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ABSTRACT

Background: Human papilloma viruses (HPV s) have been considered to be the necessary and central agents of cervical cancer among women with nearly 80% of cases found in developing countries.

Objective: To determine HPV genotypes prevalent in Ghanaian women with cervical cancer.

Design: Retrospective study.

Setting: Pathology Department, University of Ghana Medical School, College of Health Sciences, University of Ghana.

Subjects: Ghanaian women 23 to 80 years diagnosed with cervical cancer.

Results: Of the 50 samples genotyped, 49 (98%) were determined as positive for HPV DNA. Eight out of 18 different HPV genotypes were detected and all were high-risk types. In decreasing order of prevalence, were HPV 18 in 42 (84%) samples, HPV 16 in 12 (24%) samples, HPV 45 in three (6%) samples, HPV 39 in two (4%) samples and HPV 35, 52, 56 and 66 in one (2%) sample each. Multiple infections were detected in 13 (26%) of the 50 samples, the most common co-infections being HPV types 16/18 (18%). Triple infections were found in two (4%) samples with HPV types 16, 18 and 39. HPV type 18 was more relatively frequent in adenocarcinomas and adenosquamous carcinomas than in squamous cell carcinomas.

Conclusion: Eight high-risk HPV genotypes: 16, 18, 35, 39, 45, 52, 56 and 66 were typed, with HPV type 18 being the most prevalent in Ghanaian women with cervical cancer. This is contrary to the worldwide prevalence where HPV 16 is the most common type associated with invasive cervical carcinoma.

INTRODUCTION

Cervical cancer is the second most common malignant disease among women worldwide with nearly 80% of cases occurring in less developed countries (1). About 500,000 new cases are diagnosed annually (2). Cervical cancer accounts for the death of 231,000 women annually with over 80% of these deaths occurring in developing countries. In Ghana, a study done over a 52 month period in Korle-Bu Teaching Hospital (KBTH) showed cervical cancer to account for 58.3% of gynaecological cancers (3). Studies in Ivory Coast and Gambia have revealed cervical cancer incidences of 24 and 34% respectively (4,5). Number of sexual partners, smoking, age of first sexual contact and immunological status have been

identified as significant risk factors for development of cervical cancer (6,7).

Human papillomaviruses have been identified as the central etiologic agents of cervical cancer (8). It is also clear from several case control and cohort studies that HPV infection is the main factor for the development of cervical intraepithelial neoplasia (CIN) and that risk is significantly increased by persistent infection with high risk HPV genotypes such as types 16 and 18 (9-12). More than 200 types of HPV have been identified using DNA sequence data, out of which 40 different types have been detected in the anogenital area (13). Based on their oncogenic potential, genital HPVs have been divided into high and low risk. High-risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66 and 68 are associated

with high grade CIN and invasive cervical cancer (13). Other genotypes such as HPVs 6, 11, 34, 40, 42, 43 and 44 are classified as low risk types, detected in benign lesions such as condyloma accuminata (14).

Detection of high-risk HPV infections helps to identify women who are at increased risk of development or the progression of a cervical lesion (15, 16). Several HPV targeted therapies and vaccines have been developed, however, it would be better to know the HPV genotypes that are prevalent in each geographical region of the world so that type specific or globally effective vaccines can be produced. A previous study in Ghana showed a 10.7% prevalence of HPV in Ghanaian women attending gynaecological clinics (17). In this present study we determined the prevailing genotypes of HPV present in Ghanaian women with cervical cancer.

MATERIALS AND METHODS

Tissue Materials and Histological Analysis: Formalin-fixed paraffin embedded blocks of 50 patients with histologically proven cervical cancer obtained from archival specimens of the Pathology Department, Korle-Bu Teaching Hospital, Accra, between January and December 2003 were used in this study. Ethical approval to use the samples was granted according to ethical guidelines established in the University of Ghana Medical School. The first and last sections (5 μ m) of each tissue were stained with haematoxylin and eosin. These were used as microscopic controls to ensure that the tissue sections used in the PCR contained malignant tissue. To avoid carried-over contamination from one block to another during sectioning, a new disposable microtome knife was used to cut sections from each block.

