Original Article

The Diagnostic and Prognostic Value of Kinesin-4A Protein, β-Tubulin Protein, and Exosomes as Non-Invasive Biomarkers in Iraqi Women with Breast Cancer

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Abstract

Background: Breast cancer (BC) is a disease in which cells in the breast grow out of control. There are different kinds of BC. The kind of BC depends on which cells in the breast turn into cancer. BC can begin in different parts of the breast. **Objectives:** The current study aimed to investigate using Kinesin-4A (KIF4A) protein as a Diagnostic and Prognostic biomarker in Iraqi Women with Breast Cancer. **Materials and Methods:** This study involved 70 Iraqi women with (BC), who had BC diagnosed for the first time. The study also included a control group (CG) of 70 participants. The period the study extended from February 2021 to October 2022. The research was carried out in Iraq, at the Department of Biochemistry in the College of Medicine at the University of Babylon, as well as at The Oncology Center at Marjan Teaching Hospital in Hilla City, Iraq. Determination of kinesin-4A, β-tubulin, and exosomes was done by a fully automated Elisys Uno Analyzer before and after mastectomy. Various circulating biomarkers and tumor markers were investigated including liver function test. **Results:** First-time diagnosis show that kinesin-4A and β-tubulin were found to be increased in women with BC ($P < 0.01$), whereas the levels of exosomes were extremely high in BC women compared to the control group ($P < 0.001$). Two weeks after the mastectomy, the results of kinesin-4A, β-tubulin, and exosomes dropped dramatically near normal levels. **Conclusion:** These results suggest that different types of BC can alter several aspects of host immunity causing increased production of specific immune products. And these products can use as diagnostic and prognostic markers to reduce invasive procedures such as surgeries or radiation exposure.

Keywords: Breast cancer, exosomes, kinesin-4A, mastectomy, selenium, β-tubulin

Introduction

Breast cancer (BC) is a disease in which cells in the breast grow out of control. There are different kinds of BC. The kind of BC depends on which cells in the breast turn into cancer. BC can begin in different parts of the breast.[1] Within the Middle East Region, cancer is the fourth-ranked cause of death after cardiovascular diseases, infectious diseases, and injuries. According to the International Agency for Cancer Research and GLOBOCAN, the Age Standardized Incidence Rate in Iraq was (31.1/100,000).[2]

According to Data from the Iraqi Cancer Registry for Cancer, the estimated population of 38 million, the total number of new cancer cases reached (31,502) cases. The total number of deaths due to cancer was (10,293) deaths in 2018. BC incidence is gradually increasing in Iraq, and the total number of cases is 6094 (34.06% of all cancer types), while the number of deaths has reached 1166 cases (23.02% of all cancers). The highest death rate was among women in the age group of 70 years and over.[3]

The causes of BC are multifactorial and include a combination of genetic predisposition, hormonal factors, lifestyle choices (such as alcohol consumption, obesity, and lack of physical activity), reproductive history (early menstruation or late menopause), and exposure to certain

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environmental factors and radiation.[4] BC disrupts the normal physiology of breast tissue. Abnormal cell growth and division occur, leading to the formation of tumors. These tumors can interfere with the function of the breast, affecting the production and regulation of hormones, milk production, and lymphatic drainage. Metastasis may occur, spreading cancer cells to other organs.[5] There are several types of BC, including ductal carcinoma *in situ* (DCIS), invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), triple-negative breast cancer (TNBC), HER2-positive BC, inflammatory breast cancer (IBC), and male BC. DCIS is non-invasive, while IDC and ILC involve the invasion of surrounding tissues.[6] TNBC lacks estrogen, progesterone, and HER2 receptors. HER2-positive BC overexpresses the HER2 protein. IBC is characterized by breast redness and swelling. Although rare, BC can also occur in men. ADditional classifications consider hormone receptor status, grade, and stage, guiding treatment decisions and prognosis for individuals with BC.[7]

Several risk factors contribute to the development of BC, including advanced age, family history of the disease, certain genetic mutations (BRCA1 and BRCA2), early onset of menstruation or late menopause, hormone replacement therapy, dense breast tissue, previous radiation exposure, and lifestyle factors such as obesity and alcohol consumption.[8]

