Int J Clin Exp Pathol 2020;13(8):2115-2122
www.ijcep.com /ISSN:1936-2625/IJCEP0114261

Original Article

Expression of CD117, CD34, and VEGF proteins in progression from endometrial hyperplasia to endometrioid carcinoma

Wael Abdo Hassan[1,2], Rehab Ibrahim[1,3]

[1]Department of Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt; [2]Department of Basic Sciences, Sulaiman Al Rajhi University, Al-Bukayriyah, KSA; [3]Department of Pathology, Collage of Medicine, Jouf University, Sakaka, KSA

Received May 13, 2020; Accepted June 29, 2020; Epub August 1, 2020; Published August 15, 2020

Abstract: Background: The risk of endometrial hyperplasia progressing into endometrioid carcinoma ranges from 1% for benign hyperplasia to 46.2% for endometrial intra-epithelial neoplasia. Differentiation between both types of hyperplasia is thus crucial for optimal management. The present study investigates the expression of the following immune-histochemical markers, for their potential roles in differentiating between both types of endometrial hyperplasia; as well as their expression in endometrial carcinoma: VEGF, CD34 and CD117. Methods: Tissue samples were obtained, fixed, processed, stained by hematoxylin and eosin for diagnosis, and then imunohistochemically stained using anti CD117, CD34, and VEGF antibodies. Results: In benign endometrial hyperplasia, the cells show weak expression to VEGF and CD34, and absent CD117. In endometrial intra-epithelial neoplasia, the cells show strong expression of VEGF and weak expression of CD34 and CD117. In case of endometrioid carcinoma, all cases showed strong reaction for VEGF and CD34, and moderate expression to CD117. Conclusion: Our data suggests a role for CD117, CD34, and VEGF in progression from hyperplasia to carcinoma.

Keywords: Endometrial hyperplasia, endometrioid carcinoma, CD117, CD34, VEGF

Introduction

Endometrial hyperplasia (EH) is a well-known precursor lesion to endometrioid carcinoma (EC) of the endometrium. It is the second most common gynecological malignancy world-wide [1]. It is presented with abnormal uterine bleeding, usually in the early postmenopausal years [2].

EH has been linked to continuous exposure of estrogen, unopposed with progesterone [3, 4]. The degree of risk increases in obese nulliparous postmenopausal females; especially if diabetic and hypertensive [5].

Pathologically, EH represents a heterogeneous group of lesions, characterized with an increase in the gland to stroma ratio compared to proliferative endometrium [6]. Progression of EH to EC is related to the degree of architectural complexity and cytological atypia. The latter is considered the principal histological determinant for malignant potential [7]. Moreover, aberrant expression of the VHL gene is associated with progression of EH to EC [8].

The pathological diagnosis and classification of EH has been through many changes and updates. Prior to 2014, the World Health Organization (WHO) classification system used cellular complexity, crowding of the endometrial gland, and the presence of cytological atypia to classify EH as simple or complex and with or without atypia [9]. This system suffered from significant interobserver variation [10]. Accordingly, the new WHO classification, accepted by the International Society of Gynecological Pathologists, divided EH into only two groups: benign hyperplasia and atypical hyperplasia/endometrial intraepithelial neoplasia (EIN) [11-13].

Table 1. Antibodies for immunocytochemistry. References, quantities and working dilutions are indicated

Primary antibodies	Lot	Working dilution
VEGF	M7273	1:100
CD34	QBEnd-10	1:100
CD117	A4502	1:100

The risk of EH progressing into EC ranges from 1% for benign EH to 46.2% for EIN [14]. Differentiation between both types of EH is crucial for optimal management. The distinction between benign and atypical EH is based on certain microscopic diagnostic criteria determined from a hematoxylin and eosin (H&E) stained endometrial section. However, such histopathological assessment can lead to subjective inter-observer variation especially in the diagnosis of atypical EH. This necessitates the possible use of certain immunohistochemical (IHC) markers for further confirming the diagnosis and identifying 'at risk' EH patients for malignant progression [15]. Several markers have been proposed, as estrogen receptor (ER), progesterone receptor (PR), Cyclooxygenase-2 (COX-2), Phosphatase and tensin homologue (PTEN), P53, B-cell lymphoma 2 (Bcl-2), E-cadherin, and CD10 [16-21]. However, contradictory data about the usefulness of these makers have been reported [15]. The present study investigates the expression of the following IHC markers, for their potential roles in differentiating benign EH form EIN and EC, and thereby identify groups of interacting proteins that could be involved in the progression of EH to EC: vascular endothelial growth factor (VEGF), CD34, and CD117.

