

Detection of Cytomegalo Virus in Cervical Tumors among Sudanese Women

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Abstract:

This study aimed to detect the expression of CMV Ag in cervical tumors using immunohistochemical method. Fifty formalin fixed paraffin blocks (FFPB) previously diagnosed as cervical tumors (35 of them were malignant cervical tumors and 15 were benign cervical tumors) were used in this study. Blocks were cut and stained by immunohistochemical method (New indirect method) for detection of CMV Ag. The data obtained were analyzed using SPSS program. The age of patients ranged between 20 and 80 years. Out of thirty five malignant samples, 31 samples were squamous cell carcinoma, 2 were micropapillary adenocarcinoma and 2 were chordosaroma. The immunohistochemical expression of CMV Ag was detected in 13 (26%) samples and negative in 37 (73%) samples. 6/13 positive samples were malignant and the remaining 7/13 samples were benign and there was statistical association between CMV expression and malignant tumors of the cervix ($P=0.029$). The relation between histological differentiation and CMV infection revealed that 0/6 sample was well differentiated tumors, 1/4 sample was moderately differentiated tumors and 5/24 samples were poorly differentiated tumors with no significant statistical association ($P=0.459$).

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Conclusion: *This study concludes that there is association between CMV infection and tumors of the cervix with no association with tumor grade.*

Key words: CMV, squamous cell carcinoma, chordosarcoma, micropapillary adenocarcinoma.

INTRODUCTION:

Cervical cancer is cancer that starts in the cervix. The cervix is the lower part of the uterus (womb) that opens at the top of the vagina ⁽¹⁾.

Worldwide, cervical cancer is both the fourth most common cause of cancer and deaths from cancer in women. It is the second most common cause of female specific cancer after breast cancer accounting for around 8% of both total cancer cases and total cancer deaths in women. Approximately 80% of cervical cancers occur in developing countries ⁽²⁾.

Cancer of the cervix is the second most prevalent cancer of women to date in the Sudan, in a concerted review of the records of the hospital-based cancer registry of the Radiation & Isotope Centre of Khartoum (RICK) ⁽³⁾.

There are many risk factors for cervical cancer which include HPV infection, early age of first sexual intercourse, increase number of sexual partners, long term use of oral contraceptives, smoking, history infertility, intrauterine device, high parity, Trauma with pregnancy, low education, infection by other sexually transmitted diseases (STI) (Human Immunodeficiency Virus, Chlamydia trachomatis, Cytomegalovirus, etc.) And low socioeconomic level ⁽⁴⁾.

Confirmation of the diagnosis of cervical cancer or pre-cancer requires a biopsy of the cervix. This is often done through colposcopy, a magnified visual inspection of the cervix aided by using a dilute acetic acid (e.g. vinegar) solution to highlight abnormal cells on the surface of the cervix. Medical

devices used for biopsy of the cervix include punch forceps, Spira Brush CX, Soft Biopsy or Soft-ECC ⁽⁵⁾.

The most common treatments for cervical cancer are surgery (Hysterectomy or Trachelectomy) or chemoradiation which is a combination of chemotherapy and radiotherapy (internal radiotherapy or External radiotherapy). Palliative treatment helps to improve people's quality of life by alleviating symptoms of cancer without trying to cure the disease. It is particularly important for people with advanced cancer. However, it is not just for end-of-life care and it can be used at any stage of cancer ⁽⁶⁾.

Cytomegalovirus is a genus of viruses in the order Herpesvirales, in the family Herpesviridae, in the subfamily Beta herpes virinae. Human and monkeys serve as natural hosts. There are currently eight species in this genus including the type species human herpesvirus 5. Diseases associated with HHV-5 include mononucleosis, and pneumonias^{(7) (8)}.

Immunohistochemical staining using an antibody specific for CMV, a marker for CMV infection.

This study aimed that there is association between CMV expression and tumors of uterine cervix and with histological differentiation of tumor. Cytomegalovirus should be investigated as a possible cause of cervical cancer.

MATERIALS AND METHODS:

Sample collection:

Paraffin embedded tissue blocks previously diagnosed as cervical tumors were collected for this study from different centers in Khartoum state.

Slide preparation:

One section of 5µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for

immunohistochemistry which is then taken in thermal coated slides and dried in hot plate oven at 80°C for one hour.

Immunohistochemical stain:

Sections were brought to water and retrieved using water bath retrieval technique at 97°C, then treated with hydrogen peroxide solution for 15 minutes, then washed in phosphate buffer saline (PH 7.4) for 5 minutes, then treated with anti CMV primary antibody for 30 minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for 30 minutes, then rinsed in phosphate buffer saline, then treated with DAB for 7 minutes, then washed in phosphate buffer saline for 5 minutes, then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) 2 minutes for each then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media ⁽⁹⁾.

Result Interpretation:

Results obtained were detected by researcher and confirmed by experienced histopathologist. Negative and positive controls were used for evaluation of the test sections.

Data analysis:

The data were analyzed using version 16.0 SPSS computer program; frequencies, means and chi-square correlations were calculated.