The kinesin superfamily proteins (KIFs), classified into 14 subfamilies, are ATP-dependent motor proteins with microtubule-dependent plus-end motion ability. Among the KIFs, KIF4A controls spindle organization, chromosome alignment, and kinetochore-microtubule dynamics with a protein regulator of cytokinesis.[9] Dysregulation of KIF4A induces abnormal spindle separation and causes aneuploidy of daughter cells. Several studies have suggested a potential relationship between KIF4A and cancer.[10] Increased expression of KIF4A has been observed in various types of cancer, including breast, lung, colorectal, ovarian, and gastric cancers. Elevated levels of KIF4A have been associated with poor prognosis and advanced tumor stage in these cancers.[11]

β-Tubulin, the protein to which all clinical agents that disrupt microtubules bind, is encoded by multiple genes and represented by several pseudogenes. β-Tubulin is the most divergent at the amino acid sequence level.[12] It is expressed exclusively in megakaryocytes and platelets in humans and appears to play an important role in the formation of platelets.[13] When β-tubulin is expressed in mammalian cells, it disrupts the microtubule network and microtubule fragment formation, and can ultimately cause marginal-band-like structures present in megakaryocytes and platelets.[14]

Exosomes are extracellular vesicles generated by all cells and they carry nucleic acids, proteins, lipids, and metabolites. They are mediators of near and long-distance intercellular communication in health and disease and affect various aspects of cell biology.[15] Exosome biogenesis is a mechanism of protein quality control, and once released, exosomes have activities as diverse as remodeling the extracellular matrix and transmitting signals and molecules to other cells.[16] This pathway of intercellular vesicle traffic plays important roles in many aspects of human health and disease, including development, immunity, tissue homeostasis, cancer, and neurodegenerative diseases.[17]

Cancer antigen (CA-125) is an antigenic determinant on a high-molecular-weight glycoprotein recognized by a monoclonal antibody. The highest serum levels of CA-125 are found in ovarian cancer patients, but elevation of serum CA-125 may also be associated with other malignancies and benign and physiological states, including pregnancy, endometriosis and menstruation.[18] Cancer antigen 15-3 (CA-15-3) is a tumor-associated antigen used as serum marker for BC surveillance in patients and for monitoring the response to treatment. CA-15-3 is a protein made by a variety of cells, particularly BC cells. The protein moves into the blood, where it can be measured.[19]

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are commonly used biomarkers for liver disease because of their abundance in hepatocytes.[20] Besides, because of their presence in other tissues, these enzymes may also be potential biomarkers for various diseases. Previous studies suggested a correlation between serum liver enzymes levels and visceral adiposity. A possible role of liver enzymes as markers of all-cause mortality and mortality from non-liver diseases, such as oral and oropharynx squamous cell carcinomas.[21]

Materials and Methods

Date and duration

The period extended from February 2021 to October 2022. The study was carried out in Iraq, at the Department of Biochemistry in the College of Medicine at the University of Babylon, as well as The Oncology Center at Marjan Teaching Hospital in Hilla City, Iraq.

Study design

The study design was a case–control study.

Patients and control

In this study, 70 patients with BC were included additionally, and 70 apparently healthy participants were included as the control group (CG).

Inclusion criteria

All patients with BC first time diagnosed (all stages included). All cases of BC were diagnosed by Mammography and Histopathological examination.

Exclusion criteria

BC with recurrence.

BC metastasis from another tissue such as lung or esophageal cancer.

Determination of kinesin-4A protein, β-tubulin protein, exosomes

Determination of markers levels in patient and control group were done 1-plate Fully Automated Elisys Uno Analyzer technology for immunoassay analysis.

Determination of liver enzyme and tumor markers

Determination of markers levels in patient and control group were done by Abbott architect c4000 and Cobas e411.

Ethical issues

The study was conducted in compliance with ethical principles based on the Declaration of Helsinki. Patients provided both verbal and written consent before the sample was taken. The study protocol, as well as the subject information and consent form, were reviewed and approved by a local Ethics Committee.

Results

First-time diagnosis show that kinesin-4A (KIF4A) was found to be increased in women with BC ($P < 0.01$). β-Tubulin (TUBB) levels were very high in BC women (221.3 ± 17.2) compared to controls (33.4 ± 3.1; $P < 0.001$) while the levels of exosomes were extremely high in BC women compared to the control group ($P < 0.001$). Two weeks after the mastectomy, the results of kinesin-4A, β-tubulin, and exosomes dropped dramatically near normal levels. Also results show that KIF4A, TUBB, and EVs were decreased to normal levels two weeks after the mastectomy [Table 1].

