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ORIGINAL ARTICLE

# Prevalence of human papilloma virus (HPV) and type distribution of genotypes (6, 11, 16 and 18) among Egyptian women

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## ABSTRACT

***Background****: Human papillomavirus (HPV) is the most common viral infection of the reproductive tract and the causative agent of cervical cancer and genital warts.* ***Objectives****: to study prevalence of HPV infection and type distribution of genotypes (6, 11, 16 and 18) among Egyptian females.* ***Methodology****: 65 Egyptian women were subjected to conventional Pap cytology, HPV DNA testing by polymerase chain reaction (PCR) and genotyping by multiplex PCR for genotypes (6, 11, 16 and 18) during the period from May 2018 until October 2018.* ***Results****: The prevalence of HPV among participants was (23.1%). only 20% of HPV positive cases, were infected by single HPV genotype and 80% were co-infected by more than one genotype.* ***Conclusion****: These data expand the knowledge concerning HPV prevalence and type distribution in Egypt which may help to create a national HPV prevention program.*

## INTRODUCTION

Human papillomavirus (HPV) is the most common viral infection of the genital tract and the causative agent of cervical cancer and genital warts [1]. More than 100 types of HPV exist. Some types are more likely to cause complications than others. The epidemiological classification of cervical cancer-associated HPV types classifies 15 HPV types as carcinogenic or high-risk (HR) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) and 12 HPV types as low-risk (LR) types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108)[2].

Low-risk HPV types such as (HPV-6 and HPV-11) cannot cause cervical cancer but can cause the common and highly infectious genital warts. On the other hand high-risk HPV types are greatly associated with cancer cervix. HPV-16 and HPV-18 are the two most common HR HPV types. They are responsible for 70% of cervical cancer cases worldwide [3].

Cervical cancer is the fourth most prevalent cancer in women worldwide [4]. The highest mortality of cervical cancer occurs in developing countries. It has been estimated that 969 women are diagnosed with cervical cancer and 631 die from the disease every year. Cervical cancer is as the 14th most prevalent cancer among Egyptian women and the 11th most prevalent cancer among Egyptian women aged between 15 and 44 years[5].

Fortunately HPV infection and its complications can be prevented by the bivalent HPV vaccine against (HPV-16 and HPV-18), the quadrivalent HPV vaccine against (HPV-6, HPV-11, HPV-16 and HPV-18) and nonavalent HPV vaccine against (HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV 45, HPV 52 and HPV 58)[6]. Although these vaccines are licensed in Egypt, they are not included in the national immunization program [7].

Baseline epidemiological data are required to measure the benefits of implementing a nationwide policy for HPV vaccination. However, epidemiological data for HPV in Egyptian women are limited [8].

The aim of this study was to study prevalence of HPV infection and type distribution of genotypes (6, 11, 16 and 18) in cervical specimens from Egyptian females attending gynaecological and family planning outpatient clinics in Ain shams Obstetrics and Gynaecology hospital.

## METHODOLOGY

This study was conducted on 65 Egyptian women attending gynecological and family planning clinics, Ain shams university hospital, for gynecological consultation and family planning during the period from May 2018 until October 2018. Written informed consents were taken from all participants after explanation of the aim and procedures of the study.

All patients were subjected to history taking regarding: Age, marital status, no of marriages, parity, smoking, contraception, symptomatic partner and the presenting symptoms.


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**Specimen collection**: Cervical scrapings were collected by a gynecologist via a cytobrush from the ecto and endo-cervix. A slide was prepared for Pap smear cytology and the cytobrush was then placed in RPMI viral transport medium (GIBCO diagnostics, USA), transported to the lab and stored at -80°C until processed for HPV-DNA detection.

**Cytological examination:** Cytological examination was performed at the obstetrics and gynecology Ain shams university hospital pathology lab and the findings were classified according to the 2004 Bethesda classification system [9] into normal and atypical squamous cells with either low- or high-grade squamous intraepithelial lesions (LSIL or HSIL).

**Detection of HPV-DNA by polymerase chain reaction**:

***DNA extraction:*** was done using QIAGEN DNA extraction Kit® (QIAGEN, USA), for DNA purification from cervical samples according to manufacturer's instructions.

***DNA amplification:*** Amplification of eluted DNA for detection of HPV DNA was done in thermal cycler (Thermo ELECTRON CORPORATION, MILFORD, MA 01757 USA) according to *Hassan et al* [10]**,** using MY09 and MY11 primers. Initial denaturation was done at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute then annealing at 55°C for 1 minute, then extension at 72 ◦C for 1 minute. This was followed by a final extension at 72 ◦C for 10 minutes. The sequence of primers and the amplicon size are summarized in table (1).

