DISCUSSION

Endometrial cancer is the most frequent malignancy of the female genital tract, and the fourth most common site after breast, lung, and colorectal cancers. The incidence is rising as life expectancy increases. [1]Developing countries and Japan have incidence rates four to five times lower than western industrialized nations, with the lowest rates being in India and South Asia. [2] The death rate has increased during recent decades, probably due to coexisting medical comorbidities. [3] Approximately43.470 new cases of uterine cancer were recorded in 2010 worldwide, with 7950deaths from this disease. [4]

Tumor angiogenesis, i.e. formation of new blood vessels associated with a neoplasm, is essential for tumor growth and metastasis and is regarded as one of the most important events occurring in the neoplastic process. [159-162]The lymphatic system is the primary pathway of metastasis for most human cancers and the extent of lymph node involvement is a crucial prognostic factor for a patient's outcome. In contrast to the process of angiogenesis, less information is available with regards to lymphangiogenesis and its significance for lymph node metastasis. [153, 154]

Previously, it used to be difficult to discriminate between lymphatic capillaries and blood vessel capillaries based only on morphological grounds due to lack of specific lymphatic markers. This discrimination between blood and lymphatic vessel capillaries might play different roles in the progression of cancer. [170-172]

The introduction of highly specific markers, as VEGFR3, LYVE-1, Prox 1and D2-40, brought new insights into the knowledge of distribution and morphology of lymphatic vessels, and allowed their counting in histological sections.[154, 169,174]Among those, we chose D2-40 for the present study to assess lymphatic microvessel density (LMVD) as a potential prognostic indicator in a series of endometrial cancer patients because of its presumed high sensitivity and specificity in detection of lymphatic vessels, as pointed out by many preceding publications.[165,194,195]Our results showed that D2-40 stains lymphatic endothelium rather than blood vessel endothelium.

We then considered which compartment (intra or peritumoral) to evaluate for lymphangiogenesis in this study, as this is a source of great controversy in the literature. Since our knowledge of lymphangiogenesis in different types of cancer is still limited, we decided that it would be worthwhile to compile data and evaluate both intra and peritumoral LMVD. A better understanding of the role of lymphangiogenesis in tumoral dissemination will only be achieved after contributions from different research groups.

In the current work, we evaluated intratumoral and peritumoral LMVD of endometrial carcinomas in sixty patients and investigated their association with classical pathological factors, metastatic nodal involvement and relations to other prognostic parameters.

In the present study, patients' ages ranged between 35 and 75 years, with a mean of 60.07 ± 9.75and a median of 63 years. These findings were in accord with age incidence of endometrial carcinoma in other previous studies. [72, 196-198]

In the current work; twelve (20%) patients had 0-2 live births and 48 patients had more than 2 live births (80%). In a study done by Park et al; 20.2% of patients of

endometrial carcinoma were nulliparous and 79.8% had ≥1 live births. [199] Most of our patients were multipara which is not in agreement with most of the other researches. [200-202]Nulliparity could not be a strong risk factor in our country.

As regards obesity in this study, (13.3%) patients recorded normal body weights, while (26.7%) patients were overweight and (60%) were obese. So obesity was prominent in our cases and that conforms to most of the previous studies in literature. [196, 197, 199]The link between obesity and endometrial carcinoma can be explained by increased peripheral synthesis of estrone in subcutaneous fat depots leading to increased exposure to endogenous estrogen which is a predisposing factor for endometrioid type of endometrial carcinoma. [6]

As regards contraception; among 60 patients included in this study 73.3% used a contraceptive method. Among users of contraceptive methods, only 20% used pills versus 53.3% used IUD. Pills are known to confer a protective effect against endometrial carcinoma. Oral contraceptive users for a period of at least one year are protected against endometrial carcinoma by 30-50% over a period of 10-20 years on condition that the pills are of combined type (progesterone effect).[6]

