Molecular Localization of Latent Epstein – Barr Virus Early Repeats (EBERS) in Cervical Tissues with Adenocarcinoma by RNA-In Situ Hybridization

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Abstract:

Epstein-Barr virus (EBV) has an etiological relevancy to the pathogenesis of an increasing number of cancers. Cervical carcinogenesis is a multifactorial stepwise process that has a possible link with many infective factors, including EBV infections where the standard procedures in their histopathological diagnosis rely on in situ hybridization. This retrospective research was designed to(1) investigate the frequency of EBV infections in correlation with the age of patients with cervical adenocarcinoma, (2)validate the impact this virus on the histopathological grade expression of those cancers; and(3)rating the EBV-infections by evaluating the signal scores and intensities of RNA -in situ hybridization (RISH) reactions. A total number of 49 patients, who had undergone hysterectomies or punch biopsies from their cervices were enrolled in this study. Twenty-one tissues were collected from cervical adenocarcinoma where as the control groups comprised 28 blocks from cervical tissues either without any significant pathological changes or from chronic cervicitis. Molecular detection of Latent Epstein-Barr Virus Early Repeats (EBERS) was performed by using ultra-sensitive versions of RNA-in situ hybridization (RISH) technique. The mean age of this group of Iraqi patients with cervical adenocarcinoma was 46.7 ± 11.6 years and histopathologically, well and poor grades of cervical adenocarcinoma each constituted 14.3% whereas 71.4% was moderately differentiated. The EBV was detected in 38.1% (8 out of 21) of cervical adenocarcinoma tissues while EBERs were neither detected in chronic cervicitis tissues nor in those healthy cervical tissues. Seven out of eight cases (87.5%) with positive EBERs–ISH reactions have well and moderate differentiation grades while only one case (12.5%) has poor differentiation. It may conclude that EBV infections in cervical adenocarcinoma could point for an initiating and/or cofactor roles, along with other important oncogenic viruses, in cervical oncogenesis. The observed impact of EBV on the differentiation of cervical adenocarcinoma could possibly shade light on an early- eventual occurrence of such molecular attack in cervical carcinogenesis.

Keywords: *Cervical adenocarcinoma, Epstein-Barr virus, EBERS,RNA-In Situ Hybridization.*

Introduction:

Invasive cervical carcinoma is the third most common cancer in women worldwide. In developed nations, it is the fifth among the10 most common female cancers whereas in developing countries it ranks on the top of them and in Iraq is out of these 10 ranks (1-4).

Globally, the total cases of cervical cancer in 2011 were 529,800, with age-adjusted and sex-adjusted rates of 9.0/100,000 and 17.8/100,000, respectively, in more developed and less developed countries (1-4).On reviewing Iraqi cancer registry during the period (2009-2012), 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).

Due to effective screening programs, the incidence of cervical squamous cell cancers relative to adenocarcinomas has decreased over the last few decades. However, cervical adenocarcinoma stands out, because its incidence has been increasing in recent years, particularly in younger women (5% in 1950's to10-25% in the current accounts) (5-12).

Cervical malignant transformation is a complex multifactorial stepwise process in which a number of factors with possible carcinogenic influences such as genetic, chemical, infective, immunological, hormonal, and many occupational factors, can play a part on cervical tissues (13). However,

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the exact extent of participation of any of them to induce malignant changes is still unknown (14).

The integration of DNA of high-risk types of human papillomavirus into cellular genome is widely accepted as the major contributing factor in cervical carcinogenesis (15). However, recent evidences indicated that some other Herpes viruses, other than Herpes Simplex Virus 2, such as Epstein-Barr virus (EBV) and Cytomegalovirus might contribute to cervical carcinogenesis (16).

