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UP-REGULATION OF MATRIX METALOPROTEINASES AND THEIR LATENT ROLE TO DEVELOP ENDOMETRIOSIS IN YOUNG FEMALES OF REGRESSIVE MENESTRUATION

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ABSTRACT

Endometriosis is defined as the presence of endometrium outside of uterine cavity and thought to develop through reverse menstruation of endometrial tissue into the peritoneal cavity. Endometrial cell implantation into peritoneal cavity is a crucial process in the onset of endometriosis. Interestingly during implantation some features of malignancy are demonstrated by endometrial cells, as they have the ability to attach and to invade the peritoneum or ovary structure. This invasion is effectuated with a group of enzymes known as matrix metalloproteinases (MMPs) which may contribute in extracellular matrix (ECM) degradation. ECMs and protein degrading activity of cell surface proteins play a critical role in pathophysiology of endometriosis. During inflammation activated neutrophils, macrophages and eosinophils stimulate the production of MMPs in peritoneal cavity of female with endometriosis. The objective of present study was to access the expression of MMP-2, MMP-7 and MMP-9 in glandular epithelial cells of endometriotic tissue and normal endometrium to elucidate the role of MMPs in the pathogenesis of endometriosis.

Keywords: Matrix metalloproteinases (MMPs), Extracellular matrix (ECM), Matrix metalloproteinase-2 (MMP-2), Matrix metalloproteinase-7 (MMP-7), and Matrix metalloproteinase-9 (MMP-9)

INTRODUCTION

Endometriosis a benign gynaecological disorder occurs in menstruating women and characterised by several symptoms such as dysmenorrhea, pelvic pain and infertility. It is very common with an incidence of 10-15% in females of reproductive age and 40-50% in women with infertility **[1]**. Classically endometriosis is defined as the presence of endometrium outside of uterine cavity. Endometriosis is thought to develop through reverse menstruation of endometrial tissue into the peritoneal cavity. Though retrograde menstruation frequently occurs often in every female of reproductive age, it is assumed that some additional factors are essential that influence the development and progression of endometriosis **[2]**. Endometrial cell implantation into peritoneal cavity is a crucial process in the onset of endometriosis. Interestingly during this process some features of malignancy are demonstrated by endometrial cells, as they have the ability to attach and to invade the peritoneum or ovary structure, in comparable means as cancer cell cause metastasis. This invasion is effectuated with a group of enzymes known as matrix metalloproteinases (MMPs) that are involved in tissue remodelling **[1]**. Current study was designed to analyse the influence of MMPs in endometriosis initiation and progression.

A broad family of zinc binding endopeptidases (MMPs) are responsible for extra cellular matrix components degradation including collagen, fibronectin, proteoglycan and laminin **[3]**. ECMs and protein degrading activity of cell surface proteins play a critical role in pathophysiology of endometriosis. Cell invasion process is associated with breakdown and reconstruction of surrounding microenvironment comprising ECM components hence degradation of ECM components by MMPs may trigger an increase in uterine endometrial fragments invasion in women with endometriosis **[4]**. A number of studies indicated that endometriosis is an invasive disease. The major group of proteases suggested to play crucial role in these invasive processes. Therefore the etiology and pathogenesis of endometriosis is the major group of proteases known as MMPs. Endometrial tissue are a source of MMPs production but this pattern of MMPs production is changed compared to endometriosis. Substantial data designates that MMPs play a dynamic role in endometriosis formation and progression **[5]**. Accumulating evidence propose that endometrial stromal cell secrete MMP-2 that play very vital role in the development of endometriosis through degrading main

component of ECM such as collagen and basement membrane. Several studies shows an increased expression of MMP-2 in uterine endometrium of women with endometriosis **[6]**. Another member of MMP family such as MMP-7 are responsible to cleave the components of ECM in both physiological as well as pathological process **[7]**. A number of studies have demonstrated that MMP-7 is involved in the initial phase of endometriosis development by stimulating an increase in ECM degradation **[8, 9]**.

