



Assessment of the Healthy Women by Detection and Determination of Cells in Conventional Pap Stained Cervical Smear Images

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ABSTRACT

Introduction: A Pap test can detect pre-cancerous and cancerous cells in the vagina and uterine cervix. Cervical cancer is the easiest gynecologic cancer to be prevented and diagnosed using regular screening tests and follow-up. This study aimed to estimate the cytological changes and the precancerous lesions using Pap smear test and visual inspection of the cervixes of Iraqi women, and also to determine the possible relationship of this cancer with patients' demographic characteristics. **Methods:** The study included 140 women aged (18-67) years old referred to the National Cancer Research Center (NCRC), Baghdad, Iraq, during the period 2011-2016. Both visual inspections of the uterine cervix and Papanicolaou smear screening were performed for all of the participants. **Results:** Only 14% of the women under study were in postmenopausal, and 86% were in premenopausal period. Visual inspection of the cervix showed that 48.6% of the women had erosion lesions. Upon cytology examination, 92.8% of the women showed non-specific inflammation, 70% revealed reactive squamous metaplasia, 27% had Koilocytotic atypia, and 17% suffered from cervical intraepithelial neoplasia (CIN1) or low grade squamous intraepithelial lesion (LGSIL). Contraception was used by 68% of those women, while 34.3% of them used pills. Most women, 79%, had multiple births. The abnormal vaginal discharge occurred in 34% of the participants that is why they attended the center compared with only 25.7% who came for routine checking. Finally, 67% of the participated women did not make this test previously. **Conclusion:** We conclude routine screening and Pap smear testing for uterine cervix and vagina might be useful especially for sexually active women for preventing the occurrence of precancerous and later cancerous lesions in these organs.

Keywords: Pap smear, Precancerous lesion, Cervical cancer, Iraqi women health

INTRODUCTION

Cervical cancer is the third most diagnosed cancer and the fourth leading cause of cancer death in women worldwide. The incidence and mortality rates of cervical carcinoma have been reduced in developed countries owing to using cytology screening programs [1]. Yet, the vast majority of deaths occur in women living in low- and middle-income countries because of poor access to screening and treatment services [2]. In addition to lacking recognition of this cancer until the appearance of symptoms, this includes vaginal bleeding, foul-smelling discharge, pelvic or leg pain, or kidney failure. The possibility that cervical cancer will be healed as soon as symptoms arise is much lower than when the disease is diagnosed earlier [3].

The majority of women who acquire cervical cancer tend to have one or more risk factors that predispose them to the disease. The risk factors involve ethnic factors, multiple sexual partners, younger age at first sexual intercourse, earlier age at first birth, steroid contraception, and infection with, e.g. human papillomavirus (HPV), herpes simplex virus type 2, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, immunosuppression, changing sexual behavior, cigarette smoking and human immunodeficiency virus (HIV)-infected women [4-8].

Importantly, 99.7% of the cervical precancerous changes occur due to the infection with HPV, which has many oncogenic subtypes, including: 16,18,31,33,35,39,45,51,52,56 and 58 [6,7]. In spite of the majority of HPV infections are curable spontaneously and don't lead to precancerous cell changes, chronic infection with specific types of HPV

(particularly types 16 and 18) which may cause abnormalities in the cervical cells [9]. Up to one-third of these lesions could proceed to cervical cancer if they are left untreated [10,11].

The high mortality of cervical cancer in developing countries is basically attributed to the unsuccessful screening programs, limited access to cervix carcinoma screening, and reduced levels of follow-up treatment following abnormal test results [12,13].

Recently, two types of diagnostic tests have been used for cervical cancer screening including, Papanicolaou test (also known as the Pap smear) and HPV test [9]. The last one, HPV testing, detects infections caused by types of virus that might cause cancer as well as providing a reproducible profile of women who are at high risk of emerging precancerous or cancerous lesions [9]. However, HPV testing currently is of limited use in low-income countries due to trained technicians, laboratory infrastructure, and storage facilities requirements [14,15]. Concerning the Pap smear screening, it allows for the detection and treatment of precancerous cervix lesions [16,17]. However, gross cervical lesions are not always seen in patients with abnormal Pap smear. Hence, this test is followed by colposcopy as well as therapy interaction is common cytology-based screening. Unfortunately, these assays are not always possible in most low and middle-income countries because of their high cost and fewer healthcare services [18].

