hu) ELISA Kits, the sera and contents of kits were prepared at room temperature. For each kit, the Standards, samples and Washing Buffer were diluted. The diluted Standards and samples were added respectively to the ELISA plate, covered, incubated and washed. After adding of HRP-conjugate, the plate was covered, incubated, washed, and the Chromogens A and B were added. After incubation, the Stop solution was added, and the absorbance was read at 450 nm Optical density (OD). After setting the OD value of Blank Control well to zero, ODs and concentrations of Standards and ODs of sera were plotted on the Standard Curve to calculate the concentration of a marker in tested samples.

### *Statistical analysis*

Chi-Square ( $\chi^2$ ) test and One-Way ANOVA in the GraphPad Prism Software (version 6.01) were applied to assess variation in values [Mean  $\pm$  Standard Error (M $\pm$ SE)] of Cath-D and Nestin in early and advanced BCPs, and to detect association of each marker to different to age groups at P<0.05 (AL-Shaeli et al., 2022).

## Results

The quantitative findings of ELISA showed that the values of Cath-D and Nestin in BCPs were  $283.997 \pm 18.12$  pg/ml and  $66.258 \pm 2.559$  pg/ml, respectively (Figure 1). Moreover, significant higher values of Cath-D (P $\leq$ 0.035) and Nestin (P $\leq$ 0.0271) were detected in advanced stage ( $395.683 \pm 14.737$  pg/ml and  $78.581$  pg/ml, respectively) when compared

to those of early stage ( $172.31 \pm 6.33$  pg/ml and  $53.934 \pm 2.36$  pg/ml, respectively), (Figures 2, 3). According to age of study patients, insignificant variation ( $P \leq 0.0$ ) was reported in values of  $\leq 40$  and  $\geq 41$  years old in both Cath-D ( $305.319 \pm 31.55$  pg/m and  $270.027 \pm 21.81$  pg/ml, respectively) and Nestin ( $67.357 \pm 2.81$  and  $64.532 \pm 3.85$  pg/ml, respectively), (Figures 4, 5).

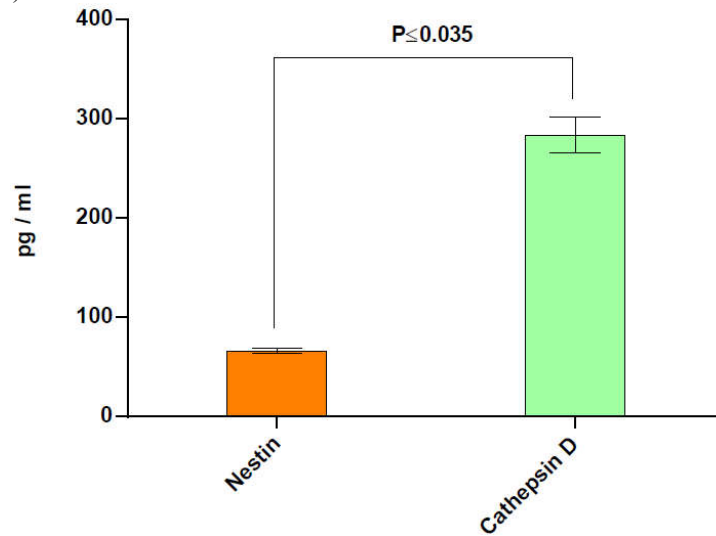


Figure (1): Total results of Cath-D and Nestin in breast cancer patients (Total No. 48)