The efficient EBV infection is restricted to primary human B lymphocytes leading to infectious mononucleosis via C3d receptor (CD21) (17). Mainly, EBV associated with undifferentiated nasopharyngeal carcinoma and Burkett lymphoma. However, it has also been detected in T-cell lymphoma, Hodgkin's disease, gastric carcinoma and smooth muscle tumors (18). Moreover, EBV can replicates in the epithelial cells of oropharynx, parotid gland and uterine cervix (17). In addition, epithelial cell lines from gastric, biliary, laryngeal, hepatocellular, colon, and bladder carcinoma can be infected and transformed by EBV with efficiencies that approach those of B lymphocytes (18). In addition, many observations have supported a role for EBV in cervical tumorigenesis and a clear linkage of EBV with epithelium-originated tumors (18); documentation of C3d receptor in normal cervical epithelia (13); C3d-independent mechanisms for EBV-cervical infections (19); and an association between EBV and cervical carcinoma as suggested by Landers et al(20). However, other researchers denied supporting a role for EBV in cervical tumorigenesis (21). Singh et al (22) described cervical adenocarcinoma in females with nasopharyngeal carcinoma and proposed a same or related etiologic factor between nasopharyngeal carcinoma and carcinoma of cervix. Zhenghe and co-workers (23) suggested a role for EBV in the initiation or further development of cervical squamous-cell carcinoma. Other researchers concluded significant roles for co-infection of EBV, CMV with HPV16 in etiopathogenesis of uterine cervical cancer (24).

In Iraq, cervical cancer constituted 0.96% of all women cancers (Iraqi cancer registry,2012)(4). Worldwide, the main viral etiology of this cancer was strongly related to HPV. Some authors proposed a role for EBV in the carcinogenesis of cervical tumors (25) while others did not support such hypothesis (21). However, in our country the first who studied the role of HPV in cervical neoplasia was Mohammed Ali in (2001) (26).In addition, the present research work, and up to our best knowledge is also the first that study the forth type of herpes viruses (EBV) in Iraqi female patients with invasive cervical adenocarcinoma. This study is aiming to assess whether EBV 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 cervices. 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 diagnoses were based on their accompanied pathological reports 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 EBERS - RNA in situ hybridization (RISH) detection system using biotinylated-labeled oligonucleotides probe which targets EBERS by ISH kit that was purchased from (Zyto Vision GmbH. Fischkai, Bremerhaven. Germany).The details of methods for performing ISH reaction with this probe were conducted according the instructions of the manufacturing company, in the Research Laboratory of the Clinical Communicable Diseases Research Unit, at College of Medicine, University of Baghdad.

The main steps for the in situ hybridization procedure are to place the slides in 60c hot-air oven over night then the tissue sections were de-paraffinized and treated by graded alcohols (according to the standard methods of rehydration) via twice times treatment for 5 minutes with xylene, twice times and for 5 minutes treatment with 100% ethanol, twice times and for 5 minutes treatment with 96% ethanol, once time and for 5 minutes treatment with 70% ethanol, can be used. Air drying of sections was then done. Then application (dropwise) of pepsin solution(ES1) to the tissue sections and incubation for 20-30 minutes at 37°C in a humidity chamber. Then immersion the slides in distilled water and draining off the water and air drying these sections were done. Then, the probe was added to the center of a cover slip and the cover slip was turned upside down on the target area.

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. Denaturation of the slides at 75°C for 5 minutes, on hot plate, then transferring the slides to a humidity chamber and hybridization of the probes was done for 60 minutes at 55°C. Post-hybridization and detection processes were to remove the cover slip (by submerging in 1x wash buffer TBS) , then washing for 5 minutes in 1x TBS wash buffer at 55°C (prepared by using WB5) (but this step was not performed on slides hybridized with Zytofast RNA (+)

control probe (PF6) as this will reduce signal intensity). Then application of AP-Streptavidin(AB9) drop wise (3-4 drops per slide) and incubation for 30 minutes at 37°C in a humidity chamber. Then washing in wash buffer TBS (prepared by usingWB5) and then twice times for 1 minute in distilled water and application of NBT/BCIP(SB4) drop wise (4 drops per slide) to the slides and incubation for 40 minutes at 37°C in humidity chamber. Lastly, washing three times for min in distilled water and covering the sections. Then the sections were mounted in an aqueous embedding medium.

Analysis:

According to the specification of the kit, proper use of this ISH detection system gives an intense blue signal at specific sites of the hybridization probe in positive test tissues (by using light microscope).

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) (27). 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 $P < 0.05$.

