Archives of Razi Institute, Vol. 77, No. 5 (2022) 1659-1671

DOI: 10.22092/ARI.2022.358329.2194

Original Article

Effect of Serum Level of Human Epididymis Protein 4 and Interleukin-6 as Biomarkers in Patients with Adnexal Mass

Hameed, S[1], Abdulqader Jasim, H[1]*, Sharief, M[2]

1. Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
2. Department of Gynecology and Obstetrics, College of Medicine, University of Basrah, Basrah, Iraq

Received 1 April 2022; Accepted 16 April 2022
Corresponding Author: hanadi.jasim@uobasrah.edu.iq

Abstract

Ovarian carcinoma is one of the most common types of neoplasms in women and the fifth leading cause of cancer death among women worldwide. Adnexal masses are classified as simple or complicated and can be benign or malignant. No single biomarker has demonstrated high sensitivity and specificity for detecting early ovarian cancer. Therefore, the current study was designed to investigate the influence of using two biomarkers as a tool for diagnosis in patients with an adnexal mass. This prospective case-control study was carried out on female patients diagnosed by ultrasound and magnetic resonance imaging with adnexal masses and scheduled for surgery and healthy women as a control group (n=50 each). The patients were in the age range of 16-80 years old and had attended the surgical rooms of Basrah hospitals, Basrah, Iraq, from January to July 2021. The levels of serum biomarkers were quantitatively assessed using the enzyme-linked immunosorbent assay. The serum concentration of the human epididymis protein 4 (HE4) biomarker exhibited significant differences between females with adnexal mass and healthy women. There was no significant association between neither the patient's age nor the menopausal state and the serum level of HE4. The serum level of HE4 had a sensitivity of 92% and a specificity of 66% as a serum marker for the presence of adnexal mass with a positive predictive value of 73% and a negative predictive value of 89%. In this study, serum interleukin-6 (IL-6) had a sensitivity of 30% and specificity of 64% in determining patients with adnexal mass pathology. It was found that the level of IL-6 was similar in all patients, compared to that in the control group. The median levels of serum HE4 showed high value in patients in the age groups of 21-40, 41-50, and >50 than in the control group; however, it was not statistically different (P=0.413). Human epididymis protein 4 was the top biomarker representing a higher concentration in adnexal mass; moreover, it demonstrated the highest performance in all samples with Adnexal mass. The results of our study showed that combining more than one marker measurement increased both the sensitivity and specificity of distinguishing patients with adnexal mass pathology.

Keywords: Adnexal mass, HE4, IL-6, Ovarian cancer

1. Introduction

Ovarian carcinoma is the seventh most prevalent malignancy among women globally, and it is one of the most common forms of neoplasms. More than 295 thousand new cases of cancer in women are detected each year, accounting for 3.6% of all cancers in women (1). It is one of the main causes of death in women with nearly 185 thousand deaths per year, and it has been reported that more than 70% of women present at the advanced stage of the disease (2, 3). Abood, Abdahmed (4) stated that ovarian cancer is one of the most frequent cancer types in females in Basra, Iraq. Moreover, it has the highest case fatality rate among all gynecological malignancies (5). Due to its highly metastatic nature, the prognosis is so poor. Therefore, it is still needed to perform research focused on the pathogenesis, detection, and treatment of this disease (6).

Given that the early detection of the disease is difficult due to the absence of recognized physical symptoms and the lack of sensitive screening tools, ovarian carcinomas have higher morbidity and fatality rates than other gynecological cancers. The American Cancer Society projected that a total of 22,000 new cases and over 15,000 deaths would happen in 2012 Tarver (7). The risk of ovarian cancer has been linked to late conception, a smaller family size, obesity, and the use of hormone replacement therapy. Data from a meta-analysis suggest that women who use hormone therapy for 5 years from the age of 50 have about one extra ovarian cancer case per 1,000 women (8).

Notably, an adnexal mass or an ovarian cyst will be diagnosed in approximately 20% of all women at some point in their life, yet only a small percentage of these masses represent an ovarian malignancy (9). Adnexal masses comprise approximately 18% of all gynecological pathologies and are found in 6-11% of perimenopausal and postmenopausal women during routine gynecological or ultrasound examination (10). Approximately 85-90% of all adnexal masses are benign (11). Adnexal masses are classified as simple or complicated and can be benign or malignant.

The current gold standard biomarker for ovarian cancer is cancer antigen 125 (CA125), a serum glycoprotein. Although it is approved for both differential diagnosis of a pelvic tumor and as a serial response measure in patients receiving treatment, it has low specificity for the condition. Other benign and malignant ovarian and non-ovarian disorders cause CA125 to rise (12). As a result, CA125 is insufficient for diagnosis or screening as a stand-alone test (13).