Material and methods

Patients

Tissue samples from 40 females presented with perimenopausal bleeding and underwent diagnostic dilatation and curettage (D&C) in the Gynecology Departments of El-Zagazig University Hospital, and Suez Canal University Hospital, in Egypt. Two pathologists reviewed all the tissue samples to confirm the diagnosis. The histological grade of EC was assessed according to the latest WHO classification [11]. A full and informed patient consent was obtained from each patient.

Histopathological evaluation: The samples were fixed with 10% formalin and embedded in paraffin. From each block, histological sections of 3 μm thickness were submitted, mounted to glass slides, stained by hematoxylin and eosin (H&E), and reviewed to confirm the diagnosis of benign EH, EIN, and EC and identify in the latter tumor grade and depth of invasion.

Immunohistochemical staining: Sections from the selected paraffin blocks were cut into 4 micrometers thick sections and mounted on poly-L-lysine glass slides for immunohistochemical staining. Primary antibodies (**Table 1**) were purchased from Dako (Glostrup, Denmark). This was followed by 60 min incubations with secondary antibodies (Envision+System-HRP Labelled Polymer, Dako) and visualization with the Liquid DAB+Substrate Chromogen System (Dako). All slides were lightly counterstained with hematoxylin for 30 s prior to dehydration and mounting.

Immunohistochemical scoring: The following semi-quantitative method to assess the staining intensity was used: a strong positive result was defined as strong or moderate immunoreactivity in 50% or more of cells (Score 2), a weak positive result was defined as weak immunoreactivity or staining of fewer than 50% of tumor cells (Score 1), and cells with no or focal minimal staining was scored as negative (Score 0).

Statistical analysis

Data were collected, coded, and entered into the computer statistical program. All statistical analysis was done using JMP9 software program (SAS Institute Inc, Cary, NC). Quantitative data were presented as mean and standard deviation. Qualitative data was presented in the form of numbers and percentages. Pearson's Chi-square test was used to test the significance of the difference between groups in qualitative variables. Fisher's exact-test was used instead, in cases that were >25% of cells had expected count <5. Mann Whitney U-test was used to test the significance of differences in quantitative variables between groups. For all statistical analysis level of significance was considered to be <0.05.

