Egypt. Acad. J. Biolog. Sci., 5(1): 101 – 106 (2013) C. Physiology & Molecular Biology
Email: egyptianacademic@yahoo.com ISSN: 2090-0767
Received: 25 / 1 / 2013 www.eajbs.eg.net

Screening for Cervical Cancer and Its Association with Human Papilloma Virus (HPV) among Sudanese Women

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ABSTRACT

This study investigates the presence of human papilloma virus (HPV) on cervical smear among women in Khartoum State. Four hundred specimens were taken from patients who attended different hospitals in Khartoum State during a period from July 2008 to July 2009. The specimens were processed and screened using cytological technique (Papanicolaou stain) and PCR for HPV detection.

The pre-cancer cells were detected in 30/400 (7.5%) specimens among which cervical glandular intraepithelial neoplasia (CGIN) was found in one cases 1/30 (0.3%), the details were as follows: mild dyskaryosis was present in 18/30 (4.5%), moderate dyskaryosis in 5/30 (1.3%), and severe dyskaryosis 6/30 (1.5%). On the other hand, screening for HPV among the enrolled subjects revealed high ratio (36.0%). HPV was detected in all cases that had cytological changes except one (29 out of 30 cases (96.7%)). Infections other than HPV were observed during cytological assessment which include *T. vaginalis* 8 (2.0%), *Candida* spp. 11(2.8%), and *Actinomyces* spp. 5 (1.3%).

The study concluded that the prevalence of HPV infection is high in Sudanese women (36%) who were revealed as 96.6% in pre-cancer cases and 83.3% in cervical cancer patients.

Keywords: cervical cancer , human papilloma virus (HPV), Sudanese women

INTRODUCTION

Cancer of uterine cervix is the second leading cause of cancer death in women world wide with more than 270000 deaths reported every year. Over 80% of these deaths occur in developing countries (Muchiri *et al*., 2006 and Kent, 2010).

Human Papilloma virus infection plays an etiological role in the development of cervical cancer, and most of cervical cancers contain HPV DNA (McMurray *et al*., 2001).

HPV is viewed as sexually transmitted infection (STI) as Snijders *et al*., (2006) and De Sanjose *et al*., (2007) reported more than 50% of sexually active women have been infected with genital HPV at some time in their life. Infection prevalence of up to 82% has been reported in adolescent and young adult women in Southern Africa (Marais *et al*., 2000).

HPV is a member of the papilloma virus family of viruses that are capable of infecting humans. Like all papilloma viruses, HPV establishes productive infections only in the stratified epithelium of the skin or mucous membranes. While the majority of the nearly 200 known types of HPV causes no symptoms in most people, some types can cause warts (verrucae), while others can–in a minority of cases–lead to cancers of the cervix, vulva, vagina, and anus in women or cancers of the anus and penis in men (Muñoz *et al*., 2003). Of these, 15 are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 as probable high-risk (26, 53, and 66), and 12 as low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) (Muñoz *et al*., 2003), but even those may cause cancer. Types 16 and 18 are generally acknowledged to cause about 70% of cervical cancer cases. Together with type 31, they are the prime risk factors for cervical cancer (Walboomers *et al*., 1999).

More than 30 to 40 types of HPV are typically transmitted through sexual contact and infect the anogenital region. Some sexually transmitted HPV types may cause genital warts. Persistent infection with "high-risk" HPV types–different from the ones that cause warts–may progress to precancerous lesions and invasive cancer (Schiffman and Wacholder, 2009).

It is quite obvious that there is a need to study the prevalence of different HPV types in different geographic areas and, particularly, in less studied regions such as Africa. In Sudan, the number of cervical cancer new cases is in dramatic increase according to Radiation and Isotope Center Khartoum records (2009). Screening for cervical cancer remains an important health and economic concern in Sudan to decline the incidence of cervical cancer and its associated mortality.