The only biomarkers that have been approved and used in clinical settings are human epididymis protein 4 (HE4) and CA125 (14). Human epididymis protein 4 is a protein that is overexpressed in ovarian carcinomas. Although the normal functions of HE4 are unknown, its specificity and sensitivity suggest that it can be used as an early detection serum marker for ovarian cancer (15). Moore, McMeekin (16) discovered HE4 as an ovarian cancer biomarker in 2008.

Interleukin 6 (IL-6) has a variety of roles in physiological settings, including attracting neutrophils; stimulating T lymphocyte migration, proliferation, activation, and differentiation, and promoting B lymphocyte differentiation to plasma cells to manufacture immunoglobulins (17). Alhumrani, Jamalludeen (18) stated the role of IL-6 as an indicator of the severity and as a predictor of complications with the acute coronary syndrome.

On the other hand, through the effects of IL-6 on various cell signaling pathways that promote the cell cycle and growth, it has been demonstrated that IL-6 has direct stimulatory effects on several cancer cells. Furthermore, IL-6 has been shown to have negative effects on immune cells at high doses by blocking the expression of IL-2, decreasing T cell activation, and boosting lymphocyte apoptosis, which can hinder immune surveillance of cancer cells Maccio and Madeddu (19). To the best of our knowledge, to present, no single biomarker has demonstrated high sensitivity and specificity for detecting early ovarian cancer, and the use of a panel of biomarkers in clinical practice is not yet practical (20).

This study aimed to investigate the accuracy of using single or combined biomarkers to diagnose ovarian cancer in patients with an ovarian mass.

2. Materials and Methods

2.1. Study Population

This prospective case-control study was carried out from January to July 2021. The samples of this study (n=100) were divided into two groups of patients and controls (n=50 each). The patients were in the age range of 16-80 years old and consisted of women referring to the surgical rooms of Basrah Women and Children Teaching Hospital, Basrah Teaching Hospital, Al-Fayhaa Teaching Hospital, and Al-Mawani Teaching Hospital, Iraq. The control group included healthy women who were 16-80 years old and attended outpatient gynecological clinics and family planning clinics. They underwent pelvic ultrasound examination,

which is a normal and a negative C-reactive protein test for all enrolled controls.

All medical information of patients and controls were recorded in a questionnaire form, including name, age, phone number, residency, occupation, marital status, number of children, being overweight or obese, breastfeeding, using birth control pills, using fertility treatment, and having a family history of ovarian cancer, breast cancer, and colorectal cancer.

All enrolled women had an ovarian mass that was confirmed by pelvic ultrasound examination or magnetic resonance imaging. Cancer antigen 125 was estimated for the whole participants who underwent laparotomy for the resection of the ovarian mass. On the other hand, any pregnant women, women with confirmed ovarian malignancy, or women under treatment were excluded from this study.

The research objectives and procedures were explained to all individuals, and informed consent was obtained from all of them. The participants agreed in writing consent to be followed up by phone for biopsy results.

2.2. Measuring Serum Levels of IL-6 and HE4 by the Enzyme Linked Immunosorbent Assay

The volumes of 5 ml of peripheral blood samples were collected in vacuum gel tubes, centrifuged to obtain serum, and stored at -36°C for use in a serological study by the enzyme linked immunosorbent assay (ELISA). Serum IL-6 and HE4 concentrations were estimated by specific commercial kits, namely the sandwich ELISA (IL-6 and HE4 ELISA Kit provided by Bio Sources, USA). Assays were measured using an ELISA reader at 450 nm. The procedure and steps of the methods depended on the manufacturer's instructions.

2.3. Statistical Analysis

The collected data were analyzed in SPSS software (version 24). Qualitative data were expressed as frequencies and percentages, while quantitative data were presented as mean±standard deviation, median, and minimum and maximum values. To investigate the statistical significance in any observed association of qualitative variables, Chi^2 was used. The presence of significant statistical differences between/among quantitative variables was examined using the Mann-Whitney U, Wilcoxon Signed Ranks, and Kruskal Wallis tests. Spearman's nonparametric correlation test was used to determine any statistically significant correlations.

3. Results

A total of 100 women (50 patients and 50 controls) were entered into this study. The age of the examined women ranged from 20 to more than 60 years for both patient and control groups. They were checked by a gynecologist and confirmed to be free from any urological and clinical problems rather than adnexal mass for patients.

All demographical and clinical features of patients and controls are presented in table 1 comparing patients having adnexal mass with the controls in terms of some sociodemographic and health characteristics. It is clear from table 1 that the only variable which differed statistically significantly in patients from the controls was the vaginal bleeding, revealing that none of the controls suffered from bleeding.

Table 2 summarizes the levels of tumor markers in both patients and control. Accordingly, the serum level of HE4 was significantly statistically higher in patients. However, there was no difference in the level of IL-6 between patients and controls.

The median levels of IL-6 and HE4 in patients and controls according to age groups are tabulated in table 3. Considering the age group, there were no statistically significant differences in the levels of the two markers of IL-6 and HE4 in all age groups. In this regard, the median levels of serum HE4 showed high values in patients in different age groups (i.e., 21-40, 41-50, and >50) than in the control group; nevertheless, it was not statistically different (P=0.413) (Table 3).

