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Molecular Detection of Human Papillomavirus Type-16 DNA in Cervical Cancer Tissue Biopsies

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Objective: The aim of the study is to detect the frequency of human papillomavirus type-16 among patients with cervical carcinoma.

Setting: Khartoum Hospital, Army Medical Hospital and Soba University Hospital, Sudan.

Design: Descriptive-cross sectional study.

Method: Fifty specimens of treated cervical biopsy sections (Paraffin embedded) were included in the study from April to October 2012. DNA was extracted followed by the detection of E6 gene of human papillomavirus type-16 using non-probed SYBER green real-time PCR.

Result: Thirty (60%) showed positive results as compared with the sigmoid curve of the positive control for HPV type-16; while 20 (40%) were negative. Most of the positive results were among the age group 31-50 years.

Conclusion: Human papillomavirus type-16 was detected in 60% of women with cervical cancer, which seems to have a strong association with cancer development.

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Human papilloma virus (HPV) infection has global distribution and has been identified as the leading etiologic agent for cervical cancer[1]. HPV is sexually transmitted which affects women after beginning the sexual life. In young women, the disease is usually transient, its prevalence decreasing around 30 years[2].

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HPVs comprise more than 120 putative virus types, of which 85 types have been fully sequenced[3].

Mucosal types of HPV are classified into high-risk and low-risk types. High-risk HPV types have been implicated in the development of squamous intraepithelial lesions (SILs) and cervical cancer[4]. About 40 HPV types identified so far can infect the cervix[5]. HPV types 16 and 18 are considered to be the most frequent HPV types worldwide and are responsible for approximately 70% of all cervical cancer cases[6]. Low-risk HPVs has been associated with benign warts of oral and urogenital epithelium[4].

Differences in carcinogenicity of cervix specific HPV types are partially related to the expression of the E6 and E7 oncogenes which among other functions interfere with tumor suppressor proteins p53 and pRb, respectively[7]. HPV testing has recently been introduced into clinical practice to identify women at risk of cervical cancer[7]. HPV testing is recommended in the triage of women with atypical squamous cells of undetermined significance. The use of HPV testing in the follow-up of women after local treatment of cervical intraepithelial neoplasia (CIN) is also strongly supported by clinical evidence[7].

The predictive value of different high-risk types of HPV might be helpful for the clinicians to decide the mode of therapy[8]. HPV type-16 is associated with invasive cervical carcinoma and persistent high-risk HPV gene expression is considered ominous sign of progression from low-grade, to high-grade and even to invasive squamous cell carcinoma. Studies demonstrated that the oncogenic potential of high-risk HPV infections was due to the viral-transforming genes E6 and E7[9].

The aim of this study is to detect the frequency of HPV type-16 among women with cervical cancer.

METHOD

Fifty specimens of treated cervical biopsy sections (Paraffin embedded) were included. Patients who were negative for cervical carcinoma or used any anti-HPV therapy were excluded.

Data were collected from patients' files. Thick sections were cut from each paraffin embedded tissue block of cervical biopsy and put in sterile container and preserved at room temperature till processed. DNA was extracted using Wizard® SV Genomic DNA Purification system[10].

PCR product was analyzed using plus/minus scoring method which is used to record the presence or absence of PCR product (qualitative analysis). A non-template control (NTC) was used to set confidence thresholds above which all unknowns were scored positive and negative. Samples between the two threshold values were scored as undetermined.

Data was analyzed by SPSS version16.

RESULT

A total of 50 cervical cancer tissue sections were collected. Patients' ages range was 31-90 years with a mean of 53 years, see table 1. Thirty (60%) were positive for HPV type-16 and 20 (40%) were negative, see figure 1. High percentage of HPV type-16 was observed among 31-50 years followed by 51-70 and 71-90 years, see figure 2. Figure 3 showed the amplification graphs of samples and controls, while figure 4 showed the product analysis by plus-minus scoring method.

Table 1: Frequency of HPV Type-16 and Age Groups

Age Group	Number (Percentage)
31-50	24 (48)
51-70	21 (42)
71-90	5 (10)
Total	**50 (100)**

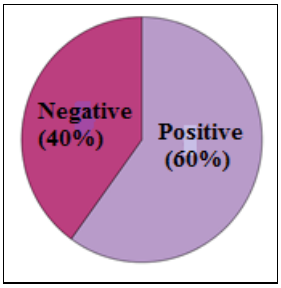

Figure 1: Percentage of HPV Type-16 Using Rt-PCR (SYBER Green Method)

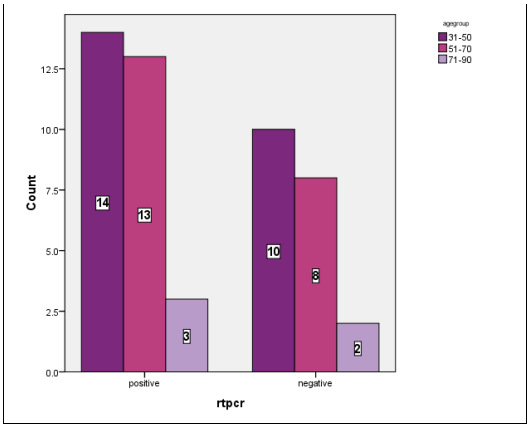

Figure 2: Frequency of HPV Type-16 and Age Using Rt- PCR (SYBER Green Method)

