RESEARCH ARTICLE

Human Papilloma Virus Genotype Distribution in Cervical lesions in Zanjan, Iran

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Abstract

Objective: Cervical cancer is one of the most common cancers among women all over the world, and main cause is persistent infection with high risk human papillomavirus (HPV) strains. It has been reported that the distribution and prevalence of HPV types varies by geographical region, so that this is important for prevention by type-specific vaccines. The aim of current study was to determine the genotype distribution of HPV using the INNO-LiPA genotyping assay in Zanjan province, North West Iran. **Methods:** A total of 112 formalin-fixed paraffin embedded (FFPE) tissue samples from cases of low-grade intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) were collected. The polymerase chain reaction (PCR) was used to amplify DNA for genotyping. **Results:** Among the 112 samples from females (ranging from 20 to 69 years, mean age 43.8 ± 10.1) tested for HPV DNA, 50 samples were positive. Based on results of genotyping, most common HPV genotypes were HPV18 (48%) followed by HPV-6 (24%), HPV73 (16%), HPV-51(8%), HPV-31(8%), HPV-16 (8%), HPV-56 (4%), HPV-44 (4%). **Conclusion:** While HPV infection is the major etiological factor for cervical cancer, presence was relatively low in our survey. In the positive cases, however, HPV18 was the most common in line with many other populations. The fact that types vary among different populations must clearly be taken into account in design of vaccines for our country.

Keywords: Human papilloma virus- INNO−LiPA- cervical cancer- Zanjan- Iran

Introduction

Cervical cancer is one of the main causes of cancer related deaths among women worldwide (Farjadian et al., 2003; Stamataki et al., 2010; Zandi et al., 2010). Today it is totally accepted that HPV is the main etiological factor for cervical cancer (Vaucel et al, 2011; Vujošević et al, 2012). With a molecular view HPV leads to cancer via inhibition of apoptosis and production of proteins that restrain P53 and retinoblastoma genes (Karimi-Zarchi et al, 2009). More than 200 HPV genotypes have been described and nearly 40 types based on their oncogenic potential in developing to cervical cancers are classified as low-risk and High-risk (Stamataki et al., 2010; Heard et al., 2013; Wang et al., 2014).

Previous studies demonstrated that 80% of new cases occur in developing countries and the rate of cervical cancer in developed countries has been decreased which seems to be associated with the screening programs in these countries (Farjadian et al., 2003). Screening tests based on cytology has decreased the mortality rate from cervical cancer in developed countries although screening tests has been performed successfully all over the world but they has lower sensitivity (30-87%), because of lower sensitivity of pap test superseded by Liquid Based Cytology (LBC) which has higher sensitivity, but this method has limitations also, HPV DNA testing is another method which has higher sensitivity than pap tests and chance for HPV detection with this method increase notably (Cooper et al., 2005). It has been reported that the distribution and prevalence of HPV types varies by geographical region and even in different area of the same country (Stamataki et al., 2010; Kim et al, 2014; Wang et al., 2014).

In fact, investigation of prevalence of different HPV types in population is necessary for: 1. planning efficient screening program 2. management of disease 3. developing vaccines based on patterns of the prevalent HPV types.

Whereas HPV genotypes prevalence varies in different geographical region, and determination of HPV types is important for prevention of cervical cancer also there are no reports on HPV prevalence in Zanjan Province, the aim of current study is to investigate prevalence and genotype distribution of HPV using INNO-LiPA assay in Zanjan province, North West of Iran.

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Materials and Methods

A total of 112 formalin-fixed paraffin embedded (FFPE) tissue samples with LSIL and HSIL and SCC between 2010-2016 time interval were obtained from pathology department of Mousavi Hospital in Zanjan city ,Hematoxylin Eosin (H and E) stained slides of these paraffin blocks were examined by pathologist to verify them.

DNA extraction

After depparafinization of blocks by xylene-ethanol method, DNA extraction performed by DNA extraction kit (CinnaClon Company, Iran, Catalog No.PR911683) based on manufacturer instruction, extracted DNA was stored at -20 ºC.

DNA detection

DNA samples were subjected to PCR by using GP5+/GP6+ primers that amplify 150 bp product from the HPV L1 ORF (Table1). The PCR amplification program comprised an initial denaturation; 94ºC for 5min, 35 cycles of 94ºC for 30 s ,55ºC for 45 s, 72ºC for 45 s and final extension at 72ºC for 5 min (Salehpour et al., 2015). We used extracted DNA from Hela cell line as positive control for HPV and our negative control contained all ingredients except DNA. After amplification, PCR products subjected to agarose gel electrophoresis (in agarose 2%) and stained with ethidium bromide and visualized on UV trans illuminator.

