Role of preoperative red cell distribution width (RDW), mean platelet volume (MPV), neutrophil to lymphocyte ratio (NLR) and CA 125 in differential diagnosis of epithelial ovarian tumors

1. Malathi Verabelly
Associate Professor
Department of Obstetrics and Gynecology, Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad, Telangana, India
9291279091
malathi1174@yahoo.com

2. Swapnarani Seedipally
Assistant Professor, Department of Obstetrics and Gynecology, Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad, Telangana, India
9440548041
swapnasuren@gmail.com

3. Darshana Shinde
Post Graduate
Department of Obstetrics and Gynecology
Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad, Telangana, India
7702733605
Drdarshanashinde0253@gmail.com

Corresponding Author: Swapnarani Seedipally
Assistant Professor
Department of Obstetrics and Gynecology, Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad, Telangana, India
9440548041
swapnasuren@gmail.com

Abstract:
Background: The discovery of early ovarian cancer indicators is critical for improving the patient diagnosis, therapy effectiveness, and prognosis of the ovarian tumors
Objective: To study the role of preoperative red cell distribution width (RDW), mean platelet volume (MPV), neutrophil to lymphocyte ratio (NLR) and CA125 in the differential diagnosis of epithelial ovarian tumors
Methods: We carried out a cross-sectional study in 90 patients diagnosed withthe ovarian pathology. They were assessed clinically and later admitted. Routine investigations along with cancer biomarkers like CA 125, RDW, MPV, and NLR were tested. Patients were posted for Laparotomy with hysterectomy/Ovarian cystectomy and specimen sent for histopathology. They were followed post operatively.
Results:60% of the cases were benign and 7.8% were malignant. All the benign cases were <45 years. Malignancy was slightly more in nulliparous women compared to multiparous

women. Biomarkers like CA 125, MPV and NLR were significantly elevated in malignant cases compared to benign cases. All the cases having RMI <25 were benign. All the biomarkers were significantly increased in borderline and malignant cases compared to the benign cases.
Conclusion: There was significant association observed between CA125, MPV, NLR and ovarian tumours. CA125, NLR are positively correlated whereas MPV was negatively correlated. The combination of these biomarkers or at least 2 or 3 biomarkers are suggested for early stage diagnosis of pelvic mass with high sensitivity and specificity. The hematological parameters such as MPV, NLR along with CA125 are useful in the detection of the malignant ovarian tumours.

Key words:biomarkers, cancer, tumor, metastasis, detection

Introduction

Ovarian tumors include a wide spectrum of neoplasms involving a variety of histological types. The most common are epithelial tumors forming 80% of all tumors. Among all malignant tumors 90% are epithelial in origin. It is the sixth most common cancer among women (Age standardized incidence rate being 6.6/100,000) and seventh leading cause of cancer deaths globally (age standardized mortality rate being 4.0/10,000). Late presentation and ineffective screening methods are impediments in its early detection. The screening tests in form of estimation of CA-125 and Transvaginal sonography are non-specific. Therefore, the diagnosis is made in late stage when cure rates are low and with increased morbidity due to limited effective treatment options.[1]

Age has a strong correlation to ovarian cancer risk and 80% cases are diagnosed after 50 years of age. [2]Advancing age increased the possibility of malignant transformation. Murthy et al reported that the disease increases from 35 years of age and reaches a peak between the ages 55-64.[3]

The risk factors associated with ovarian tumors are age, hormonal influences, positive family history, reproductive factors and genetic factors. [4]

Previous history of breast cancer and radiotherapy treatment also carried long term risk of ovarian cancer. Other factors which are related to increased risk include obesity due to increase in free biologically active estradiol, endometriosis (related to endometrioid and clear cell carcinoma), and asbestos and talc exposure.[2]

