Journal of Medicine in Scientific Research

Volume 6 | Issue 1 Article 5

Subject Area: Obstetrics and Gynecology

# Expression levels of matrix metalloproteinase 2 protein in preinvasive and invasive cervical lesions and its clinicopathological association: a North Indian study

Rekha Sachan
*Departments of Obstetrics & Gynaecology, King George Medical University, Lucknow*, drrekhasachan@gmail.com

Pushp L. Sachan
*Department of Physiology, Autonomous State Medical College, Hardoi, Uttar Pradesh, India*

Ayushi Shukla
*Departments of Obstetrics & Gynaecology, King George Medical University, Lucknow*

Malti Maurya
*Departments of Pathology, King George Medical University, Lucknow*

Munna L. Patel
*Department of Medicine, King George Medical University, Lucknow*



## Recommended Citation

Sachan, Rekha; Sachan, Pushp L.; Shukla, Ayushi; Maurya, Malti; and Patel, Munna L. (2023) "Expression levels of matrix metalloproteinase 2 protein in preinvasive and invasive cervical lesions and its clinicopathological association: a North Indian study," *Journal of Medicine in Scientific Research*: Vol. 6: Iss. 1, Article 5.
DOI: https://doi.org/10.59299/2537-0928.1023

# Expression levels of matrix metalloproteinase 2 protein in preinvasive and invasive cervical lesions and its clinicopathological association: a North Indian study

## Cover Page Footnote

Dr Uma Singh, Professor and Head, Department of Obstetrics and Gynaecology, KGMU, Lucknow had given permission to work in genital cancer unit.

ORIGINAL STUDY

# Expression Levels of Matrix Metalloproteinase 2 Protein in Preinvasive and Invasive Cervical Lesions and its Clinicopathological Association: A North Indian Study

Rekha Sachan [a,*], Pushp L. Sachan [b], Ayushi Shukla [a], Malti K. Maurya [c], Munna L. Patel [d]

[a] Department of Obstetrics & Gynaecology, King George Medical University, Lucknow, India
[b] Department of Physiology, Autonomous State Medical College, Hardoi, Uttar Pradesh, India
[c] Department of Pathology, King George Medical University, Lucknow, India
[d] Department of Medicine, King George Medical University, Lucknow, India

**Abstract**

*Background*: **Matrix metalloproteinases (MMPs) play an important role in development of female genital cancers and metastasis. The aim of this study was to analyze expression levels of MMP2 in various grades of cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC) of cervix and its association with clinicopathological variables.**

*Patients and methods*: **This prospective study was carried out in a tertiary care center in Northern India over a period of 1 year. A total of 664 participants with gynecological complaints and unhealthy cervix were recruited for colposcopy. Of these, 70 biopsy samples were obtained from higher grade colposcopy lesions, where 15 were of chronic cervicitis, 40 samples of CIN [CIN1 (14), CIN2 (eight), and CIN3 (18)], and 15 cases of SCC of cervix. All samples were enrolled for final analysis.**

*Results*: **Positive association of MMP2 expression was found with advanced age (46−55 years) and high parity (>5). Based on clinicopathological aspects, MMP2 expression had no association or 0 score with discharge per vaginum. On the contrary, postcoital bleeding even less than 6 months had a strong positive MMP2 expression score. Postmenopausal bleeding also showed positive association with MMP2 expression. Overall, 93.3% samples of chronic cervicitis were negative for MMP2. Moreover, 7.1% CIN1 biopsy samples showed MMP2 positive expression, 37.5% of CIN2 samples, and 50% of CIN3 samples showed MMP2 positive expressions, where 33.3% were strongly positive. MMP2 expression was positive in 80% of biopsy samples of the cancer of cervix, being strongly positive (score = 3) in 66.67%. Overall positive MMP2 expression in CIN was 32.5% and in SCC was 80%. As disease severity increased, the expression of MMP2 also increased.**

*Conclusions*: **In CIN lesions, MMP2 positive expressions and intensity of expression increased with increase in severity of lesions, which reached the maximum in invasive cancer.**

*Keywords:* Cervical cancer, Cervical intraepithelial neoplasia, Clinicopathological association, Immunohistochemistry, Matrix metalloproteinase 2 protein expression

## 1. Introduction

In India, cervical cancer is the second most common cancer affecting females. Globally, it is the fourth most common cancer in women. It is the most common cause of cancer-related deaths in developing countries [1−3]. In India, ~122 844 cases of cancer cervix are diagnosed per year and 67 477 deaths are reported each year. More than 80% of these cancers of the cervix present in late stages in

Received 18 September 2022; accepted 27 October 2022.
Available online 18 August 2023

