

THE DIFFERENCE BETWEEN HE4 EXPRESSION IN URINE OF WOMEN WITH OVARIAN CYST AND WOMEN WITH NORMAL OVARY

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Abstract : The aim of this research were to determine differences in HE4 urine levels in women with ovarian cyst and normal ovary. This research is a comparative analytic, using case control design. This research was conducted in RSUP H. Adam Malik Medan, dr. Pirngadi Medan and the networking hospitals of the Department of Obstetrics and Gynecology of Faculty of Medicine University of North Sumatera from September 2017 to February 2018. The sample is ovarian cyst patients which met the inclusion and exclusion criteria, compared to control group which are women with normal ovary. The mean rank of HE4 level of ovarian cyst patient is 56,35, with the median of 76,41, and min-max 42,40-92,80. Meanwhile in patient with normal ovary, the mean rank of urine HE4 is 24,64, with the median of 49,11, and min-max 30-69. With Mann-Whitney test, p value is 0,000 ($p < 0,05$), showing a significant differences. The mean urine HE4 levels of ovarian cyst is significantly higher than that of in normal ovary.

Keywords: Urine HE4, Benign epithelial ovarian tumor, Malignant epithelial ovarian tumor

I. INTRODUCTION

Ovary is a part of female reproductive organ which has an important role associated with fertility function. There are a lot of pathologic conditions may be present in ovary, such as ovarian cyst and ovarian cancer.

Ovarian cyst is one of the most frequent gynecologic problems which affects about 7% of world population. A study in United States showed that the incidence rate of ovarian cyst among postmenopausal women was approximately 18% for the last 15 years. Whereas, data from a test in Europe showed that the incidence rate of ovarian cyst among healthy menopausal women was 21,2%. In Western countries, the incidence of benign ovarian tumor lies between 75% to 80%. A study from Nepal reported the incidence of benign ovarian tumor as 90,5%. Ovarian cancer is the cause of mortality among gynecologic malignancies in United States and lead to 16.000 death in 2004. Approximately, 1 of 57 women in United States develops ovarian cancer, and mostly affects women over 50 years of age. Little is known about the incidence of ovarian tumor in Indonesia due to the lack of cases documentation. However, it's estimated that the prevalence of ovarian cyst is 60% among all ovarian abnormalities cases.¹⁻⁴

Both benign and malignant ovarian tumor remain as difficult problem to solve in clinical practice. Therefore, an early detection method of ovarian tumor is needed to established a management plan as soon as possible. Both transabdominal and transvaginal ultrasonography examinations become one of advanced examination methods which are beneficial to display the characteristic of ovarian cyst, but the facilities are not widely available in every health centers.⁴⁻⁶

The gold standard examination to diagnose an ovarian tumor including ovarian cyst is histopathologic examination. However, histopathologic examination is an invasif procedure that leads to another problem in the diagnosing process of an ovarian cyst.⁷

Various biomarkers associated with ovarian cyst have been studied, one of them is Human Epididymis Protein-4 (HE4). Serum HE4 level assay combined with or without CA-125 assay demonstrated higher diagnostic result or significant to detect malignancy in ovary even at early stage.⁹ Concentration of HE4 does not only present in serum, but it's also thought presents in urine.^{7,10}

The incidence and prevalence of ovarian cyst in Indonesia remain unclear to date. The researchs about HE4 is relatively less in number and the research about the difference between HE4 protein expression in urine of benign ovarian tumor case and normal ovary in Indonesia especially in Medan has not been found yet, therefore, we were interested to do research to increase our understanding about HE4 expressed in urine of women with benign ovarian cyst and women with normal ovary in the detection of ovarian cyst.

II. EXPERIMENTAL

This was an analitical, case control study enrolled both ovarian cyst ward patients and outpatients at Gynecology-Oncology health care unit at Adam Malik General Hospital, Dr. Pirngadi Hospital, and network hospitals in Medan.

The case group consisted of women who were diagnosed with benign ovarian cyst based on USG, CT-scan, and histopathology, and were not suffered from other type of tumors and kidney failure, and also have Risk of Malignancy Index (RMI) > 200. Whereas women who were not suffered from kidney failure and any gynecologic tumors and other non-gynecologic tumors were included as control group.