Genotyping

HPV genotyping performed by INNO-LiPA HPV genotyping system (Fujirebio Europe N.V. Technologiepark 6 9052 Gent, Belgium) based on manufacturer instruction.

This kit is capable of identification of 32 different types of HPV by detection part of L1 region of HPV by SPF-10 primers also an additional primer pair for the amplification of HLA-DPB1 is added to investigate sample quality and extraction.

Statistical Analysis

Statistical analysis was performed by SPSS statistical software package version 23, Chi-Square test was used to find any relation between HPV types and degree of Cytopathologic abnormalities. A P-Value < 0.05 statistically considered significant.

Results

Among 112 samples from females (ranging from 20 to69 years with mean age of 43.8 ± 10.1) tested for HPV DNA, 50 (44.6%) samples were positive (Figure 1). Single HPV infection was detected in 36% of HPV positive samples and also infection with multiple types detected in 36% of HPV positive samples, also other positive samples remain unknown which were positive for HPV DNA test but were negative in genotyping and it can be related to SPF-10 primer which has limitation for detecting different HPV types.

Based on results of genotyping, most common

HPV genotypes were HPV18 (48%) followed by HPV-6 (24%), HPV73 (16%), HPV-51(8%), HPV-31(8%), HPV-16 (8%), HPV-56 (4%), HPV-44 (4%) (Table 2) (Figure 2).

Among single infected samples the most frequent type was HPV-18 (55.5%) which was followed by HPV-73 (11.1%) HPV-31 (11.1%), HPV-16 (11.1%). In multiple infected samples, the most prevalent type was HPV-18

Table 1. Primers and PCR Product Characteristics

Gene	Primers	Product Size (bp)	Reference
L1 (HPV)	GP5+ TTTGTTACTGTGGTAGATACTAC	150	(Shikova et al., 2009)
	GP6+ GAAAAATAAACTGTAAATCATATTC		

Table 2. HPV Genotypes Prevalence Based on Cytological Diagnosis

HPV Types	Sample number	Age	Mono infection N=18	Multiple infection	Multiple infection		Unknown types	pathological diagnosis		
	N=50	Mean age of positive samples		N=18	Double infection	Triple infection	N=14	LSIL	HSIL	SCC
					N=8	N=10		N=32	N=16	N=2
High risk	18	24	46	10(55.5%)	14(77.7%)	4 (50%)	10 (100%)	------	18 (56.2%)	6 (37.5%)	0 (0)
	16	4	48.8	2 (11.1%)	2 (11.1%)	2 (25%)	0 (0)	------	0 (0)	2 (12.5%)	2 (100%)
	31	4	54.3	2 (11.1%)	2 (11.1%)	0 (0)	2 (20%)	------	4 (12.5%)	0 (0)	0 (0)
	51	5	43.6	2 (11.1%)	3 (16.6%)	1 (12.5%)	2 (20%)	------	2 (6.25%)	3 (18.75%)	0 (0)
	56	2	36	0 (0)	2 (11.1%)	0 (0%)	2 (20%)	------	0 (0)	2 (12.5%)	0 (0)
	73	8	42.1	2 (11.1%)	6 (33.3%)	0 (0)	6 (60%)	------	4 (12.5%)	4 (25%)	0 (0)
Low risk	6	13	44.8	0 (0)	13 (72.2%)	7 (87.5%)	6 (60%)	------	7 (21.8%)	6(37.5%)	0 (0)
	44	2	40.2	0 (0)	2 (11.1%)	0 (0)	2 (20%)	------	2 (6.25%)	0 (0)	0 (0)

Figure 1. PCR Analysis of DNA Samples Extracted Paraffin Embedded Tissues Using HPV GP5+ GP6+primers: lane 1, DNA Size marker; lane 2-11, HPV positive samples; lane 12, positive control; lane 13, negative control

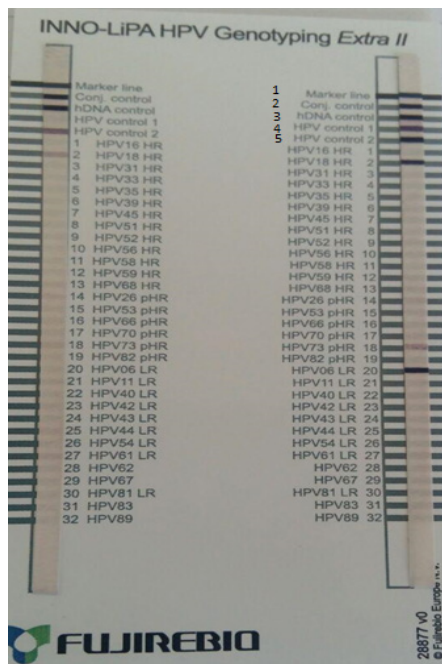Figure 3. HPV Types Prevalence Based on Pathological Diagnosis

Figure 2. HPV Genotyping Result Using INNO-LiPA Technique, line 1, Marker line; line 2, Conjugate control; line 3, hDNA control; line4, HPV control1; line5, HPV control 2; Right Strip, HPV18,6,73 genotypes; Left Strip, HPV18 genotype

(77.7%) followed by HPV-6 (72.2%), HPV-73 (33.3%), HPV-51(16.6%), HPV-56(11.1%), HPV-31 (11.1%), HPV-44(11.1%) (Table 2).