Table 1. Comparison of the sociodemographic and health characteristics between patients and control groups

Characteristic		Group		Total	Sig.*
		Control	Patient		
Age (years)	<20	8 16.0%	5 10.0%	13 13.0%	0.728
	20-30	17 34.0%	20 40.0%	37 37.0%	
	30-40	10 20.0%	11 22.0%	21 21.0%	
	40-50	13 26.0%	10 20.0%	23 23.0%	
	>50	2 4.0%	4 8.0%	6 6.0%	
Menopause	Premenopausal	42 84.0%	42 84.0%	84 84.0%	1.000
	Postmenopausal	8 16.0%	8 16.0%	16 16.0%	
Number of children	0	20 40.0%	20 40.0%	40 40.0%	0.787
	1-5	24 48.0%	26 52.0%	50 50.0%	
	>5	6 12.0%	4 8.0%	10 10.0%	
Body mass index	≤30	32 64.0%	34 68.0%	66 66.0%	0.673
	>30	18 36.0%	16 32.0%	34 34.0%	
Marital status	Single	10 20.0%	13 26.0%	23 23.0%	0.476
	Married	40 80.0%	37 74.0%	77 77.0%	
Breastfeeding	Yes	3 6.0%	7 14.0%	10 10.0%	0.182
	No	47 94.0%	43 86.0%	90 90.0%	
Pill user	Yes	5 10.0%	11 22.0%	16 16.0%	0.102
	No	45 90.0%	39 78.0%	84 84.0%	
Induction ovulation	Yes	13 26.0%	10 20.0%	23 23.0%	0.476
	No	37 74.0%	40 80.0%	77 77.0%	
History of infertility	Unmarried	9 18.0%	10 20.0%	19 19.0%	0.874
	Non-fertile	11 22.0%	9 18.0%	20 20.0%	
	Fertile	30 60.0%	31 62.0%	61 61.0%	
History of cancer	Yes	9 18.0%	11 22.0%	20 20.0%	0.617
	No	41 82.0%	39 78.0%	80 80.0%	
Vaginal bleeding	Menorrhagia	0 0.0%	14 28.0%	14 14.0%	0.0001
	No bleeding	50 100.0%	21 42.0%	71 71.0%	
	Metrorrhagia	0 0.0%	15 30.0%	15 15.0%	

Table 2. Median levels of IL-6 and HE4 in patient and control groups

Category		Serum IL-6	Serum HE4
Patients	n	50	50
	Mean±SD	0.708±2.16	859.22±2385.71
	Median	0.054	0.058
	Min-Max	0.031-11.643	0.016-9783.900
Controls	n	50	50
	Mean±SD	0.602±1.57	0.024±0.03
	Median	0.058	0.019
	Min-Max	0.031-6.86	0.000-0.244
Sig*		0.647	0.0001

*Mann-Whitney U Test

Table 3. Median levels of IL-6 and HE4 in patients and controls according to age groups

Category	Age groups		Serum IL-6	Serum HE4
Patients	<20 Years old	n	5	5
		Mean±SD	0.76±1.59	0.037±0.023
		Median	0.052	0.03
		Min-Max	0.03-3.61	0.023-0.079
	20-30 years old	n	20	20
		Mean±SD	0.85±2.148	939.64±2616.59
		Median	0.054	0.067
		Min-Max	0.036-9.2	.016-9783.9
	30-40 years old	n	11	11
		Mean±SD	1.109±3.49	1691.669±3430.37
		Median	0.056	87.92
		Min-Max	0.03-11.64	0.026-9763.4
	40-50 years old	n	10	10
		Mean±SD	0.22±0.52	503.16±1339.66
		Median	0.054	19.58
		Min-Max	0.046-1.705	0.018-4288.0
	>50 years old	n	4	4
		Mean±SD	0.053±0.017	132.02±225.52
		Median	0.054	30.237
		Min-Max	0.03-0.07	0.023-467.610
Sig*			0.908	0.490
Controls	<20 Years old	n	8	8
		Mean±SD	0.054±0.012	0.022±0.008
		Median	0.057	0.022
		Min-Max	0.03-0.067	0.008-0.034
	20-30 years old	n	17	17
		Mean±SD	0.94±1.92	0.03±0.055
		Median	0.07	0.02
		Min-Max	0.038-5.92	0.004-0.244
	30-40 years old	n	10	10
		Mean±SD	0.6±1.29	0.0156±0.0124
		Median	0.052	0.015
		Min-Max	0.03-4.022	0.000-0.04
	40-50 years old	n	13	13
		Mean±SD	0.58±1.88	0.023±0.018
		Median	0.061	0.019
		Min-Max	0.036-6.864	0.002-0.08
	>50 years old	n	5	5
		Mean±SD	0.04±0.001	0.012±0.004
		Median	0.04	0.012
		Min-Max	0.039-0.041	0.009-0.016
Sig*			0.157	0.413

* Kruskal Wallis test

Table 4 summarizes the median levels of IL-6 and HE4 according to the state of menopause. Accordingly, there were no statistically significant differences in the levels of the markers regarding the menopausal state in both patient and control groups. The levels of serum HE4 were found to be high in patients with postmenopausal than in other individuals in both patient and control groups without any statistical difference (*P*=0.419).