***The amplified products were identified using gel electrophoresis*** on 2% agarose gel stained with ethidium bromide. Band sizes were estimated by comparison with a 100 bp molecular weight marker, and gels were photographed in a UV trans-illuminator.

**HPV genotyping by multiplex PCR:** HPV positive samples were genotyped using QIAGEN Multiplex PCR kit **(Qiagen, USA),** for genotypes 6, 11, 16 and 18. Amplification of DNA was done according to *Nishiwaki, et al* [11]**.** Initial denaturation was done at 95°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds then annealing at 65°C for 90 seconds, then extension at 72 ◦C for 90 seconds. This was followed by a final extension at 72 ◦C for 10 minutes. The sequence of primers and the amplicon sizes are summarized in table 2.

**Statistical Analysis:**

The collected data were revised, coded, tabulated and introduced to a PC using statistical package for social sciences (IBM SPSS 20.0).

**Table 1: Primers' sequence for HPV screening (MY09/ MY11) [10]**

| Gene | Primer direction | Primer sequence | Amplicon Size |
|---|---|---|---|
| **HPV screening gene** | Forward<br>Reverse | 5-CGT CCA AAA GGA AAC TGA GC-3<br>5-GCA CAG GGA CAT AAC AAT GG-3 | 450bp |

**Table 2: Primer sequence for HPV genotypes 6, 11, 16 and 18 [11]**

| Gene | Primer direction | Primer sequence | Amplicon size |
|---|---|---|---|
| **Hpv-6** | Forward<br>Reverse | 5-GCT AAA GGT CCT GTT TCG AGG CGG CTA-3<br>5-GGC AGC GAC CCT TCC ACG TAC AAT-3 | 263bp |
| **Hpv-11** | Forward<br>Reverse | 5-CGC AGA GAT ATA TGC ATA TGC-3<br>5-AGT TCT AAG CAA CAG GCA CAC-3 | 472bp |
| **Hpv-16** | Forward<br>Reverse | 5-TCA AAA GCC ACT GTG TCC TGA-3<br>5-CGT GTT CTT GAT GAT CTG CAA-3 | 217bp |
| **Hpv-18** | Forward<br>Reverse | 5-CCG AGC ACG ACA GGA ACG ACT-3<br>5-TCG TTT TCT TCC TCT GAG TCG CTT-3 | 187bp |

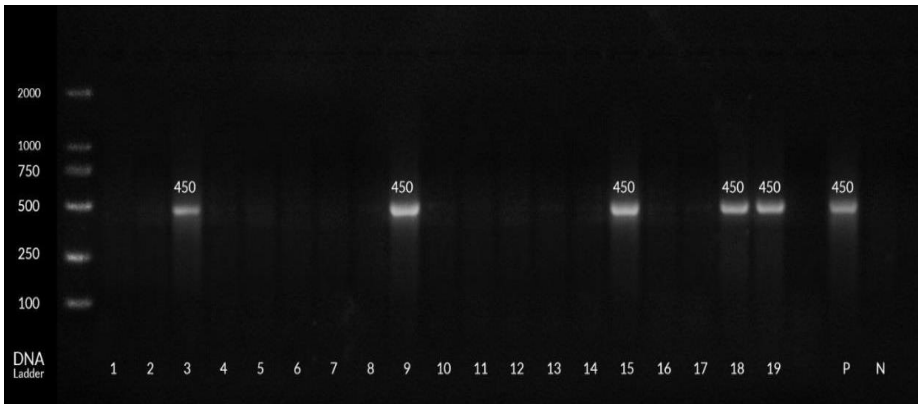


**Fig. 1:** Gel electrophoresis for detection of amplified HPV-DNA (450 bp)

## RESULTS

Among the all participants in the present study (No. 65) the prevalence of HPV infection was 15 (23.1%). only 3 cases, out of 15 HPV positive samples, were infected by single HPV genotype, two of them were infected by HPV 6 and 1 case was infected by HPV 18. The remaining 12 cases were co-infected by more than one HPV genotype. HPV 6 was detected in all cases of co-infection. The results of HPV genotyping are shown in figure (2). The percentages of the 4 studied genotypes among the 15 HPV positive samples are shown in figure (3).

