Original Article

NGAL/MMP-9 as a Biomarker for Epithelial Ovarian Cancer: A Case–Control Diagnostic Accuracy Study

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Abstract

Background: Epithelial ovarian cancer (EOC) are often diagnosed late due to lack of specific symptoms and efficient tumor markers. Neutrophil gelatinase-associated lipocalin/matrix metallopeptidase-9 (NGAL/MMP-9) complex are involved in the development and progression of various cancers and have potential as a biomarker for diagnosing ovarian cancer.

Objectives: To compare the serum NGAL/MMP-9 complex levels in patients with EOC, benign ovarian tumor, and healthy controls, and determine the potential cut-off values of NGAL/MMP-9 complex for diagnosing EOC.

Materials and Methods: The study included 50 patients each with EOC and benign ovarian tumor, along with 50 age-matched healthy controls ($N = 150$). The level of serum NGAL/MMP-9 complex was estimated based on sandwich ELISA. The mean and median of the three groups were compared, and the ROC curve was used to determine the optimum cut-off, sensitivity, and specificity of serum NGAL/MMP-9 complex levels in the diagnosis of EOC.

Results: A significant difference was found in the median values of the NGAL/MMP-9 complex (malignant EOC: 67.5 ng/ml, benign ovarian tumor: 53.7 ng/ml, controls: 29.2 ng/ml; $P < 0.01$). NGAL/MMP-9 complex level was also significantly associated with the FIGO staging (Stages I and II: 42.9 ng/ml; Stages III and IV: 70.5 ng/ml; $P < 0.003$). At a 55.0 ng/ml cut-off value, the NGAL/MMP-9 complex had 82.0% sensitivity and 78.0% specificity in diagnosing EOC.

Conclusion: The NGAL/MMP-9 complex may be a promising biomarker for determining the progression of EOC as well as in detecting advanced-stage ovarian cancer.

Keywords: Biomarker, diagnosis, epithelial ovarian cancer, FIGO staging, matrix metalloproteinase, neutrophil gelatinase-associated lipocalin

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the second most common cancer among women after cervical cancer and is the most lethal gynecological malignancy.[1] In the advanced stages of EOC, the recurrence rate is high and the overall 5-year mean survival rate is only around 45–50%, despite aggressive treatment modalities.[2,3] EOC often does not present with specific symptoms or remains asymptomatic,

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particularly in the early stages.[4] This makes its early diagnosis and the differentiation between malignant EOC and benign tumor challenging;[5] these are critical factors in decreasing mortality and improving survival rates.[6] More than 70% of EOC cases are diagnosed late because of lack of specific symptoms and efficient tumor markers.[7]

Cancer antigen 125 (CA-125) and radiological tools are currently used for the diagnosis and prognosis of EOC, but have low efficiency in making an early-stage diagnosis.[8,9] Therefore, there is a need to identify new biomarkers that identify EOC early, and in turn, potentially improve clinical outcomes. Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a promising new biomarker for diagnosing various types of cancer.[10-16]

NGAL, also known as lipocalin 2, is a small protein of 178 amino acid residues encoded by the *LCN2* gene. It is highly expressed in the presence of bacterial infections in granules of the neutrophils. It binds with gelatinase B and specific receptors at the cell surface,[17-19] and covalently with matrix metalloproteinase-9 (MMP-9) protein to form the NGAL/MMP-9 complex. The NGAL/MMP-9 complex is involved in the development and progression of cancer.[20] Normal ovarian cells do not express or have a low expression of NGAL protein, but this expression is high in ovarian cancer cells.[10] Highly expressed NGAL from ovarian cancer cells may enter circulation and could be measured in the blood by a simple test. Furthermore, it has been found that urine MMP-9 could be used to distinguish ovarian cancer patients with a normal level of CA-125 from healthy controls; therefore, urinary MMP-9 can be clinically valuable in identifying advanced or recurring ovarian cancer.[11] High expression of NGAL has also been found in breast,[12] colon,[13] rectal,[14] lung,[15] and pancreatic cancers.[16]

Despite its promise, there is currently insufficient evidence regarding the use of NGAL/MMP-9 complex as a biomarker for diagnosing ovarian cancer. Therefore, the current study analyzed the serum NGAL/MMP-9 complex in patients with benign and malignant ovarian cancer and assessed its accuracy as a diagnostic cancer biomarker. Based on the literature, the authors hypothesize that the NGAL/MMP-9 complex levels may increase in EOC, making it useful for diagnosing EOC and differentiating between benign and malignant EOC.