The results of this study show toward positive correlation (strength of the linear relationship) with different correlation degree between kinesin-4A, β-tubulin, and exosomes [Table 2].

Results at first-time diagnosis show that CA-125 and CA-15-3 were found to be increased in women with BC with P value (<0.001) and (<0.01), respectively. And these levels were significantly decreased two weeks a mastectomy with no significant difference when compared to control group (P value > 0.05) [Table 3].

Liver enzymes also show elevated levels in patients with BC at first-time diagnosis when compared to control group. These significant elevated levels decreased in the same patients 2 weeks after mastectomy with no significant different when compared to control group (P value > 0.05) [Table 4].

Discussion

The current study found elevated levels of KIF4A in first-time diagnosis with BC may associate with tumor progression, metastasis, and poor prognosis. High levels of KIF4A correlate with increased cell proliferation and migration in BC cells.[22] Inhibition of KIF4A has shown potential as a therapeutic strategy to impede BC growth and metastasis. Further research is ongoing to understand the precise mechanisms and potential targeting of KIF4A in BC treatment.[22] These results agreed with previous studies that also found high levels of KIF4A in ovarian cancer patients[23] and prostate cancer.[24] Low levels of KIF4A in the same patients were observed two weeks after metastasectomy, and these results agreed with a previous study done by Huang *et al*.[25]

Alterations in β-tubulin expression and mutations can impact microtubule dynamics and contribute to cancer development and progression.[26] One notable phenomenon associated with β-tubulin in gastrointestinal cancer is the overexpression of specific β-tubulin isotypes, such as βIII-tubulin (TUBB3). Increased expression of TUBB3 has been linked to resistance to microtubule-targeting chemotherapy drugs, such as taxanes.[27] Previous studies

Table 1: Results of study main markers among study groups

Parameter	First diagnosis	After mastectomy	Control	*P* value
Kinesin-4A (ng/mL)	25 ± 4.1	12.1 ± 2.7	8.1 ± 2.1	<0.01
β-Tubulin (ng/mL)	221.3 ± 17.2	27.8 ± 7.6	33.4 ± 3.1	<0.001
Exosomes (pg/mL)	4.6 ± 0.3	0.21 ± 0.2	0.11 ± 0.02	<0.001

Table 2: Different correlation degree between kinesin-4A, β-tubulin and exosomes

Correlation	*P* value	*r*-value	Correlation degree
Kinesin-4A and β-tubulin	<0.001	0.910	Strong positive correlation
β-tubulin and exosomes	<0.001	0.902	Strong positive correlation
Exosomes kinesin-4A	<0.05	0.417	Moderate positive correlation

found elevated levels of β-tubulin in different types of cancer such as pancreatic cancer,[28] prostate cancer,[29] and colorectal cancer.[30] As β-tubulin is normally expressed in different types of cells and tissue, recent analysis has demonstrated that β-tubulin is part of a complex, pro-survival, molecular pathway activated by hypoxia and poor nutrient supply. Induction of this pathway in cancer is associated with an aggressive phenotype in the majority of patients.[31] The result of the current study was found in agreement with Nami *et al.*[32] that also found high expression of β-tubulin in different subsets of BC types. β-Tubulin overexpression has been observed in a subset of BC patients and is associated with a poorer response to treatment and overall prognosis.[33]

Previous studies connect elevated exosomes level and different types of cancer. Tai *et al*.[34] concluded that exosomes-mediated cell–cell communication is required in remodeling tumor microenvironments and forming premetastatic niches during cancer development and molecules of exosomes that are produced from cancer cells provide the essential signals for reprogramming various cells and architectures in tumor microenvironments or premetastatic niches.[35]

The result in the current study is consistent with the result of a previous study conducted by Shtam *et al*.[36] which concluded that Plasma exosomes have a potency to stimulate the metastasis-promoting properties of BC cells. This pro-metastatic property of normal plasma exosomes may have an impact on the course of the disease and its prognosis. The exosomal contents can promote tumor cell proliferation, migration, invasion, and angiogenesis.[37,38] Exosomes released by BC cells also can modulate the immune response, suppress anti-tumor immune cells, and promote immune evasion.[18]

CA-125, also known as carbohydrate antigen 125, is a mucinous glycoprotein with a high molecular weight. It is found on the surface of ovarian cancer cells. It has been crucial in the screening, diagnosis, and treatment of ovarian cancer.[18] CA-125 is a tumor-associated antigen that is most commonly seen in advanced ovarian cancer. It is predominantly derived from coelomic epithelium, which explains elevations in benign conditions or other malignancies. Although CA-125 production has been demonstrated in the normal breast, it has been reported most often as a marker of pleural involvement with metastatic BC.[39,40] The result of the current study is found in agreement with Laffont *et al.*[41] which concluded that CA-125 can be used as a monitoring marker for BC.