A statistically significant relationship was detected between HPV infection and [age, no of marriages and partner symptoms (warts)] with p values 0.026, 0.013 and 0.05 respectively, while there is no statistically significant relationship between HPV infection and (parity, marital status and method of contraception) (Table 3).

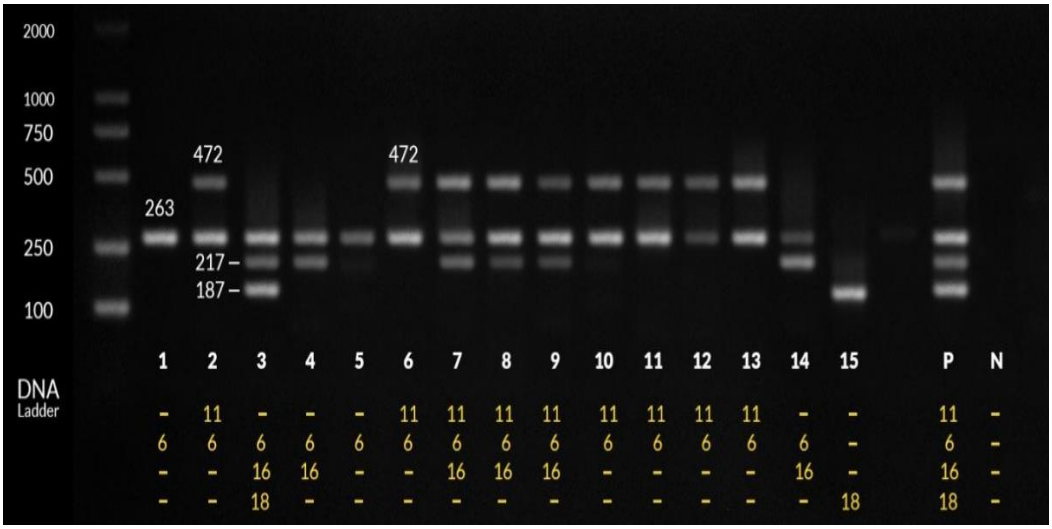


**Fig. 2:** Gel electrophoresis of amplified HPV genotypes (6, 11, 16 and 18)

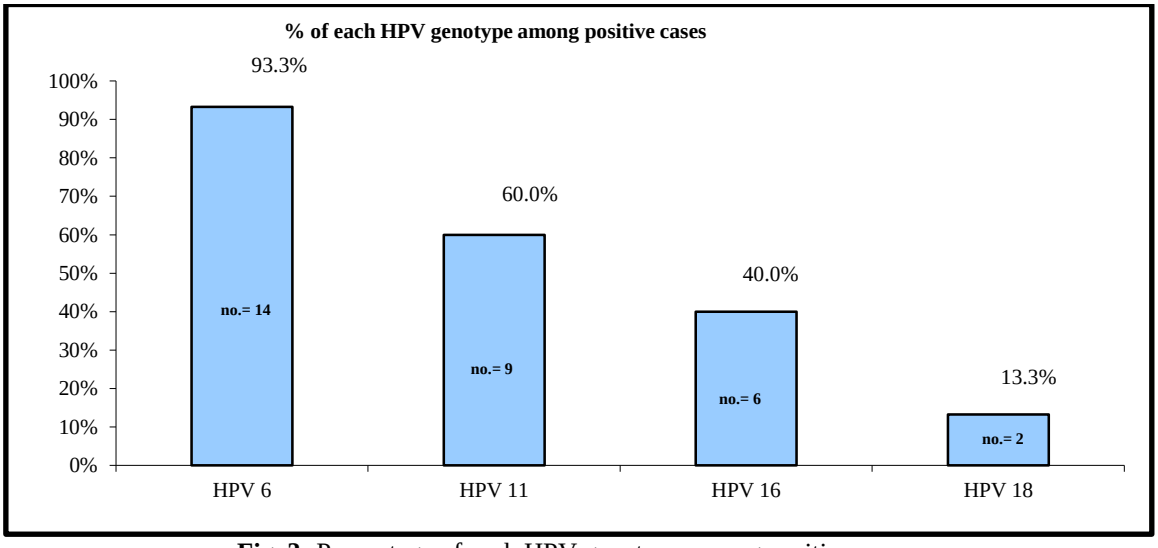


**Fig. 3:** Percentage of each HPV genotype among positive cases.

**Table 3: Relation between HPV infection and risk factors of infection**

| Variables | | HPV status | | | | Chi-square | P-value |
|---|---|---|---|---|---|---|---|
| | | Negative | | Positive | | | |
| | | No. | % | No. | % | | |
| **Age** | **18-25 years** | 2 | 28.6% | 5 | 71.4% | 11.029 FE (#) | **0.026*** |
| | **26-35 years** | 16 | 84.2% | 3 | 15.8% | | |
| | **36-45 years** | 14 | 77.8% | 4 | 22.2% | | |
| | **46-55 years** | 15 | 88.2% | 2 | 11.8% | | |
| | **≥ 56 years** | 3 | 75.0% | 1 | 25.0% | | |
| **Parity** | **<2** | 5 | 62.5% | 3 | 37.5% | 1.440 FE (#) | 0.696 |
| | **2** | 15 | 78.9% | 4 | 21.1% | | |
| | **3** | 15 | 75.0% | 5 | 25.0% | | |
| | **≥4** | 15 | 83.3% | 3 | 16.7% | | |
| **Marital status** | **Married** | 50 | 78.1% | 14 | 21.9% | 3.385 FE (#) | 0.231 |
| | **Divorced** | 0 | 0.0% | 1 | 100.0% | | |
| **No of marriages** | **1** | 47 | 82.5% | 10 | 17.5% | 7.987 FE (#) | **0.013*** |
| | **>1** | 3 | 37.5% | 5 | 62.5% | | |
| **Contraception** | **No** | 15 | 93.8% | 1 | 6.2% | 5.227 FE (#) | 0.156 |
| | **IUD** | 18 | 78.3% | 5 | 21.7% | | |
| | **COC** | 16 | 64.0% | 9 | 36.0% | | |
| | **Condom** | 1 | 100.0% | 0 | 0.0% | | |
| **Partner symptoms** | **No Symptoms** | 50 | 79.4% | 13 | 20.6% | 6.878 FE (#) | **0.050*** |
| | **Genital Warts** | 0 | 0.0% | 2 | 100.0% | | |

## DISCUSSION

In this study, the prevalence of HPV infection among Egyptian women, older than 18 year old attending gynaecological and family planning outpatient clinics was 23.3%. This result goes in accordance with the result reported by *Xu et al* [12] in China, who detected an HPV prevalence rate of 22.8%, among women visiting the Taizhou Medical Center. Also, *Hooi et al* [13] in Curacao tested HPV prevalence among women randomly selected from the national Population Register and aged between 25-65 years old by PCR and reported HPV prevalence of 19.7%.

