wjpmr, 2022,8(7), 01-10

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

OVERVIEW OF CERTAIN BIOMARKERS AS PREDICTORS AND DIFFERENTIAL AGENTS FOR BREAST AND OVARIAN CANCER

Samia A. Ahmed[1], Wessam M. Aziz[1], Omar S. Omar[2], Noha Nabil[1] and Manal A. Hamed[1]*

[1]Department of Therapeutic Chemistry, National Research Centre, Dokki, Giza, Egypt.
[2]Breast Cancer Clinic, Surgical Department, National Cancer Institute, Cairo University Hospital, Cairo, Egypt.

***Corresponding Author: Prof. Dr. Manal A. Hamed**

Department of Therapeutic Chemistry, National Research Centre, Dokki, Giza, Egypt.

Article Received on 30/04/2022 **Article Revised on 20/05/2022** **Article Accepted on 09/06/2022**

ABSTRACT

Breast cancer (BC) and ovarian cancer (OC) are the most developed malignancies in women worldwide. The goal of this study was to explore the role of certain biomarkers to predict prognosis in breast and ovarian malignancies. The research was expanded to determine the efficacy of these measures as differential factors in illness progression as well as their diagnostic specificity. Eighty females were enrolled in this study. They classified into BC, OC and healthy women. The BC and OC groups were subdivided into early cancer stage, late cancer stage of grade 2 and late cancer stage of grade 3. Cluster of differentiation 31 (CD31), vitronectin (VTN), regulated on activation normal T cell expressed and secreted (RANTES) and lysosomal-associated transmembrane protein 4β (LAPTM4β) biomarkers were estimated in all groups. The results revealed significant increase in CD31, RANTES and LAPTM4B in all stages of BC and a significant increase in VTN in the early stage of BC. Conversely, a significant decrease in CD31 and VTN in all stages of OC and slightly increases in RANTES in late stages of OC, while non-significant change was observed in the early stage. LAPTM4B showed significant increase in all stages of OC compared with the healthy women. In conclusion, vitronectin, LAPTM4B and RANTES may be considered as prognostic and diagnostic markers in BC with special emphasis to VTN as a promising marker in the early stage of BC. In addition, LAPTM4B seems to be a prognostic and diagnostic marker for OC. For the limited numbers of patients, more supportive studies are needed to confirm the efficacy and specificity of these biomarkers in prognosis or diagnosis of BC and OC.

KEYWORDS: Breast cancer; Ovarian cancer; CD31; RANTES; LAPTM4B; VTN.

1. INTRODUCTION

Cancer is the second leading cause of death in the world and responsible for about 9.6 million deaths in 2018.[1,2] In developing countries, cancers are caused 23–25% of total mortality.[3]

Breast cancer (BC) is the most frequently diagnosed cancer in women, with an estimated 1.38 million new cases per year worldwide.[4,5] Female has *BRCA1* and *BRCA2* genes, where their functions are to repair cell damage and keep breast and ovarian cells growing normally. But when these genes contain mutations, they don't function normally and thereafter both breast and ovarian cancers risk propagate.[6] Polyak[7] reported that *BRCA1* and *BRCA2* mutations may account for up to 10% of all breast cancers. Properly, genetic testing of *BRCA1* and *BRCA2* is a good tool for early detection and/or prevention of breast cancer development.[8]

Breast cancer can take many years to develop without any clinical symptoms. Therefore, early detection of the disease with mammography can greatly ameliorate the chances of survival.[9,10] However, mammography screening has generated controversy due to the risks of false-positive results.[11] It also has limited sensitivity for the detection of tumors in dense breast tissue.[12] Therefore, novel biomarkers that can predict disease prognosis and offer prognostic information for therapy stratification are urgently needed.

Ovarian cancer (OC) is the most lethal gynecological malignancy, with about 125,000 deaths worldwide per year.[14] The tumor suppressor gene (P53) and a series of genomic changes involving specific oncogenes as HER2 neu , K-ras, and c-myc enhance ovarian cancer.[13] The scarceness of specific symptoms and the lack of an effective screening method were the main causes of the tumor spread, and thereafter late diagnosis.[15] Although the initial response to treat either surgery or chemotherapy is accessible, most patients will finally relapse and die with their tumors due to the chemo resistant. In contrast to other solid tumors, the most common method for OC metastasis is spread directly

through the peritoneal. Whereas tumor cells in the ovary evade and disseminated the peritoneal fluid throughout the abdominal cavity, and then bind to the mesothelial cell lining leading to metastatic outgrowths.[16]

Tumor serum biomarkers are defined as substances synthesized by the tumor, released into the circulation and began to change quantitatively during tumor development. Biomarkers can be used as a diagnostic agent at various stages of cancer development. They can be used for cancer prevention risk assessment and for cancer diagnosis screening at an early stage, when curative treatment is still feasible. Additionally, they can be used for the diagnosis of cancer, including cancer classification, staging and grading.[17] The changes in tumor markers after malignancy make them important diagnostic tools in tumor recurrence and metastasis.[18] In particular, the early diagnosis to predict response or resistance to specific therapies is considered as an ideal tool to counteract the disease severity.[19]

Platelet endothelial cell adhesion molecule (PECAM-1) also known as cluster of differentiation 31 (CD31), is a protein encoded by the PECAM1 gene located on chromosome 17.[20] PECAM-1 plays mainly role in removing aged neutrophils from the body. It's highly expressed and has a long history of being used to control vessel density in malignant tissue.[21] Privratsky et al.[22] reported that PECAM-1 is involved in a number of processes relevant to growth and spread of primary tumors including angiogenesis, vascular permeability, and leukocyte trafficking out of the circulation. It is also present on platelets and some plasma cells, monocytes, immature mDCs, neutrophils, NK cells, lymphocytes and implicated in advanced metastatic tumor progression.[23, 24]

Vitronectin (VTN) is a glycoprotein of the hemopexin family which is abundantly found in serum, the extracellular matrix and bone.[25] VTN is composed of several independently folded domains. The N terminus (somatomedin B domain), a connecting region, and two hemopexin-like domains. The somatomedin B domain of vitronectin binds and stabilizes plasminogen activator inhibitor-1 (PAI-1) in which it interacts with urokinase plasminogen activator receptor (uPAR) that has a role in cell migration and signal transduction.[26,27] Kadowaki et al.[28] stated that vitronectin has an auxiliary role in the detection of ductal carcinoma in situ (DCIS), which is difficult to identify.