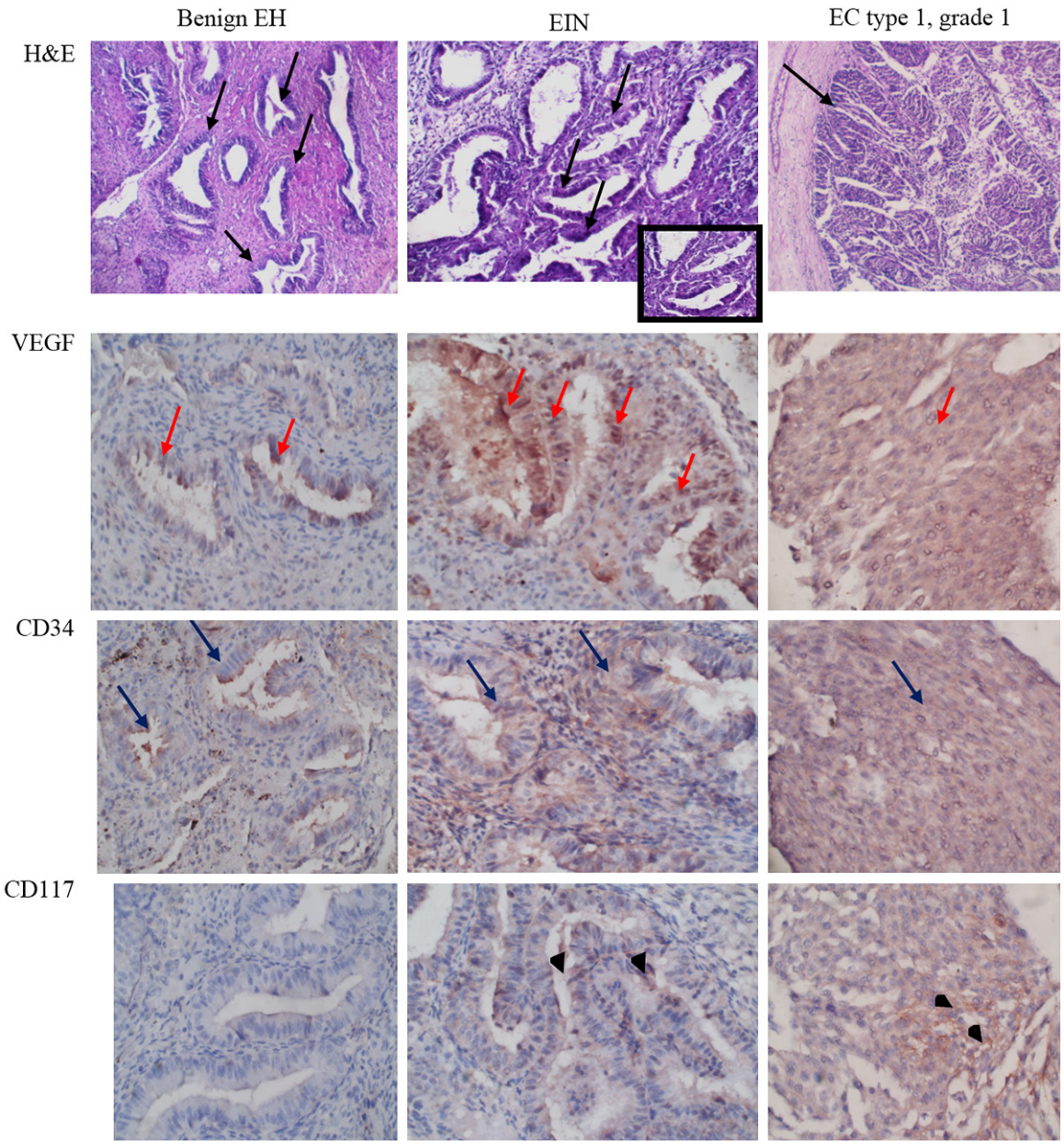

Figure 1. Expression of VEGF, CD34, and CD117 in benign endometrial hyperplasia (EH), endometrial intraepithelial neoplasia (EIN), and endometrioid carcinoma (EC); type 1. The first row shows representative H&E figures for each lesion. In benign EH, endometrial glands (black arrows) are proliferative and lined with single layer of uniform columnar epithelial cells with no atypia (H&E, 10×). Few glandular cells show weak expression of VEGF at their luminal surfaces (Red arrows), which was scored as negative (IHC, 40×). Similarly, the expression of CD34 (Blue arrows) was weak and mostly at their luminal surfaces (IHC, 40×). In case of CD117 expression, no reaction was observed in glandular cells. In EIN, the H&E figure shows a focus of glandular complexity (Black arrows), with narrow stroma in between, in which the glandular epithelial cells show atypical morphology; stratified hyperchromatic nuclei with various degrees of plemorphism (H&E, 10×; inset 40×). The expression of VEGF (Red arrows) was significantly stronger than in benign EH (IHC, 40×). CD34 expression (Blue arrows) in glandular cells showed weak positivity, similar to benign EH (IHC, 40×). CD117 (Arrow heads) showed weak focal apical staining which was scored as negative expression (IHC, 40×). In EC, the tumor (black arrow) is composed mostly of villo-glandular pattern of tumor cells arrangement, with few solid sheets (<5%), with mild degree of cellular and nuclear pleomorphism (H&E, 10×). The expression of VEGF (red arrow) and CD34 (Blue arrow) was diffuse strong cytoplasmic, in comparison to those of benign EH and EIN (IHC, 40×). CD117 (Arrow heads) showed moderate membranous reaction in more than 50% of glandular epithelial cells (IHC, 40×).

Table 2. The immunohistochemical score for expression of VEGF, CD34 and CD117 in endometrial lesions. The average score from samples ± s.d. and *p* value are indicated

	Non-atypical (Benign) EH (n=20)	Atypical EH (EIN) (n=10)	Type I EC		*p* value
			G1 (n=6)	G2 (n=4)	
VEGF	0.2 (0.4)	1 (0.44)	1.8 (0.4)	1.8 (0.4)	<0.001
CD34	0.2 (0.43)	0.4 (0.57)	1.6 (0.89)	1.6 (0.89)	<0.05
CD117	0.2 (0.45)	0.4 (0.24)	1.8 (0.4)	1.8 (0.4)	<0.05

EH, endometrial hyperplasia; EIN, endometrial intra-epithelial neoplasia; EC, Endometrioid carcinoma; G, grade; VEGF, Vascular endothelial growth factor.

