Archives of Razi Institute, Vol. 77, No. 6 (2022) 2125-2130

DOI: 10.22092/ARI.2022.359292.2397

Original Article

Expression of *HPV-16 L1* Gene Human Papillomavirus (HTLV-1) Identified by Pap Smear

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Received 7 July 2022; Accepted 6 August 2022
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Abstract

The human papillomavirus (HPV) is a crucial but not the predominant cause of cervical cancer. This study aimed to identify *HPV-16 L1* gene expression in human papillomavirus using a pap smear. A total of 120 serum samples, 60 samples were taken from infected females with papillomavirus and another 60 as healthy control. These samples were collected after pap smears were done. These women attended Al-Emam Hospital for delivery from March 1st, 2021, to February 28th, 2022. The levels of Pap-IgM and Pap-IgG were increasing among patients attacked by papilloma. The levels of viruses were higher than in levels than control groups, which was indicated by increases in the scores of mean and standard deviation (2.01±1.17, 0.11±0.02), (14.24±7.10, 0.4±0.17), respectively. Statistically, these differences between the levels of the studied groups were highly significant. The levels of the three markers Ca19.9, Ca125, and Ca15.3 were normal in levels among papilloma patients and the control group compared to the normal value of the three markers, which equaled N.V. (>37ng/ml). Statistically, these differences between the scores of the three markers, which were measured depending on mean and standard deviation, were highly significant. There is a low positive correlation between the levels of Pap-IgM (>1) with levels of Ca19.9 (>37) with (r=0.409**, *P*=0.000), while there is a moderate association between the levels of Pap-IgM (>1) with Ca125 (>35ng/ml) and Ca15.3 (>37ng/ml) levels with (r=0.574**, 0.565**, *P*=0.000, 0.000) respectively. Also, this table documents that there is a moderate positive correlation between the levels of Pap-IgG (>1) and the levels of the three tumor markers Ca19.9 (>37), Ca125 (>35), and Ca15.3 (>37) (r=0.521**, 0.592**, 0.647**). The *HPV-16 L1* gene expression was investigated in patients infected with Papillomaviruses compared to healthy controls using real-time PCR. The results showed a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration.

Keywords: Gene expression, Human Papillomavirus (HTLV-1), Pap smear

1. Introduction

The human papillomavirus (HPV) is a crucial but not the predominant cause of cervical cancer. People within the adolescent age groups who have an exposure history are susceptible to being infected with HPV, and there is an increased risk of smoking, poverty, using oral contraceptives, being simultaneously infected with other sexually transmitted microbes, recurrent and persistent inflammations, HIV or other diseases which suppress immune systems (1). These agents contribute to the pathogenesis, transmission, development, and persistence of cervical cancer and HPV-induced carcinogenesis. *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Candida species* are other sexually transmitted bacteria associated with cervical carcinomas (2). Recurrent infections facilitate epithelial shedding and cellular proliferation and promote the formation of malignant cells; chemokines, cytokines, free radicals, and growth factors aid colonization (3). Cancer occurs when cells containing viral genetic

material evade the common cell cycle regulatory mechanism (4). The co-infection and disease pathophysiology generated by these bacteria is considered a co-infection concerning their association with invasive and pre-invasive cervical malignancies (5). HTLV-1 infections increase susceptibility to other infections. Some investigations reported a co-infection between HPV and HTLV-1, with immunological responses contributing to such interactions (6). The incidence of cervical HPV infection in HTLV-1-infected women indicates a risk factor for such co-infections. Human Papillomavirus is the causative agent of the world's most prevalent sexually transmitted disease and is directly associated with cervical carcinoma, the third most prevalent cancer relationship with Trichomoniasis (7). The most prevalent HPV type in cervical squamous cell tumors is type 16 (3, 8). This study aimed to identify *HPV-16 L1* gene expression in Human Papillomavirus using a pap smear.