Results:

Mean age of the patients with cervical adenocarcinoma was (46.7 +11.6) years while the mean age of the patients with chronic cervicitis and those with healthy (normal) cervical tissues were (40.1 +15.2) and (34.7 +13.3) years, respectively. Comparative statistical analysis of cervical adenocarcinoma group versus both other patients groups has showed highly significant differences ($p<0.01$) (Table 1).

Table 1: The Age of the Patients with Cervical Adenocarcinoma

The Patients / Group	Number	Mean Age	S.D	S.E	Minimum	Maximum
Adenocarcinoma (AC)	21	46.70	11.60	1.13	28	71
Chronic Cervicitis (CC)	13	40.10	15.20	2.98	29	63
Healthy (Normal) Control (HC)	15	34.70	13.30	2.28	31	69
Comparative Analysisof Statistical significance	AC Versus CC or HC = (P Value = 0.006) =Statistically highly significant($p<0.01$)					

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.

Table2: Grading of the studied cervical Adenocarcinoma Carcinoma Group.

Grades*	N	%
Well	3	14.3
Moderately	15	71.4
Poorly	3	14.3
Total	21	100.0

* 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 RNA –ISH signal detection of the EBERs; 8/21 cervical adenocarcinoma cases (38.1%) revealed positive blue nuclear signals at the sites of sequence-complementarities. Neither chronic cervicitis nor normal healthy uterine cervix tissues presented positive signals for the EBERs RNA –ISH test. Of the positive cases, 5/8 (62.5%) adenocarcinoma tissues had score 1 (1%–25% positive cells),while 2/8 (25.0%) and 1/8(12.5%) of the examined tissues had score 3 and 2 (> 50% and 26%–50%% positive cells, respectively) .

The scoring of positive RNA –ISH blue signals of EBERs in cervical adenocarcinoma tissues (as compared to the negative ISH- microscopic appearance of their cervical healthy tissues counterparts) are illustrated in (Figure 1,2,3,4).

Table 3: Scoring of signal- detection of RNA –ISH for EBERs among tissues from cervical adenocarcinoma.

EBV signal scoring		Adenocarcinoma Tissues (N=21)		Chronic Cervicitis Tissues (n=13)		Apparently Normal Cervical Tissues (n=15)		Chi-Square Test (Statistical Significance)
		N	%	N	%	N	%	
Negative Signal		13/21	61.9	13	100.0	15	100.0	P Value**
Positive Signal		8/21	38.1	0	0.0	0	0.0	
Grade of score	I	5/8	62.5	0	0.0	0	0.0	<0.001 (Significant)
	II	1/8	12.5	0	0.0	0	0,0	
	III	2/8	25.0	0	0.0	0	0.0	

* Score 1: 1%–25%; score 2: 26%–50%; score 3: > 50%.
** On comparing the signal scoring within the group of adenocarcinoma only.

The signal intensities of EBERs -RNA –ISH signal detection were illustrated in (Figure 1, 2 ,3,4).Five out of eight (5/8 ; 62.5%) has moderate signal intensities; while 2/8 (25.0%) and 1/8(12.5%) have high and weak intensities, respectively .

Table 4: Grading of signal intensities of RNA –ISH of EBERs in cervical tissues with adenocarcinoma

Studied Groups	Positive EBV Signaling	Grades of EBV ISH-Signal Intensity*			Negative EBV Signaling	Chi-Square Test (Statistical Significance)
		Weak	Moderate	High		
Adenocarcinoma Tissues (n=21)	8/21 (38.1%)	1/8 (12.5%)	5/8 (62.5%)	2/8 (25.0%)	13/21 (61.9%)	P value =<0.001 significant (p <0.05)**
Chronic Cervicitis Tissues (n=13)	0/13 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13/13 (100.0%)	
Apparently Normal Cervical Tissues (n=15)	0/15	0/15 (0.0%)	0/15 (0.0%)	0/15 (0.0%)	15/15 (100.0%)	

* Intensity 1: 1%–25%; Intensity 2: 26%–50%; Intensity 3: > 50%
** On comparing the signal scoring within the group of adenocarcinoma only.