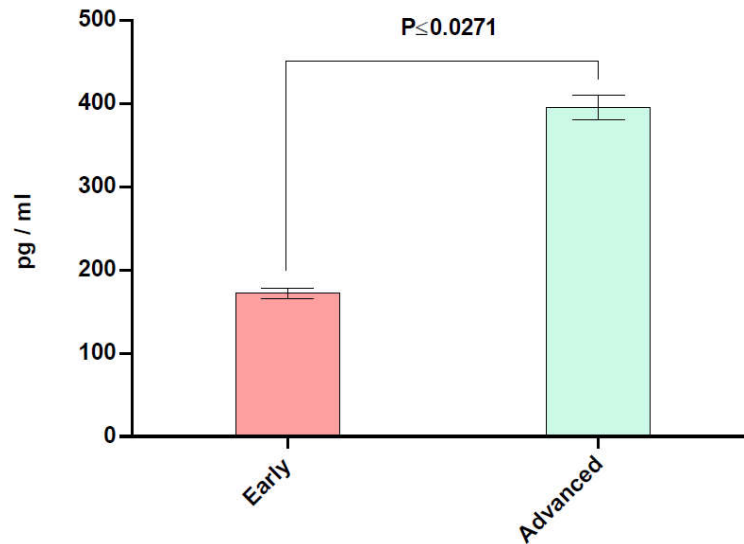


Figure (2): Results of Cath-D in early and advanced stages of breast cancer patients

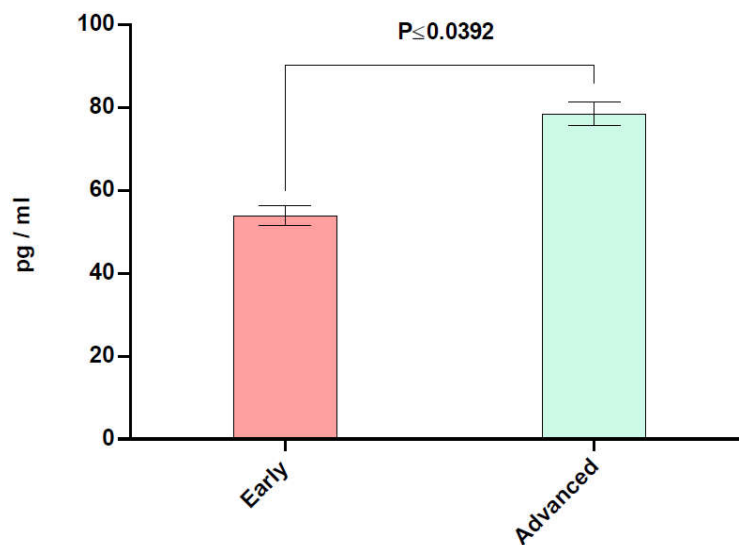


Figure (3): Results of Nestin in early and advanced stages of breast cancer patients

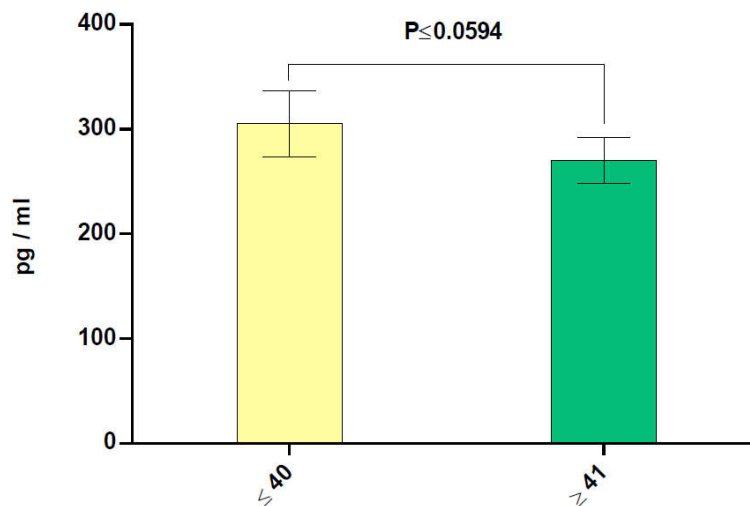


Figure (4): Distribution of Cath-D values according to age of breast cancer patients

