Rzeszów University Press 2025
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2025.1.7

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2025; 23 (1): 53–63

ORIGINAL PAPER

Prevalence and pattern of abnormalities of cervical smear examination in women attending the fertility clinic at Uniosun Teaching Hospital, Osun state, Nigeria

Ayodeji Blessing Ajileye [1], Bashiru Shola Oseni [2], Adesanmi Oladipo Adeniyi [2]

[1] Department of Biomedical Laboratory Science, College of Medicine, University of Ibadan, Ibadan, Nigeria
[2] Department of Medical Laboratory Science, Adeleke University, Ede, Nigeria

ABSTRACT

Introduction and aim. Infertility is described as the failure to conceive after one year of unprotected sexual intercourse. One of the causes of female infertility is cervical abnormalities that may be due to bacterial, parasitological, and hormonal imbalances. The purpose of this study was to determine the prevalence and Pattern of Abnormalities of cervical smear examination in women attending fertility clinic at the University of Osun Teaching Hospital, Osun State, Nigeria.

Material and methods. This study was conducted in the fertility clinic of University of Osun Teaching Hospital, Osun State. The study population consisted of 50 infertile (case group) and 50 fertile participants (control group) who are attending the gynecology clinic of the University of Osun Teaching Hospital, Osogbo. A questionnaire was used to obtain sociodemographic information and other relevant data. Cervical samples were collected using Ayre's spatula, two smears were made from each subject and stained with Papanicolaou, hematoxylin, and eosin staining techniques. The results were analyzed using a frequency table.

Results. Cervical smears revealed atypical squamous cells of undetermined significance in 15 cases (30%), while only 3 (6%) were observed among controls. Cervical cervicitis 19 (38%), *Candida* spp. (10%), *Trichomonas vaginalis* (16%), *Gardnerella vaginalis* (8%), inflammatory cell infiltrate (72%) and increased nucleo-cytoplasmic ratio (26%) were observed between cases and were significantly higher compared with the controls.

Conclusion. Abnormal pap smears in this study was significantly more often found in the case group when compared with the controls.

Keywords. infertility, estrogen, Pap smears, progesterone

Introduction

Papanicolaou smear is a useful screening tool to identify cellular alterations and anomalies in the cervix that can lead to cervical cancer and infertility.[1] Numerous factors, including hormonal fluctuations, prolonged use of birth control pills (five years or more) and infection with the Human papillomavirus (HPV), can cause abnormal changes in the cervix.[2] After the birth of three or more children, the Pap test is recommended every three years for all women between the ages of 21 and 65 years, while the HPV test is performed every five years.[3] Preinvasive cervical lesions range in prevalence from 6 to 12%, according to studies conducted in Nigeria.[4] However, women who tested positive for HIV had a prevalence of 12%, and it is well recognized that they have a higher chance of acquiring cervical cancer.[5] The current recommended screening methods in Nigeria include liquid-based cytology (LBC), visual inspection

Corresponding author: Ayodeji Blessing Ajileye, e-mail: ayobless05@gmail.com, ab.ajileye@ui.edu.ng

Received: 23.03.2024 / Revised: 17.08.2024 / Accepted: 27.08.2024 / Published: 30.03.2025

Ajileye AB, Oseni BS, Adeniyi AO. Prevalence and pattern of abnormalities of cervical smear examination in women attending the fertility clinic at Uniosun Teaching Hospital, Osun state, Nigeria. *Eur J Clin Exp Med.* 2025;23(1):53–63. doi: 10.15584/ejcem.2025.1.7.

with acetic acid (VIA), conventional (Pap-smear) tests and HPV testing for high-risk HPV types.[6] Sub-Saharan Africa has a pervasive infertility problem, according to a recent issue of Nature.[7]

In our society, infertility is stigmatized, and as a result, marital (or domestic) discord and eventual divorce are common. According to the World Health Organization.[8] It is a social public health issue. In Nigeria, the prevalence of primary infertility is 5% and secondary infertility is 8%.[8] Numerous factors including genetics, work-related stress, the environment and infectious diseases.[9] Ovulation problems, endometriosis, fallopian tube damage or obstructions, primary ovarian failure (early menopause), pelvic adhesions, uterine or cervical abnormalities, and endometriosis are some of the causes of infertility in women.[9]

The cervix is part of the female reproductive system that connects the vagina to the uterus. It has a significant impact on fertility, menstruation, pregnancy, and labor. Infertility in humans is the inability to conceive after one year of unprotected and regular intercourse between a man and a woman.[10] Infertility has a variety of causes, some of which are treatable with medications.[11] Around 5% of heterosexual couples worldwide were thought to be experiencing unresolved infertility issues in 1997. However, a significant number of couples between 12% and 28% experience infertility for at least a year.[12] It was in 1974 when a scientist named Zur Hausen established the link between HPV and cervical anomalies. Cervical cancer has been explicitly linked to the HPV virus through ongoing studies throughout subsequent years.[13] As a result, the primary risk factor for cervical cancer is high-risk HPV infection. The virus spreads through sexual activity. Studies have identified about 40 strains classified as high-risk. It has been shown that having several sexual partners and making early sexual debuts enhance the risk of HPV infection.[8] Compared to women who have only had one partner, those who have had three or more sexual partners during their lives are almost 94% more likely to have HPV.[14]

Up to 186 million people worldwide are believed to be affected by infertility. Although male infertility accounts for almost 50% of all cases of childlessness globally, infertility nonetheless places a social cost on women. Unfortunately, infertility rates tend to be higher in areas of the world with limited access to assisted reproductive technologies (ARTs).[8] In addition to not being able to conceive, the primary symptom of infertility is irregular or nonexistent menstruation, caused by hormonal dysregulation or deficiency in the proliferative follicular phase of the pre-ovulatory cycle and the secretory luteal phase of the postovulatory cycle.[15] Additionally, the sex hormones progesterone and estrogen ensure that proper anatomical and physiological conditions for ovulation and fertilization occur. After a year of unprotected sex, infertility can be indicated by unsuccessful attempts to conceive, requiring testing possibilities for both men and women. The most popular tests for female infertility are the Papanicolaou cervical test, luteinizing hormone (LH), and follicle stimulating hormone (FSH).[16]

Aim

The aim of this study was to determine the prevalence and Pattern of Abnormalities of cervical smear examinations in women attending the fertility clinic at the University of Osun Teaching Hospital, Osun State, Nigeria. The objective of the study was to examine and compare the pattern of cervical smears in women presented with infertility with those with fertility.