Table (5) shows the positive -EBERs results of RNA –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 EBERs, where as (33.3%) among moderate and poor differentiated groups (5/15 and 1/3, respectively) revealed positive-EBERs results.

Regarding histopathological grading of cervical adenocar-

cinoma and among those eight positive results of EBERs DNA –ISH reactions, seven cases (87.5%) are mainly noticed to have well and moderate differentiation grades while only one case (12.5%) has poor differentiation grade.

Table 5: Correlation of the differentiation of cervical adenocarcinoma with RNA ISH- signal scoring of EBERs.

EBV-ISH Signaling		Adenocarcinoma Grading						Chi-Square Test (Statistical Significance)
		Well differentiated (n=3)		Moderately differentiated (n=15)		Poorly differentiated (n=3)		
		N	%	N	%	N	%	
Negative Signal (n=13)		1/3	33.3	10/15	66.7	2/3	66.7	P Value 0.36 (Not significant)
Positive Signal (n=8)		2/3	66.7	5/15	33.3	1/3	33.3	
Grades of EBV-ISH Signal Scoring	I	2/2	100.0	1/5	20.0	1/1	100.0	
	II	0/2	0.0	2/5	40.0	0/1	0.0	
	III	0/2	0.0	2/5	40.0	0/1	0.0	
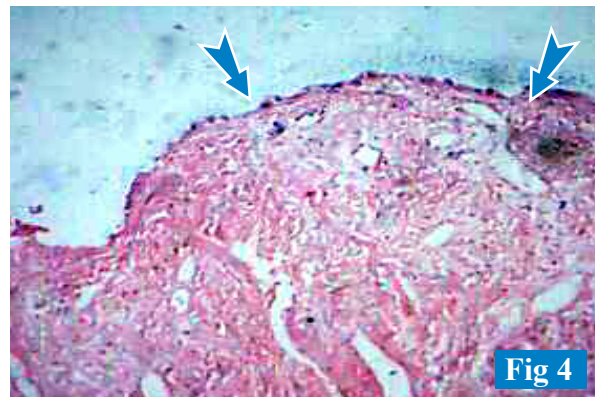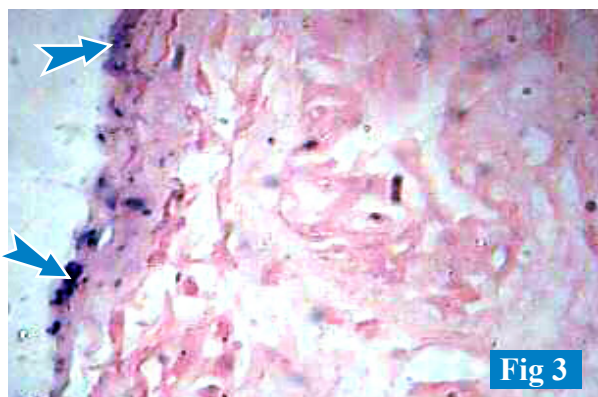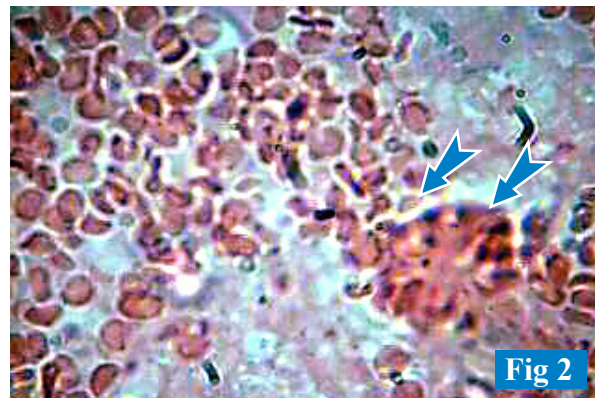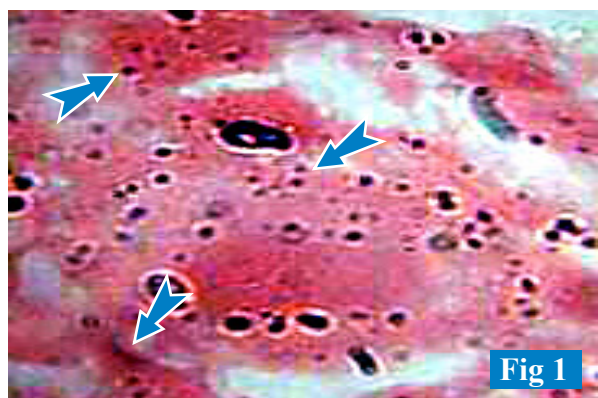
Microscopic appearance of EBV-EBERs--positive in situ hybridization reactions In endocervical malignant epithelial tissues, NBT/ BCIP stained (blue signals) and counter-stained by nuclear fast red (red signals) (400) , Figure 1:Signal score 3 and high signal intensities (400); Figure 2: Signal score 3 and moderate signal intensities (400); Figure 3: Signal score 1 and high signal intensities (400); Figure 4: Signal score 2 and high signal intensities(200).