MATERIALS AND METHODS

Four hundreds patients attended different hospitals and gynecologic clinics (out patients) with different ages and gynecological symptoms in Khartoum State (Khartoum hospital, Khartoum North hospital (Bahri), and Elneelin Clinic Center) during the period from July 2008 to July 2009 were included in this study. Written consent was obtained from every patient before they were enrolled in the study, during the interview to obtain essential identification data, cervical smears were collected by using modified Salyze cyto spatula, then they were smeared immediately on clean slides and fixed while they were still moist in 95% ethyl alcohol for 15 minutes (Papanicolaou procedure) (Bancroft and Gamble, 2002) and the remaining cells on the spatula were immersed in a plastic swab tubes containing 5 mL of Tris HCl buffer (PH 8.0). Pellets from these samples were obtained by centrifugation and then re-suspended in 3 mL Tris HCI buffer PH 8.0 and stored in cryo tube at -20°C until used (Jacobs *et al*., 1995).

Biopsies for histopathological examination were taken only from patients suggested for cervical cancer and were stained with Papanicolaou.

Isolated DNA from 10 mL of crude cell suspensions of cervical scrapes was subjected to PCR as follows: 5 x 1.25 µL master mix (Jena Bioscience) was used for the preparation of 25 ul PCR mix containing 0.25 µL of each GP5 (sequence 5" to 3": TTT GTT ACT GTG GTA GAT ACT AC), and GP6 (sequence 5" to 3": GAA AAA TAA ACT GTA AAT CAT ATT C) primers, 5.0 µL master mix, 9.5 µL nuclease free water, and 10 µL DNA template. A 4 min denaturation step at 94°C was followed by 40 cycles of amplification with a PCR processor (PE9600; Perkin-Elmer). Each cycle included a denaturation step at 94°C for 1 min, a primer annealing step at 65°C for 1.5

min, and elongation step at 72°C for 1.5 min. The final elongation step was prolonged by 4 min to ensure a complete extension of the amplified DNA. The PCR product was visualized by UV transilluminator on 1% agarose gel according to the procedure of Sambrook *et al*., (2001).

RESULTS

The ages of the study subjects ranged from 16 to 83 years, the mean age was (35.69 ± 9.0 years), most of the study group ages ranged from 20 to 39 years (Table 1). The cytological assessment of cervical smear revealed that, abnormal cells (pre-cancer cells) were detected in 30 cases (7.5%), mild dyskaryosis was present in 18 cases (4.5%), moderate dyskaryosis in 5 cases (1.3%), and severe dyskaryosis in 6 patients (1.5%), cervical glandular intraepithelial neoplasia (CGIN) was found in 1 patient (0.3%), HPV infection was found in 6 patients (1.5%), who had mild dyskaryosis, while infections other than HPV were observed during cytological assessment which include: *Trichomonas vaginalis* infection 8/30 (2.5%), *Candida* spp. 11/30 (2.8%), and *Actinomyces* spp. 5/30 (1.3%).

Table 1: Description of cytological results according age group among enrolled subjects.

Age group	**Cytological results**												**Total**	
	Negative		Mild dyskaryosis		Mild dyskaryosis + HPV infection		Moderate dyskaryosis		Severe dykaryosis		CGIN			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15-19	3	0.8%	1	0.3%	0	0%	1	0.3%	0	0%	0	0%	5	1.3%
20-24	16	4.0%	3	0.8%	0	0%	0	0%	0	0%	0	0%	19	4.8%
25-29	87	21.8%	1	0.3%	0	0%	0	0%.	0	0%	0	0 %	88	22.0%
30-39	155	38.8%	5	1.3%	4	1.0%	2	0.5%	1	0.3%	1	0.3%	168	42.0%
40-49	93	23.3%	2	0.5%	1	0.3%		0.5%	0	0%	0	0%	98	24.5%
50-59	11	2.8%	0	0%	1	0.3%	0	0%	2	0.5%	0	0%	14	3.5%
More than 59	5	1.3%	0	0%	0	0%	0	0%	3	0.8%	0	0%	8	2.0%
Total	**370**	**92.5%**	**12**	**3.0%**	**6**	**1.5%**	**5**	**1.3%**	**6**	**1.5%**	**1**	**0.3%**	**400**	**100.0%**

P value 0.05
N = Number of patients
CGIN = cervical glandular interaepithelial neoplasia

All isolated DNA were subjected to PCR, HPV was found in 144 samples (36.0%) that showed a product typical in size (150 bp) to the target band as indicated by the standard DNA marker 5 (1.3%) (Fig.1). HPV was detected in high ratio among patients with cervical intraepithelial neoplasia 29/30 (96.7%) (Table 2).

