DNA-In Situ Hybridization for Molecular Localization of Human Cytomegalovirus in Cervical Tissues from Iraqi Patients with Cervical Adenocarcinoma

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Abstract

Cervical cancer pathogenesis is a multi-factorial process where cervical epithelium is exposed to several important carcinogenic influences. On epidemiological bases, cervical cancer has the characteristics of a venereal disease and among many genital infective agents; human cytomegalovirus (HCMV) might have a role in the etiology of this neoplasm. This study aimed to investigate rate of HCMV infections in cervical adenocarcinoma using DNA -in situ hybridization (DISH) and to evaluate their impact on the expressed histopathological features of those cancers. This study was designed as retrospective research. A total number of forty-nine (49) patients who had undergone hysterectomies or punch biopsies from their cervices were included. Among these, twenty-one (21) formalin-fixed paraffin-embedded tissue blocks were from cervical adenocarcinoma while twenty-eight (28) cervical tissue blocks (either without any significant pathological changes or have chronic cervicitis) were included as a control groups for this study. Molecular detection of HCMV in those tissue blocks were performed by using ultra-sensitive versions of DISH technique. Human CMV DNA was found in 12 out of 21 (57.1 %) cervical adenocarcinoma tissues while no HCMV DNA was detected in any tissues from chronic cervicitis group or those in the histopathologically- healthy control group. Among those twelve positive results of Human CMV DNA –ISH reactions, eleven cases (91.7 %) of cervical adenocarcinoma have well or moderate differentiation while only one case (8.3%) was of poor grade.

Keywords: Cervical adenocarcinoma, HCMV, DNA-ISH.

Introduction

Worldwide, cervical cancer is the second most common type, after breast cancer. It constitutes approximately12% of all female cancers with a toll annually of 500,000 new cases and 200,000 deaths. In developing countries, it ranks top of the 10 most common female cancers whereas it is the fifth in developed nations. In Iraq, this cancer is out of these 10 ranks [1-4]. Iraqi Ministry of Health announced in 2012 an incidence of 0.46 / 100000 for cervical cancer occurrence, with an annual number of new cases of 146 (0.96 % of total) [4].

The incidence of invasive squamous cell cervical cancers has decreased over the last few decades due to effective cervical screening programs that have led to a proportional increase of cervical adenocarcinomas in relation to squamous cell carcinomas. However, an absolute increase in the rate adenocarcinoma cases was observed, too (5% in 1950’s to10-25% in the current accounts) [5-11].Age presentation of invasive adenocarcinoma is in the average of 45-55 years while it is uncommon to see adenocarcinoma in situ or invasive adenocarcinoma in younger women [12].
A number of histological subtypes of cervical adenocarcinoma have been described and the most common adenocarcinoma types are often referred to as being “End cervical”, “mucinous” or “NOS” “not otherwise”, accounting for about 90% of all cases [13]. The grade of adenocarcinoma is based on a combination of architecture and nuclear features.

The typical architecture of grade 1 adenocarcinoma is predominantly glandular and the nuclei have too atypical features so as to score as grade 1 while if nuclear atypia is out of proportion to the glandular differentiation, the grade is increased by 1. The architecture is also differentiated as grade 1 when less than 5% solid areas are noticed; while if associated with 5-50% and > 50% solid growth are recognized as grade 2 and grade 3, respectively [13]. Cervical tissues are under important effects of many possible carcinogenic influences such as genetic, chemical, infective, immunological, hormonal, religious and occupational factors. The extent of any of these factors to induce the malignant changes is still exactly unknown [14]. Malignant transformation of cervical epithelium is a complex, multi-factorial and stepwise process in which a number of these factors can play a part [15].

High-risk types of human papilloma virus are widely accepted as the major contributing factors to cervical carcinogenesis. [16]. However, recent evidences indicated that some other sexually transmitted viruses such as Epstein-Barr virus and Cytomegalovirus might contribute to cervical carcinogenesis [17].

Human CMV can replicates in vivo in epithelial cells of the kidney, parotid salivary glands and uterine cervix [18]. Recent studies have frequently reported the presence of HCMV genome and antigens in certain malignancies, such as colon cancer [19], malignant glioma [20], prostatic carcinoma [21] and cervical cancer [22].

Human CMV can induce cell transformation through the production of viral proteins [23].