Discussion:

The present results revealed that mean age of the studied group of Iraqi patients with cervical adenocarcinoma was (46.7 +11.6) years which are consistent with age of the vast majority of the females with cervical adenocarcinoma who were included in the Iraqi Cancer Registry Center in 2012 (4). These results are also consistent with worldwide-age presentation of female patients with invasive adenocarcinoma who are in the average of 45-55 years since it is uncommon neither to see adenocarcinoma in situ nor invasive adenocarcinoma in younger women (19).

Cervical adenocarcinoma is often referred to as being a mucinous type adenocarcinoma which accounts for about 90% of all cases. The typical architecture of grade 1 adenocarcinoma is predominantly glandular and the nuclei have too atypical features so as to viewed as such grade but if nuclear atypia is out of the proportion to glandular differentiation, the grade is increased by 1 (28). The current results show that 71.4% (15 out of total 21) cases of cervical adenocarcinoma were graded as grade 2 (moderately differentiated) while each of grade1 and grade3 (well and poor grades) constituted 14.3% (3 out of 21 cases).

Worldwide, the major contributing factors in cervical adenocarcinogenesis have been related to the integration of high-risk HPV types into host cell genome (29 & 30). However, the genesis of cervical carcinogenesis is a complex process that includes a possible carcinogenic role for a number of other concomitant sexually transmissible infections, other than high-risk HPV (31 & 32). Successful experiments as well as clinical studies, during the period 1960s-1990s, have incriminated Herpes simplex virus type 2, EBV and CMV as potential candidates in the cervical carcinogenesis. Some studies have evaluated the incidence of EBV infections in such cervical lesions (22-24 & 33 & 34).

In this respect, EBV was suggested as another oncogenic virus in cervical carcinogenesis, based on the clonal nature of EBV in cervical cells and presence of EBV in cervical pre-cancerous lesions.(16 & 33 - 36). Although some researchers have concluded an association of EBV with cervical carcinoma, several other authors did not reach similar conclusion (37 - 39).

In Iraq, several studies have declared an association between HPV and cervical neoplasia; Mohammed Ali (2001) (26) who found 25% of cervical carcinoma was positive for HPV by PCR then Al-Jewari et al (2007) (40) 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-partnerships are not common in our Iraqi society that 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.

Although lymphotropism of EBV is well recognized, it is becoming increasingly clear that EBV may have an epith-

eliotropic qualities supported by the established associations between EBV with nasopharyngeal and gastric cancers (16) & (33). Along that association, an indication that this virus might similarly has an oncogenic potential within the cervical epithelial cells so as to have a part to play in the etiology or pathogenesis of cervical carcinoma.

This research study, thus was designed to investigate the rate of infection with the forth type of herpes viruses (EBV) in tissues from twenty-one previously hysterectomized women for an invasive endocervical adenocarcinoma as compared to their twenty-eight counterpart tissues from women biopsied for chronic cervicitis or hysterectomized for non- malignant etiologies.

The EBV latent proteins expression contribute to most, if not all, of the transforming and immortalizing properties of this prototype DNA oncogenic viral agent. In addition to EBNA1 and the EBERs, human cancer cells, that are latently infected with this virus express the most powerful oncogenic proteins, LMP-1 and LMP-2(A and B) (18).