Material and method

Study area

This study was conducted in Osun state, specifically in the fertility clinic of the University of Osun Teaching Hospital in Osogbo, Osun State. Osun state is a state in southwestern Nigeria; bordered to the east by Ekiti and Ondo state for 84 km and for 78 km respectively, to the north by Kwara State for 73 km, to the south by Ogun State for 84 km, and to the west by Oyo State, mostly across the River Osun. Osun state is named after the River Osun – a vital river that flows through the state. Of the 36 states of Nigeria, Osun is the ninth smallest in area and 25th most populous state with an estimated population of approximately 4.7 million as of 2016.[17] Osun State is primarily inhabited by the Yoruba people, mainly of the Ibolo, Ifẹ, Igbomina, Ijesha, and Oyo. Economically, Osun state is largely based around agriculture, mainly in cocoa, cassava, millet, maize, potato and yam crops.[18] Other key industries are services, especially in urban areas, along with artisanal mining and livestock herding. Osun state is additionally noted for having the second highest literacy rate in Nigeria.[19] Yoruba and English are the official languages. People of Osun State practice Christianity, Islam, and the traditional faith. Currently, Osun State has almost 30,000 estimated people living with HIV, 13,500 of which are yet to be identified and placed on treatment. Hepatitis E virus infection is prevalent among some vulnerable groups, such as immunosuppressed individuals, pregnant women, and HBV coinfected individuals in Osun state.[20]

Uniosun Teaching Hospital (formerly known as Lautech Teaching Hospital) is a state owned medical teaching hospital located in Osogbo, Osun State, Nigeria, to provide tertiary health care and support undergraduate medical students from Osun State University, Osogbo; Adeleke University, Ede, and Fountain University, Osogbo, Osun State. Services offered at Uniosun Teaching Hospital include; clinical services,, medical laboratory services and subclinical services, it is a 400

bed hospital situated in the Idi-Seke area of station road, Osogbo. The fertility clinic is available every Tuesday.

Study population

The study population was made up of infertile women (case group) who visited the Fertility Unit of University of Osun Teaching Hospital's fertility center and fertile women (controls) who are staff of Uniosun Teaching Hospital, Osogbo, and those who have their private businesses within the premises of the University of Osun Teaching Hospital, Osogbo, Osun State. The fertile participants recruited for this study served as the control because the study design is a case control.

Study duration

This study was carried out for a period of 6 months, between January 3, 2023 to June 30, 2023.

Inclusion criteria

This study comprised participants who met the following requirements:

1. Participants diagnosed with primary infertility attended the fertility clinic at Uniosun Teaching Hospital.
2. Participants diagnosed with secondary infertility attended the fertility clinic at Uniosun Teaching Hospital.
3. Participants who had conceived and delivered 2 or more children and had never had a delay were used as controls.
4. Participants who are 20 years old and above
5. Participants who are not on any contraceptive drug
6. Participants in the case group must have had regular and unprotected sex with their spouse (male) for at least 1 year.

Exclusion criteria

The following criteria led to the exclusion of research participants:

1. Participants who did not participate.
2. Participants that were menstruating
3. Participants who just had a colposcopy
4. Participants who recently douched
5. Participants that are under 20 years of age
6. Participants in the test group who are on contraceptives

Method of sampling

A convenient sampling method was used.

Ethical approval

The protocol for this study was sought and approved by the Ethics and research committee of UNIOSUN Teaching Hospital, Osun State, with the approval number UTH/EC/2023/03/746 dated 2 March 2023.

The confidentiality and privacy of the participants was strictly respected during and after the period of data collection and collection. Serial numbers were used instead of the names of the participants to ensure confidentiality. Participants received written inform consent written in English. Participants were allowed to read their informed consent and appended their signatures before samples were collected from them.

Sample size determination

P – prevalence of abnormal Pap smear from a study done in Zaria, Nigeria (6%)

The sample size for this research work will be determined using:

$$n = \frac{Z^2P(1-P)^{21}}{d^2}$$

n – required sample size

Z confidence level at 95% (standard value 1.96)

P – prevalence of abnormal Pap smear from a study done in Zaria, Nigeria (6%)

d – accepted error

$$n = \frac{1.96^2 \times 0.06(1-0.06)}{0.05^2}$$

n=87 (minimum sample size)

A total of 100 participants that consisted of 50 cases and 50 controls.

This is a case-control study, the cervical smears obtained from controls were compared with cases.

Collection of samples

Every participant (case and control) recruited for this study was individually called into a consulting room for confidentiality purposes. Each participant was educated about the study and those who gave their verbal consent received a questionnaire and informed consent in English language to fill in privately inside the consulting room. Each question in the questionnaire was explicitly explained to the participants. The acronyms were also fully interpreted and explained to them. Participants were properly guided on how to fill in the questionnaire. The completed questionnaire and the informed consent forms were collected from the participants and kept private by the researchers. Proper care was taken to ensure names or other related personal data that could be used in tracing the completed questionnaire, and the cervical smears collected from participants were completely avoided. After this, the participants were prepared for sample collection and for those who were eligible (that is, not menstruating or not having douched for 3 days), cervical smears were collected from them. Participants were asked to sit in a lithotomy position, a disposable single use plastic spatula was used to dilate the cervix to collect sample from the participants. The Ayre spat-

ula was inserted into the cervix at the squamo-columnal junction, until only the bottom fibers was exposed. It was slowly rotated at 360° in one direction. The sample and collected was transferred immediately onto two different slides where smears were made and fixed immediately.

Laboratory procedure

Participants (infertile) for this study were recruited from individuals who visited the fertility unit of the Uniosun Teaching Hospital, Osogbo. Participants were privately called into a consulting room for verbal education and explanation of what the study entails. Verbal consent was obtained prior to the questionnaire and inform consent forms were privately given to each of the participants in the consulting room.

