Original Article

A Study of Matrix Metalloproteinase-2 and Interleukin-18 in Preinvasive and Invasive Lesions of Cancer Cervix

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ABSTRACT

Introduction: Human Papilloma-Virus infection is the major event for cervical carcinogenesis, whereas host physiological changes may confer individual susceptibility and prognosis. So here, we aimed to compare serum levels of matrix metalloproteinase-2 (MMP-2) and interleukin-18 (IL-18) between cervical cancer patients and healthy controls. **Materials and Methods:** In the present study, we enrolled 168 subjects (10 CIN I, 10 CIN II, 10 CIN III, and 54 invasive cervical cancers with 84 age-matched healthy controls). Serum levels were estimated by enzyme-linked immunosorbent assay. **Results and Discussion:** The levels of serum MMP-2 showed a characteristic pattern of increasing trend with statistically significant *P* value on comparing pre-invasive lesions and cervical cancer versus healthy controls. However, IL-18 levels showed a decreasing trend in serum levels of controls versus cases with a statistically significant *P* value ($P < 0.05$). **Conclusion:** MMP-2 accentuates tissue damage and controls many interleukins secretion, which leads invasion and malignancy. Increased levels of MMP-2 and decreased circulating levels of IL-18 were found in cases. Hence, we raise an issue to study MMP-2 and IL-18 further for their diagnostic and prognostic marker role.

Keywords: *Extracellular matrix, inflammation, interleukin-18, matrix metalloproteinase, metastasis*

Introduction

Although Cervical cancer is a largely preventable cancer still it is fourth-common cancer among women globally and the second most common cancer among Indian women. India alone bears 23% of the global cervical cancer burden.[1]

Metastasis is a hallmark of cancer. The process of metastasis includes tumor progression, extracellular matrix (ECM) degradation, cell migration, cell adhesion, invasion, and angiogenesis. Matrix metalloproteinase-2 (MMP-2) plays important role in ECM degradation. It is an active member of zinc-dependent extracellular proteinases called MMPs. It is 72 KDa enzyme also known as type IV collagenases or gelatinase A, encoded in humans by *MMP-2* gene, located on chromosome 16 at position 12.2. In normal physiological conditions, MMP's activity is highly regulated. These are synthesized in inactive form (latent enzyme) and conversion into active form is generally mediated by activators. Cancer cells also produce such activator enzymes; for example-MMP-2 and MMP-2 activate other MMPs and play an important role in ECM degradation. Besides ECM components MMP-2 also regulates some non-ECM components such as growth factors and their receptors, adhesion receptors, cell surface proteoglycans, cytokines, chemokines, and interleukins.[2]

Interleukins are a group of cytokines expressed by white blood cells. Among interleukins, interleukin-18 (IL-18) is the potent inflammatory agent, discovered as an interferon-γ-inducing factor and it has a crucial role in immunity.[3] IL-18 has pleiotropic effect, on one hand, it contributes to the immune regulatory mechanism

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that might control tumor progression and on the other hand, by producing inflammation IL-18 may stimulate tumor growth, malignant transformation, metastasis, and invasion.[4]

Therefore, in the present study, we aimed to compare serum levels of MMP-2 and IL-18 between cervical cancer cases and healthy controls to make a bridge between previous studies and upcoming studies because these two may have a prognostic role.

Materials and Methods

Subjects

The present study recruited 84 cases and 84 controls (total 168) enrolled in our Obstetrics and Gynaecology department. Recruited subjects underwent the Papanicolaou test (conventional Pap smear test) or liquid-based cytology followed by colposcopy and biopsy when required. Subjects with normal Pap smear and colposcopy served as controls. Subjects with biopsy-proven CIN or cervical cancer groped as cases. Peripheral blood samples were collected for MMP-2 and IL-18 estimation from all subjects and all the study procedures were approved by the institutional ethics committee. Informed consent was obtained from all the study subjects.

Serum collection and enzyme-linked immunosorbent assay MMP-2 and IL-18 estimation

Two ml of the blood sample was drawn and allowed to clot at room temperature. Each sample was centrifuged at 2400 g rpm for 10 min for separating serum. All the serum samples were stored at − 80°C until assayed. The levels of MMP-2 and IL-18 in serum were measured using enzyme-linked immunosorbent assay (ELISA) by commercially available kit (RayBio® Catalog #: ELH-MMP-2, RayBio® Catalog #: ELH-IL-18, respectively). The procedure was followed as per the manufacturer's protocol. The optical density of the samples was determined at 450 nm with iMark microplate absorbance reader.

Statistical analysis

Data analysis was carried out using SPSS 16.0 version (Chicago, Inc., USA). The results are presented in mean ± standard deviation. The risk was calculated at a 95% of confidence interval. The *P* value was set at <0.05 as significant.