The protective factors include pregnancy, use of contraceptive pills, non-steroidal anti-inflammatory drugs (NSAIDs), hysterectomy, and salpingo-oophrectomy. Tubal ligation decreased risk by 39% while Hysterectomy decreased by 50%. The role of diet, ovulation inducing agents, ethnicity and smoking is inconclusive as the findings are contradictory in various studies.[5] Clinical symptoms include dyspepsia, pain in abdomen and abdominal distension in majority of cases. Chronic inflammation can lead to cancer.[5]

Because of their high complication rate, ovarian tumors constitute a significant diagnostic challenge in the field of gynecological oncology.The gynecologist is put to the test by the benign nature of the tumor's ability to remain clinically quiet for an extended length of time. The five year survival rate can be increased by more than 90% if the ovarian cancers are detected and treated early. There are many methods available for the same, each having its own advantages and limitations. [6]

The discovery of early ovarian cancer indicators is therefore critical for improving patient diagnosis, therapy effectiveness, and prognosis. In this study an attempt was made to know if there was association between biomarkers such as CA-125, RDW, MPV and NLR with histomorphology of the epithelial ovarian tumors. Many biomarkers are being discovered after the discovery of CA 125. [7] CA 125 has more false positivity, even then it is commonly

used. [8]Inflammatory cytokines by various mechanisms cause increased release of immature RBCs. This increases the heterogeneity of peripheral RBCs and RDW. [9]
Low MPV can lead to ovarian tumor. But, the mechanism is not clear. There is increased immune response due to inflammation and coagulation. This mechanism leads to development of the tumor and metastasis. [10]
"Other biomarkers, especially markers of systemic inflammation, ranging from neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), to monocyte-to-lymphocyte ratio (MLR), have been revealed to possess potential to predict survival in a variety of human cancers, including Epithelial ovarian cancer (EOC)." [11]
Our study tries to find out the efficacy of CA125 biomarker in screening of EOC when combined with RDW, MPV and NLR.

MATERIAL AND METHODS

We carried out the cross-sectional study in patients diagnosed to have ovarian pathology at Malla Reddy Institute of Medical Sciences, Suraram from August 2019 to August 2021.
Based on proportion of ovarian cancer, among all the cancers in females observed in earlier publication (Murthy et al[3]) and with 95% confidence and 5% allowable margin of error minimum sample size came to be 87. Sample size was calculated using (www.calculator.net) online sample size calculator.[3]We could include a total of 90 cases.

Inclusion criteria:

1. Female patients with age >30 years.
2. Patient diagnosed to have ovarian pathology on USG
3. Patients posted for surgery and untreated.

Exclusion criteria:

1. Patients with associated systemic diseases
2. Severe anemia, recent blood transfusion, recent iron therapy
3. Venous thrombosis, advanced cases of malignancy
4. Known cases of cancer

Sample collection: Venous blood (2 mL) was collected using standard guidelines and all aseptic precautions.

Methodology

This study was conducted in Malla Reddy Institute of Medical Sciences. After patients' consent was obtained, a detailed history along with examination was done for all patients coming to gynecologyoutpatient department. Patients suspected of having adnexal pathology was clinically assessed and was admitted for further evaluation. Routine investigations like Complete blood picture including platelets, complete urine examination, random blood sugar, viral serology for HIV, HbSAg, VDRL, HCV, Thyroid profile, CA125, blood urea, serum creatinine, bleeding time, clotting time, ECG along with ultrasonography for Abdomen and Pelvis to look for size, shape, site along with Doppler (in suspicion to rule out torsion) is done. RMI Score was calculated and was noted. After getting anesthesia clearance patients were posted for Laparotomy with hysterectomy/ Ovarian cystectomy depending on patients' profile and specimen was sent for histopathological examination. Postoperatively patient was followed up.

Results

Table 1: Histopathological severity of tumors according to age groups

Age (years)	Histopathological severity of tumors

	Benign	Borderline	Malignant
< 45	54 (100%)	2 (6.9%)	0
45-65	0	23 (79.3%)	4 (57.1%)
> 65	0	4 (13.8%)	3 (42.9%)
Total	54 (60%)	29 (32.2%)	7 (7.8%)

Table 1 shows Histopathological severity according to age groups. 60% of the cases were benign and only 7.8% were malignant. All benign cases were seen in the age of less than 45 years. Age group of 45-65 years was having maximum borderline and malignant cases.

