RESEARCH ARTICLE

Evaluation of Hematological Inflammatory Markers in Differentiating Benign from Malignant Endometrial Lesions in Women with Abnormal Uterine Bleeding

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ABSTRACT

Background: Although endometrial sampling was used to confirm endometrial cancer in women with abnormal uterine bleeding (AUB) but it could be challenging due to anesthesia and surgical risks as well as due to patients' refusal. Many biomarkers were start to be studied to predict endometrial carcinoma (EC) and to avoid unnecessary intervention.

Aim: This study emphasized on the role of some hematological inflammatory markers (HIM) in differentiation of benign from malignant endometrial pathologies alone and in addition to endometrial thickness (ET).

Patients and Methods: prospective multicenter case series study of 120 patients with AUB were admitted for surgical intervention in five governmental and private hospitals in Mosul city, Iraq. Blood tests were done preoperatively in addition to ultrasound examination for ET. After obtaining histopathological results, patients were divided into two groups; those with benign endometrial lesions (105 patients) and those with endometrial carcinoma (15 patients). The results of HIM and ET were analyzed statistically. Patients with EC were older with higher body mass index (BMI) than those with benign endometrial lesions. The platelet counts, mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR), platelet- lymphocyte ratio (PLR) and endometrial thickness (ET) were all significantly higher in case of malignancy than benign lesions, lymphocyte count was significantly lower in cases of malignancy. Adding ET to each marker increase its validity indicators mainly neutrophil, lymphocyte, NLR and PLR which increase its negative predictive value significantly.

Conclusion: Combining HIM and ET measurement shows significant promise as a supportive, non-invasive way to held distinguish between benign and malignant lesions. However, larger studies are needed to fully confirm these findings before they can be used as a standard diagnostic indicator.

Keywords: Endometrial carcinoma, Endometrial thickness, Hematological inflammatory markers (HIM), Neutrophil lymphocyte ratio (NLR), Platelet-lymphocyte ratio (PLR).

INTRODUCTION

Endometrial carcinoma (EC) is recorded as the most common gynecological carcinoma and the fourth common carcinoma in women and nearly 90% of these patients have abnormal uterine bleeding (AUB) [1]. AUB is any intermenstrual bleeding (INB), postmenopausal bleeding (PMB) or disturbance in menstrual cycle regularity, frequency, duration, and/or volume of flow [2] The documents discussed the epidemiology of AUB, noting its prevalence ranges from 10% to 30% among reproductive-aged women, with heavy menstrual bleeding being particularly common [3]. PMB is the main presentation of EC in 10 – 20% of cases but the other causes of PMB are benign namely endometrial atrophy and EC may exist in the absence of bleeding [4,5]. Other EC presentation could be IMB or heavy and/or prolonged periods [5].

Risk factors of EC include excessive unopposed estrogen, early menarche, infertility, polycystic ovarian cyst syndrome, high BMI, old age, hypertension and diabetes mellitus [6]. Cancer development is linked to inflammation, as chronic inflammation plays a critical role in tumor initiation, progression, and the host's antitumor immunity. So that systemic inflammatory biomarkers might offer beneficial information concerning the presence of malignancy [7]. Such biomarkers as white blood cell count, neutrophils, lymphocytes and platelets with various ratios like the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR), provide insight into underlying causes and prognostic outcomes [8]. The host responds to

malignant tumors by systemic inflammation leading to thrombocytosis, neutrophilia and lymphocytopenia [9]. Neutrophils increase in response to pathogens. They secrete pro-inflammatory cytokines, chemokines and vascular endothelial growth factor that may play significant roles in the progression of tumor and facilitate its growth [10]. Platelets have a significant role in immune responses, cancer progression and metastasis of a malignant disease [1]. Lymphocytes are involved in regulation of tumor immunity [7]. Significant decline in lymphocytes was noticed in EC in comparison to other endometrial pathologies [11]. High NLR is closely associated with the existence of inflammation and oxidative stress and is a good indicator of systemic inflammation [12]. PLR may reflect disorder of the immune system and, consequently, abnormal anti-tumor activity [8].

PATIENTS AND METHODS

Study design

This study was a prospective multicenter case series study to evaluate some HIM in cases with EC and compared to their levels in cases with benign endometrial lesions in addition to ET.

Study setting

This prospective case series study was held between the 1st January and the 31st of August 2024 in two governmental (Al-Khansaa, Al- Batool) and three private hospitals in Mosul city/Iraq for which the patients were admitted for surgical intervention (endometrial sampling or hysterectomy) due to AUB.