Chemokine (C-C motif) ligand 5 (CCL5) is a protein which is encoded by the CCL5 gene in humans.[29] It is also known as regulated on activation, normal T cell expressed and secreted (RANTES). It is chemotactic for T cells, eosinophils and basophils that play a necessary role in recruiting leukocytes into inflammatory sites. With the help of particular cytokines (IL-2 and IFN-) released by T cells, the proliferation and activation of certain natural-killer (NK) cells can be promoted, resulting in the formation of CHAK (CC-chemokine-activated killer) cells, which have been found on human chromosome 17.[30] Datar et al.[31] demonstrated that tumor cell-derived CCL5 promoted breast cancer by recruiting macrophages into the tumor microenvironment.

Lysosomal-associated transmembrane protein 4β (LAPTM4β), a novel oncoprotein belonging to the mammalian 4-tetratransmembrane spanning protein superfamily, was originally cloned in hepatocellular carcinomas.[32] It is located in the region of chromosome 8q22.1 and contains seven exons and six introns.[33] LAPTM4β activity is increased in a number of human cancers, and its overexpression is related to cellular transformation, tumorigenesis, and metastatic progression.[34]

Therefore, the aim of this work was to explore the prognostic and diagnostic values of certain serum biomarkers in breast and ovarian cancers. The work was extended to estimate the efficiency of these parameters as differential factors in disease progression and their specificity for diagnosis.

2. SUBJECTS AND METHODS

2.1. Pathological cases

This work was conducted on eighty breast and ovarian cancer females as well as healthy control voluntaries with ages ranged from 20- 60 years. They enrolled from the Breast Cancer Clinic, Surgical Department, National Cancer Institute, Cairo University Hospital, Egypt. The patients were classified according to the histological diagnostics reports supplied by Clinical Pathology Department, National Cancer Institute, Cairo University Hospital, Egypt. The diagnosis was confirmed through routine blood investigations, chest x-ray, ECG and CT scan for all the patients. Patients were then assessed according to the pathological TMN classification.[35] The females divided into three main groups. The first main group was considered as BC patients, subdivided into three subgroups of ten patients each and considered as early stage BC females, late stage BC females with grade 2 (G2) and late stage BC females with grade 3 (G3). The second main group was considered as OC patients, subdivided into three subgroups of ten females each and considered as early stage OC females, late stage OC females with grad 2 (G2) and late stage OC females with grade 3 (G3). The second main group included 20 healthy female volunteers.

2.2. Exclusion criteria

The exclusion criteria included pregnancy and lactation, brain metastasis, radiotherapy to more than one third of the bone marrow area, severe infection or uncontrollable comorbid disease.

2.3. Specimen collection

Blood was drawn by vein puncture into Venoject tubes in the morning after an overnight fast. Blood was

centrifuged at 3,000 rpm. The clear serum was kept at 20°c for further biochemical determination. For healthy controls, blood samples were drawn during the routine medical checkup for biochemical and immunological examinations.

2.4. Clinical examination

Complete history taking and physical examination were done to BC and OC females with special emphasis on family history, contraceptive history, obstetric history, menstrual history, and locational history. Full laboratory investigations (CBC, SGOT, SGPT, albumin, bilirubin, creatinine, urea, Ca, ALP, abdominopelvic ultrasound, bone scan, estrogen, progesterone receptor, Her2neu, and KI67 levels) were done at the National Cancer Institute, Cairo University Hospital, Cairo, Egypt.

2.5. Ethics approval

The informed consents were taken from all patients according to the Medical Research Ethical Guideline Committee, National Cancer Institute, Cairo University Hospital, Cairo, Egypt.

2.6. Biochemical determinations

2.6.1. Serum CD31 level

Serum CD31 level was estimated by using ELISA kit (Abcam PLC, UK) for the quantitative measurement of CD31 in serum. A monoclonal antibody specific for CD31 had been coated onto the wells of the microtiter strips provided. Samples, including standards of known CD31 concentrations, control and tested specimens were pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for CD31 were simultaneously incubated. After washing, the enzyme streptavidin-HRP, that binds the biotinylated antibody was added, incubated and washed. A TMB substrate solution was added which acted on the bound enzyme to induce a colored reaction product. The intensity of this colored product was directly proportional to the concentration of CD31 present in the samples at 450 nm.

2.6.2. Serum vitronectin (VTN) level

Serum vitronectin level was measured by ELISA Kit (CUSA Biotechnology, LLC, USA), where the antibody specific for VTN was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any VTN present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for VTN was added to the wells. After washing, avidin conjugated horseradish peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and the color develops in proportion to the amount of VTN bound in the initial step. The color development was stopped and the intensity of the color was measured at 450 nm.

2.6.3. Serum CCL5 level

Serum CCL5 level was estimated by using ELISA kit (Abcam PLC, UK) for the quantitative measurement of RANTES protein in serum. The simple step ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. Samples or standards were added to the wells, followed by the antibody. After incubation, the wells were washed to remove unbound material. TMB substrate was added, incubated to be catalyzed by HRP and generating the blue color. The reaction was then stopped by adding stopping solution that change the color from blue to yellow. Signal was generated proportionally to the amount of bound analyte and the intensity was measured at 450 nm.

2.6.4. Serum LAPTM4B level

Serum LAPTM4B level was determined by ELISA Kit (Sandwich ELISA) (Life Span Biosciences, Inc., USA), where each well of the supplied microtiter plate was pre-coated with a target specific capture antibody. Standards or samples were added to the wells and the target antigen binded to the capture antibody. Unbound standard or sample was washed away. A biotin-conjugated detection antibody was added which binded to the captured antigen. Unbound detection antibody was washed away. An avidin-horseradish peroxidase (HRP) conjugate is then added which bind to the biotin. Unbound avidin-HRP conjugate was washed away. A TMB substrate was added and reacted with HRP enzyme resulting in color development. A sulfuric acid stop solution was added to terminate color development reaction and then the optical density (OD) of the well was measured at a wavelength of 450 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration.