Results (Figure 1 and Table 2)

The age of patients from whom the specimens were taken ranged between 45-72 years with a mean age of 48.2 years, 52 years, and 69 years in benign EH, EIN, and EC respectively.

Among the 40 specimens, 20 samples were benign EH, 10 samples were EIN, and 10 samples were EC; 6 of which were grade 1 and the other 4 were grade 2.

Evaluation of the expression of VEGF in tissue samples revealed a statistical significant difference in its expression among the cases. All cases of EC showed strong positive diffuse cytoplasmic expression, with no significant difference between grade 1 or 2 tumors. In EIN, there was moderate to strong expression of VEGF, but in fewer than 50% of glandular cells, and thus was recorded as weak cytoplasmic positive, while in benign EH, fewer than 50% of cells showed weak expression mainly at their luminal surfaces, which was recorded as negative expression. These findings suggest a role of acquisition of VEGF expression by endometrial glandular cells for progression from EH into EC.

Regarding CD34, there was a statistically significant difference in its expression between EC and the other lesions, with no differences in its expression between benign EH and EIN. 80% of cases of EC showed strong cytoplasmic expression, while its expression in benign EH and EIN showed weak to negative reactions. These findings indicate a possible role of CD34 in late stages of progression to EC, with no significant role in differentiating benign from EIN.

Regarding the expression of CD117, all cases of EC showed moderate membranous expression, with no significant difference between grade 1 or 2 tumors. In EIN, the expression of CD117 was observed in few glandular cells, at their apical luminal surfaces, and thus was recorded as negative expression, while in benign EH, it showed no expression. These findings suggest a role of acquisition of VEGF expression by endometrial glandular cells for progression from EH into EC.

Discussion

The progression of endometrial hyperplasia to carcinoma necessitates the search for certain markers for better diagnosis and understanding of such progression. Multiple non-epithelial surface and intracellular markers are associated with cancer progression [22]. We investigated the expression of CD117, VEGF, and CD34 in the progression of hyperplasia to carcinoma.

CD117 protein is a tyrosine kinase receptor which is encoded by the proto-oncogene c-kit, located on chromosome 4 [23]. Over activation of CD117 is the primary mutation seen many malignant neoplasms; GIST, mastocytosis, AML, and melanoma [24]. It is also expressed in many other solid tumors with a trend towards worse prognosis in tumors with high CD117 expression [25]. We found that CD117 expression increased significantly from benign EH to EC. Similar results were recorded in many other types of tumors; like prostatic adenocarcinoma cells which shows CD34 high/CD117 high phenotype, when compared to benign prostatic lesions [26]. Moreover, in high grade prostatic tumors, CD117 positivity was higher, compared to low grade tumors and that xenograft tumors expressing CD117 were larger and have more angiogenesis [22]. In addition, studies on phyllodes tumor of the breast as well as ovarian serous carcinoma revealed that CD117 expression was associated with higher tumor grade and poor prognosis [27-31]. A study on melano-

cytic tumors found that intradermal nevi were negative for CD117, while dysplastic nevi were 100% positive and malignant melanoma was 83.3% positive [32]. In endometrium, some studies found that CD117 expression was significant in benign endometrium, with more intense staining in hyperplastic and proliferative endometrium, as well as in endometrial carcinoma [23, 33] and that there was decreased expression of CD117 with progression from hyperplasia to carcinoma [23]. These results, albeit different from our findings, could highlight the complexity of CD117 signaling in endometrial tumorigenesis, and that it could be acted on in a time-dependent manner. Thus, further extensive studies are needed to elucidate its role.