The median levels of IL-6 and HE4 according to the presence of menstrual disturbances are presented in table 5. Regarding menstrual disturbances, it has been shown that there was no significant difference in the levels of these markers among classified groups (Table 5). However, there was an increase in the level of HE4 in patients with menorrhagia than in other groups but without any statistical difference (*P*=0.422).

Table 4. Median levels of IL-6 and HE4 according to the state of menopause

Category	Menopause		Serum IL-6	Serum HE4
Patient	Premenopausal	n	42	42
		Mean±SD	0.83±2.34	904.3±2531.89
		Median	0.054	0.036
		Min-Max	0.03-11.64	0.016-9783.9
	Postmenopausal	n	8	8
		Mean±SD	0.0561±0.015	622.57±1489.28
		Median	0.055	49.74
		Min-Max	0.032-0.078	0.023-4288.0
Sig*			0.662	0.926
Control	Premenopausal	n	42	42
		Mean±SD	0.544±1.402	0.025±0.037
		Median	0.058	0.02
		Min-Max	0.031-5.92	0.000-0.244
	Postmenopausal	n	8	8
		Mean±SD	0.909±2.405	0.017±0.006
		Median	0.062	0.015
		Min-Max	0.039-6.86	0.009-0.032
Sig*			0.801	0.419

*Mann-Whitney U test

Table 5. Median levels of IL-6 and HE4 according to the presence of menstrual disturbances

Vaginal bleeding		IL6	HE4
Menorrhagia	n	14	14
	Mean±SD	3.125±1.385	1125.878±419.36
	Median	0.059	35.445
	Min-Max	0.036-11.643	0.016-4288.0
No bleeding	n	21	21
	Mean±SD	0.725±2.107	1267.03±2894.6
	Median	0.056	0.032
	Min-Max	0.032-9.204	0.023-9783.9
Metrorrhagia	n	15	15
	Mean±SD	0.053±0.016	698.82±2510.67
	Median	0.05000	0.028
	Min-Max	0.031-0.093	0.022-9763.4
Sig*		0.170	0.422

*Kruskal Wallis test

Table 6 tabulates the median levels of IL-6 and HE4 in patients and controls lacking vaginal bleeding. It is noticed in table 6 that the HE4 level was significantly statistically different in patients from controls, considering that the study women had no history of vaginal bleeding. Furthermore, the median levels of IL-6 were found without any increase in the patients than in the controls.

Table 7 presents the median levels of IL-6 and HE4 in patients and controls according to the family history of cancer. Accordingly, it was shown that the HE4 levels were statistically significantly higher in patients than in controls in both categories, including those who had a family history of cancer and those who had not (Table 7).

Table 8 summarizes the comparison of IL-6 and HE4 serums based on the ELISA to detect an ovarian mass. The results showed that positive predictive value (PPV) and negative predictive value (NPV) were obtained at 0.6% and 0.358%, respectively, and the overall agreement was estimated at 44%.

Table 6. Median levels of IL-6 and HE4 in patients and controls lacking vaginal bleeding

	Vaginal bleeding		**Serum IL-6**	**Serum HE4**
No bleeding	Patient	n	21	21
		Mean±SD	2.107±0.725	2894.61±1267.04
		Median	0.056	0.032
		Min-Max	0.032-9.204	0.023-9783.9
	Control	n	50	50
		Mean±SD	1.578±0.602	0.034±0.024
		Median	0.05850	0.01950
		Min-Max	0.031-6.864	0.000-0.244
	Sig*		0.791	0.0001

*Mann-Whitney U test

Table 7. Median levels of IL-6 and HE4 in patients and controls according to the family history of cancer

Category	**Family history of cancer groups**		**Serum IL-6**	**Serum HE4**
Patients	Yes	n	11	11
		Mean±SD	0.05±0.01	222.68±190.769
		Median	0.05	125.4
		Min-Max	0.3-0.07	0.027-526.46
	No	n	39	39
		Mean±SD	2.42±0.89	2675.87±1047.76
		Median	0.055	0.03
		Min-Max	0.03-11.64	0.016-9783.9
	Sig*		0.266	0.206
Controls	Yes	n	9	9
		Mean±SD	2.14±2.78	0.02±0.012
		Median	0.078	0.025
		Min-Max	0.39-6.86	0.000-0.04
	No	n	41	41
		Mean±SD	1.76±0.265	0.056±0.024
		Median	0.057	0.019
		Min-Max	0.03-5.765	0.001-0.244
	Sig*		0.111	0.202

