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Original Research Article

Study of serum mesothelin in patients having endometrial cancer

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Abstract: The aim of the study is to determine serum mesothelin level in patients with endometrial cancer and its level in different histopathological subtypes. The ultimate goal is to know if we can set serum mesothelin as a marker for endometrial cancer. The method was serum samples were collected from 45women as cases attending the gynecological oncology clinic at El Shatby Maternity University Hospital, after their diagnosing as endometrial cancer. And another serum samples were collected from 45 women as a control group attending El-Shatby Maternity University Hospital for other therapeutic reasons after applying the exclusion and inclusion criteria. A sandwich ELISA tech.wes used to detect the level of mesothelin in the sera of cases and control groups. The results from the receiver operating characteristic (ROC) curve analysis, we found that mesothelin is a good indicator for diagnosis and anticipation of endometrial cancer. The best cut-off that maximizes (sensitivity + specificity) is 2.46 (ng/ml). At this level, the sensitivity is 75.56 and specificity is 86.67. In conclusion using a quantitative ELISA for serum mesothelin detection found to be elevated in 75.5% of endometrial cancer patients; these results have identified serum mesothelin a potential biomarker for these cancers. However studies involving much larger patients' samples are needed to fully characterize the sensitivity and specificity of the assay.
Keywords: Endometrial cancer, receiver operating characteristic (ROC) curve, ELISA.

INTRODUCTION

Endometrial cancer is early manifested, early diagnosed and has an excellent prognosis. There may be value in a tumor marker to aid in screening for endometrial cancer in women that are at increased risk for the development of endometrial cancer or for women already diagnosed with endometrial cancer who at high risk for recurrence or for patients with advanced stage disease [1]. Endometrial cancer is one of the most common malignancies that affect women worldwide, with nearly 287.100 new cases each year, comprising 4% of all cancers in females [2]. Endometrial cancer is the eighth leading cause of cancer deaths among women. Endometrial cancer is rare in women under the age of 45. Most cases are found in women aged 55, there are over 600,000 women who are survivors of this cancer. The majority has a good prognosis because they seek medical attention early due to vaginal bleeding and endometrial biopsy that lead to early diagnosis [3, 4].

Most endometrial cancers are adenocarcinomas, meaning that they originate from the single layer of epithelial cells that line the endometrium and form the endometrial glands. There are many microscopic subtypes of endometrial carcinoma, but they are broadly organized into two categories, type I and type II, based on clinical features and pathogenesis [5, 6].

There are many risk factors for endometrial cancer .Some, such as age, race and family history, which can't be changed. Others are related to personal choices such as smoking, exercising, body weight, drinking, or diet. Unopposed long-lasting estrogen stimulation, not counterbalanced by progesterone, is considered to be a major contributing factor to disease development [7].

Mesothelin is a cell surface glycoprotein that is normally present on the mesothelial cells lining the pleura, peritoneum and pericardium. Since mesothelin is over expressed in several cancers, it could be exploited as tumor marker [8].

Elevations of serum mesothelin specific to ovarian and other cancer patients like uterine serous carcinoma [9]. Elevated serum mesothelin was found in most patients with mesothelioma .Circulating mesothelin is reported in nearly all pancreatic cancers [10].

In this study we determine serum mesothelin level in patients with endometrial cancer and its level in different histopathological subtypes. The ultimate goal is to know if we can set serum mesothelin as a marker for endometrial cancer.

Patients and Methods

A case control study was conducted over the period from June 2014 to April 2015. The study included 90 women 45 of them recruited from the gynecological oncology clinic at El Shatby Maternity University hospital.

The patients divided into two study groups:

Group A: Forty Five women diagnosed as endometrial cancer by D&C or endometrial pipele biopsy

Group B: Forty Five women served as a control group who are recruited from inpatients wards of El Shatby Maternity University hospital seeking for other therapeutic reasons.

Inclusion Criteria: Patients diagnosed as a cases of endometrial cancer, all histopathological types of endometrial cancer.