CA-15-3 is a mucinous antigen encoded by the MUC1 gene. The function of MUC1 is not completely understood, but it might play a role in cell adhesion facilitating the detachment of malignant cells and this will lead to cancer invasion and metastasis.[42] Serum levels of CA-15-3, the most commonly used tumor marker for BC, are rarely elevated in patients with localized BC, whereas the majority of patients with metastatic breast carcinoma have elevated levels.[43]

Previously published studies have shown also higher levels of CA-15-3 when compared to normal healthy subjects.[44] The finding of the current study suggests that higher levels of CA-15-3 represent BC extent and reflect the cell differentiation and aggressiveness of the tumor. Therefore, it could be concluded that the determination of CA-15-3 before the surgical operation may be useful as a prognostic factor in BC.

The liver enzyme can be used as an independent factor in predicting the incidence of different types of cancer and could be used as a good biochemical indicator to predict the occurrence of cancer.[45-47] The result of the current study was found in agreement with a previously reported study that also found high liver enzymes in BC patients.[48]

Conclusions

Numerous factors can operate alone or in concert to cause BC, especially in women who are genetically predisposed to the disease or who are exposed to high-risk factors. Results suggest that different types of BC can alter several aspects of host immunity causing increased production of specific immune products and different proteins. And these products can use as diagnostic and prognostic

Table 3: Results of tumor markers among study groups

Parameter	First diagnosis	After mastectomy	Control	P value
CA-125 (U/mL)	70.1±12.5	13.2±2.9	10.5±2.1	***<0.001
CA-15-3 (U/mL)	55.4±7.7	21.2±3.5	18.7±5.1	**<0.01

Table 4: Results of liver enzyme among study groups

Parameter	First diagnosis	After mastectomy	Control	P value
ALT (U/L)	42±5.1	37±4.5	22±2.9	*<0.5
AST (U/L)	38±6.7	28±6.1	17±3.4	*<0.5
ALP (IU/L)	230±18.1	130±10.9	112±8.8	**<0.01

markers to reduce invasive procedures such as surgeries or radiation exposure. As the levels of kinesin-4A, *β*-tubulin, and exosomes dropped dramatically near normal levels. Two weeks after the mastectomy, these markers can be used for monitoring women with BC after mastectomy. The higher levels of study markers (kinesin-4A, *β*-tubulin, and exosomes) among specific individuals in the BC group the worse prognosis of the disease suggesting that these markers can be used as prediction markers.

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Conflicts of interest

There are no conflicts of interest.

