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Original Research Paper

Pathology

THE ROLE OF PREOPERATIVE NEUTROPHIL TO LYMPHOCYTE RATIO AND PTEN IMMUNOHISTOCHEMICAL EXPRESSION IN ENDOMETRIAL BIOPSIES – A TWO YEAR STUDY

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ABSTRACT Endometrial cancer (EC) is the most common and second most dangerous gynaecological cancer among women in the United States however in India, the rates are as low as 4.3 per 100,0001. Endometrial hyperplasia (EH) is the ideal benign counterpart for endometrioid carcinoma while atypical hyperplasia is a precursor lesion. Neutrophil-lymphocyte ratio (NLR) is known to be a marker of Inflammation. Increased NLR is found to have worse overall-survival in EC2-3.Therefore, NLR is a cheap, easy, simple, and reproducible marker to distinguish the endometrial pathologies including normal, hyperplasia without atypia, hyperplasia with atypia, and endometrial cancer. PTEN (Phosphate and TENsin homolog deleted on chromosome 10) gene expression is normally present in the cyclical endometrial conditions which are oestrogen rich.Thus its tumour suppressor function is decreased which is directly proportional to cancer risk particularly in high estrogenic states4-5. Immunohistochemistry study of PTEN expression is an effective screening method for endometrial carcinoma. In our study, NLR was found significantly higher in EC among endometrial pathologies and PTEN negative immunoreactivity was detected in the majority of EC and atypical hyperplasia. **Summary:** NLR was found significantly higher in EC among endometrial pathologies. Thus, NLR can be used to discriminate EC from other endometrial pathologies including endometrial hyperplasia and normal endometrial findings.

KEYWORDS : Endometrial Carcinoma, Endometrial Hyperplasia, PTEN Immunohistochemistry, NLR

INTRODUCTION

Endometrial cancer (EC) is the most common and second most dangerous gynaecological cancer among women in the United States however in India, the rates are as low as 4.3 per 100,000[1]. Endometrial hyperplasia (EH) is the ideal benign counterpart for EC while atypical hyperplasia is a precursor lesion.

Neutrophil-lymphocyte ratio (NLR) is known to be a marker of Inflammation. Increased NLR is found to have worse overall-survival in EC[2-3].Therefore, NLR is a cheap, easy, simple, and reproducible marker to distinguish the endometrial pathologies including normal, hyperplasia without atypia, hyperplasia with atypia, and endometrial cancer.

PTEN (Phosphate and TENsin homolog deleted on chromosome 10) gene expression is normally present in the cyclical endometrial conditions which are oestrogen rich. Thus its tumour suppressor function is decreased which is directly proportional to cancer risk particularly in high estrogenic states[4-5]. Immunohistochemistry study of PTEN expression is an effective screening method for endometrial carcinoma.

The current study aimed to investigate the normal, hyperplastic and neoplastic endometrial glands expression by using NLR and PTEN as marker and differentiate between hyperplastic and malignant endometrial glands in endometrial biopsies, thereby evaluating the role of possibility in early diagnosis of endometrial premalignant lesions.

MATERIAL AND METHODS

The study was conducted in the department of Pathology in a tertiary care hospital. The study was conducted over a period of two years. A total of 120 cases of endometrial biopsies were included in the study.

The blood samples of these patients were collected in EDTA vacutainers preoperatively and processed for complete blood picture (CBP) in a 5 part M-6200 haematology analyser. Total leucocyte counts along with absolute neutrophil count and absolute lymphocyte counts are selected for the calculation of NLR. NLR is calculated as the absolute neutrophil count divided by the absolute lymphocyte count.

A normal range of NLR is between 1-2, the values higher than 3.0 and below 0.7 in adults are pathological. NLR in a grey zone between 2.3-3.0 may serve as early warning of pathological state or process such like cancer, atherosclerosis, infection, inflammation, psychiatric disorders and stress.

Endometrial biopsies received in the department of Pathology were labelled, gross examination was done and the tissue was preserved in 10% formalin immediately. After fixation of the tissue for 24-48 hours, it was sectioned and processed for microscopy. Slides were stained with Hematoxylin and Eosin stain. Stained histopathology slides were examined and reported. Immunohistochemistry is done with PTEN immunohistochemical marker using Mouse monoclonal antibody clone 6H2.1, Diagnostic Biosystems (DBS).

