Detection the level of YKL-40 biochemical marker and vitamin D level in sera of Iraqi Uterine cancer females' patients

الكشف عن مستوى مادة (YKL-40) الكيموحياتية ومستوى الفيتامين دال عند الإناث العراقيات المصابات بسرطان الرحم

Walaa E. Jasm*
Amal R. Farhan**
Wasan T. Abed, MsC ***

*** Professor, Clinical chemistry, College of Medicine, University of Baghdad**
**** Assistant Professor, Clinical chemistry, College of Medicine, University of Al-mustanseria**
***** Department of Medical Laboratory, College of Health and Medical Technology/Baghdad**

المستخلص:

الهدف: الكشف عن مستوى مادة**YKL-40** الكيموحياتية ومستوى الفيتامين دال عند الإناث العراقيات المصابات بسرطان الرحم.

المنهجية: وشملت الدراسة ٩٠ متطوعة، كان ٣٠منهم متطوعين غير مصابات اعتبرن كمجموعة ضابطة، في حين تم جمع عينات مصل من ستين مريضة من النساء اللواتي يعانين من أورام الرحم (٣٠منها من الأورام الرحمية الخبيثة و٣٠ منهن للورم الليفي الذي هو من الأورام الرحمية الحميدة)،حيث اعتبرت مجموعة الأورام الحميدة كمجموعة ضابطة للمرض. كان متوسط عمر هؤلاء الإناث ٣٠-٧٥سنة، والذي يقابل المجموعة الضابطة .تم جمع جميع العينات المذكورة أعلاه من مستشفى أزادي التعليمي في كركوك والقسم الخاص بأمراض النساء في المدينة الطبية في بغداد خلال شهر أكتوبر / ٢٠١٢ إلى مارس. وقد خضعت جميع العينات المصل لتقدير مستويات YKL-40 الكيموحياتية، و 25 (OH) vitamin D فيتامين باستخدام تقنية,ELISA, كما جمعت بيانات الوزن والطول . وتمت مقارنة نتائج عينات المرضى مع البيانات المناظرة لها من المجموعة الضابطة وثم تم تحليلها إحصائيا.

النتائج: تقدير مستويات YKL-40 في مصل الدم أن هناك ٢٨ مريضة (٣٣,٩٣٪) من مرضى سرطان بطانة الرحم كان عندهم مستوى عال من YKL-40بالمصل، في حين أن ٢٦ مريضة (٨٦.٦٧٪) من مرضى الورم الليفي (ورم حميد) وجد عندهم انخفاض مستوى YKL-40 بالمصل، و١٥امرأة (٥٠.٠٠٪) من الأصحاء كان عندهم المستوى منخفض من YKL-40 بالمصل. وظهر هناك فرق كبير في مستوى مادة YKL-40 في مرضى سرطان بطانة الرحم بالمقارنة مع مرضى الورم الليفي (ورم حميد) والأصحاء (p=٠,٠٠٠١ ، p=٠,٠٠٠١) على التوالي. كان أعلى نسبة من النساء ذوات سرطان بطانة الرحم والنساء ذوات الورم الليفي (ورم حميد)عندهم نقص في مستوى ٢٥هيدروكسي الفيتامين (د) (٦٦.٦٧٪). في حين أن أعلى نسبة من النساء الأصحاء لديهم مستوى الاكتفاء من٢٥هيدروكسي الفيتامين (د) (٥٦.٦٧٪). إحصائيا كان هناك فرق كبير بين مجموعات الدراسة (p=٠,٠٠٠١) ، بينما لا يوجد فرق كبير بين مرضى سرطان بطانة الرحم والورم الليفي (ورم حميد) (P=٠,٨٢٢).

التوصيات: أوصت الدراسة إلى المقارنة بين مادة ykl-40 الكيموحياتية ومواد أخرى في الكشف عن أورام الرحم.دراسة المستوى التشخيصي لهذه المادة وعلاقتها بأورام أخرى في الجسم. أوصت أيضا بقياس مستوى الفيتامين دال مع طرق أخرى احدث وملاحظة علاقته مع المرض.

