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Article

Evaluation of Serum Midkine Levels as a Diagnostic Differentiator of Malignant from Benign Breast Tumors

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Abstract: Introduction. Breast cancer (BC) is a disease of breast cells that can grow out of control and result in tumors. BC holds the distinction of being the most significant and commonly diagnosed cancer among women globally. Midkine (MDK) is a simple heparin-binding low molecular weight growth factor and is highly induced during oncogenesis, inflammation, and tissue repair. This study aimed to measure MDK levels in women with BC and whether they can be used as a potential diagnostic biomarker for distinguishing between malignant and benign breast tumors. **Methods.** The study included 90 participants, 60 of them patients (female) with BC, divided into two groups first group included 30 patients with malignant breast tumors (G1), while the second group included 30 patients with benign breast tumors (G2), and also the control group included 30 healthy individuals (G3). A blood sample was obtained from the patient before surgery, and the practical part of this study was carried out over a period of eleven months at Oncology Teaching Hospital in Medical City, Baghdad. Group comparisons were performed using one-way ANOVA, and diagnostic performance was assessed via ROC curve analysis. **Results.** The findings of the study revealed that there was an increase in MDK levels in two groups of women with BC compared with the control group, with a significant difference between G1 and G2 P-value (0.01), G2 and G3 P-value (0.01), whereas a highly significant difference between G1 and G3 P-value (0.001). The AUC for all group comparisons (malignant vs. benign, malignant vs. control, and benign vs. control) was 1.00 ($p<0.001$), indicating perfect discrimination for MDK as a diagnostic biomarker. **Conclusions.** The levels of MDK were abnormally elevated in women with breast tumors when compared to the control group. Moreover, MDK levels in malignant conditions are markedly higher than in benign conditions. Therefore, MDK could be used as a potential diagnostic biomarker for distinguishing between malignant and benign breast tumors. Furthermore, future studies are required for larger cohorts.

Keywords: Breast Neoplasms, Medkine, Breast Malignant Neoplasms, Breast Benign Neoplasms.

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1. Introduction

Breast cancer (BC) is a disease of breast cells that can grow out of control and result in tumors. If the tumors go untreated, they can metastasize throughout the body and lead to death. BC is the leading and most frequently encountered cancer in women in the world. BC represents about 24.2% of all newly diagnosed cases of cancers in women worldwide, which means one in four newly diagnosed cases is breast cancer, and is responsible for the largest cause of cancer deaths in women [1]. BC is the most common type of female cancer; incidence rates in women are particularly elevated today. Known risk factors are: (A) menstrual and reproductive history, (B) family history of breast cancer, (C)

postmenopausal obesity, (D) genetics, (E) tobacco smoking, and (F) exposure to ionizing radiation. More than half of the BC risk was unexplained. In recent times, various innovative approaches have been developed to address cancer treatment, with a particular focus on breast cancer, by aiming to minimize the adverse side effects often associated with conventional therapies, which include surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy. BC is histologically classified as in situ or invasive carcinoma [2]. Each type can be further divided into ductal and lobular forms. After invasive BC is diagnosed, the primary focus is on determining which patients should undergo chemotherapy and which should receive adjuvant therapy, as well as identifying those who do not require additional treatment. If adjuvant therapy is chosen, the subsequent challenge is to determine the most suitable treatment or combination of treatments for each patient [3]. BC was demonstrated that miRNAs play a dual role in mammary gland development, growth, and proliferation, which may be relevant to BC biology, where all patients diagnosed with BC can take the first step toward personalized treatment by combining approved prognostic/predictive biomarkers and conventional prognostic factors. CtDNA is a dependable method for tracking tumor load dynamics in patients with metastatic BC receiving systemic therapy, and it can help choose the best sequencing of treatments in metastatic disease [4].