2.7. Statistical analysis

The results were expressed as mean ± SD of ten females reading in each group. All data were analyzed using the Costat Software Computer Program, where an unshared letter was significant at $p < 0.05$. The correlations between all markers and tumor grade in BC and OC were analyzed using Pearson's correlation analysis test. Sensitivity, specificity and accuracy were also estimated by receiver operating characteristic (ROC) curve.

3. RESULTS

The present results showed that CD31 level in BC patients was slightly increased in early and late stages with grade 2 and grade 3 by 105, 111 and 116%, respectively ($p < 0.05$) as compared to the normal healthy subjects. The levels of CD31 in OC females were significantly decreased ($p < 0.05$) by 93, 87.5 and 79% in

early and late stages with grade 2 and grade 3, respectively (Table 1 and Fig.1a).

Concerning to serum vitronectin level in BC females, it showed significant increase in the early and late stages with grade 2 and grade 3 by 120, 115 and 112 %, respectively as compared to the control group. In the early stage of BC group, the level of VTN increases by 1.2 fold than in the late stages as compared with control group. In case of OC females, the serum vitronectin level recorded significant decrease in the early and late stages (G2 and G3) by 83.3, 76.6 and 72.9%, respectively as compared to the control groups at $p < 0.05$ (Table 2 and Fig. 1b).

Regarding to RANTES level, it showed highly significant increase in early and late stages (G2 and G3) of breast cancer patients by 300.7, 388.2 and 419%, respectively as compared to normal healthy patients. In case of ovarian cancer females, it was increased in late stages either in G2 and G3 by 128 and144 %, respectively (Table 3 and Fig. 2a).

Regarding LAPTM4B level, the current study recorded a significant elevations in its level in BC females at all stages as compared to the healthy control. Breast cancer recorded highly significant elevation in LAPTM4B level by 381, 480 and 568 % in early and late stages with G2 and G3, respectively. In addition, ovarian cancer females recorded elevation by 140, 205 and 244% in early and late stage (G2-G3), respectively as compared to the healthy control ($p<0.05$) (Table 4, Fig. 2b). A significant positive correlation was also observed between those biomarkers and the tumor grades in BC (Table 5) accompanied with negative correlation in RANTES and LAPM4B in OC (Table 6).

The validity of a particular marker in BC and OC was seen in Tables 7 and 8. The calculated area of CD31, vitronectin, RANTES and LAPTM4B proved the validity of using them as markers in diagnosis of breast cancer. The ROC curve proved CD31, vitronectin and LAPTM4B as markers in ovarian cancer diagnosis, while RANTES recorded minimal diagnosis. The impressive finding was the sensitivity of CD31, vitronectin, RANTES and LAPTM4B which recorded 100% sensitivity and specificity at its optimal cutoff values reached to7.29 ng/ml, 84.35ng/ml, 308.7ng/ml and 13.30ng/ml, respectively in BC. In ovarian cancer, the cutoff values of CD31, vitronectin and LAPTM4B reached to 6.79ng/ml, 73.47ng/ml and 7.05ng/ml, respectively. We also noticed that the area under the ROC curve (AUC) of all investigated biomarkers had a good accuracy in diagnosis expect RANTES in OC.

Table 1: Levels of CD31 in normal, breast and ovarian cancer females with different grades.

Normal	Breast cancer (BC)			Ovarian cancer (OC)		
	Early stage	Late stage G2	Late stage G3	Early stage	Late stage G2	Late Stage G3
7.045^{d} ± 0.136	7.456^{c} ± 0.089	7.87^{b} ± 0.072	8.18^{a} ± 0.041	6.59^{e} ± 0.088	6.16^{f} ± 0.064	5.59^{g} ± 0.178

Data are mean ± SD of 10 subjects in each group. Values are expressed as ng/ml. Statistical analysis is carried out using one-way analysis of variance (ANOVA) , Co-stat software Computer Program, accompanied with *post-hoc* test at least significance difference (LSD) between groups at $p < 0.05$. Unshared superscript letters between groups are significantly difference at $p < 0.001$.

Table 2: Levels of vitronectin in normal, breast and ovarian cancer females with different grades.

Normal	Breast cancer (BC)			Ovarian cancer (OC)		
	Early stage	Late stage G2	Late stage G3	Early stage	Late stage G2	Late Stage G3
79.62^{d} ± 1.46	95.80^{a} ± 1.07	92.15^{b} ± 0.721	89.40^{c} ± 0.964	66.11^{e} ± 2.28	61.05^{f} ± 1.103	58.11^{g} ± 0.495

Data are mean ± SD of 10 subjects in each group. Values are expressed as ng/ml. Statistical analysis is carried out using one-way analysis of variance (ANOVA) , Co-stat software Computer Program, accompanied with *post-hoc* test at least significance difference (LSD) between groups at $p < 0.05$. Unshared superscript letters between groups are significantly difference at $p < 0.001$.

Table 3: Levels of RANTES in normal, breast and ovarian cancer females with different grades.

Normal	Breast cancer (BC)			Ovarian cancer (OC)		
	Early stage	Late stage G2	Late stage G3	Early stage	Late stageG2	Late stageG3
163.91^{e} ± 4.57	502.03^{c} ± 57.02	648.02^{b} ± 22.03	700.68^{a} ± 3.90	168.87^{e} ± 5.14	209^{d} ± 15.45	232.37^{d} ± 13.19

Data are mean ± SD of 10 subjects number in each group. Values are expressed as ng/ml. Statistical analysis is carried out using one-way analysis of variance (ANOVA) , Co-stat software Computer Program, accompanied with *post-hoc* test at least significance difference (LSD) between groups at $p < 0.05$. Unshared superscript letters between groups are significantly difference at $p < 0.001$.

Table (4): Levels of LAPTM4B in normal, breast cancer and ovarian cancer in Egyptian female patient with different grades.

Normal	Breast cancer (BC)			Ovarian cancer (OC)		
	Early stage	Late stage G2	Late stage G3	Early stage	Late stage G2	Late Stage G3
5.66^{g} ± 0.48	21.60^{c} ± 0.915	27.26^{b} ± 0.4108	32.15^{a} ± 1.18	7.96^{f} ± 0.06	10.72^{e} ± 0.174	12.74^{d} ± 0.163

Data are mean ± SD of 10 subjects in each group. Values are expressed as ng/ml. Statistical analysis is carried out using one-way analysis of variance (ANOVA) , Co-stat software Computer Program, accompanied with *post-hoc* test at least significance difference (LSD) between groups at $p < 0.05$. Unshared letters are significantly at $p < 0.001$. Unshared superscript letters between groups are significantly difference at $p < 0.001$.