The sample collection was carried out by the medical personnel team (Medical Doctors, Nurses and Medical Laboratory Scientists) at the fertility center of the University of Osun Teaching Hospital and laboratory analyzes were exclusively done by the Cytoscientists at the Cytopathology Laboratory of the University of Osun Teaching Hospital, Osun State. The collected samples were smeared onto clean slides and immediately fixed with 95% alcohol. A portion of the smears were stained with the Papanicolaou staining technique, while the second portion of the smears were stained with hematoxylin and eosin staining techniques. The stained smears were viewed, analyzed, and captured on a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

Papanicolaou staining technique

Slides were fixed in 95% alcohol for 30 minutes and rinsed with tap water. The smears were flooded with Harris Hematoxylin solution for 4 minutes before being briefly differentiated in 1% acid alcohol, then rinsed in running tap water for 10 seconds. The smears were briefly dipped in 70% alcohol briefly, then 95% alcohol for 10 seconds. The smears were flooded with Orange G 6 for 1 minute, then briefly dipped in 95% alcohol briefly. Smears were stained with Eosin Azure 50 for 2 minutes. Thereafter, briefly dipped into two changes of 95% alcohol. The smears were dipped in absolute alcohol (100%) for 1 minute, cleared in Xylene, covered with a slip and examined microscopically.[22] The stained cervical smears were viewed and captured on a Brunel light microscope, 20 mega pixels (Brunel SP35 Trinocular).

Hematoxylin and eosin staining technique (H&E)

Cervical smears were fixed in 95% alcohol for 30 minutes before adding water. Hydrated smears were stained in Harris hematoxylin for 4 minutes, rinsed in tap water and briefly differentiated in 0.5% acid alcohol. The smears were rinsed in water and blued in tap water for 10 minutes. Stained smears were counterstained with 1% Eosin for 2 minutes, rinsed in water and dehydrated in ascending grades of alcohol, cleared in xylene and mounted with DPX.[18] The stained cervical smears were viewed and captured on a Brunel light microscope, 20 mega pixels (Brunel SP35 Trinocular).

Data Analysis

Statistical analysis for Social Sciences (SPSS, IBM, Armonk, NY, USA) version 25 was the statistical package used to analyze all data obtained from the questionnaire and cervical smears. Data obtained from this study were captured from the questionnaire and cervical smears obtained from cases and controls. The statistician handled the statistical analysis of this study. The Student's t test and Pearson's correlation were used to compare the mean of the different analytes with $p < 0.05$ statistical significance.

Variables captured from the questionnaire administered prior to sample collection in this study include: the age of the participants, educational level, years of marriage, occupation, days of menstrual flow, intercourse frequency, knowledge of ovulation, timing of ovulation, history of past pregnancy, previous results obtained from pap smears and family history of infertility. The variables captured from the cervical smears collected included distribution of cell morphology in the smears of both the case group and controls, distribution of cytomorphological characteristics, and infections among the participants.

Results

Approximately 100 participants consisting of 50 in the case group and 50 controls were recruited for this study, with ages ranging from 20 years to 60 years and older. Among the cases recruited for this study, there were 8 participants between the ages of 20 to 29 years (16%), 15 between 30 and 39 years(30%), 16 between 40 and 49 years (32%), 8 between 50 and 59 years (16%), and 3 participants that were 60 years and above (6%). Among the controls recruited for this study, there were 6 participants between the ages of 20 to 29 years old (12%), 29 between the age of 30 and 39 years (58%), 12 between the age of 40to 49 years (24%), 2 between 50 and 59 years (4%), and 1 participant that was 60 years old (2%). The number of years that the participants have been married differs and are listed in Table 1.

The educational level of the participants recruited for this study differs between the cases and controls. About 5 (10%) participants in the case group have no formal education, 17 (34%) have a primary school leave certificate; 15 (30%) have a secondary school certificate while 13 (26%) have a tertiary certificate. Among the control group, 3 (6%) have no formal education; 6 (12%) have a primary school leaving certificate; 17 (34%) have

a secondary school leaving certificate, and 24 (48%) had a tertiary certificate. However, a significant difference was revealed between the age of participants (p=0.042), educational status (p=0.0034) and the years of marriage (p=0.014) between the case group and controls.

Table 1. Sociodemographic characteristics of study participants

Variables	Case group n=50, frequency (%)	Control group n=50, frequency (%)	p
Age (years)			
20–29	8 (16)	6 (12)	
30–39	15 (30)	29 (58)	0.042
40–49	16 (32)	12 (24)	
50–59	8 (16)	2 (4)	
60 and above	3 (6)	1 (2)	
Years of marriage			
≤5	8 (16)	12 (24)	
6–15	37 (74)	22 (44)	0.014
16–25	4 (8)	11 (22)	
26 and above	1 (2)	5 (10)	
Education			
None	5 (10)	3 (6)	
Primary	17 (34)	6 (12)	0.0034
Secondary	15 (30)	17 (34)	
Tertiary	13 (26)	24 (48)	
Occupation			
Artisan	5 (10)	8 (16)	
Civil servants	10 (20)	23 (46)	
Public servants	3 (6)	1 (2)	0.06
Traders	15 (30)	8 (16)	
Unemployed	14 (28)	8 (16)	
Others	3 (6)	2 (4)	

The occupations of the participants recruited for this study are different, with 5 (10%) being artisans, 10 (20%) civil servants; 3 (6%) public servants; 15 (30%) traders; 14 were unemployed (28%), while others were 3 (6%) the least among the cases. Among the controls, 8 (16%) were artisans, 23 (46%) civil servants, 1 (2%) public servants, 8 (16%) traders, 8 (16%) were unemployed while those who did not disclose their occupation and were placed under "others" were 2 (4%). However, no significant difference observed in occupation (p=0.06) between the case group and controls (Table 1).

Note: Not all variables on socio-demographic status detailed in the answered questionnaire were included in the table above. The variables above are very important and relevant to this study.

Among the cases recruited for this study, 42 (84%) have their menstrual flow for 4-6 days, 7 (14%) have menstrual flow for 13 days, while only 1 (2%) had menstrual flow for 7 days and more. For controls, 49 (98%) always observed menstrual flow between 1–3 days, while only 1 (2%) observed 4–6 days of menstrual flow and none had menstrual flow for 7 days and above.

Among the cases recruited for this study, 42 (84%) have their menstrual flow for 4-6 days, 7 (14%) have menstrual flow for 13 days, while only 1 (2%) had menstrual flow for 7 days and more. For controls, 49 (98%) always observed menstrual flow between 1–3 days, while only 1 (2%) observed 4–6 days of menstrual flow and none had menstrual flow for 7 days and above.