*Mann-Whitney U test

Table 8. Comparison of IL-6 and HE4 serums based on the ELISA to detect ovarian mass

		HE4 cutoff point		Total
		Normal	Patient	
IL-6 cutoff point 2	Normal	24 64.9%	43 68.3%	67 67.0%
	Patient	13 35.1%	20 31.7%	33 33.0%
Total		37 100.0%	63 100.0%	100 100.0%

Positive predictive value=20/33=0.6%
Negative predictive value=24/67=0.358%
Overall agreement=24+20=44%

4. Discussion

The results of numerous studies have reported the disadvantages of relying on a single tumor marker to diagnose ovarian cancer. In this study, IL-6 and HE4 biomarkers were investigated to enhance the specificity and sensitivity of an adnexal mass evaluation. It was found that 98% of the patients had benign adnexal disease during the period of the study, while only one case had malignant ovarian disease. Despite that, a high percentage of the patients had changes in the studied serum markers; this highlighted the advantages as well as the efficacy of monitoring these markers in benign ovarian disease.

Human epididymis protein 4 was designated as a new biomarker since it was shown to be strongly elevated in the early stages of ovarian cancer. However, blood HE4 levels were elevated in benign gynecologic diseases (21, 22). The results of the present study revealed that the mean serum HE4 level was significantly higher in the patients with adnexal mass (859.22±2385.71) than in the healthy control group (0.024±0.03) (Table 2). Based on the findings of previous research, 32% of ovarian cancer patients had high HE4 levels in their blood (23). It has been reported that HE4 lacks sensitivity similar to CA125; however, it has greater specificity for ovarian cancer (24). Previously published works reported that combining HE4 with other tumor markers was successful in the early detection of ovarian cancer, including both primary and recurrent cancer scenarios (25, 26).

Even though HE4 is a sensitive predictor for epithelial ovarian malignancy with a higher specificity than CA125, relatively little research has been reported on HE4 overexpression in benign gynecological tumors and diseases (9). To evaluate HE4 differences between benign and malignant diseases, Huhtinen, Suvitie (27) compared 129 women having endometriosis with 14 ovarian cancers, 16 endometrial carcinoma, and 66 control participants in terms of HE4. The results of the mentioned study showed that women with ovarian cancer had greater mean serum HE4 levels than those with endometriosis, which had a significantly greater level than the control group (27).

To elaborate on the role of HE4 in benign ovarian disease in contrast to CA125, Moore, Miller (22), in their study regarding benign ovarian tumors, found that CA125 levels were raised in 29% of cases, whereas HE4 levels were elevated in only 8% of patients. Serous cystadenomas and cystadenofibroma are among the most prevalent neoplasms identified in both premenopausal and postmenopausal females. Such tumors are frequently manifested as cystic and solid ovarian masses, making them difficult to be distinguished from ovarian cancers using traditional imaging technology. The comparison of premenopausal and postmenopausal women with women having a high CA125 level showed that relatively few cases had an increased HE4 level, notably in the premenopausal age group. In mucinous tumors, on the other hand, there was no significant difference in the proportion of females who had biomarker increased (22). Zhang,

Qiao (28) evaluated the levels of HE4 according to the histological classification of the benign ovarian disease and reported that HE4 levels were elevated in certain histological varieties, such as endometriosis/endometriomas, cystadenoma, and germ cell tumors, but not in others, such as those with inflammatory conditions (e.g., abscess, hydrosalpinx, and pelvic inflammatory disease). Considering findings from the mentioned studies as well as those of our study, HE4 seems to have a role in benign adnexal cases as it has in ovarian cancer patients.

In this study, HE4 had a sensitivity of 92% and a specificity of 66% as a serum marker for the presence of adnexal mass with PPV of 73% and NPV of 89%. Moore, McMeekin (16) found that HE4 had a sensitivity and a specificity of 72.9% and 95%, respectively, as a diagnostic biomarker for early identification of ovarian cancer; furthermore, the sensitivity elevated at 76.4% if both HE4 and CA125 were identified. As a consequence of the identification of two or more markers, HE4 sensitivity increased by 3.5% (16). In comparison, Hellström, Raycraft (24) reported that plasma HE4, extracted from individuals with benign ovarian tumors, showed the highest sensitivity of 67% and the best specificity of 96% as a diagnostic biomarker. The risk of ovarian malignancy algorithm estimates the likelihood of whether an ovarian cystic lesion or pelvic tumor is cancer by combining serum HE4 and CA125 values with menopausal status. It has a sensitivity of 94% and a specificity of 75% in two prospective multicenter trials for the identification of malignant epithelial ovarian tumors (16, 29).

