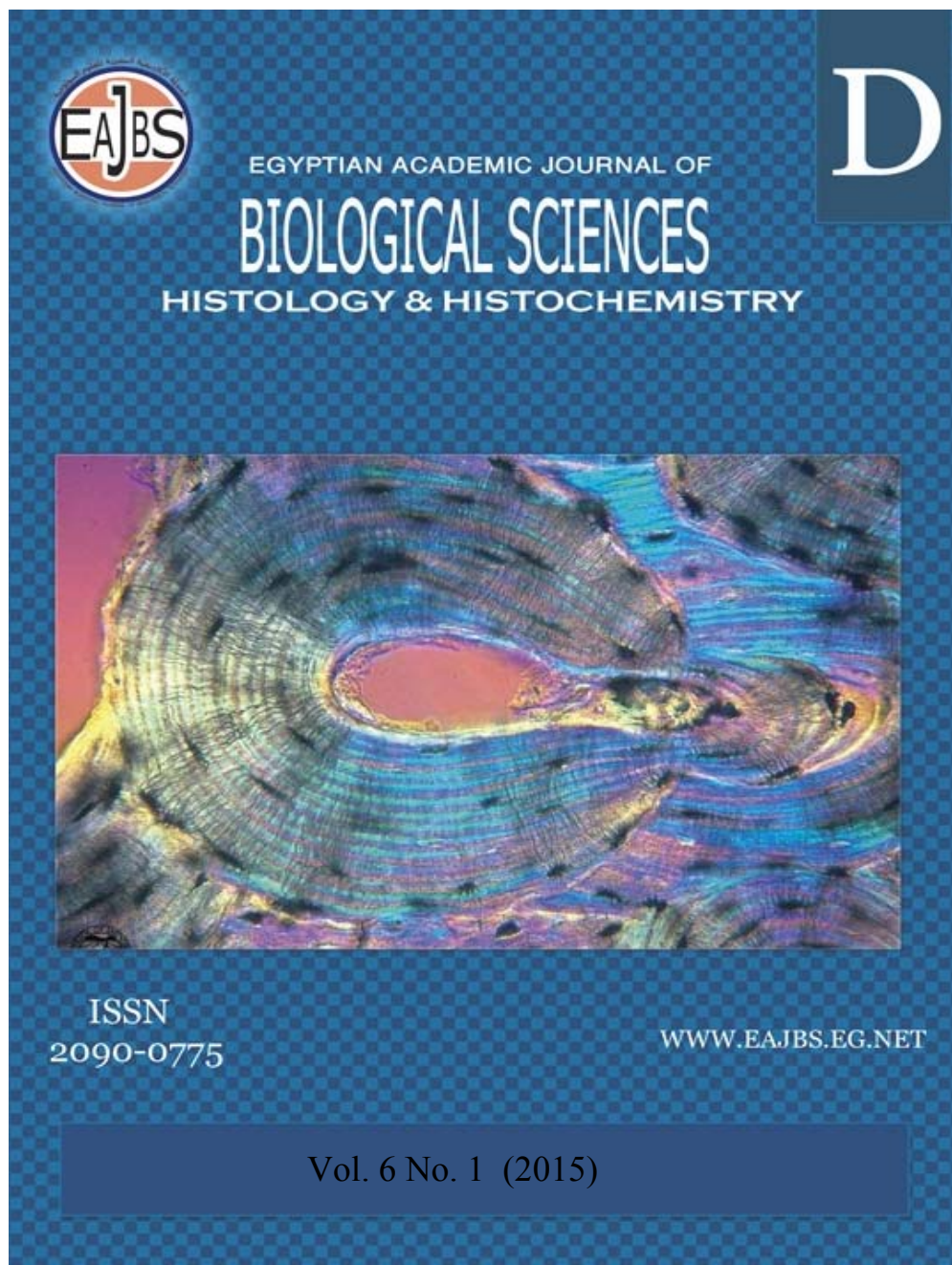


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Cytological pattern of vaginal swab among *multiple sexual partners*' females with genital warts

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ABSTRACT

Background: Genital warts are one of the most common types of sexually transmitted infections, which are mostly caused by Human Papilloma Virus (HPV). Therefore, the objective of this study was to assess the cytological evidence of possible causes of genital warts among multi-sexual partners.

Methodology: One hundred and fifty sexually active multi-partner women complaining from genital warts were randomly selected for this study. Cytological smear was prepared from each participant and subsequently stained using Papanicolaou method.