* Corresponding author at: Department of Obstetrics & Gynaecology, King George Medical University, C-28, Sec-J Aliganj, Lucknow, 226024, Uttar Pradesh, India. Fax: +0522 22575.
E-mail address: drrekhasachan@gmail.com (R. Sachan).

https://doi.org/10.59299/2537-0928.1023

India [4,5]. Screening has been used to identify precancerous lesions to reduce morbidity and mortality caused by invasive diseases. Various screening modalities, including cytological examination (pap smear), HPV DNA testing, visual inspection techniques, and primary biomarkers, have been evaluated to identify premalignant lesions of the cervix [6]. Cervical intraepithelial neoplasia (CIN) is a precancerous lesion characterized by abnormal cellular proliferation, maturation, and nuclear atypia. These lesions may regress to normal, persist, or progress to invasive disease [7,8]. Despite availability of various screening modalities, it is not possible to predict which CIN lesion will regress, persist, or progress. Often histopathological differentiation of CIN lesion is difficult. Moreover, colposcopy-guided biopsy patients the patients to an unnecessary surgical intervention. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. Various physiological and pathological disease processes in human tissues are mediated by MMPs. Maintenance and reconstruction of extracellular matrix (ECM) is an important function of MMPs. Various authors have described their important role in development of female cancers and metastasis [9]. Different level of expressions of these MMPs was found in different types of cancers. Histopathological diagnosis of CIN is relevant for the management of disease. Severity of CIN is expressed by its microscopic grade, which influences the treatment of patients. Drawbacks of histopathological classification system are the presence of three different grades of CIN that give faulty static impression of the disease, whereas CIN is a dynamic lesion that can either persist, progress, or regress with time. Intraobserver and interobserver variability and difficulty in distinguishing CIN lesions from nonneoplastic lesions ultimately result in overtreatment or undertreatment. MMP2 is the most common metalloproteinase involved in tumorigenesis and metastasis of carcinoma of the cervix [10]. In cancer of the cervix, cancer cell invasion and tumor metastasis might be detected early by detecting MMP2 expression levels. Moreover, a few studies have shown expression of these biomarkers increases with increasing severity of CIN. According to a few studies, MMPs have been associated with the stages of malignancy and grades of tumor as well as recurrence of the tumor [11,12]. MMP2 was found to be associated with high-grade tumor with unfavorable outcomes [13,14]. Only few studies have evaluated the expression levels of MMP2 in various grades of CIN [15,16]. Hence, this study was planned to analyze expression levels of MMP2 in various grades of CIN and squamous cell carcinoma (SCC) of cervix and its association with clinicopathological variables.

## 2. Patients and methods

This prospective study was carried out in a tertiary care center in Northern India over a period of 1 year from August 2018 to September 2019. After informed consent and ethical clearance from institutional ethics committee (Ethical Clearance Ref. code: 90th ECM II B-Thesis/P40), total 664 participants with gynecological complaints and unhealthy cervix were recruited for colposcopy. Before doing colposcopy and guided biopsy, all patients were explained about the procedure. Colposcopy-guided biopsy and tissue samples for histopathological examination were obtained from those women who showed higher grade lesions on colposcopy. After histopathological confirmation of chronic cervicitis, CIN, and cervical cancer in tissue biopsies, these tissues samples were further subjected to immunohistochemistry (IHC) analysis to observe MMP2 protein expression.

Of 70 biopsy samples obtained from higher grade colposcopy lesions, 15 were of chronic cervicitis, 40 samples were of CIN [CIN1 (14), CIN2 (eight), and CIN3 (18)], and 15 cases were of SCC of the cervix. All samples were enrolled for final analysis.

### *2.1. Immunohistochemistry*

#### *2.1.1. Procedure for immunohistochemistry analysis for matrix metalloproteinase 2 expressions*

IHC was completed with standard procedures. Four-micrometers-thick paraffin sections were cut on coated microscopy slides. These sections were first deparaffinized and rehydrated in graded alcohols. Antigen retrieval was done in Tris−EDTA buffer by maintaining pH 9 at 98 °C for 25 min in a microwave oven followed by Tris buffered saline washing, and peroxidase blocking was done. For using primary antibody of MMP2 (Abcam ab37150, 152 Grove Street, Waltham, MA 02453, USA), manufacturer's protocol was followed. Sections were incubated with primary antibody for 90 min at room temperature followed by secondary antibodies (Dako REAL EnVision Detection System, Peroxidase/DAB + Rabbit/Mouse) for 30 min at room temperature. Expressions were localized by incubation with diaminobenzidine. Finally, slides were stained with hematoxylin. Negative controls were similarly processed by omitting primary antibody, whereas normal liver tissue was used as the positive control.