References

1. Alwan NA, Tawfeeq FN, Mallah N. Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. J Contemp Med Sci 2019;5:14-9.
2. Alrawi N. A review on breast cancer in Iraq and future therapies insights. Baghdad J Biochem Appl Biol Sci 2022;3:4-16.
3. Al-Gburi AS, Alwan NA, Al-Tameemi EA, Al-Dabbagh WH. Opportunistic screening for early detection of breast cancer in Iraq. Int Med J 2021;28:28-32.
4. Lefta SF, Kadhim A. Association of pregnancy loss with breast cancer in Babil Governorate's women. Med J Babylon 2022;19:15.
5. Al-Jenabi NA, Kadhem AA, Abbas HF. Prevalence of breast cancer women in Babylon Province, Iraq. Med J Babylon 2019;16:369.
6. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—Epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—An updated review. Cancers 2021;13:4287.
7. Goff SL, Danforth DN. The role of immune cells in breast tissue and immunotherapy for the treatment of breast cancer. Clin Breast Cancer 2021;21:e63-73.
8. Feruza X. Current concepts of breast cancer risk factors. Int J Philos Stud Soc Sci 2021;1:57-66.
9. Hu G, Yan Z, Zhang C, Cheng M, Yan Y, Wang Y, *et al*. FOXM1 promotes hepatocellular carcinoma progression by regulating KIF4A expression. J Exp Clin Cancer Res 2019;38:1-7.
10. Gao H, Chen X, Cai Q, Shang Z, Niu Y. Increased KIF4A expression is a potential prognostic factor in prostate cancer. Oncol Lett 2018;15:7941-7.
11. Hou PF, Jiang T, Chen F, Shi PC, Li HQ, Bai J, *et al*. KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. Cell Death Dis 2018;9:477.
12. Ti SC, Alushin GM, Kapoor TM. Human β-tubulin isotypes can regulate microtubule protofilament number and stability. Dev Cell 2018;47:175-190.e5.
13. Wloga D, Joachimiak E, Fabczak H. Tubulin post-translational modifications and microtubule dynamics. Int J Mol Sci 2017;18:2207.
14. Campesi I, Marino M, Montella A, Pais S, Franconi F. Sex differences in estrogen receptor α and β levels and activation status in LPS-stimulated human macrophages. J Cell Physiol 2017;232:340-5.
15. Ruivo CF, Adem B, Silva M, Melo SA. The biology of cancer exosomes: Insights and new perspectives biology of cancer exosomes. Cancer Res 2017;77:6480-8.
16. Meldolesi J. Exosomes and ectosomes in intercellular communication. Curr Biol 2018;28:R435-44.
17. Rajagopal C, Harikumar KB. The origin and functions of exosomes in cancer. Front Oncol 2018;8:66.
18. Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res 2019;12:1-9.
19. Marques RC, Costa-Rama E, Viswanathan S, Nouws HP, Costa-García A, Delerue-Matos C, *et al*. Voltammetric immunosensor for the simultaneous analysis of the breast cancer biomarkers CA 15-3 and HER2-ECD. Sens Actuat B 2018;255:918-25.
20. Mansour-Ghanaei F, Pourmasoumi M, Hadi A, Joukar F. Efficacy of curcumin/turmeric on liver enzymes in patients with non-alcoholic fatty liver disease: A systematic review of randomized controlled trials. Integr Med Res 2019;8:57-61.
21. Zhu Z, Zhou J, Xiong X, Liu D, Zheng Y, Ding X, *et al*. Prognostic value of serum liver enzymes in oral and oropharynx squamous cell carcinomas. J Oral Pathol Med 2019;48:36-42.
22. Jin Y, Yang L, Li X, Liu F. Circular RNA KIF4A promotes cell migration, invasion and inhibits apoptosis through miR-152/ZEB1 axis in breast cancer. Diagn Pathol 2020;15:1-9.
23. Feng S, Luo S, Ji C, Shi J. miR-29c-3p regulates proliferation and migration in ovarian cancer by targeting KIF4A. World J Surg Oncol 2020;18:1-8.
24. Cao Q, Song Z, Ruan H, Wang C, Yang X, Bao L, *et al*. Targeting the KIF4A/AR axis to reverse endocrine therapy resistance in castration-resistant prostate cancerKIF4A promotes prostate cancer endocrine therapy resistance. Clin Cancer Res 2020;26:1516-28.
25. Huang J, Deng X, Chen X, Chang Z, Lu Q, Tang A, *et al*. Circular RNA KIF4A promotes liver metastasis of breast cancer by reprogramming glucose metabolism. J Oncol 2022;2022:1-10.
26. Lopes D, Maiato H. The tubulin code in mitosis and cancer. Cells 2020;9:2356.
27. Höflmayer D, Öztürk E, Schroeder C, Hube-Magg C, Blessin NC, Simon R, *et al*. High expression of class III β-tubulin in upper gastrointestinal cancer types. Oncol Lett 2018;16:7139-45.
28. Kashyap VK, Wang Q, Setua S, Nagesh PK, Chauhan N, Kumari S, *et al*. Therapeutic efficacy of a novel βIII/βIV-tubulin inhibitor (VERU-111) in pancreatic cancer. J Exp Clin Cancer Res 2019;38:1-4.
29. Maahs L, Sanchez BE, Gupta N, Van Harn M, Barrack ER, Reddy PV, *et al*. Class III β-tubulin expression as a predictor of docetaxel-resistance in metastatic castration-resistant prostate cancer. PLoS ONE 2019;14:e0222510.
30. Ruksha K, Mezheyeuski A, Nerovnya A, Bich T, Tur G, Gorgun J, *et al*. Over-expression of BII-tubulin and especially its localization in cell nuclei correlates with poorer outcomes in colorectal cancer. Cells 2019;8:25.
31. Radakovic A, Boger DL. High expression of class III β-tubulin has no impact on functional cancer cell growth inhibition of a series of key vinblastine analogs. Bioorg Med Chem Lett 2018;28:863-5.
32. Nami B, Wang Z. Genetics and expression profile of the tubulin gene superfamily in breast cancer subtypes and its relation to taxane resistance. Cancers 2018;10:274.
33. Subedi L, Teli MK, Lee JH, Gaire BP, Kim MH, Kim SY. A stilbenoid isorhapontigenin as a potential anti-cancer agent against breast cancer through inhibiting sphingosine kinases/tubulin stabilization. Cancers 2019;11:1947.
34. Tai YL, Chen KC, Hsieh JT, Shen TL. Exosomes in cancer development and clinical applications. Cancer Science 2018;109:2364-74.
35. Wee I, Syn N, Sethi G, Goh BC, Wang L. Role of tumor-derived exosomes in cancer metastasis. Biochim Biophys Acta 2019;1871:12-9.