Table 5: Pearson's correlations between tumor grade, CD31, vitronectin, RANTES and LAPTM4B in breast cancer.

	CD31	VTN	RANTES	LAPTM4B	Tumor grade
CD31	1	0.504^{*}	0.918^{**}	0.943^{**}	0.979^{**}
VTN	0.504^{*}	1	0.727^{**}	0.705^{**}	0.474^{*}
RANTES	0.918^{**}	0.727^{**}	1	0.981^{**}	0.931^{**}
LAPM4B	0.943^{**}	0.705^{**}	0.981^{**}	1	0.952^{**}
Tumor grade	0.979^{**}	0.474^{*}	0.931^{**}	0.952^{**}	1

* Correlation is significant at $p< 0.05$ (2-tailed).

** Correlation is significant at $p< 0.01$ (2-tailed).

Table 6: Pearson's correlations between tumor grade, CD31, vitronectin, RANTES and LAPTM4B in ovarian cancer.

	CD31	VTN	RANTES	LAPTM4B	Tumor grade
CD31	1	0.901^{**}	-0.788^{**}	-0.975^{**}	-0.976^{**}
VTN	0.901^{**}	1	-0.626^{**}	-0.933^{**}	-0.931^{**}
RANTES	-0.788^{**}	-0.626^{**}	1	0.831^{**}	0.774^{**}
LAPM4B	-0.975^{**}	-0.933^{**}	0.831^{**}	1	0.994^{**}
Tumor grade	-0.976^{**}	-0.931^{**}	0.774^{**}	0.994^{**}	1

** Correlation is significant at $p <0.01$ (2-tailed).

Table 7: ROC curve of different biomarkers in breast cancer.

Biomarkers	AUC	Cut-off value	Sensitivity %	Specificity %	95% confidence interval
CD31	1	7.29	100	100	1.000-1.000
Vitronectin	1	84.35	100	100	1.000-1.000
RANTES	1	308.7	100	100	1.000-1.000
LAPTM4B	1	13.30	100	100	1.000-1.000

Table 8: ROC curve of different biomarkers in ovarian cancer.

Biomarkers	AUC	Cut-off value	Sensitivity %	Specificity %	95% confidence interval
CD31	1	6.79	100	100	1.000-1.000
Vitronectin	1	73.47	100	100	1.000-1.000
RANTES	0.862	193.3	66.7	100	0.708-0.982
LAPTM4B	1	7.05	100	100	1.000-1.000

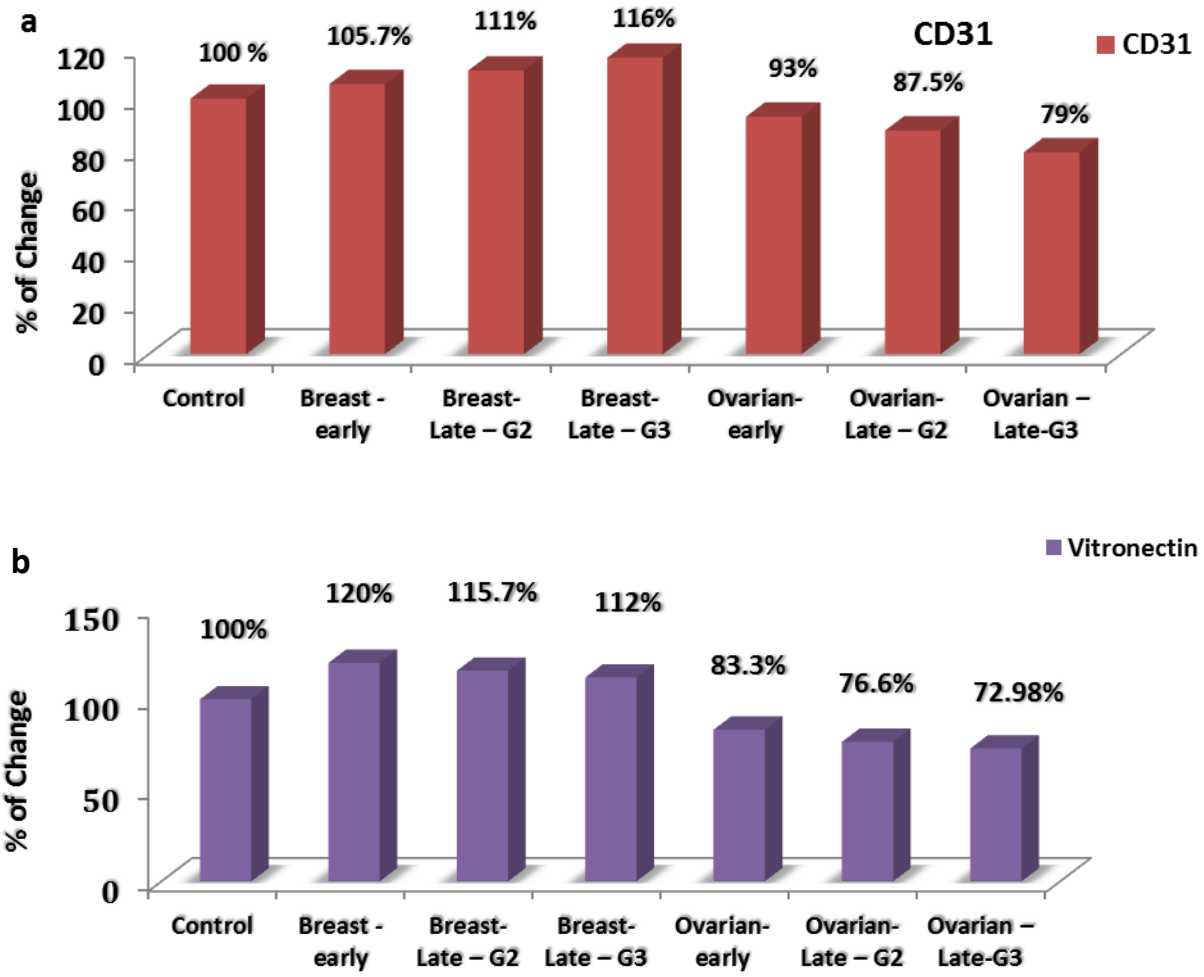
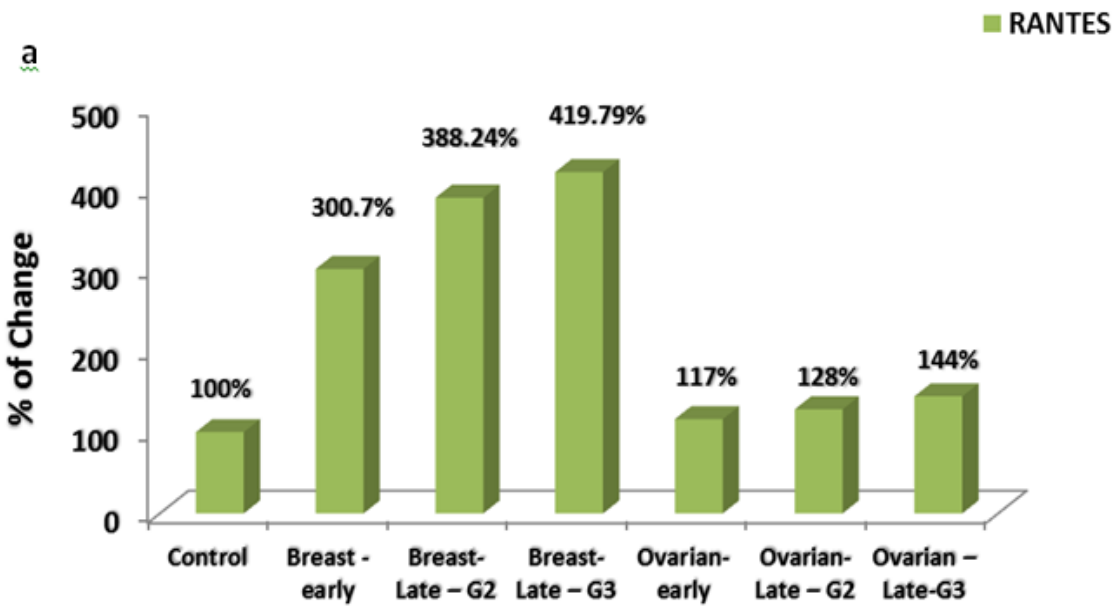


