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ESTIMATION OF THE LEVEL OF BASIC FIBROBLAST GROWTH FACTOR (BFGF) IN THE SERUM OF WOMEN WITH OVARIAN CANCER

Hiba Nadhom Kadhom and Anwar Abdul-Ameer Mohammed Karim

Department of Life Science, College of Science, Diyala University, Iraq.

Abstract

Ovarian cancer is the seventh status among cancer types in the world and the eighth one among cancer types that leads to death annually. The level of basic fibroblast growth factor (bFGF) rises in diagnosed women with ovarian cancer compared to other women with borderline tumors for healthy women with ovarian tumors so bFGF is biomarker to predict the appearance of the tumor through its correlation with angiogenesis. This study has been conducted to evaluate the role of basic fibroblast growth factor (bFGF) and Cancer antigen (CA125) in ovarian cancer progression. Samples of this study were collected from the Teaching Oncology Hospital and Baghdad Teaching Hospital , Medical City. 110 blood samples were collected& divided into 40 samples for healthy women (control group) 16 samples for women with ovarian cysts, 39 samples for women with ovarian cancer, 15 samples for women with ovarian cancer after chemotherapy, their ages at diagnosis were between 15- 60 years.Enzyme-Linked immune sorbent assay (ELISA) was used to measure the level of bFGF and CA125 in serum of all groups of the study. Results showed a high level of bFGF and CA125, a significant increase in the group of women with ovarian cancer compared to other groups of the study ($P<0.05$). Surface Epithelial Cells (SEC) was the most common type of tumors (87%), while Sex cord- Stromal tumorwere about 7.30% and the Germ Cells Tumors were about 5.70%. The 55≤ age group was the most age group of women with ovarian cancer. bFGF is an important predictive factor in early detection of ovarian cancer and CA125 also rises, but is less sensitive than bFGF in detecting ovarian cancer, so it is useful to use as tumor marker for early detection of ovarian cancer.

Key words: OC : Ovarian cancer, bFGF, CA125, Angiogenesis.

Introduction

Ovarian cancer (OC) is an inherently hidden disease that arises and develops without prominent signs or symptoms. The disease is often diagnosed in advanced stages (Gubbels *et al*., 2010; Vargas., 2014). OC is divided into three types and the most cases of the tumor arise from one of the three types of cells in the ovary: Surface Epithelial Cells, which are multipotent, Germ Cells (Totipotent) and Sex Cord Stromal Cells which are multipotent (Chen et al., 2003; Kumar *et al*., 2007). Depending on genetic changes. OC is separated into two types: Type 1: characterized by its slow growth, as the tumor is often diagnosed on the ovary and develops from what is called Borderline tumor; Type2: that grows rapidly and tends to spread, unlike the type 1, it is difficult to determine the precursor that evolved from it (Kurman and Shih., 2008). There are many risk factors related to the disease, many of them are genetic, other related to age, Ethnicity, familial history of cancer and environmental factors (Karst and Drapkin., 2009). Controlling of this disease and its treatment remains a major challengebecause ovarian cancerincludes a heterogeneous group of cancers that affect the ovary often differ in their symptoms and methods of diagnosis (Pieterse *et al*., 2019). The most dangerous side in ovarian cancer is to return the disease even after the eradication of the tumor surgically where the disease returns again to spread rapidly in other organs (Carpenter and Cohen., 1990). Basic fibroblast growth factor bFGF is sometimes referred to as the second fibroblast growth factor-fgf-2, it represents one of the growth factors (GFs) which in turn include a variety group of polypeptides (Böhen *et al.,* 1984). bFGF was purified from Pituitary Gland extracts in cows and was diagnosed as a polypeptide consisting of approximately on 146 amino acids (Beenkin

***Author for correspondence :** E-mail : riri88hiba@gmail.com

and Mohammedi., 2009). It belongs to a family of growth factors that includes approximately 23 elements called the fibroblast growth factor family (FGFs) (Steringer and Nickel., 2018). Most tissues produce and release it outside the cell via its plasma membrane (Bikfalvi *et al*., 1997). Four receptors are described for bFGF, the first called (Flg) FGFR1, the second is called (bek) FGFR2, FGFR3 and FGFR4 (1Madsen *et al*., 2012). The level of bFGF rises in diagnosed women with ovarian cancer compared to other women with borderline tumors and healthy women with ovarian tumors, so bFGF is an important biomarker to predict the appearance of the tumor through its correlation with angiogenesis (Müller and Werner., 2012). It plays an important role in many biological activities including cell proliferation, cell migration, survival and angiogenesis as well as cell differentiation (Bottoni and Scatena., 2015). In the other side, Cancer antigen (CA125) is also known as Mucin 16 (MCU16), which is Glycoprotein. It belongs to a large group that includes a number of mucus types and is expressed by the *Mucin16* gene (Babic *et al*., 2017). It is of high molecular weight and is most often produced in the Epithelial Cell (Bast *et al*., 1983). CA125 is one of the tumor markers as it rises in the serum level in many malignant tumors, as in the cases of Lung Cancer, Breast C., Colon C., pancreas C., and Ovarian Cancer (Sturgeon., 2002). The level of CA125 is influenced by many factors, including age, frequent reproduction as well as the use of oral contraceptives (Khalid *et al*., 2015). The studies focused on measuring the level of CA125 among women with ovarian cancer that the level of antigen is high in the advanced stages of OC in about revealed 90% of cases, while its level appears low in about 20-50% of OC cases in its early stages (Prat., 2012).