#### *2.1.2. Microscopic interpretation of immunohistochemistry*

Brown granular cytoplasm staining for MMP2 was considered positive, and scoring was done on

*Table 1. Matrix metalloproteinase 2 scoring.*

| MMP2 score | Description |
|---|---|
| 0 | Negative |
| 1 | +Weak positive |
| 2 | ++Moderately positive |
| 3 | +++Strong positive |

MMP, matrix metalloproteinase.

the basis of intensity of staining pattern and grading of stained tumor cells (Table 1) [17]. MMP2 positivity on IHC was taken as score of 1 or above (Figs. 1–3).

Statistical analysis was done using SPSS (Statistical Package for the Social Sciences), version 21.0. SPSS Inc. 233 S. Wacker Drive 11th Floor Chicago, IL 60606, USA. The values were represented in number and mean ± SD. MMP2 expression was shown using *n* (%) and compared among the groups using $\chi^2$ test. Differences at *P* value less than 0.05 were considered statistically significant.

## 3. Results

While correlating age with MMP2 expression, maximum women [66.6% ($n = 8$)] were in the age group of 46–55 years who had biopsy samples with positive MMP2 expression (score 1 or above), and among them, 58.3% ($n = 7$) showed a strong positive expression. Overall, 79.2% ($n = 19$) women in age group 23–35 years had a negative expression (score 0) on biopsy samples.

While correlating parity with MMP2 expression, maximum women [58.3% ($n = 7$)] with high parity (>5) had biopsy samples with positive expression (score 1 and above). However, association of MMP2 expression with age and parity was not statistically significant on $\chi^2$ test analysis (Table 2).

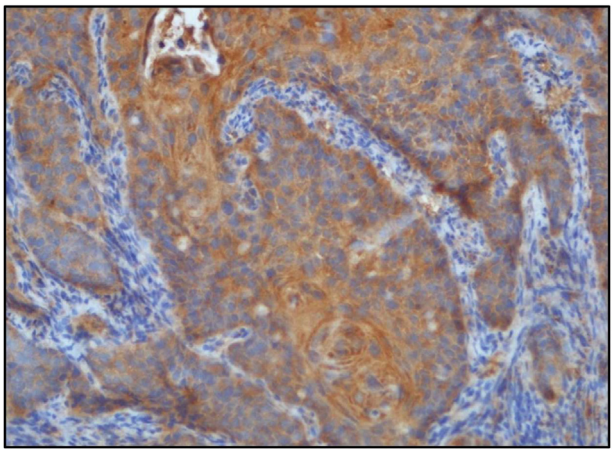

*Fig. 1. Strong positive MMP2 expression (grade 3). Strong cytoplasmic expression of MMP2 in squamous cell of carcinoma of uterine cervix ( × 200). MMP, matrix metalloproteinase.*

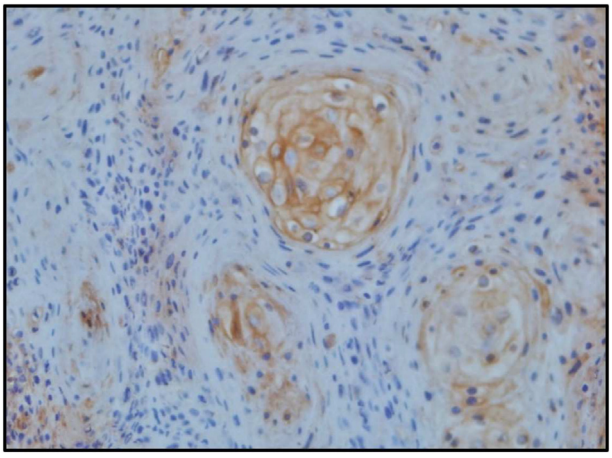

*Fig. 2. Moderately positive expression MMP2 (grade 2): less than 50% of cells show cytoplasmic expression of MMP2 in squamous cell of carcinoma of uterine cervix ( × 200). MMP, matrix metalloproteinase.*

The binary logistic regression was used for analysis in age (years), parity, and MMP2 score to determine the risk factors for cervical cancer, and MMP2 score was a significant independent risk factor for cervical cancer (Table 3).