In the present study diabetes mellitus alone was present in 60% of cases and hypertension in 66.7%. Both diabetes mellitus and hypertension together were present in 53.3% of cases. In a study done by Yin et al in patients diagnosed with endometrial carcinoma; 15% of patients were diabetic and 24% were hypertensive. [196]In another study done by Willis et al; 45% of cases had either diabetes mellitus or hypertension. [203]There is an extraordinary increased incidence of diabetes mellitus and hypertension in Egypt and other developing countries. The prevalence of diabetes mellitus in Egypt adjusted to world and national population in 2010 was 11.4% and 10.4% respectively. [204]The estimated total number of adults with hypertension in 2005, 333 million (329-336 million) in economically developed countries and 639 million (625-654 million) in economically developing countries. [205]These diseases are commonly associated with endometrial carcinoma because both are squelae of obesity an environment of excess estrogen. [6]

In the present work; sensitivity of ultrasonography in relation to histopathology in diagnosis of myometrial invasion was 70%, specificity 80% , PPV 87.50%, NPV 57.14% and accuracy 73.33%.While, sensitivity of CT in relation to histopathology in diagnosing myometrial invasion was 50%, specificity 60% PPV 71.43 %, NPV 37.50 % and accuracy 53.33%. These results were in accordance with other previous studies. [206-208]In a study done by Ivanov, comparing the accuracy, sensitivity and specificity of US, CT and MRI in detection of myometrial invasion in endometrial cancer; concluded that the ultrasound examination had 80% accuracy. The accuracy, sensitivity and specificity of MRI were 85%, 77% and 87% respectively. The CT scan examinations were not so convincing. [207]

In the present study, Sensitivity of CT in relation to histopathology in assessment of preoperative lymph node status was 66.67%, specificity 66.7% and accuracy 66.67%. This is in contrast to a study done by Rossard et al on 244 cases of endometrial carcinoma, where the sensitivity of CT in lymph node assessment was 50% and specificity was 80%.[209]That could be due to change in the sample size.

In the current work, transvaginal U.S endometrial thickness ranged between 3.0 and 20.0 mm with a mean of 12.0 ± 5.26 mm.

The relation between increased endometrial thickness by transvaginal US and higher tumor grade was strongly statistically significant. The relation between increased endometrial thickness by transvaginal US and higher tumor stage was statistically significant. That came in accordance with most of the previous studies where thicker endometrium was significantly associated with higher tumor grade and stage. [210, 211]

In a study done by Eitan et al on 29 endometrial cancer patients; the median preoperative sonographic endometrial stripe was 12.0 mm (range, 5.0-32.0 mm). Tumor stage was only marginally associated with endometrial thickness (correlation 0.23; P = 0.07). No correlation was found between endometrial thickness and tumor grade. [212]This difference may be attributed to change in the sample size.

Malignant cells in peritoneal washings may be the result of trans-tubal dissemination of primary tumor, tumor extension via myometrium/serosal lymphatics, or exfoliation of cells from disease at other extrauterine sites [16]. In the present study, positive peritoneal cytology for malignant cells was detected in 13.3% of patients. That was in accordance with a study done by Kawamura et al where positive peritoneal cytology for malignant cells was detected in 11% of patients. [213]

Our results indicated that positive peritoneal cytology was significantly associated with poor prognostic factors as; older patient's age, deeper myometrial invasion, presence of adnexal infiltration, lymph node positivity, higher tumor stage and presence of vaginal vault recurrence after 6 and 12 months postoperatively. That came in agreement with a majority of other previous studies. [47, 214-217] However, positive peritoneal cytology was insignificantly associated with non endometroid type tumors, higher tumor grade and presence of lymphovascular space invasion. These findings might seem paradoxical. Logically serous histotype and increased LVSI might be expected to demonstrate extrauterine spread more often than do their counterparts. However, Kadar et al reported that malignant cytology was associated with an adverse effect on survival only when accompanied by disease at other extrauterine sites and not if endometrial cancer was still limited to the uterus. [47] Others have speculated that malignant cytology serves as an indicator of aggressive tumor behavior rather than intraperitoneal disease spread. [46, 218]

In the current work; Tumors were of the endometroid type in 73.3% of cases and non endometroid (serous) in 26.7%.These figures conform with the worldwide incidence of type II endometrial cancer which revolves around the figure of 25%. [5,213, 219]

As regards tumor grade in this study; tumors were of grade I in 20% of cases, grade II in 33.3% and grade III in 46.7%. That was in contrast to several previous studies where lower grades (I&II) constituted the majority of cases, around (60-70%) versus only 30% being of high grade (III). [199, 219, 220]

It is worth noting that the aforementioned figures [199, 219, 220] are obtained from studies conducted mainly on endometroid histotypes, unlike the case in our work where including sixteen (26.7%) serous carcinoma cases (by default grade III) resulted in automatic upgrading of the studied cohort.