In addition to that growth and maintenance of endometriosis is reliant on blood vessel recruitment to endometrial lesions from previous ones to assure the supply of indispensable nutrients as well as oxygen **[10]**. It has been shown that tumor implant survival depends on neovascularization and that via inducing angiogenesis endometrial lesions recruit blood vessels. New blood vessel formation is a complex process characterised by corresponding sequence of humoral and cellular interactions. Angiogenic growth factor after stimulation destabilise the mature blood vessels by extracellular matrix degradation and mural cells detachment that is a primitive step for new vessels formation **[11]**. Increased expression of MMPs in endometrium has been reported by Chen et al., this will lead to the migration of epithelial cell into surrounding intersititium and further results in capillary buds and sprouts formation **[12]**. The length and diameter of the newly formed blood vessels increase constantly as epithelial cell proliferate behind the migrating sprouts endothelium. Lastly newly formed blood vessels are stabilised via mural cells attachment comprising smooth muscle cells and pericytes as well as ECM compound production **[13]**. MMPs play an active role in ECM turnover and degradation. These enzymes such as collagenases (MMP-1, MMP-8 and MMP13), gelatinases (MMP-2 and MMP-9) and stromelysins (MMP-3, MMP10 and MMP-11) are able to degrade all components of ECM. Previous studies suggest that MMPs may allow endometrium tissue to digest into the ECM of peritoneum as well as underlying connective tissue. Endometrium stroma, epithelium and polymorphic mononuclear leukocytes are a good source of MMPs production. Additionally inflammation induced activation of neutrophils, macrophages and eosinophils also stimulate the production of MMPs in peritoneal cavity of female with endometriosis **[14]**. The objective of present study was to access the level of MMP-2,

MMP-7 and MMP-9 in females with endometriosis.

MATERIALS AND METHODS

The endometriosis group of current study comprised fifty (50) women with endometriosis confirmed by clinical reports of ultra sound of stage IV endometriosis and histological examination at the Jinnah Hospital Lahore, Pakistan. All women provided informed consent. The control group comprised fifty (50) healthy women. There was no significant difference in age among the women with endometriosis and healthy women. All the contestants had not any immune system disorder, the history of taking alcohol, smoking and hormones as well as had not received immunosuppressive or anti-inflammatory therapy. Current study was approved by the "Research and Ethics Committee" at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan. 5ml of blood was collected from study population. After centrifugation at 4000 rpm supernatant plasma was stored at -70°C. For further processing sample was transferred in laboratory.

Biochemical analysis

Matrix metalloproteinases (MMPs) belongs a large family of $Zn^{2+}$and Ca^{2+}dependent endopeptidases released by various immune cells and are involved in tissue remodelling, chronic inflammation and endometriosis. Several MMPs including MMP-2, MMP-7 and MMP-9 were quantified by commercially available ELISA kit (Sensolyte). By following its possible protocol and absorbance was accessed with the help of ELISA reader at 412nm.