Preparation of Genomic DNA: Genomic DNA was prepared using the method described by Dabic *et al.* (18) with slight modifications. Briefly, 10 μ m section was cut from each paraffin block into a 2.0ml micro-centrifuge tube. These sections were incubated in 250 μ l lysis buffer, [1mg/ml Proteinase K (Sigma, USA), in 50mM Tris-HCL pH 8, 1mM EDTA, and 0.5% Tween-20 (Sigma, USA)] for 16 hours at 56°C. Proteinase K was inactivated at 100°C for five minutes and the lysate centrifuged at 13000 rpm for five minutes. 0.2-2 μ l of the supernatant was then used for PCR amplification and the rest of the DNA lysate stored at -20°C until further use.

HPV Nested Multiplex PCR: Primer sets (Eurogenetics, Belgium) and the method described by Sotlar *et al.* (19) was used. The HPV genome was amplified using consensus forward primer (GP-E6-3F) and two consensus back primers (GP-E7-5B and GP-E7-6B). The PCR reaction mix of 50 μ l contained 10x

PCR, buffer (Promega, USA), 2.5mM MgC₂, 200 μ M of each of the four oligonucleotide triphosphates (dNTPs) (Promega, USA), 15pmols of each E6/E7 consensus primer and 1.25 units of Taq Polymerase enzyme (Promega, USA). 0.2-2 μ l of DNA lysate was used as template for the amplification reaction and sterile double distilled water used to make up the volume to 50 μ l. The DNA amplification was carried out using a Techne thermal cycler (Progene, Princeton, NJ, USA). The cycling parameters for the first round PCR with the E6/E7 consensus primers were as follows: 94°C for four minutes followed by 40 cycles of 94°C for one minute, 40°C for two minutes, 72°C for two minutes and a single final elongation step of 72°C for ten minutes. For each reaction positive controls (HPV 16 and 18 plasmid DNA) and a negative control (double distilled water) were included. In the second round nested PCR. 1 μ l of first round PCR product, 15pmols of forward and reverse primers for genotyping were used. The other parameters above remained the same. The genotyping cycling parameters were as follows: 94°C for four minutes followed by 35 cycles of 94°C for 30seconds. 56°C for 30 seconds, 72°C for 45 seconds and a single final elongation step of 72°C for four minutes.

Primers for the identification of high-risk genotypes: 16,18,31,33,35,39,45, 51, 52, 56, 58, 59, 66 and 68 and low-risk genotypes 6/11, 42, 43 and 44 were used. The primers were used in four cocktails each containing four to five different primer pairs. The PCR products were run on a 2% agarose gel and stained with 0.5 μ g/ml ethidium bromide to detect the presence of amplified DNA fragments with a hundred base pair DNA molecular weight marker was run along side.

RESULTS

The mean age of HPV positive patients was 54.1 years (S.D = 7.6years) ranging from 23 to 80 years. The oldest case was a squamous cell carcinoma and the youngest, an adenocarcinoma (Table 1). All samples were positive for HPV DNA. Forty nine (98%) of the 50 samples could not be typed based on the primers used. In one case a squamous cell carcinoma, we could determine the genotype. Table 2 shows all the HPV genotypes identified, multiple infections were counted more than once. A total of eight different HPV genotypes of the 18 possible genotypes were detected and all were high risk types. One HPV positive sample did not genotype with any of the primers used. HPV 18 was the most prevalent as this was found in 42 (84%) of the 50 samples. The prevalence of other genotypes was as follows: HPV 16 in 12 (24%) samples. HPV 45 in three (6%) samples. HPV 39 in two (4%) samples. and HPV 35,52,56 and 66 in one (2%) sample each.

Table 1
Age Distribution of Types of Cancers in Genotyped Samples

Age	SCC	ADC/ASC	Total
20-29	0	1	1
30-39	4	1	5
40-49	10	3	13
50-59	12	3	13
60-69	6	1	7
70-79	6	1	7
80-89	1	0	1
Total	39	10	49

Key:

SCC: Squamous cell carcinoma

ADC: Adenocarcinomas

ASC: Adenosquamous carcinomas

DISCUSSION

The diagnosis of HPV infection in recent times has been based on the detection of viral DNA using PCR assays (10,15). In this work a nested multiplex PCR developed by Sotlar *et al.* (19) was used in the detection and genotyping of HPV types in paraffin embedded cervical carcinomas in Ghanaian women.