Materials and Methods

A. Samples collection: 110 blood samples were collected from diagnosed women with cancer at Teaching Oncology Hospital and Baghdad Teaching Hospital / Medical City. Samples are divided into 16 samples for women with ovarian cysts, 39 samples for women with ovarian cancer, 15 samples for women with ovarian cancer after chemotherapy. The age periods were between 15- 60 years, 40 samples for healthy women (control group). An amount of 5 ml of venous blood was collected from each woman and placed in a gel tube and centrifuging at a rate of 4000rpm for 10 minutes and separating serum.

B. Measurement levels of bFGF& CA125: The levels of bFGFand CA125 in serum were quantified using ELISA sandwich test according to the manufacturer's instructions given in the Abkam equipped test kit.

C. Statistical analysis: Data of current study were analyzed by using Chi-square (X^2) test to compared between percentages. also, measured sensitivity and specificity of diagnostic tests (detection the best test for diagnosis) Numeric data were described by (Mean ± SD and St. Error). T test used to compare between two numeric variable or more. The least significant difference-LSD test to the comparative between means. Pearson correlation (r) accounted to explain type and strength of relationship between variables. A level of significance of α=0.05 was applied to test. (SPSS v.22 and Excel 2013) programs used to analyze current data.

Result and Discussion

OC is age related and is mainly considered as a postmenopausal disease where an increased incidence of (OC) is observed over the age of 65 years (Momenimovahed *et al*., 2019). Also, (Wentzensen *et al*., 2016) reported that the disease increases by age 35 and reaches its peak among women aged 55-64 years. OC is affects all age groups of women. In the current study, the most affected age group was 55≤ years, with a rate of 40.7% and the lowest age group between 34-25 years, with a rate of 1.9%. The results of a study showed that 48% of OC affects the age group of 65 years. The infection rate of OC increases with age Progress (Yancik., 1993), where OC affects women after 50 years and 64% (Hussein and Salai., 2019). The results of table 1 showed that the age index gave a high and significant increase in this age group and these results are agreed with study (Rott and Evans., 2009).

Another study revealed that 75% of ovarian cancer cases were diagnosed in women over 55 years of age (Sundar *et al*., 2015). This is due to the fact that when women get older, they suffer from many hormonal disorders, as well as the ovulation process, as the more women age, this increases the risk of ovarian cancer (Brinton *et al*., 2015), where the theory of continuous ovulation states without interruption to the development of (OC), causing damage to the ovarian surface epithelium, many studies indicated a relationship between an increased risk of ovarian cancer and the use of treatments

Table 1: Age groups comparison in patients groups.

Age	No.	Percentage %	Statistics
15-24	5	9.3%	X^2=29
25-34	1	1.9%	DF=4
35-44	8	14.8%	P=0.001***
45-54	18	33.3%	
≥55	22	40.7%	
Total	54	100%	

Table 2: Comparison of mean age and biomarkers in the groups under study.

Parameter		NO.	Mean	Std. Error	Statistics
Age	ControlOvarian cystsMalignant	401654	$29.33^{b}30.31^{b}50.63^{a}$	1.562.232.03	$F=38.22DF=2P=0.001^{***}$
bFGFpg/ml	ControlOvarian cysts Malignant	401654	$66.81^{b}86.44^{b}145.63^{a}$	4.204.075.09	$F=76.11DF=2P=0.001^{***}$
CA125 U/mL	ControlOvarian cysts Malignant	401654	$17.65^{b}57.08^{a}68.01^{a}$	0.686.892.60	$F=101.11DF=2P=0.001^{***}$

that stimulate the ovulation process, such as Clomiphene Citrate (Reigstad *et al*., 2017). In table 2 the results showed an increase in the age level index in the group of women with ovarian cancer compared to the control group (2.03.63 ± 50 and 1.5629.33 ±) the rise was very high and very significant (P<0.005).