Overall, 87.09% ($n = 27$) of biopsy samples of women with discharge per vaginum with any duration (less than or more than 6 months) had no MMP2 expression (score 0). Moreover, 54.5% ($n = 6$) of samples of women with postcoital bleeding had positive MMP2 expression. In addition, 50% ($n = 4$) of samples of women with postcoital bleeding with duration of less than 6 months had strong MMP2 expression (score 3). Of 18 cases, 66.67% ($n = 12$) of samples of postmenopausal bleeding showed positive MMP2 expression. In cases with PMB less than 6 months, 72.7% ($n = 8$) cases had positive MMP2 expression. Overall, 60% ($n = 6$) of cases with AUB had negative expression. Among the group with strongest MMP2 expression (score 3), 47% ($n = 8$) had postmenopausal bleeding followed by 23.5% having postcoital bleeding as the presenting complaint (Table 4).

Positive MMP2 expression was defined as a score of 1 or above. Overall, 93.3% ($n = 14$) of samples of chronic cervicitis were MMP2 negative. In CIN3 group, 50% of biopsy samples ($n = 9$) were MMP2 positive, with 33.3% of samples ($n = 6$) being strongly positive. Only 37.5% of cases ($n = 3$) of CIN2 and one (7.1%) case of CIN1 were MMP2 positive. MMP2 expression was positive in 80% ($n = 12$) of biopsy samples of cervical cancer, being strongly positive (score = 3) in 10 (66.67%) cases (Table 5).

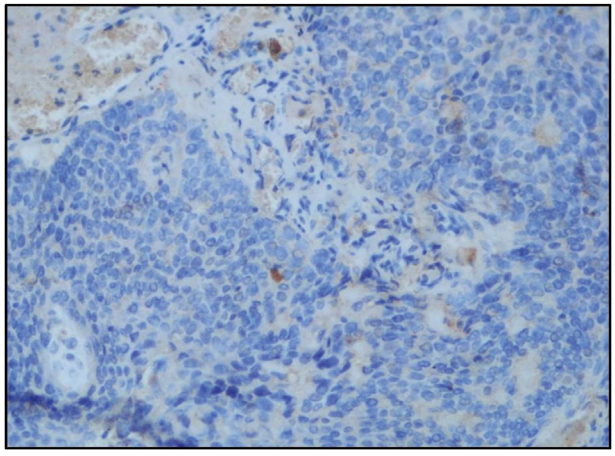

*Fig. 3. Negative expression MMP2 (grade 0): no cytoplasmic expression of MMP2 in squamous cell of carcinoma of uterine cervix ( × 200). MMP, matrix metalloproteinase.*

Overall positive MMP2 expression in CIN was 32.5% and in SCC was 80%. As the disease severity increased, the expression of MMP2 also increased, and this association was statistically significant.

The receiver operating characteristic analysis to determine diagnostic accuracy, for cervical cancer, at a cutoff of MMP2 score 2, 95% confidence interval, and area under the curve of 0.829, had 85.7% sensitivity and 80.4% specificity (Fig. 4).

## 4. Discussion

Transformation from preinvasive lesions to invasive carcinoma begins with focal disruption of the subepithelial basement membrane. MMPs are well-known potent proteolytic enzymes that have a key role in pathophysiology of cervical malignancy [18]. In the present study, presence and pattern of MMP2 protein expression levels were evaluated by IHC analysis in chronic cervicitis, CIN lesions, and SCC cervix to identify progressive nature of dysplasia and correlation of MMP2 with clinicopathological variables. MMP2 can regulate, synthesize, and release angiogenesis factors; hence, it plays an important role in infiltration and metastasis of whole tumor by providing adequate space for growth of blood vessels and release of angiogenic factors. It also strengthens the vascular signal system and promotes angiogenesis by activating cell surface receptors [19,20]. In our study, MMP2 expression increased with advancing age (20.8% in 25–35 age group to 66.6% in 46–55 years), as opposed to the findings of Yu et al. [21] Increased MMP2 expression was seen in women with high parity, similar to our study. Ghosh et al. [15] reported in their study on patients with CIN3 and SCC a higher trend of MMP2 expression in woman above the age of 40 years.

Most patients with parity of 3–5 and maximum women with parity more than 5 had positive MMP2 expression.

Two major complaints, that is, postmenopausal bleeding and postcoital bleeding, are very often found in women with cervical cancer. In the present study, a strong positive MMP2 expression score was observed with complaints of less than 6 months of postcoital bleeding and postmenopausal bleeding. However, no association of MMP2 expression was found in long-standing discharge per vaginum and abnormal uterine bleeding, though these two major complaints also very often associated with cancer cervix.

In CIN lesions, intensity of expression increased with increasing severity of lesions (progressive lesion) and reached maximum in invasive cancer. In our study, 93.3% of cases of chronic cervicitis were negative for MMP2 expression, which is similar to the study done by Yu et al. [21] (90%). Gaiotto et al. (>99.58%) [22], Branca et al. [23] (85.7%), and Sheu et al. [24] (87%) also reported similar rates for negative MMP2 expression in normal epithelium.