Abstract:

Objective: Detection the level of YKL-40 biochemical marker and vitamin D level in sera of Iraqi uterine cancer females' patients.

Methodology: This study included 90 female volunteers, 30 of them were healthy volunteers who were considered as a control group, while sixty serum samples were collected from women patients suffering from uterine tumors (30 malignant and 30 fibroid benign tumors), benign cases were considered as a disease control group for malignant tumors. The average age of those females was 30-75 years, which matched the control group. All the samples were collected from Azady hospital in Kirkuk and the gynecologic department at Medical City in Baghdad during October /2012 to May /2013. All the serum samples were undergone biochemical estimation for the levels of YKL-40, and 25 (OH) vitamin D using ELISA technique, and BMI data were collected.

Results: Estimation of YKL-40 levels showed that there were 28 No. (93.33%) of EC patients had high level of YKL-40, while 26 no. (86.67%) of fibroid (benign tumor) patients had low level, and 15(50.00 %) of healthy control had low. There was a significant difference found in YKL-40 level in EC patients when compared with the fibroid (benign tumor) patients and healthy control (Pvalue= 0.0001), (Pvalue= 0.0001) respectively. The highest percent of women with EC and the women with fibroid (benign tumor) had deficiency of 25 (OH) vitamin D levels (66.67%). While the highest percent of healthy control had sufficiency of 25(OH) vitamin D level (56.67%). statistically there was significant difference among study groups (p=0.0001). Were as no significant difference between EC patients and fibroid (benign tumor) patients (P-value =0.822).

Recommendations: Comparing between the ykl-40 marker and other tumor marker diagnostic levels in the detection of uterine tumors. For further studies, we recommended study the diagnostic levels of ykl-40 marker and its correlation with other body tumors. It is recommended to do estimation of vitamin D levels with more advanced method and correlation of its with disease.

Key words: Uterine cancer, Gynecologic tumor, YKL-40, Vitamin D, Uterine fibroid, Tumor marker.

Introduction:

Endometrial carcinoma is the commonest type of female genital tract malignancy in the developed countries, accounting for nearly 50 percent of all new gynecologic cancers diagnosed in the Western world and Arab regions [1, 2].

Cancer is caused in all or almost all instances by mutation or by some other abnormal activation of cellular secretion that control cell growth and cell mitosis. The abnormal cellular secretions called biomarker. As many as different biomarker has been discovered [3, 4].

YKL-40 (cartilage glycoprotein-39) or chitinase 3-like 1, it was originally defined in 1989 as a biomarker [5].

However, recent studies have revealed that YKL-40 has elevated serum level in several solid tumors and it is a potential biomarker in the detection and management of adenocarcinoma of several types of cancers including a diagnostic and prognostic role in the uterine cancer [6].

It was denoted that YKL-40 is subjected to tight regulation at multiple levels. In cancer cells, its role can be compromised by various mechanisms that's include proliferation and differentiation of malignant cells, protects the cancer cells from undergoing apoptosis, stimulates angiogenesis, and stimulates fibroblasts surrounding the tumor [7].

There is another factor which was observed to play a crucial role in cancer development, which a limited data are available that regarding the association of vitamin D with endometrial cancer risk [8]. Vitamin D deficiency is extremely common in patients with all types of cancer.

Therefore, it is important for cancer patients to be sure that do not have vitamin D deficiency. A specific type of blood test (that measures 25-hydroxyvitamin D) is the only way a person can find out if he has enough vitamin D [9].

Methodology:

This study was case control study included 90 female**s** samples were collected from Azady hospital in Kirkuk (39 samples) and the gynecologic department in Medical City Baghdad (51 samples) during October /2012 to May /2013. The patients' age ranged from (30-70) years, these samples included 30 malignant cases and 30 fibroid benign uterine tumors. Benign cases were considered as a disease control group for malignant uterine tumors, the results of these investigations have been compared with the analogue results of 30 serum samples of apparently healthy volunteers' females. All serum samples had been tested for YKL-40, and vitamin D levels using YKL-40 (human GP-39) kit from creative diagnostic company USA, and 25 hydroxyvitamin D (25 0H vitamin D) kit from Eruoimmune company Turkya by ELISA technique. All data collected and analyised by using personal computer through the statistical package of social sciences (SPSS) program (version -18) and excel application.