Prevention, early detection, control and treatment of the pre-invasive uterine cervical lesions are essential methods to limit cervical cancers and are imperative ways in decreasing early death throughout the productive period in a woman's life [1]. Teaching and effective screening techniques have been developed, including visual inspection after the application of 3-5% acetic acid solution (VIA) on the cervix, which does not need any laboratory equipment [19-21]. VIA is easy to learn, in which health care workers can be taught within 5-10 days [22]. This test has a similar sensitivity or even better when compared with conventional cytological testing, and in low-resource countries, VIA is the best choice for testing for cervical cancer. Nevertheless, its specificity is lower adding to the risk of overtreatment [23,24]. Vaccination of young females against HPV-16 and HPV-18 infections has been found to be an active new way of reducing cervical cancer, although this vaccine is costly and not applicable in poor countries [25].

Although in the Middle East and other parts of the developing countries cervical cancer rates have been decreased which might be due to the decreasing HPV infections or could be societal disapproval of extramarital sexual activity [26]. However, cervical carcinoma still represents a major public health problem in the developing countries [1].

Numerous studies about Pap smear test have been conducted in Iraq mainly to detect the reproductive system troubles including specific or non-specific infections, adding to the cytological changes defined as precancerous lesions [27-29]. Reports in Iraq indicate that every year 311 women are diagnosed with cervical cancer with roughly 212 of them die from the disease [30]. Additionally, in the latest report of the Iraqi cancer board, which was published in 2017, 197 cases of cervix cancer were registered among Iraqi patients (25-70 years old) living in different provinces during the year 2013. In Iraq, cervical cancer is 12th cancer among women, and the 10th among those aged between 15 and 44 years old [31]. Generally, data on Iraqi population having HPV infection is not yet available, but in Asia, the continent where Iraq belongs, approximately 2.5% of females are exposed to have cervical HPV-16/18 infections [7,30].

Despite the incidence rates of cervical cancer in Iraqi hospitals has been found to be very low, but if this cancer could occur, it would develop into an advanced stage, which prognoses very poor survival [32]. To our knowledge, we could not find many studies that relate demographic and clinical features of the patients with the incidence of cervical cancer. Therefore, because of the importance of this disease on Iraqi women, this study aimed to estimate the visual inspection of the cervix along with studying cytological changes of precancerous lesions using the Pap smear test in order to determine whether a correlation exists or not between cervix carcinoma and demographic characteristics of the patients.

PATIENTS AND METHODS

Patients

Total 140 women aged 18-67 years (mean 39.5 ± 10.8) referred to the National Cancer Research Center (NCRC), which belongs to University of Baghdad, Baghdad, Iraq during the period from December 2011 to December 2016. These women either suffered from genital health problems or just attended the aforementioned center for a routine checkup. All patients were aware of Pap smear and its purpose. Patients who were sexually active were included in the study. Demographic variables and databases taken from each participant included age, parity, history of sexually transmitted diseases, hormone replacement therapy, history of contraception, menopausal status, and smoking.

Pap Smear Test

The Pap smear test is a microscopic examination of cells scraped from the uterine cervix and is used to detect cancerous or pre-cancerous conditions of the cervix along with other medical conditions. Firstly, all the patients were subjected to the visual inspection of the cervix and vagina at NCRC center. Secondly, the samples were collected from the participants using Ayres spatula or a brush with appropriate instructions to the women [18]. The smaller end of the Ayres spatula was introduced through the external os and the squamocolumnar junction was scraped by rotating the spatula to 360°. Then, the scraping was evenly spread onto a glass slide to be stained for cytological diagnosis according to the method of Papanicolaou stain [33]. The Pap staining procedure was done according to the procedure described by Manal, et al., which involved fixation of all slides in 95% ethanol prior to staining for 20-30 min, and then the slides were dipped in descending concentrations of ethanol, each for 10 dips, including 95%, 80%, 70% and 50% ethanol. Next, the slides were dipped 10 times in distilled water, followed by 1-2 min staining with Haematoxylin. The excessive stain was removed by subjecting slides to running tap water shortly. Later, ascending concentrations of ethanol (50%, 70%, 80% and 95%) were applied to dip the slides 10 times in each concentration. Subsequently, the slides were put in orange-G stain for 2 min, followed by 10 dips in 95% ethanol. Eosin stain was also used to stain the samples for 2 min. Afterward, the slides were immersed in 95% ethanol for 10 dips, and this was repeated for another 10 dips in another 95% ethanol. Then, absolute ethanol was used for 10 dips. The slides were later put in a 50:50 mixture of absolute ethanol: Xylene for 5 min. Finally, the slides stayed in Xylene for 20 min to be mounted with DPX (a mixture of distyrene, a plasticizer, dissolved in xylene).

