Evaluation of WT1 gene expression as a diagnostic and prognostic marker in epithelial ovarian cancer

Jawdat N. Gaaib
Department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq.

Received Date: 5 / 3 / 2017
Accepted Date: 13 / 4 / 2017

الخلاصة

جين WT1 هو أحد الجينات الكاتبة للسرطان والذي يشفر لتصنيع عامل استنساخ (zinc finger) والذي يلغى تنشيطه في ورم ويلمزWilms tumor. يلعب جين WT1 دورا اساسيا في نمو وتطور وتكاثر بعض اعضاء الاجنة والبالغين. يكون تعبير جين WT1 كمستضد مرتبط بالورم في انواع مختلفة من سرطانات الدم والانسجة الصلبة وبذلك يعتبر معلم مفيد لمراقبتها ومراقبة العلاجات المناعية لها. تهدف الدراسة الحالية الى تحديد مستويات التعبير الجيني للجين WT1 باستخدام تقنية النسخ العكسي- تفاعل سلسلة البلمرة الكمي (RT-PCR) في عينات نسيج الورم المأخوذة من 86 مريضة مصابة بمراحل مختلفة من سرطان المبيض المشخص حديثا و14 عينة من مريضات مصابات بورم المبيض الحميد والتي استخدمت كمجموعة سيطرة. اظهرت النتائج ان (79٪) من العينات كانت موجبة للتعبير الجيني (68 عينة من مجموعة المرضى و11 عينة من مجموعة السيطرة) واظهرت النتائج فروقا معنوية احصائيا (p value= 0.00238, p <0.01) في التعبير الجيني بين المجموعتين. ولم تظهر النتائج فروقا معنوية بين مستوى التعبير الجيني بالمقارنة مع اعمار المرضى وسن اليأس وتاريخ العائلة المرضي. عكست نتائج الدراسة الحالية امكانية تحديد التعبير الجيني للجين WT1 في المرضى المصابين بأورام حميدة والمصابين بأورام خبيثة ولكن بفروق معنوية والتي تعكس بدورها قيمة جين WT1 كأداة مفيدة للتفريق بين اورام المبيض الحميدة والخبيثة. ومن جهة اخرى فان ارتفاع مستوى التعبير الجيني لجين WT1 في المرحلة الاولى من سرطان المبيض يعكس فائدته في التشخيص المبكر للمرض.

الكلمات المفتاحية

جين WT1، سرطان المبيض، واسمة للتشخيص.

Abstract

The Wilms tumor gene 1 (WT1) is a tumor suppressor gene that encodes a zinc finger transcription factor that is inactivated in a subset of Wilms tumors. It plays a crucial role in growth, development and proliferation of some embryonic and adult organs. In various types of hematopoietic and solid malignancies, WT1 is expressed as a tumor associated antigen (TAA) and it can also consider as a useful marker for monitoring of minimal residual disease (MRD) and targeted immunotherapy. The aim of the present study is to evaluate the expression levels of the WT1 consider mRNA in the Paraffin-embedded tissue blocks from 86 patients with different stages of newly diagnosed ovarian cancer and 14 samples of patients with benign ovarian tumors tissues were used as a control group using a reverse transcriptase polymerase chain reaction (RT-PCR). The present study showed that WT1 mRNA was expressed in 79 (79%) of samples, 68 with ovarian cancer and 11 benign ovarian tum or. According to malignancy status the percentage of patients with WT1-positive gene expression was significantly higher in comparison with benign tumor patients. The present study showed no significant correlation between WT1 expression level and age, menopausal state and family history. In correlation to the histopathologic tumor types, the endometriod ovarian tumors showed statistically significant differences in the level of WT1 gene expression comparing with other tumor types. In correlation with tumor stages, although samples with stage I showed the highest level of WT1 gene expression comparing with stage III, but statistically no significant difference was found between stages. The present study results reflected the possibility of detecting of that gene transcript in benign as well as the malignant tumor samples but with wide differences in the sample percentages and level of gene expression which in turn reflect the value of WT1 gene as a useful tool for discriminating malignant ovarian tumor from non-malignant ones, on the other hand the elevated levels of expression of WT1 gene in stage I sample may reflect the usefulness of WT1 for early detection of ovarian cancer.

Keywords

Ovarian cancer, Wilms' tumor 1, Diagnostic marker.