Table 2. Distribution of days of menstrual flow and number of times of sexual intercourse

Variables	Case group n=50, frequency (%)	Control group n=50, frequency (%)	p
Menstrual flow			
1–3 days	7 (14)	49 (98)	0.0002
4–6 days	42 (84)	1 (2)	
7 days and above	1 (2)	0 (0)	
Numbers of intercourse			
Daily	2 (4)	1 (2)	
Once a week	5 (10)	28 (56)	0.0002
2–5 times weekly	41 (82)	20 (40)	
6 times weekly	2 (4)	1 (2)	

Regarding the number of times of sexual intercourse among the case group, 41 (82%) always had intercourse with their spouse 2–5 times a week, 2 (4%) always had intercourse daily and 6 times a week, respectively. For the control group, 28 (56%) always had sexual intercourse once a week, 20 (40%) had 2–5 times a week while 1 (2%) had sexual intercourse daily and 6 times a week, respectively. Significant differences were observed on the days of menstrual flow (p=0.0002) and number of sexual intercourse instances (p=0.0002) between the case and control group (Table 2).

Table 3. Knowledge of ovulation, timing of ovulation, history of previous pregnancy, and previous results of Pap smear among participants

Variables	Case group n=50, frequency (%)	Control group n=50, frequency (%)	p
Knowledge of ovulation			
Yes	21 (42)	39 (78)	0.0005
No	29 (58)	11 (22)	
Ovulation timing			
Yes	17 (34)	29 (58)	0.0273
No	33 (66)	21 (42)	
History of past pregnancy			
Yes	11 (22)	50 (100)	0.0006
No	39 (78)	0 (0)	
Previous Pap smear result			
Yes	4 (8)	12 (24)	0.0462
No	46 (92)	38 (76)	
Family history of infertility			
Yes	18 (36)	8 (16)	0.0402
No	32 (64)	42 (84)	

Among the cases, 29 (58%) of the participants had no knowledge of ovulation while 21 (42%) had knowledge of

ovulation. Among the controls, 39 (78%) of the participants had knowledge of ovulation, while 11 (22%) had no knowledge. A significant difference (p=0.0005) was observed in ovulation knowledge of ovulation among the case group and controls. Among the case group, only 17 (34%) knew their ovulation time, while 33 (66%) did not know their ovulation periods. Among the controls, 29 (58%) knew their ovulation time, while 21 (42%) did not knowledgeable about their ovulation time. About 11 (22%) had a history of previous pregnancy, while 39 (78%) had no history of previous pregnancy among the case group. For the controls, all participants had history of pregnancy 50 (100%). Only 4 (8%) out of the participants had a Pap smear test previously, while the majority 46 (92%) of the participants had no history of a Pap smear test among the cases. For the controls, approximately 12 (24%) had a previous Pap smear test, while 38 (76%) had no previous Pap smear test. A majority of the cases, 32 (64%) had no family history of infertility, while 18 (36%) have a family history of infertility. For the controls, 8 (16%) had a history of infertility, while 42 (84%) had no family history of infertility. Significant differences in ovulation timing were observed (p = 0.0273), history of past pregnancy (p=0.0006), previous results of Pap smear results (p=0.0462) and family history of infertility (p=0.0402) between the case group when compared with the controls (Table 3).

Table 4. Distribution of cell morphology among participants*

Variables	Case group n=50, frequency (%)	Control group n=50, frequency (%)
Normal cell	34 (68)	47 (94)
ASC-US	15 (30)	3 (6)
LGSIL	1 (2)	0 (0)
HGSIL	0 (0)	0 (0)
SQC	0 (0)	0 (0)
AGC	0 (0)	0 (0)
AIS	0 (0)	0 (0)

* ASC-US – atypical squamous cells of unknown significance, LGSIL – low-grade squamous, intraepithelial lesion, HGSIL – high-grade squamous intraepithelial lesion, SQC – squamous cell carcinoma, AGC – atypical glandular cells, AIS – adenocarcinoma in situ

The morphology of cervical smears among the case group revealed that 34 (68%) of the participants had normal cell morphology, 15 (30%) of the participants had atypical squamous cells of unknown significance, and only 1 (2%) had low-grade squamous intraepithelial lesion. Although the morphology of the control group revealed that 47 (94%), 3 (6%) and 0 (0%) had normal cell morphology, atypical squamous cells of undetermined significance, and low grade squamous intraepithelial lesions, respectively.

Table 5. Distribution of cytomorphological features and infections among participants

Variables	Case group n=50		Control group n=50		p
	Yes (%)	No (%)	Yes (%)	No (%)	
Cervicitis	19 (38)	31 (62)	8 (16)	42 (84)	0.0243
Candida spp.	5 (10)	45 (90)	1 (2)	49 (98)	0.2065
T. vaginalis	8 (16)	42 (84)	1 (2)	49 (98)	0.036
G. vaginalis	4 (8)	46 (92)	2 (4)	48 (96)	0.674
Infiltrate of inflammatory cells	36 (72)	14 (28)	12 (24)	38 (76)	0.00001
Increased nucleo-cytoplasmic ratio	13 (26)	37 (74)	2 (4)	48 (96)	0.034

The distribution of cytomorphological characteristics and infections among the case group was observed as follows; cervicitis 19 (38%), yeast cell (*Candida* spp.) 5 (10%), *T. vaginalis* 8 (16%), *G. vaginalis* 4 (8%), infiltrate of inflammatory cells 36 (72%) and increased nucleocytoplasmic ratio 13 (26%). On the other hand, cervicitis 8 (16%), yeast cell 1 (2%), *T. vaginalis* 1 (2%), *G. vaginalis* 2 (4%), infiltrate of inflammatory cells 12 (24%) and increased nucleocytoplasmic ratio 2 (4%) were observed among the control group (Fig. 1–10). Infection with bacteria, fungi, and protozoa may be one of the significant causes of infertility in women (Table 5).