MATERIALS AND METHODS

Study design and setting

This is a hospital-based, prospective, case–control diagnostic accuracy study conducted in the Biochemistry and the Obstetrics & Gynecology departments of Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India, between 2012 and 2015. The study was approved by the Ethics Committee of Maulana Azad Medical College, New Delhi, India, and was performed in accordance with the Declaration of Helsinki, 2013. This manuscript was prepared following the STARD guideline.

Study population

The study included a total of 150 participants who were selected conveniently and divided into the following three groups: Group I (50 histopathologically diagnosed patients of ovarian cancer), group II (50 patients with benign ovarian conditions), and group III (50 age-matched healthy controls with no indication of benign or malignant ovarian pathology), as determined by clinical examination and relevant investigations [Figure 1].

On histopathological examination of the biopsy samples, patients diagnosed with non-malignant ovarian conditions were included in group II. Patients of malignant ovarian tumors were categorized according to the International Federation of Gynecology and Obstetrics (FIGO) staging, and any FIGO stage of EOC was considered eligible for inclusion in this study. Healthy controls were randomly enrolled from the outpatient department of Lok Nayak Hospital, and were neither on any medications nor diagnosed with any acute or chronic disease. Those presenting with any other malignancy, on steroid therapy, with kidney disease, and chronic inflammation were excluded from the study.

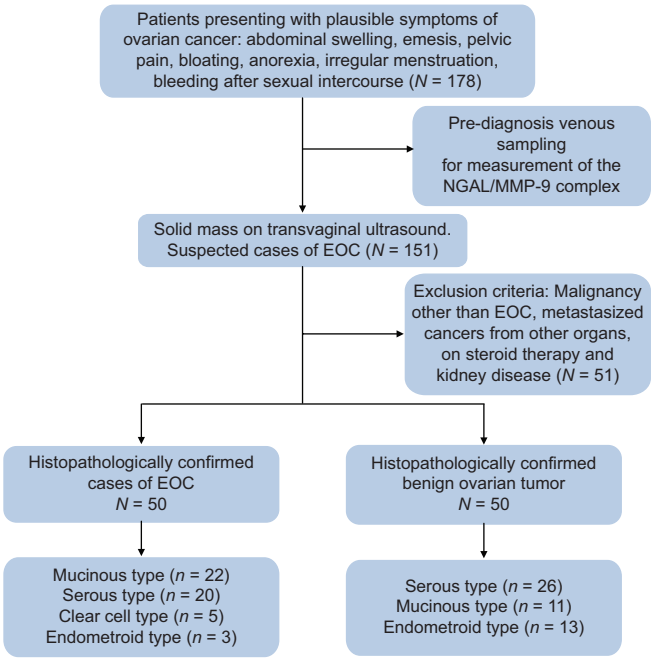

Figure 1: Flow of participants included in the study

Sample size calculation

The authors first assessed the NGAL/MMP-9 complex levels in 10 EOC patients, of which 2 patients were found to have had increased NGAL/MMP-9 complex (i.e., prevalence = 20.0%). Based on this and using the formula $N = (Z2)(pq)/L2$ (Z = Confidence interval, P = prevalence, q = 1-prevalence and L = absolute error of 10%), the sample size was calculated as 64. For increased statistical power, the authors included 150 participants across the three groups.

Neutrophil gelatinase-associated lipocalin/matrix metallopeptidase-9 complex estimation

About 4 ml blood was collected before surgery from patients of EOC and benign ovarian tumors and healthy controls in plain vacutainer to estimate the NGAL/MMP-9 complex. Serum NGAL/MMP-9 complex was estimated by commercially available enzyme-linked immunosorbent assay (ELISA) kit of R & D Systems (Minneapolis, MN, USA) based on the principle of sandwich ELISA.