Although biomarker levels can be measured using a variety of methods (e.g., ELISA and chemiluminescent microparticle immunoassay), Ruggeri, Bandiera (30) recently proved that chemiluminescent immunoassays are much more appropriate and efficacious in increasing the sensitivity and specificity of the test than commonly available ELISA kits, which have intraassay imprecision percentages (coefficient of variation percent) varying from 6.8% to 10.3%, in comparison to 4% for electrochemiluminescence immunoassay (ECLIA). Those findings encourage the use of the ECLIA method for regular HE4 measurements. Furthermore, the difference in accuracy between ELISA and ECLIA can be attributable to the fully automated format of ECLIA, whereas ELISAs are manual assays that require double testing.

The results of this study showed that there was no significant association between neither the patient's age nor the menopausal state and the serum level of HE4; nevertheless, women near the age of 40 tended to have a higher level of HE4 than younger age groups. Similar findings were obtained from the study by Moore, Miller (31), which found that there was no statistically significant difference in mean serum HE4 concentrations between premenopausal women aged 40 years and older and postmenopausal women aged 60 years and younger; this revealed that the menopausal state was not the determining factor in rising median serum concentrations, rather, the age was the major determining factorl. These results were consistent with those of a study that looked at a group of females at high risk for developing ovarian cancer and found that their HE4 levels increased with age (32). The results of a study conducted on people of various Asian nations indicated that HE4 levels were linked to age and ethnicity and the level of HE4 rose with age, notably in women over the age of 50 years. The levels of HE4 were significantly different between Malaysians and Indians, however, not across Malaysians and Chinese (33). As serum HE4 usually increases with age, patients' baselines would be critical in following established ovarian cancer, especially in postmenopausal females. In comparison to CA125, boosted CA125 concentrations in postmenopausal females have also been connected to increased age in healthy females (34).

Based on the results of the present study, there was a significant difference in the bleeding history whether in the form of menorrhagia or metrorrhagia between the

patients with adnexal mass and the controls as 58% of the patients with adnexal mass had a history of vaginal bleeding, while none of the control cases had a similar history. Nonetheless, there was no significant difference in the HE4 concentration between cases that suffered from bleeding and those without bleeding among patients with an adnexal mass. Dubé (35) reported that vaginal bleeding might be associated with adnexal pathologies, especially endometrioma, although it was an uncommon presentation for ovarian cancer (36).

The results of our study revealed that there was no significant correlation between family history of malignant disease and serum level of HE4 in different patient groups. This finding might be attributed to the fact that most of our studied patients had benign adnexal disease. In comparison to other studies, family history of ovarian cancer was associated with the ovarian cancer discovery in the studied patients; therefore, it indirectly had a relationship with HE4 (37).

The findings of the study conducted by Moore, Miller (31) showed that marital status, the number of children, and breastfeeding had no clinical differences in serum HE4 levels in the studied patients. However, pregnancy was linked with a decreased serum level of HE4 (31).

For the creation and application of appropriate therapy in the treatment and recovery of individual quality of life, it is critical to determine the disease etiology and relevant prediction parameters. From this point of view, we realized that it is important to investigate the challenging role of IL-6 in the adnexal mass pathology. The results of this study showed that there was no significant association in serum IL-6 between patients with adnexal mass and the control group. However, a slightly higher level of serum IL-6 was recorded in patients with an adnexal mass. This finding could be explained that 98% of the included patients had benign ovarian pathology and only 2% of the cases had malignant ovarian disease. Kampan, Madondo (38) found that women with advanced ovarian malignancy had a mean IL-6 level that was 24-fold more than that in women with healthy ovaries. Although in all cases with advanced ovarian carcinoma, the IL-6 concentration remained strongly detectable following treatment, it was abolished in 23.8% of women with healthy ovaries (38). Cancerous cells or intraperitoneal mesothelial cells, both substantial sources of IL-6 synthesis within the tumor environment, may be the responsible causes for this (39). The presence of IL-6 in the cancerous ascites, sera, and plasma of women with ovarian cancer has been linked to advanced disease and poor prognosis (40). In evaluating the role of IL-6 in benign ovarian neoplasms, Darai, Detchev (41) reported that although endometrioma had higher IL-6 concentrations, the sera IL-6 levels were lower in endometrioma patients than in ovarian carcinoma patients. Similarly, Block, Maurer (42) have found that, compared to a sample of women with benign ovarian lesions, ovarian cancer patients had greater IL-6 serum concentrations. This was in line with the findings of studies by Chudecka-Glaz, Cymbaluk-Ploska (43), (44) in evaluating the difference in serum levels between benign and malignant ovarian pathologies.

In the current study, serum IL-6 had a sensitivity of 30% and specificity of 64% in determining patients with adnexal mass pathology. Moreover, age, menopausal state, parity, body mass index (BMI), and other patient characteristics showed no statically significant differences with serum IL-6. This can be explained by the evidence that the IL-6 level was associated with the degree of inflammation and pathology advancement rather than other parameters (45).