Fig 1: (a) % change of CD31 level in normal, breast and ovarian cancer females with different grades. (b) % change of vitronectin level in normal, breast and ovarian cancer females with different grades. Values are expressed as % change related to normal control. Normal control consider as 100%.



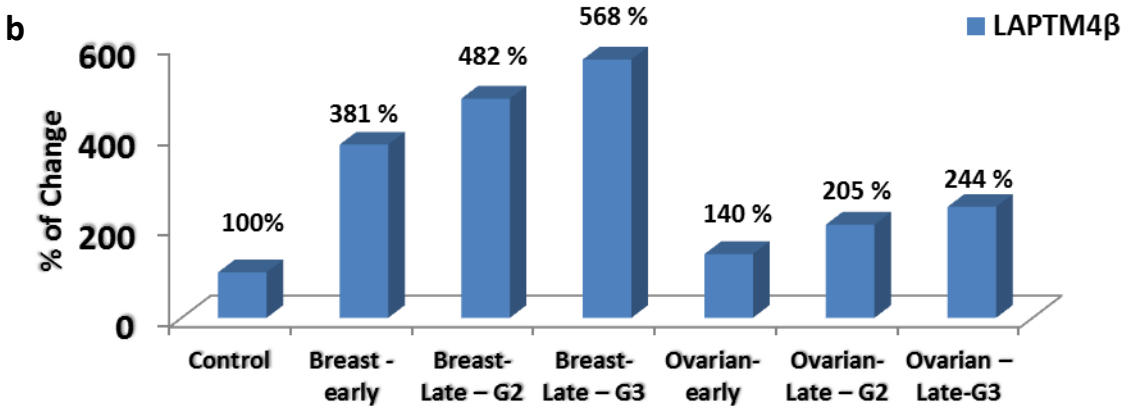


Fig. 2: (a) % change of RANTES level in normal, breast and ovarian cancer females with different grades. (b) % change of LAPTM4β level in normal, breast and ovarian cancer females with different grades. Values are expressed as % change related to normal control. Normal control consider as 100%.

4. DISCUSSION

Tumor biomarkers are substances found in or produced by a tumor or the host microenvironment as a result of the tumorigenesis and progression phase. They contain proteins, hormones, enzymes, and oncogene products, among other biochemical entities. Chemical, immunological, and molecular biological techniques can detect these substances qualitatively or quantitatively in cells, tissues, and body fluids.[36] Serum tumor markers may be helpful in cancer diagnosis, prognosis and treatment.[37]

CD31 is a 100kDA glycoprotein in endothelial cells and 130kD in platelets.[38] Sapino et al[39] showed that endothelial cells, leukocytes, and platelets all express CD31, which is used in surgical pathology as a marker of normal and neoplastic vascularization. Its expression was also high in nuclear grade ductal carcinoma. Also, El Agouza et al[40] confirmed that significant increase in CD31 level among breast cancer. In this study and in line with the observation of Kumar et al[41] and Dales et al[42], serum CD31 showed slightly increased in early and late (G2- G3) stages of BC patients when compared to the normal healthy control. Also, Montrucchio et al[43] stated that CD31 is widely used in breast pathology as a marker of angiogenesis and this may be due to a high PECAM-1 immunoreactivity that related to the formation of tubules, histological grade of malignancy and clinical stage. Moreover, Kim et al[44] stated that the high specificity of PECAM-1 stimulates tumor angiogenesis and metastasis of the macrophages. In ovarian carcinoma, CD31 showed slightly decrease in both early and late (G2-G3) stages of the disease. These results were parallel to previous study of Darai et al[45] who demonstrated that, CD31 immunostaining may have prognostic importance in ovarian carcinoma, however its serological levels had no diagnostic significance in ovarian tumors.