Statistical analysis

All data were evaluated using SPSS PC version 17 (SPSS Inc., Chicago, IL, USA). Non-skewed data were expressed as mean and standard deviation (SD), and skewed data were expressed as median and range. Significant differences of mean and median in different groups were calculated by the ANOVA and the Kruskal–Wallis tests, respectively. The receiver operating characteristic (ROC) curve was used to detect the optimum cut-off of the parameters, their sensitivity, and specificity in predicting the diagnosis of ovarian cancer. $P < 0.05$ was considered statistically significant.

RESULTS

The mean (±SD) ages of patients with malignant EOC, benign ovarian tumors, and healthy controls were 50.1 (±10.7), 43.6 (±15.9), and 48.5 (±9.2) years, respectively; there was no significant difference in the age across the three groups ($P = 0.274$). Baseline characteristics of benign and malignant ovarian tumors are described in Table 1. The incidence was high in the age group 30–50 years in both benign (54%) and malignant (66%) ovarian tumors. In terms of the FIGO staging, most patients were stage III (40%), followed by stage IV (24%), stage II (20%), and stage I (16%). The incidence of serous-type histopathology (52%) was high in benign ovarian tumors, while mucinous-type histopathology (44%) was high in malignant ovarian tumors.

The median values of NGAL/MMP-9 complex in those with malignant EOC, benign ovarian tumor, and healthy controls were 67.5 ng/ml, 53.7 ng/ml, and 29.2 ng/ml, respectively. Data were found to be non-parametric by the Kolmogorov–Smirnov analysis. The median values between the three groups differed significantly ($P < 0.001$) [Table 2]. In addition, the NGAL/MMP-9 complex levels were significantly high in malignant ovarian conditions compared with benign ovarian conditions and healthy controls [Table 3 and Figure 2].

The median values of NGAL/MMP-9 complex were 42.9 ng/ml and 70.3 ng/ml in the early and late stages, respectively [Table 4]. The area under of curve for NGAL was 0.827 [Figure 3]. The cut-off value of NGAL/MMP-9 complex was 55.0 ng/ml, at which the complex had 82.0% sensitivity and 78.0% specificity to detect EOC [Table 5].

Table 1: Baseline characteristic of benign and malignant ovarian tumor

Variables	Benign ovarian tumor, *n* (%)	Malignant ovarian tumor, *n* (%)
Age (years)		
<30	9 (18)	5 (10)
30-50	27 (54)	33 (66)
>50	14 (28)	12 (24)
FIGO staging		
Stage I	-	8 (16)
Stage II	-	10 (20)
Stage III	-	20 (40)
Stage IV	-	12 (24)
Histopathology type		
Mucinous	11 (22)	22 (44)
Serous	26 (52)	20 (40)
Endometroid/cyst	13 (26)	3 (6)
Clear cell	-	5 (10)

FIGO – International federation of gynecology and obstetrics staging

Table 2: Serum level of NGAL/MMP-9 complex in study participants

Study groups	NGAL/MMP-9 complex (ng/ml)	
	Median	Range
Healthy controls	29.2	20.7-41.8
Benign ovarian tumor	53.7	25.6-77.8
Malignant ovarian tumor	67.5	35.2-99.2
*P**	0.001	

**P* value calculated by Kruskal–Wallis test. NGAL – Neutrophil gelatinase-associated lipocalin; MMP-9 – Matrix metallopeptidase-9

Table 3: Result of *post hoc* Dunn test for NGAL/MMP-9 complex in study participants

Study groups	Median (ng/ml)	*P**
Healthy controls	29.2	<0.01
Benign ovarian tumor	53.7	
Healthy controls	29.2	<0.01
Malignant ovarian tumor	67.5	
Benign ovarian tumor	53.7	<0.01
Malignant ovarian tumor	67.5	