Results: Cytological evidences of presence of HPV were identified among 4.7% of the study population. According to the state of personal hygiene, 68% of the study population was found to have poor hygiene practice, and the rest 32% had good hygiene practice.

Conclusion: Genital warts associated with HPV infection is relatively very low in the studied group; consequently confirmatory study using more specific detection is needed to verify this relationship.

INTRODUCTION

Female genital tract is exposed to many sexual transmitted diseases that results in genital wart (Richards, 2014). Genital warts caused by Human papillomavirus (HPV) have global distribution. Out of over 100 types, HPV types 6 and 11 are responsible for most genital warts (Leszczyszyn *et al*, 2014). Genital herpes is most commonly due to herpes simplex virus type 2 (HSV – 2; 70 – 80%). Primary infection due to HSV – 2 is associated with systemic signs and symptoms and painful vesicles that ulcerate on the labia, vulva, and cervix. Vesicles heal within 3 weeks and may reappear every 4 – 6 weeks without systemic signs or symptoms (Edwards, 1998).

The cytological presentation of genital herpes very characteristic; by being faintly basophilic and opaque homogenization of the nuclear contents.

This ground-glass appearance is due to invasion of the nucleus by the virus. HPV warts vary in size; from small, flat-topped papules to large, cauliflower-like lesions on the anogenital mucosa and surrounding skin (Karnes and Ustie, 2014). HPV infects the skin via areas of minimal trauma. Skin-to-skin or mucosa-to-mucosa contact is enough to transmit the virus, which presents high tropism for those tissues (Leszczyszyn *et al.* 2014). HPV warts are diagnosed clinically while atypical lesions are confirmed by histology (Loo and Tang, 2009). HPV vaccination may be an effective approach for primary prevention of genital warts (GWs) (Giuliano, *et al.* 2011, Gabriella, *et al.* 2011).

Risk factors are early onset of sexual activity, multiple sexual partners, a history of STDs, an early age of first pregnancy and tobacco use (Loo and Tang, 2009). The Papillomaviridae family includes over 120 viruses, some of which have high malignant transformation rates. The most common malignancy connected to HPV is uterine cervix cancer and anal canal cancer. Therapeutic modalities often seek to eliminate macroscopic changes rather than focus on the cause of the infection, which leads to a high recurrence rate (Leszczyszyn *et al.*, 2014).

Because of the individual variability in disease, treatment should be determined on a case-by-case basis (Lynde *et al.*, 2013). No current treatment completely eradicates the human papillomavirus virus. Wart therapies involve methods of targeted lesion destruction, as well as selective immunologic modification. As therapeutic modalities often seek to eliminate macroscopic changes rather than focus on the cause of the infection, which leads to a high recurrence rate. Individuals, who are essentially more

susceptible to HPV infections and less responsive to treatment (Fathi, *et al.* 2014).

Therefore, the aim of the present study was to demonstrate the cytological changes in the squamous epithelium cells in female genital tract among sexually multi-partner women complaining from genital warts.

MATERIALS AND METHODS

In this cross-sectional descriptive study, 150 vaginal swabs were collected from multiple sexual partners' females presenting with genital warts. Participants were selected from different geographical regions with multiple social and religious backgrounds. Since, multiple sexual partners is prohibited in the areas where the study was performed, the participant required not to publish their identifications.

Sample processing: Smear was made from each swab and immediately fixed using 90% ethyl alcohol for 15 minutes then sent to the laboratory for subsequent staining with Papanicolaou Method.

Papanicolaou Method: The smears were stained using the Papanicolaou staining method. Ethyl alcohol fixed smears were hydrated in descending concentrations of 95% alcohol through 70% alcohol to distilled water, for two minutes in each stage. Then the smears were treated with Harris' hematoxylin for five minutes to stain the nuclei, rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for a few seconds, to remove the excess stain. They were then immediately rinsed in distilled water, to stop the action of discoloration.

Then the smears were blued in alkaline water for a few seconds and dehydrated in ascending alcoholic concentrations from 70%, through two changes of 95% alcohol for two minutes for each change. The smears were next

treated with Eosin Azure 50 for four minutes. For cytoplasmic staining, they were treated with Papanicolaou Orange G6 for two minutes, rinsed in 95% alcohol and then dehydrated in absolute alcohol. The smears were then cleared in Xylene and mounted in DPX (Distrene Polystyrene Xylene) mount. All the reagents used were from Thermo Electron Corporation, UK.