The average age in the current study was 50.63 years and this Consistent with the study (Reigstad *et al*., 2017). In their study, the mean age at diagnosis was varied between 52.2 and 59.5 years, and both Doufekas and Olaitan (Doufekas and Olaitan., 2014) stated that the average age at diagnosis was 63 years in the United Kingdom. Results of table 2 showed a high significant increase in level of bFGF in the same group compared to the control group (145.20 ± 5.09 and ± 4.2066.81 and the benign cyst group 4.07 ± 86.44 respectively), as the levels of bFGF were high in women with ovarian cancer compared with the benign cyst group and these results are consistent with results of study (Müller *et al*., 2012), the level of bFGF rises in the serum of women with ovarian cancer compared to the control group and this is due to its production by ovarian cancer cells, it is possible that this indicator is also a useful sign in monitoring disease progression and responding to treatment (Lepag *et al*., 2006). High significant increase of level ofCA125showed in table 2 in the same group of patients where it was 19.12 ± 68.1 compared to the control group 17.65 ± 4.28 and these results are also agreed with the study (Lepag *et al*., 2006). What explains the rise of this indicator is to monitor Chemotherapy for women with ovarian cancer

Table 3: Comparing the level of bFGF and CA125 with age groups in the patient group.

Perameter	Age groups	No.	Mean	Std. Error	Statistics
bFGFpg/ml	15-24	8	118.88^{b}	15.27	F = 3.5
	15-24	11	118.88^{b}	15.27	Df = 4
	25-34	10	97.00^{b}	7.97	$P = 0.01^{**}$
	35-44	10	135.60^{a}	15.80	
	45-54	19	150.37^{a}	8.73	
	55≤	22	136.50^{a}	8.13	
CA125	15-24	8	62.43	7.97	F = 0.78
	25-34	11	65.12	8.57	Df = 4
	35-44	10	65.69	7.83	P = 0.54
	45-54	19	59.92	4.51	
	55≤	22	71.59	3.80	

(Gupta and Lis., 2009).

When comparing the level of biomarkers under study with patients age groups, bFGF showed the highest level in the 45-54 years and it was 150.37 ± 8.37 ,this increase was high and significant (P<0.05)compared to other age groups in the patients group, as shown in table 3.

A study (Študent *et al*., 2018) indicated there is no difference between the levels of bFGF and age, but its levels were high in the serum of ovarian cancer patients compared to the ovarian cysts group and the control group, its level was also high before chemotherapy. Studies were shown that cancer cells have the ability to secrete a peptide growth factor and its receptors through the mechanism of Autocrain and thus, cells will maintain their proliferation naturally (Di *et al*., 1995). CA125 showed its highest level in the 55≤ age group and it was 71.59 ± 3.80 and there was no significant difference in its level in this category compared to other age groups. This agreed with the study (Johnson *et al*., 2008), CA125 levels are high with age. Although CA125 is a prediction factor now. there is an inverse relationship between its levels and the survival rate of ovarian cancer patients. Low levels indicate a positive response to treatment and survival. High levels indicate a tumor recurrence and a low level of survival (Gupta and Lis., 2009). The current study included various types of OC: Epithelial cells are the most common and considered a major problem as 75% of patients experience recurrence of the injury after the initial treatment. This type is often Chemo resistance MDijkgraadf *et al*., 2012). The results of table 4 showed that the type of epithelial surface cell carcinoma (SECs) showed the highest percentage among other types (87.00%).

These results were consistent with the study (Momenimovahed *et al*., 2019) that (SECs) accounted for 95% of other species (OC) As for its types, the type of Serous is 44.4%. The serous type is the most common species due to its rapid spread and its malignant capabilities are the highest (Doufekas and Olaitan., 2014). The subtype Mucinous about 14.80% (), the subtype Endometriod with 11.10% agreed with (21) and the subtype Clear cell shape ratio is about 3.70% and also the subtype Transitional cell with 3.70%, while Carcinoma constitutes a ratio of about 9.30% and these results are consistent with the results of a study (Jumaah., 2013).

Table 4: Distribution of the main and subtypes of ovarian cancer in the patient group only.

Tumor type		No.	Percent%	Statistic
Surface epithelial tumor		42	87.00%	
	Serous	24	44.40%	
	Endometriod	6	11.10%	
	Mucinous	8	14.8%	
	Clear cell	2	3.70%	
	Carcinoma	5	9.30%	X^2=81
	Transitional cell	2	3.70%	DF=9
Sex cord-stromal tumor		4	7.30%	P=0.001***
	Granuolosa	4	7.30%	
Germ cell tumor	Choriocarcinoma	3	5.70%	
		1	1.90%	
	Dysgerminoma	1	1.90%	
	Yolk sac tumor	1	1.90%	