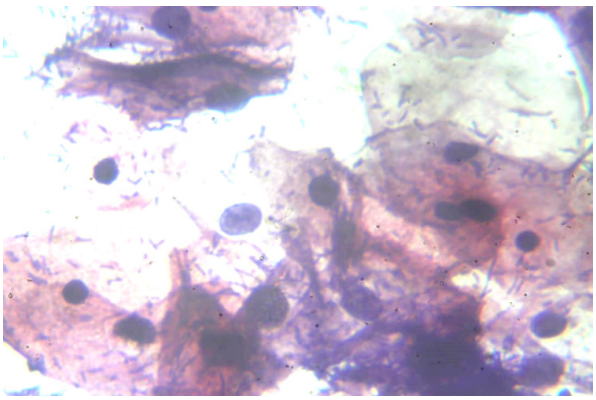

Fig. 1. Cervical smear from a participant (control group) (6-15 years of marriage) revealing *Candida* spp. and slight increase in nuclear-cytoplasmic ratio (Pap stain, 400X)

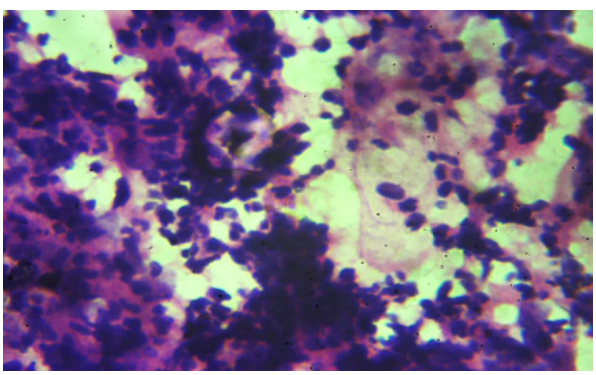

Fig. 2. Cervical smear from a participant (case group) (6–15 years of marriage) revealing LGSIL (Pap stain, 400×)

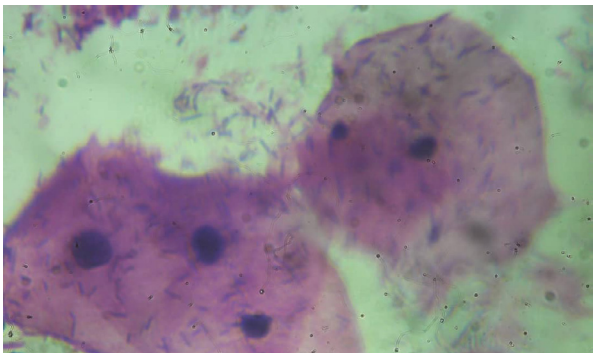

Fig. 3. Cervical smear from a participant (case group) (<5 years of marriage) revealing *Candida* spp. (Pap stain, 400×)

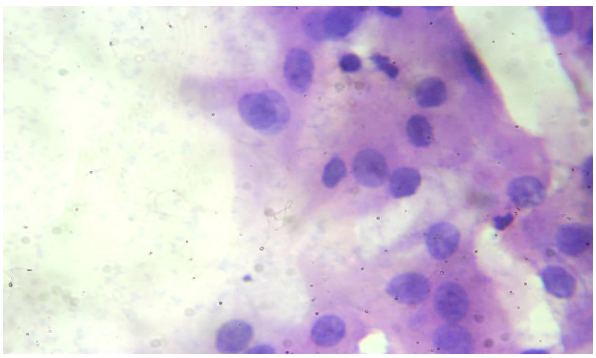

Fig. 4. Cervical smear of a participant (case group) (16-25 years of marriage) revealing LGSIL (Pap stain, 400×)

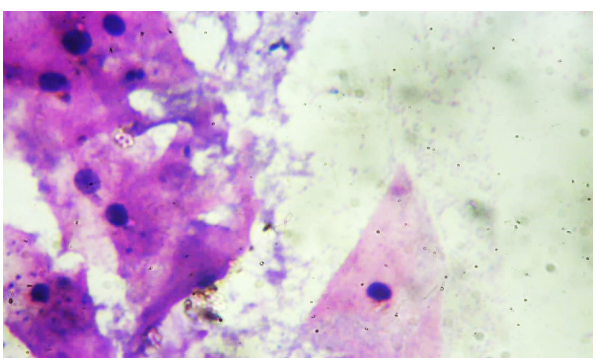

Fig. 5. Cervical smear from a participant (control group) (6–15 years of marriage) revealing normal squamous cells (H&E, 400×)

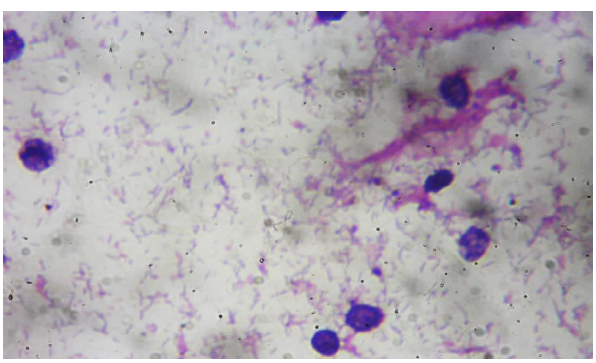

Fig. 6. Cervical smear from a participant (case group) (6–15 years of marriage) revealing *Candida* spp. and cytolysis (H&E, 400×)

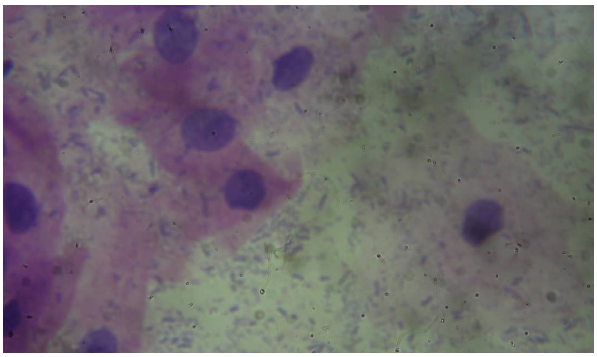

Fig. 7. Cervical smear from a participant (case group) (6–15 years of marriage) revealing ASC-US also with *Candida* spp. (H&E, 400×)

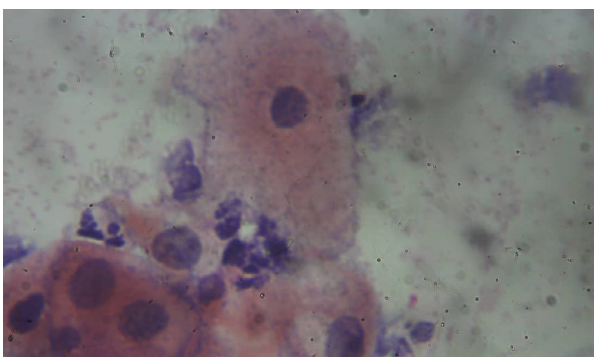

Fig. 8. Pap smear of a participant (case group) (26 years and older of marriage) revealing LGSIL (H&E, 400×)