All the slides were examined under the light microscope using 40X magnification. Cervical Pap smears were then examined by a specialist pathologist in the Department of Pathology in NCRC.

According to the 2001 Bethesda classification, the smears were characterized as negative, atypical squamous cells (ASC), atypical glandular cells (AGC), low-grade intraepithelial lesion, high-grade intraepithelial lesion, and cancer [34].

Statistical Analysis

The statistical program SPSS V.22 for Windows was used to analyze the data, which were represented as frequency, percentage, means and standard deviations (SD) of the variables.

RESULTS

Demographic characteristics

Most women, 96.4%, in this study were from Baghdad province, with only 3 cases from Anbar province (2.14%) and one case (0.71%) from each Babylon and Najaf provinces. Approximately, half of the participants (56.4%) were housewives, followed by 40.7% were an employee and the lowest (0.7%) were students. The married women constituted 95.7%, whereas both divorced and widows were 2.14% each. Around 68% of the participants used different types of contraceptives, while pills were the most frequently (34.3%) used among women, injection accounted for 2.9%. In contrast, 32.14% of women did not use contraceptives. Regarding menstrual period, 69% of the women had regular versus 31% had an irregular period. Finally, women's answers varied concerning their reasons for visiting NCRC, while 33.6% of them had abnormal vaginal discharge, 25.7% attended for a routine check-up, and other 20.7% complained of post-coital bleeding (Table 1).

Table 1 Demographic characteristic of the patients

Variables	Frequency (N)	Percent (%)
Area		
Baghdad	135	96.40%
Anbar	3	2.14%
Babel	1	0.71%
Najaf	1	0.71%
Total	140	100.00%
Family history		
Housewife	79	56.40%
Employed	57	40.70%

Student	1	0.71%
Retired	3	2.14%
Total	140	100.00%
Marital status		
Married	134	95.70%
Widowed	3	2.140%
Divorced	3	2.14%
Total	140	100.00%
Methods of contraception		
Without contraception	45	32.14%
Pill	48	34.30%
Condom	14	10.00%
Coitus interruptus	6	4.30%
Loop	23	16.40%
Injection	4	2.90%
Total	140	100.00%
Taking contraception		
Yes	95	68.00%
No	45	32.00%
Total	140	100.00%
Menstrual period		
Regular	96	69.00%
Irregular	44	31.00%
Total	140	100.00%
Cause of visit		
Checking	36	25.70%
Post-coital bleeding	29	20.70%
Dyspareunia	19	13.60%
Irregular vaginal bleeding	9	6.40%
Abnormal vaginal discharge	47	33.60%
Total	140	100.00%

Table 2 includes information about numbers of occurrence of pregnancy, abortion, having Pap smear before as well as dates for attendance to the NCRC. Our data show that 13% of the participants were nulliparous, 20% of the women gave birth to three children, whereas the least percent (0.7%) included women who had 7 or 8 children. Women who never been aborted comprised 55% compared to 23% who had one aborted fetus and only 2% suffered from five abortions. Approximately, half of the cases referred to NCRC in 2012 in comparison with only 9% of cases attended in 2016. Most of the participants (67%) did not try Pap smear before related to 33% who did (Table 2).

Table 2 Frequencies of pregnancy, abortion, having Pap smear before and dates for attendance to the NCRC

Pregnancy no.	Total pregnancies	
	Women (N)	Percent (%)
0	18	13.0%
1	11	8.0%
2	32	23.0%
3	28	20.0%
4	21	15.0%
5	14	10.0%
6	9	6.0%
7	1	0.7%
8	1	0.7%
9	3	2.1%
10	2	1.4%
Total	140	100.0%