In Egypt, a lower prevalence rate was reported by *Abdel Aziz et al* [14] who conducted a study on 166 females attending Kasr El Aini obstetrics and gynecology outpatient clinics, detected HPV-DNA by PCR, performed HPV genotyping by hybrid capture II assay and reported HPV prevalence of 15.06 %.

A much lower prevalence rate in Egypt was reported by *Shaltout et al* [15] who conducted their study on 443 Egyptian women attending gynaecological and family planning outpatient clinics at Ain Shams university hospital, detected HPV-DNA by PCR, genotyped HPV-DNA by Microarray technique and reported HPV prevalence of 10.3%.

Similarly the prevalence rate in this study was higher than the results reported in Northern Africa by *Ogembo et al* [16] who reported HPV prevalence of 12.8 % and also higher than results reported by *Su et al* [17] in Dali-Bai, China who reported HPV prevalence of 7.6 %.

In contrast to the previous low prevalence reports, a much higher prevalence rate was reported in Egypt in the study conducted by *Yousef et al* [8]**.** They examined 152 Egyptian women attending for routine gynecologic care at the family planning and gynecological outpatient clinics, Kasr Al Aini University Hospital, Cairo, and the outpatient clinics of the Obstetrics and Gynecology Department at El Shatby Maternity Hospital, Alexandria University, tested their samples for HPV by PCR and genotyped them by Microarray. They reported HPV prevalence of 40%. Our results were also lower than results reported in Kazakhastan by *Niyazmetova et al* [18], who reported HPV prevalence of 43.8% among 140 patients aged 18-59, who visited the outpatient gynecological clinic by PCR and genotyped HPV positive samples by multiplex PCR. Similarly, *Kovacevic et al* [19], in Serberia, reported HPV prevalence of 51.8%. Also, *Zhang et al* [20] in Whenzhou and *Martora et al* [21] in Naples reported HPV prevalence of 49.6% and 44.5% respectively.