According to the findings of our study, combining more than one marker measurement increased the sensitivity and specificity of distinguishing patients with adnexal mass pathology. Terry, Sluss (46) found that in differentiating benign from malignant ovarian tumors, IL-6 alone was not higher than the prognostic value of other classical biomarkers and assays, whereas when serum CA125 or risk of malignancy index was administered alone, the rate of false positives was higher. The coupling of IL-6 and CA125 could be

particularly beneficial in discriminating between ovarian endometrioma and malignancy with erroneously increased CA125 (46).

It is important to notify that this study included only one patient with a diagnosis of malignant ovarian cancer, who had a normal IL-6 level (0.063) with elevated serum HE4 level (60.45). She presented with metrorrhagia, her age was 55 years, and her BMI was more than 30.

One of the limitations of this study was primarily the very limited number of cases with ovarian cancer which limited their involvement in the study and affected some of the study goals. Secondly, the exact diagnosis of the benign adnexal pathology was not included in the study design; therefore, the association between the elevated serum level of HE4 and each type of benign adnexal neoplasm was not assessed.

Authors' Contribution

Study concept and design: H. A. J.
Acquisition of data: H. A. J.
Analysis and interpretation of data: S. H.
Drafting of the manuscript: Hameed, S
Critical revision of the manuscript for important intellectual content: H. A. J.
Statistical analysis: M. S.
Administrative, technical, and material support: M. S.

Ethics

Ethical approval was obtained from the Scientific Research and Ethics Committee of the College of Medicine, University of Basrah, Basrah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors are grateful to all members of the Department of Microbiology, College of Medicine, University of Basrah. They also thank the staff of Basrah Women and Children Teaching Hospital, Basrah Teaching Hospital, Al-Fayhaa Teaching Hospital, and Al-Mawani Teaching Hospital, especially the laboratory and operation staff for their generous support. Our special thanks are forwarded to Assistant Professor Dr. Alaa Hussein Abed, Basrah University College of Science and Technology, for his help in statistical analysis.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):359-86.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
3. Cohen JG, White M, Cruz A, Farias-Eisner R. In 2014, can we do better than CA125 in the early detection of ovarian cancer? World J Biol Chem. 2014;5(3):286-300.
4. Abood R, Abdahmed K, Mazyed S. Epidemiology of Different Types of Cancers Reported in Basra, Iraq. Sultan Qaboos Univ Med J. 2020;20:295.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7-30.
6. Lee SW, Lee HY, Bang HJ, Song HJ, Kong SW, Kim YM. An Improved Prediction Model for Ovarian Cancer Using Urinary Biomarkers and a Novel Validation Strategy. Int J Mol Sci. 2019;20(19).
7. Tarver T. Cancer Facts & Figures 2012. American Cancer Society (ACS). J Consum Health Internet. 2012;16(3):366-7.
8. Collaborative Group on Epidemiological Studies of Ovarian C. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. Lancet. 2015;385(9980):1835-42.
9. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol. 2008;108(2):402-8.
10. Conway C, Zalud I, Dilena M, Maulik D, Schulman H, Haley J, et al. Simple cyst in the postmenopausal

patient: detection and management. J Ultrasound Med. 1998;17(6):369-72; quiz 73-4.
11. Dorum A, Blom GP, Ekerhovd E, Granberg S. Prevalence and histologic diagnosis of adnexal cysts in postmenopausal women: an autopsy study. Am J Obstet Gynecol. 2005;192(1):48-54.
12. Sevinc A, Adli M, Kalender ME, Camci C. Benign causes of increased serum CA-125 concentration. Lancet Oncol. 2007;8(12):1054-5.
13. Fotopoulou C, Gabra H, Blagden SP. Surgical and Medical Management of Epithelial Ovarian Cancer. Dewhurst's Textbook of Obstetrics & Gynaecology2018. p. 884-904.
14. Kumari S. Serum Biomarker Based Algorithms in Diagnosis of Ovarian Cancer: A Review. Indian J Clin Biochem. 2018;33(4):382-6.
15. Hellstrom I, Hellstrom KE. SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. Adv Exp Med Biol. 2008;622:15-21.
16. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2009;112(1):40-6.
17. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. Cancer Res. 2005;65(23):10794-800.
18. Alhumrani SA-R, Jamalludeen NM, Abdulhameed AA. Study of Interleukin 6 as marker of inflammation and a predictor of in-hospital complications in patients with acute coronary syndrome. Med J Basrah Univ. 2020;38(2):115-9.
19. Maccio A, Madeddu C. Inflammation and ovarian cancer. Cytokine. 2012;58(2):133-47.
20. Bast RC, Jr., Skates S, Lokshin A, Moore RG. Differential diagnosis of a pelvic mass: improved algorithms and novel biomarkers. Int J Gynecol Cancer. 2012;22 Suppl 1:5-8.
21. Granato T, Midulla C, Longo F, Colaprisca B, Frati L, Anastasi E. Role of HE4, CA72.4, and CA125 in monitoring ovarian cancer. Tumour Biol. 2012;33(5):1335-9.
22. Moore RG, Miller MC, Steinhoff MM, Skates SJ, Lu KH, Lambert-Messerlian G, et al. Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. Am J Obstet Gynecol. 2012;206(4):351 e1-8.
23. Rosen DG, Wang L, Atkinson JN, Yu Y, Lu KH, Diamandis EP, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol. 2005;99(2):267-77.
24. Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003;63(13):3695-700.
25. Havrilesky LJ, Whitehead CM, Rubatt JM, Cheek RL, Groelke J, He Q, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol. 2008;110(3):374-82.
26. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol. 2008;110(2):196-201.
27. Huhtinen K, Suvitie P, Hiissa J, Junnila J, Huvila J, Kujari H, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. Br J Cancer. 2009;100(8):1315-9.
28. Zhang Y, Qiao C, Li L, Zhao X, Li Y. Serum HE4 is more suitable as a biomarker than CA125 in Chinese women with benign gynecologic disorders. Afr Health Sci. 2014;14(4):913-8.
29. Moore RG, Miller MC, Disilvestro P, Landrum LM, Gajewski W, Ball JJ, et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstet Gynecol. 2011;118(2 Pt 1):280-8.
30. Ruggeri G, Bandiera E, Zanotti L, Belloli S, Ravaggi A, Romani C, et al. HE4 and epithelial ovarian cancer: comparison and clinical evaluation of two immunoassays and a combination algorithm. Clin Chim Acta. 2011;412(15-16):1447-53.
31. Moore RG, Miller MC, Eklund EE, Lu KH, Bast RC, Jr., Lambert-Messerlian G. Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age. Am J Obstet Gynecol. 2012;206(4):349 e1-7.
32. Lowe KA, Shah C, Wallace E, Anderson G, Paley P, McIntosh M, et al. Effects of personal characteristics on serum CA125, mesothelin, and HE4 levels in healthy postmenopausal women at high-risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2008;17(9):2480-7.