In the present study, invasion of less than half the myometrial thickness was noted in 33.3% of cases versus 66.7% with invasion of equal to or more than half the myometrial thickness. That was in accordance with a study done by Kawamura et al. [213] But in

contrast to other previous studies where invasion of less than half the myometrial thickness was more than the deeper invasion [199, 219] That can be explained by that 46.7% of our cases were of grade III included non endometroid histotype that represented 26.7% of our cases. These cases are known to be associated with invasion of equal or more than half the myometrial thickness. [5]

In the current study, adnexal infiltration was confirmed in 13.3% of cases. That was in accordance with other internationally recorded figures of adnexal involvement. [213, 220]

In the present study, 40% of patients were positive for lymphovascular space invasion (LVSI). That was in contrast to other previous studies where positive LVSI ranged between 10% and 30%. [199, 213, 219] that can be due to that a large group of our cases were high risk group with higher tumor grade (46.7%) and deeper myometrial invasion (33.3%).

LVSI has been evaluated as a predictor of nodal metastasis and poor survival in endometrial carcinoma.[40, 41]The present study concluded that higher tumor grade, deeper myometrial invasion, lymph node positivity and higher tumor stage were all significantly associated with positive LVSI. While, adnexal infiltration, non endometroid type tumors and positivity of peritoneal fluid cytology were insignificantly associated with positive LVSI. Also in the present study; LVSI was significantly associated with presence of vaginal vault recurrence after 6 months postoperatively. Most of these results came in accordance with other previous studies. [221-226]

Currently, positive lymph node metastasis was detected in 20% of cases. That was similar to a study done by Gao et al. [219] conversely; Park et al recorded 4.7 %.[199] In literature, there is great variation in nodal involvement in endometrial carcinoma ranging between 4% and 40%. [199, 213, 219] That could be due to variations in the surgical skills concerning lymph node dissection or the occurrence of some intraoperative complications preventing the completion of the procedure.

Lymph node metastasis in endometrial carcinoma is an important prognostic factor and a required element for cancer staging. [67] As regards positivity of retroperitoneal lymph nodes for metastasis, the present study concluded that deeper myometrial invasion, adnexal infiltration, non endometroid histotype, higher tumor stage, LVSI, and positive peritoneal cytology were significant predictors of retroperitoneal lymph node involvement. Also in the present study, lymph node positivity was associated with vaginal vault recurrence after 6 and 12 months postoperatively. These results were in accordance with other previous studies [39, 213, 223, 224, 227, 228]

However, in the current study, the relation between tumor grade and lymph node positivity was statistically insignificant. Despite the international acknowledged importance of tumor grade in preoperative lymphadenectomy decision making. [67, 68]Yet our failure to recapitulate the same dogma can be ascribed to the wide range of variation in incidence of nodal involvement in endometrial carcinoma. [199, 213, 219]

In the current work, cases were assigned stage I in 60% of cases, stage II in 6.7% and stage III in 33.3%. In our work, a stage III was assigned to cases with adnexal invasion (8 cases), nodal metastasis (12 cases) and /or parametrial involvement (4 cases). Such stage distribution is in concert with that reported in previous studies [207, 219, 220] but contrasted

with a study by Park et al who recorded a higher figure for stage I and II (92%). [199]) it is worth noting that, in endometrial carcinoma, higher tumor stage is known to be associated with higher tumor grade [3, 229]. In view of this, the over represented higher tumor stage (stage III) in our study material can be considered a reflection of the over represented higher tumor grade.