Many epidemiological studies indicate that human cancer develops through different molecular biologic pathways. Currently, there is compelling evidence that the development of human cervical cancer without the involvement of specific HPV is exceptional or impossible (15). HPV DNA has been found to be in 46.9 to 100% of archival cervical carcinoma specimens using PCR based methods (16). In this study, HPV DNA prevalence was 98%, which was comparable with other studies (15,16,18). These studies detected HPV in between 65 to 87.6% of patients with invasive cervical cancer in formalin

Table 2
HPV Genotypes in Relation to Histologic Types

Hpv Genotypes	Histologic Type			Relative Frequency
	Squamous cell carcinomas	Adenocarcinomas	Adenosquamous carcinomas	
HPV-16	11	1	0	18.8%
HPV-18	32	7	3	65.6%
HPV-35	1	0	0	1.6%
HPV-39	2	0	0	3.1%
HPV-45	3	0	0	4.7%
HPV-52	1	0	0	1.6%
HPV-56	1	0	0	1.6%
HPV-66	1	0	0	1.6%
HPV-X ¹	1	0	0	1.6%

¹Untypable sample based on the 18 primers used for genotyping though positive for HPV DNA

Thirteen (26%) of the 50 specimens had multiple infections of which 11 were double infections with HPV types 16 and 18 in nine and one each with 16 and 35 and 18 and 45. Two samples had triple infections with HPV 16, 18 and 39. Thirty-nine of the HPV positive samples were squamous carcinomas, eight were adenocarcinomas and three were adenosquamous carcinomas. HPV 18 was present relatively more frequently in tumours with glandular component (10 out of 11) than in pure squamous cell carcinomas whereas HPV 16 was detected relatively more frequently in squamous cell carcinomas (11 of 39) than adenocarcinomas (1 of 11).

fixed and paraffin-embedded tumour tissues using PCR analysis. To our knowledge, this study is the first genotyping of cervical carcinomas using paraffin embedded specimens in Ghana.

A total of 85 studies using PCR to estimate HPV prevalence in invasive cervical cancer in different parts of the world revealed that the common HPV types, in order of decreasing prevalence were 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 80 (2). In these studies, for squamous carcinomas (SCC), HPV 16 was the predominant type followed by HPV 18, 45, 31 and 33 in all regions except Asia, where HPV types 58 and 52 were more frequently identified.

Also for adenocarcinoma (ADC) and adenosquamous carcinoma (ASC), HPV prevalence was significantly lower than in SCC and HPV 18 was the predominant type in every region, followed by 16 and 45 (10). The international biological study on cervical cancer also reported HPV 16 as the most prevalent type in 22 countries with the exception of Indonesia, where HPV 18 was the most prevalent type (10). However, in this work the most prevalent HPV type was HPV 18, followed by 16, 45 and 39 respectively.

Data collected from several studies had shown that HPV 31 and 33 were frequently found in cervical carcinomas (10) but these genotypes were absent in the 50 samples we used. This probably supports suggestion that there are different types of HPV causing cancers in different parts of the world. In these samples we genotyped, no low risk HPV types were detected in any of HPV positive samples obtained supporting the fact that only high risk HPVs are integrated into the host cell genome (20). This present study strengthens the argument of causal association between adenocarcinoma/ adenosquamous carcinomas and HPV 18 (8). Three cases of HPV 45 were detected which agreed with some clusters of HPV 45 previously found in West Africa (10).

Multiple infections were present in about 12.8% of the cervical cancer samples used in this work. Recent studies also indicated that the presence of multiple HPV infections might contribute to the development or progression of cervical dysplasia (21). The complex interaction of concomitant HPV infections in cervical carcinogenesis remains to be investigated in the future.

In conclusion, this study provides data about the HPV genotypes associated with cervical cancer in Ghanaian women. Eight high-risk HPV genotypes: 16, 18, 35, 39, 45, 52, 56 and 66 were detected and this supported the fact that only high risk HPV's are integrated into the host cell chromosome in cervical carcinogenesis. HPV type 18 was the most common HPV genotype associated with cervical cancer in Ghanaian women for the samples genotyped.

We believe that this paper provides baseline information on current prevalence of high risk HPV types in cervical cancer in Ghanaian women and this will serve as the basis for comparison of all future studies on the subject.