More than 200 proteins encoded by Human CMV [24]. Immediate- early (IE) proteins could induce malignant transformation of human cervical epithelial cells if they were non-permissive for full replication cycle of this virus [23]. Over the past few years, it is well established in model systems and latency studies that the both latency and reactivation as well as regulation of major IE genes of the cytomegalovirus’s are directly influenced by either the inflammatory cytokines or TNF.

Many studies concluded that the co-infection of EBV and CMV with HPV16 have potential significance in etiopathogenesis of uterine cervical cancer [25-26].

In Iraq, cervical cancer constituted 0.96% of all women cancers (4). Worldwide, the main viral etiology of this cancer was strongly related to HPV. However, in our country the first who studied the role of HPV in cervical neoplasia by PCR was [27] and by ISH were Al-Jewari et al [28].

Some authors proposed a role for CMV in the carcinogenesis of cervical tumors while others did not support such hypothesis. The present study, and up to our best Knowledge is also the first that study the fifth type of herpes virus (HCMV) in Iraqi female patients with invasive cervical adenocarcinoma. This study is aiming to assess whether HCMV is associated with such female genital tract neoplasms.

Materials and Methods

Tissue Samples

This retrospective study used a total number of forty-nine [49] selected formalin-fixed, paraffin-embedded blocks from cervical tissue samples from patients who had undergone hysterectomies or punch biopsies from their cervixes. Twenty-one [21] patients have cervical adenocarcinoma and thirteen [13] patients were diagnosed to have chronic cervicitis. A further fifteen [15] blocks from normal cervical tissues samples were labeled as a control group for this study (i.e. normal healthy cervical tissues without any significant pathological changes).

The age range of the patients was 28–71 years. The specimens were collected during the period 2001–14 from major hospitals and private histopathological laboratories in Baghdad. The initial diagnoses were based on their accompanied pathological records of the corresponding patients.
Laboratory Methods

Following trimming process of these tissue blocks, a consultant pathologist reexamined all these cases to further confirm their diagnoses. One paraffin embedded (4 mm) thick-tissue section was prepared and mounted on ordinary glass slide and stained with hematoxyline and eosin, while another (4 mm) thick-tissue section was stuck onto positively charged slide to be used for CMV-DNA in sit hybridization (DISH) detection system using biotinylated-labeled DNA oligonucleotides probe which targets CMV-DNA by ISH kit that was purchased from (US Biological, USA; C9011-01G, Lot. No.:L13112165 & C13112165).

The details of methods for performing ISH reaction with this probe were conducted according the instructions of the manufacturing company. For the in situ hybridization procedure, the slides were placed in 60°C hot-air oven overnight then the tissue sections were de-paraffin zed and via then incubation of slides for 15 minutes (twice times) in xylene then treatment by graded alcohols via incubation for 5 minutes in 100% ethanol(twice times).

The same dewaxing protocols were routinely used for immunohistochemistry procedures, e.g. 15 minutes xylene (twice times), 5 minutes 100% ethanol(twice times), 5 minutes 96% ethanol(one time), 5 minutes 70% ethanol(one time), were used. Finally, immersion in distilled water for 5 minutes was done for removing any residual alcohol. After that, slides were allowed to dry completely by incubating them at 37°C for 5 minutes. Then digestion process was done by adding proteinase K to the slides, and then the slides were incubated at 37°C for 15 minutes. Then the slides were dehydrated by immersing them sequentially in the following solution at room temperature for the indicated times, distilled water for 1 minute, 70% ethanol for 1 minute, 95% ethanol for 1 minute and 100% by incubating them at 37% for 5 minutes.

All reagents used during hybridization and detection were pre-warmed to room temperature and the slides were not allowed to dry out at any time during the hybridization and staining. Then we add the 20 µl of cDNA probe added to each section and slides were covered by cover slips be careful to avoid trapping any air bubbles. Positive-control reaction was performed by replacing the probe with a biotinylated housekeeping gene probe. For the negative-control reaction, all reagents were added except the diluted probe. After that probe and target DNA were denatured by placing the cover slipped-slides in pre-warmed oven at 95°C for 8-10 minutes, slides were transferred to a pre-warmed humid hybridization chamber and incubated at 37°C for overnight.

At the next day, slides were soaked in pre-warmed protein block at 37°C until the cover slips fell off, then the slides were allowed to remain in the buffer for 3 minutes, at 37°C after cover slips were removed. After that streptavidin-alkaline phosphatase conjugate reagent was added to tissue sections. Then slides were kept in a humid chamber at 37°C for 20 minutes. Slides were then rinsed in detergent wash buffer for 5 minutes and then drained. After that one to two drops of 5-bromo-3-chloro-3-indolyl / phosphate/nitro blue tertrazolium (BCIP/NBT) substrate-chromate solution were placed on tissue section. Slides were incubated at 37°C for 30 minutes or until color was completely developed.