Ethical consent: Although, the specimens were taken as a part of diagnosis requirement, each participant was asked to sign a written ethical consent before obtaining of the sample.

RESULTS

This study was conducted among sexually multi-partner women who were complaining from genital warts. One hundred and fifty sexually multi-partner women their ages ranging from 15 to 55 years with mean age of 35 years have participated in this study. In this descriptive study, the cytological changes were evaluated 150 smears prepared

from 150 cervical scrapings, as indicated in Microphotograph 1, 2,3. As shown in Fig. 1, the majority of the study population were at age group 21-30 which constituting 70 women (46.7%), followed by the age group (31-40), (41-50) which constituting 35 women 23.3%, and 20 women 13.3 respectively.

Fig. 2 shows the description of study population by cytological change the cytological changes koilocytosis was 4.7% among them and 95.3% of them showed no cytological changes. Fig. 3 shows the degree of good hygiene practice in the study population; 68 % of the study population was found to have poor hygiene practice, and the rest 32 % had good hygiene practice. Classification of the study population by cytological changes and ages group the cytological changes are presented in Fig. 4. It was highest in age group 10-21 accounting 10% and lowest in age group 31-40 accounting (2.9%) and absent in age group 51-60.

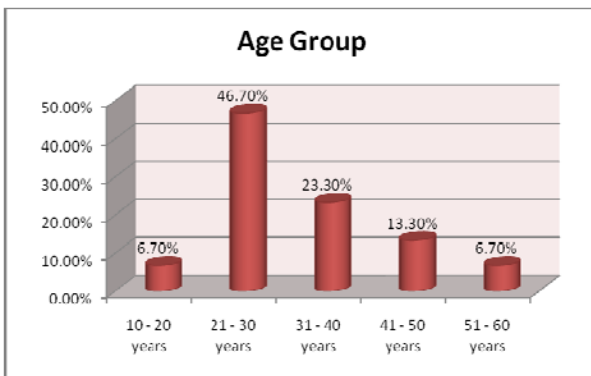


Fig. 1: Study population by age.

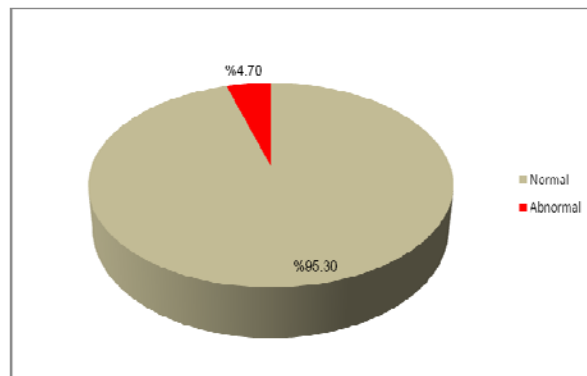


Fig. 2: Cytological changes observed in the study population.