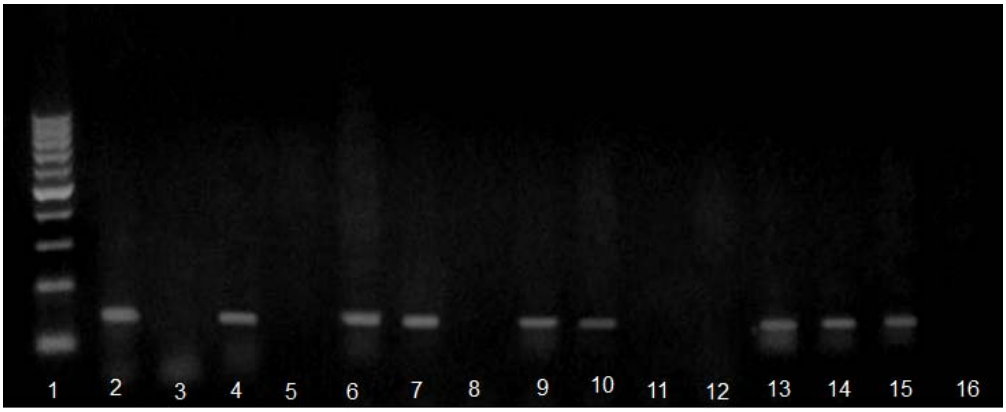

Fig. 1: The amplicon of HPV after PCR run on 1% agarose gel: Lane 1 = 100 bp marker; 2 = posative control (150 bp); 4, 6, 7, 9, 10, 13, 14, and 15 are positive for HVP; 16 = negative control; 3, 5, 8, 11, and 12 are negative samples for HVP.

Table 2: Correlation between HPV and cytological findings among enrolled subjects.

HPV result	**Cytological result**												**Total**	
	Negative		Mild Dyskaryosis		Mild Dyskaryosis + HPV infection		Moderate Dyskaryosis		Severe Dykaryosis		CGIN			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Positive	115	28.8%	12	3.0%	6	1.5%	5	1.3%	5	1.3%	1	0.3%	144	36.0%
Negative	225	63.8%	0	0%	0	0%	0	0%	1	0.3%	0	0%	256	64.0%
Total	**370**	**92.5%**	**12**	**3.0%**	**6**	**1.5%**	**5**	**1.3%**	**6**	**1.5%**	**1**	**0.3%**	**400**	**100.0%**

P value 0. 000
N = Number of patients
CGIN = cervical glandular interaepithelial neoplasia

DISCUSSION

The present study showed that the incidence of cervical intraepithelial neoplasia (CIN) is low by cytological investigations, which were (7.5%) cases out of the study group. This result agrees with Hassan and Khirelseed (2009) who have conducted a community-based survey in 256 Sudanese women living in Khartoum, during the period from 2003 to 2008. Indicators of cervical cancer screening participation were examined: at least one previous Pap smear and Pap testing in the last 1 year, their findings confirm low levels of cervical cancer screening among Sudanese women using pap stain.

Also, the present study showed that cervical malignancy was (1.5%), HPV infection (1.5%), and *Trichomonas vaginalis* infection in 2.0% cases of study subjects. These results agree with Misra *et al*., (2006) who studied the results of long term hospital-based cytological screening in asymptomatic women, they found that the incidence of squamous intraepithelial lesion (SIL) was 5.9% of cases studied, while cervical malignancy was seen in 0.6% of cases, HPV infection was seen in (0.4%) cases, and *T. vaginalis* in (2.6%) of the cases.

Revzina and Diclemente (2005) reported that the estimates of HPV prevalence vary from 14% to more than 90%.

The present study showed that the prevalence of HPV is 144 (36.0%) out of 400 subjects with age ranging from 16-83years, this prevalence is relatively high, Also the present study showed that the prevalence of HPV is 34.8% (139 out of 400 cases) among patients at age ranging 15-59 years. These results are not far away from the results reported by Aggarwal *et al*., (2006) which was (36.8%), they studied the prevalence of high risk human papilloma virus infections in women with benign cervical cytology (hospital based study from North India), and Dunne *et al*., (2007) found HPV in (26.8%) in 14-59 age group patients.

