SENRA Academic Publishers, British Columbia
Vol. 8, No. 1, pp. 2671-2676, February 2014
Online ISSN: 1920-3853; Print ISSN: 1715-9997

Canadian Journal of pure&applied sciences

# DETECTION OF HUMAN PAPILLOMAVIRUS-21 GENOTYPES IN A SAMPLE OF IRAQI WOMEN WITH CERVICAL ABNORMALITIES AND CANCER

*Hula Y Fadhil[1], Dhuha S Saleh[1] and Faisal G Al-Hamdani[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad-Al-Jadiria
[2]Department of Virology, National Central Public Health Laboratory
Ministry of Health, Baghdad-Al-Andlus Street, Iraq

## ABSTRACT

To determine the prevalence and genotypes of cervical HPV infection in Iraq by newly developed technique. In this study, 188 women with cervical dysplasia (CIN I, II/III), 7 women with squamous cervical cancer, 40 women with atypical squamous cell and cervicitis and 25 healthy women as control group were collected. For Detection of HPV types, DNA extracted from cervical exfoliated cells was evaluated by polymerase chain reaction and typing with genoarray test. HPV-DNA positive was found 28.94% (68/235) in the cases but was absent in the control group. In the cases, the detection rate of HPV DNA in cytological categories atypical squamous with chronic cervicitis, LSIL (CIN I), HSIL(CIN II-III), and cancer was 5% (2/40), 30.34% (44/145), 34.88%(15/43), and 100%(7/7), respectively. Seventeen different HPV genotypes were investigated among 68 infected women. Moreover, ten different HPV for the first time in Iraqi women were recorded. Our finding demonstrated a predominance of HPV-59 (14.4%) followed by HPV-16 (13.3%) of all infection, but HPV-16 the most common type in HSIL and cancer was observed. Our results provide evidence the genoarray using for detection was increasing the number of isolates. Since 28% of infected women had HPV-16 and -18, the HPV vaccine is importance to introduction. Moreover, HPV-16, - 45 and -18 were highly associated with increasing severity of the disease, thus the strongest risk factor for persistence of infection was the presence of these types.

**Keywords**: Human Papillomavirus (HPV), genotyping, genoarray test, Iraqi women.

## INTRODUCTION

Human papillomaviruses (HPVs) have been recognized as etiologic factors in cervical carcinoma, precancerous lesions of the cervix uteri, and several other anogenital cancers in females and males (Bosch *et al*., 2002; Mun˜oz *et al*., 2006). In addition; about 26% of head and neck cancers are linked to HPV infection (Gillison and Lowy, 2004). HPVs represent an extremely heterogeneous group of DNA viruses. Until now, more than 100 HPV types have been identified and fully sequenced (de Villiers *et al*., 2004). Approximately 40 HPV types infecting the anogenital epithelium are classified as either low risk (LR) or high risk (HR) on the basis of their oncogenic potentials. A recent meta-analysis has designated 15 anogenital HPV types as HR, with an additional 3 HPV types designated as probable HR types (Mun˜oz *et al*., 2003). A number of PCR-based HPV genotyping assays have been developed to amplify HPV DNA, followed by reverse hybridization against immobilized genotype-specific probes, allowing the simultaneous identification of a broad range of anogenital HPV genotypes. However, the different assays have potential variations in their abilities to detect different HPV types due to their different analytical sensitivities and specificities and their failure to detect specific variants (Brink *et al*., 2007; Molijn *et al*., 2005; Sabol *et al*., 2008). We used HPV GenoArray (Macroarray) test is a newly developed PCR-based HPV genotyping assay. It utilizes L1 consensus primers to simultaneously amplify 21 HPV genotypes, followed by flow through hybridization with immobilized genotype-specific probes (Grisaru *et al*., 2008). It is marked as Conformite´ Europe´enne (CE) for use in Europe and is currently being used in some hospitals in China. HPV GenoArray test kit (GA) and the Roche Linear Array (LA) assays were showed no significant difference in the rates of detection of both HPV genotyping assays and oncogenic genotypes (Liu *et al*., 2010). The results showed that there was a high level (93.8%) of the agreement between the results of that assay and those of the Amplicor HPV test (Grisaru *et al*., 2008). Currently, HPV infections are increased significantly among Iraqi women without vaccine and many studies detected some HPV genotypes with different samples, but as our knowledge there is no previous study investigated HPV-21 genotype. Thus, our study was designed to investigate the prevalence of HPVgenotypes that associated with different cervical histological changes in Iraqi women using the GenoArray assay for the first time in Iraq.