Inclusion and Exclusion Criteria

Patients with AUB who were eligible in this study were free from any acute or chronic inflammatory disorders, not taking medication for the previous three months (corticosteroid, anti-inflammatory drugs, progesterone, hormonal replacement therapy (HRT)) and no blood transfusion. No history of previous or current malignancies other than EC. They were not pregnant and accepted to be participated in this study. Patients who didn't meet the above-mentioned criteria were excluded.

Data Collection and Sampling

of A total of 120 patients aged 35 years and over were enrolled in the study using convenience sampling method during the study period. A detailed history was taken from the patients followed by physical examination. Trans-vaginal ultrasound examination for ET were performed. Preoperative blood samples were collected from patients to measure the neutrophil, lymphocyte, platelets count and mean platelet volume (MPV) then neutrophil lymphocyte count (NLR) and PLR ratios were obtained by dividing absolute neutrophil count by absolute lymphocyte count and dividing absolute platelet count by absolute lymphocyte count respectively [10]. Fifty-six (56) patients underwent total abdominal hysterectomy and sixty-four (64) patients underwent endometrial sampling. The specimens were obtained and sent for histopathological examination. According to the final results, the cases were divided into two groups; patients with benign endometrial lesions were (105) and patients with EC (15).

Statistical Analysis

Data coding and tabulation was achieved by Microsoft Excel-2010. Descriptive and analytic statistics was performed using Minitab version 18 software statistical program. The descriptive statistics consist of mean ± Standard Deviation (SD) for measurable variables and frequencies and percentages for categories variables. Independent T-test of two means was used for quantitative variables. Validity markers (Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) were used to evaluate the diagnostic accuracy of HIM and ET in detecting EC**.** Using Exact Wilson Score method to calculate 95% confidence interval (CI) of validity indicators. P-values < 0.05 were considered significant statistically throughout data analysis.

Ethical Approval

All patients were informed clearly prior to process and documented agreement was taken to be part of the study. They were informed that the data would be used exclusively for the purpose of this study. Official approval was obtained from The Iraqi Council of the Iraqi Board of Medical Specializations and the agreement of the Nineveh Health Directorate.

RESULTS

A total of 120 patients with AUB were enrolled in this study. Regarding age; the patients with EC were significantly older (59.9±10.13 years) than those with benign endometrial pathology (50.4 ± 7.73 years) with P=0.001. The mean BMI (kg/m²) was significantly higher in patients with malignancy with P=0.015, Mean age of menarche was lower with significant statistical difference in EC. Mean years since last labour, mean duration of AUB, mean parity, mean abortion and mean age of menopause did not show significant statistical difference as showed in Table **1**. Neutrophil count didn't show significant statistical differences between cases of malignancy and those of benign lesions. Lymphocyte count was significantly lower in case of malignancy than benign P=0.009. Meanwhile, platelet count, NLR, PLR and MPV were all significantly higher in cases of malignancy than benign lesions (P=0.041), (P=0.008), (P= 0.001) and (P=0.001) respectively. ET was significantly higher in case of malignancy than benign lesions with (P=0.042) as seen in Table **2**.

Table 1
Comparison in sociodemographic characteristics between the benign endometrial lesions and EC in the study sampled population

Characteristics	Benign endometrial lesions [n = 105]	Endometrial carcinoma (EC) [n = 15]	All [n = 120]	P-value*
Mean age [years]	50.4 ± 7.73	59.9 ± 10.13	51.6 ± 8.8	0.001
Mean BMI [kg/m^2]	29.5 ± 5.25	32.97 ± 4.14	29.9 ± 5.3	0.015
Mean age of menarche	12.8 ± 1.18	11.9 ± 1.06	12.7 ± 1.20	0.005
Mean duration of AUB, months	4.7 ± 2.64	4.00 ± 2.95	4.1 ± 1.37	0.549
Mean parity	6.1 ± 2.25	6.3 ± 3.22	6.1 ± 2.60	0.772
Mean abortion	1.4 ± 0.86	1.1 ± .92	1.4 ± 0.80	0.519
Mean years since last labor	14.1 ± 7.07	21.6 ± 6.92	15.0 ± 7.5	0.001
Mean age of menopause	49.4 ± 3.20	50.8 ± 3.49	49.8 ± 2.20	0.177

*Independent T-test of two means was used for quantitative variables.

Table 2
The relationship between histopathological results and HIM and ET in the study sampled population

Hematological inflammatory markers	Histopathological results		P-value*
	Benign endometrial lesions [n = 105]	Endometrial carcinoma (EC) [n = 15]	
Neutrophil ×10^9/L	5.1 ± 1.37	5.8 ± 1.83	0.085
Lymphocyte ×10^9/L	2.4 ± 0.71	1.9 ± 0.60	0.009
Platelets ×10^9/L	298.0 ± 68.4	335.0 ± 95.0	0.041
NLR	2.3 ± 0.78	3.4 ± 1.44	0.001
PLR	136 ± 53	190 ± 78	0.001
MPV [fL]	9.4 ± 1.00	10.1 ± 1.13	0.008
ET [mm]	11.9 ± 4.23	14.5 ± 6.60	0.041

*Independent T-test of two means was used

Table 3
Validity indicators and their 95% CI of HIM and ET in the diagnosis of EC in the study sampled population*.