CD34 is a transmembrane phosphoglycoprotein, first identified on hematopoietic stem and progenitor cells, and is expressed also by mesenchymal stem cells, muscle satellite cells, corneal keratocytes, interstitial cells, epithelial progenitors, and vascular endothelial progenitors [34, 35]. The present data shows that there was a statistically significant difference in CD34 expression between EC and the other lesions, with no differences in its expression between benign EH and EIN. 80% of cases of EC showed strong cytoplasmic expression, while its expression in benign EH and EIN showed weak to negative reaction. These findings indicate a possible role of CD34 in late stages of progression to EC, with no significant role in differentiating benign from atypical EH. To our knowledge, this is the first report of identifying CD34 protein expression in glandular cells in endometrial lesions. Some studies showed that CD34 expression in vessels in endometrial carcinoma was significantly higher than in normal proliferative endometrium and its expression increased in higher grades endometrial carcinoma [36-38]. Our findings are similar to the results of other studies in other tumors, like hepatocellular carcinoma, which show positive CD34 staining, in comparison to hepatic adenoma and focal nodular hyperplasia, which show incomplete positive staining and to normal or cirrhotic liver, which show negative expression [39]. Similar results were found in colorectal adenomas and carcinomas [40, 41], head and neck squamous cell carcinoma [42, 43], malignant melanoma [44, 45], oral and esophageal squamous cell carcinoma [46], prostatic carcinoma [47], and NUT midline carcinoma [48]. Similarly, CD34 expression was significantly higher in malignant compared to benign surface epithelial tumors of ovary and in high grade compared to low grade tumors [49].

VEGF is a potent pro-angiogenic factor and a key mediator of neovascularization, a process which is involved in the pathogenesis of malignant tumors, as its overexpression in tumor cells stimulate tumor growth and metastasis in many cancers [50]. The current work revealed that all cases of EC showed strong positive diffuse cytoplasmic expression of VEGF, with no significant difference between grade 1 or 2 tumors. In EIN, there was weak cytoplasmic positive staining, while in benign EH, the expression was negative. These findings suggest a role of VEGF in the progression of EH into EC. Similar results were obtained by previous studies which reported significantly increased expression of VEGF, with progression from normal endometrium to simple endometrial hyperplasia without atypia to complex hyperplasia without atypia to complex hyperplasia with atypia [50, 51]. Moreover, high expression of VEGF correlates with higher grade and stage of endometrial cancer [52]. Studies in other cancers revealed similar results, as ovarian serous carcinoma [53], granulosa cell tumor [54], breast invasive ductal carcinoma [55], gastric adenocarcinoma [56, 57], colorectal adenocarcinoma [58], and prostatic carcinoma [59].

Summary and conclusion

In conclusion, our findings demonstrated that the expression of CD117, CD34, and VEGF were significantly increased when progressing from EH to EC, indicating that acquisition of these proteins could be involved in progression from EH to EIN and then to EC.

Acknowledgements

Special thanks to Dr. Yasser Saraya; Faculty of medicine Zagazig University, Sulaiman Al Rajhi University for providing related pathological specimens. Special thanks to Mr. M. Saad for his technical assistance in preparing slides. This study was fully funded by the authors.

The study was approved by The Ethics Committee of Suez Canal University (Research approval number #357). Informed consent was

obtained from patients. Information of each patient was anonymized prior to analysis.

Disclosure of conflict of interest

None.

Address correspondence to: Wael Abdo Hassan, Department of Basic Sciences, Sulaiman Al Rajhi University, Al Bukairiyah 51941, PO Box 777, Kingdom of Saudi Arabia. Tel: 00966-507091876; Fax: 00966-163169090; E-mail: w.hassan@sr.edu.sa

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J cancer 2015; 136: E359-E386.

[2] Yuk JS. The incidence rates of endometrial hyperplasia and endometrial cancer: a four-year population-based study. PeerJ 2016; 4: e2374.

[3] Antunes CM, Strolley PD, Rosenshein NB, Davies JL, Tonascia JA, Brown C, Burnett L, Rutledge A, Pokempner M and Garcia R. Endometrial cancer and estrogen use. Report of a large case-control study. N Engl J Med 1979; 300: 9-13.

[4] Montgomery BE, Daum GS and Dunton CJ. Endometrial hyperplasia: a review. Obstet Gynecol Surv 2004; 59: 368-78.

[5] Heller DS, Mosquera C, Goldsmith LT and Cracchiolo B. Body mass index of patients with endometrial hyperplasia: comparison to patients with proliferative endometrium and abnormal bleeding. J Reprod Med 2011; 56: 110-2.

[6] Mills AM and Longacre TA. Endometrial hyperplasia. Semin Diagn Pathol 2010; 27: 199-214.

[7] Ellenson LH, Ronnett BM and Kurman RJ. Precursor lesions of endometrial carcinoma. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. Springer, Boston, MA; 2011.