Midkine (MDK) is a simple heparin-binding low molecular weight growth factor and, along with pleiotrophin (NEGF1; 46% identical with MDK), is classified in a family of heparin-binding growth factors consisting of 121 amino acids with two disulfide-bonded domains, both non-glycosylated proteins [5]. This gene product, known as MDK, plays a crucial role in development and is strongly induced by retinoic acid during mid-gestation. Normal adult MDK is primarily limited to specific tissues; however, MDK is highly induced in tissues during processes such as angiogenesis, inflammation, and repair. Pleiotropic activity of MDK influences various processes such as cell proliferation, migration, angiogenesis, and fibrinolysis [6]. MDK was originally detected because it is highly expressed during early mouse embryogenesis. Over time, MDK has come to be recognized as a pleiotropic protein that, along with pleiotrophin (PTN), forms a growth factor family with a characteristic structure (the unusual form in the latter case), primarily the binding to heparin, which suggests that they play major roles in development and in many biological processes [7]. MDK is a soluble secreted protein whose level increases in many types of diseases, such as cancer, and is, as a result, a promising marker for diseases. The research findings indicate that MDK is commonly up-regulated in most cancers. MDK expression in tumors tends to increase as cancer stages progress. MDK expression within tumors can be accessed via blood, urinary, and tumor studies [8].

BC is a complex disease with great genetic inheritance and frequency. While a great number of biomarkers are investigated in detail, only a few of them are accepted for clinical application. The heterogeneity of breast cancer may have differences in clinical outcome and prognosis. Therefore, it is high time to search for novel molecules acting as novel prognosis biomarkers as well as novel therapeutic targets. Measurements of plasma and tissue MDK levels in breast cancer patients showed an abnormal increase compared with those in control subjects [9]. These findings suggest that MDK might be deregulated during the early stages of the disease process. The diagnosis accuracy of breast cancer was significantly improved with the combination of raised plasma MDK level and classical markers CA15-3 and CEA. A combination of these markers gave more precise results, potentially leading to earlier detection and more effective treatment strategies. Plasma MDK levels in the patients who survived the sepsis were elevated when compared to the patients who did not survive. It has been found that inhibition of MDK has significantly beneficial effects on lung injury in sepsis in humans, yet there still exists a wide discrepancy in understanding the role of MDK in critical illness, including sepsis, requiring further investigation in a clinical setting focused on its regulation. However, distinguishing benign from malignant breast tumors remains a clinical challenge and also requires invasive biopsy. Therefore, a non-invasive serum biomarker could reduce

unnecessary procedures. The objectives of this study were to assay MDK levels in BC women and determine whether it can serve as a promising diagnostic marker differentiating between malignant and benign breast tumors [10].

2. Materials and Methods

2.1 Study design and participants

All patients recruited for the present study were diagnosed at the Oncology teaching hospital of Medical City of Baghdad according to the WHO classification of a sample biopsy from the Breast for cancer cells, and according to the Tumor, Node, Metastasis (TNM) classification. Two groups of patients' sera were prepared. Group 1 contains 30 serum samples from malignant breast tumors (G1), whereas group 2 contains 30 serum samples from benign breast tumors (G2), and group 3 contains 30 serum samples from healthy subjects (G3). This study was approved by the College of science /, Mustansiriyah University. The ethics committee of the Ministry of Health and Environment of Iraq also approved our study. In turn, ethical consent was taken from all the individuals, and all the study groups were adequately informed about our study, including purpose, methods, possible advantages of the study, and how the results would be kept confidential. MDK levels were measured using enzyme-linked immunosorbent assays (ELISA) kits.

2.2 Exclusion Criteria

Excluded from this study were the following groups of people: premenopausal women (< 45 yrs); patients with metabolic diseases, acute and chronic infections, renal diseases, muscle diseases, and malignancies, or benign tumors at other sites which could affect the plasma level of MDK.

2.3 Blood Sample Collections

In this current study, Blood was collected using a syringe in the vein for five milliliters from both healthy women and breast cancer patients before surgery, and put into a gel tube to coagulate for ten minutes, and then was centrifuged at 3000 rpm for ten minutes, and serum was obtained at room temperature and then kept at -25 °C until we measure its concentration using an ELISA kit.