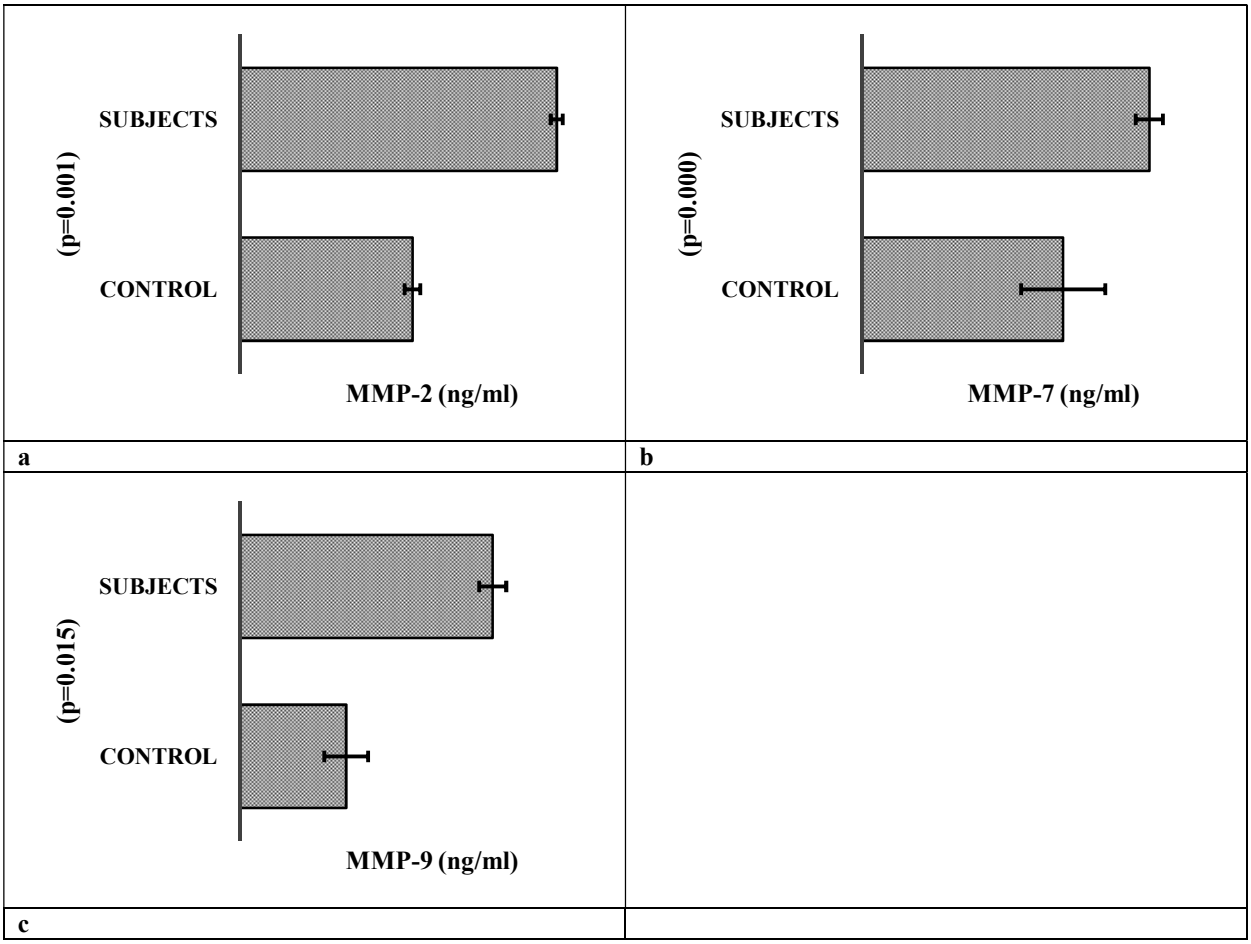
Statistical analysis

All data from ELIZA assay were managed with Statistical Package for Social Sciences (SPSS) Version 17.0 for Windows (SPSS, Chicago, IL) and are presented as the mean ± S.D. Statistical significance was analysed by independent sample t-test and P-values <0.05 were considered significant.

RESULTS

Current study has been conducted to determine the association of serum concentrations of matrix metalloproteinases with the incidence of endometriosis. The results of measurements of serum concentration of MMP-2, MMP-7 and MMP-9 in study population showed a mean serum levels of MMP-2 of 659.35±12.59 ng/ml, MMP-7 of 112.26±5.29 ng/ml and MMP-9 of 98.59±5.29 ng/ml in women with endometriosis and MMP-2 of 358.29±16.35 ng/ml, MMP-7 of 78.59±16.5 ng/ml and MMP-9 of 41.26±8.59 ng/ml in women without endometriosis. Serum concentrations

of MMP-2, MMP-7 and MMP-9 endometriosis women were higher than the women without endometriosis and there is a significant difference (p=0.001, p=0.000 and p=0.015) between the two groups respectively.