RESULTS:

The age of study subjects revealed that 20 to 40 years were 14 (28%), 41 to 60 years were 18 (36%) and 61 to 80 years were 18 (36%) Table (1). Table (2) showed the tumor types which

represent that squamous cell carcinoma in 31 (62%) samples, micropapillary adenocarcinoma in 2 (4%) samples, chordosaroma in 2 (4%) samples and benign tumors in 15 (30%) samples. Table (3) showed that malignant cervical tumors revealed positive expression of CMV in 6/35 samples, while benign tumors showed positive result in 7/15 samples (P=0.029). Histological differentiation and CMV infection revealed that 0/6 sample was well differentiated tumors, 1/4 sample was moderately differentiated tumors and 5/24 samples were poorly differentiated tumors with no significant statistical association (P=0.459) Table (4). Figure (1): cervix cervicitis showed positive expression of CMV Ag. Figure (2): poorly differentiated squamous cell carcinoma showed positive expression of CMV Ag.

Table (1): Age among the study population:

Age group(year)	Frequency	Percent %
20-40	14	28
41-60	18	36
60-80	18	36
Total	50	100%

Table (2): Histopathology diagnosis of study samples population:

Histopathology diagnosis	Frequency	Percent %
Squamous cell carcinoma	31	62
Micropapillary adenocarcinoma	2	4
Chordosaroma	2	4
Benign tumors	15	30
Total	50	100%

Table (3): Relation between immunohistochemical expression of CMV Ag and tumor type:

Histopathology diagnosis	CMV expression		Total
	Positive	Negative	
Malignant tumors	6	29	35
Benign tumors	7	8	15
Total	13	37	50

P= 0.029

Table (4): Relation between immunohistochemical expression of CMV Ag and tumor grade:

Histological differentiation	CMV expression		Total
	Positive	Negative	
Well differentiated tumor	0	6	6
Moderately differentiated tumor	1	3	4
Poorly differentiated tumor	5	19	24
Total	13	37	50

P= 0.459

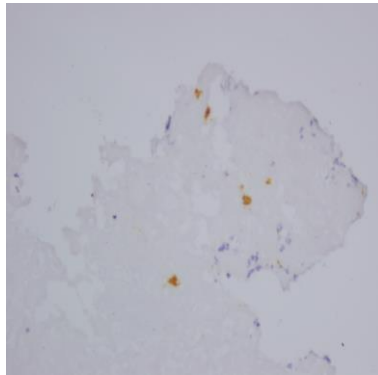


Figure (1): cervix cervicitis showed positive expression of CMV Ag.

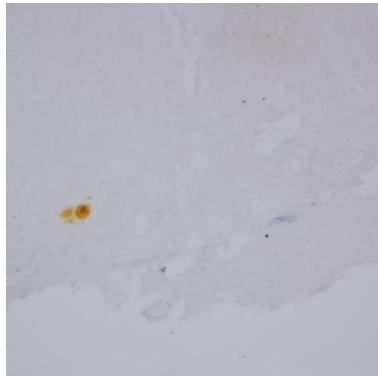


Figure (2): poorly differentiated squamous cell carcinoma showed positive expression of CMV Ag.

DISCUSSION:

Cervical cancer (CC) as a single diagnostic entity exhibits differences in clinical behavior and poor outcomes in response to therapy in advanced tumors ⁽¹⁰⁾.

Cervical cancer is one of the most common neoplastic diseases affecting women, with a combined worldwide incidence of almost half a million new cases annually, second only to breast cancer ⁽¹¹⁾.

The present study includes samples containing 32 (60.8%) samples were squamous cell carcinoma (SCC), 2 (3.9%) samples were micropapillary adenocarcinoma and 2 (3.9%) samples were chordosaroma and cervix cervicitis were 15 (29.4%) samples, indicating that SCC is the predominant type of the cervical cancers identified in Sudanese patients. Our study includes patient from different age groups, with no relation between the prevalence of the cervical tumors and specific age group.

CMV has been shown to induce transformation of cells in culture. Because of the high prevalence of genital infections with this pathogen and evidence that may have oncogenic effect on the cervix. CMV encodes a protein, UL16, which is involved in the immune evasion of NK cell responses. It binds to ligands ULBP1, ULBP2 and MICB of NK cell activating receptor NKG2D, which prevents their surface expression. These ligands are normally upregulated in times of cellular stress, such as in viral infection, and by preventing their upregulation, CMV can prevent its host cell from dying due to NK cells ⁽¹²⁾.

Immunohistochemical detection of CMV Ag showed that 13/50 (26%) positive samples of all cases of cervical lesions. 6/35 positive samples were malignant tumor and 7/15 positive samples were benign tumors with significant association between CMV infection and tumor behavior (P=0.029). This finding is consistent with Marinho-Dias and Sousa⁽¹³⁾ result

who reported that the frequency of CMV is 18.9% of all types of cervical samples. Also our finding is compatible with Odida and Schmauz⁽¹⁴⁾, they reported that CMV was detected in 5/34 cases of cervical cancer. Also Chin-ping *et al.*,⁽¹⁵⁾ they reported that CMV was detected in 67% of cervical samples. This finding is disagreeing with the study of Chen-Yang *et al.*,⁽¹⁶⁾ they reported that CMV infection alone without co-infection with other viruses was associated with no increased risk for cervical cancer.

Concerning the relation between CMV infection and the grade of tumor the study showed that the positive expression was not related to the grades observed ($P=0.459$), indicating the aggressiveness of tumor is not initiated by the presence or absence of the infective agent.

CONCLUSION

The study concludes that there was association between CMV infection and tumor behavior with no association with tumor differentiation.

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