Vitronectin is an adhesion protein that interacts with urokinase plasminogen activator receptor and certain integrin receptors. It was thought to be a key chemoattractant present in diluted plasma/serum that specifically stimulates cancer cell migration, in addition to its pro-adhesive properties.[46] Cho et al[47] and Hao et al[48] found that serum levels of vitronectin were elevated in patients with breast cancer. The present study supported their results by the elevation of vitronectin expression in the early and advanced breast cancer patients as compared to healthy controls. Harris et al[27] attributed this phenomenon to its interact between plasminogen activator inhibitor type-1 (PAI-1) and urokinase plasminogen activator (uPA) that binds to the ECM component and plays a pivotal role in tumor invasion and metastasis.

Regarding to the ovarian cancer, vitronectin showed a significant decrease in the early and late stages as compared to the control group. These observations was in parallel with the result of Heyman et al[49] who demonstrated that vitronectin and its receptors have a crucial role in the ability of ovarian carcinoma cells to bind to peritoneal mesothelium. Additionally, Turan et al.[50] attributed the decrease in serum VTN level to the matrix metalloproteinase-2 (MMP2) that secreted by tumor cells in ECM. Kenny et al[51] revealed increased OvCa cell peritoneal adhesion by cleaving the ECM protein vitronectin into small fragments which marked up OvCa cells binding to these VTN fragments and its receptors resulting in a decrease in VTN.

RANTES is a target gene of NF-κB activity, whereas different stimuli activate NF- κB such as CD40L or IL-15 to induce RANTES production.[52-55] Karnoub et al[56] found a significant correlation between disease progression, relapse, metastasis and the high level of RANTES in breast tumor cells. The same authors asserted that RANTES expression by breast tumor cells

represents a valuable prognostic factor for detection of stage 2 breast cancer patients who are at risk through disease progression. These results were in full consistence with our results which showed highly significant increase in early and late stages of breast cancer as compared to normal healthy patient. We also noticed a positive correlation between RANTES and tumor grade in BC females. These results were in line with the previous work of Lv et al[57] and Kalimutho et al.[58]

Concerning to RANTES in ovarian cancer, non-significant difference in the early stage was observed. In the late stage of the disease, we noticed an increase in RANTES level as compared with the control group. A positive correlation between RANTES and tumor grade in OC was recorded. Our results were in parallel with the results of Tsukishiro et al[59] who observed a high RANTES level in OC patients comparing with benign ovarian cysts patients. Recently, Long et al[60] showed that RANTES is expressed in OC stem cells and characterized by CD133 antigen expression that, through its migration and invasion, identifies a particular subpopulation of ovarian carcinoma cells and tissues.

LAPTM4B is located on late endosomes and lysosomes.[61] Its expression is increase in breast, liver, lung, ovary, uterus, and gastric cancers.[62] LAPTM4B also fosters autophagy, a cell survival mechanism mediated by lysosomes that protects tumor cells from metabolic and genotoxic stress that promotes tumour growth.[63] A highly significant elevation in LAPTM4B levels were observed in BC and OC females as compared to the healthy control. Our results were in line with the study of Meng et al[64] and Fan et al[65] who demonstrated that LAPTM4B was overexpressed in endometrial and breast cancers. Li et al[63] also found that LAPTM4B expression is significantly correlated with breast cancer that may be predictive in prognosis and effectiveness in therapeutic target.

Therefore, the present study recorded the validity of CD31, vitronectin, RANTES and LAPTM4B for using them as markers in diagnosis of breast cancer. Additionally, CD31, vitronectin and LAPTM4B can be used as markers in ovarian cancer, while RANTES recorded minimal diagnostic level.

CONCLUSION

Vitronectin, LAPTM4B and RANTES are considered as prognostic and diagnostic markers in breast cancer patients with special emphasis to vitronectin as a promising marker in the early stage. LAPTM4B had low prognostic and diagnostic value in ovarian cancer detection. More supportive studies are needed to validate the efficacy and specificity of these biomarkers in prognosis or diagnosis of BC and OC due to the small number of patients enrolled in this study.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to National Research Centre for facilitating this work.

Data availability statement

Data in this manuscript is available to any reader upon acceptance.

Disclosure statement

The authors declared no conflict of interest.

REFERENCES

1. Armstrong N, Ryder S, Forbes C, Ross J, Quek R G. A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin. Epidemiol*, 2019; 11: 543.
2. Xue H, Lu B, Lai M. The cancer secretome: a reservoir of biomarkers. J Transl Med, 2008; 17: 6: 52.
3. Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, Casey DC, Charlson FJ, Chen AZ, Coates MM , Coggeshall M. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The lancet, 2016; 388(10053): 1459-1544.
4. Ferlay J, Shin HR, Bray F, Forman D, Mathers C , Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer, 2010; 127(12): 2893-2917.
5. Boulos S, Gadallah M, Neguib S, Essam EA, Youssef A, Costa A, Mittra I and Miller AB. Breast screening in the emerging world: high prevalence of breast cancer in Cairo. The Breast, 2005; 14(5): 340-346.
6. Colditz GA, Wolin KY, Gehlert S. Applying what we know to accelerate cancer prevention. Science translational medicine, 2012; 4(127): 127rv4.
7. Polyak K. Breast cancer: origins and evolution. The Journal of clinical investigation, 2007; 117(11): 3155-3163.
8. Allison KH. Molecular pathology of breast cancer: what a pathologist needs to know. American journal of clinical pathology, 2012; 138(6): 770-780.
9. Chen Y, Brock G, Wu D. Estimating key parameters in periodic breast cancer screening—application to the Canadian national breast screening study data. *Cancer Epidemiol*, 2010; *34*(4): 429-433.
10. Gøtzsche PC, Jørgensen KJ. Screening for breast cancer with mammography. Cochrane database of systematic reviews, 2013; (6).
11. 11.Welch H G, Prorok P C, O'Malley A J, Kramer B S. Breast-cancer tumor size, overdiagnosis, and mammography screening effectiveness. N. Engl. J. Med.", 2016; *375*(15): 1438-1447.
12. Boyd N F, Guo H, Martin L J, Sun L, Stone J, Fishell E, Yaffe M J. Mammographic density and the risk and detection of breast cancer. N. Engl. J. Med*.",* 2007; *356*(3): 227-236.