Number of abortion		
Abortion no.	Women (N)	Percent (%)
0	77	55.0%
1	32	23.0%
2	21	15.0%
3	4	3.0%
4	3	2.0%
5	3	2.0%
Date		
Attendance	Women (N)	Percent (%)
2011	29	21.0%
2012	72	51.5%
2013	13	9.0%
2014	5	3.5%
2015	8	6.0%
2016	13	9.0%
Total	140	100.0%
Having Pap smear before		
Having test	Women (N)	Percent (%)
Yes	46	33.0%
No	94	67.0%
Total	140	100.0%

Regarding ages associated with the reproductive status of the participants, 86% of the women were in premenopausal status compared with 14% in postmenopausal. The youngest in postmenopausal age was 38 years old and the oldest was 55 years old. Ages at marriage ranged between 12 to 45 years old (with a mean of 22.3 ± 6.3). Women's ages at the first birth were 15-39 years old with a mean of 20.7 ± 7 years (Table 3).

Table 3 Participants' age at the different reproductive status

Reproductive status and Age	Total no.	Minimum	Maximum	Mean	Std. Deviation
Current age	140	18	67	39.5	10.8
Age at marriage	140	12	45	22.3	6.3
Age at 1 st pregnancy	129	15	39	20.7	7
Age at 1 st child birth	129	16	39	22.5	6.8
Age at menopause	20	38	55	46.5	8.5
Age at premenopause	120	10	54	32	22

Pap Smear

According to Bethesda Terminology, smears of 92.8% of cases showed a severe degree of non-specific cervicitis/endocervicitis, and 70% of the samples had reactive squamous metaplasia and atypical cellular changes. Some smears showed severe chronic cervicitis/endocervicitis with atypical changes in endocervix and benign-looking epithelial and metaplastic cells in favor of cervical intraepithelial neoplasia (CIN1), which ranged between atypical squamous cells of undetermined significance (ASCUS) (10% of cases) to low grade squamous intraepithelial lesion (LGSIL) (17% of smears) with possibility of polyp. In addition, mild koilocytotic like atypia with mild dysplasia was observed in 27% of the smears. Other smears showed infiltration of clusters of squamous epithelial cells indicating different degrees of maturation, some with degenerative changes. Some cases had evidence of cellular changes consistent with tissue repair. Furthermore, 7.2 % of cases showed specific cervicitis extending to the endocervix with Candidiasis by *Candida albicans* or Trichomoniasis by *Trichomonas vaginalis* (Table 4, Figure 1).

Table 4 Visual inspection and cytological changes of the cervix

Clinical and cytological findings	No.	%
Visual inspection of the uterine cervix		
Normally looking	71	50.7%
Genital warts	2	1.4%

Erosion	67	47.9%
Cytological changes		
Atrophy	7	5.0%
Endometrial hyperplasia	11	9.0%
Reactive squamous metaplasia	98	70.0%
Atypical squamous cells of undetermined significance (ASCUS)	14	10.0%
CIN1 or LGSIL (Cervical Intraepithelial Neoplasia or Low grade squamous intraepithelial lesion)	24	17.0%
Koilocytotic atypia (Possibility of Human Papilloma Virus infection)	38	27.0%
Clusters and sheets of benign-abnormal exfoliated endometrial cells	20	14.3%
Inflammation		
Non-specific inflammation (vaginitis, cervicitis, endocervicitis)	130	92.8%
Specific inflammation by <i>Candida albicans</i> or <i>Trichomonas vaginalis</i>	10	7.2%