As regards recurrence rate in endometrial carcinoma, approximately 22% of treated endometrial cancer patients die within 5 years. [7] About one fourth of patients treated for early endometrial cancer develop recurrent disease. More than one half of the recurrences develop within 2 years, and about three fourths occur within 3 years of initial treatment[77, 119, 124, 229]In the present study; the follow up visits for detection of disease recurrence were scheduled after 3, 6 and 12 months postoperatively. No cases had vaginal vault recurrence after 3 months postoperatively, while 6.7% of cases recorded such recurrence after 6 months postoperatively and 20% after 12 months. The overall recurrence rate was 26.7% which conform with the internationally published figures for the overall recurrence rate for early endometrial carcinoma. [77, 119, 124, 191, 229] In our work that happened within the first one year of follow up mainly due to unavailability of vaginal brachytherapy during the period of the present study.

Recurrent disease after 6 and 12 months was significantly associated with positive peritoneal cytology and adnexal infiltration. The relation of vaginal vault recurrence after 6 months postoperatively to non endometroid histotypes, presence of lymphovascular space invasion and lymph node positivity was statistically significant. While, was insignificantly associated with deeper myometrial invasion and higher tumor grade.

Recurrent disease after 12 months was significantly associated with higher tumor grade and lymph node positivity While, was insignificantly associated with deeper myometrial invasion, non endometroid histotypes and positivity of lymphovascular space invasion.

Most of our results came in accordance with the findings of previous studies in literature except for the findings concerning deeper myometrial infiltration and higher tumor grade with their relation to the recurrence rate. As in contrast to the findings of the current study, most of the previous studies found that deeper myometrial invasion and higher tumor grade were associated with significant increased recurrence rate in endometrial carcinoma. [230, 231]

This difference could be attributed to the prospective nature of the present study with a follow up period of a maximum 12 months whereas most of the other studies retrospective analyzed the recurrence rate with a follow up periods of a median of 67 months (range, 12-183 months). [230, 231]So this difference could possibly be reversed if longer period of follow up was conducted.

Lymphatic microvessel density (LMVD) has been studied in several types of cancers employing various methods. For example, cases of cutaneous melanoma metastasis. [175], cases of metastatic breast cancer [232], non-small cell lung cancer [233], gastric carcinoma [234], breast invasive lobular carcinoma [235], oral squamous cell carcinoma [236] and cases of colorectal cancer. [237]

Various specific markers have been used as VEGFR3, LYVE-1, Prox 1and D2-40 to highlight the lymphatic endothelium. [154, 169, 174]

In the field of gyne-oncology, few studies about LMVD are present. Saptefrați et al used D2-40 immunostaining to highlight lymphatic vessels in preinvasive and invasive cervical cancer lesions. [238]They found a significant increase in the values of LMVD from patients with squamous metaplasia to intraepithelial neoplasia, so they speculated that lymphangiogenesis is an early event during cervical carcinogenesis. They also found that increased peritumoral (not intratumoral) LMVD was associated with higher tumor grade, lymphovascular invasion and lymph node metastasis. [238]

In another study by Zhang et al, LYVE-1 (lymphatic vessel endothelial hyaluronan receptor-1) was used as the lymphatic marker in early stage cervical carcinoma. [239]They found that both intratumoral and peritumoral high LMVD values were associated with lymph node metastasis and adverse clinicopathological features of the tumors. [239]

Gombos et al. studied intra- and peritumoral LMVD using D2-40 immunostaining in cervical squamous cell carcinomas in comparison to vascular endothelial growth factor (VEGF)-C expression. They found only high peritumoral LMVD values to be associated with poor overall survival. [194]

In a conflicting report, Birner et al studied LMVD in cases of stage pTIb cervical carcinomas and observed a more favorable prognosis among tumors with high LMVD values. [240]

LMVD studies in endometrial cancer are extremely limited. To investigate the relation of whether increased peritumoral and intratumoral LMVD was related to patient prognosis, Gao et al used LYVE-1 as the lymphatic marker. They concluded that peritumoral and not intratumoral LMVD was an independent risk factor for progression-free survival and overall survival. [219]

Maghraby et al used D2-40 as the lymphatic marker. They found that peritumoral LMVD was associated with poor outcome. [220]