Exclusion Criteria: Patients suspected or diagnosed as a condition which cause elevation in mesothelin level as (mesothelioma, ovarian cancer, pancreaticcancer, abnormal renal function and patients on chemotherapy or radiotherapy).

METHODS

All cases of endometrial cancer and control group included in the study subjected to:

1) Detailed history taking.

2) Full physical examination (general and gynecological): Carried out in patients with suspected endometrial cancer with emphasis on: BMI, uterine abnormality, other pelvic pathology

3) Pelvic ultrasonography: To assess uterine size, shape and endometrial thickness.

4) Computerized tomography scan on pelvis: To assess the spread of the tumor to other organs also used to detect other pelvic pathology, which done on the main university hospital. (PhilipsCT scanner brilliance 64-slice).

5) Surgical treatment: Total abdominal hysterectomy with bilateral salpingo-oophorectomy ± pelvic lymphadenectomy.

6) Measurement of mesothelin in the sera

Sandwich ELISA using antibodies reacting with two different epitopes on human mesothelin, used to quantitate serum mesothelin levels, a standard curve was generated using a mesothelin-Fc fusion immunoglobulin protein. Two ml. blood samples collected using serum separator tubes and allow samples to clot for 2 hours before centrifugation for 20 minutes to obtain the serum.

Sera from group A&B collected preoperatively and analyzed the level of mesothelin in the serum is less than nine ng/mL in normal population.

The results collected and analyzed in correlation with:

-Histopathological type and grading of the tumor.

-Tumor staging.

- Lymph vascular invasion.

Collected data were statistically analyzed [11, 12].

RESULTS

The age in patients group range between (45-73years) and in control group range between (17-39years) ,the main complaint of studied group was vaginal bleeding (86.7%) while only(13.3%)of cases complaining of leukorria unresponsive to medical treatment.

Mesothelin level:

Table (1) and figure (1) showing a comparison between mesothelin level in both groups. The level of mesothelin in studied group ranges between (0.44 – 51.48 ng) with a mean (7.13 ± 9.42 ng) while its level in control group was ranging between (0.46 – 4.53 ng) with a mean (1.53 ± 1.04 ng) that shows a statistically significance difference.

Table 1: Comparison between both groups according to serum level of (mesothelin)

	Cases (n = 45)	Control (n = 45)	Z	p
Tumor marker (mesothelin)				
Min. – Max.	0.44 – 51.48	0.46 – 4.53	6.177*	<0.001*
Mean ± SD.	7.13 ± 9.42	1.53 ± 1.04		
Median	4.19	1.07		

Z: Z for Mann Whitney test

*: Statistically significant at $p \leq 0.05$

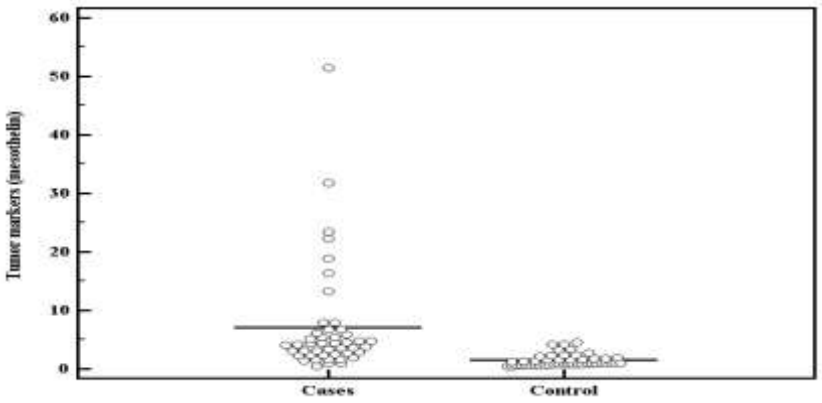

Fig 1: Comparison between both groups according to tumor markers (mesothelin)

Histopathology:

Table number (2) shows distribution of the studied group according to tumor type in studied group and the mean of mesothelin level in each grade as showed the grade 1 include 20 (44.4%) of cases the mean level of mesothelin was (4.74ng) while in grade 2 include 13 (28.9%) of cases the mean mesothelin level was (10.01ng) while grade 3 include 8 (17.8%) of cases the mean mesothelin level was(10.14ng).While type 2 which include one case of papillary carcinoma with mesothelin level(6.74ng) and the same level in also one case of serous type. Two cases of other subtypes (mixed cell carcinoma) with a mean mesothelin level (4.4ng).