The description of HPV results by age in this study showed that, although the highest HPV prevalence was detected in patients aged 30-39 years (which is a large study group number), the incidence of HPV in age 15-19 years is higher 4 (80.0%) out of 5 cases, and also in age more than 50 it was present in 12 (54.5%) out of 22 patients. These results confirm the findings of Stamataki *et al*. (2010) who found that the prevalence of HPV in female aged 16 to 20 years was 57.1% and Dunne *et al*. (2007) who found that the age of 50 more than 80% of American women will have contracted at least one strain of genital HPV, while Zhang *et al*., (2010) noted that age group more than 50 years had a higher risk than other age groups.

The high prevalence in age group 15-19 may partially be attributed to the few cases (5 cases) examined during the present investigation.

Most patients attending to hospitals were at the reproductive age, so the age group from 30-39 years was more frequent in the present study.

Steben and Durate-Franco (2007) noted that the National Health and Nutrition Examination Survey determined that the prevalence of HPV infection in a representative's sample of women was highest in those aged 20-24 years (44.8%); the same result was reported by Dunne *et al*., (2007); who studied the prevalence of cervical cancer in relation to age. They noted that prevalence decreases with age. This may be due to HPV infection being cleared by the immune system or sinking to undetectable levels while still presents in the body. These results agree with the present result (47.4%) as HPV was present in 9 out of 19 patients.

CONCLUSION

The pre-cancer and cancer cells are low while the prevalence of HPV is high and always in association with CIN in young women and women with cervical squamous carcinoma.

REFERENCES

Aggarwal, R., Gupta, S., Nijhawan, R., Suri, V., Kaur, A., Bhasin, V., Arora, S.K. (2006). Prevalence of high-risk human papillomavirus infections in women with benign cervical cytology: a hospital based study from North India. *Indian J. Cancer*. 43(3):110-6.

Annual Statistical Records. (2009). Radiation and Isotope Center Khartoum (RICK).

Bancroft, J.D and Gamble, M. (2002). Theory and practice of histological technique. 5th ed : Churchill living stone –London New York.pp: 361-362.

De Sanjosé, S., Diaz, M., Castellsague, X., Clifford, G., Bruni, L., Muñoz, N., Bosch, F.X. (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis.* 7(7):453-9.

Dunne, E.F., Unger, E.R., Sternberg, M. (2007). "Prevalence of HPV infection among females in the United States". *Journal of the American Medical Association* (JAMA): 297 (8): 813–9.

Hassan, F.M and Khirelseed, M. (2009). Cervical Cancer Screening among Sudanese Women. *Gulf J. Oncolog*. (6):28-34.

Jacobs, M. V.; DE Roda Husman, A. M.; Van den Brule, A. J. C. Snijdersc, P. J. F. Meijer, J. L. M. and J. M. M. Walboomers. (1995). Group-Specific Differentiation between High- and Low-Risk Human Papillomavirus Genotypes by General Primer-Mediated PCR and Two Cocktails of Oligonucleotide Probes. J. Clinical Microbiology., 3(34):901-905.

Kent, A. (2010). HPV Vaccination and Testing. *Rev in Obstetrics and Gynecology*. 3 (1): 33-34.

Marais, D.J.; Vardas, E.; Ramjee. G.; Allan, B.; Kay, P.; Rose, R.C. and Williamson, A. L. (2000). The impact of human immunodeficiency virus type 1 status on human papillomavirus (HPV) prevalence and HPV antibodies in serum and cervical secretions. *J. Infect. Dis*. 182:1239–1242.

McMurray H R, Nguyen D, Westbrook T F. (2001). Biology of human papilloma-viruses. *Int J Exp Pathol*. 82(1): 15-33.

Misra, J.S., Singh, U. (2006). Results of longterm hospital based cytological screening in asymptomatic women. *Diagn Cytopathol*. 34(3):184-7.

Muchiri L, Korir A and Ribeiro M. (2006). Cervical cancer and HIV in sub-Saharan Africa: Potential impact of a HPV vaccine, *J. Med. Ed. Res*.

