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Research Article

POSSIBILITIES OF MATRIX METALLOPROTEINASES AS KEY ROLE IN CERVICAL CARCINOGENESIS: A NORTH INDIAN STUDY

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ABSTRACT

Objectives- We aimed to evaluate the levels of circulating MMP-2, MMP-9 and TIMP-2 in pre-invasive stage patients, cervical carcinoma patients and healthy women.
Methods - It was hospital based case-control study conducted at Department of Obstetrics and Gynaecology, King George's Medical University, Lucknow, India. Included numbers of cases were 84 with equal number of controls. Diagnosis of cases was made through biopsy – proven cervical intra-epithelial neoplasia or cervical cancer. Peripheral blood samples were collected from cases and controls. Expression was evaluated in serum samples by ELISA method.
Results- Significant differences were found in the serum levels of total MMP-2 in cases as compared to healthy controls ($P<0.05$). (CIN I 200.70 ± 2.45 ng/ml = 137.77±9.59, p value= 0.000), CIN II (257.00±23.96 = 137.77±9.59, p value= 0.000), CIN III (249.40±5.21 = 137.77±9.59, p value= 0.000) and in CC (318.41± 10.17 = 137.77±9.59, p value= 0.000). Significant differences were also found in the serum levels of total TIMP-2 in CIN I (315.90 ± 6.40 pg/ml = 332.94± 14.46, p value= 0.000), CIN II (225.10±11.90 = 332.94 ± 14.46, p value= 0.000), CINIII (189.80± 4.96 = 332.94± 14.46, p value= 0.000) and in CC (148.94 ± 11.00 = 332.94± 14.46, p value= 0.000).
Conclusion- In the present study we conclude that mmp-2 and TIMP-2 may play role in progression in cervical carcinogenesis in North Indian population.
Keywords: MMP-2= matrix metalloproteinases-2, MMP-9= matrix metalloproteinases-9, TIMP-2 = tissue inhibitor of metalloproteinases-2, CC=cancer cervix, CIN= cervical intraepithelial neoplasia.

INTRODUCTION

Cervical Cancer is the third leading malignancy among women in the world after breast cancer and colorectal cancer and according to 2012 report 527,624 new cases and 265,653 deaths were estimated [(1)]. Increased access to health services, successful cytology-based screening programs, improvements in treatment, and awareness about risk factors for cervical cancer results in declining in mortality rate up to 1.6 percent in worldwide [(2)]. But the developing countries still account more than 80 percent of death [(3)]. High-risk of human papillomavirus infections (HPV 16 and HPV18) have been well established as a primary cause of cervical cancer with little geographic variation in the predominant HPV types associated with cervical cancer, however HPV infection is very common and usually initiate with sexual activity and clear spontaneously within one to

two years and not all HPV infection leads to cancer. So there may be some physiological changes inside the body which may lead HPV infection to cervical carcinogenesis in women [4]. Therefore it is important to identify these changes for better diagnosis and prognosis of disease. Inflammatory conditions can initiate oncogenic transformation, genetic or epigenetic changes in cellular environment which further leads tumor progression and matrix metallo-proteinases (MMPs) have been found as pivotal players in inflammation and extracellular matrix (ECM) degradation [5] [6].

Moreover, the ability of cancer cells to spread from their principal origin tissue to other place through metastasis process is essential for tumor progression, therefore MMPs became point of concern [7] [8] [9]. These are the family of metal dependent (Zn^{2+}, Ca^{2+}) endopeptidases [10]. In normal physiological condition the activities of MMPs are highly regulated by their inhibitors called tissue inhibitor of metallo-proteinases (TIMPs) [11] [12]. But the question arise are they also well regulated in cervical cancer patients? This will have to be finding out. In present study we aimed to evaluate the levels of circulating MMP-2, MMP-9 and TIMP-2 in pre-invasive stage patients, cervical carcinoma patients and healthy women.

MATERIALS AND METHODS

Subjects

The present study included 84 cases with equal number of controls. Patients were enrolled at the OPD (Outpatient Department) and IPD (Inpatient Department) of the Department of Obstetrics and Gynaecology, King George's Medical University, Lucknow, India. Diagnosis of cases was made through biopsy – proven cervical intra-epithelial neoplasia or cervical cancer. Papanicolaou test (Pap smear test) or Liduid Based Cytology (LBC) followed by colposcopy and biopsy are the steps for diagnosis. Peripheral blood samples were collected from cases, classified according to the International Federation of Gynecology and Obstetrics (FIGO) staging system and the control group were consisting of those with normal Pap smear (or LBC) and normal colposcopic finding. All the study procedures were approved by the institutional ethics committee. Informed consent was obtained from all the study subjects.

Serum collection and ELISA: Five ml of blood sample was drawn carefully and allowed to clot at room temperature. Each sample was centrifuged at 2400 g rpm for 10 minutes to separate the serum. All the serum samples were stored at -80oC until assayed. The levels of total MMP-2, MMP-9 and TIMP-2 in serum were measured using enzyme-linked immunosorbent assay (ELISA) by commercially available kit (RayBio® Catalog #: ELH-MMP2, RayBio® Catalog #: ELH-MMP9 and RayBio® Catalog #: ELH-TIMP2 respectively). Procedure was followed as per manufacturer's protocol. The optical density of the samples was determined at 450 nm with iMark microplate absorbance reader.