Results:

Table 1. Distribution of study groups according to age groups

Age groups	Groups							
	EC		Fibroid (benign tumor)		Healthy control		Total	
	No	%	No	%	No	%	No	%
30-39	0	.00	7	23.33	7	23.33	14	15.56
40-49	1	3.33	20	66.67	8	26.67	29	32.22
50-59	13	43.33	3	10.00	6	20.00	22	24.44
≥60	16	53.33	0	.00	9	30.00	25	27.78
Total	30	100.00	30	100.00	30	100.00	90	100.00

EC: endometrial cancer, %: percent, No: number

Table and figure shows the distribution of study groups according to age, these results showed that the majority of patients with EC is (≥60) years old with 16 patients (53.33%), while 20 patients (66.67%) for fibroid (benign tumor) in age (40-49) years old and 9 subjects (30.00%) for healthy control with age of (≥60) years old .

Table 2. Distribution of study groups according to BMI

BMI	EC		Fibroid (benign tumor)		Healthy control		Total		P
	No	%	No	%	No	%	No	%	
Normal	1	3.33	5	16.67	7	23.33	13	14.44	0.001*
Overweight	5	16.67	15	50.00	14	46.67	34	37.78	
Obese	24	80.00	10	33.33	9	30.00	43	47.78	
Total	30	100.00	30	100.00	30	100.00	90	100.00	

BMI: body mass index, EC: endometrial cancer, P: probability level at ≤0.05, %: percent, No: number
χ^2=19.475

Table data demonstrated the distribution of study groups according to BMI, that shows the majority of patients with EC had obese 24 patients (80.00%), and normal weight 1 patient (3.33%), while in patients with fibroid (benign tumor) the highest percent 15 patients (50.00%), and 14 patients (46.67%) in the healthy control were overweight. A significant difference was found between the EC patients when they were compared with that of control groups (p= 0.001).

Table 3. Distribution of EC and fibroid (benign tumor) according to serum YKL-40 level

Serum YKL-40 (ng/ml)	EC		Fibroid (benign tumor)		Total		p-value
	No	%	No	%	No	%	
High level	28	93.33	4	13.33	32	53.33	0.0001*
Low level	2	6.67	26	86.67	28	46.67	
Limit level	0	.00	0	.00	0	.00	
Total	30	100.00	30	100.00	60	100.00	

EC: endometrial cancer, YKL:[tyrosine (Y), lysine (K), and leucine (L)], Ng:nanogram, ml:millileter, P:probability level at ≤0.05, %:percent, No: number

Table represented the distribution of EC and fibroid (benign tumor) according to serum YKL-40 level, the table showed that EC patients who had high level were 28 patients (93.33%), and fibroid (benign tumor) patients who had low level were 26 patients (86.67%). There was a significant difference found in serum YKL-40 level in EC patients when compared with the fibroid (benign tumor) patients (P. value= 0.0001).

Table 4. Distribution of fibroid (benign tumor) and healthy control according to serum YKL-40 level

Serum YKL-40 (ng/ml)	Fibroid(benign tumor)		Healthy control		Total		p-value
	No	%	No	%	No	%	
High level	4	13.33	0	.00	4	6.67	0.056
Low level	26	86.67	15	50.00	41	68.33	
Limit level	0	.00	15	50.00	15	25.00	
Total	30	100.00	30	100.00	60	100.00	

YKL: [tyrosine (Y), lysine (K), and leucine (L)], Ng: nanogram, ml: millileter, P: probability level at ≤0.05, %:percent, No: number

Data demonstrated in table the distribution of fibroid (benign tumor) according to serum YKL-40 level in comparison with healthy control. The table shows that the majority of patients with Fibroid (benign tumor) had low level 26 patients (86.67%), and high level 4 patients (13.33%), while limit level were 0(0%). A non significant difference was found between the patients when they were compared with that of control (P. value>0.05).