Donughue et al evaluated LMVD using D2-40 as the lymphatic marker. They observed a tendency towards increased intratumoral LMVD in grade 3 tumors compared with grade 1 tumors. [241]

Kawamura et al used D2-40 as the lymphatic marker. They found that high intratumoral LMVD is a characteristic of endometrial carcinomas with lesser extrauterine extension and is associated with better outcome. [213]

In our study, Intratumoral lymphatics were quantitatively less than peritumoral lymphatics. The mean number of intratumoral lymphatic vessels ranged between 0 and 3. The median value was 1. The median was the cutoff point below which readings were considered of low intratumoral LMVD and above which or equal readings were considered of high intratumoral LMVD respectively. The mean number of peritumoral lymphatic vessels ranged between 1.60 and 5.40 with a median value of 3.2.The median was the cutoff point below which readings were considered of low peritumoral LMVD and above which or equal readings were considered of high peritumoral LMVD. Intratumoral lymphatics were detected in 66.7% of cases while peritumoral lymphatics were present in

100% of cases. Intratumoral lymphatics had flattened or closed lumena, contrasting the widely opened lymphatics in the peritumoral regions.

Maghraby et al found that the mean number of intratumoral lymphatics in endometrial carcinoma was 5 ± 3. The mean number of peritumoral lymphatics was 15 ± 7. Intratumoral lymphatics were detected in only 29% of endometrial cancer cases because the cut off value was higher than that of our results and peritumoral lymph-angiogenesis occurs in 100% of cases. [220]

The lack of or decreased number of immunohistochemically identifiable intratumoral lymphatics could be explained by the fact that incorporated intratumoral lymphatics can hardly survive and tend to regress or vanish. In concert with this notion, Giatromanolaki et al demonstrated poor survival of vessels in the inner tumor areas and that the vessel density rapidly decreased in tumor areas located 4–6 mm deeper from the invading tumor front.[242] The same findings were reported by other researches. [157, 169, 220, 243-245]

In the present study, decreased intratumoral and peritumoral LMVD were associated with poorer favorable prognostic factors including higher FIGO stage and grade, non endometroid histotypes, deeper myometrial invasion, positive peritoneal cytology for malignant cells, positive adnexal infiltration and high recurrence rate. Decreased peritumoral LMVD did not show significant correlation with lymph node positivity and LVSI.

We originally expected increased intra or peritumoral LMVD to be associated with a dismal prognosis as that provides easier access of cancer cells to the lymphatic system and, hence, raises the opportunity for lymphatic spread.

Several explanations have been attempted by different research groups: It is well known that cancer cells can be successfully destroyed by an immunological response. [246, 247] A pre-requisite for the initiation of primary immune responses is that cancer cell antigens are brought in contact with lymphoid system by blood, lymph or mobile antigen-presenting cells namely the dendritic cells. [248-250]These dendritic cells appear to move through the lymphatic system to the site of T cell-rich areas (lymph nodes and spleen) and facilitate the presentation of tumor cell antigens to these specific T cells leading to initiation of primary T cell–mediated antitumor immune response against cancer cells.[249-251]It was speculated that high peritumoral or intratumoral LMVD enhances migration of dendritic cells to lymphatic organs, leading to more potent antigen presentation, resulting in an improved T cell–mediated immune response against cancer cells and consequently better outcome, hence the apparently discrepant relation between high LMVD and better outcome. [239,252-254]

In view of this, dendritic cells are nowadays studied as potential cancer vaccines in a variety of human cancers. [255-258]

Another explanation was proposed by Koukourakis et al who argued that the newly formed intra or peritumoral lymphatics are functionless. They speculated that tumors may use their blood vasculature for lymphatic drainage. [174]This can explain why in our work there was no significant association between LMVD and positivity of lymphovascular space invasion or lymph node metastases.