Based on pathological diagnosis most common
HPV types in LSIL specimens was HPV18(56.2%) followed by HPV-6 (21.8%),most common HPV types in HSIL specimens was HPV-18(37.5%) and HPV-6 (37.5%) followed by HPV-73 (25%) and the most common HPV in SCC specimens was HPV-16 (100%). Based on Chi-square test result there is a relation between Cytopathologic degree and HPV types P-value< 0.001 (Figure 3).

Discussion

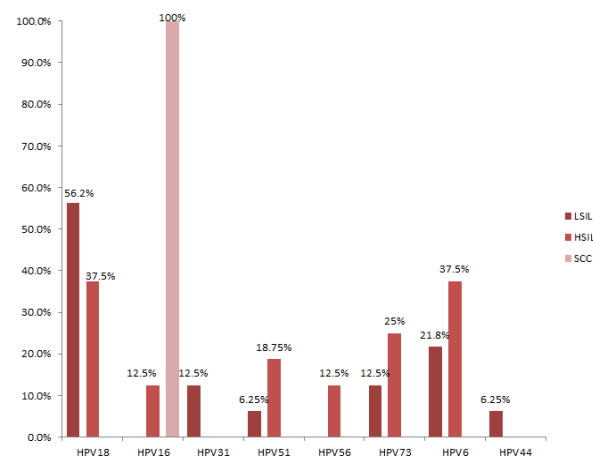Cervical cancer is one of the most common cancer among women all over the world and its relation with HPV has been well established (Farjadian et al., 2003; Karimi-Zarchi et al, 2009; Stamataki et al., 2010).

Pap smear test has been used as routine screening test which has important role in detection of abnormal cells, finally leading to cervical cancer but its sensitivity is questionable, So using molecular test in conjunction with cervical cytology could help to improve cervical cancer screening (Das et al., 2000; Kulasingam et al., 2002).

In this study we used PCR method for detection of HPV DNA in our samples and INNO-LiPA assay for identification of HPV genotypes.

In our study among 112 samples, 50 (44.6%) were positive for HPV, 36% of HPV positive samples has been infected with multiple types of HPV.

HPV prevalence rate in our study was compatible to several studies in Iran and other countries, in one study in turkey in 2009 on samples of women with normal and abnormal cytology, HPV prevalence has been reported 36% in women with abnormal cytology and 20% in woman with normal cytology (Dursun et al, 2009).

In a study carried out in Iran, Tehran in 2016 on samples of patients with different degrees of abnormality include ASCUS, LSIL, HSIL, HPV prevalence has been reported 45.4% (Salehi-Vaziri et al., 2016).

In other study that performed in Tehran in 2013 on women, who attended regular gynecological visits, and most of them has different degrees of abnormality, HPV prevalence has been reported 31.1% (Shafaghi et al., 2013).

The similarity of these studies result to our findings can be related to type of samples since most of these samples were from patient with LSIL and HSIL, primer sets which has been used .

In contrast to our study higher prevalence and lower prevalence of HPV in several studies from different countries also different parts of Iran has been reported.

In an investigation in Qatar in 2014 on samples from women with normal and abnormal cytology, HPV prevalence has been reported 18.4% in women with abnormal cytology and 5.9% in women with normal cytology (Bansal et al., 2014).

In a study in Iran, Bushehr city in 2010 HPV

prevalence has been reported 5.5% in women who subjected to routine pap smear test (Zandi et al., 2010).

Lower prevalence of HPV can be related to geographical region, life style some cultural limitation and type of sample.

In one study in brazil in 2013 on samples from patients with cervical cancer 98.9% of samples were HPV positive, (De Oliveira et al, 2013).

In study from japan in 2012 on samples from patients with different degrees of abnormality 94% of samples were positive for HPV (Kondo et al., 2012).

In study from Iran Shiraz province on samples with cervical cancer 87.1% of samples were HPV positive (Farjadian et al., 2003).