## MATERIALS AND METHODS

### Patients and specimens

Cervical exfoliated cells were prospectively collected from 188 women with cervical dysplasia (CIN I, II/III), 7

*Corresponding author email: hulayounis@scbaghdad.edu.iq

women with squamous cervical cancer, 40 women with atypical squamous cell and cervicitis and 25 healthy women as control group attending Women Health Center-Al-Alwia hospital and Al-kadhmia teaching hospital in Baghdad during 2011. Control group mainly depended on the absence of clinical symptoms and cervical cytological changes were referred for Pap smear examination. Pap smear swabs were inserted into the endocervix by a clinician and rotated in both directions to collect the exfoliated cervical samples. These samples were stored at -20°C until use for HPV detection and genotyping.

**Genomic DNA isolation from cervical swab samples**

Genomic DNA isolation was performed using the HPV DNA extraction kit according to the manufacturer's protocol (Hybribio Limited Corporation, Hong Kong). Briefly, 0.5ml of the cervix of the uterus cell preservation fluid was centrifuged at 14000rpm for 1min.The supernatant was discarded, and the HPV DNA was extracted with the HPV DNA extraction reagent.

**HPV genotyping test**

The genoarry test is an L1consensus primer-based PCR assay and is capable of amplifying 21 HPV genotypes, including 13 HR types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,and 68), 2 probable HR (PHR) types (types 53 and 66), and 6 low-risk (LR) and unknown-risk (UR) types (types 6, 11, 42, 43, 44, and CP8304 [HPV-81]). The assay was performed according to the manufacturer's protocol. Briefly, PCR was performed with a reaction volume of 25µl containing 5µl of DNA template, 19.25µl of the master mixture provided, and 0.75µl of DNA *Taq*po lymerase in thermocycler apparatus (Techine-UK). The amplification protocol was as follows: 9 min of denaturation at 95°C and 40 cycles of 20 s of denaturation at 95°C, 30 s of annealing at 55°C, and 30 s of elongation at 72°C, followed by a final extension for 5min at 72°C. The amplicon was subsequently denatured and subjected to hybridization. The assay utilized a flow through hybridization technique by actively directing the targeting molecules toward the immobilized probes within the membrane fibers, with the complementary molecules beingretained by the formation of duplexes. After a stringent wash, the hybridswere detected by the addition of the streptavidin-horseradish peroxidaseconjugate (provided with the kit), which binds to the biotinylated PCR products, and a substrate (nitrobluetetrazolium–5-bromo-4-chloro-3-indolylphosphate) to generate a purple precipitate at the probe dot.

## STATISTICAL ANALYSIS

The data and graphs were carried out using SPSS program version 20 IBM. The proportion and their frequencies were checked by applying the chi - square test. The P-values <0.05 considered statistically significant.

## RESULTS

**HPV Detection**

In the present study, HPV-DNA positive was found 28.94% of the cases but was absent in the control group. In the cases, the detection rate of HPV DNA in cytological categories atypical squamous with chronic cervicitis, LSIL(CIN I), HSIL (CIN II-III), and cancer was 5% (2/40), 30.34% (44/145), 34.88% (15/43), and 100% (7/7), respectively. Significant differences of positively HPV with cytological changes were observed ($P<0.05$) in women with cervical dysplasia and cancer from those with chronic cervicitis.

**HPV Genotypes of infected women**

Seventeen different HPV genotypes were investigated among 68 infected women. Fourteen of these HPV genotypes comprised 82.35% were HR-types and 17.56% were LR-type. The present study indicates presence of ten different HPV genotypes (HPV-39, -45, -51, -52, -53, -58, -59, -66, -68 and -44) were recorded for first time in Iraqi women with cervical lesions. Our finding demonstrated a predominance of HPV-59(14.4%) followed by HPV-16 (13.3%) (Table 1). On the other hand, genotypes-59, -16, -6, -39 and -11 were the five most common types detected in 62.2% of the types and 82.4%of the HPV positive cases. In the present study, type 16 was detected alone in 7/68 (10.3%) cases and in association with type -18 in one (1.5%) case. Type -18 was detected as monotype in three (4.4%) case. Thus, type -16 and -18 were detected alone and in association with each other types in 28% of the 68 cases. Type 59 was detected alone and in association with other types in 19.1% of cases.