13. Boyle P, Levin B. World cancer report 2008. IARC Press, International Agency for Research on Cancer, 2008.
14. Stepanenko A, Kavsan V. Cancer genes and chromosome instability. Oncogene and Cancer-From Bench to Clinic, 2013; 151-182.
15. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun M J. Cancer statistics, 2009. CA: CA-CANCER J CLIN, 2009; *59*(4): 225-249.
16. Shield K, Ackland M L, Ahmed N, Rice G E. Multicellular spheroids in ovarian cancer metastases: Biology and pathology. Gynecologic oncology, 2009; 113(1): 143-148.
17. Sawyers C L. The cancer biomarker problem. *Nature*, 2008; 452(7187): 548.
18. Sawant P, Ishii M, Hazuku T, Takamasa T, Mori M. Properties of disturbance waves in vertical annular two-phase flow *NUCL ENG DES*, 2008; *238*(12): 3528-3541.
19. Bates S E. Clinical applications of serum tumor markers. Ann. Intern. Med, 1991; 115(8): 623-638.
20. Xie Y, Muller W A. Fluorescencein SituHybridization Mapping of the Mouse Platelet Endothelial Cell Adhesion Molecule-1 (PECAM1) to Mouse Chromosome 6, Region F3–G1. Genomics, 1996; 37(2): 226-228.
21. Schlüter A, Weller P, Kanaan O, Nel I, Heusgen L, Höing B, Haßkamp P, Zander S, Mandapathil M, Dominas N, Arnolds J. CD31 and VEGF are prognostic biomarkers in early-stage, but not in late-stage, laryngeal squamous cell carcinoma. BMC cancer, 2018; *18*(1): 272.
22. Privratsky JR, Newman DK, Newman PJ. PECAM-1: conflicts of interest in inflammation. Life sciences, 2010; *87*(3-4): 69-82.
23. Bergom C, Gao C, Newman PJ. Mechanisms of PECAM-1-mediated cytoprotection and implications for cancer cell survival. Leukemia & lymphoma, 2005; *46*(10): 1409-1421.
24. DeLisser H, Liu Y, Desprez PY, Thor A, Briasouli P, Handumrongkul C, Wilfong J, Yount G, Nosrati M, Fong S, Shtivelman E. Vascular endothelial platelet endothelial cell adhesion molecule 1 (PECAM-1) regulates advanced metastatic progression. Proc. Natl. Acad. Sci, 2010; *107*(43): 18616-18621.
25. Boron W F, Boulpaep EL. Medical physiology E-book. Elsevier Health Sciences, 2016.
26. Huai Q, Zhou A, Lin L, Mazar AP, Parry GC, Callahan J, Shaw DE, Furie B, Furie BC, Huang M. Crystal structures of two human vitronectin, urokinase and urokinase receptor complexes. Nat Struct Mol Biol, 2008; 15: 422–423.
27. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol, 2007; 25: 5287–5312.
28. Kadowaki M, Sangai T, Nagashima T, Sakakibara M, Yoshitomi H, Takano S, Sogawa K, Umemura H, Fushimi K, Nakatani Y, Nomura F. Identification of vitronectin as a novel serum marker for early breast cancer detection using a new proteomic approach. J CANCER RES CLIN, 2011; *137*(7): 1105-1115.
29. Donlon TA, Krensky AM, Wallace MR, Collins F S, Lovett M, Clayberger C. Localization of a human T-cell-specific gene, RANTES (D17S136E), to chromosome 17q11. 2-q12. *Genomics*, 1990; *6*(3): 548-553.
30. Maghazachi A A, Al-Aoukaty A, Schall T J. CC chemokines induce the generation of killer cells from CD56+ cells. EUR J IMMUNOL, 1996; 26(2): 315-319.
31. Datar I, Qiu X, Ma HZ, Yeung M, Aras S, de la Serna I, Al-Mulla F, Thiery JP, Trumbly R, Fan X, Cui H, Yeung K C. RKIP regulates CCL5 expression to inhibit breast cancer invasion and metastasis by controlling macrophage infiltration. Oncotarget, 2015; 6(36): 39050-39061.
32. Liu XR, Zhou RL, Zhang QY, Zhang Y, Jin YY, Lin M, Rui JA, Ye DX . Structure analysis and expressions of a novel tetra transmembrane protein, lysosome associated protein transmembrane 4 beta associated with hepatocellular carcinoma. World J Gastroenterol, 2004; 10: 1555–1559.
33. Shao GZ, Zhou RL, Zhang QY, Zhang Y, Liu JJ, Rui JA, Ye DX. Molecular cloning and characterization of LAPTM4B, a novel gene upregulated in hepatocellular carcinoma. Oncogene, 2003; 22(32): 5060.
34. Zhou L, He XD, Cui QC, Zhou WX, Qu Q, Zhou RL, Rui JA, Yu JC. Expression of LAPTM4B-35: a novel marker of progression, invasiveness and poor prognosis of extrahepatic cholangiocarcinoma. Cancer Lett, 2008; 264(2): 209–217.
35. Sobin LH, Wittekind. CInternational Union against Cancer (UICC). TNM classification of malignant tumors. 6th ed. New York (NY): Wiley-Liss, 2002.
36. Wu L, Qu X. Cancer biomarker detection: recent achievements and challenges. Chem. Soc. Rev, 2015; 44(10): 2963-2997.
37. Sturgeon CM, Lai LC, Duffy MJ. Serum tumour markers: how to order and interpret them. Bmj, 2009; *339*: b3527.
38. Gumina RJ, Schultz JE, Yao Z, Kenny D, Warltier DC, Newman PJ, Gross GJ. Antibody to platelet/endothelial cell adhesion molecule-1 reduces myocardial infarct size in a rat model of ischemia-reperfusion injury. Circulation, 1996; *94*(12): 3327-3333.
39. Sapino A, Bongiovanni M, Cassoni P, Righi L, Arisio R, Deaglio S and Malavasi F. Expression of CD31 by cells of extensive ductal in situ and invasive carcinomas of the breast. J Pathol, 2001; 194: 254-261.
40. El Agouza IM, Eissa S S, El Houseini M M, El-Nashar D E, El Hameed O A. Taurine: a novel tumor marker for enhanced detection of breast