1. Introduction:

Ovarian cancer is one of the five leading causes of cancer related mortality in women in developed countries [1]. About)70%(of epithelial ovarian cancers are detected at a late stage because of the lack of reliable screening methods. Consequently, there is a serious need to develop novel diagnostic, prognostic and predictive biomarkers for development of improved personalized treatment for ovarian cancer. Most of human malignancies are due to accumulation of mutations within tumor-suppressor genes and oncogenes [2]. The Wilms' tumor 1 (WT1) gene locus on DNA is 11p13, it was first cloned in 1990 as a suppressor in Wilms' tumor [3]. The protein product of this gene is a zinc finger transcription factor participated in tissue development, proliferation and differentiation, and in a programmed cell death (apoptosis), and is classified as a tumor suppressor gene [4]. Subsequent studies indicated that WT1 gene might play an oncologic role in hematologic malignancies like leukemia and in a variety of solid tumors like ovarian cancer, breast cancer, soft tissue sarcoma and glioblastoma [5, 6, 7 and 8].

WT1 expression has been detected in epithelial type of ovarian tumor [9]. It has limited reactivity in ovarian serous carcinomas, and is not found in mucinous carcinomas [10, 11]. It's believed that the origin of epithelial ovarian tumors is the normal epithelium of ovary. Many factors including Type I collagen, are play a big role in metastasis of ovarian tumor [12]. Accumulation of collagen- binding integrins trigger expression of the transcription factor called early growth response 1 (EGR1) [13] and following initiation of membrane type 1 matrix metalloproteinase (MT1-MMP) activity which increase the effect of collagen invasion [14, 15], and the anchoring of metastatic lesions occurred in consequence of that. The protein product of WT1 gene is a zinc-finger transcriptional activator factor that binds to a DNA sequence element common to the Early Growth Response family [16]. The WT1 gene expression is positively linked with epithelial ovarian cancer [17]. The aim of this study, therefore, was to determine the levels of WT1 gene expression and evaluate the role of this gene in ovarian cancer diagnosis and prognosis.

2. Materials and Methods:

The study group included 86 Paraffin-embedded tissue blocks from patients with different stages of newly diagnosed Invasive ovarian cancer were provided by certain Iraqi hospitals (including Al-Kadhemia, AL -Yarmouk Teaching Hospital, Baghdad Hospital, the Teaching Laboratories of Medical City, Nuclear Medical Hospital in Baghdad and Alsader Hospital in Misan) after patients underwent to total abdominal hysterectomy and bilateral salpangio-oopherectomy (TAH-BSO), subtotal abdominal hysterectomy, vaginal hysterectomy, and endometrial biopsy, 14 samples of patients with benign ovarian tumors tissues were used as a control. The required tumor's and patient's information was

recorded from the patients' files. The Paraffin-embedded tissue blocks were sectioned into 10µm in DNase-RNase free tubes for molecular evaluation. Samples subjected to RNA extraction and molecular study by using Real Time Reverse Transcription-PCR at Molecular Oncology Unit in Guy´s hospital – Kings College/London.

2.1. RNA extraction and RT-PCR assay:

The total RNA was extracted using the RNeasy FFPE kit which designed for purifying total RNA from FFPE tissue sections (Qiagen- USA) following the protocol provided by the manufacturer. Total RNA was reversely transcribed using Thermo-Scrip Reverse Transcription kit (Invitrogen/USA). The procedure was carried out in a reaction volume of 20 µl (2 µl RT buffer, 0.8 µl dNTPs mix, 0.2 µl RT random primers, 1 µl reverse transcriptase, 1 µl RNase inhibitor and 15 µl total RNA). Reverse transcription protocol was as follows: stage 1; 25ºC for 10 minutes, stage 2; 37ºC for)120(minutes, stage 3; 85ºC for 5 minutes, stage 4; 4ºC for ∞. cDNA was stored at -80 °C until use.