[8] Xu JY, Zhu WJ, Cao XZ, Li XF and Wu J. Aberrant expression of the von Hippel-Lindau gene in human endometrial hyperplasia and endometrial carcinoma. Int J Gynecol Cancer 2011; 21: 430-4.

[9] Armstrong AJ, Hurd WW, Elguero S, Barker NM and Zanotti KM. Diagnosis and management of endometrial hyperplasia. J Minim Invasive Gynecol 2012; 19: 562-71.

[10] Allison KH, Reed SD, Voigt LF, Jordan CD, Newton KM and Garcia RL. Diagnosing endometrial hyperplasia: why is it so difficult to agree? Am J Surg Pathol 2008; 32: 691-8.

[11] Kurman R, Carcangiu M, Herrington C and Young R. World Health Organisation classification of tumors of female reproductive organs. 4th edition. Lyon France: International Agency for Research on Cancer (IARC) Press; 2014.

[12] Sobczuk K and Sobczuk A. New classification system of endometrial hyperplasia WHO 2014 and its clinical implications. Prz Menopauzalny 2017; 16: 107-111.

[13] Ordi J, Bergeron C, Hardisson D, McCluggage WG, Hollema H, Felix A, Soslow RA, Oliva E, Tavassoli FA, Alvarado-Cabrero I, Wells M and Nogales FF. Reproducibility of current classifications of endometrial endometrioid glandular proliferations: further evidence supporting a simplified classification. Histopathology 2014; 64: 284-92.

[14] Laas E, Ballester M, Cortez A, Gonin J, Canlorbe G, Daraï E and Graesslin O. Supervised clustering of immunohistochemical markers to distinguish atypical and non-atypical endometrial hyperplasia. Gynecol Endocrinol 2015; 31: 282-5.

[15] Sanderson PA, Critchley HO, Williams AR, Arends MJ and Saunders PT. New concepts for an old problem: the diagnosis of endometrial hyperplasia. Hum Reprod Update 2017; 23: 232-254.

[16] Hu K, Zhong G and He F. Expression of estrogen receptors ERalpha and ERbeta in endometrial hyperplasia and adenocarcinoma. Int J Gynecol Cancer 2008; 15: 537-541.

[17] Pieczyńska B, Wojtylak S, Zawrocki A and Biernat W. Analysis of PTEN, estrogen receptor α and progesterone receptor expression in endometrial hyperplasia using tissue microarray. Pol J Pathol 2011; 62: 133-138.

[18] Nasir A, Boulware D, Kaiser HE, Lancaster JM, Coppola D, Smith PV, Hakam A, Siegel SE and Bodey B. Cyclooxygenase-2 (COX-2) expression in human endometrial carcinoma and precursor lesions and its possible use in cancer chemoprevention and therapy. In Vivo 2007; 21: 35-43.

[19] Steinbakk A, Gudlaugsson E, Aasprong OG, Skaland I, Malpica A, Feng W, Janssen EA and Baak JP. Molecular biomarkers in endometrial hyperplasias predict cancer progression. Am J Obstet Gynecol 2011; 204: 357, e1-e12.

[20] Nunobiki O, Nakamura M, Taniguchi E, Utsunomiya H, Mori I, Tsubota Y, Mabuchi Y and Kakudo K. Adrenomedullin, Bcl-2 and microvessel density in normal, hyperplastic and neoplastic endometrium. Pathol Int 2009; 59: 530-536.