In the current study, HPV-16, -45, and -56 were comprised of 57.14 % (4/7), 28.57% (2/7), and 14.29% (1/7) of SCC cases, respectively. Regarding the HPV-6 was detected as co-infector with HPV-45 in one case of SCC. It is remarkable the HPV-66 was detected as monotype in one case suffering from severs dysplasia, because these changes are the last stage for developing invasive cervical cancer. Although, the prevalence of most HPV types were non-significantly ($P>0.05$) among LSIL and HSIL, HPV-45 in HSIL and HPV-6, -11 in LSIL were significantly present ($P<0.0000001$) and ($P<0.05$), respectively.

Eleven cervical samples from women with genital wart as typical criteria of HPV infection were tested. Seven different HPV genotypes have been identified in these specimens, which included: HPV-6 (36.4%), HPV-11(18.2%), and HPV-16, -59,-39,-66 and 44 (9.09% of each).

**High and Low Risk Genotypes among infected women**

The frequency of different HPV genotypes accounted in 68 women was 90 subtypes where 74.4%were HR types

Table 1. HPV Genotypes Detected by Genoarray Technique.

| HPV genotype | No. of infected women [a] | % of total infected[b] |
|---|---|---|
| 16 | 12 | 13.3 |
| 18 | 7 | 7.8 |
| 33 | 1 | 1.1 |
| 35 | 2 | 2.2 |
| 39 | 9 | 10 |
| 45 | 3 | 3.3 |
| 56 | 1 | 1.1 |
| 58 | 5 | 5.5 |
| 51 | 1 | 1.1 |
| 59 | 13 | 14.4 |
| 52 | 1 | 1.1 |
| 66 | 8 | 8.9 |
| 53 | 2 | 2.2 |
| 68 | 2 | 2.2 |
| 44 | 1 | 1.1 |
| 6 | 13 | 14.4 |
| 11 | 9 | 10 |
| Women with Single genotype | 49 | 72.06 |
| Women with Mixed genotype | 19 | 27.94 |

Character (a) in the table indicates to number of infected women that harbor with either single or associated with other genotypes, (b) refers to genotype percentage of total genotypes frequency of all infected women.

and 25.6%LR types (Table 2). Forty five out of 68 infected women (66.18%) had HR types alone, whether single or multiple types, whereas14.7% (10/68) of infected women had HR types in association with LR types and 19.12% (13/68) had LR types alone. However, 80.9% of the positive women had HR-HPV types, thus those women are at risk of developing high-grade squamous intra epithelial lesions or invasive cervical cancer.

**Single and Multiple HPV Infection**

Figure 1 demonstrates that 72.06% (49/68) of infected women had single HPV types, they included that 60.29% (41/68) of the women with dysplasia (CIN I, II/III), compared with 8.83% (6/68) of patients with SCC, and 2.94% (2/68) had chronic cervicitis. As shown in fig. 1, infection with multiple HPV types was 27.94% (19/68) of the study patients; they are distributed to 26.47% (18/68) with dysplasia and 1.47% (1/68) with SCC cases. Appearance of mixed infection in CIN I more than CIN II/III and cancer was observed, whereasthe significant occurrence of single infection with HR-types in CIN II/III and SCC was reported ($P<0.01$).

On the other hand, out of single infections, 26.53% (13/49) were LR-types, HR-types other than HPV-16 constituted 59.18% (29/49), whereas HPV-16 comprised of 14.29% (7/49). Of the multiple infections, 26.32% (5/19) associated with HPV-16, 21.05% (4/19) were infected with HR-types other than HPV-16, and 52.63% (10/19) were infected with LR and HR-HPV types.

**DISCUSSION**

The close correlation between HPV infection and cervical cancer is well established, but there is a wide difference between the prevalence of infection and the occurrence of actual cancer (Yumin *et al*., 2012). The present study was demonstrated ten genotypes for the first time in our country which some of these were recorded in neighboring countries like Saudi Arabia, Iran and Turkey (Alsbeih *et al*., 2011; Zandi *et al*., 2010; Dursun *et al*., 2009). In this study, HPV-16 the most common type in SCC was explained the clearance more slowly than infections caused by other high-risk types (Kulmala *et al*., 2006). Moreover, other HR-HPV types were present in cervical cancer HPV-45 and HPV-56. Although, HPV-33 is one of the most types of cancer, our finding that low percentage (1.1%) of HPV types was present in CIN I. Contrast, Clifford *et al*. (2005a) reported that HPV-16,-18,-33 and -45 were the most four genotypes present in cervical cancer in North America. Also, Young *et al*. (2009) indicated that approximately 95% of cervical cancers in Korean women have been found to contain DNA of HR-HPV types, most commonly HPV-16, followed by HPV types -18, -31 and -45.Geographic differences in the relative prevalence of HPV genotypes may be related to the complex interplay among different