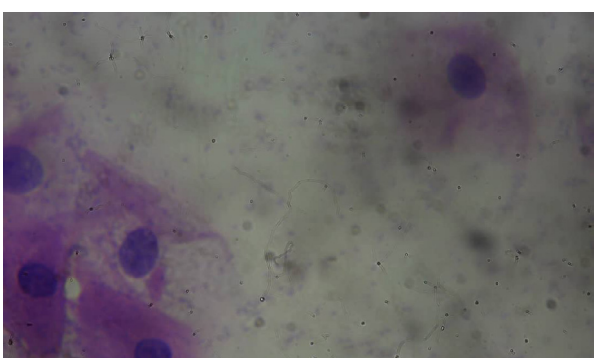

Fig. 9. Cervical smear from a participant (case group) (16–25 years of marriage) revealing ASC-US also with *Candida* spp. (H&E, 400×)

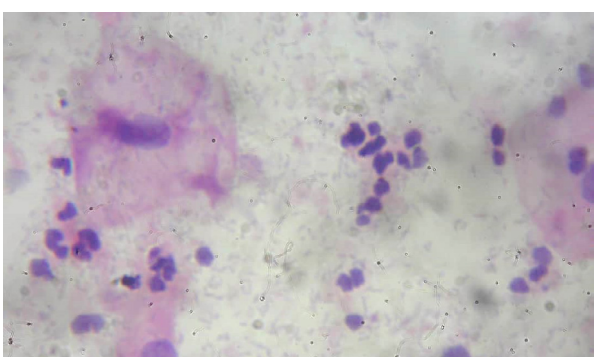

Fig. 10. Cervical smear from a participant (case group) (6–15 years of marriage) revealing cervical cervicitis (H&E, 400×)

Discussion

Infertility is a global problem that affects between 8–10% of couples.[23] Infertility can be primary or secondary. Primary infertility is when a person has never been achieved by a pregnancy, and secondary infertility is when at least one prior pregnancy has been achieved.[24] Generally, pelvic inflammatory disease, which is frequently brought on by sexually transmitted infections (STDs), particularly Chlamydia trachomatis (CT) is more common than infertility.[23] The idea that women experiencing infertility are at increased risk of developing cervical intraepithelial lesions or even carcinoma, was reinforced by the fact that these women are typically treated with exogenous hormones and are exposed to sexually transmitted infections (STDs) and HPV.[25,26] Ovulation failure is the most frequent reason for infertility in women.[23] Cervical cell abnormalities can be detected early with Pap smear screening, which also triggers intervention according to established criteria. It has been demonstrated that screening is associated with a considerable decline in the prevalence of invasive cervical cancer.[27]

In this study, about 50 cases and 50 controls were screened for Pap smear test using Papanicolaou and hematoxylin and eosin staining techniques. Participants between the ages of 40–49 were observed to be the highest number of cases randomly recruited for this study, while participants between the ages of 30 39 were observed to be the highest number of controls randomly recruited for this study. This study observed a significant association on the age of the participants (p=0.042) and years of marriage (p=0.014) between the case and control group. Among the cases recruited for this study, most had a menstrual flow for 4–6 days, while among the controls, majority had a menstrual flow for 1–3 days. This is in parallel with the study carried out in Sudan by Almobarak et al., who revealed that bad menstrual flow among Sudanese is the main cause of infertility among women.[28] In terms of the number of times sexual intercourse with their spouses in the cases, majority always had intercourse 25 times a week. Regular intercourse among cases may be due to the eagerness of having conception. This finding is in tandem with the study conducted in Saudi Arabia by Al-Jaroudi and Hussain.[26]

This study revealed that most cases had no previous Pap smear result, as evident by the fact that many of them did not know what the test entails. Most of those who had heard about it were unaware of the recommended frequency of Pap smear tests within a year. This research contradicts the findings conducted in Niger and Iran by Owoeye and Ibrahim and Tran et al. which found that only 50.6% and 44.3% of the participants, respectively, were aware of cervical cancer screening tests.[29,30] This discrepancy may be due to low health awareness in the study area.

The results of this study observed that some of the cases did not know about ovulation, this could be as a result of a low level of education among the participants. Only 34% of the cases in this study had a good understanding of when they usually ovulate. This low percentage is consistent with a study by Wolde et al. that found that 23% of Ethiopian women of childbearing age know about their fertile period and its determinants.[31] The study was based on a multilevel analysis of data from the Ethiopian demographic and health survey of 2016.

Among the cases recruited for this study, there was a higher prevalence of abnormal cervical smear compared to controls. This abnormal prevalence of Pap smear is consistent with the 29.5% of subfertile participants in the study conducted in Saudi Arabia by Al-Jaroudi and Hussain who examined the prevalence of abnormal cervical cytology among subfertile Saudi women.[26] Also, in the study by Lundqvist et al. on the cytological screening and human papilloma virus test in women undergoing artificial fertilization.[32] Their findings revealed different results, reporting aberrant cytology in 4.1% of the control and 2.3% of the infertile participants. However, this study had numerous drawbacks, including a small sample size of 100 participants.

Cervical cervicitis was observed in cervical smears of the case group. However, cervical cytology is meant to be one of the diagnostic tools to examine gynecological infections, additional microbiology and immunology research is usually advised in microbiology and immunology. This study observed that 30% of the case group had atypical squamous cells of unknown significance; 2% had low-grade squamous intraepithelial lesion, while none of the case group had high grade squamous intraepithelial lesion. These findings contrast with the study by Pushp et al. who reported ASCUS to be 2.9% of screened women, LSIL 5.09% and HSIL to be 0.48%.[33] These findings are similar to those of Verma et al. who found LSIL in 5.5% and HSIL in 2.5% of their women screened at King Georges Medical University, Lucknow, UP, India.[34] In addition, Padmini et al. conducted a study on the cytological and colposcopic evaluation of unhealthy cervix in women who attended the gynecological outpatient department of Sri Siddartha Medical College, India.[35] The study revealed that 5% of the participants had LSIL and 3% had HSIL. A study by Nayani and Hendre reported a higher percentage of LSIL (8.6%) and HSIL (3.8%) lesions.[36] Cytologists disagree on the threshold for the ASC-US diagnostic category, which characterizes cellular abnormalities more marked than those caused by reactive alterations, but which do not quantitatively or qualitatively support a conclusive diagnosis of low-grade squamous intraepithelial lesion. The high prevalence of ASCUS cytological abnormalities seen in this study may be caused by variations in age, in-

cidence of associated infections, awareness of screening, and the existence or nonexistence of cervical screening programs across the nation. The epithelial pathological diagnosis of SIL has a 4.9% detection rate, according to a study conducted in Saudi Arabia by Magdy et al.[37] Lack of screening programs and low awareness of screening contributed to the low SIL rate. According to Saha et al.[38] ASCUS is the most prevalent cytological anomaly, which is consistent with these findings. Infiltrate of inflammation cells were seen in 72% of the study participants, while 26% of Pap smears revealed an increased nucleocytoplasmic ratio. This finding is contrary to the studies conducted by Atilgan et al. and Kulkarni et al., both observed a higher prevalence (95%) of infiltrates of inflammatory cells among Turkish and Indian women.[39,40] The low prevalence observed in this study suggests that participants had less genital tract infections, which are common in women of reproductive age and have a significant financial impact. Many women with cervicitis or vaginitis have been found to not exhibit any symptoms. According to studies of Bhutia et al. and Barouti et al., women who have prolonged inflammation should receive the proper care because failing to do so raises the risk of developing intraepithelial cervical lesions.[41,42]