**P* value calculated by *post hoc* Dunn test

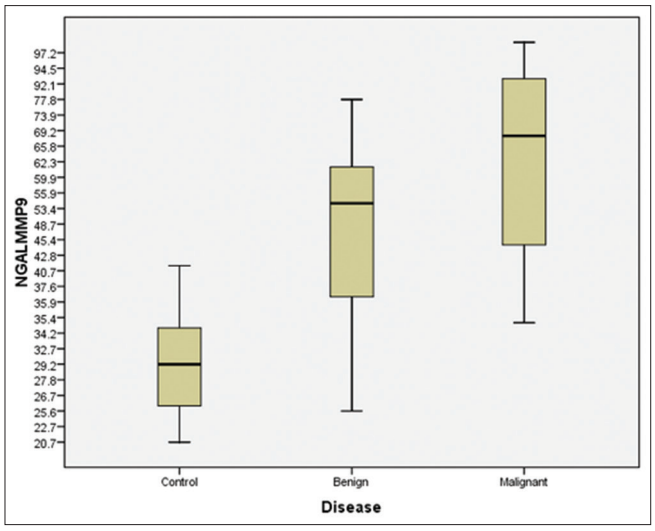

Figure 2: Median values of neutrophil gelatinase-associated lipocalin/matrix metallopeptidase- complex in controls, benign and malignant epithelial ovarian cancer groups

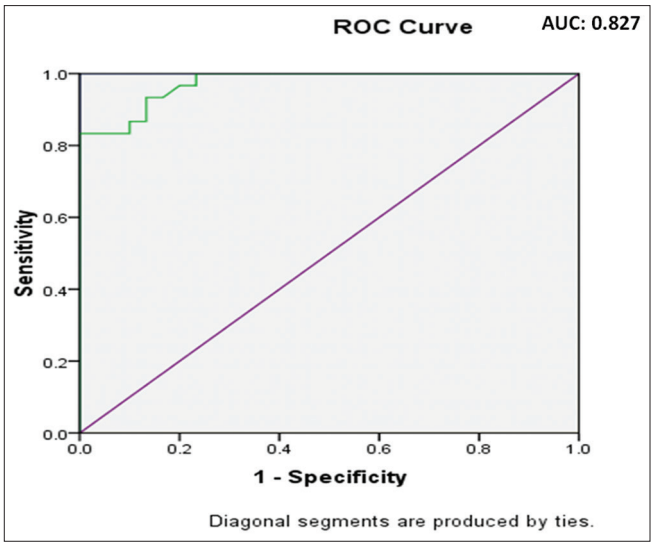

Figure 3: Receiver operating characteristic curve for neutrophil gelatinase-associated lipocalin/matrix metallopeptidase- complex to differentiate epithelial ovarian cancer from the benign ovarian tumor

DISCUSSION

This study found that the NGAL/MMP-9 was significantly higher in those with malignant EOC and benign ovarian tumors than the healthy controls. In addition, the serum levels of the NGAL/MMP-9 complex was significantly lower in those with benign ovarian tumors than EOC cases. At a 55.0 ng/ml cut-off value, NGAL has 82.0% sensitivity and 78.0% specificity in detecting EOC. These findings suggest that estimating the serum levels of NGAL/MMP-9 has potential as a biomarker to diagnose EOC and differentiate between the malignant EOC and benign ovarian tumor cases.

NGAL/MMP-9 complex was also significantly associated with the FIGO staging: the higher the stage, the higher the level of the NGAL/MMP-9 complex. This suggests that the complex may have a direct role in the progression of ovarian cancer. NGAL plays a key role in cell adhesion and cell growth in the cell of malignancy. It has been associated with increased invasiveness of cancer cells.[21] Some researchers observed that NGAL has an important role as a tumor oncogene and tumor suppressor gene.[22] Matrix metalloproteinases are groups of proteinases that require zinc as a cofactor. It degrades the extracellular matrix and plays a key role in the invasion and metastasis of cancers.[23] NGAL forms a homodimer with MMP-9 and prevents degradation of MMP-9. MMP-9 further degrades collagen type I, IV, and gelatin type I in the basement membrane. Basement degradation is also found during the development of ovarian carcinogenesis.[24] Therefore, the increased NGAL/MMP-9 complex may be one of the reasons for the development of EOC.