Serial dilutions of cDNA were used for preparing of standard curve. Standard curve were prepared for both target and endogenous control gene Phospho Glycerate Kinase 1 (PGK1). Quantitative real-time PCR assays were performed in triplicate using the Applied Bio systems 7900. SYBR Green master mix and Real time PCR primer designed with Primer3 (http: //www. ncbi.nlm.nih.gov/tools/primer-blast/) (Table 1) were used for quantitative assessment. The 20 µl of reaction volume containing10 µl of SYBR Green master mix, 1 µl of primer mixes, 5µl of RNase free water and 4µl of cDNA template. Real-Time PCR protocol was as follows; stage 1: 50ºC for 2 minutes, stage 2: 95ºC for 10 min, stage 3: in a two-step cycle procedure (95ºC for 15 Sec. and 65ºC for 1 min) repeated for 6 cycles and stage 4: in a two-step cycle procedure (95ºC for 15 Sec. and annealing 61ºC for 1 min) repeated for 40 cycles. The slope of a standard curves was used to evaluate the PCR amplification efficiency of the real-time PCR reaction.

For each sample, the Ct value is used to compare across all samples. The Ct is inversely proportional to the amount of starting mRNA of the target gene (WT1) as well as the endogenous control gene, Phospho Glycerate Kinase 1 (PGK1). The relative fold change ratio of the target gene in the sample was calculated as described below:

Log copy (endogenous control gene) = (Ct -32.85) /-3.3592

Copy number (endogenous control gene) = 10^Log copy

Log copy (WT1) = (Ct -34.82) /-3.5126

Copy number (WT1) = 10^Log copy

Fold change = Copy number (WT1)/Copy number (endogenous control gene)

Table (1): Primers sequences

Primer	Sequence
*WT*1-*F*	5′- AGGCTTTGCTGCTGAGGAC -3′
*WT*1-*R*	5′- CAGGTCATGCATTCAAGCTG -3′
*PGK*1-*F*	5′- GGGAAAAGATGCTTCTGGGAA -3′
*PGK*1-*R*	5′- TTGGAAAGTGAAGCTCGGAAA -3′

3. Statistical Analysis

The Statistical Analysis System-SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and least significant difference – LSD test was used to significant compare between means in this study.

4. Results:

The patients' age range was 14-70 years and the median was 47 years. According to the family history for ovarian cancer, all samples were negative. Clinical features of ovarian cancer samples are listed in table 2. In regard to the menopausal state of ovarian cancer patients, 40(46.5%) of samples were premenopausal, while 46(53.5%) of them were postmenopausal. According to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system, most of samples 70(81.4%) came with stage I, while the other samples 16 (18.6%) with stage III. According to the tumor histological types, the samples were divided into three clinical groups; surface epithelial tumors 76(88.3%) samples, sex cord tumors 6(6.9%) samples, and germ cell tumors 4(4.65%) samples.

By using the mean value of WT1 gene expression in benign tumors (5.302) as the cutoff value to separate tumor samples into WT1-positive and WT1-negative, the present study showed that out of 86 samples with ovarian cancer, WT1 was positively expressed in 68(79.1%) of samples, which showed statistically significant differences (p value= 0.00238, $p < 0.01$) in compare with WT1-negative samples 18(20.9%). According to malignancy status the ovarian cancer patients with WT1-positive gene expression showed the highest levels of WT1 gene mRNA comparing with benign tumor patients, which was statistically significant (p value= 0.0081). Relation between WT1 gene expression and clinicopathologic parameters, are listed in table 3. The present study showed statistically no significant differences in the levels of gene expression with age, menopausal state, and family history. In correlation to the histopathologic tumor types, the endometriod ovarian tumors showed statistically significant differences in the level of WT1 gene expression comparing with other

tumor types (p value= 0.0473 p<0.05). In correlation with tumor stages, although samples with stage I showed the highest level of WT1 gene expression comparing with stage III, but statistically no significant difference was found between stages (P=0.8914).

Table (2): Clinical features of ovarian cancer samples.

Age groups	
children age 0-14 years	(4.65%)4
Teenagers and young adults aged 15-24 years	(2.32%)2
Adults aged 25-49 years	(37.2%)32
Adults aged 50-74 years	(55.8%)48
Menopausal state	
Premenopausal no. (%)	(46.5%)40
(%) .Postmenopausal no	(53.5%)46
Family history	
(%) .Positive no	0
(%) .Negative no	86(100%)
International Federation of Gynecology and Obstetrics (FIGO) surgical stage	
(%) .Stage I no	(81.4%) 70
(%) .Stage III no	(18.6%) 16
Tumor histological types	
Surface epithelial tumors	(88.37%)76
Sex cord tumors	(6.9%)6
Germ cell tumors	(4.65%)4

Table (3): Effect of clinic-pathological features on WT1 gene expression in ovarian cancer patients.