The research was started with sample collection after receiving approval from Ethical Committee. Sample that met inclusion criterias were collected based on medical record. Then 10 mL mid-stream urine was obtained from the sample and brought to Integrated Laboratorium of Faculty of Medicine University of North Sumatera to be examined for urine HE4 level measurement. HE4 level was measured by quantitative ELISA, using the manufacturer's manual. Mid-stream urine sample should be diluted to be 1:40 prior to testing and then phosphate buffered saline, detergent, inert blue dye and preservative were added. The sample plate was read at 405 nm wave-length using a microplate reader in 15 minutes.

After 60 minutes of incubation and washing, rabbit anti-HE4 antibody labeled-biotin was added and incubated for 60 minutes along with the trapped HE4. After another washing process, streptavidin-HRP conjugate was added. After 30 minutes of incubation and the last washing step, the remaining conjugate was let to have reaction with substrate solution (TMB). The reaction was stopped by adding acid solution and the absorbance from the yellow product of the reaction was measured. The absorbance was comparable to HE4. The standard curve was made by planning the absorbance value to the standard concentration, and the unknown sample concentration was determined by using the standard curve.

The data was analyzed descriptively to see the frequency distribution of the research subject based on the charateristic. The difference among variables was anayzed using independent t-test for normally distributed data and using Mann-Whitney U test for not normally distributed data. The analysis result was considered normal if p value <0,05 with confidence interval of 95%.

III.RESULT

This research involved 40 urine samples of women with ovarian cyst and 40 urine samples of women with normal ovary which were examined to measure the HE4 level. Then, statistical test was performed to know the magnitude of significancy of the difference between the two groups.

Tabel 1 shows the characteristic of the research subjects consisted of women with ovarian cyst and women with normal ovary. Among 40 patients with ovarian cyst, 21 patients aged 20-40 years (52,5%), 19 patients aged 41-60 years (47,5%). Whereas 21 patients are nulliparity (52,4%), 36 patients were married (72,5%), 28 patients were menopause (70%) and 26 patients have normal BMI (65%).

Table 1. Characteristic of Research Subjects

Characteristic	Ovarian Cyst	Percentage (%)	Normal Ovary	Percentage (%)
	n (40 women)		n (40 women)	
Age				
20-40 years	21	52,5	23	57,5
41-60 years	19	47,5	17	42,5
Parity				
Nulliparity	21	52,5	6	15

Primiparity	10	25	15	37,5
Secundiparity	5	12,5	12	30
Multiparity	4	10	7	17,5
Marital Status				
Married	36	90	38	95
Unmarried	4	10	2	5
Menopause Status				
Premenopause	12	30	8	20
Menopause	28	70	32	80
BMI				
Underweight	0	0	0	0
Normoweight	26	65	25	62,5
Overweight	10	25	15	37,5
Obese	4	10	0	0

Tabel 2 shows urine HE4 level in ovarian cyst patients and normal ovary for each characteristic. For age category, the median of HE4 level for the 21-40 years group was 69,7, with min-max 42,40-92,60, whereas the median of 41-60 years group was 81,6, min-max 67,70-92,80. In the normal ovary group, the median was 37,8 and 64,3 for 20-40 years and 41-60 years group, respectively. The median of HE4 level for nulliparity and multiparity ovarian cyst patients was 66,6 and 90,7, respectively. The median of HE4 for married and not unmarried ovarian cyst patient was 74,9 and 76,3, respectively. The median of HE4 level for menopausal and non menopausal ovarian cyst patients was 83,4 and 71,2, respectively. The median of HE4 for normoweight, overweight, and obese ovarian cyst was 74,9, 82,7, and 72,6 respectively.

Table 2. Urine HE4 Level in Ovarian Cyst and Normal Ovary for Each Characteristic (Median)

HE4 for Characteristic	Each	Ovarian Cyst		Normal Ovary	
		Median	Min-Max	Median	Min-Max
Age					
20-40 years		69,7	42,40-92,60	37,8	30-58
41-60 years		81,6	67,70-92,80	64,3	42-69
Parity					
Nulliparity		66,6	42,40-89,70	36,9	30-68
Primiparity		81,5	68,20-89,80	37,8	30-52
Secundiparity		90,5	85,30-92,80	57,8	31-69
Multiparity		90,7	69,50-92,60	63,7	50-67
Marital Status					
Married		74,9	42,40-92,80	49,4	30-69
Unmarried		76,3	51,80-89,70	30,4	30-31
Menopause Status					
Non menopause		71,2	42,40-92,60	42,1	30-65
Menopause		83,4	68,20-92,80	66,6	63-69
BMI					
Underweight		0	0	0	0
Normoweight		74,9	42,40-92,80	38,7	30-63
Overweight		82,7	46,89-90,10	64,5	41-69
Obese		72,6	62,50-87,50	65,4	30-69

Table 3 and Graphic 1 shows that the most common type of histopathology of ovarian cyst patient was serous cystadenoma of 18 (45%), followed by mucinous cystadenoma of 15 (37,5%) and dermoid cyst of 7 (17,5%).