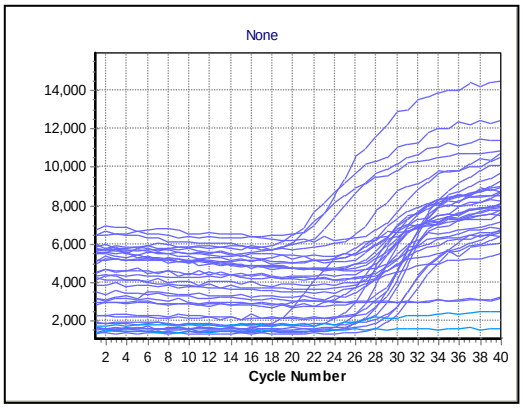

Figure 3 (Left)

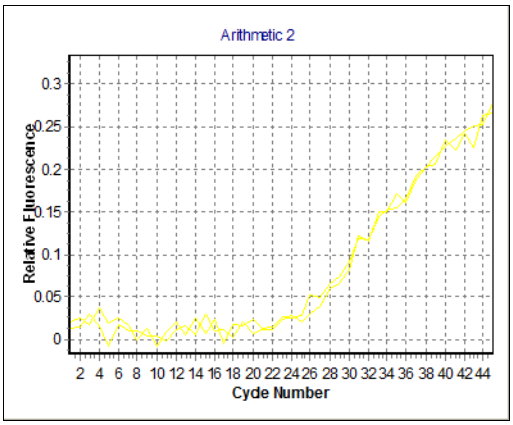

Figure 3 (Right)

Figure 3 (Right): A Real Sigmoid Curve of Non-Template Control and Specimens (Including Positive and Negative Results). (Left): A Real Sigmoid Curve of Positive Control

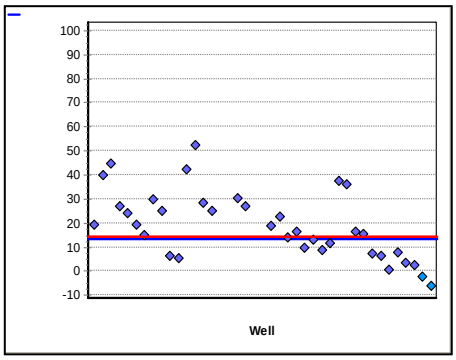

Figure 4 (Left)

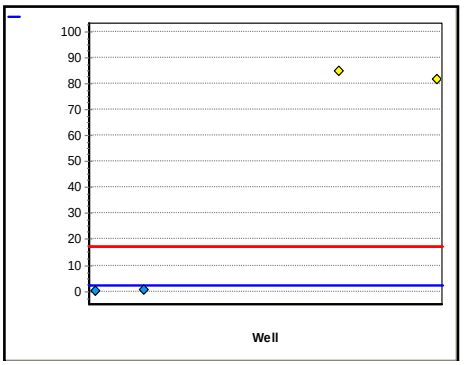

Figure 4 (Right)

Figure 4: Plus/Minus Score Graphs for Detection of HPV Type-16. (Right): Reaction of Positive Control and Non-Template Control. (Left): Reactions of Positive and Negative Samples for HPV Type-16

DISCUSSION

Human papillomavirus has been considered as the most significant risk factor for cervical cancers. HPV is recognized as a public health hazard for its role as a factor in the pathogenesis of cervical cancer[11].

In this study the result showed that the frequency of HPV type-16 among women with cervical cancer was 60%. A similar study showed that the frequency of infection with HR-HPV subtypes 16 and 18 was high among Sudanese women with cervical lesions but correlation between cervical and oral HPV infection was not found[12].

However, de Sanjose et al found that the HPV prevalence in women was about 70.1% in those who had cervical cancer.

Another study revealed that 99.7% of cervical cancer patients had history of persistent infection of high risk-HPV. The high risk types are most frequently seen in cervical cancer which includes HPV type-16 which accounts for over 70% of squamous cell carcinoma of the uterine cervix[13].

In this study, the mean age was 53 years; high percentage of HPV type-16 was observed among ages 31-50. A study in Saudi Arabia showed that the mean age of women with cervical carcinoma was 37 years; this difference may be due to a different sexual behavior such as early sexual practice or other factors[14].

CONCLUSION

HPV Type-16 was detected in 60% of women with cervical cancer, which seems to have a strong association with cancer development.

The study emphasizes the need for early detection and screening of cervical cancer specimens for HPV-16 for successful treatment and control.

Further research applying probed real-time PCR method is warranted.

Author contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

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Competing interest: None. **Sponsorship:** None.

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