Results

One hundred and ninety subjects were recruited of which 22 subjects refused to participate in the study and finally 168 subjects were analyzed. There were 84 cases (including preinvasive (CIN) and invasive cancer cervix proven by cervical biopsy) and 84 age-matched controls who were cancer-free, healthy controls. Clinical staging of cervical cancer cases was performed as per the guidelines by the International Federation of Gynaecology and Obstetrics. There were 30 cases of pre-invasive cervical lesions and 54 cases of invasive cancer cervix.

Demographic analysis of cases showed that 91% (77/84) were Hindus and 9% (07/84) were Muslims and Sikhs. 83% of cases (70/84) were educated till middle school. Majority of the cases (76%) belonged to low socioeconomic status, had poor personal hygiene and poor dietary intake. The demographic information was obtained by personal interview through well-designed questionnaire and socioeconomic status was according to the kuppuswamy scale.

The serum levels of MMP-2 and IL-18 in cervical cancer cases and control groups were measured and analyzed. Mean values of MMP-2 among control and cases showed a characteristic pattern of increasing trend with statistically significant *P* value. However, mean values of IL-18 among controls and cases showed a characteristic pattern of decreasing trend with a statistically significant *P* value. The details are given in Table 1.

We also measured serum levels of MMP-2 and IL-18 in CIN I, CIN II, and CIN III. Our result showed a gradual increment in MMP-2 serum level from CIN I to CIN III and *P* value was found to be statistically significant. However, IL-18 serum levels in CIN I, II, III showed opposite trend to that observed in MMP-2 serum levels. The levels of IL-18 gradually decreased from CIN I to CIN III. The *P* values obtained were insignificant [Table 2].

We also calculated MMP-2 and IL-18 levels in stage II, III, IV of invasive cervical cancer. The serum levels of MMP-2 and IL-18 were similar and were not statistically different [Table 3].

Discussion

The previous two decades have witnessed lot of research related to molecular events occur during carcinogenesis. The microenvironment around tumor cells have very important role in this process. Many studies conducted during past decades have concluded MMP as the principal player of microenvironment alteration during carcinogenesis. These are zinc-dependent endopeptidases family involved in many normal physiological processes like uterine involution, organogenesis, and wound healing. These are also involved in disastrous events such as auto-immune disorders, inflammation, and carcinogenesis. MMPs have been considered as potential

Table 1: Comparison of matrix metalloproteinase-2 and interleukin-18 serum levels in cases (preinvasive lesions and invasive lesions of cervical cancer) and controls

Parameter	Mean±SD			Preinvasive lesions versus control (*P*)	Invasive cervical cancer versus control (*P*)
	Control (*n*=84)	Preinvasive lesions (*n*=30)	Invasive cervical cancer (*n*=54)		
MMP-2	137.77±9.59	235.70±28.85	318.41±10.18	0.001	0.001
IL-18	340.52±11.91	325.77±12.90	292.79±6.24	0.001	0.001

Values represent mean±SD (range) and significant *P* values are highlighted. SD: Standard deviation, MMP: Matrix metalloproteinase, IL: Interleukin

Table 2: Comparison of matrix metalloproteinase-2 and interleukin-18 serum levels in cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III

Marker	Case stage	Case (serum level), mean±SD	*P*
MMP-2	CIN I (10)	200.70±2.45	0.01
	CIN II (10)	249.40±5.21	0.01
	CIN III (10)	257.00±23.96	0.001
IL-18	CIN I (10)	327.80±12.97	0.78
	CIN II (10)	325.80±14.45	0.49
	CIN III (10)	323.70±12.22	0.99

Significant *P* values are highlighted. *P* calculated by ANOVA between preinvasive clinical groups (CIN I, CIN II, CIN III). Significant level=<0.05. SD: Standard deviation, MMP: Matrix metalloproteinase, IL: Interleukin, CIN: Cervical intraepithelial neoplasia

Table 3: Comparison of matrix metalloproteinase-2 and interleukin-18 serum levels in different stage of invasive cervical cancer

Marker	Case stage	Case (serum level), mean±SD	*P*
MMP-2	Stage II (19)	319.89±12.14	0.39
	Stage III (31)	318.29±9.20	0.17
	Stage IV (4)	312.25±5.67	0.23
IL-18	Stage II (19)	291.95±5.85	0.75
	Stage III (31)	293.19±6.44	0.60
	Stage IV (4)	293.75±7.84	0.47

P calculated by ANOVA between invasive clinical groups (Stage II, Stage III, Stage IV); Significant level=<0.05. SD: Standard deviation, MMP: Matrix metalloproteinase, IL: Interleukin

diagnostic and prognostic biomarkers in many types of cancers.