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REFERENCES

1. Parkin, D.M., Pisani, P. and Ferlay, J. Estimates of worldwide incidence of 25 major cancers in 1990. *Int. J. Cancer.* 1999; **80**: 827-841.
2. Waggoner, S.E. Cervical Cancer. *Lancet.* 2003; **361**: 2217-2225.
3. Nkyerkyer, K. Pattern of Gynecological cancers in Ghana. *East Afr. Med. J.* 2000; **77**: 12-16.
4. Echimane, A.K, Ahnoux, A.A., Adoubi, I., *et al.* Cancer incidence in Abidjan, Ivory Coast: first results from the cancer registry, 1995- 1997. *Int. J. Cancer.* 2000; **89**: 653-663.
5. Bah, E., Parkin, D.M., Hall, A.I., *et al.* Cancer in Gambia: 1988-97. *Br. J. Cancer.* 2001; **84**:1207-1214.
6. Burger, M.P., Hollema, H., Gouw, A. S., *et al.* Cigarette smoking and HPV in patients with reported cervical cytological abnormality. *Br. Med. J.* 1993; **306**:749-752.
7. Gopalkrishna, V., Murthy, N.S., Sharma, J.K., *et al.* Increased HPV infection with increasing number of pregnancies in Indian women. *J. Infect. Dis.* 1995; **171**: 254-255.
8. Walboomers, J.M.M., Jacobs, M.V., Manos, M.M., *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999; **189**: 12-19.
9. Koutsky, L.A., Holmes, K.K., Critchlow, C.W., *et al.* A cohort study of the risk of intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N. Engl. J. Med.* 1992; **327**: 1272-1278.
10. Bosch, F.X., Munoz, N., Sherman, M., *et al.* Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. International biological study on cervical cancer (IBSCC) study group. *J. Natl. Cancer Inst.* 1995; **87**: 796-802.
11. Liaw, K.L., Hsing, A.W., Chen, C. J., *et al.* Human papillomavirus and cervical neoplasia: A case-control study in Taiwan. *Int. J. Cancer.* 1995; **62**: 565-571.
12. Schiffman, M.H. New Epidenliology of Human Papilloma virus infection and cervical neoplasia. *J. Natl. Inst.* 1995; **87**:1345-1347.
13. zur Hausen, H. Papillomavirus in human cancers. *Proc. Assoc. Am. Physicians.* 1999; **111**: 581-587.
14. Lorincz, A.T., Reid, R, Jenson, A.B., *et al.* Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet. Gynecol.* 1992; **79**: 328- 337.
15. Cox, J.T., Lorincz, A.T., Schiffman, M.H., *et al.* Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous, cells of undetermined significance. *Am. J. Obstet. Gynecol.* 1995; **172**: 946-954.

16. Cuzick, J., Szarenwski, A., Terry, G., *et al.* Human papillomavirus testing in primary cervical screening. *Lancet.* 1995; **345**: 1533- 1536.
17. Domfeh, A.B. The prevalence of cervico-vaginal papilloma virus in women visiting the gynaecological department of the Korle-Bu Teaching Hospital. MPhil. Thesis, University of Ghana Medical School, Pathology Department; 2003.
18. Dabic, M.M., Hlupic, L., Babic, D., *et al.* Comparison of polymerase chain reaction and catalyzed signal amplification *in situ* hybridation methods for human papillomavirus detection in paraffin-embedded cervical pre-neoplastic and neoplastic lesions. *Arch. Med. Res.* 2004; **35**: 511-516.
19. Sotlar, K., Diemer, D., Dethleffs, A., *et al.* Detection and typing of human papillomavirus infection by E6 Nested Multiplex PCR. *J. Clin. Microbiol.* 2004; **42**: 3176-3184.
20. Jacobs, M.V., de Roda Husman, A.M., van den Brule, A.J., *et al.* Group-specific differentiation between high and low risk human papilloma virus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. *J. Clin. Microbiol.* 1995; **33**:901-905.
21. Fife, K.H., Cramer, H.M., Schroeder, H.M. *et al.* Detection of multiple human papilloma-virus types in the lower genital tract correlates with cervical dysplasia. *J. Med. Virol.* 2001; **64**: 550-559.