*Table 2. Association of matrix metalloproteinase 2 score with age and parity of patients.*

| | MMP2 score [*n* (%)] | | | | Total | *P* value |
|---|---|---|---|---|---|---|
| | 0 ($N = 44$) | 1 ($N = 45$) | 2 ($N = 44$) | 3 ($N = 417$) | | |
| Age (years) | | | | | | |
| 23–35 | 19 (43.18) | 1 (20.00) | 3 (75.00) | 1 (5.88) | 24 | $\chi^2 = 19.274$, $P = 0.082$ |
| 36–45 | 13 (29.55) | 1 (20.00) | 0 | 4 (23.53) | 18 | |
| 46–55 | 4 (9.09) | 1 (20.00) | 0 | 7 (41.18) | 12 | |
| 56–65 | 7 (15.91) | 2 (40.00) | 1 (25.00) | 5 (29.41) | 15 | |
| >65 | 1 (2.27) | 0 | 0 | 0 | 1 | |
| Parity | | | | | | |
| 0–2 | 16 (36.36) | 1 (20.00) | 1 (25.00) | 4 (23.53) | 22 | $\chi^2 = 4.011$, $P = 0.675$ |
| 3–5 | 23 (52.27) | 2 (40.00) | 2 (50.00) | 9 (52.94) | 36 | |
| >5 | 5 (11.36) | 2 (40.00) | 1 (25.00) | 4 (23.53) | 12 | |

MMP, matrix metalloproteinase.

*Table 3. Association of matrix metalloproteinase 2 score with symptoms of patients.*

| Symptom (presenting complaint) | MMP2 score [*n* (%)] | | | | Total ($N = 70$) |
|---|---|---|---|---|---|
| | 0 ($N = 44$) | 1 ($N = 5$) | 2 ($N = 4$) | 3 ($N = 17$) | |
| Discharge PV ≤ 6 months | 12 (27.3) | 0 | 0 | 1 (5.9) | 13 (18.6) |
| Discharge PV > 6 months | 15 (34.1) | 0 | 1 (25.0) | 2 (11.8) | 18 (25.7) |
| PCB ≤6 months | 2 (4.5) | 2 (40.0) | 0 | 4 (23.5) | 8 (11.4) |
| PCB >6 months | 3 (6.8) | 0 | 0 | 0 | 3 (4.3) |
| PMB ≤6 months | 3 (6.8) | 3 (60.0) | 0 | 5 (29.4) | 11 (15.7) |
| PMB >6 months | 3 (6.8) | 0 | 1 (25.0) | 3 (17.6) | 7 (10.0) |
| AUB ≤6 months | 4 (9.1) | 0 | 1 (25.0) | 2 (11.8) | 7 (10.0) |
| AUB >6 months | 2 (4.5) | 0 | 1 (25.0) | 0 | 3 (4.3) |
| *P* value | $\chi^2 = 38.914$, $P = 0.010$[a] | | | | |

MMP, matrix metalloproteinase.
[a] Significant ($P < 0.05$).

*Table 4. Association of matrix metalloproteinase 2 score with histopathology of biopsy tissue.*

| HPE | MMP2 score | | | | | | | | Total | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ($N = 44$) | | 1 ($N = 5$) | | 2 ($N = 4$) | | 3 ($N = 17$) | | | |
| CC | 14 | 31.82 | 1 | 20.00 | 0 | 0.00 | 0 | 0.00 | 15 | |
| CIN1 | 13 | 29.55 | 0 | 0.00 | 1 | 25.00 | 0 | 0.00 | 14 | $\chi 2 = 31.58$, $P = 0.002$[a] |
| CIN2 | 5 | 11.36 | 1 | 20.00 | 1 | 25.00 | 1 | 5.88 | 8 | |
| CIN3 | 9 | 20.45 | 2 | 40.00 | 1 | 25.00 | 6 | 35.29 | 18 | |
| SCC | 3 | 6.82 | 1 | 20.00 | 1 | 25.00 | 10 | 58.82 | 15 | |

MMP, matrix metalloproteinase; SCC, squamous cell carcinoma.
[a] Significant (P < 0.05).

Overall, 92.9% of women with CIN1 showed negative expression for MMP2 (0 score) in the present study. Similar conclusion was obtained from other studies done by Gaiotto et al. [22] (96.4%) and Branca et al. [23] (72.2%).

Overall, 37.5% of biopsy samples of CIN2 demonstrated positivity for MMP2 expression, and 25% showed a strong positive score in our study, but Branca et al. [23] reported 75% and Hong et al. [25] reported 66.7%.