2.4 Statistical Analysis

Statistical data on biochemical variables of the cancer patients (patients with breast cancer) and the healthy subjects were evaluated using the Statistical Package for Social Sciences (SPSS). The values were shown as mean ± SD (standard deviation) using the method of one-way ANOVA and post-hoc test for pairwise comparisons. The ROC curve analysis was performed to calculate the area under the curve (AUC), sensitivity, and specificity. Highly significant and significant values were given with $P < 0.001$ and $P < 0.05$, respectively.

3. Results

In the two BC women groups and the control group, MDK levels were found to be elevated, as presented in **Table I**. Comparing the different BC groups with the control and among BC groups: highly significant difference between G1 with G2 (984.23 ± 153.34), (576.66 ± 77.56), P-value (0.01), highly significant difference between G2 with G3 (576.66 ± 77.56), (290.46 ± 49.01), P-value (0.01), and very highly significant difference between G1 with G3 (984.23 ± 153.34), (290.46 ± 49.01), P-value (0.001) respectively [11]. Because of this significant difference, it has been determined using receiver operating characteristic curve (ROC) analysis to decide on the usefulness of the biomarker curves for MDK for discriminating between benign and malignant breast tumors from the control one, thus they are good candidates for differentiation.

Table 1. Serum levels of (MDK) in Three Groups

Parameters	(G1) Mean ± SD	(G2) Mean ± SD	(G3) Mean ± SD	P-Value G1 vs. G2	P-Value G1 vs. G3	P-Value G2 vs. G3
MDK	984.23 ± 153.34	576.66 ± 77.56	290.46 ± 49.01	0.01	0.001	0.01

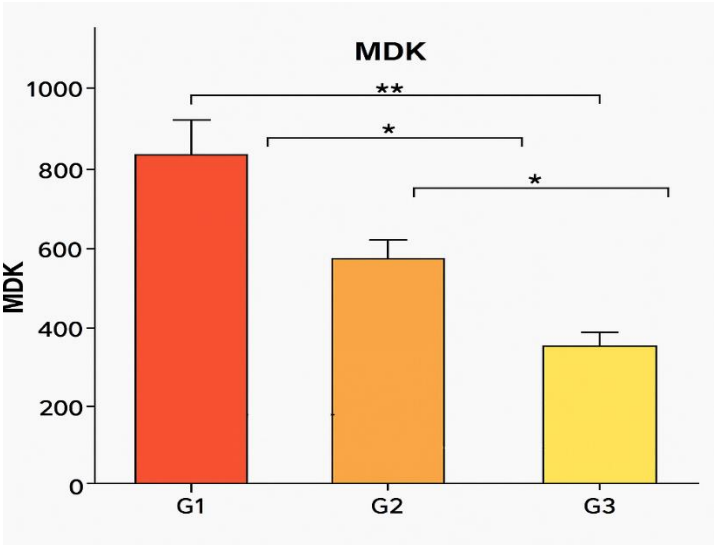


Figure 1. Serum levels of (MDK) in Three Groups

Table 2. Diagnostic Performance Metrics for Group Comparisons (AUC, Sensitivity, Specificity)

Comparison	AUC	Sensitivity	Specificity	P-value
Malignant vs. Benign	1.00	1.00	1.00	0.001*
Malignant vs. Controls	1.00	1.00	1.00	0.001*
Benign vs. Controls	1.00	1.00	1.00	0.001*