Table 1: Mean serum levels of MMP-2, MMP-9 and TIMP-2 in cases and controls

Characteristic	Control (n=84)	CIN I (n=10)	P value	CIN II (n=10)	P value	CIN III (n=10)	P value	CC (n=54)	P value
MMP-2	137.77 ± 9.59	200.70 ± 2.45	**0**	257.00 ± 23.96	**0**	249.40 ± 5.21	**0**	318.41 ± 10.17	**0**
MMP-9	79.40 ± 20.42	79.70 ± 24.63	0.96	82.70 ± 23.91	0.63	87.40 ± 19.63	0.24	59.24 ± 11.85	**0**
TIMP-2	332.94 ± 14.46	315.90 ± 6.40	**0**	225.10 ± 11.90	**0**	189.80 ± 4.96	**0**	148.94 ± 11.00	**0**

Values are expressed as mean ± SD. The p-value in bold was set at <0.05 as significant.

Mean level of total MMP-2 in ng/ml, in study subjects but mean level of total MMP-9 and TIMP-2 in pg/ml, in study subjects.

MMP-2= matrix metalloproteinases-2, MMP-9= matrix metalloproteinases-9, TIMP-2 = tissue inhibitor of metalloproteinases-2, CIN I= cervical intra-epithelial neoplasia I, CIN II= cervical intra-epithelial neoplasia II, CINIII=cervical intra-epithelial neoplasia III CC= cervical cancer subjects, n= Number.

STATISTICAL ANALYSIS

All the analysis was carried out using SPSS 16.0 version (Chicago, Inc. USA). The results are presented in mean±SD. The continuous variables were compared with t-test. The risk was calculated at 95 % of confidence interval. The p-value was set at <0.05 as significant.

RESULTS:

Significant differences were found in the serum levels of total MMP-2 in cases as compared to healthy controls. (CIN I 200.70 ± 2.45 ng/ml = 137.77±9.59, p value= 0.000), CIN II (257.00±23.96 = 137.77±9.59, p value= 0.000), CIN III (249.40±5.21 = 137.77±9.59, p value= 0.000) and in CC (318.41± 10.17 = 137.77±9.59, p value= 0.000). Despite this no significant differences were found in serum levels of total MMP-9 in CINI (79.70±24.63 pg/ml = 79.40± 20.42, p value=0.966), CINII (82.70± 23.91 = 79.40± 20.42, p value- 0.637) and CIN III (87.40± 19.63 = 79.40± 20.42, p value-0.243) but significant difference were found in CC patients (59.24± 11.85 = 79.40± 20.42, p value= 0.000). Significant differences were also found in the serum levels of total TIMP-2 in CIN I (315.90 ± 6.40 pg/ml = 332.94± 14.46, p value= 0.000), CIN II (225.10±11.90 = 332.94 ± 14.46, p value= 0.000), CINIII (189.80± 4.96 = 332.94± 14.46, p value= 0.000) and in CC (148.94 ± 11.00 = 332.94± 14.46, p value= 0.000).

DISCUSSION:

Cervical cancer, a largely preventable disease but accounts third number of deaths by cancer in women worldwide. Although epidemiological and experimental studies have provided evidence that human papillomavirus (HPV) infection is a main player in cancer cervix. High risk oncogenic HPV are major cause of almost all cases of carcinoma cervix. Among these HPV 16 is the most prevalent one [13]. However, focussing on invasion, migration, angiogenesis of cancer cells from the origin tissue to surrounding or distant organs, through degradation of the ECM is essential, and MMPs play critical role.

It was a case–control study on North Indian population. We aimed to evaluate the serum protein levels of MMP-2, MMP-9 and TIMP-2 in pre-invasive and invasive lesions of cancer cervix cases as compared to healthy controls. Our findings demonstrated a positive association between these markers with study disease. As shown in table no-1, protein level of MMP-2 significantly increases from pre-invasive to invasive state of cancer cervix (<0.05). No significant association was found in protein level of MMP-9 in their pre-invasive stage although invasive stage is significantly associated with the cancer cervix. Moreover we observed that TIMP-2 is gradually decreasing as cancer cervix reached on their advance stage.

Similar to the present study, Talvensaari-Mattila Anne et al conducted a study in year 2010 on 12 cervical carcinoma patients and 27 healthy volunteer control patients in Finnish population. They showed that the mean levels of serum TIMP-2 and MMP-2-TIMP-2 complex were higher in the healthy controls compared to those with a malignant tumor. Serum TIMP-2 values decreased significantly from healthy controls (median 323 µg/l, range 305–342 µg/l) to malignant (median 136 µg/l, range 120–151 µg/l) cervical carcinoma patients (P < .000). Also, serum proMMP2-TIMP2 complex values decreased from control patients to cervical carcinoma patients (P < .006)[14].

Another study in Taiwanese population, two hundred one blood samples were collected from 52 patients with early cervical carcinoma, 41 with high-grade cervical intraepithelial neoplasia (CINII, CINIII), 27 with low-grade cervical intraepithelial neoplasia (CINI), and 81 healthy individuals also showed that patients with low- and high-grade CIN were found to have significantly different plasma MMP-9 levels (P < .001) but not MMP-2 levels. Elevation of plasma MMP-9 levels and the MMP-9 : MMP-2 ratio was found in high-grade CIN and CC patients manifests a stage point of high-grade CIN in cervical carcinogenesis [15]. Besides this significant association between MMP-2, MMP-9, TIMP-1 and TIMP-2 expression with lesions on cervical tissue was reported in south Indian population [16].

The strength of the study is that biopsy proven cases have been recruited whereas less sample size is the drawback of the study. Therefore more studies with large sample size are required for better diagnosis and prognosis of the disease. We conclude that mmp-2 and TIMP-2 may play role in progression in cervical carcinogenesis in North Indian population.

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Conflict of Interest: There is no conflict of interest.

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