[21] Ahmed AR and Muhammad EM. E-cadherin and CD10 expression in atypical hyperplastic and malignant endometrial lesions. J Egypt Natl Canc Inst 2014; 26: 211-217.
[22] Harris KS and Kerr BA. Prostate cancer stem cell markers drive progression, therapeutic resistance, and bone metastasis. Stem Cells Int 2017; 2017: 8629234.
[23] Yilmaz E, Celik O, Simsek Y, Turkcuoglu I, Celik E, Gül M, Hascalik S and Aydin NE. c-Kit protooncogene expression in endometrial hyperplasia and endometrial cancer. Arch Gynecol Obstet 2012; 286: 197-200.
[24] Foster BM, Zaidi D, Young TR, Mobley ME and Kerr BA. CD117/c-kit in cancer stem cell-mediated progression and therapeutic resistance. Biomedicines 2018; 61: 31.
[25] Medinger M, Kleinschmidt M, Mross K, Wehmeyer B, Unger C, Schaefer HE, Weber R and Azemar M. c-kit (CD117) expression in human tumors and its prognostic value: an immunohistochemical analysis. Pathol Oncol 2010; 16: 295-301.
[26] Foroozan M, Roudi R, Abolhasani M, Gheytanchi E and Mehrazma M. Clinical significance of endothelial cell marker CD34 and mast cell marker CD117 in prostate adenocarcinoma. Pathol Res Pract 2017; 213: 612-618.
[27] Chougule A, Bal A, Das A, Kohli PS and Singh G. In phyllodes tumour of the breast expression of c-kit but not of ALDH1A1 is associated with adverse clinico-pathological features. Virchows Arch 2016; 469: 651-658.
[28] Tan WJ, Thike AA, Tan SY, Tse GM, Tan MH, Bay BH and Tan PH. CD117 expression in breast phyllodes tumors correlates with adverse pathologic parameters and reduced survival. Mod Pathol 2015; 28: 352-358.
[29] Burgos-Ojeda D, Rueda B and Buckanovich R. Ovarian cancer stem cell markers: prognostic and therapeutic implications. Cancer Lett 2012; 322: 1-7.
[30] Luo L, Zeng J, Liang B, Zhao Z, Sun L, Cao D, Yang J and Shen K. Ovarian cancer cells with the CD117 phenotype are highly tumorigenic and are related to chemotherapy outcome. Exp Mol Pathol 2011; 91: 596-602.
[31] Stemberger-Papić S, Vrdoljak-Mozetic D, Ostojić DV, Rubesa-Mihaljević R, Krigtofić I, Brncić-Fisher A, Kragević M and Eminović S. Expression of CD133 and CD117 in 64 serous ovarian cancer cases. Coll Antropol 2015; 39: 745-753.
[32] Shamloulaa MM, Gheidab SF and El-sakka AM. Immunohistochemical expression of CD-117 and CD34 as stem cell markers in intradermal nevi, dysplastic nevi, and malignant melanomas. Journal of the Egyptian Women's Dermatologic Society 2013; 10: 10-17.
[33] Elmore L, Domson K, Moore J, Kornstein M and Burks R. Expression of c-kit (CD117) in benign and malignant human endometrial epithelium. Arch Pathol Lab Med 2001; 125: 146-51.
[34] Sidney LE, Branch MJ, Dunphy SE, Dua HS and Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells 2014; 32: 1380-1389.
[35] Barbera M, di Pietro M, Walker E, Brierley C, MacRae S, Simons BD, Jones PH, Stingl J and Fitzgerald RC. The human squamous oesophagus has widespread capacity for clonal expansion from cells at diverse stages of differentiation. Gut 2015; 64: 11-19.
[36] Kamat AA, Merritt WM, Coffey D, Lin YG, Patel PR, Broaddus R, Nugent E, Han LY, Landen CN Jr, Spannuth WA, Lu C, Coleman RL, Gershenson DM and Sood AK. Clinical and biological significance of vascular endothelial growth factor in endometrial cancer. Clin Cancer Res 2007; 13: 7487-7495.
[37] Aybatlı A, Sayın C, Kaplan PB, Varol F, Altaner S and Süt N. The investigation of tumoral angiogenesis with HIF-1 alpha and microvessel density in women with endometrium cancer. J Turk Ger Gynecol Assoc 2012; 13: 37-44.
[38] Amr E, Shaima A, Ghalia A and Fawzia H. Enhanced expression of vascular endothelial growth factor and increased microvascular density in women with endometrial hyperplasia: a possible relationship with uterine natural killer cells. Rom J Morphol Embryol 2015; 56: 725-734.
[39] Coston WM, Loera S, Lau SK, Ishizawa S, Jiang Z, Wu CL, Yen Y, Weiss LM and Chu PG. Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. Am J Surg Pathol 2008; 32: 433-444.
[40] Cesare R, Francesco F, Elisabetta T, et al. Vascular Endothelial growth factors and CD34 expression can implement NICE (NBI international endoscopic) classification in colorectal polypoid lesion diagnosis. Gastroenterology 2019; 156: 1421.
[41] Behbehani A, Ranjbari N, Rahim F and Jazayeri N. Evaluating the expression of CD34 marker in colorectal adenocarcinoma and its relationship with clinicopathologic factors. Asian Journal of Cell Biology 2015; 10: 80-86.
[42] Ettl T, Hautmann M, Reichert T and Bauer R. Cycling CD34 expression in subpopulations of head and neck squamous cell carcinoma cell lines is involved in radioresistance and change in cytokeratin expression profile. Clin Exp Med 2017; 17: 565-574.
[43] Young MR, Wright MA, Lozano Y, Prechel MM, Benefield J, Leonetti JP, Collins SL and

Petruzzelli GJ. Increased recurrence and metastasis in patients whose primary head and neck squamous cell carcinomas secreted granulocyte-macrophage colony-stimulating factor and contained CD34+ natural suppressor cells. Int J Cancer 1997; 74: 69-74.

