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

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Abstract

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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

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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 grouped 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 (n=30) | Invasive cervical cancer (n=54) | Preinvasive lesions versus control (P) | Invasive cervical cancer versus control (P) |
|-----------|---------|----------------------------|---------------------------------|--------------------------------------|------------------------------------------|
| 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.

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