36. Shtam T, Naryzhny S, Samsonov R, Karasik D, Mizgirev I, Kopylov A, *et al*. Plasma exosomes stimulate breast cancer metastasis through surface interactions and activation of FAK signaling. Breast Cancer Res Treat 2019;174:129-41.
37. Meng Y, Sun J, Wang X, Hu T, Ma Y, Kong C, *et al*. Exosomes: A promising avenue for the diagnosis of breast cancer. Technol Cancer Res Treat 2019;18:1533033818821421.
38. Wang M, Ji S, Shao G, Zhang J, Zhao K, Wang Z, *et al*. Effect of exosome biomarkers for diagnosis and prognosis of breast cancer patients. Clin Transl Oncol 2018;20:906-11.
39. Li X, Dai D, Chen B, He S, Zhang J, Wen C, *et al*. Prognostic values of preoperative serum CEA and CA125 levels and nomograms for young breast cancer patients. Onco Targets Therapy 2019;12:8789.
40. Lian M, Zhang C, Zhang D, Chen P, Yang H, Yang Y, *et al*. The association of five preoperative serum tumor markers and pathological features in patients with breast cancer. J Clin Lab Anal 2019;33:e22875.
41. Laffont A, Goussot V, Berriolo-Riedinger A, Riedinger JM. CA 125 assay in the extension assessment of newly diagnosed breast cancers: Why and how? Ann Biol Clin (Paris) 2018;76:553-61.
42. Hing JX, Mok CW, Tan PT, Sudhakar SS, Seah CM, Lee WP, *et al*. Clinical utility of tumour marker velocity of cancer antigen 15–3 (CA 15–3) and carcinoembryonic antigen (CEA) in breast cancer surveillance. The Breast 2020;52:95-101.
43. Savitri M, Bintoro SU, Sedana MP, Muhammad MN, Romadhon PZ, Amrita PN, *et al*. Circulating plasma miRNA-21 as a superior biomarker compared to CA 15-3: Assessment in healthy age matched subjects and different stage of breast cancer patients. Indonesian Biomed J 2020;12:185-8.
44. Hussain AM, Ali AH, Mohammed HL. Correlation between serum and tissue markers in breast cancer Iraqi patients. Baghdad Sci J 2022;19:0501.
45. Zhou J, He Z, Ma S, Liu R. AST/ALT ratio as a significant predictor of the incidence risk of prostate cancer. Cancer Med 2020;9:5672-7.
46. Lee H, Choi YH, Sung HH, Han DH, Jeon HG, Jeong BC, *et al*. De Ritis ratio (AST/ALT) as a significant prognostic factor in patients with upper tract urothelial cancer treated with surgery. Clin Genitourin Cancer 2017;15:e379-85.
47. Knittelfelder O, Delago D, Jakse G, Reinisch S, Partl R, Stranzl-Lawatsch H, *et al*. The AST/ALT (De Ritis) ratio predicts survival in patients with oral and oropharyngeal cancer. Diagnostics 2020;10:973.
48. Finnsdottir S, Sverrisdottir A, Björnsson ES. Hepatotoxicity associated with ribociclib among breast cancer patients. Acta Oncol 2021;60:195-8.