cancer among female patients. *Angiogenesis*, 2011; *14*(3): 321.
41. Dales JP, Garcia S, Carpentier S, Andrac L, Ramuz O, Lavaut MN, Allasia C, Bonnier P, Charpin C. Long-term prognostic significance of neoangiogenesis in breast carcinomas: comparison of Tie-2/Tek, CD105, and CD31 immunocytochemical expression. Hum Pathol, 2004; 35: 176-183.
42. Kumar P, Wang JM, Bernabeu C. CD105 and angiogenesis. J Pathol, 1996; 178: 363–366.
43. Montrucchio G, Sapino A, Bussolati B, Ghisolfi G, Rizea-Savu S, Silvestro L, Lupia E, Camussi G. Potential angiogenic role of platelet-activating factor in human breast cancer. Am J Pathol, 1998; 153*:* 1589–1596.
44. Kim OH, Kang GH, Noh H, Cha JY, Lee HJ, Yoon JH, Mamura M, Nam JS, Lee DH, Kim YA. Proangiogenic TIE2+/CD31+ macrophages are the predominant population of tumor-associated macrophages infiltrating metastatic lymph nodes. Mol Cells, 2013; 36: 432-438.
45. Darai E, Bringuier AF, Walker-Combrouze F, Fauconnier A, Couvelard A, Feldmann G, Madelenat P, Scoazec JY. CD31 expression in benign, borderline, and malignant epithelial ovarian tumors: an immunohistochemical and serological analysis. Gynecol Oncol, 1998; 71(1): 122-127.
46. Schneider G, Suszynska M, Kakar S, Ratajczak MZ. Vitronectin in the ascites of human ovarian carcinoma acts as a potent chemoattractant for ovarian carcinoma: Implication for metastasis by cancer stem cells. J. Cancer Stem Cell Res.", 2016; 4.
47. Cho W, Jung K, Regnier FE. Sialylated Lewis x antigen bearing glycoproteins in human plasma. J Proteome Res, 2010; 9: 5960–5968.
48. Hao W, Zhang X, Xiu B, Yang X, Hu S, Liu Z, Duan C, Jin S, Ying X, Zhao Y, Han X. Vitronectin: a promising breast cancer serum biomarker for early diagnosis of breast cancer in patients. TUMOR BIOL, 2016; 37(7): 8909-8916.
49. Heyman L, Kellouche S, Fernandes J, Dutoit S, Poulain L, Carreiras F. Vitronectin and its receptors partly mediate adhesion of ovarian cancer cells to peritoneal mesothelium in vitro. *TUMOR BIOL*, 2008; 29(4):231-244.
50. Turan T, Torun M, Atalay F, GÖNENÇ A. Assessment of vitronectin, soluble epithelial-cadherin and TGF-β1 as a serum biomarker with predictive value for endometrial and ovarian cancers. Turk J Pharm Sci, 2017; 14(2): 141.
51. Kenny H A, Kaur S, Coussens LM, Lengyel E.The initial steps of ovarian cancer cell metastasis are mediated by MMP-2 cleavage of vitronectin and fibronectin. J CLIN INVEST, 2008; *118*(4): 1367-1379.
52. Aldinucci D, Colombatti A. The inflammatory chemokine CCL5 and cancer progression. MEDIAT INFLAMM, 2014 Oct; 2014.
53. Makarov SS. NF-κB as a therapeutic target in chronic inflammation: recent advances. "Mol. Med. Today, 2000; *6*(11): 441-448.
54. Wietek C, O'Neill LA. Diversity and regulation in the NF-κB system. Trends Biochem. Sci, 2007; *32*(7): 311-319.
55. Balkwill FR. The chemokine system and cancer. J. Pathol, 2012; 226(2): 148–157.
56. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature, 2007; 449(7162): 557-563.
57. Lv D, Zhang Y, Kim HJ, Zhang L, Ma X. CCL5 as a potential immunotherapeutic target in triple-negative breast cancer. CELL MOL IMMUNOL, 2013; *10*(4): 303.
58. Kalimutho M, Parsons K, Mittal D, López JA, Srihari S, Khanna KK. Targeted therapies for triple-negative breast cancer: combating a stubborn disease. TRENDS PHARMACOL SC.I, 2015; *36*(12): 822-846.
59. Tsukishiro S, Suzumori N, Nishikawa H, Arakawa A, Suzumori K. Elevated serum RANTES levels in patients with ovarian cancer correlate with the extent of the disorder. Gynecol. Oncol, 2006; *102*(3): 542-545.
60. Long H, Xie R, Xiang T, Zhao Z, Lin S, Liang Z, Chen Z, Zhu B. Autocrine CCL5 signaling promotes invasion and migration of CD133+ ovarian cancer stem-like cells via NF-κB-mediated MMP-9 upregulation. Stem cells, 2012; 30(10): 2309-2319.
61. Meng Y, Wang L, Chen D, Chang Y, Zhang M, Xu JJ, Zhou R, Zhang QY. LAPTM4B: an oncogene in various solid tumors and its functions. Oncogene, 2016; *35*(50): 6359.
62. Kasper G, Vogel A, Klaman I, Gröne J, Petersen I, Weber B, Castaños-Vélez E, Staub E, Mennerich D. The human LAPTM4b transcript is upregulated in various types of solid tumours and seems to play a dual functional role during tumour progression. Cancer letters, 2005; 224(1): 93-103.
63. Li Y, Zhang Q, Tian R, Wang Q, Zhao JJ, Iglehart JD, Wang ZC, Richardson AL. "Lysosomal transmembrane protein LAPTM4B promotes autophagy and tolerance to metabolic stress in cancer cells". Cancer Res, 2011; 71(24): 7481–7489.
64. Meng FL, Yin MZ, Song HT, Yang H, Lou G, Zhou RL. LAPTM4B-35 overexpression is an independent prognostic marker in endometrial carcinoma. INT J GYNECOL CANCER, 2010; *20*(5): 745-750.
65. Fan M, Liu Y, Zhou R, Zhang Q. Association of LAPTM4B gene polymorphism with breast cancer susceptibility. Cancer Epidemiol, 2012; *36*(4): 364-368.