***WT*1 gene expression**	
Tumor group	**Mean ± SE of *WT*1 gene**
Benign tumors	5.302 ± 1.28
Malignant tumors	752.61 ± 233.13
P-value	0.0081
(P<0.01).	
Histological tumor type	**Mean ± SE of *WT*1 gene**
Mucinous	10.81 ± 3.62
Serous	11.32 ± 5.67
Endometriod	38.34 ± 25.96
Clear cell	14.68 ± 12.81
Germ cell tumor	15.57 ± 0.00
Sex-cord	0.115 ± 0.02
P-value	0.0473
(P<0.05).	
Tumor stage	**Mean ± SE of *WT*1 gene**
Stage 1	20.44 ± 8.42
Stage 3	17.99 ± 10.55
P-value	0.8914
Non-significant.	

5. Discussion

WT1 was identified initially as one of the most important gene that plays a key role in the childhood kidney neoplasm Wilms tumor occurrence [18]. Subsequent to the discovery of WT1, an increasing number of studies have demonstrated its participation in other origins neoplasm [19]. WT1 acts as a transcriptional regulator, but may act as either a repressor or an activator based on cellular context [19]. In parallel, in tumors of different origin, it has been reported that WT1 act as an oncogene and tumor suppressor gene. For example, in the formation of Wilms tumors it act as a tumor suppressor gene, whereas in acute myeloid leukemia and breast cancer, high WT1 expression has been reported which indicating it's correlation with poor prognosis of tumors [20,21]. Poor outcome is correlated with epigenetic silencing of WT1 in clear cell ovarian adenocarcinoma patients [22]; however, unfavorable prognosis is indicative due to expression of WT1 in women with serous epithelial ovarian carcinoma [23].

During development, it has been conjectured that WT1 is involved in the maintenance of a mesenchymal epithelial balance [19]; therefore, it is interesting to theorize that abnormal regulation of WT1 gene expression in ovarian tumors may act to enhance the mesenchymal-epithelial transition observed in early ovarian carcinogenesis [24]. On the other hand, breast and ovarian cancers are important diagnostic considerations for women who have metastatic lesions of unknown primary origin [25]. Standard immune-histochemical and histopathological techniques often cannot differentiate between these two tumor types. It have been shown that gene expression profiles can be used to accurately and precisely differentiate breast from ovarian carcinomas, clarifying the ability of this approach both in recognizing genes of biological interest and classifying cancers. In the present study we examined the possibility of using WT1 gene as a diagnostic and prognostic marker depending on the levels of gene expression in ovarian tumor tissues by using qRT-PCR technique. The results of the present study showed that the WT1-positive ovarian cancer showed the highest levels of WT1 gene expression, which was significantly higher when compared with benign tumor patients, these findings indicates high specificity of WT1 gene as a diagnostic marker to discriminate malignant from benign ovarian tumor. The present study also showed that WT1 positively expressed in 68(79.1%) of samples, which showed statistically significant differences in compare with WT1-negative samples 18(20.9%).These results have some similarity to that reported by other studies including Lilac et al, who found that the expression of WT1 was positive in 61(71%) cases, and negative in 24(27%) cases of ovarian cancer [26], Kriplani and Patel, who found that (82%) of primary ovarian serous adenocarcinomas showed positivity with WT1[27], and Hylander et al., who detected the expression of WT1 in 78(78%) of epithelial ovarian cancer samples[28]. On the other

hand, the present study results were different from results that reported by Barbolina, et al, who found that only(56%) of all tested primary and metastatic ovarian carcinoma samples were positive for WT1 by using immunohistochemistry technique [29], and Netinatsunthorn et al., who found that only 50(50%) of advanced serous ovarian carcinoma patients were reactive for WT1[30].

