Research Article

Mullerian Inhibiting Substance type II Receptor (MISIIR) Expression in Premalignant and Malignant Cervical Lesions

Mohammed M. Amin, Mahmoud S. Mahmoud, Mohammed S. Mohammed, Sahar M. Aboel-Eion and Ahmad S. Abd El-Malik
Department of Microbiology and Immunology,
El-Minia Faculty of Medicine.

Abstract
Cancer cervix is the third most common neoplasm of the female genital tract, cervical cancer accounts for ١٠٪ of all cancers in women and resulted in ٤,٨٠٠ deaths in ١٩٩٩ in the United States. The study started in April ٢٠١١ and ended in December ٢٠١٣ it included ٦٠ female patients with CIN and cancer cervix managed in Minia university maternity hospital and ٤٠ women with no cervical lesions as controls. Correlation data between MISIIR gene expression and risk factors revealed that. Risk factors (age at marriage, parity and age at first pregnancy) were considered as protective values, there is weak correlation between MISIIR gene expression and risk factors (age, duration of marriage, number of marriage, menopausity, age at menopause , type of contraception and family history of cervical malignancy), there is moderate correlation between MISIIR gene expression and recurrence of disease and there is strong correlation between MISIIR gene expression and risk factors (number of lesions, size of lesion, grade of lesion and treatment of lesion).

Keywords: CIN; cervical intraepithelial neoplasia
MISIIR; Mullerian inhibiting substance type II receptor

Introduction
The risk of cervical neoplasia is most strongly related to persistent genital high risk human papilloma virus (HR-HPV) infection[٢١]. Early onset of sexual activity, multiple sexual partners has role in CIN and cervical cancers[٧]. Increasing parity, progesterone exposure, Immune suppression, and physical trauma related to vaginal deliveries have been suggested as etiologic factors associated with the development of cervical CIN[١٦]. Low-grade Squamous Intraepithelial Lesions (SIL) encompasses the cytologic features of HPV infection and CIN ١, ١٥- to ٣٠-% risk of CIN ٢ or ٣[٢٤]. High-grade SIL cytology encompasses features of CIN ٢ and CIN ٣ or invasive cancer [١٣].Most cervical cancers originate from cells infected with HPV, which is sexually transmitted. Parity and combination oral contraceptive (COC) pill use has a significant association with cervical cancer[١٦]. An increased number of sexual partners and early age of first intercourse have been shown to increase cervical cancer risks[١١]. Squamous cell carcinoma of the cervix typically arises at the squamocolumnar junction, which in most cases follows infection with HPV[٤]. The two most common histologic subtypes of cervical cancer are squamous cell and adenocarcinoma. Of these, squamous cell tumors predominate, comprise ٧٥ % of all cervical cancers, and arise from the ectocervix[٢٣]. Several lines of evidence suggest that MIS/AMH inhibits the growth in tissue and cell lines of other MIS/AMH receptor-expressed gynecological malignancies such as cervical, endometrial and breast cancers[٢٠]. MIS can inhibit the growth of both ovarian and cervical neoplasms. Malignant cells contained in ascites collected from ovarian-cancer patients express MISRII, human ovarian cancer cell lines express the MISRII, and their proliferation is inhibited in vitro and in vivo by recombinant MIS [٩].

Subject and method
A total of ١٠٠ female subjects participated in the research, and ١١١ cervical smears were taken. ٦٠ of them were women with (CIN and cervical squamous cell carcinoma) and ٤٠ women without cervical lesions as controls. All subjects were enrolled from Minia maternity university hospital in the period between April ٢٠١١, and December ٢٠١٣. A written informed

consent was taken from every participant in this study. Full history and gynecological examination were done. The aim of the present study is the Detection of Mullerian Inhibiting Substance Type II Receptor (MISIIR) mRNA By RT- PCR and Its quantitative measures in premalignant and malignant cervical lesions. Cervical tissue samples were taken in barafen for histopathology and in RNAlater (protect RNA from disintegration) for MISIIR gene expression by RT-PCR.