Table 2: Distribution of cases according to Parity and Histopathological severity of tumors

Age (years)	Histopathological severity of tumors		
	Benign	Borderline	Malignant
Nulliparous	4 (7.4%)	7 (24.1%)	4 (57.1%)
Multipara	50 (92.6%)	22 (75.9%)	3 (42.9%)
Total	54 (60%)	29 (32.2%)	7 (7.8%)

Table 2 shows distribution of cases according to Parity and Histopathological severity of tumors. The malignancy rate was slightly more in nulliparous women. The multiparous women had significantly higher proportion of benign tumor compared to the nulliparous.

Table 3: Association between biomarkers values and Histopathological severity of tumors

Biomarkers		Histopathological severity of tumors		p
		Benign/borderline	Malignant	
CA 125 (U/ml)	< 35	49	1	**0.0115**
	≥ 35	34	6	
RDW	< 14.5	39	1	0.0944
	≥ 14.5	44	6	
MPV	< 12	80	2	**< 0.001**
	≥ 12	3	5	
NLR	< 5.25	74	1	**< 0.001**
	≥ 5.25	9	6	

Table 3 shows association between biomarkers values and Histopathological severity of tumors. The biomarkers like CA 125, MPV and NLR were significantly elevated in malignant cases compared to benign/borderline cases. But, not for RDW.

Table 4: Distribution of 'Risk of Malignancy Index' (RMI) according to severity of histo-pathological types

Severity of histo-pathological types	Risk of Malignancy Index' (RMI)		
	< 25	25 – 250	> 250
Benign	24 (100%)	30 (73.2%)	0 (0%)
Borderline	0 (0%)	6 (14.6%)	23 (92%)
Malignant	0 (0%)	5 (12.2%)	2 (8%)
Total	24 (26.7%)	41 (45.6%)	25 (27.8%)

Table 4 shows distribution of 'Risk of Malignancy Index' (RMI) according to severity of histo-pathological types. All cases having RMI <25 were benign. Among 41 cases with RMI

25-250, majority were benign. Among 25 cases with RMI >250; 92% were borderline and 8% were malignant and no case was benign.

Table 5: Mean values of biomarkers in different types of tumors

Type of tumor	CA 125	RDW	MPV	NLR
Benign	21.96 ± 10.28	13.33 ± 1.53	13.15 ± 0.71	2.82 ± 0.40
Borderline	165.57 ± 93.19	18.78 ± 0.84	13.19 ± 1.14	5.11 ± 0.55
Malignant	123.42± 177.88	18.21 ± 1.92	10.57 ± 1.58	5.44 ± 0.61
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 5 shows mean values of biomarkers in different types of tumors. All the biomarkers were significantly increased in borderline and malignant cases compared to the benign cases.

DISCUSSION

The discovery of early ovarian cancer indicators is therefore critical for improving patient diagnosis, therapy effectiveness, and prognosis. In this study an attempt was made to know if there was association between biomarkers such as CA-125, RDW, MPV and NLR with histomorphology of the epithelial ovarian tumors.

Age has a strong correlation to ovarian cancer risk and 80% cases are diagnosed after 50 years of age. [2] Advancing age increased the possibility of malignant transformation. Murthy et al reported that the disease increases from 35 years of age and reaches a peak between the ages 55-64.[3]

According to Suk Hee Heo et al., the age-adjusted incidence of ovarian malignancy in women older than 20 years is reported to be 11.446 per 100,000.[12] Only 93 percent of epithelial tumors arose between the ages of 20 and 59 years. Mucinous tumors appear to affect people of various ages, ranging from 10 to 59 years old.