In the present study, we measured the serum level of MMP-2 and IL-18 in 168 numbers of pre-invasive, invasive cervical cancer patients and in control subjects. We found a positive association of MMP-2 with increasing cervical cancer stages with a statistically significant difference (<0.05). A similar study has been done in Brazil by Gaiotto *et al*., where they evaluated 60 cervical tissues for MMP-2 expression in CIN I, CIN II, CIN III, cervical cancer and control subjects. The expression of MMP-2 gradually increased with an increasing degree of cervical intraepithelial neoplasia and was maximum in cervical cancer (control < CIN I < CIN II < CIN III < cervical cancer). Their result suggested that expression of MMP-2 can help in discriminating CIN I, CIN II, CIN III and cervical cancer from healthy individuals.[5]

Sheu *et al*., in 2003 explored the role of various MMPs in pre-invasive and invasive cervical cancer development in the Taiwanese population. By immunohistochemical study, they demonstrated that MMP-2 was expressed in more than 90% of invasive cervical cancer and 83%–100% of high-grade squamous intraepithelial lesions but was less frequently expressed in low-grade squamous intraepithelial lesions and normal epithelium (13%).[6]

A study on the north Indian population by Srivastava *et al*., evaluated the role of MMP-2 polymorphism in cervical cancer susceptibility. They recruited 200 cervical cancer cases and 200 age-matched, cancer-free healthy controls. Their findings demonstrated no significant association between MMP-2 gene polymorphisms and the risk of cervical cancer in the study population.[7]

Tumour preparing inflammation and immune escape for avoiding tumor cells destruction are the hallmarks of cancer and some previous research demonstrated that pro-inflammatory cytokine IL-18 are involved in this process. Firstly IL-18 was discovered as an immune activator for NK cells and T-cells, which eliminate cancer cells or pathogen-infected cells. Contrary to the anti-cancer role of IL-18, its pro-cancerous characteristics have also been suggested in squamous cell carcinoma (SCC), gastric cancer, and skin tumor in melanoma.[3,4]

In the present study, IL-18 serum levels were compared between pre-invasive, invasive, and control groups. We found gradual decrease in serum IL-18 as the severity of disease increased. Similarly, Cho *et al*. conducted a study in the year 2001 on cervical carcinoma cells. They found that the cells expressed E6 oncogene downregulate IL-18 to evade immune surveillance; many more studies explained the same like Cho *et al*.[8]

In 2008, Qi *et al*., investigated the correlations between serum IL-18 and IL-18 gene promoter polymorphism.

Five SNPs of the IL-18 gene were detected by means of sequences analysis in cervical cancer cases, and in normal control, their serum IL-18 level was tested using ELISA. They found IL-18 gene polymorphism and IL-18 serum level was related to cervical carcinogenesis and serum IL-18 level was significantly lower than that of the normal controls.[9]

Conclusion

In the present era, cervical cancer prevention and its cure has become an important need. MMP-2 has a vital role in tissue pathology in many organs as well as the cervix. It accentuates tissue damage and controls many interleukins secretion, which leads to invasion and malignancy. Increased levels of MMP-2 and decreased circulating levels of IL-18 were found in the serum samples of pre-invasive and invasive cases. Future studies will determine the prognostic value of MMP-2 and IL-18 in cervical cancer.

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Conflicts of interest

There are no conflicts of interest.

References

1. Mishra GA, Pimple SA, Shastri SS. Prevention of Cervix Cancer in India. Oncology 2016;91 Suppl 1:1-7.
2. Libra M, Scalisi A, Vella N, Clementi S, Sorio R, Stivala F, *et al.* Uterine cervical carcinoma: Role of matrix metalloproteinases (review). Int J Oncol 2009;34:897-903.
3. Park H, Byun D, Kim TS, Kim YI, Kang JS, Hahm ES, *et al.* Enhanced IL-18 expression in common skin tumors. Immunol Lett 2001;79:215-9.
4. Park S, Cheon S, Cho D. The dual effects of interleukin-18 in tumor progression. Cell Mol Immunol 2007;4:329-35.
5. Gaiotto MA, Focchi J, Ribalta JL, Stavale JN, Baracat EC, Lima GR, *et al*. Comparative study of MMP-2 immune expression in normal uterine cervix, intraepithelial neoplasias, and squamous cells cervical carcinoma Bottom of Form. Am J Obstet Gynecol 2004;190:1278-82.
6. 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.
7. Srivastava P, Pandey S, Mittal B, Mittal RD. No association of matrix metalloproteinase [MMP]-2 (2735C > T) and tissue inhibitor of metalloproteinase [TIMP]-2 (2418G > C) gene polymorphisms with cervical cancer susceptibility. Ind J Clin Biochem 2013;28:13-8.
8. Cho YS, Kang JW, Cho M, Cho CW, Lee S, Choe YK, *et al.* Down modulation of IL-18 expression by human papillomavirus type 16 E6 oncogene via binding to IL-18. FEBS Lett 2001;501:139-45.
9. Qi T, Wang Q, Zheng L, Yang HL, Bao J. Correlation of serum IL-18 level and IL-18 gene promoter polymorphisms to the risk of cervical cancer. Nan Fang Yi Ke Da Xue Xue Bao 2008;28:754-7.