In this context, the current research work found that the percentage of Epstein-Barr virus-EBERs in the cervical adenocarcinomatous tissues was 38.1% (8 / 21 cases revealed positive blue nuclear signals at the sites of sequence-complementarities). Neither chronic cervicitis nor normal healthy uterine cervix tissues presented positive signals for the EBERs RNA –ISH test. Up to our best knowledge this study is the first that search to assess whether EBV is associated with such invasive cervical adenocarcinoma in a group of Iraqi female patients.

The present result is compatible with the results of Landers et al 12 in 1993(20) who have detected EBV DNA in 44.4% (8 of 18) of cases with cervical squamous cell carcinoma by polymerase chain reaction and also consistent with their results (using DNA- in situ hybridization) where EBV DNA have been detected in 27.8% (5 of 18) of the same cases. This precludes the comparism to other Iraqi literatures in that respect. In this respect, the present results have also supported many other studies who have found that the expression rate of genes, such as EBNA-2, LMP-1 and EBER-1, were also significantly higher in cervical carcinoma and CIN than in the normal (25&41). However, the current result is incompatible with the results of Se Thoe et al (42) who detected EBV DNA in 63% of cervical carcinomas but are completely compatible with their results where none of normal cervices were positive by using DNA- in situ hybridization. These results may, in part, related the occurrence of EBV to a certain extend to the development of these carcinomas.

The differences among these results and ours could be frankly related to the criteria of PCR as the most sensitive technique for DNA amplification than in situ hybridization for detection of viral DNA so that even one particle of viral DNA in a tissue section would be theoretically detectable by PCR. It is possible that those tissues with negative results by the present in situ hybridization study may not have an adequate copy numbers of this virus to permit detection and could show further positive results if PCR would be used.

In this study, the direct ultra-sensitive in situ hybridization kit used for EBV DNA detection has been in most of the studied cases shown the presence of viral DNA within the nuclei of these cervical malignant cells and since this virus appeared to have an oncogenic role in the development of other malignancies, this strongly suggests that EBV in the studied tissues which integrated into host genome has making an oncogenic effect possible if not probable .It is likely that this EBV, in a manner similar to other herpes viruses and some types of HPV, can be transmitted sexually, although it is not known whether EBV can replicate in the male genital tract (33).

Herein, such human seminal plasma was found to activate EBV in a lymphoblastoid cell line and also had raised the possibility that human semen might activated a latent EBV in the uterine cervical tissues from patients with cervical carcinoma.

However, much debate has been evoked because of divergent results including the relatively high prevalence rate of EBV-positive non-neoplastic cervical tissues, which shed doubt on that possibility .This controversy is present regarding those heterogeneous reports on lymphoepithelioma-like carcinoma of the uterine cervix when Noel et al(2001) (43) in Asian women showed a higher EBV infection where as Seo et al., (2005) (28) found that EBV plays little role in cervical carcinogenesis in Korean women and lastly Yang 2004(44) reported no association between them at all.

Many scientific observations could suggest the same or related etiologic factors between nasopharyngeal carcinoma and cervical lymphoepithelioma-like carcinoma. Hachisuga et al (23) have described an intriguing finding of a lymphoma-like EBV-positive lesion of the uterine cervix from a woman with serologic findings of acute EBV infec-

tion. Other intriguing findings are the frequent detection of EBV in lymphoepithelioma-like carcinomas of stomach(45) which are quite similar to undifferentiated nasopharyngeal carcinoma, clearly an EBV-associated neoplasm. In addition, Singh et al (22) described cervical adenocarcinoma in female patients with nasopharyngeal carcinoma.

However, other researchers did not find a similar EBV DNA - positive cervical lymphoepithelioma-like carcinoma or - EBER RNA -positive cervical pre-invasive lesions, by using in situ hybridization and PCR, respectively (46). Although EBV DNA have equally detected in both carcinomatous and non neoplastic lesions, they could be related to EBV-positive lymphocytes and stromal cells but not necessarily to positive- tumor cells (38&47).For the criticism of RNA integrity in the examined tissues, one can not completely rule out false-negative carcinomatous and non neoplastic lesions in the present study and as stated by (de Oliveira et al,1999) (46).