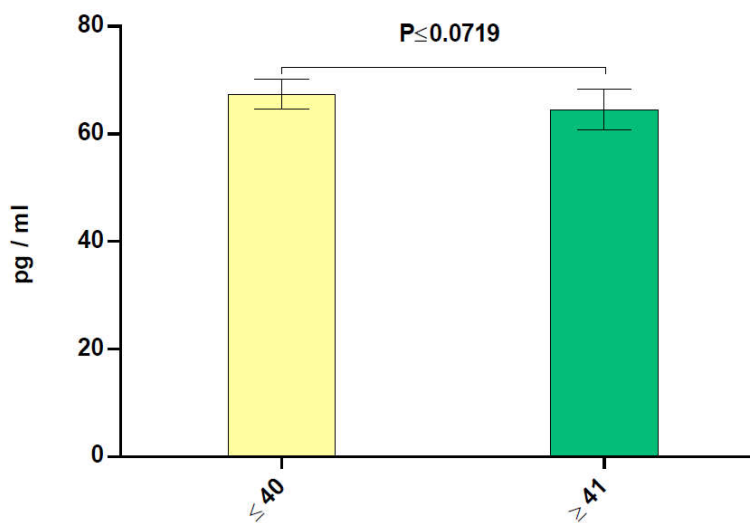


Figure (5): Distribution of Nestin values according to age of breast cancer patients

**Discussion**

In last three decades, several researchers show that Cath-D (Rocheftort et al., 1987; Wilson et al., 1991) and Nestin (Parry et al., 2008; Liu et al., 2010) are preferentially expressed in basal / myoepithelial cells of mammary gland, and might have value as a prognostic factor in breast cancer. In this study, the findings show the high concentrations of Cath-D and Nestin in BCPs in particular in advanced cases.

Cath-D, first described in 1979 by Westley and Rocheftort, is an estrogen-induced lysosomal protease, which found to be produced constitutively in different pathological processes (Karamanos et al., 2021; Kos et al., 2022; Jiang et al., 2023). Capony et al. (1989) demonstrated the higher expression of Cath-D at 2- to 50- fold in comparison with its concentration in normal mammary glands or fibroblasts. Using a standardized and validated cytosolic immunoassay, Rocheftort (1990, 1992) indicated that the Cath-D level in primary breast cancer cytosol is an independent prognostic parameter correlated to clinical metastases and shorter survival. Later studies demonstrated that this marker is both housekeeping enzyme and a regulated enzyme induced by estrogens and growth factors (Schwartz-Roberts et al., 2013; Dian et al., 2014; Malik et al., 2016). In a meta-analysis study, authors have suggested that the overexpression Cath-D is concerned to a poorer prognosis in BCPs, and a therapeutic target for breast cancer (Kang et al., 2020).

Since 2007, Nestin has received attention as a primeval angiogenesis protein marker of newly proliferating endothelial cells in colorectal cancer (Teranishi et al., 2007); and then, as a myoepithelial marker in breast cancer (Li et al., 2007; Parry et al., 2008). Liu et al. (2010) detected that Nestin expression is significantly correlates with lymph node metastases, and is associated with poor prognosis in the lymph node positive patients; suggesting the concept that Nestin-positive cancer cells may have higher lymphatic metastases capability. Zhao et al. (2014) indicated that Nestin positively regulates the proliferation, survival and invasiveness of breast cancer stem cells by enhancing the Wnt/ $\beta$ -catenin activation. In 2017, a study demonstrated that Nestin-positive microvessel density was higher in cases with lymph node metastases, advanced stage disease and higher histological grade; suggesting that this increases in Nestin is related to a greater aggressive course and a poor prognosis (Nowak et al., 2017). Nowak and Dziegiel (2018) have found the expression of Nestin in breast cancer stem cells and newly-formed tumor vessels; and that Nestine acts as a factor of promoting invasion and metastasis. Recently, concluded that Nestin is a valuable biomarker for unfavorable clinic-pathological features and tumor angiogenesis of breast cancer (Zhang et al., 2020).

Our results reported insignificant differences ( $P>0.05$ ) between values of both Cath-D and Nestin in BCPs aged  $\leq 40$  years old and those of  $\geq 41$  years old. These findings were in agreement with those detected by other researchers (Gao et al., 2014; Sun et al., 2016) but in contrast with others (Malik et al., 2016; Zhang et al., 2020).

### Conclusion

This might be the first Iraqi study determined serologically Cath-D and Nestin as tumor markers in BCPs. This study demonstrates that these markers were provided additional information about the level of cancer activity in patients at early and advanced stages of disease. However, the identification of more accurate predictors of release or survival in patients with breast cancer might improve the selection of therapy and guide further investigation into basic biologic questions concerning such tumors. In Iraq, furthermore studies appear necessary to estimate the association of other tumor markers in BCPs or other diseased cancers.

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### Conflicts of interests

There is conflict to be interested.

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