In CIN3, 50% of biopsy samples showed a positive expression for MMP2. Of these, 33.3% had a strong positive expression score for MMP2. Branca et al. [23] reported positive scores in 86% and Hong et al. [25] reported 31.6% only.

Overall, 80% cases of SCC showed positive expression for MMP2. Of these, 66.7% showed high score (≥3), whereas Yu and Li [17] reported the expression in 74% and Branca et al. [23] reported 95.6% positive expression. A study done by Westin et al. [7] showed that the percentages of both tumor and stromal cells immunopositivity for MMP2 were

*Table 5. Binary logistic regression (enter) analysis performed in each patient group for age and parity to determine the independent risk factors for cervical cancer.*

| Variables | | | | | | 95% CI | |
|---|---|---|---|---|---|---|---|
| | β | SE | Wald | *P* | Odd ratio | Lower | Upper |
| Age (years) | −0.21 | 0.16 | 1.81 | 0.179 | 0.81 | 0.60 | 1.10 |
| Parity | −0.65 | 0.57 | 1.30 | 0.255 | 0.52 | 0.17 | 1.60 |
| MMP2 score | 1.41 | 0.61 | 5.28 | 0.022[a] | 4.08 | 1.23 | 13.56 |

MMP, matrix metalloproteinase.
[a] Significant ($P < 0.05$).

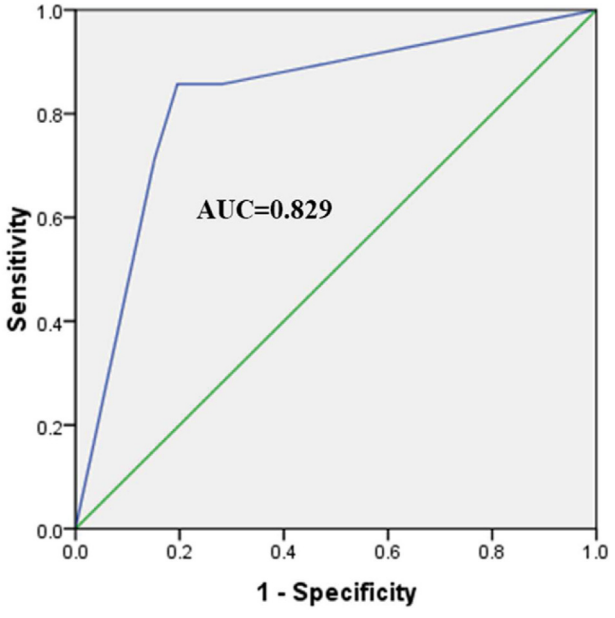


*Fig. 4. The receiver operating characteristic analysis to determine diagnostic accuracy, for cervical cancer, at cutoff of MMP2 score 2, 95% confidence interval, and area under the curve (AUC) of 0.829, had 85.7% sensitivity and 80.4% specificity. MMP, matrix metalloproteinase.*

*Table 6. Matrix metalloproteinase 2 expression comparative analysis.*

| Authors | Negative expression | | | Positive expression | |
|---|---|---|---|---|---|
| | Chronic cervicitis | CIN1 | CIN2 | CIN3 | SCC |
| Yu and Li [17] | – | – | – | – | 74.0% |
| Yu et al. [21] | 90.0% | – | – | – | – |
| Gaiotto et al. [22] | >99.58% | 96.4% | – | – | – |
| Branca et al. [23] | 85.7% | 72.2% | 66.7% | 86.0% | 95.6% |
| Sheu et al. [24] | 87.0% | – | – | – | – |
| Hong et al. [25] | – | – | 75.0% | 31.6% | |
| Present study | 93.3% | 92.9% | 37.5% (strong positive –25.0%) | 50.0% (33.3% strong positive) | 80.0% (High score >3–66.7%) |

SCC, squamous cell carcinoma.

significantly higher in invasive carcinoma compared with CIN3 (Table 6).

In our study, receiver operating characteristic analysis for cervical cancer, at a cut-off of MMP2 score 2, 95% confidence interval, and area under the curve of 0.829, had 85.7% sensitivity and 80.4% specificity. However, Ghosh et al. [15] found that sensitivity and specificity for active MMP2 score were 0.85 and 0.90, respectively.

MMP2 causes degradation of ECM and fibronectin, which results in destruction of basement membrane and might be responsible for the pathophysiology behind the development of invasive carcinoma [26,27]. In our study, MMP2 expressions were elevated in cervical cancer compared with CIN and chronic cervicitis. This was similarly reported by Yu et al. [21].