Then the slides were rinsed in distilled water for 5 minutes, then counter staining process by immersion of the slides in nuclear fast red stain for 30 seconds, then washing process was followed by immersion the slides for 1 minute in distilled water. After that sections were dehydrated by ethyl alcohol, (95%, once for one minute then, 100% twice times for 2 minutes each); cleared by xylene, then mounted with permanent mounting medium (DPX).

Color development was monitored by viewing the slides under the light microscope. According to the specification of the kit, proper use of this ISH detection system gives an intense blue signal at the specific complementary sites of the probe in the positive test cells and tissues.

Analysis

The signal was evaluated under light microscopy using × 100 lens for counting the positive cells. The ISH results were given intensity and percentage scores based on intensity of positive signals and number of cells that gave these signals, respectively.
Positive cells were counted in 10 different fields of 100 cells for each sample and the average percentage of positive cells within the 10 fields was determined. A scale of 0-3 was used for relative intensity with 0 corresponding to no detectable ISH reaction, and 1, 2, 3 equivalents to low, moderate, and high intensity of reaction respectively. Cases were assigned to one of the following percentage score categories: 1%–25% (score 1), 26%–50% (score 2) or > 50% (score 3) (29).

The chi-squared test was used to detect the significance between groups. The statistical analysis was done using SPSS program, version 17, and differences were considered significant when $P < 0.05$ (13).

The Results

The age recognitions were (46.7 +11.6), (40.1 +15.2) and (34.7 +13.3) years, for those patients with cervical adenocarcinoma, chronic cervicitis and those with healthy (normal) cervical tissues, respectively (Table 1).

Table 1: The Age distribution of the patients with cervical adenocarcinoma

<table>
<thead>
<tr>
<th>Studied Tissues Taken from:</th>
<th>Number</th>
<th>Mean Age (Years)</th>
<th>S.D</th>
<th>S.E</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma Patients (AC)</td>
<td>21</td>
<td>46.70</td>
<td>11.60</td>
<td>1.13</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>Chronic Cervicitis Patients (CC)</td>
<td>13</td>
<td>40.10</td>
<td>15.20</td>
<td>2.98</td>
<td>29</td>
<td>63</td>
</tr>
<tr>
<td>Control Patients With Apparently Healthy Cervices (HC)</td>
<td>15</td>
<td>34.70</td>
<td>13.30</td>
<td>2.28</td>
<td>31</td>
<td>69</td>
</tr>
</tbody>
</table>

Table (2) shows that each of well and poor grades of cervical adenocarcinoma constituted 14.3% (3 out of 21 cases) whereas the rest tissue blocks (15 out of total 21 cases; 71.4%) were moderately differentiated.

Table 2: Grading of the studied cervical Adenocarcinoma Carcinoma Group

<table>
<thead>
<tr>
<th>Tissue Grading*</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>3</td>
<td>14.3</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>71.4</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The statistical analysis shows significant differences ($p<0.05$) between moderately differentiated grade and each of well and poorly differentiated cervical adenocarcinoma, while non-significant difference was noticed between poorly and well differentiated cervical adenocarcinoma.

Table (3) shows the positive results of the Human CMV DNA –ISH signal detection; 12/21 adenocarcinoma cases (57.1%) revealed positive blue nuclear signals at the sites of sequence-complementarities. None of the chronic cervicitis and normal healthy uterine cervix tissues presented positive signals for the Human CMV DNA –ISH test. Of the positive cases, 6/12 (50.0%) adenocarcinoma tissues had score 2 (26%–50% positive cells), while 3/12 (25.0%) of the examined tissues had either score 1 or 3(1%–25% or > 50%. positive cells, respectively).