Table 3. Histopathology Type in Ovarian Cyst Patients

Histopathology	Number	Percentage (%)
Musinous Cystadenoma	15	37,5
Serous Cystadenoma	18	45
Dermoid Cyst	7	17,5
Total	40	100

Tabel 4 shows that the median of HE4 level for serous cyst was 78,70 with min-max 47,60-92,80, whereas for mucinous cyst was 85,30 with min-max 42,40-92,60, and for dermoid cyst was 67,60, with min-max 46,40-92,80.

Table 4. Median of Urine HE4 Level in Ovarian Cyst Patients for Each Histopathology Type

HE4 Urin	Median	Min-Max
Serous Cystadenoma	78,70	47,60-92,80
Musinous Cystadenoma	85,30	42,40-92,60
Dermoid Cyst	67,70	46,40-92,80

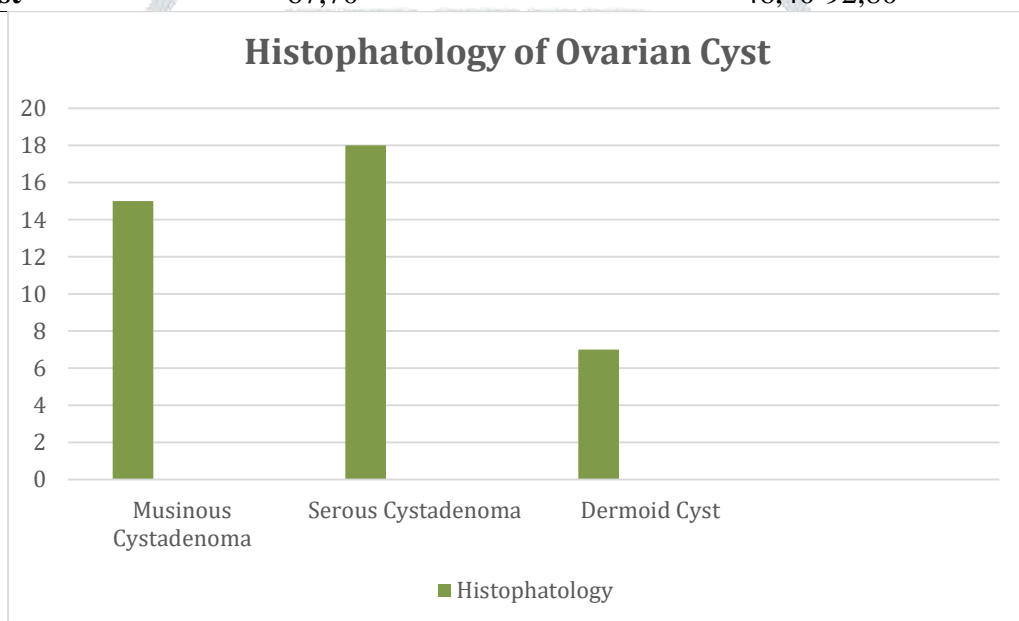


Figure 6. Histopathology Type in Ovarian Cyst Patients

Tabel 5 shows the analysis of urine HE4 level in ovarian cyst patient and normal ovary groups. Mann-Whitney test was performed because the data was not normally distributed according to the statistic test result. Data analysis of ovarian cyst patient for HE4 level shows that the mean rank was 56,35, median 76,41, min-max 42,40-92,80, whereas the analysis performed for normal ovary patients shows the mean rank was 24,65, median 49,11, min-max 30-69. The p value = 0,000, consistent for p<0,05.