This present study demonstrated a positive MMP2 expression in CIN and expression levels increased with increasing grade of intraepithelial neoplasia. Nair et al. [28] have shown in their study that the severity of cervical neoplasia increased with increasing MMP2 positivity.

MMP2 is especially important in ECM degradation, cancer cell invasion, and metastasis [29]. Fernandes et al. [30] have shown in their study that higher the stromal cells show MMP2 positivity, higher are the chances of invasion. Brummer et al. [31] have shown that lesions with focal high-grade intraepithelial neoplasia with increased MMP2 positivity are at high risk for invasion.

Identification of malignant behavior of premalignant and malignant lesions would be helpful in management of preinvasive lesions of the cervix to reduce the morbidity and mortality associated with cervical cancer.

A limitation of our study was the small sample size. Although the results were encouraging, for final comments, larger studies are required.

### *4.1. Conclusions*

Our study suggests that MMP2 can be adequately used as a biomarker to differentiate difficult histopathology reports and to identify biological behavior of premalignant and malignant lesions correctly. Monitoring of MMP2 expression in cervical lesions either preinvasive or invasive can be used to identify the severity of disease and prognosis in cervical cancer. In CIN lesions, positive MMP2 expression increased with increase in severity of lesions, reaching maximum in invasive cancer. Two major complaints very often found in women with cancer cervix, that is, postcoital bleeding and postmenopausal bleeding, were strongly associated with positive MMP2 expressions. No such type of evaluation has been reported in any study.

## Authors contribution

All authors contributed equally. Prof. Rekha Sachan was involved in concept design, definition of intellectual content, and manuscript preparation. Dr Ayushi Shukla was involved in literature search, data collection, data acquisition, and statistical analysis. Dr Munna L. Patel was involved in literature search and manuscript review and editing. Dr Malti Maurya was involved in MMP2 expression analysis.

## Conflict of interest

There are no conflicts of interest.

## Acknowledgements

Dr Uma Singh, Professor and Head, Department of Obstetrics and Gynaecology, KGMU, Lucknow had given permission to work in genital cancer unit. We are acknowledging Research Cell, King Georges Medical University, Lucknow who had funded this project (Total Grant 35000).

## References

[1] Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. Int J Womens Health 2015;7:405–14.