The sex cord-stromal type has a rate of about 7.30%, and its subtype form Granulosa in this study has a rate of 7.30% and these results are consistent with the results of (Jumaah., 2013; Gaaib., 2018). As for the Germ cell type, the ratio of 5.70% is the lowest type Common, where the type Choriocarcinoma appeared by 1.90%, the type of Dysgerminom by 1.90%, followed by the type Yolk sac tumor by 1.90% as well, respectively, these results were consistent with the results of (2018) Gaaib and (2019) Hussein and salai and the difference was very high and significant between species ($p \leq 0.01$). Results of table 5 showed that there were differences in the levels of bFGF concentration between study groups and these differences were very high and very significant ($P<0.05$), where control group was 4.2066.81 ± and the ovarian cyst group 4.0786.44 ± they had differences compared with the group of malignant tumors where they were 145.20 ± 5.09 and did not show a difference between it and the malignant tumors group after chemotherapy.

We observed from the results differences between the four groups. The levels of bFGF were high in serum of infected women with OC compared to the group of ovarian cysts, and this is consent with the results of (2016) Szubert *et al.*, while a low level of bFGF is observed after chemotherapy, as mentioned (2018) Študent *et al*., The level of bFGF in the serum of infected women (OC) was high before chemotherapy, where the levels of bFGF in patients before chemotherapy were high compared to patients showing a response to chemotherapy (Dirix *et al*., 1997). And not only in OC patients, where the level of bFGF in patients' serum decreases to Multiple Myeloma (Sezer *et al*., 2001). Reproduction, migration and survival of cancer cells (Hu *et al*., 2016). However, targeting this process by blocking FGFR receptors will lead to stopping the blood flow to feed the cancer cell and thus lead to its death. These receptors are important targets for the treatment of different types of cancer (Dai *et al*., 2019).

Table 5: Comparing bFGF level among the groups under study.

Groups	No.	Mean	Std. Error	Statistics
Control	40	66.81[c]	4.20	F = 55
Ovarian cysts	16	86.44[b]	4.07	DF = 3
Malignant	54	145.20[a]	5.09	P = 0.001***
After chemotherapy	15	14.00[a]	9.06	

Table 6: Comparison between biomarkers bFGF, CA125.

Parameter		bFGF	CA125
bFGF pg/ml	R	1	0.244*
	P		0.042
CA125 U/ml	R	0.244*	1
	P	0.042	

Table 6 shows the correlation between the two biomarker under study, where the results showed a high significant relationship between the two indicators (R = 0.244), as the bFGF was correlated with the CA125 directly, and the reason for this is that both indicators rise with the disease OC and this is agree with (Madsen *et al*., 2012).

While a study indicated that there is no correlation between the levels of the two biomarkers in the serum before chemotherapy (Študent *et al*., 2018). Since bFGF rises in serum of patients (OC) due to its important role in the Angiogenesis , as it stimulates the movement and reproduction of endothelial cells (EC), this factor is the basis of this process (Kumer *et al*., 2007). In addition to the mechanism by which FGF works, through Autocrine and Paracrine, cells that secrete bFGF(Kumer *et al*., 2007). When comparing the current study with another study on the Vascular endothelial growth factor (VEGF), this factor also rises in the serum of female patients (OC) because of their important role in the Angiogenesis process as they are considered pro-angiogenesis factors (Jumaah., 2013), bFGF is higher in other types of cancer except OC, such as Thyroid cancer (Pasieka *et al*., 2003).

Calculating the sensitivity and specificity of both biomarkers in the patient and healthy groups came as shown in table 7, where the sensitivity of the bFGF indicator to disease is very high (98%), corresponding to

Table 7: Calculation of sensitivity and specificity of bFGF&CA125 biomarkers, using the ROC curve.

Parameter	Sensitivity%	Specificity%	Cut off	AUS	P value
bFGF pg/ml	98%	81%	60≤	0.938	0.028*
CA125 U/mL	61%	37%	35≤	0.657	0.102

the specificity (81%), while CA125 received a sensitivity of 61%, with a specificity of 37%, the difference between sensitivity and specificityfor the two biomarkers was significant (P<0.05).

bFGF is an aid in predicting OC because it contributes to the first step of cancer cell formation through its role in the Angiogenesis process which is the most important step in the development of cancer (Madsen *et al*., 2012). In addition, CA125 is low sensitivity in the early stages of OC because it is raised in normal conditions such as menstruation and endometriosis (Endometerosis) (Dochez *et al*., 2019), Which makes it insensitive and also ineffective in early detection of OC (Helzlsouer *et al*., 1993; Bast *et al*., 2005). CA125 is also used to monitoring chemotherapy in patients to evaluate the response to treatment as it is low sensitivity (Duffy *et al*., 2005) Therefore, it is not recommended to use CA125 alone unless it is linked to another biomarker such as osteopontin (Mohammed *et al*., 2018).

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