Table 5. Distribution of EC and healthy control according to serum YKL-40 level

Serum YKL-40 (ng/ml)	EC		Healthy control		p-value
	No	%	No	%	
High level	28	93.33	0	.00	0.0001*
Low level	2	6.67	15	50.00	
Limit level	0	.00	15	50.00	
Total	30	100.00	30	100.00	

EC: endometrial cancer, YKL:[tyrosine (Y), lysine (K), and leucine (L)], Ng:nanogram, ml:millileter, P:probability level at ≤0.05, %:percent, No: number, χ^2=48.82

Table show that there is a no significant difference in the distribution of serum YKL-40 level between both EC patients and control group (p=0.0001). In this study the majority of patients with EC had high level 28 patients (93.33%), and low level 2 patients (6.67%), while limit level were 0(0%).

Table 6. Distribution of EC and fibroid (benign tumor) according to serum 25(OH) vitamin D level

Serum 25(OH) vitamin D (ng/ml)	EC		Fibroid(benign tumor)		Total		p-value
	No	%	No	%	No	%	
Deficient (< 20)	20	66.67	20	66.67	40	66.67	0.822
Insufficient (20-32)	8	26.67	9	30.00	17	28.33	
Sufficient (32 -100)	2	6.67	1	3.33	3	5.00	
Total	30	100.00	30	100.00	60	100.00	

EC: endometrial cancer, OH: hydroxy, Ng: nanogram, ml: millileter, P:probability level at ≤0.05, %:percent, No: number

Results in table revealed the distribution of EC patients and fibroid (benign tumor) patients according to serum 25(OH) vitamin D level. It was obvious that EC patients and fibroid patients who had deficiency of serum 25(OH) vitamin D level were (66.67%) . statistically there was no significant difference between EC patients and fibroid (benign tumor) patients (P-value =0.822).

Table 7. Distribution of EC patients and healthy control group according to serum 25(OH) vitamin D level

Serum 25(OH) vitamin D (ng/ml)	EC		Healthy control		p-value
	No	%	No	%	
Deficient (< 20)	20	66.67	4	13.33	0.0001*
Insufficient (20-32)	8	26.67	9	30.00	
Sufficient (32 -100)	2	6.67	17	56.67	
Total	30	100.00	30	100.00	

EC: endometrial cancer, OH: hydroxy, Ng: nanogram, ml: millileter, P:probability level at ≤0.05, %:percent, No: number

The pattern of distribution of EC patients and healthy control group according to serum 25(OH) vitamin D level is noticeable in table, which demonstrate that the majority of EC patients were had deficiency of 25(OH) vitamin D 20 patients (66.67%), while in healthy control group were had sufficiency of serum 25(OH) vitamin D 17 subjects (56%). Moreover, a significant difference was found between EC and healthy control (P_{value}=0.0001).

Table 8. Distribution of fibroid (benign tumor) patients and healthy control subjects according to serum 25(OH) vitamin D level

Serum 25(OH) vitamin D level (ng/ml)	Fibroid (benign tumor)		Healthy control		Total		p-value
	No	%	No	%	No	%	
Deficient (< 20)	20	66.67	4	13.33	24	40.00	0.0001*
Insufficient (20-32)	9	30.00	9	30.00	18	30.00	
Sufficient (32 -100)	1	3.33	17	56.67	18	30.00	
Total	30	100.00	30	100.00	60	100.00	

OH: hydroxy, Ng: nanogram, ml: millileter, P: probability level at ≤0.05, %: percent, No: number

Table shows the distribution of fibroid (benign tumor) patients and healthy control subjects according to serum 25(OH) vitamin D level. These results show that 20 patients (66.67%) of fibroid (benign tumor) have deficiency of serum 25(OH) vitamin D level , while 17 subjects (56.67%) have sufficiency of serum 25(OH) vitamin D level . Statistically, there is a significant difference (p_{value}=0.0001).