Higher prevalence of HPV in these studies can be explain by this point that samples were obtained from patients with cervical cancer, so HPV detection possibility increase other reason is method sensitivity, increase in method sensitivity has direct relation with HPV detection. These differences can be explained by variation in geographical region, type of sample and size of samples, different method used and these methods sensitivity (Akcali et al, 2013; Salehi-Vaziri et al., 2016).

Overall the reason of the lower prevalence of HPV in our study can be related to type and size of sample which majority of our sample were from patients with LSIL so chance for detection of HPV decrease.

Type of Sample is important also, samples we used were paraffin-embedded tissues, this type of samples can lead to lower HPV positivity rate because the fixation process degrades DNA (De Oliveira et al, 2013) other reason is differences in geographical region (Akcali et al, 2013), in addition type of primer set which were used is important because some kind of primer-sets are less sensitive for detection of special HPV types (Karimi-Zarchi et al., 2015).

In some studies has mentioned that negative results in PCR of samples with histopathological diagnosis of cancer or precancerous state, may be due to the way of viral circular genome integrate in host chromosome, based on some of thesis in this field viral genome integrate in genome in status that lead to changes in part of gene which is PCR or amplification target (Gallo et al., 2003).

Based on results of genotyping, most common HPV genotypes were HPV18 (48%) followed by HPV-6 (24%), HPV73 (16%), HPV-51 (8%), HPV-31(8%), HPV-16 (8%), HPV-56 (4%), HPV-44 (4%), is in contrast to many studies that reported HPV16 as most common type (Farjadian et al., 2003; Dursun et al, 2009; Zandi et al., 2010; Turki et al, 2013).

In a research on samples from patients with invasive cervical cancer in Brazil, in 99% of samples HPV DNA has been detected as mentioned above, and the most common types in this study were HPV-16 (77.6%), HPV-18 (12.3%), HPV-31(8.8%), HPV-33(7.1%), HPV-35 (5.9%) (De Oliveira et al, 2013).

In an investigation in Iran on samples from patients with SCC 49% of patients were positive for HPV DNA, and the most common types in this study were HPV-16(62%), HPV-18 (12%) and other types HPV-31, 33, 45, 52 (26%) (Eslami et al., 2008).

In addition higher prevalence of HPV-16 from different parts of Iran has been reported, these differences in HPV type's prevalence can be explain by differences in geographical region, and sample types.

There are several studies that they results are compatible with our findings, in one study from north of Iran HPV18 (41%) has been the most common type followed by HPV16 (29.5%) (Haghshenas et al., 2013) which explain the importance of geographical region in HPV genotypes distribution, in another study from Kayseri in Turkey HPV18 was the most common type followed by HPV16 (Eroglu et al., 2011), in addition in one study performed in Tehran HPV53 has been reported as most common type followed by HPV-16 that shows that prevalent genotypes of HPV vary by geographical region (Pouryasin et al., 2014).

These variations can explain by several reasons; HPV genotypes distribution vary by geographical region (Stamataki et al., 2010; Kim et al, 2014), for example in one study has performed to determination of HPV-16 prevalence in eleven province of Iran, Kerman province (South of Iran) has highest prevalence (75%), but Qazvin province (Northwest of Iran) has lowest prevalence (0%) (Mobinikeshe et al., 2013). Genotypes prevalence may influenced by host genetical factors, in this case higher HPV16 titers has been reported in carriers of a specific haplotype of HLA-DRB1-DBQ1, also based on report on the frequency of HLA class II alleles in patients with cervical cancer, it has been determined that there is an association between HLA-DQB10601 and squamous cell carcinoma of cervix (Beskow and Gyllensten, 2002; Dehaghani et al, 2002). Sample type is also related to frequency of type, it has been observed HPV18 is more common in precancerous lesions and LSIL and with increase in severity of abnormalityHPV18 frequency decreases (Vujošević et al, 2012; Leinonen et al., 2013) it is also necessary to mention that majority of our samples were related to patients with LSIL.

In addition it has been revealed that studies using histological samples have reported higher prevalence of HPV-18 (Stamataki et al., 2010).

In our study 36% of HPV positive samples has been infected with multiple HPV types, in other studies from Iran similar results has been reported. In a study in Tehran multiple infection has been reported in 50% of HPV positive samples (Pouryasin et al., 2014), in other study in Tehran infection with multiple HPV types has been reported in 33.8% of HPV positive samples (Salehi-Vaziri et al., 2016). This higher multiple infection rate may be due to method for genotyping, since they has been used INNO-LiPA assay too.

In conclusion, our study demonstrated that using molecular methods beside routine Pap smear test could help to early diagnosis and efficiently prevention of cervical cancer, also prevalence of HPV and its types vary among different population with considering this point that vaccine immunity is type specific the need for further studies in all part of our country is recommended.

Acknowledgments

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