Table 2. Frequency of HPV genotypes according to infection type.

| HPV genotype | Frequency of genotypes as: | | *HPV genotype Co-infectors in mixed infection(NO. of infections) |
|---|---|---|---|
| | Single infection | Mixed infection | |
| 16 | 7 | 5 | 39(1),33(1),52(1),18+66(1),58+59(1) |
| 59 | 7 | 6 | 6(3), 39(1), 16+58(1), 11(1) |
| 66 | 5 | 3 | 11(1), 16+18(1), 18+51(1) |
| 6 | 7 | 6 | 59(3), 39(2), 45(1) |
| 11 | 5 | 4 | 66(1), 58(1), 18(1), 59(1) |
| 39 | 5 | 4 | 16(1), 59(1), 6(2) |
| 35 | 2 | 0 | - |
| 56 | 1 | 0 | - |
| 44 | 1 | 0 | - |
| 68 | 1 | 1 | 53(1) |
| 18 | 3 | 4 | 51+66(1), 16+66(1), 53(1), 11(1) |
| 45 | 2 | 1 | 6(1) |
| 58 | 3 | 2 | 11(1), 16+59(1) |
| 53 | 0 | 2 | 68(1), 18(1) |
| 33 | 0 | 1 | 16(1) |
| 51 | 0 | 1 | 66+18(1) |
| 52 | 0 | 1 | 16(1) |
| Total | 49 | 41 | |

Asterisk indicates that genotypes which appeared with detection genotype in the same infected women.

HPV genotypes and/or variants with host immunogenetic factors (e.g., HLA polymorphisms) (Hildesheim and Wang, 2002). Alternatively, a recent study showed that HPV 16 appeared less influenced by immune status than other HPV genotypes (Strickler *et al*., 2003). However, regional differences appear to become less pronounced with increasing severity of lesions, as HPV 16 becomes increasingly dominant (Clifford *et al*., 2005b). The strongest risk factor for persistence of infection is the presence of HPV-16 (Molano *et al*., 2003). This may explain HPV-16 in women with chronic cervicitis was observed. On the other hand, only one case (14.28%) of SCC genotypes has high-risk type infection in co-infection with HPV-6 was found. Based on the accepted model of cervical carcinogenesis, HPV-6 rarely integrates in the human genome (Coutlee *et al*., 2009). The investigators Garcia *et al*. (2011) suggested that the LR-HPVs persist in cancer lesions possibly as commensals and presence of other HPV types in the same lesions may represent concomitant infections. In contrary, HPV18 is unlikely to cause HSIL or worse cytologies despite its importance in causing 37% to 41% of cases of cervical adenocarcinoma. Moreover, Schiffman *et al*. (2005) showed that no one has been able to explain exactly why HPV18 tends to be ‘‘occult’’ at the stage of high-grade intraepithelial lesions (precancer), the target of cervical cancer screening. This may compare with the low prevalence of HPV-18 in HSIL of present study. The co-infection of HPV, that is, including two or more than two types of HPV in the same patient, is one of the main unsolved problems associated with the current HPV diagnosis and HPV vaccination. Our finding demonstrated that high rate 73.7% (14/19) of women with multiple infections was constituted CIN I in comparison with 26.3% (5/19) in CIN II/III and SCC. It remains to be determined if viral load of multiple HPV infections is indicative of an immunological response due to a combined effect among HPV types present in the infected area and whether or not it would predict a higher risk of infection persistence (Ahn *et al*., 2003). Furthermore, another study showed that mixed infections with HR-HPV genotypes are less likely to progress to cervical cancer than infections with single HR-HPV genotypes (Zuna *et al*., 2004). Thus, the high rate of single infections with HR-HPV types (approximately 53%) in this study population may also predict the higher risk of progression to invasive cancer. Nevertheless, Schellekens *et al*. (2004) reported significantly higher amount of multiple HPV infections in adenosquamous carcinoma in comparison with squamous cell carcinoma. These hypothesizes are confirmed by other study which showed that the infections with multiple HPV types may increase the risk