Hematological inflammatory markers	Sensitivity % 95% CI	Specificity % 95% CI	PPV %* 95% CI	NPV %* 95% CI**
Neutrophil ×10^9/L	20.0 [7.1-44.3%]	95.2 [89.3-98%]	37.5 [18.2 - 61.4%]	89.3 [80.97 - 94.30%]
Lymphocyte ×10^9/L	100.0 [79.6-100%]	3.8 [1.5-9.4%]	12.9 [8.0% - 20.2%]	100.0 [95.65% - 100%]
Platelets ×10^9/L	20.0 [7.1-44.3%]	94.28 [88.1-97.4%]	33.3 [21.4% - 47.9%]	89.2 [81.30 - 94.06%]
NLR	80.0 [54.8-93%]	43.8 [43.8-53.4%]	16.9 [10.5% -26.0%]	93.9 [87.31 - 97.18%]
PLR	93.3 [70.2-98.8%]	18.1 [11.9-26.4%]	14.0 [8.7% - 21.9%]	95.0 [86.30 - 98.29%]
MPV [fL]	46.7 [24.8-69.9%]	86.7 [78.9-91.9%]	33.3 [21.4% - 47.9%]	91.9 [84.12 - 96.03%]
ET [mm]	93.3 [70.2-98.8%]	44.8 [35.6-54.3%]	19.4 [13.23-27.53%]	97.9 [92.83 - 99.41%]

* PPV and NPV calculated at apparent prevalence rate in the present study [12.5%] of ET
**95% CI and statistical significance were calculated for validity rate using Exact Wilson Score method

Table 4
Validity indicators and their 95% CI of combined HIM and ET in the diagnosis of EC in study sampled population.

Hematological inflammatory markers	Sensitivity % 95% CI	Specificity % 95% CI	PPV %* 95% CI**	NPV %* 95% CI**
Neutrophil ×109/L +ET	93.3 [70.2% - 98.8%]	67.8 [58.2 - 75.8%]	42.5 [28.5-57.7%]	97.9 [92.3-99.4%]
Lymphocyte ×109/L +ET	100.0 [79.6% - 100%]	25.6 [18.3 -34.8%]	45.3 [23.2-70.8%]	100.0 [96.5-100%]
Platelets ×109/L +ET	40.0 [19.8% - 64.3%]	96.3 [90.6 - 99.0%]	51.5 [35.2-67.5%]	91.2 [84.1-95.3%]
NLR +ET	85.0 [62.1% - 96.3%]	46.2 [37.4 - 56.2%]	25.0 [8.89-53.2%]	94.5 [88.5-97.5%]
PLR +ET	93.8 [68.1% - 99.8%]	31.2 [23.3-40.8%]	34.0 [19.9-52.65%]	96.0 [90.2-98.4%]
MPV [fL] +ET	55.6 30.1% - 75.2%	88.0 [80.0 - 92.6%]	38.5 [17.7- 64.48%]	93.0 [86.3-96.6%]

* PPV and NPV calculated at apparent prevalence rate in the present study [12.5%] of ET.
**95% CI and statistical significance were calculated for validity rate using Exact Wilson Score method

The highest sensitivity and NPV were seen with lymphocyte count and ET followed by PLR, NLR, MPV and platelets with narrow range of 95% CI among NPV. The highest specificity and PPV were reported in neutrophil and platelet count with narrow range of 95% CI as showed in Table 3.

When combining the ET with every marker among HIM as showed in Table **4**, there were increase in validity indicators in all marker except specificity of neutrophil count which decreased from 95.2% to 67.8%. The calculated range of 95%CI for all markers combined with ET were narrower than that reported for markers alone.

DISCUSSION

Differentiating benign from malignant endometrial lesions before surgical intervention remains a major concern for all gynecologist all over the world therefore, simple and accessible tools like HIM and US are utilized for this purpose.

Aging is associated with changes in the immune system causing low-grade inflammation which predispose to various diseases, including malignancies [7]. In the current study; mean age was higher in malignant group than benign; this result was agreed by other studies [7,13].

In this study; malignancy was more among those with high BMI which matched other studies [7,14] as high BMI acts through endometrial proliferation caused by estrogen (which is produced by adipose tissue) in the absence of progesterone [6]. Early menarche was significantly associated with malignancy as reported in other studies [13,15].