Results

The study subjects were classified into cases (women with CIN or cervical squamous cell carcinoma, (NO. = ٦٠) and controls (without cervical lesion, (NO. = ٤٠). The study revealed that, the mean frequency of age at marriage, In cases, ٢٦.٨٣, while in controls, ٢٣.٤٥, (p=٠.٠٧٧). The mean frequency of duration of marriage, In cases, ١٥.١٠, while in controls ٢٠.٧٥, (p=٠.٠١٢) . The mean frequency of age at first pregnancy, In cases, ٢٨.٤٩, while in controls ٢٥.٠٥, (p=٠.٠٠١). Nullipara women were excluded from this table in cases (n = ١) and in controls (n=٣). The mean frequency of parity, In cases, ٣.٥٣, while in controls ٣.٣٠, (p=٠.٥٣٨). The mean frequency of age at menopause among, In cases, ٤٦.٢١, while in controls ٤٦.٧٨. Regarding to methods of contraception, In cases, ٣٨.٥٪ of women were using hormonal contraception, while in controls ٥٢.٥ % of women were using hormonal contraception. Regarding to family history of cervical malignancy, In cases, ١٠٪ of women were with positive family history of cervical malignancies, while in controls all women with negative family history of cervical malignancies (p=٠.٠٤٢) .

Of the ٦٠ subjects investigated for MISIIR in the cases, ١٢ (٢٠٪) showed positive gene expression. and were named subgroup I, while ٤٨ (٨٠٪) of cases showed Negative gene expression, named subgroup II. In terms of number of lesions, In subgroup I, ٢ (١٦.٧٪) women had one lesion, one woman (٨.٣٪) had two lesions, ٢ (١٦.٧٪) women had three lesions and ٧ (٥٨.٣٪) had four lesions. While in the subgroup II, ٤٣ (٨٩.٦٪) women had one lesion, ٢ (٤.٢٪) women had two lesions and ٣ (٦.٢٪) women had three lesions. (r=٠.٧٣٨). In terms of size of lesion, In subgroup I, ٢ (١٦.٧٪) had lesion of ٢ mm, ٦ (٥٠٪) had lesion of ٣ mm in size and ٤ (٣٣.٣٪) had lesion of ٤ mm in size. While in subgroup II, ٣١(٦٤.٦٪) had lesion of ١ mm, ٨ (١٦.٧٪) had lesion of ٢ mm in size, ٥ (١٠.٤٪) had lesion of ٣ mm in size and ٤ (٨.٣٪) had lesion of ٤ mm in size. (r=٠.٦٧٨) In terms of grades of lesions, in subgroup I, ٩ (٧٥٪) had CIN lesion of (grade III) and ٣(٢٥ %) had squamous cell carcinoma. While in subgroup II, ١٦(٣٣.٣٪) had CIN lesion of (grade I), ٢٠(٤١.٧٪) had CIN lesion of (grade II), ١٢ (٢٥٪) had lesion of (grade III). (r=٠.٧٩٨). In terms of MISIIR gene expression, in subgroup I, mean +SD: ٢٨٠٣.٤٣٤+٤٥٢.٨٦٩, while in subgroup II, mean + SD: ٤١٨.٥٧٣ +٤٠١.٦٩٢, (p=٠.٠٠١)

Discussion

Regarding the age of first marriage the mean age was ٢٦.٨٣ which were more than the recorded by Reich ٢٠٠٥, who said that the young age at first intercourse (less than ١٦ years) is a risk factor for cancer cervix [١٩]. Martyn Plummer et al., ٢٠١١ [١٤], found a nonsignificant, slightly reduced risk of cancer among women, who had first sexual intercourse within a year of menarche. Short interval between births was associated with an increased risk of CIN٣ in postmenopausal GM women [٢]. Regarding to parity the mean was ٣.٥٣. Munoz et al., ٢٠٠٢[١٦], they found that high parity increases the risk of squamous-cell carcinoma of the cervix among HPV-positive women. A general decline in parity might therefore partly explain the reduction in cervical cancer recently seen in most countries. Early first full-term pregnancy and high parity are associated with a reduction in risk of breast, ovarian, and endometrial cancer.[١٧] but with an increase in cervical cancer risk [١١]. Regarding to the age at menopause, the mean frequency of age was ٤٦.٢١; it is lower than described by Belsey and Pinol ١٩٩٧ [٣], which was ٥٢ years. Regarding to methods of contraception, In cases, ٣٨.٥٪ of women were using hormonal contraception, while in controls ٥٢.٥ % of women were using hormonal contraception, Franceschi et al., ٢٠٠٣ [٨], were found statistically significant findings of both increased_and decreased risk associated with earlier final menstrual period. The human cervix is strongly altered by hormonal changes,[٦], and cervical cancer incidence rates are transiently increased by oral contraceptive use[١٠]. Moodley, ٢٠٠٤[١٥], found that hormonal contraception can lead to up to twofold to