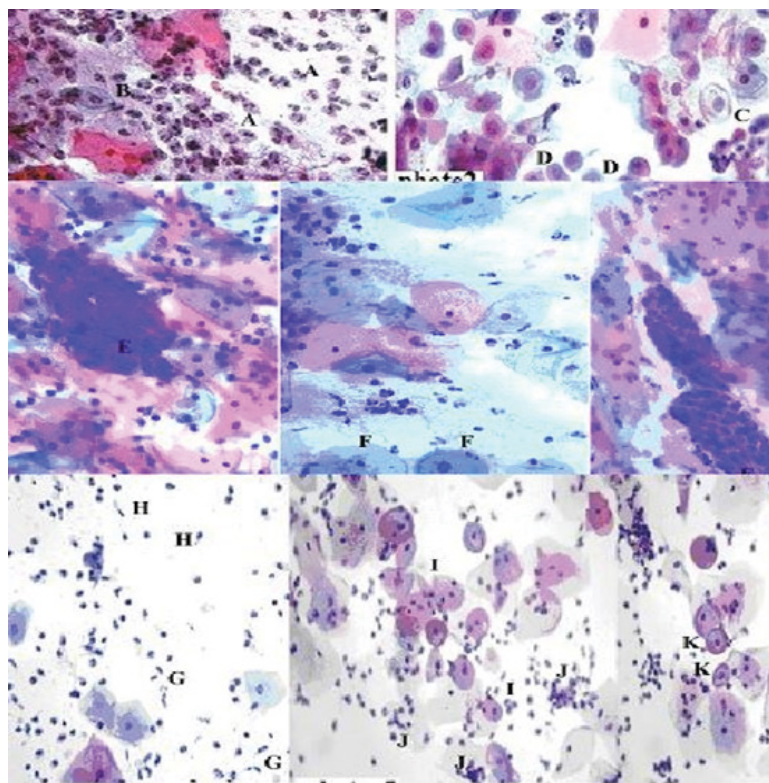


Figure 1 Pap smear showing different cellular changes in the cervix. (A) Upper left image: Non-specific cervicitis with benign squamous metaplasia exhibiting inflammatory-associated cellular changes, (B) Upper right image: Reactive squamous metaplasia, (C) and endocervical glandular dysplasia (i.e., CIN1 or LGIL), (D) The middle images: Cervical smear from a woman of 38 years old shows a moderate-severe degree of non-specific cervicitis extending to the endocervix, numerous sheets of benign abnormally looked exfoliated endocervical cells are observed (endocervical polyp), (E) Evidence of minimal atypia within squamous and endocervical cells is also shown, (F) Lower left image: Cervical smear from a woman of 45 years old shows both *Candida albicans*, (G) and *Trichomonas vaginalis* (H) infections with dysplastic cells, (I) Lower right image: Cervical smear from a woman of 50 years old shows a severe degree of non-specific cervicitis extending to the endocervix, (J) Numerous clusters of moderate aplastic endocervical cells are shown, (K) with presence of koilocytotic atypia possibility due to Human Papilloma Virus infection

DISCUSSION

Approximately 99.7% of cervical cancer cases occur due to the infection with human papillomavirus infection (HPV) [6,7]. This infection can recover without any therapeutic intervention in most women, however, those with persisting HPV infection are at a risk for CIN II, III and cervical cancer [29,35]. In developing countries, there is a tendency for increasing incidence of the disease attributed to the early beginning of sexual activities, certain sexual behaviors like a

high number of multiple partners, infrequent use of condoms, multiple pregnancies, immunosuppression and infectious agents mainly HPV [36]. Adding to the oral contraceptives, occupational factors, and dietary habits [11,30,37].

Therefore, this study was performed in order to determine the potential impact of the above risk factors for uterine cervix health or disease of Iraqi women. Our findings show that most infected women (96%) were married but those without a partner were only 4%. This means that sexual activity has a major role in the exposure of the female reproductive system to many health problems [38]. It was reported that almost all sexually active women will be infected with HPV at some point throughout their lives while some of them may be infected repeated. The peak time for infection is soon after becoming sexually active [2].

Reports state that young women possess large areas of immature metaplastic cervical epithelium, which look more susceptible to infection by carcinogenic HPV than other squamous epithelia. If sexual activity begins at an early age, especially with multiple partners harboring carcinogenic HPV, then the women will face a high risk for cervical cancer development [39-41]. Cervix carcinoma induced by HPV can progress within a couple of years post-infection of unstable squamous epithelia of the endocervix. However, most cancers arise from or neighboring to precursor lesions that proceed from one stage to another throughout 10-30 years. Over time, uninfected metaplastic squamous epithelia become mature and appear more susceptible to viruses of no-risk or low-risk, e.g. HPV-6 [42].

On the other hand, postmenopausal women without previous cervical disease have been demonstrated with low risk of cervical cancer [43]. This is consistent with our data where only 14% of women were registered in this study belonged to the postmenopausal stage.

Furthermore, in this study, residence, occupation or regularity of menstrual cycle did not have a significant influence on the occurrence of the cervical carcinoma. Concerning contraceptive, approximately, two-thirds (68%) of total participants used different types of contraceptives, with pills were the most frequently used (34.3%) among others. It has been reported that oral contraceptives constituted the major risk factors for cervical cancer [5,44]. Administration of oral contraceptives for more than 5 years has been estimated to double the risk, whereas more than 10 years uptake has quadruple risk [45].