The lack of detailed clinical information about these patients with the attached clinical as well as histopathological reports with those cervical tissue specimens in the present study compels this study leaves many questions to be answered in further studies: How does this EBV initially infect these cervical tissues? Is this integrated virus or in an active or latent phase in those malignant cells and what is its exact role in cervical adenocarcinogenesis? To incriminate EBV in the pathogenesis of cervical carcinoma and to clarify issues related to many biological aspects of EBV in cervical carcinogenesis, such questions and others remain to be answered by future works directed towards establishing whether these viruses can directly transform cervical epithelial cells, and their mechanisms of infection, whether sexually hematogenously, or both.

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الموضعة الجزيئية للترددات المبكرة الكامنة لفيروس الابشتاين- بار في أنسجة سرطان الرحم الغدية بأستخدام تقنية التهجين الموقعي للحامض النووي (رنا)

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الخلاصة:

أشارت الدراسات إلى احتمال وجود ارتباطات سببية بين فيروس الابشتاين- بار وامراضية عدد متزايد من السرطانات. عملية السرطنة لانسجة عنق الرحم متعددة الخطوات وهنالك احتمالية ارتباطها بالمسببات الخمجية بضمنها العدوى بفيروس الابشتاين- بار كما وان تقنية التهجين الموقعي للحامض النووي هي الطريقة القياسية لتشخيصه نسيجيآ. صممت هذه الدراسة كبحث بأثر رجعي (١) للتحري عن نسبة العدوى بفيروس الابشتاين-بار وعلاقتها بأعمار المريضات بسرطان عنق الرحم الغدية (٢) للتثبت من تأثير هذا الفيروس في درجة التغيرات النسيجية المرضية لهذه السرطانات (٣) ولتحديد شدة الاصابة بفيروس الابشتاين- بار من خلال تقييم درجات وشدة الاشارات اللونية لنتائج تفاعلات التهجين الموقعي للحامض النووي (رنا).

وقد جندت لهذه الدراسة ٤٩ عيّنة نسيجية أخذت من عنق الرحم , ٢١ لمريضات شُخِّصَ لديهن سرطان غدي خبيث في انسجة عنق الرحم، إلى جانب ٢٨ مريضة كشواهد شُخِّصَ لديهن التهابات مزمنة في انسجة عنق الرحم اوالانسجة الرحمية التي لاتبدو فيها علامات مرضية. وقد استخدمت لغرض الموضعة الجزيئية للترددات المبكرة الكامنة لفيروس الابشتاين- بار في أنسجة سرطان عنق الرحم الغدية اجيال فائقة الحساسية لتقنية التهجين الموقعي للحامض النووي (رنا). فقد كشف الباحثون بان متوسط اعمار فريق المصابات بسرطان عنق الرحم كانت ٤٦,٧ ± ١١,٦ سنة وان كل من التمايز النسيجي الجيد والفقير قد شكل ١٤,٣٪ بينما بقية ٧١,٤٪ للتمايز النسيجي المتوسط. كماأظهرت الدراسة وجود لفيروس الابشتاين-بار في أنسجة سرطان عنق الرحم الغدية بنسبة ٣٨,١٪ (٨ من اصل ٢١ قطعة نسيجية محفوظة) ولم يكن هناك أي عينة من أنسجة عنق الرحم السليمة او ذات الالتهابات المزمنة إيجابية له. سبعة أنسجة من اصل ثمانية (٨٧,٥٪) من التي احتوت للترددات المبكرة الكامنة لفيروس الابشتاين- بار كانت ذات تمايز نسيجي جيد و متوسط بينما (١٢,٥٪) لها تمايز نسيجي فقير. يمكن الاستدلال من كشف فايروس الابشتاين- بار في أنسجة المريضات المصابات بسرطان عنق الرحم الغدي وتاثيره في التمايز النسيجي دعمآ لنظرية أداء الفيروس لدور سَبَبي مبكر , مولدآ و / أو عاملآ موافقآ في إحداثية السرطنة الغدية لعنق الرحم.