Table 5. The Difference Between Urine HE4 Level in Ovarian Cyst and Normal Ovary Group

Concentration of HE4 Level	Mean Rank	Median	Min-Max	p value*
Ovarian Cyst	56,35	76,41	42,40-92,80	0,000
Normal Ovary	24,65	49,11	30-69	

*Mann-Whitney U Test

IV. DISCUSSION

1. Age

Among 40 patients with ovarian cyst, 21 patients aged 20-40 years (52,5%). A study which was conducted by Zahra on ovarian cyst tumor in Qatar demonstrated the average ovarian cyst patients were women at their 16-58 years of age, with mean of 32 years. A study by Abduljabbar *et al* on 22 ovarian cyst patients found the range of age lied between 23-47 years with mean of 35 years. Some studies stated that the incidence of ovarian cyst was more common among productive women.¹¹⁻¹⁴

2. Parity

The biggest parity group in this research was nulliparity consisted of 21 women (52,5%). Both Gameraddin and Mandiwa, stated in their research that most of ovarian cyst patients were nulliparity and primiparity. This was consistent with the result of this research which stated that the higher the parity number, the lower the ovarian cyst incidence rate. This is because parity is a protective factor for ovarian cyst incidence, which is consistent with a study conducted by Hartge *et al* stated that women who had gave birth more than five times would have a decreased risk of 32% for ovarian cyst incidence. However, according to Wei from Italia, increased parity number had a little association with ovarian cyst incidence. The mechanism of association between parity and decreased ovarian cyst incidence is still unclear to date. It's thought that women who have higher parity number are less exposed to gonadotropin, so it decreases the formation of ovarian cyst. In addition to this, pregnancy and lactation can also decrease the incidence rate of ovarian cyst, due to the suppressing effect of ovarian activity which can lower the ovarian cyst formation.^{13,15,16}

3. Marital Status

In this research, the married group was dominant for both case and control groups which consisted of 36 women (90%) and 38 women (95%), respectively. A study by Abduljabbar *et al* obtained 67,6% ovarian cyst patients were married. A study by Holt *et al* also demonstrated that 48,4% ovarian cyst patient were married. This was consistent with the result of this research that mostly ovarian cyst patients were married women.^{12,17}

4. Menopause Status

In this research, menopause women was 28 (70%) among ovarian cyst patients group and 32 (80%) among normal ovary group. A study by Mandiwa revealed that 82,2% ovarian cyst patients were menopausal women. A study by Moszynski *et al* of 167 ovarian cyst patients found that 131 ovarian cyst patients (78%) were in premenopause group. The incidence rate of ovarian cysts among premenopause patients was approximately 8% and 14-18% among postmenopause patients with annual incidence was approximately 8% according to Ross *et al*. A study conducted by Alaa *et al* found that there was no significant difference of benign ovarian cyst incidence, endometrioma, and malignant ovarian lesion between menopause and premenopause patients.^{15,18-20}

5. Body Mass Index (BMI)

For both ovarian cyst and normal ovary groups, the number of normoweight women was 26 (65%) and 25 (62,5%), respectively. A study by Abduljabbar *et al* reported that the average BMI among ovarian cyst patients was 27,76 kg/m². Whereas Wright *et al* conducted a study on 668 ovarian cyst patients and found that 248 patients (37%) were obese, 244 patients (36,5%) were normoweight, and 176 patients (36,3%) were overweight. The study concluded that the higher BMI, the lower the risk of low potency ovarian tumor.^{12,15,21}

6. HE4 Level in Urine of Ovarian Cyst Patient

Analysis of urine HE4 level in patients for the age group showed that the median of HE4 level in group of 41-60 years of age was 81,6 min-max 67,70-92,80. The result was consistent with the previous researchs by Karlsen *et al* and Wanglei *et al* that there was increased HE4 level related to increased age. This is due to the reduction of Glomerular Filtration Rate (GFR) related to increased age, that results to reduction of HE4 clearance rate.^{22,23}

Analysis of urine HE4 level in ovarian cyst patients for parity showed that the median of HE4 level in multiparity group was 90,7, min-max 69,50-92,60, and HE4 level was higher on higher number of parity. Lowe stated in his study that there was increased HE4 level in women who have gave birth.²⁴

Analysis of urine HE4 level in ovarian cyst patients for marital status revealed that the median of HE4 level in women who were not married was 76,3, min-max 51,80-89,70. There is not any research had been performed about the link between marital status and HE4 level.