cervical cancer but only for women who were both long-term users.In the current study, ١٠ % of cases had positive family history of cervical lesions, while in controls all women had negative family history of cervical malignancy. American Cancer Society (ACS). ٢٠١٣ [١], showing that cervical cancer may run in some families. If a woman's mother or sister had cervical cancer, her chances of developing the disease are ٢ to ٣ times higher than if no one in the family had it. Some researchers suspect some instances of this familial tendency are caused by an inherited condition that makes some women less able to fight off HPV infection than others. In the present study regarding to the number of cervical lesions, in subgroup I , ٢ (١٦.٧٪) women had one lesion, one woman (٨.٣٪) had two lesions, ٢ (١٦.٧٪) women had three lesions and ٧ (٥٨.٣٪) had four lesions. While in the subgroup II, ٤٣ (٨٩.٦٪) women had one lesion, ٢ (٤.٢٪) women had two lesions and ٣ (٦.٢٪) women had three lesions. With high significant statistical difference between two groups and strong correlation, regarding to the size of cervical lesions, In subgroup I, ٢ (١٦.٧٪) had lesion of ٢ mm, ٦ (٥٠٪) had lesion of ٣ mm in size and ٤ (٣٣.٣٪) had lesion of ٤ mm in size. While in subgroup II, ٣١(٦٤.٦٪) had lesion of ١ mm, ٨ (١٦.٧٪) had lesion of ٢ mm in size, ٥ (١٠.٤٪) had lesion of ٣ mm in size and ٤ (٨.٣٪) had lesion of ٤ mm in size. With high significant statistical difference between two groups and strong correlation. Piver et al., ١٩٩٧[١٨], found that Excellent ٥-year survival rates for women with cervical carcinoma were associated with cervical lesions measuring less than ٣ cm and resected pelvic lymph nodes which did not contain metastatic cancer. Van Nagell et al., ١٩٧٧ [٢٢], found that the incidence of lymph nodal metastases and tumor recurrence was more directly related to lesion size than to tumor-cell type. The incidence of metastatic disease was significantly increased and survival was reduced in those patients whose tumors were greater than ٢ cm in diameter. Regarding to the grades of cervical lesions, in subgroup I, ٩ (٧٥٪) had CIN lesion of (grade III) and ٣(٢٥ %) had squamous cell carcinoma. While in subgroup II, ١٦(٣٣.٣٪) had CIN lesion of (grade I), ٢٠(٤١.٧٪) had CIN lesion of (grade II), ١٢ (٢٥٪) had lesion of (grade III). With high significant statistical difference between two groups and strong correlation. Regarding to the MISIIR gene expression in cervical lesions, in subgroup I, (mean +SD: ٢٨٠٣.٤+٤٥٢.٨), while the in subgroup II, (mean + SD: ٤١٨.٥+٤٠١.٦), with highly significant statistical difference between the two groups. Cases with positive gene expression were ٩ (٧٥ %) in CIN grade III and in all ٣ (٢٥ %) cases of squamous cell carcinoma. while in CIN grade I and CIN grade II cases gene expression was negative.Jae Yen et al., ٢٠١٢ [١٢], work on ٣٠ cervical cancer tissues (٦ squamous cell carcinoma in situ, ٢ microinvasive squamous carcinoma, ١١ squamous cell carcinoma, ٢ adenocarcinoma in situ, ٢ microinvasive adenocarcinoma, ٧ adenocarcinoma), RT-PCR. Analyzed MISRII mRNA expression in a squamous cell carcinoma of cervix, and an adenocarcinoma of cervix by RT-PCR and all tissues showed Positive gene expression at ٣٦١ bp band which was confirmed to be identical to a segment of human MISRII cDNA sequence.

Conclusion

The age at marriage, duration of marriage, age at first child, family history of cervical malignancy, number of marriage, size of lesion, recurrence and MISIIR and gene expression , had role in increasing the incidence of cervical CIN and cervical malignancy. MISIIR gene expression is positive in all cervical squamous cell carcinoma while it is positive in CIN grade III only. This indicating that follow up of cases of CIN I and II is recommended for early diagnosis of CIN III and Cancer cervix.