Table 3: Frequency of ISH- signals scoring of Human CMV DNA detection in tissues with cervical adenocarcinoma

<table>
<thead>
<tr>
<th>CMV-ISH Signaling</th>
<th>Adenocarcinoma Tissues (N=21)</th>
<th>Chronic Cervicitis Tissues (N=13)</th>
<th>Apparently Normal Cervical Tissues (N=15)</th>
<th>Chi-Square Test (Statistical Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Negative Signal</td>
<td>9/21</td>
<td>42.9</td>
<td>13</td>
<td>100.0</td>
</tr>
<tr>
<td>Positive Signal</td>
<td>12/21</td>
<td>57.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Grades of ISH-Signaling</td>
<td>Score 1: 1%–25%; score 2: 26%–50%; score 3: &gt; 50%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score 1</td>
<td>3/12</td>
<td>25.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Score 2</td>
<td>6/12</td>
<td>50.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Score 3</td>
<td>3/12</td>
<td>25.0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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The signal intensities of Human CMV DNA –ISH signal detection were illustrated in (Figure 2) and detailed in (Table 4). Five out of twelve (5/12 ; 41.7%) has high signal intensities; while 4/12 (33.3%) and 3/12(25.0%) have moderate and weak intensities, respectively.

Table 4: Frequency distribution of Human CMV DNA-ISH signal intensity grades of cervical tissues with adenocarcinoma

<table>
<thead>
<tr>
<th>Studied Groups</th>
<th>Positive CMV Signaling</th>
<th>Grades of CMV ISH-Signal Intensity*</th>
<th>Negative CMV Signaling</th>
<th>Chi-Square Test (Statistical Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Adenocarcinaoma (N=21)</td>
<td>12/21 (57.1%)</td>
<td>9/12 (25.0%)</td>
<td>4/12 (33.3%)</td>
<td>5/12 (41.7%)</td>
</tr>
<tr>
<td>Chronic Cervicitis (n=13)</td>
<td>0/13 (0.0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apparently Normal Cervical Tissues (N=15)</td>
<td>0/15 (0.0%)</td>
<td>15 (0.0%)</td>
<td>0/15 (0.0%)</td>
<td>0/15 (0.0%)</td>
</tr>
</tbody>
</table>

Intensity 1: 1%–25%; Intensity 2: 26%–50%; Intensity 3: > 50%.

Figure 1: In Situ Hybridization(ISH) for HCMV Deduction Infiltrative end cervical malignant epithelial tissues Using Biotinylated -Labeled HCMV Probe; Stained with NBT BCIP (Blue)and Counter Stained by Nuclear Fast Red (Red). A-Signal score 1 and high signal intensities (400); B-Signal score 2 and high signal intensities (200). C-Signal score 3 and high signal intensities (400); D-Signal score 3 and moderate signal intensities (40).

Table 5 shows the positive - Human CMV results of DNA –ISH signaling in relation to the examined histopathological grading of cervical adenocarcinoma. Among well differentiated group, 2/3 of adenocarcinoma cases (66.7%) revealed positive blue nuclear signals for CMV- DNA, whereas among moderate and poor differentiated groups, 9/15 (60.0%) and 1/3 (33.3%) revealed positive-Human CMV results.

Among those twelve positive results of Human CMV DNA–ISH reactions, eleven cases (91.7 %) of cervical adenocarcinoma have well or moderate differentiation grades while only one case (8.3%) was of poor differentiation grade.

Table 5: Correlation of the differentiation of cervical adenocarcinoma with ISH- signal scoring of Human CMV- DNA

<table>
<thead>
<tr>
<th>CMV-ISH Signaling</th>
<th>Adenocarcinaoma Grading</th>
<th>Chi-Square Test (Statistical Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well differentiated (n=3)</td>
<td>Moderately differentiated (n=15)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Negative Signal (n=9)</td>
<td>1 / 3</td>
<td>33.3</td>
</tr>
<tr>
<td>Positive Signal (n=12)</td>
<td>2 / 3</td>
<td>66.7</td>
</tr>
<tr>
<td>Grades of CMV-ISH Signal Scoring</td>
<td>I</td>
<td>0 / 3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2 / 3</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0 / 3</td>
</tr>
</tbody>
</table>

Discussion

Viral relation to cervical squamous cell carcinoma as well as adenocarcinoma remains a controversial issue [23–24]. However, strong cumulative evidences incriminated HPV as the principal etiological agent in cervical carcinogenesis (30-31).
Malignant cervical transformation depends on the integration of high-risk HPV types into the host cell genome as well as too many other factors such as the duration of HPV infection, patient age, hormonal abnormalities, immunological competency, additional genomic mutations and other concomitant infections. Apart from Herpes simplex virus type 2 (HSV-2), which has long been suspected to act as a cofactor in the development of cervical cancer, other herpes viruses are also potential candidates, such as EBV and CMV may contribute to the cervical carcinogenesis [32].