The variation in the prevalence rates of HPV reported by different researchers can be explained by the nature of HPV infections being transient and resolving on their own, so the prevalence of HPV might change over time. Secondly, different inclusion criteria for participant women may lead to variation in HPV prevalence as most studies with high HPV prevalence performed the study on women with abnormal cytological finding leading to a possibility of over estimation of infection. Tertiary, difference in sensitivity of the tests used to measure the prevalence might be a contributing factor. Fourthly, different

geographic distribution may be a cause of different HPV prevalence rate. Fifthly, low prevalence rate could be due to implementation of HPV vaccine in national vaccination programs in some countries as shown in a study implemented in Canada by *Steben et al* [3] who compared HPV prevalence between HPV vaccinated and HPV non vaccinated women and the result was 1.5% versus 11% respectively.

In the present study, HPV-6 was the most prevalent low risk HPV type and HPV-16 was the most prevalent HR HPV type. These results were consistent with that reported in Egyptian studies by *Shaltout et al* [15] and *Yousef et al* [8] and also consistent with the results reported by *Kim Y et al* [22], *Santos Filho et al* [23] and *Bray et al* [24].

The current study showed that 80% of HPV positive women were co-infected with more than one HPV genotype while 20% were infected with single genotype. This finding was consistent with that reported by *Yousef et al* [8] who reported 63% for co-infection and 37% for single infection. On the other hand, this result was different from that reported by *Xu et al* [12] who reported that co-infection represented only 25.3% of HPV positive women. Also our results were different from that reported by *Cilingir et al* [25] in Turkey who reported HPV co-infection prevalence of 6% and *De Vuyst et al* [26] in Kenya who reported HPV co-infection prevalence of 46%.

This study found that the prevalence of HPV was highest in women aged 18–25 years, which is the age of high sexual activity and this was consistent with that reported by *Shaltout et al* [15], *Su Y et al* [17] and *Martora et al* [21].

Our study also reported that there is a statistically significant relationship between positive HPV infection and number of lifetime partners, which is concordant to that reported by *Oakeshott et al* [27] and *Bray et al* [24]. This could be due to increased chances for infection with increased number of lifetime partners.

Our study also reported that there is a statistically significant relationship between positive HPV infection and abnormal cytological finding. This is similar to that reported by *Santos Filho et al* [23] in Brazil, *Abdel Aziz et al* [14] in Egypt.

On the other hand, this study reported that there is no statistically significant relationship between HPV infection and parity or use of contraceptives. This was contrary to the results reported by *Kovacefic et al* [19], *Santos Filho et al* [23] and by *Amaral et al* [28] who reported a statistically significant relationship between HPV infection and (use of hormonal contraception and high parity). Use of contraception and high parity increases the probability of HPV infection. However, the absence of significant relationship in our study could be due to lower sample size.

This study also found that there is no statistically significant relationship between HPV infection and marital status, which is similar to that reported by *De Vuyst et al* [26].

## CONCLUSIONS & RECOMMENDATIONS

This study provided important reference data for public health officials when formulating future strategies, including implementation of national vaccination programs, to prevent HPV- associated complications in Egypt.

Further studies are recommended on wider scale including females from other areas and governorates. In addition, we recommend further studies testing other HPV genotypes especially other high-risk HPV genotypes.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

## REFERENCES

1. Yusupov A. et al. The nonavalent vaccine : a review of high-risk HPVs and a plea to the CDC. Am J Stem Cells. 2019; 8, 52–64.
2. Muñoz N. et al. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. N Engl J Med. 2003; 348, 518–527.
3. Steben M. et al. A Review of the Impact and Effectiveness of the Quadrivalent Human Papillomavirus Vaccine : 10 Years of Clinical Experience in Canada INTRODUCTION. J. Obstet. Gynaecol. Canada. 2019; 40, 1635–1645.
4. Arbyn M. et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob. Heal. 2020; 8, 191–203.
5. Ghorab AE.-MMI., Mohamed MAE.-H, Eid SMM., Saleh MMA & Semary SE.-DSO. Detection of

human papillomavirus genotypes in cancer cervix patients : damietta governorate , EGYPT By. Al-Azhar Med. J. 2019; 48, 407–420.