Muñoz, N., Bosch, F.X., de Sanjosé, S., Herrero, R, Castellsagué, X., Shah, K.V., Snijders, P.J., Meijer, C.J. (2003). Epidemiologic classifi-cation of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med*., 348-354.

Revzina, N.V and Diclemente, R. J. (2005). Prevalence and incidence of human papilloma virus infection in women in the USA: a systematic review. Int. J. of STD and AIDS. 16 (8): 528–37.

Salih M M, El Safi M, Hart K, Tobi K, Adam I (2010). Genotypes of human

papilloma virus in Sudanese women with cervical pathology. *Journal Infectious Agents and Cancer*. 5:26.

Sambrook, J.; Russell, D. and Gola, J. (2001). Molecular cloning, third ed, A laboratory manual., 1: 32-34.

Schiffman, M., and Wacholder. S. (2009). From India to the World A Better Way to Prevent Cervical Cancer The New England J. Medicine., 360:1453-1455.

Snijders, P.J, Steenbergen, R.D, Heideman, D.A., Meijer, C.J. (2006). "HPV-mediated cervical carcinog-enesis: concepts and clinical implications. J. Pathol. 208 (2): 152–64.

Stamataki, P., Papazafiropoulou A, Elefsiniotis, I., Giannakopoulou, M., Brokalaki, H., Apostolopoulou, E, Sarafis, P., Saroglou, G. (2010). Prevalence of HPV infection among Greek women attending a gynecological outpatient clinic. *BMC Infect Dis*. 15; 10:27.

Steben, M., Duarte-Franco, E. (2007). Human papillomavirus infection: epidemiology and pathophysiology. *J. Gynecol Oncol*. 107(2 Suppl 1):S2-5.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer J A, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol*. 189:12–19.

Zhang, P.J., Deng, X.X., Bai, G.R., Jiang, S.F., Lu, C.L,, Zhang, X.J., Tong, H.L., Du, Y.N., Fu, H.Y., Huang, P., Ma, Y., Tian, Y.P. (2010). A new method of screening human papillomavirus genotypes and clinical validation. *Front Biosci*. 2:1015-27.

ARABIC SUMMARY

الكشف عن سرطان عنق الرحم وإرتباطه مع فيروس الورم الحليمي البشري بين نساء سودانيات

منيرة ع منصور[1,5]، مجدي م صالح[2] ، أحمد إ شمو[3] ، أمل ع بخيت[4] ، مجاهد م الحسن [1,5]

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هدفت هذه الدراسة للكشف عن فيروس الورم الحليمي البشري (HPV) على لطاخة عنق الرحم بين النساء في ولاية الخرطوم . أخذت اربعمائة عينة من المرضى الذين حضروا لمستشفيات مختلفة في ولاية الخرطوم ؛ خلال الفترة من يوليو 2008 إلى يوليو 2009م. تم تجهيز العينات و فحصها باستخدام التقنية الخلوية (صبغة بابانيكولا) و PCR للكشف عن الحمض النووي (الدنا) لفيروس الورم الحليمي البشري.

تم الكشف عن الخلايا ما قبل السرطانية في 400/30 (7.5 ٪) من العينات من بينهن وجدت حالة واحدة من الأورام الغدية الظهارية البينية لعنق الرحم 1/ 30 (0.3%) ، و كانت التفاصيل على النحو التالي : كان هناك شذوذ نووي طفيف في 30/18 (4.5٪) ، شذوذ نووي معتدل في 5 /30 (1.3 ٪) ، شذوذ نووي شديد 30/6 (1.5 ٪). وقد لوحظت إصابات غير عدوي فيروس الورم الحليمي البشري و ذلك من خلال التقييم الخلوي شملت تلك الإصابات المشعرة المهبلية 8 (2.5%)، أنواع المبيضات .11 (2.8%) و أنواع الشعية النيابة 5 (1.3%) .

خلصت الدراسة إلى أن معدل انتشار عدوى فيروس الورم الحليمي البشري في ارتفاع عند النساء السودانيات. إذ كان معدل انتشار فيروس الورم الحليمي البشري 36 ٪ ، في حالات ما قبل السرطان (96.6 ٪)، و(83.3 ٪) في المرضى الذين يعانون من سرطان عنق الرحم .