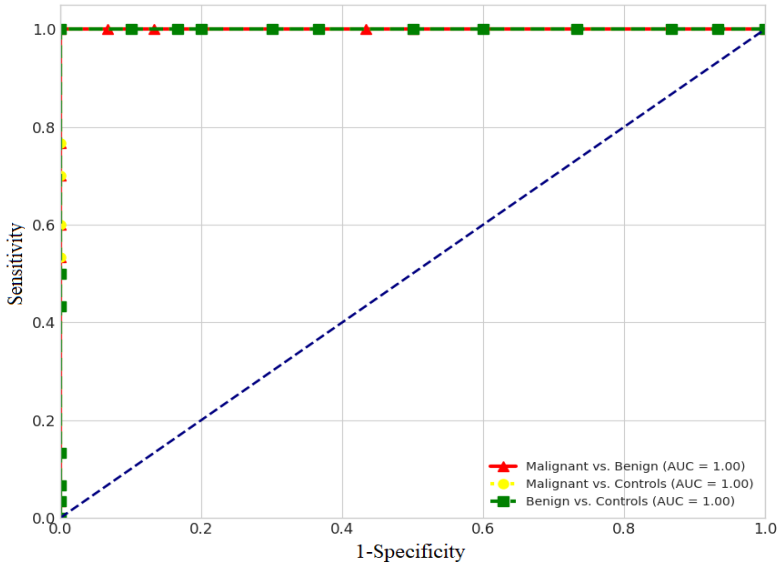


Figure 2. Display ROC curve for midkine between 3 groups as discrimination of benign vs malignant breast tumors (BC)

AUC (Area Under the Curve): The AUC for all comparisons (Malignant vs. Benign, Malignant vs. Controls, and Benign vs. Controls) is 1.00. This is the highest possible value and indicates that a perfect classification threshold exists [12].

4. Discussion

We observed that the levels of MDK in cancer patients are considerably higher than normal in the current study, and in the same way, G1 had significantly higher MDK than G2 P-value (0.01), and G1 had significantly higher MDK than G3 P-value (0.001), and G2 had a significantly higher MDK than G3 P-value (0.01), consistent with prior research [13]. Multiple biological and immunologic factors may be associated with high MDK in patients with malignant BC, in contrast to benign BC. MDK is a secreted protein in which cell in different situation like growth, inflammation, or wound healing secrete it. And the reason why MDK links with cancer is its roles in several vital processes. Angiogenesis (which refers to the formation of new blood vessels) is regulated by MDK and can contribute to tumor growth and metastasis by supplying the tumor with oxygen and nutrients [14]. MDK promotes the migration and dispersal of the cancer cell, and this is part of the pathway enabling the tumor cell to spread to adjacent tissue or to more remote organs. MDK can influence the immune response in cancer; this protein may reduce the immune system's ability to recognize and destroy cancerous cells [15]. On the other hand, most benign tumors do not spread from the initial site. Since new blood vessel formation is not required to the same extent as in malignant tumors, the MDK value is often lower in benign tumors. Therefore, possible that MDK is only indicative of malignant rather than benign proliferation [16]. MDK is a useful cancer marker in other studies at different time points of the disease, i.e., during early diagnosis, response to treatment, and monitoring after recurrence [17] [18].

The analysis of the ROC curve revealed that AUC between G1 and G2, G1 and G3, and G2 and G3 were 1.00, $p<0.001$, 1.00, $p<0.001$, and 1.00, $p<0.001$, respectively. Based on these results, the AUC value of MDK was statistically significant, proving that MDK has good performance in the diagnosis of breast tumors in terms of discriminating between benign and malignant tumors from the control group [19].

5. Conclusion

The findings of this study suggest that the MDK level is elevated significantly in women having breast tumors (malignant and benign) as compared to the controls, with a much higher level in malignant than benign disease. Hence, MDK could be a possible diagnostic marker for differentiation of malignant and benign breast tumors. As far as benign tumors are concerned, it was concluded that MDK can not only be a marker for cancerous disease but also for detecting benign ones, which may be beneficial for treatment planning and patient management. Our appreciation and gratitude are to the members of the College of Science, Mustansiriyah University, and the Oncology Teaching Hospital in Medical City for their valuable contribution in all steps of this study. This work was conducted without receiving any financial support. The researchers conducted this work on their own funding. The authors do not have any competing interests. All the operations undertaken in this research with human involvement were conducted according to the ethical procedures accepted by the National Research Ethics Committee of the College of science /, Mustansiriyah University. In addition, the ethics committee from the Ministry of Health and Environment in Iraq gave us permission for our study (20241108 at 20/2/2024), along with the Helsinki declaration from 1964 and amendments or alternative accepted standards of ethics. Informed voluntary consent was received from each volunteer within the study.

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