PTEN immunohistochemistry is considered positive when brown staining is seen in nuclei or cytoplasm of glandular cells. Interpretation of PTEN Immunohistochemical Expression Negative- <10% staining of glandular cells, 1+ - 10% - 50% staining of glandular cells, 2+ - >50% staining of glandular cells.

OBSERVATIONS AND RESULTS

In our study a total of 120 cases were studied. Most of the patient's biopsies were in the age range of 41-50 years. Range of the age varied from maximum of 62 years and minimum of 28 years. The most common symptom seen is abnormal

uterine bleeding with endometrial causes (AUB-E) around 32 cases out of 120 cases. Endometrial Thickness (ET) of all the cases ranged from 4mm to 36mm.

Table 1: Complaints of the Patients

Symptoms	Number of Cases
Post-menopausal bleeding (PMB)	15 (12.5%)
AUB-L	23 (19.2%)
AUB-E	32 (26.7%)
AUB	24 (20%)
HMB	19 (15.8%)
AUB-O	02 (1.7%)
Prolapse	04 (3.3%)
AUB-A	01 (0.8%)

In this study, abnormal uterine bleeding with endometrial cause is the most common complaint (AUB-E) registered and least common is abnormal uterine bleeding with adenomyosis (AUB-A).

Table 2: Histopathological Diagnosis with Endometrial Thickness

Histopathological Diagnosis	No. of Cases	Endometrial Thickness (ET in mm)
Proliferative Endometrium	16 (13.3%)	13.5 (8.9 – 30)
Secretory Endometrium	24 (20%)	12.0 (4.0 – 20)
Hyperplasia without Atypia	42 (35%)	19.45 (8.9 – 30)
Atypical Endometrial Hyperplasia	30 (25%)	19.0 (8.0 – 30)
Endometrial Carcinoma	08 (6.7%)	21.5 (7.0 – 36)

In our study we found that out of 120 cases, 42 cases (35%) were diagnosed as Hyperplasia without atypia and 6 cases (6.7%) were diagnosed as EC. It was found that ET was maximum in endometrial carcinoma with mean of 21.5 mm (7-36mm).

Table 3: Neutrophil to Lymphocyte Ratio (NLR Ratio) in Various Endometrial Pathologies

Histopathological Diagnosis	NLR (Mean)
Proliferative Endometrium	2.0 (2.5 – 1.3)
Secretory Endometrium	1.92 (1.04 – 2.8)
Hyperplasia without Atypia	2.49 (1.09 – 3.9)
Atypical Endometrial Hyperplasia	2.8 (1.3 – 7.09)
Endometrial Carcinoma Grade 1	3.74 (2.48 – 5.0)
Endometrial Carcinoma Grade 2	3.5 (3.2 – 3.8)
Endometrial Carcinoma Grade 3	3.75

In our study we found that NLR ratio seem to be increasing from premalignant to malignant conditions that is NLR ratio is more in endometrial carcinoma and less in benign phases of endometrium.

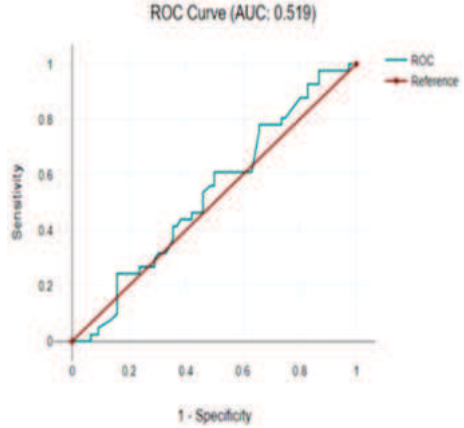

Figure 1: ROC-Hyperplasia Without Atypia

When Receiver Operating Characteristic (ROC) curve plotted between Sensitivity and Specificity for Atypical Endometrial hyperplasia with NLR showing true positive rate.