2. Materials and Methods

Sixty samples were obtained from papillomavirus-infected females and sixty from healthy females. These samples were taken following the completion of pap smears. These women were delivered to Al-Emam Ali Hospital between March 1, 2021, and February 28, 2022. According to the manufacturer's instructions, IgM and IgG antibodies against *HPV-16* antigens are detected in patient samples using an ELISA kit for *HPV-16* antibodies (Cussabio, Wuhan, China). The kit included a microtiter plate coated with an antigen specific to HPV. Ca19.9, Ca125, and Ca15.3 were determined using the min-vides method. qPCR was utilized for RNA and DNA extraction and quantification of all *HPV-16* genes. Using 1 l for each DNA (10 ng) and 1 l for cDNA, quantitative PCR (qPCR) was performed (Go-Taq q-PCR system assays, Promega, Southampton, U.K.).

2.1. Distribution of Study Group according to Residency Status

Table 1 shows the distribution of the study group according to residency status.

Table 1. Distribution of study group according to residency status

Residency status		Study group		Total
		Pap. V.	Control	
Rural	Count	30	30	60
	%	50.0%	50.0%	50.0%
Urban	Count	30	30	60
	%	50.0%	50.0%	50.0%
Total	Count	60	60	120
	%	100.0%	100.0%	100.0%

2.2. Statistical Analyses

Statistical analyses were performed using SPSS. Data was introduced as mean±S.D. or numbers and percentages. The Student t-test was used to compare abortions and the normal group.

3. Results

Table 2 revealed that most cases of papilloma infection were found in the age group of 27–36 years, 16 (26.7%) of total study cases (n=60), while fewer cases of papilloma infection were found in the age group (>67), 4 (6.7%) of total study cases (n=60). This difference was statistically non-significant (*P*-value=0.9).

Table 2. Distribution of study group according to categorical age group/years

Categorical age group/Years		Study group		Total
		Pap. patient	Control	
(17-26)yrs	Count	12	12	24
	%	20.0%	20.0%	20.0%
(27-36)yrs	Count	16	16	32
	%	26.7%	26.7%	26.7%
(37-46)yrs	Count	7	9	16
	%	11.7%	15.0%	13.3%
(47-56)yrs	Count	13	13	26
	%	21.7%	21.7%	21.7%
(57-67)yrs	Count	8	6	14
	%	13.3%	10.0%	11.7%
>67	Count	4	4	8
	%	6.7%	6.7%	6.7%
Total	Count	60	60	120
	%	100.0%	100.0%	100.0%
Chi-square		0.53		
P-value		0.9		

In table 3, we discovered that the concentrations of Pap-IgM and Pap-IgG were rising in papilloma

patients. Virus concentrations were more significant in control groups than in control groups, as shown by increases in mean and standard deviation scores (2.011.17, 0.110.02), (14.247.10, 0.40.17), respectively. These variations between the levels of the studied groups were statistically highly significant.

Table 4 demonstrates that the levels of the three indicators Ca19.9, Ca125, and Ca15.3 were normal in papilloma patients and the control group compared to the normal value (>37ng/ml) of the three markers. These disparities between the scores of the three markers, as measured by mean and standard deviation, were statistically highly significant.

Table 5 revealed there is a low positive correlation between the levels of Pap-İgM (<1) with levels of Ca19.9 (<37) with (r=0.409**, *P*=0.000), while there is a moderate association between the levels of Pap-İgM (<1) with Ca125 (<35) and Ca15.3 (<37) levels with (r=0.574**, 0.565**, *P*=0.000, 0.000) respectively. Also, this table documents that there is a moderate positive correlation between the levels of Pap-İgG (<1) and the levels of the three tumor markers Ca19.9 (<37), Ca125 (<35) and Ca15.3 (<37) with (r=0.521**, 0.592**, 0.647**) respectively.

The *HPV-16 L1* gene expression was investigated in patients infected with papillomavirus compared with the healthy controls by using real-time PCR. The results showed a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration, as shown in figure 1.