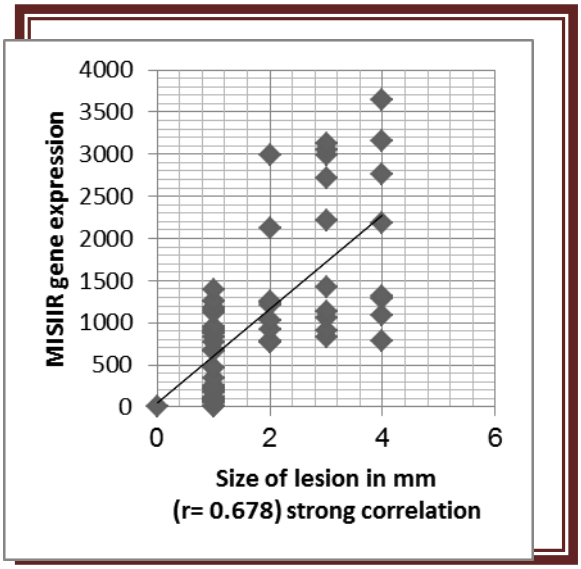

Figure (١): Amplification blot of MIS IIR Gene expression by Real Time PCR

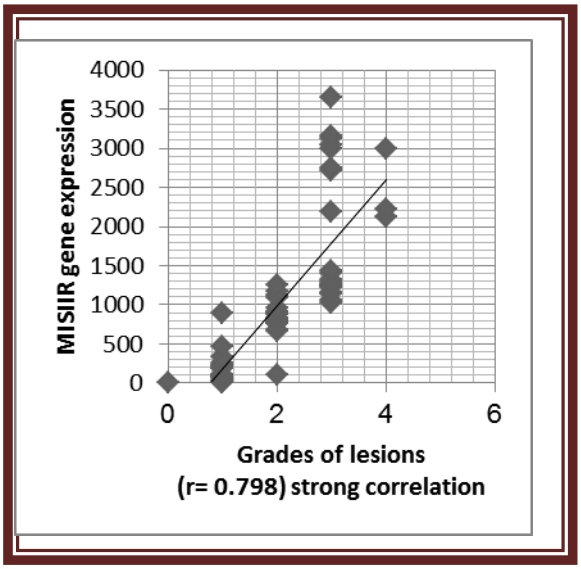

Figure (٢): Number of lesions among subgroup I and subgroup II

Recommendations: Suitable screening programs needed to be implemented for any woman above ٣٠ years and women with a risk factor for cancer cervix. Therapeutic modalities other than surgery need to be available. After treatment of patients with cancer cervix, patients needed to be educated that follow up is as important as the treatment they had. The oncology unit needs to improve on patient's data recording. This may require the introduction of computerized data recording systems. This would not only help patient's management but would also help in the use of these data for research purposes. This leads to improvement of the methods of treatment through comparing the outcomes of different treatments modalities. Follow up of low and intermediate CIN (CIN types I and II) by MISIIR is recommended for early detection of CIN grade III and squamous cell carcinoma.

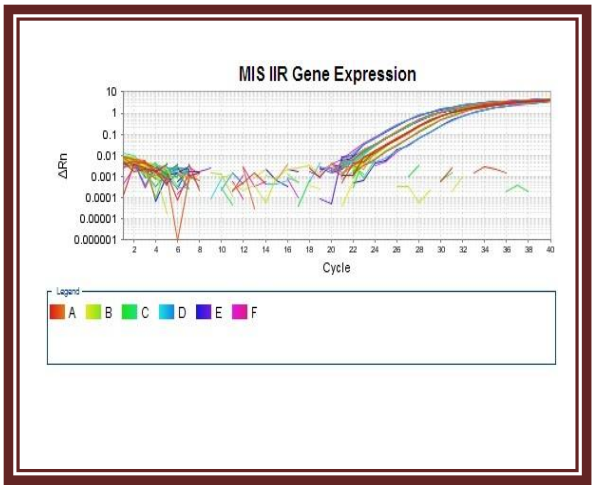

Figure (٣): size of lesions among subgroup I and subgroup II

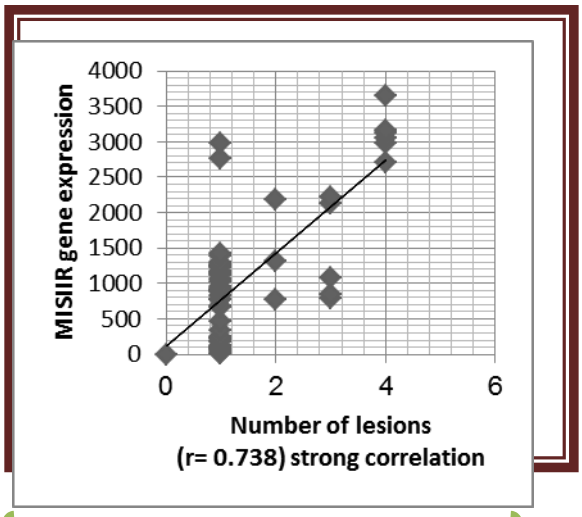

Figure (٤): grade of lesions among subgroup I and subgroup II

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