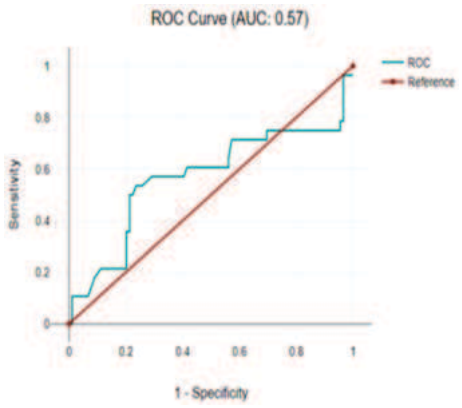

Figure 2: ROC-Atypical Endometrial Hyperplasia with NLR

When Receiver Operating Characteristic (ROC) curve plotted between Sensitivity and Specificity for Atypical Endometrial hyperplasia with NLR showing true positive rate.

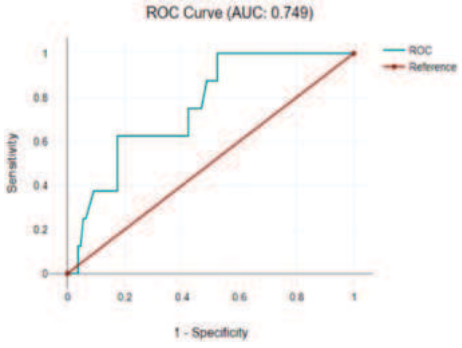

Figure 3: ROC-Endometrial Carcinoma with NLR

When Receiver Operating Characteristic (ROC) curve plotted between Sensitivity and Specificity for Endometrial carcinoma with NLR showing true positive rate.

Table 4: PTEN Expression

Histopathological Diagnosis	Negative	1+	2+	Total
Proliferative Endometrium	0	2 (12.5%)	14 (87.5%)	16
Secretory Endometrium	0	4 (16.7%)	20 (83.3%)	24
Hyperplasia without Atypia	30 (71.4%)	2 (4.8%)	10 (23.8%)	42
Atypical Endometrial Hyperplasia	27 (90%)	2 (6.7%)	1 (3.3%)	30
Endometrial Carcinoma	8 (100%)	0	0	8

In our study we found that PTEN expression was negative in EC and positive in proliferative and secretory endometrium. Out of 42 cases of Hyperplasia without atypia, 30 cases (71.4%) were negative and 12 cases (28.6) were positive for PTEN immunohistochemistry. Out of 30 cases of Atypical Endometrial Hyperplasia, 27 cases (90%) were negative and 3 cases (10%) were positive for PTEN immunohistochemistry.

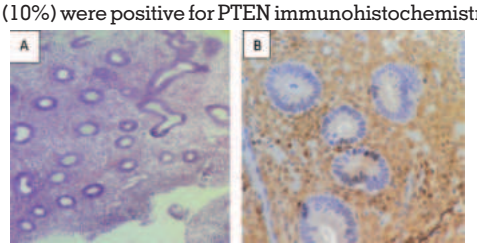

Figure 4: A) H & E stained picture shows endometrium in

Proliferative phase (20X) B) PTEN IHC positively stained glands in Proliferative phase (40X).

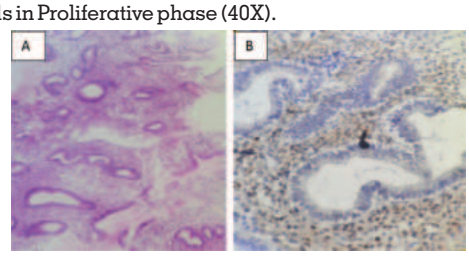

Figure 5: A) H & E stained picture shows endometrium in Secretory phase (20X) B) PTEN IHC positively stained glands in Secretory phase (40X).

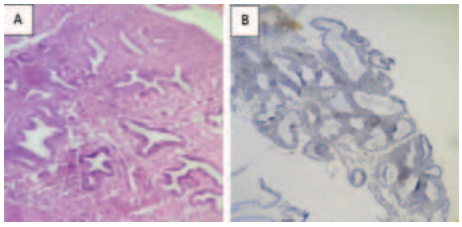

Figure 6: A) H & E stained picture shows Atypical Endometrial Hyperplasia (20X) B) PTEN IHC negatively stained glands in Atypical Endometrial Hyperplasia (20X).