Because of close relationship between endometrial pathologies and inflammations [7,16] therefore, several studies have pointed that HIM may play a significant role in predicting EC [17]. Also, the tumor cells produce cytokine and chemokine that induce inflammation which appear as alteration in HIM so it can be used to aid as suspicion of diagnosis [18].

The platelet count is a clue to systemic inflammation caused by the tumor. Some cytokines promote megakaryocyte proliferation with consequence in thrombocytosis [10]. The increased MPV reflect the platelets activation in case of inflammation which may be associated with cancer occurrence [17]. This study manifested, both platelets count and MPV were significantly higher in case of malignancy than in benign lesions which was in line with other studies [10,17,19]. Lymphocytes play an important role in the natural immune defense against malignant tumors. So, decrease of lymphocytes suggests a reduced function of killing tumor cells and the anti-tumor ability of the body is reduced [20]. This study showed that in case of EC there was a statistically significant decrease in lymphocyte count which was consistent with other studies [1,19,21].

In this study, neutrophil count was higher in patients with EC than those with benign endometrial pathologies; however, the difference was not statistically significant which didn't correspond to Temur M. et al study [21] which possibly due to the different sample size.

The NLR reflects the burden of the tumor and the state of immunity [22]. This study coincided other studies [10,21,23] which noticed that NLR were significantly higher in patients with EC than benign endometrial lesions. The elevated PLR observed in EC indicates increased platelet activity, which plays an important role in tumor biology. Platelets help tumor cell survival and shelter them from immune-mediated destruction [24]. This study demonstrated that PLR was significantly higher in carcinoma group than benign, the result matched other studies [1,24]. Maungto et al study [18] concluded; neither NLR nor PLR showed significant statistical difference between benign endometrial lesions and EC and they attributed that to differences in the clinical characteristics of EC cases.

Sonography is considered a proper and noninvasive technique for an initial evaluation of endometrium for an

early diagnosis of EC [25]. In this study, it was found that ET was significantly higher in case of EC which came in line with other studies [21,25].

The validity indicator of neutrophil count in the diagnosis of EC showed in this study a diagnostic sensitivity of 20.0%, specificity 95.2 %, PPV% 37.5% and NPV 89.3%. while Song Y. et al [7] reported 82.1%, 45.7%, 58.0% and 73.7 respectively. In this study regarding NLR for detection of EC; the sensitivity was 80%, specificity 43.8%, PPV% 16.9, NPV% 93.9 while other study recorded sensitivity, 52.4%; specificity, 68.5%, PPV% 60.3 and NPV% 61.2 [7]. In this study; PLR had achieved sensitivity 93.3% and specificity18.1% as in another study achieving high sensitivity and low specificity [24]. Another study reported sensitivity of 88%, and specificity 66% [1]. Thrombocytosis in the current study showed specificity 94.3% and NPV 89.2%. there was no other study in this field to compare with. The validity indicator of MPV in the diagnosis of EC showed in this study a diagnostic sensitivity of 46.7%, specificity 86.7%, PPV% 33.3 and NPV 91.9%. Other study showed sensitivity 76.2%, specificity 55.4%, PPV% 61.0% and NPV 71.8% [7]. In the current study; ET efficacy in detecting EC showed the sensitivity was 93.3%, specificity 44.8%, and that was very close to another study which reported a sensitivity of 94.8% and a specificity of 46.7 for EC detection [6] but was somewhat different from other study where sensitivity was 75% and specificity 83.6% [21].

The differences in HIM validity indicators among different studies could be due to patient's number, cut-off values dependent and accuracy of laboratory tests.

The validity indicators of combined HIM and ET in the diagnosis of EC in this study had a higher value than each factor individually. There was no other study could be found to compare with it.

Study strength and limitations

A prospective multicenter study in addition to direct interview preoperatively as well as restricting malignant lesion to endometrial carcinoma were an important point in increasing strength of this study. The main study limitation was the commitment to the assigned timeframe which affected the number of patients meant for inclusion in the study particularly women with endometrial carcinoma.

CONCLUSIONS

Normal lymphocyte count and thin endometrial thickness below the cut value could aid in decreasing suspicion of EC in cases with AUB. Evaluation of each HIM (mainly lymphocyte count, neutrophil count, NLR and PLR) in combination with ET increase its value in decreasing suspicion of EC. The HIM difference between benign and malignant lesions supported the theory of inflammation in malignancy initiation.

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Conflict of interest

All authors declare that they have no conflict of interest.

Author contribution

The 1st and 2nd authors designed the study; the first author collected the data, and the 1st and 2nd authors analyzed the data; drafted the manuscript and provided critical revisions.

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