SUMMARY

Endometrial carcinoma is the most common type of female genital tract. The most important prognostic factors in endometrial cancer are histological type, grade, lymph node status, deep myometrial invasion, estrogen receptor (ER) and progesterone receptor (PR) status, tumor stage and Tumor DNA ploidy. Clinical and pathological observations suggest that for many carcinomas, transport of tumor cells via lymphatics is a common pathway of initial dissemination, with patterns of spread via afferent lymphatics following routes of natural drainage. It is unclear whether lymphatic dissemination occurs as a result of cancer cell infiltration of pre-existing lymphatic vessels or newly formed ones, originating from those of the normal surrounding tissues. Lymph-angiogenesis has been difficult to investigate because there was a lack of specific antibodies recognizing the lymphatic endothelium. D2-40 was reported to be a specific marker for lymphatic endothelium in normal and neoplastic tissue.

The aim of this work was to assess intratumoral and peritumoral lymphatic microvascular density (LMVD) in endometrial carcinomas and to investigate their association with classical pathological factors, risk of lymph node (LN) metastasis and theirrelation to other prognostic parameters.

This study was a prospective study done in tertiary education and research hospital (El-Shatby Maternity University Hospital) on sixty patients presented with endometrial carcinoma underwent complete surgical staging, histopathological examination reporting tumor histotype, tumor grade, tumor stage, LVSI, adnexal infiltration, lymph node status, degree of myometrial infiltration and cytological examination of peritoneal fluid aspirate.Tumor sections mounted onto positively charged slides were immunostained using monoclonal antibodies against Podoplanin. (A marker specifically expressed in lymphatic endothelial cells) to highlight lymphatic vessels.Stained histologic sections were analyzed using standard light microscopy. Under low magnification, the most vascularized intratumoral and peritumoral areas were identified. The numbers of immunostained lymphatic vessels found in 10 hot spot areas at 400X magnification were counted to calculate peritumoral and intratumoral lymphatic microvessel density (LMVD).

All the patients were scheduled for follow up visits three, six, and twelve months postoperatively looking for stump and pelvic recurrence by physical and gynecological examination. Multi-slice CT scanning of the abdomen and pelvis was done at six and twelve month intervals.

The result shows that, high LMVD was associated with endometroid type of tumors, lesser myometrial, adnexal and peritoneal infiltration, lower tumor grade and stage and lesser recurrent cases. There is lower lymph node involvement among cases with high intratumoral LMVD and cases of high peritumoral LMVD; that reach statistical significance only among cases of high intratumoral LMVD. No association was seen between LMVD and lymphovascular space invasion. On the other hand, low LMVD was associated with poor outcome.

The results suggested that increased LMVD was associated with favorable prognosis in endometrial cancer patients.

CONCLUSIONS

1. Endometroid type of endometrial carcinoma is the most common histologic type.
2. Ultrasonography is an accurate method for detection of myometrial invasion in cases of endometrial carcinoma.
3. Increased endometrial thickness by transvaginal US had a significant relation with higher tumor stage and grade.
4. Lymph node metastasis in endometrial carcinoma is an important prognostic factor and a required element for cancer staging.
5. Deeper myometrial invasion, adnexal infiltration, non endometroid histotype, higher tumor stage, LVSI, and positive peritoneal cytology were all predictors of retroperitoneal lymph node involvement. Lymph node positivity was significantly associated with vaginal vault recurrence after 6 and 12 months postoperatively.
6. Higher tumor grade, deeper myometrial invasion, lymph node positivity and higher tumor stage had an association with positive LVSI. LVSI was significantly associated with presence of vaginal vault recurrence.
7. Positive peritoneal cytology was a poor prognostic factor associated with deeper myometrial invasion, presence of adnexal infiltration, lymph node positivity, higher tumor stage and presence of vaginal vault recurrence after 6 and 12 months postoperatively.
8. D2-40 stains lymphatic endothelium rather than blood vessel endothelium.
9. Intratumoral lymphatics were quantitatively less than peritumoral lymphatics.
10. Intratumoral lymphatics had flattened or closed lumena, contrasting the widely opened lymphatics in the peritumoral regions.
11. Low intratumoral and peritumoral LMVD were associated with poorer prognostic factors including higher FIGO stage and grade, non endometroid histotypes, deeper myometrial invasion, positive peritoneal cytology for malignant cells, positive adnexal infiltration and high recurrence rate.
12. Understanding the molecular biology of cancer is a principle resource leading to the identification of new potential therapeutic targets and options in gynecologic cancer.