Table 4: Distribution of NGAL/MMP-9 according to FIGO staging in patients of epithelial ovarian cancer

FIGO staging	NGAL/MMP-9 complex level (ng/ml)		*P**
	Median	Range	
Early stage (I and II)	42.9	35.2-60.9	<0.003
Late stage (III and IV)	70.3	39.2-99.2	

**P* value calculated by Mann–Whitney test. FIGO – International federation of gynecology and obstetrics; NGAL – Neutrophil gelatinase-associated lipocalin; MMP-9 – Matrix metallopeptidase-9

Similar to our study, Lim *et al*.[25] reported that NGAL concentration was higher in those with benign ovarian tumours and malignant EOC compared with healthy controls. Cho *et al*.[26] have also reported significantly higher level of lipocalin 2 and its correlation with tumor differentiation in EOC. Manenti *et al*.[27] have also found an increased serum level of MMP-9 in EOC compared with benign ovarian cases and healthy controls. MMP-9 overexpression has also been found in ovarian cancer cell lines and ascitic fluid of patients diagnosed with advanced ovarian cancer. Furthermore, expression of MMP-9 has been shown to have significant correlation with the severity of invasiveness in ovarian cancer lines.[28]

NGAL expression was found to be low in benign ovarian tissue and high with moderate staining in borderline ovarian tumors. NGAL could be a valuable marker for monitoring the transition of benign lesions to malignant ovarian cancer, and it may be involved in the progression of EOCs.[22] The incidence of serous type histopathology (52%) was high in benign ovarian tumors, and mucinous type histopathology (44%) was high in malignant ovarian tumors. High incidence of mucinous histopathology may influence study results, and it remains to be investigated

Table 5: Distribution of cases according to the cut-off value of NGAL/MMP-9 complex

NGAL/MMP-9 complex	Benign ovarian tumor (%)	Malignant ovarian tumor (%)	Measures
≥55.0 (ng/ml)	11 (24.0)	41 (82.0)	Sensitivity=82.0%
<55.0 (ng/ml)	39 (78.0)	9 (18.0)	Specificity=78.0%
χ^2, df, *P*	36.05, 1, <0.00001		

NGAL – Neutrophil gelatinase-associated lipocalin; MMP-9 – Matrix metallopeptidase-9

whether these findings can be generalized to other patient populations, including higher rates of patients with low-grade or high-grade serous ovarian carcinoma.

NGAL/MMP-9 complex has been found to be elevated in various cancer. For example, Tsakogiannis *et al*.[29] reported that the NGAL/MMP-9 complex might be a promising biomarker for detecting breast cancer in premenopausal obese women. In addition, MMP-9 and NGAL has been found to be expressed significantly higher in gastric cancer tissues than in normal tissues in immunohistochemistry analysis ($P < 0.001$), based on which the NGAL/MMP-9 complex was considered a novel biomarker to diagnose gastric cancer at an early stage.[30] Similarly, based on the results of the current study, NGAL/MMP-9 complex can be considered a viable biomarker for determining the development and progression of EOC.

Limitations

The limitations of our study are the small sample size, lack of follow-up or survival, no follow-up data from the control patients to determine if they developed EOC in the future, and no comparison with CA-125 serum levels. Therefore, a multicenter, large-scale, follow-up study that measures the NGAL/MMP-9 complex levels in the blood and serum of patients of EOC and healthy controls should be conducted to substantiate the findings of this study. Furthermore, along with NGAL/MMP-9 complex measurement, real-time polymerase chain reaction (RT-PCR) and immunohistochemistry can be performed to detect the correlation between blood level and tissue expression.

CONCLUSION

The serum level of NGAL/MMP-9 complex was significantly higher in EOC patients than benign and healthy controls. In addition, the level of NGAL/MMP-9 complex was significantly associated with the staging of EOC. Therefore, the NGAL/MMP-9 complex may be a promising biomarker for determining the progression of EOC and detecting advanced-stage ovarian cancer.

Ethical considerations

The protocol for the study was approved by the Ethics Committee of Maulana Azad Medical College, New Delhi (Ref No: 11; Date: October 10, 2012). All subjects volunteered to participate in the study and provided signed informed consent before inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, 2013.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Peer review

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Conflict of interest

There are no conflicts of interest.

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