Conclusion

The need to screen for cervical cytology in infertile participants is emphasized by the high frequency of abnormal cervical cytology among this group of participants. However, compared to participants who are fertile, this study also found that infertile participants had abnormal pap smears far more frequently. Governments and non-governmental organizations should encourage the general awareness among women of regular cervical screening should be encouraged by governments and non-governmental organizations.

Declarations

Funding

The three authors provided funding for this study.

Authors' contributions

Conceptualization, A.B.A., B.S.O. and A.O.A; Methodology, A.B.A., B.S.O. and A.O.A.; Software, A.B.A., B.S.O. and A.O.A.; Validation, A.B.A., B.S.O. and A.O.A.; Formal Analysis, A.B.A.; Investigation, A.B.A., B.S.O. and A.O.A.; Resources, A.B.A., B.S.O. and A.O.A.; Data Curation A.B.A. and A.O.A.; Writing – Original Draft Preparation, A.B.A. and A.O.A.; Writing – Review & Editing, A.B.A.; Visualization, A.B.A. and B.S.O.; Supervision, A.B.A. and B.S.O.; Project Administration, A.B.A., B.S.O. and A.O.A.; Funding Acquisition, A.B.A., B.S.O. and A.O.A.;

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

All data and materials collected during this study are available with the corresponding author upon reasonable request.

Ethics approval

The protocol for this study was sought and approved by the Ethics and research committee of UNIOSUN Teaching Hospital, Osun State, with the approval number UTH/EC/2023/03/746 dated 2 March 2023.

References

1. Ali KE, Mohammed IA, Difabachew MN, et al. Burden and genotype distribution of high risk Human Papillomavirus infection and cervical cytology abnormalities at selected obstetrics and gynecology clinics of Addis Ababa, Ethiopia. *BMC Cancer.* 2019;19:768. doi: 10.1186/s12885-019-5953-1
2. Bruni L, Albero G, Serrano B, et al. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Benin. *Summary Report.* 2019;19:34-45.
3. ACOG Committee on Practice Bulletins-Gynecology. ACOG Practice Bulletin No. 109: cervical cytology screening. *Obstet Gynecol.* 2009;114(6):1409-1419. doi: 10.1097/AOG.0b013e3181c6f8a4
4. Bakari F, Abdul M, Ahmed S. The prevalence and course of preinvasive cervical lesions during pregnancy in a Northern Nigerian Teaching Hospital. *Ann Afr Med*. 2017;16(2):74-80. doi: 10.4103/aam.aam_35_16
5. Daniel GO, Musa J, Akindigh TM, et al. Prevalence and predictors of precancerous cervical lesions among HIV--positive women in Jos, north-central Nigeria. *Int J Gynecol Obstet.* 2020;151(2):253-259. doi: 10.1002/ijgo.13312
6. Kayode OA, Julia CG, Akinfolarin CA, et al. A Population--Based Study of Visual Inspection with Acetic Acid (Via) for Cervical Screening in Rural Nigeria. *Int J Gynecol Cancer.* 2013;23(3):507-512. doi: 10.1097/IGC.0b013e318280f395
7. Olufunmilayo L, Lola A, Zainab T, et al. Cervical cancer screening outcomes in public health facilities in three states in Nigeria. *BMC Public Health.* 2023;23:1688. doi: 10.1186/s12889-023-16539-1
8. World Health Organization (WHO). Comprehensive Cervical Cancer Control; A guide to essential practice 2 edition https://www.who.int/publications/i/item/9789241548953. Accessed January 10, 2024.
9. Siristatidis C, Pouliakis A, Sergentanis TN. Special characteristics, reproductive, and clinical profile of women with unexplained infertility versus other causes of infertility: A comparative study. *Journal of Assisted Reproduction and Genetics.* 2020;37(8):1923-1930. doi: 10.1007/s10815-020-01845-z