Table 3. Levels of Pap- İgM and Pap- İgG among the studied group (Papilloma N=60) and (Control=60)

Type of antibody	Study group	N	Mean	S.D.	t-test	*P-value
Pap- İgM	Papilloma patient	60	2.01	1.17	12.2	0.000
N.V(<1ng/ml)	Control	60	0.11	0.02		(**H.S)
Pap- İgG	Papiloma patient	60	14.24	7.10	14.9	0.00(H.S)
N.V(<1ng/ml)	Control	60	0.4	0.17		

*; Independent sample test,**; highly –significant

Table 4. Levels of tumor marker among studied groups (Papilloma N=60) and (Control=60)

Markers	Study group	N	Mean	Std. Deviation	t-test	*P-value
Ca19.9	Papilloma patients	60	35.31	14.81	7.8	0.000
N.V(<37ng/ml)	Control	60	19.31	5.72		(H.S.)
Ca125	Papiloma patients	60	32.23	10.84	10.6	0.000
(<35ng/ml)	Control	60	16.15	4.24		(H.S.)
Ca15.3	Papilloma patients	60	31.04	11.27	10.5	0.000
N.V(<37ng/ml)	Control	60	14.49	4.94		(H.S.)

*; Independent sample test,**; highly –significant

Table 5. Correlation between IgM and IgG levels with tumor marker parameters

Parameter	Test	Pap-İgM(<1)	Ca19.9(<37)	Ca125(<35)	Ca15.3(<37)	Pap-İgG(<1)
	r	1	0.409**	0.574**	0.565**	0.564**
Pap-İgM (<1)	*P*-value		.000 (H.S)	.000 (H.S)	.000 (H.S)	.000 (H.S)
	N	120	120	120	120	120
	r	.564**	0.521**	0.592**	0.647**	1
Pap-İgG (<1)	*P*-value	.000	.000 (H.S)	.000 (H.S)	.000 (H.S)	
	N	120	120	120	120	120

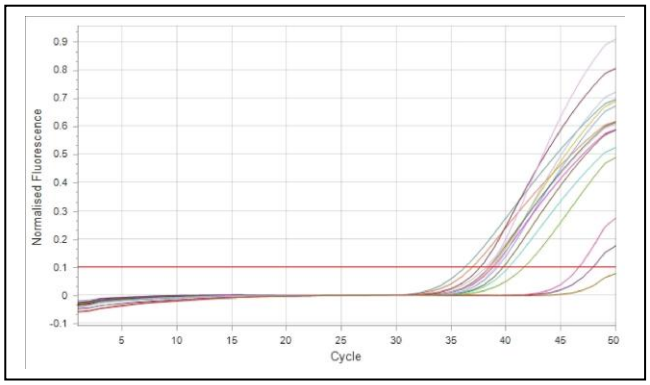

Figure 1. Expression of *HPV-16 L1* gene of papillomavirus in cervix inflammatory by using real-time PCR