The identification of WT1 expression positivity according to the age groups, family history, and menopausal state showed no significant correlation. In regarding to the histopathologic tumor types, the endometriod ovarian tumors showed statistically significant differences in the level of WT1 gene expression comparing with other tumor types. The present study results were different from that reported by Liliac et al, who found that according to the histologic subtypes, WT1 was mainly expressed in serous carcinoma, regardless of their differentiation degree [26]. The present study results also different from that reported by Barbolina, et al, who demonstrated an elevated level of WT1 gene expression in serous type of ovarian carcinomas, with more constricted expression recognized in other histopathologic types [29]. In correlation with stages, statistically no significant difference was found between examined stages, nevertheless, samples with stage I which the highest level of expression than samples with stage III. The data of the current study incompatible with most of the previous study which indicated that the elevated levels of WT1 gene expression were related with advanced FIGO stages, lymph node status and omentum metastasis, Liu et al., who reported that the levels of WT1 expression in patients with late stages disease(III and IV) increased significantly compared to early stage patients [31], Barbolina, et al., also showed that the WT1 gene expression is most prevalent in late stage tumors (stage III and stage IV) as well as in 100% of metastatic regions [29]. On the other hand, the present study results similar to that reported by Netinatsunthorn et al., who did not show any relation with tumor stage, size of residual tumor or histologic grade [30]. This difference might be explained by the fact that the present study included a small number of samples with stage III (16 sample), comparing with stage I (70 sample).

In conclusion the results reflected the possibility of detecting of that gene transcript in benign as well as cancer samples but with wide differences in the level of gene expression which in turn reflect the value of WT1 gene as a useful tool for discriminating malignant ovarian tumors from non-malignant ones. The present study also indicated the diagnostic value of WT1 gene for diagnosis of ovarian cancer since the gene had been expressed in most of the ovarian cancer samples regardless tumor stage and histological type. The prognostic value of that gene may be not proved in this study due to a small number of samples with stage III, so further studies are recommended with large number of samples with advanced stages.

6. Acknowledgment

The author thanks Dr. Khalid Tubal / Head of Molecular Oncology Department/ Guys hospital / London, and his staff for their co-operation in part of the study related with molecular analysis.

References:

[1] La Vecchia C. Epidemiology of ovarian cancer: a summary review. Eur J Cancer Prev; 10: 125-129, (2001).

[2] Blum H.E. Molecular therapy and prevention of liver diseases. Adv Med Sci.; 52:29-36, (2007).

[3] Call, K. M. et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 60, 509–20, (1990).

[4] Menke, A. L., A. J. Van der Eb, and A. G. Jochemsen. The Wilms' tumor 1 gene: oncogene or tumor suppressor gene? Int. Rev. Cytol. 181:15, (1998).

[5] Miwa, H., Beran, M. & Saunders, G. F. Expression of the Wilms' tumor gene (WT1) in human leukemias. Leukemia 6, 405–9, (1992).

[6] Oji, Y., Ogawa H, Tamaki H, Oka Y, Tsuboi A, Kim EH, Soma T, Tatekawa T, Kawakami M, Asada M, Kishimoto T, and Sugiyama H.Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. Jpn J Cancer Res 90, 194–204, (1999).

[7] Menssen, H. D. Bertelmann E, Bartelt S, Schmidt RA, Pecher G, Schramm K, and Thiel E. Wilms' tumor gene (WT1) expression in lung cancer, colon cancer and glioblastoma cell lines compared to freshly isolated tumor specimens. J Cancer Res Clin Oncol 126, 226–32, (2000).

[8] Nakatsuka, S., Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, Kawano K, Kuwae Y, Yamauchi A, Okumura M, Kitamura Y, Oka Y, Kawase I, Sugiyama H, Aozasa K. Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol 19, 804–14, (2006).

[9] Shimizu M, Toki T, Takagi Y, Konishi I, Fujii S. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. Int J Gynecol Pathol, 19:158-163, (2000).

[10] Goldstein NS, Bassi D, Uzieblo A. WT1 is an integral component of an antibody panel to distinguish pancreaticobiliary and some ovarian epithelial neoplasms. Am J Clin Pathol, 116:246-252, (2001).

[11] Hwang H, Quenneville L, Yaziji H, Gown AM. Wilms tumor gene product: sensitive and contextually specific marker of serous carcinomas of ovarian surface epithelial origin. Appl Immunohistochem Mol Morphol, 12:122-126, (2004).

[12] Zhu GG, Risteli J, Puistola U, Kauppila A, Risteli L. Progressive ovarian carcinoma induces synthesis of type I and type III procollagens in the tumor tissue and peritoneal cavity. Cancer Res.; 53:5028–5032, (1993).