[44] Hoang MP, Selim MA, Bentley RC, Burchette JL and Shea CR. CD34 expression in desmoplastic melanoma. J Cutan Pathol 2001; 28: 508-512.

[45] Breza TS and Magro CM. CD34 expression in primary cutaneous malignant melanoma: apropos of a case and review of the aberrant melanoma phenotype. J Cutan Pathol 2005; 32: 685-689.

[46] Fatemeh S, Sareh F, Donia S and Marzieh S. Evaluation of microvascularity by CD34 expression in esophagus and oral squamous cell carcinoma. J Contemp Dent Pract 2015; 16: 458-462.

[47] Aissar N and Renato F. Immunohistochemistry expression of tumor markers CD34 and P27 as a prognostic factor of clinically localized prostate adenocarcinoma after radical prostatectomy. Rev Col Bras Cir 2010; 37: 338-344.

[48] Weijie L and Katherine C. NUT midline carcinoma with leukemic presentation mimicking CD34-positive acute leukemia. Blood 2018; 132: 456.

[49] Arjunan A, Thiriveni B, Mani R, Sudha B, Narmadha D and Malaichamy V. Expression of p53 and CD34 in surface epithelial tumors of ovary. 2016; 15: 1-13.

[50] Guşet G, Costi S, Lazăr E, Dema A, Cornianu M, Vernic C and Păiuşan L. Expression of vascular endothelial growth factor (VEGF) and assessment of microvascular density with CD34 as prognostic markers for endometrial carcinoma. Rom J Morphol Embryol 2010; 51: 677-82.

[51] Yokoyama Y, Charnock-Jones DS, Licence D, Yanaihara A, Hastings JM, Holland CM, Emoto M, Sakamoto A, Sakamoto T, Maruyama H, Sato S, Mizunuma H and Smith SK. Expression of vascular endothelial growth factor (VEGF)-D and its receptor, VEGF receptor 3, as a prognostic factor in endometrial carcinoma. Clin Cancer Res 2003; 9: 1361-1369.

[52] Sunita B, Sen A and Suhag V. To evaluate immunoreactivity of cyclooxygenase-2 in cases of endometrial carcinoma and correlate it with expression of p53 and vascular endothelial growth factor. J Can Res Ther 2018; 14: 1366-72.

[53] Ranjbar R, Nejatollahi F, Nedaei AS, Hafezi H and Safaie A. Expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) in patients with serous ovarian carcinoma and their clinical significance. Iran J Cancer Prev 2015; 8: e3428.

[54] Färkkilä A, Anttonen M, Pociuviene J, Leminen A, Butzow R, Heikinheimo M and Unkila-Kallio L. Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 are highly expressed in ovarian granulosa cell tumors. Eur J Endocrinol 2011; 164: 115-22.

[55] Cimpean A, Raica M, Suciu C, Tatuco D, Sarb S and Muresan A. Vascular endothelial growth factor A (VEGF A) as individual prognostic factor in invasive breast carcinoma. Rom J Morphol Embryol 2008; 49: 303-308

[56] Li C, Ma Y, Wei Y, et al. Relationship between VEGF, EGF and invasion, metastasis of gastric cancer cells. Chin J Cancer Res 2009; 21: 122-129.

[57] Zheng H, Takahashi H, Murai Y, Cui Z, Nomoto K, Niwa H, Tsuneyama K and Takano Y. Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma. Anticancer Res 2006; 26: 3579-3583.

[58] Wang Y, Yao X, Ge J, Hu F and Zhao Y. Can vascular endothelial growth factor and microvessel density be used as prognostic biomarkers for colorectal cancer? A systematic review and meta-analysis. ScientificWorldJournal 2014; 2014: 102736.

[59] Roberts E, Cossigny DA and Quan GM. The role of vascular endothelial growth factor in metastatic prostate cancer to the skeleton. Prostate Cancer 2013; 2013: 418340.