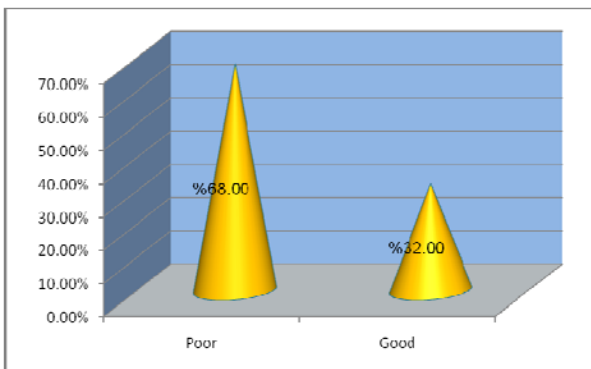


Fig. 3: Practice of hygiene among study population

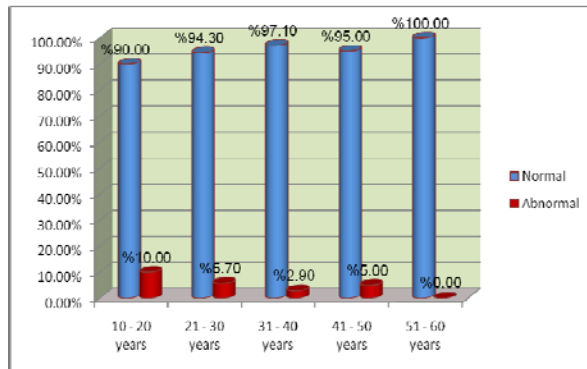
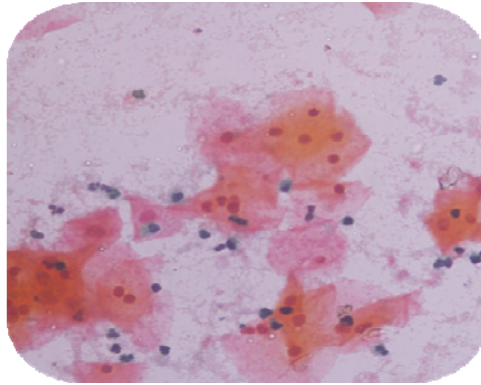
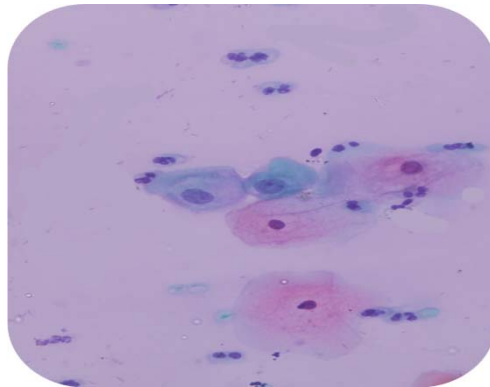


Fig. 4: Cytological changes observed among different age groups of the study population

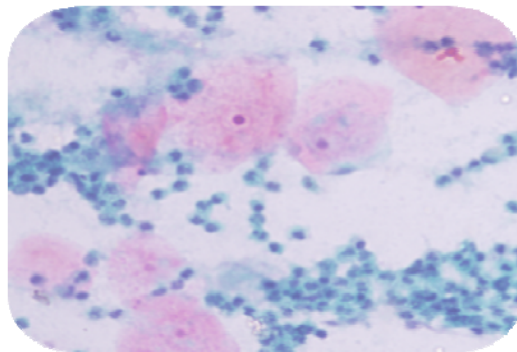
Fig. 5 shows microphotograph (A) Normal epithelial cells in Pap-stained cervical smear ($\times 40$) and microphotographs (B & C) koilocytes cells in Pap-stained cervical smear ($\times 40$).



Microphotograph A: Normal epithelial cells, Pap. smear $\times 40$.



Microphotograph B: koilocytes cells, and inflammatory cells Pap. smear $\times 40$.



Microphotograph C: Koilocytes cells, and intense inflammatory cells infiltrate, Pap. smear $\times 40$.

Fig. 5: Shows (A) Normal epithelial cells in Pap-stained cervical smear ($\times 40$) and (B & C) koilocytes cells in Pap-stained cervical smear ($\times 40$).

DISCUSSION

Genital warts are a common sexually transmitted infection that is frequently associated with Human Papilloma Virus (HPV) infection. Although the warts are usually painless,

but if it is linked to HPV, it may have harmful subsequent effects (Richards, 2014). To date, more than 120 distinct subtypes of human papillomavirus have been identified. Human papillomavirus types 6 and 11 rarely give rise to cervical

cancers, but are responsible for 90 percent of the cases of genital warts (Valerie, *et al.* 2012).

In the present study, cytological assessment was performed from vaginal smears obtained from multi-partner women. Cytological evidences of HPV (presence of koilocytes was only detected among 4.7% of the study population.

This percentage is very low in comparison to proportions report in most part of the world. In a study, genital lesions consistent with exophytic condylomata acuminata were removed by excision biopsy from 65 patients, HPV types associated with an increased risk of dysplasia (high-risk types) were detected in 42 (64.6%) of the total of 65 specimens (Darron, *et al.* 1999).

External genital warts and their associated HPV infections are considered among the most common sexually transmitted diseases affecting the general population. It is estimated that one percent of the sexually active population of the United States, or 3 to 6 million people, acquire symptomatic genital wart infections each year (Oriol, 1971). Approximately 90 percent of genital warts are related to infection with HPV subtypes 6 and 11, which have a very low malignant potential and rarely progress to cancerous lesions. However, those warts associated with HPV subtypes 16 and 18 may be predisposed to oncogenic transformation (Lombard, 1998).

However, although information on the prevalence of genital warts is scarce, it is estimated to be approximately 1% among the sexually active populations in some high-income countries. Data also suggest that the cumulative lifetime risk of genital warts can reach around 10% (Kjaer, *et al.* 2009; Insinga, *et al.* 2009; Koutsky, 1997). In addition, several epidemiological studies show that the prevalence of genital warts seems to be increasing (Koutsky, 1997).