The mean age of the studied group of patients (46.7 +11.6 years) was consistent with that declared by Iraqi Cancer Registry Center in 2012 for vast majority of the Iraqi females with cervical adenocarcinoma (4). These results are also compatible with the global average age –presentation (45-55 years) of those female patients with invasive adenocarcinoma where it is uncommon neither to see adenocarcinoma in situ nor invasive adenocarcinoma in their younger-counterpart women [19].

The current results show that each of well grade (grade 1) and poor grade (grade 3) constituted 14.3% (3 out of 21 cases) while 15 out of total 21 cases (71.4%) of cervical adenocarcinoma were graded as moderately differentiated (grade 2). The cervical adenocarcinoma is often graded based on the typical architecture of adenocarcinoma where grade 1 is predominantly glandular and has less than 5% solid areas where as if 5-50% solid areas present and the nuclei have too atypical features will be viewed as grade 2; and as grade 3 when more than 50% solid growth is found while nuclear atypia is out of the proportion to glandular differentiation [28].

Although the relation of viruses with cervical carcinogenesis is a controversial issue, strong cumulative evidence incriminated high- oncogenic risk types of HPV as its principal etiological agents [30-31]. However, these high-risk HPV types are considered necessary but not sufficient for the development of cervical cancer [33].

Apart from Herpes simplex virus type 2 (HSV-2), which has long been suspected to act as a cofactor in the development of cervical cancer, previous studies have pointed to other herpes viruses, such as cytomegalovirus (CMV) and Epstein-barr virus (EBV), as potential candidates contributing as co-factors in the HPV-associated cervical carcinogenesis [32-33].

Invasive carcinoma of the cervix is one of the commonest malignant tumors of the female genital tract. Much controversy surrounds its etiology. The possibility of a sexually transmissible infectious carcinogen in the genesis of cervical neoplasia has received widespread attention [34-36]. World-wide, genital HSV-2 infection was the chief suspect in late 1960s and early 1970s. However, this association was not upheld to be causative in human. Since mid-1970s, a compelling evidences implicating human papilloma virus in many genital cancers including cervical neoplasia as the principal etiological agent [37].

In Iraq, several studies declared an association of HPV with cervical neoplasia; the first was done by Mohammed Ali [27] who found 25% of cervical carcinoma was positive for HPV by PCR then Al-Jewari et al [32] who found 28.4% HPV-positive cervical neoplasia by ISH. These lower percentages are a reflection of low prevalence of HPV in our general population since sexual multi-partners are not common in our society and thus may constitute a probable cause for the differences between all Iraqi and world-wide studies. Therefore, multiple other infecting agents might synergistically play a role in initiation, co-factoring and promotion of cervical carcinogenesis in our country.

In the present research work, it was decided to investigate the rate of infection with the fifth type of herpes viruses (CMV) in tissues from twenty-one previously hysterectomies women for an invasive endo cervical adenocarcinoma as compared to their twenty-eight counterpart tissues from women biopsied for chronic cervicitis or hysterectomies for non-malignant etiologies.

Cytomegalovirus has the largest genetic content of all human herpes viruses where more than 200 proteins encode by this virus [24]. The immediate-early protein of human CMV could induce transformation of infected cells in human cervical epithelial cell. If these cells were not permissive for the full replication cycle of the virus, the cell could become malignant [23]. Previous evidence
indicates the frequent presence of genome and antigens of HCMV in certain malignant tumors, such as colon cancer [19], malignant glioma [20], EBV-negative Hodgkin’s lymphoma [38], prostatic intraepithelial neoplasia and carcinoma [21] and cervical cancer [39].

Because of the ubiquitous distribution of HCMV and the high seroconversion rates, an etiological association between HCMV infection and human cancer has been difficult to establish. However, evidence based on virologic, epidemiologic, and molecular studies which have demonstrated the presence of viral DNA or antigens in tumor tissues suggests its involvement in specific cancers.

While HCMV has been isolated from cervical cancer biopsy specimens and their derived cell cultures, seroepidemiologic studies linking HCMV infection to cervical cancer have yielded conflicting results. Some investigators have found significantly higher levels of antibodies to HCMV in patients with cervical carcinoma than in controls, while other groups have found no correlation [40-41].

Huang et al., [42] and Fletcher et al., [43] have detected HCMV DNA in cervical cancer specimens. However, DNAs of several other viruses, including HSV-2 and human papilloma virus (HPV), have also been detected in these tumors [44]. It is possible that synergistic interactions among these viruses in the infected cell lead to the development of cervical cancer. Members of the herpes virus family have been implicated in the etiology or associated with human cervical carcinoma: the Epstein-Barr virus (EBV), in the study of Mohammed Ali [27]; and HCMV which is associated with cervical carcinoma [45-46].