DISCUSSION

Although it is largely accepted that refluxed endometrial tissue implantation leads to the development of endometriosis, this process occurs by which mechanism is not known yet. Endometrial tissue must attach and invade a different environment, proliferate and form a blood supply for its implantation and to flourish in an ectopic place. Recent studies have provided evidence for the role of MMPs in endometrial remodelling, in invasion, as well as in development and progression of endometriosis **[15]**. In current study we investigated the level of several MMPs such as MMP-2, MMP7 and MMP-9 in females with endometriosis and found that expression levels of all these MMPs were significantly

increased in endometriotic females than those in normal. These findings are likely as previous studies suggested that upregulation of MMP activity may contribute to invasion and progression of endometriosis **[16]**. For degradation of basement membrane and collagen type IV MMP-9 play a crucial role in different diseases such as embryogenesis, rheumatoid arthritis and cancer metastasis **[17]**. Hence the observed elevation in the activity of proMMP-9 and expression may have important association for the vascular complication development which further leads to the development of endometriosis. For example, Collette and his colleagues proposed that an increased activity of proMMP-9 is responsible for increase in proteolytic activity as well as play very vital role in vascular remodelling during endometrial tissue invasion at ectopic location in endometrioses **[18]**. Several studies have also demonstrated that establishment of angiogenesis depends upon MMP activity **[19]**. Thus increased activity of MMP-9 may be crucial for neovascularisation and proliferation of endothelial cells as well as for continuous development and stabilization of endometriotic lesions at ectopic positions **[17]**. In current study upregulation in MMP-9 activity was observed in women with endometriosis as compared to women without endometriosis and identifies MMP-9 as diagnostic marker for endometriosis. The results of current study support the idea of previous studies that overexpression of MMP-9 in endometrium of women with endometriosis might be involved in the progression of endometriosis. Frequently a wide range of cells express MMP-2 and is thought to play an important role in the maintenance of basic functions for ECM remodelling thus increased activity of MMP-2 results in pathological process **[20]**. Herein we discuss the role of MMP-2 in angiogenesis formation during the development and progression of endometriosis. Present study found a significant increase in MMP-2 activity in uterine endometrium of women with endometriosis as compared to normal women. Recently, several studies have provided circumstantial evidence suggesting the role MMPs in adhesion and proliferation of endometrial tissue, as the pathogenesis of endometriosis **[21]**. Tissue remodelling during endometriosis development and progression cause an increase in MMP-2 circulation in women with endometriosis, as a result of increased MMP-2 and aromatase activity endometrial tissue can trigger the increased production of MMP-2 that may

contribute in the invasion and progression of endometriosis **[22]**. Previous studies have demonstrated that in addition to migration and invasion MMP-2 may also contribute in tumor progression by inducing cell growth and angiogenesis **[23]**. Current study suggest that upregulation of MMP-2 expression was positively associated with angiogenesis during endometriosis. These findings determine that to predict the prognosis of patients MMP-2 is a potential useful marker. Several studies have identified the role of MMP-7 in endometriosis. Current study also found the increase activity and expression of MMP-7 in endometriosis. It has been reported that increased expression of MMP-7 is associated with increased invasiveness. In endometriosis the presence of MMP-7 specifies its increased invasive nature **[24, 25]**. Several studies demonstrates that glandular epithelial cells in ovarian endometriosis as well as epithelial cells in uterine endometriosis are the main source of MMP-7 expression in endometriosis where MMP-7 response is influenced by stromal progesterone signalling **[26-37]**. The results of current study are in accordance with this one as significantly increased MMP-7 expression was observed in endometrial epithelial cells from women with endometriosis as compared to women without endometriosis. The results of current study suggest further evidence that increased expression of MMPs including MMP-2, MMP-7 and MMP-9 and elevation in their activity may play a key role in etiology of endometriosis. Hence it is possible that all these MMPs are responsible for the pathogenesis of endometriosis and these findings indicate functional involvement of MMP-2, MMP-7 and MMP-9 in endometriosis but their precise mechanism cannot be elucidated yet.

CONCLUSION

Present study concluded that there is a significant increase in levels of MMPs (MMP-2, MMP-7 and MMP-9) in women suffering from endometriosis as compared to women without endometriosis. The increased expression level of MMPs is highly associated with the prevalence of endometriosis. These findings may contribute to a better understanding of disease as well as upkeep clinical evaluation of endometriosis.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

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