Nevertheless, in the studied population multi partner sexual practice is considered illegal by the law, this in addition to the fact that it is considered as social stigma; consequently all practices are done in hide. Thus, women practicing these sexual behaviors are not exposed to any mean of health checkup.

Since, cytological evidences alone will not be able to detect all positive cases; reasonable cases might be missed in this study. However, Polymerase chain reaction or in situ hybridization is highly specific techniques but they were not affordable in this study.

Furthermore, other causes of genital warts might be contributed, but were not revealed due to the absence of their evidences in cytological smears. Cervical carcinoma and cervical intra-epithelial neoplasia (CIN) are likely to be associated with all sexually transmitted diseases (STDs). To help discover which (if any) of the recognized STDs might actually cause these conditions, a key question is whether one particular such association is much stronger than the others. In a study in which, only women newly attending an STD clinic, and compares the prevalence rates of cytological abnormalities of the cervix among 415 women attending with genital warts, 135 with genital herpes, and 458 with trichomoniasis or gonorrhoea. Significantly more genital wart patients (8.1%) than trichomoniasis or gonorrhoea patients (1.9%) showed dyskaryotic changes (adjusted relative risk (RR) = 5.8 with 95% limits 2.5-13.5) at, or a few months before, first attendance, while no excess whatever was seen in women with genital herpes. Moreover, half the women had a subsequent smear (at an average of 3-4 years after first attendance) and, although the diagnosis at first attendance was not related to the onset rate of dyskaryotic changes observed in these subsequent smears, it was related to the onset rate of grade III cervical intra-epithelial

neoplasia (CIN III), which was found in 7 previous genital wart patients, in 2 previous trichomonas patients, but in 0 previous genital herpes patients. Thus, our findings suggest that herpes is not directly relevant to dyskaryotic change, but that one or more of the human papilloma viruses that cause genital warts may be (Franceschi, *et al.* 1983).

However, this study has several limitations including the use of cytological methods only rather than other more sophisticated techniques such as PCR and ISH. Also, the absence of demographical characteristics as well as investigation of other possible causes of genital tract. But what merits this study is its stimulation of further research in this area in communities among whom such practices are prohibited by law, as well as, considered as social stigma.

In conclusion: Prevalence of HPV in association with genital warts is low in this study. Further studies using more specific detection techniques is important to identify the exact prevalence of HPV that associated with genital warts in such population.

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ARABIC SUMMERY

دراسة الأنماط الخلوية للتأليل المهبلي لدى مجموعة من النساء متعددات العلاقة الجنسية

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كلية العلوم التطبيقية - جامعة حائل - حائل - المملكة العربية السعودية

خلفية الدراسة: تعد التأليل التناسلية واحدة من أكثر أنواع الأمراض المعدية المنتقلة عبر الجنس، والتي تحدث في معظمها نتيجة للإصابة بفيروس الورم الحليمي البشري والمرتبطة في أغلب الأحيان بالعلاقات الجنسية المتعددة. لذلك، فقد هدفت الدراسة إلى تقييم الجوانب الخلوية المحتملة للتسبب في التأليل التناسلية لدى مجموعة من النساء متعددات العلاقة الجنسية.

طرائق البحث: شملت الدراسة عينة عشوائية مكونة من ١٥٠ امرأة متعددة العلاقات الجنسية ممن يشتكين من وجود التأليل التناسلية. تم أخذ مسحة مهبليّة لكل من أفراد الدراسة، وتم عمل الاختبار الصبغى باتباع طريقة بابانيكولاو للكشف عن وجود فيروس الورم الحليمي البشري.

النتائج: أظهرت نتائج الدراسة أن فيروس الورم الحليمي البشري تم تحديده في ٤.٧% من أفراد الدراسة، وأن ما نسبته ٦٨% من أفراد الدراسة لم يكونوا يتبعون الممارسات الصحية وإجراءات النظافة الشخصية، في حين كان ٣٢% من أفراد الدراسة يتبعون تلك الممارسات والإجراءات.

الخلاصة: خلصت الدراسة إلى أن نسبة التأليل التناسلية المرتبطة بالفيروس الحليمي البشري كانت منخفضة جداً، مما يعني أن سبب التأليل قد لا يكون مرتبطاً بهذا الفيروس، مما يتطلب إجراء المزيد من الدراسات واستخدام مؤشرات أكثر دقة لتأكيد تلك العلاقة بين الفيروس والمرضى.