Importantly, the Papanicolaou cytology screening was used in this study. It was named after Dr. George N. Papanicolaou who first described it in 1928. This test has dramatically decreased cervical cancer incidence and mortality rates by 75%. It is a screening tool that looks for changes in the transformation zone of the cervix, which most often are caused by HPV [18,46]. Previous studies found that there might be a significant correlation between abnormal Pap smears with hormonal contraception, postcoital bleeding and vaginal discharge that make the women seeking for a clinic to check up her cervix status. In this paper, women who suffered from abnormal vaginal discharge were 34%, with 20% complained of postcoital bleeding. Abnormalities including vaginal secretions, abdominal pain, and itching are the most important signs of cervical inflammation [21,47].

In this research, there were mild and moderate cervix problems, such as erosion in 48.6% of participants and warts in 1.4%. In addition, the microscopic examination revealed non-specific vaginitis, cervicitis and endocervicitis in 92.8% of the women, extending to the endocervix with reactive squamous metaplasia and cervical intraepithelial neoplasia (CIN1) or low grade squamous intraepithelial lesion (LGSIL) in 70% and 17%, respectively, which can be termed as precancerous lesions. These cases could be treated at low cost and a short time to avoid progression to cervical cancer [18]. It is known that cervical cancer is preceded by a long stage of a pre-invasive disease called cervical intraepithelial neoplasia (CIN), which is classified into grades I, II, and III. If these lesions are left untreated, particularly the high-grade, CIN II and III, they largely might progress into cancer. Using Papanicolaou's technique for the cervicovaginal smear, these alterations can be detected [48]. Moreover, in the present study, mild koilocytotic atypia of cervical squamous cells with mild dysplasia was demonstrated in 27% of the participated women. Thus, the possibility of HPV infection cannot be excluded.

Likewise, our findings showed that 7.2% of women had a severe degree of cervicitis/endocervicitis due to infection with *Candida albicans* and *Trichomonas vaginalis*, which were observed via the Pap smear test. This technique has been demonstrated for its ability to diagnose some microbiological agents such as *Actinomyces spp.*, Herpes Simplex virus or HPV along with *C. albicans* and *T. Vaginalis* [49].

It is vital to apply tests such as the Papanicolaou technique for cervical cancer screening to prevent unnecessary

treatment. The standard treatment program for cervical cancer is radical hysterectomy with dissection of a pelvic lymph node for early-stage disease along with radiation, chemotherapy, or both for higher stages [50-52]. However, some doctors do not administer chemotherapy unless it is necessary to avoid suppression to the immune system of the patient [53].

Molecular detection of HPV DNA or RNA is currently the gold standard for identification of HPV [54], however, these tests are rather cost-effective in developing countries. In addition, vaccination against HPV, whether available or not, does not replace cervical cancer screening [2]. Thus, in the absence of a well-organized screening program, a reliable and cheap approach such as the Pap smear test is required for the least preliminary diagnosis of uterine cervix problems. This has to be consistent with increasing women's awareness for attending clinics and hospitals for routine checkup against gynecologic diseases including cervical carcinoma [37,55-57].

The screening programs based on cytology faced numerous logistical difficulties in various countries. Of these, enrollment of eligible screeners who can appropriately establish laboratories and introduce programs of quality assurance, which might represent one of the largest struggles [42]. Furthermore, it is necessary to develop a control strategy for cancer that integrates cancer screening and diagnosis with the treatment of pre-invasive and invasive cancers [3].

According to the latest guidelines of the American Cancer Society, screening must start at the age of 21 [58], and young women should be screened neither with Pap test nor with HPV test. However, Pap test should be performed every three years for women aged 21-29 years, while the HPV test should be used only if Pap test revealed abnormal findings. Women between 30-65 years should be tested every five years with both Pap test and HPV test together. This type of screening is desirable, but the continuing of Pap test screening every 3 years is also suitable [59].

CONCLUSION

The Pap smear is one of the most important methods for the early detection of cervical cancer, which requires follow-up to detect genital problems, especially pre-cancerous and cancer lesions. If lesions and infections could be diagnosed and treated, then the prevention occur and later the development of a serious pathology problem might be stopped. We strongly recommend the sexually active women to undergo routine cervical smear screening because they are more prone to contact infection than others.

DECLARATIONS

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Conflict of interest

The authors declare that they have no conflict of interest.

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