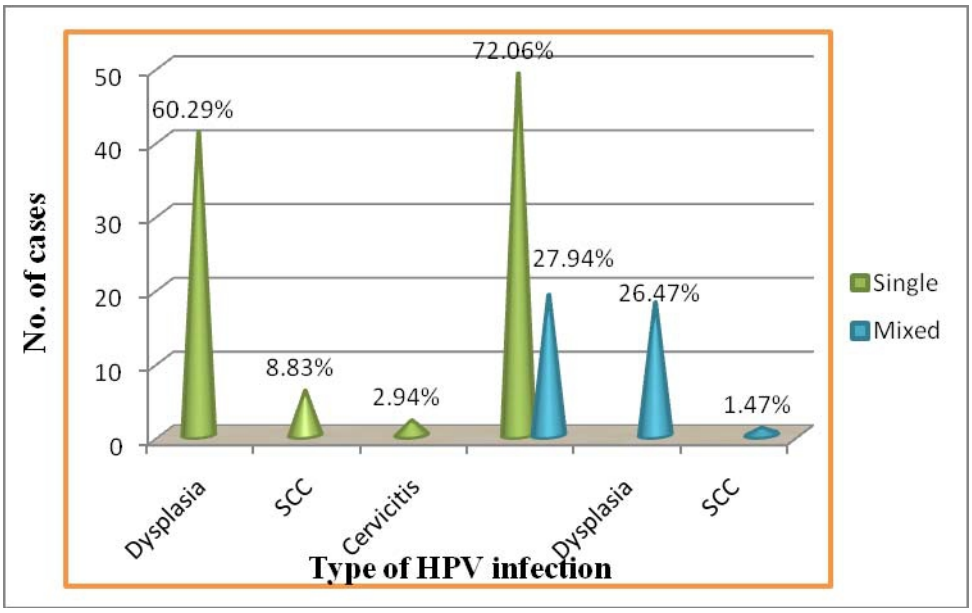


Fig. 1. Distribution Single and Mixed HPV infections according to cytological categories of infected women, term dysplasia indicates women with CIN I, and CIN II/III.

of cervical disease but their effect on SCC pathogenesis is unclear (Rong Sun *et al*., 2010).

In Conclusion: Our results provide evidence the genoarray using for detection was increasing the number of isolates. Since 28% of infected women had HPV-16 and -18, the HPV vaccine is importance to introduction. Moreover, HPV-16, - 45 and -18 were highly associated with increasing severity of the disease, thus the strongest risk factor for persistence of infection was the presence of these types.

## ACKNOWLEDGEMENT

We deeply thank to director Faiza L. Tome/virologist and all staff members of the General viruses Laboratory/National Central Public Health Laboratory for their help.

## REFERENCES

Ahn, WS., Bae, SM. and Kim, TY. 2003. A therapy modality using recombinant IL-12 adenovirus plus E7 protein in a human papillomavirus 16 E6/E7-associated cervical cancer animal model. Human Gene Therapy. 14(15):1389-1399.

Alsbeih, G., Ahmed, R., Al-Harbi, N., Venturina, LA., Tulbah, A. and Balaraj, K. 2011. Prevalence and genotypes distribution of human papillomavirus in invasive cervical cancer in Saudi Arabia. Gynecol. Oncol. 121(3):522-526.

Bosch, FX., Lorincz, A., Munoz, N., Meijer, CJ. and Shah, KV. 2002. Thecausal relation between human papillomavirus and cervical cancer. J. Clin. Pathol. 55:244-265.

Brink, AA., Snijders, PJ. and Meijer, CJ. 2007. HPV detection methods. Dis. Markers. 23(4):273-281.

Clifford, GM., Gallus, S., Herrero, R., *et al*. 2005[a]. HPV Prevalence Surveys Study Group: Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 366(9490):991-8.

Clifford, GM., Rana, RK., Franceschi, S., Smith, JS., Gough, G. and Pimenta, JM. 2005[b]. HPVs genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol. Biomarkers. 14(5):1157-1164.

Coutlee, F., Trottier, H., Gagnon, S., *et al*. 2009. Low-risk HPV type 6 DNA load and integration in cervical samples from women with Squamous intraepithelial lesion. J. Clin.Virol. 45(2):96-99.

de Villiers, EM., Fauquet, C., Broker, TR., Bernard, HU. and ZurHausen, H. 2004. Classification of papillomaviruses. Virol. 324(1):17-27.

Dursun, P., Senger, SS., Arslan, H., Kuscu, E. and Ayhan, A. 2009. Human papillomavirus (HPV) prevalence and types amoung Turkish women at a gynecology outpatient unit. BMC Infect. Dis. 9:191-195.