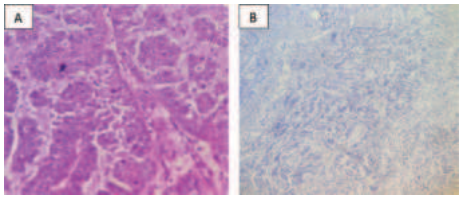

Figure 7: A) H & E stained picture shows Endometrial Carcinoma (40X) B) PTEN IHC negatively stained glands in Endometrial Carcinoma (40X).

DISCUSSION

EH which is usually a result of continuous unopposed oestrogen exposure is a predisposing factor for the development of EC with rates of up to 30%. Although inflammation is known to play a critical role in the etiopathogenesis of cancer by many pathways such as initiation, progression and metastasis, the underlying mechanism between chronic inflammation and cancer has not yet been fully elucidated [6-9].Tumour cells weaken the immune system and accelerate the inflammatory process. This leads to tumour growth and progression of inflammation. Considering this vicious circle between inflammation and cancer, many systemic inflammatory markers have recently been investigated in relation to malignancies. There are very few studies in the literature that determine the relationship between cancerous and precancerous endometrial pathologies and white blood cells, lymphocytes and neutrophil-to-lymphocyte ratio (NLR)[5-9].

Higher NLR association with prognosis can be explained by these: i) immune-response to the tumour is mainly via lymphocytes ii) neutrophils are secreting the vast majority of vascular endothelial growth factor (VEGF) to the circulation which enhances the tumour progression[10].According to Aksoy et al[11] study that investigates the NLR differences between endometrial pathologies revealed that NLR was higher in EC patients rather than patients with non-cancerous endometrial pathologies which is similar to our study.

In a study conducted by Orkun Ilgen et al[12],it was determined that higher NLR is associated with EC as compatible with our study, however, no difference was found between endometrial hyperplasia with atypia and endometrial hyperplasia without atypia patients in terms of NLR, which was in discordance with our study. In the present study, ROC curve analysis was performed to detect a cut-off value to distinguish EC among groups and higher values of NLR were determined associated with cancer. Therefore, it can be said that NLR can be used to discriminate EC from other endometrial pathologies according to the findings of the present study.

Microsatellite instability, mutations of PTEN and K-ras, and nuclear accumulation of β-catenin are the most characteristic molecular alterations associated with these tumours. The tumour suppressor gene named PTEN (Phosphatase and Tensin Homologue) also called MMAC1, is located on chromosome 10q23. It is somatically mutated in several types of tumour. In present study, there is negative expression of PTEN in 71.4%, 90% and 100 % respectively in Hyperplasia without atypia, AEH and EC's respectively.

Table 5: Comparative Studies Showing Loss of Pten Expression

Study	Hyperplasia without atypia	Atypical Endometrial Hyperplasia	Endometrial Carcinoma
Soheila Sarmadi et al13	-	25%	52%
Erkanli S et al14	60%	70%	80%
Shanumugapriya et al15	35%	60%	70%
Present Study	71.4%	90%	100%

CONCLUSION

NLR is systemic immune response parameter that can be easily evaluated from routine blood tests with no additional cost. NLR was found significantly seen on higher side in EC when compared to other endometrial pathologies. Thus, NLR potentially might be used in the future to discriminate EC from other endometrial pathologies including endometrial hyperplasia and normal cyclical endometrium. NLR is used as a reliable and cheap marker of ongoing cancer-related inflammation and a valid indicator of prognosis in solid tumours. NLR has independent prognostic role regarding overall, cancer free and cancer-specific survival.

PTEN is well expressed in cyclical endometrium of both proliferative and secretory phase and showed altered expression in hyperplasia with atypia compared with hyperplasia without atypia. PTEN immunoreactivity is negative in the majority of EC and AEH. PTEN loss appears barely a moderately accurate marker of premalignant hyperplasia. Therefore, its usefulness in the common practice should be further investigated.

Conflicts of Interest: NONE

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