The percentage of benign tumors in this study was 60% which is in agreement with majority of the studies in the past which showed percentage ranging from 66% to 76%. There were also some studies like Vora S et al[13] where the percentage of the benign tumors was 38%. The probable reason for the lower percentage of the benign would have been smaller study population and selection bias.

This probable mismatch in percentages of borderline and malignant tumors between prior research and ours might be attributed to the widespread utilization of early screening investigations such as imaging and biomarkers. As a result, we think that tumor screening enhanced the discovery of tumors at an early stage of malignancy rather than full-fledged malignancy. Therefore, this might have led to increase in number of borderline tumors at the expense of malignant tumors. This hypothesis needs further validation with bigger and longer studies.

Among surface epithelial ovarian tumors, serous cystadenoma (61 cases 67%) were common, which was followed by mucinous cystadenoma (25 cases 27%). The malignant tumors were seen more in serous tumors (2 cases 2.2%), as compared to mucinous (1 case 1.1%)

It has been hypothesized that ovarian cancer risk is increased with a greater number of lifetime ovulatory cycles, which results in more mitotic events and chances for genetic mutation. [14] Several studies have suggested that the reason for nulliparity (such as infertility

or subfertility) may be important in ovarian cancer.[15] In our study, maximum number of cases were seen in multiparous women around 83.3% when compared to nulliparous women (16.7%). But risk of malignancy in nulliparous women is around 26.6% when compared to multiparous women (4%).

Despite a substantial amount of research examining its function, the significance of CA125 in health and illness is still poorly understood. CA125 may have a function in cell-mediated immune response based on the peculiar characteristics of the oligosaccharides connected to it. [16]In women with OC, median CA125 levels were 18.0 over 5 years before to diagnosis, but only 10.9 in healthy women. The median CA125 level was 27.2, months after diagnosis. CA125 is >30 U/ml in 50 percent and 24 percent of patients up to 18 and 60 months before diagnosis, according to a case-control study utilizing the same data. [17] The mean CA-125 levels in our cohort with benign OC was 21.96 U/ml, little higher than the previous study by Zurawski et al. [17]

Using the JANUS databank and a nested case-control approach, preclinical CA125 levels were determined for 668 ovarian cancer patients (478 invasive and 190 borderline) and 1989 matched controls with a longer follow-up. Over the course of the study, 15% of the 478 ovarian cancer patients had increased CA125 at the time of blood collection, compared to 6% of the controls. Women with increased CA125 had a statistically significant greater incidence of OC (OR = 3.1). Importantly, limiting the study to patients with serum sample within two years after diagnosis and matched controls resulted in a higher OR of 13.0 (2). Similar study was done in UK with similar outcomes. [18] Similarly in our study the mean CA-125 levels in borderline and malignant were 165±93 and 123±177 U/ml levels respectively, much higher than benign levels

Although the relevance of RDW levels is well recognized, there was no significant association found in our present study between tumor histopathological severity and RDW levels (p: 0.09).

Platelets are important regulators of inflammation. Platelet activation is increased by released inflammatory mediators, which results in a shift in MPV. The reduction in MPV might be due to platelet consumption at the inflammatory site. Furthermore, an inflammatory environment can inhibit megakaryopoiesis, causing tiny platelets to be released from the bone marrow. [19]

Inflammation may be responsible for the association between MPV and survival. There is a strong linkage between inflammation and cancer. [20] Moreover, platelet plays an essential role in inflammation and cancer. MPV is an early parameter of activated platelets. Large platelets are more reactive than their smaller counterparts in releasing a variety of pro-inflammatory cytokines, and are more likely to aggregate. The aggregation at sites of inflammation leads to the intensive infiltration of large platelets into vascular and intestinal wall, and the reduction of platelet size. [21] Therefore, lower MPV values could be suggestive of an enhanced consumption of large platelets in inflammatory states. [19] These data are also consistent with the current knowledge that anti-platelet is considered to be a part of cancer adjuvant therapy. [22]