33. Mokhtar N, Thevarajah M, Ma N, M I. Human epididymis protein 4 reference intervals in a multiethnic asian women population. Asian Pac J Cancer Prev. 2012;13(12):6391-5.
34. Dehaghani AS, Ghiam AF, Hosseini M, Mansouri S, Ghaderi A. Factors influencing serum concentration of CA125 and CA15-3 in Iranian healthy postmenopausal women. Pathol Oncol Res. 2007;13(4):360-4.
35. Dubé L-R. Approach to Gynecological Adnexal Masses. Mcgill J Med. 2022;20(1).
36. Dubé L-RJMJoM. Approach to Gynecological Adnexal Masses. 2022;20(1).
37. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, et al. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer. 2011;104(5):863-70.
38. Kampan NC, Madondo MT, Reynolds J, Hallo J, McNally OM, Jobling TW, et al. Pre-operative sera interleukin-6 in the diagnosis of high-grade serous ovarian cancer. Sci Rep. 2020;10(1):2213.
39. Mikula-Pietrasik J, Uruski P, Matuszkiewicz K, Szubert S, Moszynski R, Szpurek D, et al. Ovarian cancer-derived ascitic fluids induce a senescence-dependent pro-cancerogenic phenotype in normal peritoneal mesothelial cells. Cell Oncol (Dordr). 2016;39(5):473-81.
40. Sanguinete MMM, Oliveira PH, Martins-Filho A, Micheli DC, Tavares-Murta BM, Murta EFC, et al. Serum IL-6 and IL-8 Correlate with Prognostic Factors in Ovarian Cancer. Immunol Invest. 2017;46(7):677-88.
41. Darai E, Detchev R, Hugol D, Quang NT. Serum and cyst fluid levels of interleukin (IL) -6, IL-8 and tumour necrosis factor-alpha in women with endometriomas and benign and malignant cystic ovarian tumours. Hum Reprod. 2003;18(8):1681-5.
42. Block MS, Maurer MJ, Goergen K, Kalli KR, Erskine CL, Behrens MD, et al. Plasma immune analytes in patients with epithelial ovarian cancer. Cytokine. 2015;73(1):108-13.
43. Chudecka-Glaz AM, Cymbaluk-Ploska AA, Menkiszak JL, Pius-Sadowska E, Machalinski BB, Sompolska-Rzechula A, et al. Assessment of selected cytokines, proteins, and growth factors in the peritoneal fluid of patients with ovarian cancer and benign gynecological conditions. Onco Targets Ther. 2015;8:471-85.
44. Lane D, Matte I, Garde-Granger P, Laplante C, Carignan A, Rancourt C, et al. Inflammation-regulating factors in ascites as predictive biomarkers of drug resistance and progression-free survival in serous epithelial ovarian cancers. BMC Cancer. 2015;15:492.
45. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: a magnificent pathway. J Gerontol A Biol Sci Med Sci. 2006;61(6):575-84.
46. Terry KL, Sluss PM, Skates SJ, Mok SC, Ye B, Vitonis AF, et al. Blood and urine markers for ovarian cancer: a comprehensive review. Dis Markers. 2004;20(2):53-70.