Analysis of urine HE4 level in ovarian cyst patients for menopause status revealed that the median of HE4 level in menopause group was 83,4, min-max 68,20-92,80, which was higher than non menopause group. This was consistent with a previous research by Wanglei *et al* which stated that HE4 level was increased in menopause patients (57,6 pM) compared to premenopause patients (46,6 pM).²³

Analysis of urine HE4 level in ovarian cyst for BMI showed that the median of HE4 level of normoweight and obese group was 74,9, min-max 42,40-92,80 and 72,6, min-max 87,50, respectively. This demonstrated that higher body weight was associated with reduction in HE4 level. In a study conducted by Bolstad, HE4 level was also found lower in higher BMI group for 5% in BMI of 25 kg/m², and 10% in BMI of 30kg/m².^{23,25}

7. The Association Between Urine HE4 Level in Ovarian Cyst and Normal Ovary

The result of this research stated that the median of urine HE4 level in ovarian cyst and normal ovary was 76,41, min-max 42,40-92,80 and 49,11, min-max 30-69, respectively. P value was 0,000 9p < 0,005), so it can be concluded that there was difference between urine HE4 level in ovarian cyst and normal ovary.

Alaa *et al* and Bandierra *et al* stated in their researchs that urine HE4 level had high specificity to distinguish between benign and malignant ovarian lesion.^{20,26} According to research conducted by Partheen *et al*, HE4 was biomarker which increased progressively as the disease developed and the level was lower in women with normal ovary compared to women with benign ovarian cyst.^{23,27-29}

V. CONCLUSION

1. Dominant characteristics among ovarian cyst patients were women aged 20-40 years (52,5%), nulliparity (52,5%), married (90%), menopause (70%), and normoweight (65%)
2. The median of HE4 level in ovarian cyst patient was higher in women aged 41-60 years (81,6), multiparity (90,7), unmarried (76,3), menopause (83,4), obese (72,6).
3. The most common histopathology type in ovarian cyst patient was serous cystadenoma which was found in 18 women (45%)
4. There was significant difference between urine HE4 level between ovarian cyst and normal ovary.

VI. SUGGESTION

Further research should be performed to find any biomarkers which can be used as specific marker for ovarian cyst, included urine HE4 level.

REFERENCES

1. Cunningham FG, Gant NF, Leveno KJ, Gilstrap LC, Hauth JC, Wenstrom KD. *Obstetri Williams Edisi ke-21 Vol. 2*. Jakarta : ECG; 2004. p. 934, 1035-7.^[1]
2. Pudasaini, S, M. Lakhey, S. Hirachand, J. Akhter, & B. Thapa. A Study of Ovarian Cyst in a Tertiary Hospital of Kathmandu Valley. 2011. Nepal Med Coll J. ; 13(1):39-41.
3. Winarto H, Laihadi BJ, Nuranna L. *Modification of Cutoff Values for HE4, CA125, the Risk of Malignancy Index, and the Risk of Malignancy Algorithm for Ovarian Cancer Detection in Jakarta, Indonesia*. Asian Pac J Cancer Prev, 2014;15(5):1949-53.
4. Zalud, Ivica, Raydeen, Bussee, & Biserka, Kurjak. Asymptomatic Simple Ovarian Cyst in Postmenopausal Women : Syndrome of ‘Visible Ovary’. 2013. Jaypee.
5. Kurman RJ, Visvanathan K, Roden R, Wu TC, Shih Ie M. *Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis*. Am J ObstetGynecol 2008;198:351-6.
6. Zolton JR, Maseelall PB. *Evaluation of ovarian cysts in adolescent*. Open J Obs Gyn 2013; 3: 12-16.
7. Y.T. Zou, J.Y. Gao, H.L. Wang, Y. Wang, H. Wang, danP.L. Li. *Downregulation of microRNA-630 inhibits cell proliferation and invasion and enhances chemosensitivity in human ovarian carcinoma*. Genetics and Molecular Research 14 (3): 8766-8777 (2015).
8. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, Hecht JL. *Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas*. Cancer Res 2005;65:2162-9