The NLR is an inflammatory and immune-related measure that is computed by dividing the total number of neutrophils in the blood by the total number of lymphocytes in the blood. This reflects both the tumor microenvironment's neutrophilia and lymphopenia. The increased number of neutrophils aids cancer cell invasion, migration, and angiogenesis, all of which contribute to cancer growth.[23]

NLR is an inflammatory marker that is used to assess a patient's health. In epithelial ovarian cancer, pre-treatment NLR was raised, and it had predictive prognostic relevance for survival following therapy. Antigen and antibody against the antigen create immune complexes (ICs),

and circulating immune complexes (CICs) are free ICs that circulate through bodily fluids. Large CICs are normally removed by macrophages, whereas tiny CICs are filtered out of the body by the renal filtration system. Some middle-sized CICs, on the other hand, are unable to be cleared and linger in the circulatory system. These CICs have the potential to trigger the inflammatory response, which is a key component of immunological complex illness. Daniel et al. showed the presence of CA125 CICs in 2010 and proposed that CA125 CICs might explain ovarian cancer with low CA125 values.[24]

For individuals with low CA125 concentrations, CA125>7.65 U/ml and NLR >1.72 would be an effective maker. A novel cancer screening technique with two-step evaluation that is easy to administer and cost-effective must be developed to prevent missing a diagnosis of ovarian cancer owing to a false negative during regular physical examination.[25]

In our study with 90 patients with ovarian pathology NLR was raised in malignant ovarian tumors when compared to benign with p value <0.001.

Limitations:

In this study they were few limitations, like it was small sample size as compared to the prevalence of the disease, and they were no follow up done during the study period. Study sample consisted of patients of varied age groups. Biomarker values are not as sensitive as it can get raised in other co morbid conditions. In our study few malignant conditions had normal blood parameters hence larger studies needs to be done.

Conclusion:

Benign tumours constituted majority of the cases in the cohort (61%), followed by borderline tumours 32% and malignant tumours 7%.There was no clear correlation seen between RDW and ovarian tumours (i.e., P value not significant). There was significant association observed between CA125, MPV, NLR and ovarian tumours. There was no significant association noted between biomarkers and cell type (serous/mucinous) of the ovarian tumours. According to our study CA125, NLR are positively correlated whereas MPV was negatively correlated. According to the results of this study, the combination of these biomarkers or at least 2 or 3 biomarkers are suggested for early stage diagnosis of pelvic mass with high sensitivity and specificity. The haematological parameters such as MPV, NLR along with CA125 is useful in the detection of malignant ovarian tumours have been evaluated as useful new markers.

References:

1. Jindal D, Sahasrabhojanee M, Jindal M, D'Souza J. Epidemiology of epithelial ovarian cancer: a tertiary hospital based study in Goa, India. Int J Reprod Contracept Obstet Gynecol. 2017 May 25;6(6):2541.
2. Olaitan A, Doufekas K. Clinical epidemiology of epithelial ovarian cancer in the UK. Int J Women's Health. 2014; 6:537-45.
3. Murthy NS, Shalini S, Suman G, Pruthvish S, Mathew A. Changing trends in incidence of ovarian cancer - the Indian scenario. Asian Pac J Cancer Prev 2009;10(6):1025–30.
4. Quirk JT, Mettlin CJ, Moysich KB, Swede H. Risk factors for invasive epithelial ovarian cancer by histological subtype. JHAS 2004;3. Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.566.3800&rep=rep1&type=pdf Accessed on 24-8-2021
5. McLemore MR, Miaskowski C, Aouizerat BE, Chen L, Dodd MJ. Epidemiological and Genetic Factors Associated With Ovarian Cancer: Cancer Nurs. 2009 Jul;32(4):281–8.
6. Li J, Dowdy S, Tipton T, Podratz K, Lu W-G, Xie X, et al. HE4 as a biomarker for ovarian and endometrial cancer management. Expert Rev Mol Diagn. 2009 Sep;9(6):555–66.
7. Huang J, Hu W, Sood AK. Prognostic biomarkers in ovarian cancer. 2011 Oct 14;8(4–5):231–51.