Table 2: Distribution of the studied cases according to tumor type in studied group (n = 45)

	No.	**%**	**Mean of mesothelin level**
Type I(endometriod type)			
Grade I	20	44.4	4.74
Grade II	13	28.9	10.01
Grade III	8	17.8	10.14
Type II			
Papillary	1	2.2	6.74
Serous	1	2.2	6.74
Others	2	4.4	3.10

Staging:

Table (3) shows the distribution of the studied group according to tumor type in cases group as:28 (62.2%)of cases are in stage 1,while stage 2 include :7(15.6%) of cases, stage 3 include :4(8.9%) of cases and stage 4 include: 6(13.3%) of cases.

Table 3: Distribution of the studied cases according to staging in studied group (n = 45)

	No.	**%**
Staging		
Stage I	28	62.2
Stage II	7	15.6
Stage III	4	8.9
Stage IV	6	13.3

Tables (4) show the correlation between mesothelin level in patients and its affection by different measurement taken during the study. And as shows in the table there is no significant correlation between mesothelin level and different parameters present on the patients.

Table 4: Relation between mesothelin with different parameters

	N	Tumor markers (mesothelin)			Test of Sig.	p
		Min. – Max.	Mean ± SD.	Median		
DM						
No	**26**	0.44 – 31.85	7.09 ± 8.06	4.37	Z=0.023	0.982
Yes	**19**	0.93 – 51.48	7.18 ± 11.25	3.85		
HTN						
No	**25**	0.44 – 31.85	6.82 ± 7.99	3.85	Z=0.377	0.706
Yes	**20**	0.94 – 51.48	7.51 ± 11.16	4.37		
Myomtrial Invasion						
No	**28**	0.44 – 22.23	5.01 ± 5.0	3.58	Z=1.686	0.092
Yes	**17**	0.93 – 51.48	10.61 ± 13.46	5.47		
Lymph Nodes						
No	**41**	0.44 – 31.85	5.93 ± 6.66	4.10	Z=1.675	0.094
Yes	**4**	2.77 – 51.48	19.35 ± 22.17	11.58		
Staging						
Stage I	**28**	0.44 – 22.23	4.87 ± 4.83	3.50	$^{KW}\chi^2$= 3.324	0.344
Stage II	**7**	0.93 – 31.85	10.81 ± 11.91	5.19		
Stage III	**4**	1.56 – 13.24	5.99 ± 5.04	4.59		
Stage IV	**6**	1.87 – 51.48	14.12 ± 19.02	6.11		
Type I						
Grade I	**20**	0.93 – 23.54	4.74 ± 4.71	3.73	$^{KW}\chi^2$=2.068	0.356
Grade II	**13**	0.44 – 51.48	10.01 ± 14.11	4.84		
Grade III	**8**	1.50 – 31.85	10.14 ± 10.25	6.11		

Z: Z for Mann Whitney test

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

ROC CURVE:

For determination of sensitivity &specificity of mesothelin biomarker in detection of endometrial cancer ROC curve was done with that result:

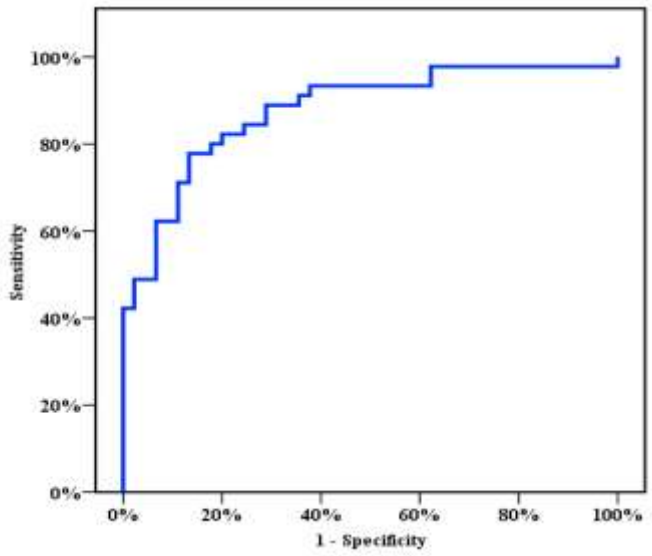

Fig 2: ROC curve for mesothelin to diagnose cases

Sensitivity, specificity and accuracy for mesothelin

Table (5) shows sensitivity, specificity and accuracy for mesothelin; theSensitivity of mesothelin was 75.56%, Specificity of mesothelin was 86.67% and the cutoff value is 2.46 ng

Table 5: (sensitivity, specificity and accuracy) for mesothelin

		Control	Cases	Sensitivity	Specificity	PPV	NPV	Accuracy
Mesothelin	**≤2.46**	39	11	75.56	86.67	85.0	78.0	81.11
	>2.46	6	34					

DISCUSSION:

In the present study, we aimed to determine serum mesothelin level in patients with endometrial cancer and its level in different histopathological subtypes. The ultimate goal is to know if we can set serum mesothelin as a marker for endometrial cancer.

From the receiver operating characteristic (ROC) curve analysis, we found that mesothelin is a good indicator for diagnosis and anticipation of endometrial cancer. The best cut-off that maximizes (sensitivity + specificity) is 2.46 (ng/ml). At this level, the sensitivity is 75.56 and specificity is 86.67.

Obulhasim G. *et al.;* [13] found that in endometrial samples, endometrioid uterine adenocarcinoma was frequently positive for mesothelin (8 of 16; 50%). In the present study, we found that 7 (14%) of cases showed positive results for mesothelin (>9 ng/ml), this may be due to the adoption of high level (9 ng/ml) for diagnosis of positive cases.

In our study 5 (11.1%) of cases were pre-menopause and (40) 88.9% of cases were post-menopause. Lentz G.M. *et al.;* reported that endometrial cancer may affect pre-menopausal women (20-25%) of cases [14].

Dashti S.G. *et al.;* [15] reported that 90 out of 133 (67.7%) of endometrial cancer patients were premenopausal. This finding increases the importance of screening for endometrial cancer even in premenopausal women. They also reported that 16 out of 133 who were postmenopausal were below 50 years of age.

Hassan R. *et al.;* [16] in a study that serum obtained from 24 healthy volunteers reported no mesothelin was detected in 14 (58%), <5 ng/mL in 7 (29%) and levels between 5 and 9 ng/mL in 3 (13%). Based on this analysis, a serum level of 9 ng/mL was used as the upper limit of the reference range for normal values of serum mesothelin. 43 random hospitalized patients with no information regarding these patients including diagnosis. Out of these 43 patients, 38 (88%) had levels below 9 ng/mL and 5 (12%) had serum mesothelin levels between 9 and 15 ng/mL.

Obulhasim G. *et al.;* [17] reported in a study with16 case of uterine endometrial carcinoma, and normal tissue specimens were used. Monoclonal antibody (5B2) was employed for the immunohistochemical analysis. The methylation-sensitive single-nucleotide primer extension (Ms-SNuPE) technique was used to quantify the methylation/hypomethylation status at 20 CpG sites in the mesothelin promoter region. Fifty percent of endometrial carcinoma was immunoreactive for mesothelin [18].

In our study mesothelin shows elevation in about 75.5% of patients with cutoff value of 2.46 ng/ml .which agreed with previous mentioned studies also our results showed no significant relation between level of mesothelin and type of tumor or its staging, may be due to small sample size and most of our cases was in grade 1 with early staging.

Conclusion:

From this study, we concluded that:

Using a quantitative ELISA for serum mesothelin detection found to be elevated in 75.5% of endometrial cancer patients; these results have identified serum mesothelin a potential biomarker for these cancers. However studies involving much larger patient's samples are needed to fully characterize the sensitivity and specificity of the assay.

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