García, DA., Cid-Arregui, A., Schmitt, M., Castillo, M., Briceño, I. and Aristizábal, FA. 2011. Highly Sensitive Detection and Genotyping of HPV by PCR Multiplex and Luminex Technology in a Cohort of Colombian Women with Abnormal Cytology. The Open Virol. J. 5:70-79.

Gillison, ML. and Lowy, DR. 2004. A causal role for human papilloma-virus in head and neck cancer. Lancet.363:1488-1489.

Grisaru, D., Avidor, B., Niv, J., *et al*. 2008. Pilot study of prevalence of high-risk papilloma virus genotypes in Israeli Jewish women referred for colposcopic examination. J. Clin. Microbiol. 46(5):1602-1605.

Hildesheim, A. and Wang, SS. 2002. Host and viral genetics and risk of cervical cancer: a review. Virus Res. 89(2):229-40.

Kulmala, SMA., Syrjanen, SM., Gyllensten, UB., *et al*. 2006. Early integration of high copy HPV-16 detectable in women with normal and low grade cervical cytology and histology. J. Clin. Pathol. 59(5):513-517.

Liu, SS., Leung, RC., Chan, KK., Cheung, AN. and Ngan, HY. 2010. Evaluation of a newly developed GenoArray human papillomavirus (HPV) genotyping assay and comparison with the Roche Linear Array HPVgenotyping assay. J. Clin. Microbiol. 48(3):758-764.

Molano, M., Brule, V., Plummer, M., *et al*. 2003. Determinations of clearance of HPV infections in Colombian women with normal cytology: A population-based, 5-years follow up study. Am. J. Epidemiol. 158(5):486-494.

Molijn, A., Kleter, B., Quint, W. and Van Doorn, LJ. 2005. Molecular diagnosis of human papillomavirus (HPV) infections. J. Clin. Virol. 32(Suppl. 1):S43-S51.

Muñoz, N., Bosch, FX., de Sanjosé, S., *et al*. 2003. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 348(6):518-527.

Muñoz, N., Castellsague´X., de Gonza´lez, AB. and Gissmann, L. 2006. HPV in the etiology of human cancer. Vaccine. 24(S3):S3/1-S3/10.

RongSun, Z., Ji, YH., Zhou, WQ., Zhang, SL., Jiang, WG. and Ruan, Q. 2010. Characteristics of HPV prevalence among women in Liaoning province, China. Int. J. Gynecol. Obstet. 109(2):105-109.

Sabol, I., Salakova, M., Smahelova, J., *et al*. 2008. Evaluation of different techniques for identification of human papillomavirus types of low prevalence. J. Clin. Microbiol. 46(5):1606–1613.

Schellekens, MC., Dijkman, A., Aziz, MF., *et al*. 2004. Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecol. Oncol. 93(1):49-53.

Schiffman, M., Herrero, R., Desalle, R., *et al*. 2005. The carcinogenicity of human papillomavirus types reflects viral evolution. Virol. 20(337):76-84.

Strickler, HD., Palefsky, JM., Shah, KV., *et al*. 2003. HPV type 16 and immune status in human immunodeficiency virus-seropositive women. J. Natl. Cancer Inst. 95(14):1062-1071.

Youngkim, J., Park, S., Nam, BH., *et al*. 2009. Low initial HPV Viral load implicates worse prognosis in patients with uterine cervical cancer treated with radiotherapy. J. Clin. Oncol. 27(30):5088-5093.

Yumin, W., Jie, C., Wenhui, Z., Wangdong, H. and Fangyou, Y. 2012. Study of the prevalence of human Papillomavirus infection in Chinese women with cervical cancer. Afri. J. of Microbiol. Res. 6(5):1048-1053.

ZandiK, E., Ghbali, SS., Hamkar, R., *et al*. 2010. Prevalence of various Human Papillomavirus (HPV) genotypes amoung women who subjected to routine Pap smear test in Bushehr city (South West of Iran) 2008-2009.Virol. J. 7(1):65-68.

Zuna, RE., Allen, RA., Moore, WE., Mattu, R. and Dunn, ST. 2004. Comparison of HPV genotypes in high-grade squamous intraepithelial lesions and invasive cervical carcinoma: evidence for differences in biologic potential of precursor lesions. Mol. Pat hol. 17:1314-1322.

Received: Sept 24, 2013; Accepted: Oct 26, 2013