8. Mai PL, Wentzensen N, Greene MH. Challenges Related to Developing Serum-Based Biomarkers for Early Ovarian Cancer Detection. Cancer Prev Res (Phila Pa). 2011 Mar 1;4(3):303–6.
9. Qin Y, Wu Y, Xian X, Qin J, Lai Z, Liao L, et al. Single and combined use of red cell distribution width, mean platelet volume, and cancer antigen 125 for differential diagnosis of ovarian cancer and benign ovarian tumors. J Ovarian Res. 2018 Jan 22;11(1):10.
10. Babu SN, Chetal G, Kumar S. Macrophage Migration Inhibitory Factor: a Potential Marker for Cancer Diagnosis and Therapy. Asian Pac J Cancer Prev. 2012 May 30;13(5):1737–44.
11. Li Z, Hong N, Robertson M, Wang C, Jiang G. Preoperative red cell distribution width and neutrophil-to-lymphocyte ratio predict survival in patients with epithelial ovarian cancer. Sci Rep. 2017 Feb 22;7:43001.
12. Heo SH, Kim JW, Shin SS, Jeong SI, Lim HS, Choi YD, et al. Review of Ovarian Tumors in Children and Adolescents: Radiologic-Pathologic Correlation. RadioGraphics. 2014 Nov;34(7):2039–55.
13. Vora S, Bhargava VL. Study of Ovarian Neoplasms. J Obstet Gynecol India. 1969;19:358.
14. Fathalla MF. Incessant ovulation--a factor in ovarian neoplasia? Lancet Lond Engl. 1971 Jul 17;2(7716):163.
15. Riman T, Persson I, Nilsson S. Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence. Clin Endocrinol (Oxf). 1998 Dec;49(6):695–707.
16. Kui Wong N, Easton RL, Panico M, Sutton-Smith M, Morrison JC, Lattanzio FA, et al. Characterization of the Oligosaccharides Associated with the Human Ovarian Tumor Marker CA125. J Biol Chem. 2003 Aug;278(31):28619–34.
17. Zurawski VR, Orjaseter H, Andersen A, Jellum E. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: Relevance for early detection of ovarian cancer. Int J Cancer. 1988 Nov 15;42(5):677–80.
18. Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, et al. Screening for ovarian cancer: a pilot randomized controlled trial. The Lancet. 1999 Apr;353(9160):1207–10.
19. Yuri Gasparyan A, Ayvazyan L, Mikhailidis D, Kitas G. Mean Platelet Volume: A Link Between Thrombosis and Inflammation? Curr Pharm Des. 2011 Jan 1;17(1):47–58.
20. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008 Jul;454(7203):436–44.
21. Gasparyan AY, Sandoo A, Stavropoulos-Kalinoglou A, Kitas GD. Mean platelet volume in patients with rheumatoid arthritis: the effect of anti-TNF-alpha therapy. Rheumatol Int. 2010 Jun;30(8):1125–9.
22. Mezouar S, Frère C, Darbousset R, Mege D, Crescence L, Dignat-George F, et al. Role of platelets in cancer and cancer-associated thrombosis: Experimental and clinical evidences. Thromb Res. 2016 Mar;139:65–76.
23. Powell DR, Huttenlocher A. Neutrophils in the Tumor Microenvironment. Trends Immunol. 2016 Jan;37(1):41–52.
24. Cramer DW, O'Rourke DJ, Vitonis AF, Matulonis UA, DiJohnson DA, Sluss PM, et al. CA125 Immune Complexes in Ovarian Cancer Patients with Low CA125 Concentrations. Clin Chem. 2010 Dec 1;56(12):1889–92.
25. Zhang H, Huo Q, Huang L, Cheng Y, Liu Y, Bao H. Neutrophil-to-Lymphocyte Ratio in Ovarian Cancer Patients with Low CA125 Concentration. BioMed Res Int. 2019 Jun 25;2019:1–7.