9. Macuks R, Baidekalna I, Donina S. *Urinary concentrations of Human Epididymis Secretory Protein 4 (HE4) in The Diagnosis of Ovarian Cancer: A Case Control Study*. Asian Pacific J Cancer Prev, 13 (9), 4695-4698.
10. Van Gorp I, Cadron I, Despierre E, et al. *HE4 and CA-125 as a diagnostic test in ovarian cancer: prospective evaluation of the Risk of Malignancy Algorithm*. Br J Cancer. 2011;104:863–870.
11. Zahra, Fatimah. 2016. Pattern of Benign Ovarian Cyst in Qatari Women. Qatar Medical Journal. (17).
12. Abduljabbar, H. Yasir, AB. Estabraq, GA, Ghazal, SA, Afnan, AA., et al. Review of 244 Ovarian Cyst. Saudi Med Journal. 2015; 36(7) : 834-838.
13. Gameraddin, MB, & Nagla, KB., Characterisation of Benign Ovarian Lesion in Among Sudanese Women Undergoing Pelvic Ultrasound Scans : Impact of Parity and Age. Journal of Clinical and Diagnostic Research. 2018;12(5).
14. Rofe, Guy, Ron, A, Martha, D., Benign Ovarian Cyst in Reproductive Age Women Undergoing Assisted Reproductive Technology Treatment. 2013. Open Journal of Obstetrics and Gynecology;3;17-22.
15. Mandiwa, C, Li, JS., Yao, HT., Lu, LS., Gui QX., et al. Parity and Risk of Ovarian Cysts : Cross Sectional Evidence from the Dongfeng-Tongji Cohort Study. 2016. J Huazhong Univ Sci Techol :36(5);767-771.
16. Leone Roberti Maggiore U, Scala C, Venturini PL, et al. Endometriotic ovarian cysts do not negatively affect the rate of spontaneous ovulation. Hum Reprod, 2015,30(2):299-307.
17. Holt, V., Kara, LCH., & Jane, RD. Risk of Functional Ovarian Cyst : Effect of Smoking and Marijuana Use According to Body Mass Index. American Journal of Epidemiology. 2004 :161 (6).
18. Moszynski, R., Patrick, Z., Andrzej, W., Sebastian S., Stefan S., et al. Menopausal Status Strongly Influences the Utility of Predictive Models in Differential Diagnosis of Ovarian Tumors : an External Validation of Selected Diagnostic Tools. Ginekol Pol. 2014 ; 85 (892-895).
19. Ross, EK., & Medhi, K. Incidental Ovarian Cyst : When to Reassure, When to Reassess, When to Refer. Cleveland Clinical Journal of Medicine. 2013; 80(8).
20. Alaa NBjorg K, Amr W, Sherif N, Amal H, Manal K, et al. The use of human epididymis protein 4 for differentiation between benign and malignant ovarian neoplasm. 2013. Evidence Based Women's Health Journal. 3:173-177.
21. Wright JD, Powell MA, Mutch DG, Rader JS, Gibb RK., et al. Relationship of Ovarian Neoplasms and Body Mass Index. J Reprod Med. 2005. 50(8):595-602.
22. Karlsen, NS., Mona AK., Claus HK, & Estrid VS., HE4 Tissue Expression and Serum HE4 Levels in Healthy Individuals and Patients with Benign or Malignant Tumors: A Systematic Review. 2014. American Association for Cancer Research. 2285-2295.
23. Wanglei, Qu., Li Jinping., Duan Ping., Tang Zuoqing., et al. Physiopathological factors affecting the diagnostic value of serum HE4-test for gynecologic malignancies. 2016;16 (12):1271-1282.
24. Lowe, Kimberly., Chirag S., Erin W., Garnet A., Pamela P., et al. Effects of Personal Characteristics on Serum CA125, Mesothelin, and HE4 Levels in Healthy Post-menopausal Women at High-Risk for Ovarian Cancer. 2008. Cancer Epidemiol Biomarkers ; 17(9): 2480–2487.
25. Bolstad, Nils., Miriam O., Kjell N., Johan N., Human epididymis protein 4 reference limits and natural variation in a Nordic reference population. 2012. Springer. 33:141-148.
26. Elisabett Bandiera, Chiara Romani, Claudia Specchia, Laura Zanotti, dkk. *Serum Human Epididymis Protein 4 and Risk for Ovarian Malignancy Algorithm as New Diagnostic and Prognostic Tools for Epithelial Ovarian Cancer Management*. Cancer Epidemiol Biomarkers Prev; 20(12); 2496–506, 2011 AACR.
27. Partheen, Karolina., Bjorg, K., & Karin, S., Evaluation of ovarian cancer biomarkers HE4 and CA-125 in women presenting with a suspicious cystic ovarian mass. 2011. Journal of Gynecologic Oncology; 22(4) : 244-252.
28. Moore RG, Miller MC, Steinhoff MM, et al. Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. Am J Obstet Gynecol. 2012;206(4):351e1–8.
29. Anastasi E, Granato T, Falzarano R, et al. The use of HE4, CA125 and CA72-4 biomarkers for differential diagnosis between ovarian endometrioma and epithelial ovarian cancer. J Ovarian Res. 2013;6(1):44