4. Discussion

The human papillomavirus (HPV) is an essential but not the leading cervical cancer cause. Most cases of papilloma infection were found in the age group (27-36) years, with 16 (26.7%) of total study cases (n=60), while fewer cases of papilloma infection were found in the age group. Laantri, Attaleb (9) reported that the average age of the patients was 39.65 years, with a range of 10 to 87 years. Figure 2 depicts the age distributions of our patients with bimodal trends. The levels of Pap-IgM and Pap-IgG were rising in papillomavirus-infected patients (9). Papillomavirus was higher in levels than in control groups, which was indicated by increases in the scores of mean and standard deviation (2.01±1.17, 0.11±0.02), (14.24±7.10, 0.4±0.17), respectively. Statistically, these differences between the levels of the studied groups were highly significant. These findings agree with Ma and Yang (10). Kerishnan, Gopinath (11) showed that an HPV-16 specific ELISA test did the evaluation of patients and controls for detecting (IgM) and (IgG) antibodies. According to the ELISA test, 197/206 OSCC patients and 89/134 controls showed positive IgG to HPV, while only 42/206 OSCC patients demonstrated positive IgM to HPV-16 (11). The levels of the three markers Ca19.9, Ca125, and Ca15.3 were normal in levels among papilloma patients and the control group compared to the normal value of the three markers, which equaled N.V. (> 37ng/ml). Statistically, these differences between the scores of the three markers, which were measured depending on mean and standard deviation, were highly significant. These findings were consistent with those of Katar (12) w, who demonstrated that CA-125 levels were normal in 1 of 14 (7.1%) patients and above 65 U/mL in 13 instances (92.9 percent). 8 percent of patients with benign pelvic tumors were postmenopausal, with a median age of 35,7 years. S. CA-125 values were normal in 54/75 cases (72 percent) and elevated in 21 instances (28 percent). Our investigation revealed that the sensitivity of CA-125 was 92.9%, while its specificity was 72% (12). There is a moderate positive correlation between the levels of Papilloma-İgG and the levels of the three tumor markers Ca19.9, Ca125 (<35), and Ca15.3 with cervicitis. Ayhan, Guven (13) stated that the favorable CA-125 rates among the severe group were significantly higher than the mucinous group. In contrast, the positive CA-19-9 rate among the mucinous histology was significantly higher than the serous tumor. In the case of tumor size grouping as <4, 4.1-10, and >10 cm, there was a significant increase in mean levels of serum tumor markers with tumor size increase (P<0.05 for CA-125 and CA-19-9, P>0.05 for CA-15-3 (13). According to figures 1 and 2, the Pap smear was made clear to women with papilloma who had inflammation in the cervix, as revealed by examination under the microscope (14). There was no Neoplasia, although the examination of tumor makers showed that it was elevated in affected women, this confirms the infection immunologically, and no cancerous cells appear. This may be attributed to this case; the appearance of Neoplasia may be delayed because the pathological condition may be acute and has not reached the chronic state, or maybe it was not detected under the microscope and needs more confirmatory tests. Also, in the expression of the *HPV-16* gene, there was a unique creation of SAF via the replacement of the h4 helix of *HPV-16* capsid L1 proteins with the L2 peptides. Two different feeding strategies in the fed-batch culture of *P. pastoris* Mut^s have been assessed: the pre-

determined feed rates vs. feeding according to O_2consumptions through constant dissolved O_2 level maintenance (D.O. stat). The cultures exhibited a significant biomass elevation after feeding methanol when the D.O. stat method was used. In *P. pastoris,* the concentration of SAF was higher among Mut^s strains than those in Mut^+ strains. Nevertheless, the highest SAF level, at 132.10 mg L−1 culture, was produced by *H. polymorpha,* whereas only 23.61 mg L−1was produced by *P. pastoris* Mut^s. *H. polymorpha* demonstrated a higher potential to express *HPV-16 L1/L2* chimeric protein despite the *P. pastoris* track record as a high-level heterologous protein. To expirations of quantified the *HPV-16* viral transcripts by using RNA sequencing and comparing them with qPCR results. The values of 40-ΔΔCt were evaluated by qPCR for RNA and DNA and shown together with the alignment of the *HPV-16* genome using RNA sequencing. The cases revealed higher amounts of the *HPV-16* genes with RNA transcripts evaluated using the qPCR and the RNA sequencing alignment methods.

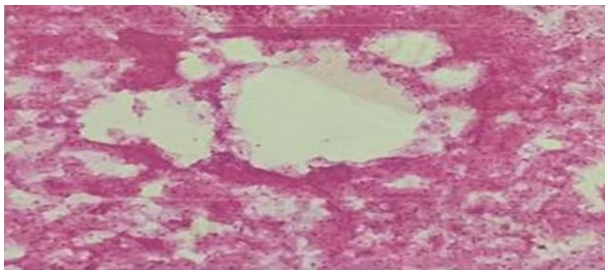

Figure 2. Pap smear conventional type stained with H&E stains, the microscopical description the smear showed benign epithelial cell with acute inflammatory cells and harmful malignancy

Authors' Contribution

Study concept and design: S. I. J.
Acquisition of data: S. I. J.
Analysis and interpretation of data: B. Q. D.
Drafting of the manuscript: B. Q. D.
Critical revision of the manuscript for important intellectual content: S. I. J.
Statistical analysis: S. I. J.
Administrative, technical, and material support: S. I. J.

Ethics

The human study was approved by Middle Technical University, Baghdad, Iraq Review Board and written informed consent obtained from each participant.

Conflict of Interest

The authors declare that they have no conflict of interest.

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