[2] GLOBOCAN Cancer Fact Sheets. Cervical cancer. 2016. Available at: http://www.globocan.iarc.fr/old/FactSheets/cancers/cervix-new.asp. [Accessed 18 September 2016].
[3] Kaarthigeyan K. Cervical cancer in India and HPV vaccination. Indian J Med Paediatr Oncol 2012;33:7−12.
[4] WHO. 1st ed.. 2016. Available at: http://www.screening.iarc.fr/doc/WHO_India_CCSP_guidelines_2005.pdf. [Accessed 28 September 2016].
[5] Singh N, Bannur H. A cross-sectional study of p53 expression in patients with squamous cell carcinoma cervix: a hospital-based study. Indian J Health Sci Biomed Res 2017;10:203−7.
[6] Tjalma WA, Weyler JJ, Bogers JJ, Pollefliet C, Baay M, Goovaerts GC, et al. The importance of biological factors (bcl-2, bax, p53, PCNA, MI, HPV and angiogenesis) in invasive cervical cancer. Eur J Obstet Gynecol Reprod Biol 2001;97:223−30.
[7] Westin MC, Rabelo-Santos SH, Ângelo-Andrade LA, Derchain S, Pinto GA, Morais SS, et al. Expression of MMP-2, MMP-9, MMP-14, TIMP-1, TIMP- 2 in intraepithelial and invasive cervical neoplasia. J Cytol Histol 2015;S3:19.
[8] Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. J Pathol 2006;208:152−64.
[9] Kallakury BV, Karikehalli S, Haholu A, Sheehan CE, Azumi N, Ross JS. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clin Cancer Res 2001;7:3113−9.
[10] Wang PH, Ko JL, Tsai HT, Yang SF, Han CP, Lin LY, et al. Clinical significance of matrix metalloproteinase-2 in cancer of uterine cervix: a semiquantitative study of immunoreactivities using tissue array. Gynecol Oncol 2008;108:533−42.
[11] Ahmed MI, Salahy EE, Tawfiq H, Khalifa A, Hassan MM. Matrix metalloproteinase-2, squamous cell carcinoma antigen, and tissue polypeptide-specific antigen expression in Egyptian patients with cervical carcinoma: relationship with prognosis. Dis Markers 2004;20:333−43.
[12] Sato T, Ota T, Watanabe M, Imada K, Nomizu M, Ito A. Identification of an active site of EMMPRIN for the augmentation of matrix metalloproteinase-1 and -3 expression in a coculture of human uterine cervical carcinoma cells and fibroblasts. Gynecol Oncol 2009;114:337−42.
[13] Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. Chem Biol 1996;3:895−904.
[14] Curran S, Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. J Pathol 1999;189:300−8.
[15] Ghosh A, Moirangthem A, Dalui R, Ghosh T, Bandyopadhyay A, Dasgupta A, et al. Expression of matrix metalloproteinase-2 and 9 in cervical intraepithelial neoplasia and cervical carcinoma among different age groups of premenopausal and postmenopausal women. J Cancer Res Clin Oncol 2014;140:1585−93.
[16] Gaiotto MA, Focchi J, Ribalta JL, Stávale JN, Baracat EC, Lima GR, et al. Comparative study of MMP-2 (matrix metalloproteinase 2) immune expression in normal uterine cervix, intraepithelial neoplasias, and squamous cells cervical carcinoma. Am J Obstet Gynecol 2004;190:1278−82.
[17] Yu CZ, Li ZX. MMP-2 expression and MVD in invasive cervical carcinoma. Biomed Res 2017;28:5269−72.
[18] Wu YC, Wang PH, Tsai A, Yang SF, Chen SC. Semi-quantitative expression of tissue inhibitor of matrix metalloproteinase-2 in cancer of uterine cervix. J Surg Oncol 2011;104:210−5.
[19] Moberg M, Gustavsson I, Wilander E, Gyllensten U. High viral loads of human papillomavirus predict risk of invasive cervical carcinoma. Br J Cancer 2005;92:891−4.
[20] Bai X, Guan B, Liu M, Zhu Q, He Y, Wang P, et al. The antitumor effect of hederagenin on tumors growth of hepatocarcinoma (H22) tumor-bearing mice. Lat Am J Pharm 2017;36:142−50.
[21] Yu J, Xie Q, Zhou H, Peng Y, Lu H, Yao T, et al. Survivin, MMP-2, and MMP-9 expression in different types of cervical lesions and correlation analysis. Int J Clin Exp Pathol 2016;9:5445−51.
[22] Gaiotto MA, Focchi J, Ribalta JL. Comparative study of MMP2 immune expression in normal uterine cervix, intraepithelial neoplasias and squamous cell cervical carcinoma. Am J Obstet Gynecol 2004;190:1278−82.
[23] Branca M, Ciotti M, Giorgi C. MMP-2 and TIMP-2 are prognostic factors in cervical cancer, related to invasive disease but not to high risk HPV or virus persistence after treatment of CIN. Anticancer Res 2006;26(2B):1543−56.
[24] Sheu BC, Lien HC, Ho HN, Lin HH, Chow SN, Huang SC, et al. Increased expression and activation of gelatinolytic matrix metalloproteinases is associated with the progression and recurrence of human cervical cancer. Cancer Res 2003;63:6537−42.
[25] Hong J, Jo H, Soo K. Expression of MMP2, MMP9, and urokinase type plasminogen activator in CIN. Ann N Y Acad Sci 2009;1171:100−4.
[26] Hagemann C, Anacker J, Ernestus RI, Vince GH. A complete compilation of matrix metalloproteinase expression in human malignant gliomas. World J Clin Oncol 2012;3:67−79.
[27] Kato H, Duarte S, Liu D, Busuttil RW, Coito AJ. Matrix metalloproteinase-2 (MMP-2) gene deletion enhances MMP-9 activity, impairs PARP-1 degradation, and exacerbates hepatic ischemia and reperfusion injury in mice. PLoS One 2015;10:e0137642.
[28] Nair AS, Karunagaran D, Nair MB, Sudhakaran PR. Changes in matrix metalloproteinases and their endogenous inhibitors during tumor progression in the uterine cervix. J Cancer Res Clin Oncol 2003;129:123−31.
[29] Zhang X, Wang Y, Yamamoto G, Tachikawa T. Expression of matrix metalloproteinases MMP-2, MMP-9, and their tissue inhibitors TIMP-1 and TIMP-2 in the epithelium and stroma of salivary gland pleomorphic adenomas. Histopathology 2009;55:250−60.
[30] Fernandes T, de Angelo-Andrade LA, Morais SS, Pinto GA, Chagas CA, Maria-Engler SS, et al. Stromal cells play a role in cervical cancer progression mediated by MMP-2 protein. Eur J Gynaecol Oncol 2008;29(4):341−4.
[31] Brummer O, Böhmer G, Hollwitz B, Flemming P, Petry KU, Kühnle H, et al. MMP-1 and MMP-2 in the cervix uteri in different steps of malignant transformation−an immunohistochemical study. Gynecol Oncol. 2002 Feb;84(2):222−7.