[13] Barbolina MV, Adley BP, Ariztia EV, Liu Y, Stack MS. Microenviron-mental regulation of membrane type 1 matrix metalloproteinase activity in ovarian carcinoma cells via collagen-induced EGR1 expression. J Biol Chem.; 282: 4924–4931, (2007).

[14] Ellerbroek SM, Fishman DA, Kearns AS, Bafetti LM, Stack MS. Ovarian carcinoma regulation of matrix metalloproteinase- 2 and membrane type 1 matrix metalloproteinase through beta1 integrin. Cancer Res.; 59:1635–1641, (1999).

[15] Ellerbroek SM, Wu YI, Overall CM, Stack MS. Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. J Biol Chem.; 276:24833– 24842, (2001).

[16] Drummond IA, Madden SL, Rohwer-Nutter P, Bell GI, Sukhatme VP, Rauscher FJ. Repression of the insulin like growth factor II gene by the Wilms tumor suppressor WT1. Science.; 257:674–678, (1992).

[17] Acs G, Pasha T, Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, fallopian tube, ovary, and endometrium. Int J Gynecol Pathol.; 23:110–118, (2004).

[18] Pelletier J, Bruening W, Li FP, Haber DA, Glaser T, Housman DE. WT1 mutations contribute to abnormal genital system development and hereditary Wilms' tumour. Nature.; 353:431–434, (1991).

[19] Hohenstein P, Hastie ND. The many facets of the Wilms' tumour gene, WT1. Hum Mol Genet.;15(2): 196–201, (2006).

[20] Galimberti S, Guerrini F, Carulli G, et al. Significant coexpression of WT1 and MDR1 genes in acute myeloid leukemia patients at diagnosis. Eur J Haematol.;72:45–51, (2004).

[21] Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, Sugiyama H, Noguchi S. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res.; 8:1167–1171, (2002).

[22] Kaneuchi M, Sasaki M, Tanaka Y, Hiroaki Shiina, Hideto Yamada, Ritsu Yamamoto, Noriaki Sakuragi, Hideki Enokida, Mukesh Verma and Rajvir Dahiya, WT1 and WT1-AS genes are inactivated by promoter methylation in ovarian clear cell adenocarcinoma. Cancer.;104:1924–1930, (2005).

[23] Netinatsunthorn W, Hanprasertpong J, Dechsukhum C, Leetanaporn R, Geater A. WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study. BMC Cancer.; 6:90, (2006).

[24] Kurrey NK, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol Oncol.;97:155–165, (2005).

[25] Greco, F.A., Gray, J., Burris, H.A., Erland, J.B., Morrissey, L.H., and Hainsworth, J.D. Taxane-based chemotherapy for patients with carcinoma of unknown primary site. Cancer J 7, 203–212, (2001).

[26] Liliac L., Carcangiu M. L., Canevari S., Caruntu I., Apostol D.G., Danciu M., M. Onofriescu M., Amalinei C. The value of PAX8 and WT1 molecules in ovarian cancer diagnosis. Rom J Morphol Embryol, 54(1):17–27, (2013).

[27] Kriplani D.and Patel M.M. Immunohistochemistry: A diagnostic aid in differentiating primary epithelial ovarian tumors and tumors metastatic to the ovary. South Asian J Cancer.; 2(4): 254–258, (2013).

[28] Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, Singhal P, Lele S, Odunsi K. Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer, Gynecol Oncol,, 101(1):12–17, (2006).

[29] Barbolina M.V., Adley B.P., Shea L.D., Stack M.SH. Wilms Tumor Gene Protein 1 Is Associated with Ovarian Cancer Metastasis and Modulates Cell Invasion. American cancer society,112 (7), (2008).

[30] Netinatsunthorn W., Hanprasertpong J., Dechsukhum C., Leetanaporn R. and Geater A. WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study. BMC Cancer: 90(6):1471-2407, (2006).

[31] Liu Z., Yamanouchi K., Ohtao T., Matsumura S., Seino M., Shridhar V, Takahashi T, Takahashi K, Kurachi H. High Levels of Wilms' Tumor 1 (WT1) Expression Were Associated with Aggressive Clinical Features in Ovarian Cancer. Anticancer research 34: 2331-2340, (2014).