The results presented herein demonstrate that 12 out of 21(57.1%) adenocarcinoma tissues from patients with cervical adenocarcinoma (who were treated with radical hysterectomy) revealed positive blue nuclear signals for CMV at the sites of sequence-complementarities. Although a previous sero-epidemiological study in Iraq done by Abdul Kareim et al., [47] reported a high seroprevalence as well as endemity of HCMV in Iraqi population through their different age groups, in the present study none of the cervical tissues from our patients with chronic cervicitis and normal healthy uterine cervix tissues could present such CMV infection at the examined cervical tissues.

A systematic review done by Marinho-Dias & Sousa in [33] has summarized the frequency of HCMV in cervical samples collected between 1980 and 2011 and its relation to the development of cervical lesions and invasive cervical cancer. This review revealed that the overall rate of HCMV infection varied from 1.58% to 61.0% with an increased incidence in less developed countries. In addition, Cytomegalovirus infection was present in all different types of lesions and that the worldwide crude frequency of this virus in the cervix was 18.9% and 36.5% in HPV-positive women.

Although our results are in inconsistency with Marinho-Dias Jet al [48] and with Marinho-Dias and Sousa (33) studies who detected a prevalence of 22.2% and 44.4%, respectively for CMV in tissues from patients with in situ carcinoma/ invasive cervical adenocarcinoma, yet our conclusions are running compatibly with and with studies who revealed that CMV infection was associated with an increased risk for in situ/invasive carcinogenesis since none of the cervical tissues from our patients with chronic cervicitis and normal healthy uterine cervix tissues could present such positive signals for CMV at the sites of complementarities.

In this study and among those twelve patients who showed positive results of Human CMV DNA –ISH reactions in their tissues, eleven cases (91.7 %) of cervical adenocarcinoma have well or moderate differentiation grades while only one case (8.3%) was of poor differentiation grade. In addition, and among well differentiated group, two-thirds of adenocarcinoma cases (66.7%) revealed positive blue nuclear signals for CMV- DNA, whereas among moderate and poor differentiated groups, 9 out of 15 (60.0%) and one-third (33.3%) revealed positive- Human CMV results, respectively. The trend of CMV DNA –ISH scores is decreased proportionally with the deterioration of differentiation grades of the studied cases of adenocarcinoma from the well-, through moderate- to poorly-differentiated groups. This might indicate
that the role of this virus could occur at an early event in the cervical adenocarcinogenesis.

Cytomegalovirus was first isolated from (20%) biopsies from CMV-sero positive patients with advanced stage cervical cancer (75% was also HSV 2-seropositive) (49). Among 951 who were CMV-seropositive women at a sexually transmitted disease (STD) clinic, CMV had isolated from 13.6% of their cervixes [50]. Cervical CMV infection is related to sexual activity, acquisition of other STDs, or exogenous re-infection [50]. Since none of the examined cervical tissues with chronic cervicitis and normal healthy uterine cervix tissues has presented such positive signals for CMV-DNA, these facts are against the possibility that this CMV infection has occur as a long term persistent infection that expressed in the neoplastic precursors of these cervical cancers so as to play an important promotive role in the oncogenic events directly implicated in malignant progression [19].

The increasing percentage of detection of CMV with the progression of cervical lesions from neoplastic precursors of cancer to invasive cervical cancers could point for a role for this virus via its immediate-early gene products which can trans activate other viral and cellular genes so as to let such concurrent genital infections with CMV and HPV to increase the risk of cervical cancer, as these successful results of induction of experimental infection with inactivated human cytomegalovirus reported by Heggie et al. [51].

It can be concluded that the high rates of human cytomegalovirus and their evidently correlated with the grading of cervical adenocarcinoma could point for a molecular role for HCMV in the etiology of these cancers which could probably early occurred, along with other important oncogenic viruses. More extensive studies along with inclusion of high cumulative numbers to unravel their exact role in end cervical ontogenesis.

Conclusions

The high rate of cytomegalovirus infections as well as their evident correlation with the differentiation of cervical adenocarcinoma could point for a role for HCMV in these cancers as a molecular attack which probably occurred at an early event, along with other important oncogenic viruses. However, their exact role as a causative, initiating or cofactor role in end cervical ontogenesis remain to be determined.

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