10. Chowdhury SH, Cozma, AI, Chowdhury JH. Infertility. Essentials for the Canadian Medical Licensing Exam: Review and Prep for MCCQE Part I. 2nd edition. 2017
11. Makar RS, Toth TL. The evaluation of infertility. *American Journal of Clinical Pathology*. 2002;117:95-103. doi: 10.1309/w8lj-k377-dhra-cp0b
12. Azra L, Nenad S, Sonja S, Aleksandra N, Orhan S. The Prevalence of the Most Important Risk Factors Associated with Cervical Cancer. *Mater Sociomedical*. 2018;30(2):131-135. doi: 10.5455/msm.2018.30.131-135
13. Chin'ombe N, Sebata NL, Ruhanya, V, Matarira HT. Human papillomavirus genotypes in cervical cancer and vaccination challenges in Zimbabwe. *Infect Agent Cancer*. 2014;9:16-19. doi: 10.1186/1750-9378-9-16
14. Yu H, Xinzhi W, Ying, Lin W, Li JL, Baozhi S. Multiple-sexual partners and vaginal microecological disorders are associated with HPV infection and cervical carcinoma development. 2020;20(2):1915-1921. doi: 10.3892/ol.2020.11738
15. Jose-Miller AB, Boyden JW, Frey KA. Predominance of Ovarian Pathologies as Etiologies of Hypofertilités. Infertility. *American Family Physician*. 2007;75:849-856.
16. Marcela A, Paula CB, Juan PG, Mérida R, Daniela N, Hernán DV. Women's critical experiences with the pap smear for the development of cervical cancer screening devices. *Heliyon*. 2023;9(3): e14289. doi: 10.1016/j.heliyon.2023.e14289
17. National Population Estimates. Population 2006 to 2016. https://data.worldbank.org/indicator/SP.POP.TOTL. Accessed July 9, 2024.
18. Olatomide WO, Omowumi AO. Sources of Technical Efficiency among Smallholder Maize Farmers in Osun State of Nigeria. *Research Journal of Applied Science*. 2010;5(2):115-122. doi: 10.3923/rjasci.2010.115.122
19. Ige RA, Omodunbi OO, Omolade AS. Policy reform in the education sector: Osun state as a case study (2011–2018). *Acta Politica Polonica*. 2021;2(52):5-16. doi: 10.18276/ap.2021.52-01
20. Osundare FA, Klink P, Akanbi OA, et al. Hepatitis E virus infection in high-risk populations in Osun State, Nigeria. *One Health*. 2021;13:100256. doi: 10.1016/j.onehlt.2021.100256
21. Naing L, Winn T, Rusli BN. Practical issues in calculating sample size for prevalence studies. *Arch Orofac Sci*. 2006;2:9-14.
22. Ochei J, Kolhatkar, A. Medical Laboratory Science Theory and Practice, London. 2005:450-521.
23. Ahmed O, Almobarak MH, Elhoweris HM, Nour M, Abd-allah M, Ahmed A. Frequency and patterns of abnormal Pap smears in Sudanese women with infertility: What are the perspectives? *Journal of cytology*. 2013;13:376-737. doi: 10.4103/0970-9371.112651
24. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018. https://www.who.int/news-room/fact-sheets/detail/infertility#:~:text=Infertility%20can%20be%20primary%20or,diagnosis%20and%20treatment%20of%20infertility. Accessed January 15, 2024.
25. Van Hamont D, Nissen LH, Siebers AG, Hendriks JC, Melchers WJ, Kremer JA. Abnormal cervical cytology in women eligible for IVF. *Human Reprod*uction. 2006;21:2359-63. doi: 10.1093/humrep/del132
26. Al-Jaroudi D, Hussain TZ. Prevalence of abnormal cervical cytology among subfertile Saudi women. *Annal of Saudi Medicine*. 2010;30:397-400. doi: 10.4103/0256-4947.68550.
27. Joy J, Mafiana SD, Mohamednour H, Xiaohui W. Barriers to uptake of cervical cancer screening among women in Nigeria: a systematic review. *Afr Health Sci*. 2022;22(2):295-309. doi: 10.4314/ahs.v22i2.33
28. Almobarak AO, Elhoweris MH, Nour HM, Ahmed MM, Omer AA, Ahmed MH. Frequency and patterns of abnormal Pap smears in Sudanese women with infertility: What are the perspectives?. *Journal of Cytology*. 2013;30:100-103. doi: 10.4103/0970-9371.112651
29. Owoeye IOG, Ibrahim IA. Knowledge and attitude towards cervical cancer screening among female students and staff in a tertiary institution in the Niger Delta. *Int J Med Biomed Res*. 2013;2(1):48-56.
30. Tran NT, Choe SI, Taylor R, Ko WS, Pyo HS, So HC. Knowledge, attitude and practice (KAP) concerning cervical cancer and screening among rural and urban women in six provinces of the democratic People's Republic of Korea. *Asian Pacific Journal of Cancer Prevention*. 2011;12(11):3029-3033.
31. Wolde M, Kassie A, Shitu K, Azene ZN. Knowledge of Fertile Period and Its Determinants Among Women of Childbearing age in Ethiopia: A Multilevel Analysis Based on 2016 Ethiopian Demographic and Health Survey. *Front Public Health*. 2022;10:828967. doi: 10.3389/fpubh.2022.828967
32. Lundqvist M, Westin C, Lundkvist O, et al. Cytologic screening and human papilloma virus test in women undergoing artificial fertilization. *Acta Obsteerict Gynecology Scand*; 2002;81:949-953. doi: 10.1034/j.1600--0412.2002.811009.x
33. Pushp LS, Meenakshi SM, Lal P, Rekha S. A Study on Cervical Cancer Screening Using Pap Smear Test and Clinical Correlation. Asia Pac J Oncol Nurs. 2018;5(3):337-341. doi: 10.4103/apjon.apjon_15_18
34. Verma A, Verma S, Vashist S, Attri S, Singhal A. A study on cervical cancer screening in symptomatic women using pap smear in a tertiary care hospital in rural area of Himachal Pradesh, India. *Middle East Fertility Society Journal*. 2017;22:39-42. doi: 10.1016/j.mefs.2016.09.002
35. Padmini CP, Indira N, Chaitra R, et al. Cytological and colposcopic evaluation of unhealthy cervix. *J Evid Med Healthc*. 2015;2:6920-6927. doi: 10.18410/jebmh/2015/925
36. Nayani ZS, Hendre PC. Comparison and correlation of pap smear with colposcopy and histopathology in eva-

luation of cervix. *J Evol Med Dent Sci.* 2015;4:9236-9247. doi: 10.14260/jemds/2015/1341

37. Magdy HB, Mohammed SM, Naema G, Souad O. Cytological pattern of cervical papanicolaou smear in eastern region of Saudi Arabia. *Journal of Cytology.* 2011;28:173-177. doi: 10.4103/0970-9371.86343
38. Saha D, Ghosh S, Nath S, Islam H. Utility of pap smear screening for prevention of cervical cancer – A 3yrs study from rural Tripura- A northeastern state of India. *International Journal of Medical and Dental Science*. 2017;6:1456-146. doi: 10.19056/ijmdsjssmes/2017/v6i2/149896
39. Atilgan R, Celik A, Boztosun A, Ilter E, Yalta T, Ozercan R. Evaluation of cervical cytological abnormalities in Turkish population. *Indian Journal of Pathology Microbiology*. 2012;55(1):52-55. doi: 10.4103/0377-4929.94856
40. Kulkarni PR, Rani H, Vimalambike MG, Ravishankar S. Opportunistic screening for cervical cancer in a tertiary hospital in Karnataka, India. *Asian Pacific journal of Cancer Prevention* 2013;14(9):5101-5105. doi: 10.7314/apjcp.2013.14.9.5101
41. Bhutia K, Puri M, Gami N, Aggarwal K1, Trivedi SS. Persistent inflammation on Pap smear Does it warrant evaluation? *Indian Journal of Cancer.* 2011; 48(2):220-222. doi: 10.4103/0019-509X.82901
42. Barouti MD, Farah F, Azadeh AS, Zohreh T, Bahar J. The Pathogenic Microorganisms in Papanicolaou Vaginal Smears and Correlation with Inflammation. *Journal of Family and Reproductive Health.* 2013;7:23-27.