6. Maver PJ & Poljak M. Progress in prophylactic human papillomavirus ( HPV ) vaccination in 2016 : A literature review q. Vaccine. 2018; 36, 5416–5423.
7. Gohar A, Abdeltawab NF, Shehata N & Amin MA. Preclinical study of safety and immunogenicity of combined rubella and human papillomavirus vaccines: Towards enhancing vaccination uptake rates in developing countries. Papillomavirus Res. 2019; 8, 100172.
8. Youssef MA et al. Prevalence of human papilloma virus (HPV) and its genotypes in cervical specimens of Egyptian women by linear array HPV genotyping. Infect. Agent. Cancer. 2016; 11.
9. Nayar R. & Solomon, D. Second edition of 'The Bethesda System for reporting cervical cytology' - Atlas, website, and Bethesda interobserver reproducibility project. Cytojournal. 2004; 1, 1–2.
10. Hassan RA et al. Evaluation of urine as a potential alternative sample for the diagnosis of Human Papillomavirus (HPV) related cervical lesions: A pilot study. Nat. Sci. 2012; 10, 38–44.
11. Nishiwaki M et al. Genotyping of human papillomaviruses by a novel one-step typing method with multiplex PCR and clinical applications. J. Clin. Microbiol. 2008; 46, 1161–1168.
12. Xu HH et al. Prevalence characteristics of cervical human papillomavirus (HPV) genotypes in the Taizhou area, China: A cross-sectional study of 37 967 women from the general population. BMJ Open. 2017; 7.
13. Hooi DJ et al. Human papillomavirus ( HPV ) prevalence and associated risk factors in women from Curac ¸ ao. PLoS One. 2018; 35, 25–34.
14. Abdel-Aziz, M. T. et al. Screening for human papillomavirus ( HPV ) in Egyptian women by the second-generation hybrid capture ( HC II ) test. Med Sci Monit. 2006; 12, 43–49.
15. Shaltout, M. F. et al. Prevalence and type distribution of human papillomavirus among women older than 18 years in Egypt: A multicenter, observational study. Int. J. Infect. Dis. 2014; 29, 226–231.
16. Ogembo RK et al. Prevalence of Human Papillomavirus Genotypes among African Women with Normal Cervical Cytology and Neoplasia: A Systematic Review and Meta-Analysis. PLoS One. 2015; 10.
17. Su Y. et al. Prevalence and genotype distribution of human papillomavirus infection among women : A population - based study in Dali Bai Autonomous Prefecture , Yunnan Province , China. J Med Virol. 2019; 91, 1553–1561.
18. Niyazmetova L, Aimagambetova G & Stambekova, N. International Journal of Infectious Diseases Application of molecular genotyping to determine prevalence of HPV strains in Pap smears of Kazakhstan women. Int. J. Infect. Dis. 2017; 54, 85–88.
19. Kovacevic G. et al. Biologicals Prevalence of oncogenic Human papillomavirus and genetic diversity in the L1 gene of HPV16 HPV 18 HPV31 and HPV33 found in women from Vojvodina Province Serbia ☆. Biologicals. 2019; 58, 57–63.
20. Zhang C, Huang C, Zheng X & Pan D. Prevalence of human papillomavirus among Wenzhou women diagnosed with cervical intraepithelial neoplasia and cervical cancer. Infect. Agent. Cancer. 2018; 8, 1–8.
21. Martora F et al. Seven years prevalence and distribution of high and low risk HPV genotypes in women living in the metropolitan area of Naples. Cancer Epidemiol. 2019; 63, 101625.
22. Kim Y., Serrano B, Lee J, Lee H & Lee S. Burden of Human papillomavirus ( HPV ) -related disease and potential impact of HPV vaccines in the Republic of Korea. Papillomavirus Res. 2019; 7, 26–42.
23. Santos Filho MVC et al. Prevalence of human papillomavirus (HPV), distribution of HPV types, and risk factors for infection in HPV-positive women. Genet. Mol. Res. 2016; 15.
24. Bray F, Ferlay J & Soerjomataram I. Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA CANCER J CLIN 2018; 68, 394–424.
25. Çilingir IU et al. Microarray detection of human papilloma virus genotypes among Turkish women with abnormal cytology at a colposcopy unit. J Turkish-German Gynecol Assoc. 2013; 14, 23–27.
26. De Vuyst H. et al. The prevalence of human papillomavirus infection in Mombasa, Kenya. Cancer Causes Control 2010; 21, 2309–2313.
27. Oakeshott P, Aghaizu A, Reid F, Beddows S & Soldan K. Frequency and risk factors for prevalent, incident , and persistent genital carcinogenic human papillomavirus infection in sexually active women : community based. BMJ. 2012; 344, 1–10.
28. Amaral CMM et al. MDM2 polymorphism associated with the development of cervical lesions in women infected with Human papillomavirus and using of oral contraceptives. Infect. Agent. Cancer. 2014; 9, 1–8.

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