Discussions:

In the current study, it was observed that the age of disease initiation was earlier among benign tumors' subjects (in the 3rd decade) in comparison with malignant cases (at 6th decade). In spite of that, the current findings referred to development of malignant cases as delayed as 50 years which is comparable with those estimated for American women during 2013 [10]. Results in present study are in disagreement with previous result in China by Yuan He, who observed that highest percentage (56.2%) for patients with fibroid (benign tumor) and (44.3%) for healthy controls [11].

Considering BMI, it was referred that obesity acts as a risk factor for endometrial carcinoma in women. These results were in agreement with the observation of Charlotte Atkinson et al in USA, which showed that there was a significant difference between patients with fibroid (benign tumor) in comparison to healthy controls (P-value <0.01) [12]. The illustration for this controversy may be attributed to the fact that the age of most patients with malignant endometrial cancer was above 50 years (i.e. at age of menopause) in which most Iraqi women gain weight, perhaps due to hormonal disturbances. Regarding benign subjects and healthy women, the reason for over-weight may be related to the fact that they were not

engaged in any exercise and not to mention the effects of diet.

A study of Crosbie et al. [13], confirms that BMI is strongly associated with an increased risk of incident endometrial cancer which extends our previous meta-analysis demonstrating that the association becomes stronger above BMI 27 kg/m^2, and that the association is particularly strong in women who have never been exposed to HRT. Finally, it shows that menopausal status and histologic subtype did not significantly impact upon these associations.

YKL-40 has been regarded as a "biomarker" or "possible cancer marker" for detecting some malignant tumors [14]. In vivo proof it has been suggested that YKL-40 may have an important effect on the proliferation and differentiation of cancer cells, and on the role in extracellular tissue remodeling, and stimulate angiogenesis and fibroblasts surrounding the tumor. Here, it was sought to determine the diagnostic value of YKL-40 in endometrial cancer using ELISA technique. The interpretations of the present results can be summarized in the following aspects; Different groups of authors indicate that the serum level of YKL-40 in healthy individuals is 42 micrograms [15].

In our control group of healthy women (without family history of endometrial or ovarian cancer, without hormonal or contraceptive therapy, without inflammatory diseases) the level slightly, but insignificantly, increased with age. Our study showed that the serum level of YKL-40 in nonproliferating benign uterine diseases was near that of healthy women.

According to Johansen and co. [16] serum concentration of YKL-40 above 20% is indicative of increased serum level. In our study, pre-operative serum YKL-40 levels in EC patients were significantly higher than that in benign (fibroid) and healthy women, while there was no statistical difference between benign (fibroid) patients and healthy women, that's compatible with other studies [17]. These results show that the increasing of serum concentration of YKL-40 can be a sign of increasing risk of endometrial cancer development.

Circulating 25(OH) vitamin D level has been accepted as the best available biomarker for assessing vitamin D status, in spite of The relevant anticancer dose of vitamin D might be best quantified by average long term exposure level, in which case a single plasma 25(OH)D measurement may not adequately capture the vitamin D and cancer association [18]. Analyses of predicted and circulating 25(OH)D have already been done in separate studies for colorectal [19]. The level of 25(OH) vitamin D was analyzed in the sera of patients with endometrial cancer. Difference in the level of 25(OH) vitamin D in patients' sera of endometrial cancer and uterine fibroid in comparison with healthy control group referring to there was no association between vitamin D status and endometrial cancer incidence and development. Which compatible with the study by Liu et al. that fined no association between vitamin D status and endometrial cancer incidence in the NHS study population. However the same study thought that the protective benefits of vitamin D against endometrial cancer may not manifest unless 25(OH) D levels are significantly higher than 30 Ng/ ml and the range of exposure is much wider [20].

Recommendations: Comparing between the ykl-40 marker and other tumor marker diagnostic levels in the detection of uterine tumors**.** It is recommended to histopathologic study as soon as with the tumor marker in order to determine its prognostic role with tumors in other studies. For further studies, we recommended study the diagnostic levels of ykl-40 marker and its correlation with other body tumors**.** It is recommended to do estimation of vitamin D levels with more advanced method and correlation of its with disease. To conduct a large cohort study we recommended that a larger sample should be examined to confirm the work.

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