RESEARCH ARTICLE

Cervical cancer screening practices in HIV positive females – a missing link in health care delivery in Pakistan

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Abstract

Objective: To probe cervical cancer screening practices in local women positive for human immunodeficiency virus, and to determine the cervical cytological changes in them.

Method: The serial cross-sectional study was conducted at the Jinnah Hospital and Services Hospital, Lahore, Pakistan, from April 2019 to October 2020, and comprised female patients aged 18-45 years who were positive for human immunodeficiency virus or acquired immunodeficiency syndrome and were registered with the relevant programme being run by the provincial government in Punjab. Blood samples of all the patients were collected for the determination of human immunodeficiency virus viral load and cluster of differentiation 4+ count. Cervical smears were taken for cytopathological analysis, while the swabs were analysed for culture sensitivity. The same individuals were subjected to the same testing one year later, and the status of the disease and clinical stability or disease progression was explored. Data was analysed using SPSS 25.

Results: There were 150 women with mean age 32.08±7.13 years (range: 21-45 years). Age at marriage/sexual activity was 17.33±4.73 years in 15(10%) subjects. Cytological examination showed atypical squamous cells of undetermined significance in 6(4%) of the cases whereas 3(2%) cases showed atypical squamous cells, which cannot rule out high grade squamous intraepithelial lesion on cytology, while the rest were classified as negative for intraepithelial lesion or malignancy. Cervical microbial changes revealed methicillin-resistant staphylococcus aureus infection in 9(6%) cases, extended-spectrum beta-lactamase in 15(10%) cases, whereas fungal infection and trichomonas vaginalis infection were found in 30(20%) smears. There was a significant association between cluster of differentiation 4+ cell count and stability of high-risk patients ($p<0.001$). After one year, 84(56%) patients remained clinically stable, while 51(34%) developed some chronic illness. There was a significant association between cluster of differentiation 4+ cell count <200/mm^3 and the risk of developing a chronic illness ($p<0.001$).

Conclusion: There was a dire need to educate healthcare workers to offer regular cervical screening to patients with high-risk sexually-transmitted infections to prevent them from the morbidity and mortality related to cervical cancer.

Key Words: HIV/AIDS, Cervical cancer, Screening, Bethesda system.

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Introduction

Sexually-transmitted infections (STIs) are one of the leading causes of morbidity and mortality worldwide. Over one million STIs per day and around 357 million per year are reported globally.[1] The World Health Organisation (WHO) defines STIs as infections that spread predominately by intimate relations and are also capable of spreading through non-sex routes, such as blood and blood products and maternal transmission.[1]

Females are at higher risk of attaining STIs in case of having a history of sexual contact with any of the high-risk groups (HRGs).[2] Human immunodeficiency virus (HIV), among one of the four untreatable STIs, invades components of human immune system, thus leading to decreased cluster of differentiation 4+ (CD4+) count.[3] Resultantly, a number of disease symptoms owing to various microorganisms, like viral, bacterial, fungal etc., along with malignancies are reported in these patients with rapid progression to acquired immunodeficiency syndrome (AIDS).[4] The assessment of CD4+ cell count and viraemia is therefore imperative to stage HIV/AIDS that is clinically classified into four stages as per the WHO system. Stage-I is asymptomatic, while Stage-IV represents AIDS.[5] The 12 countries of Asia and the Pacific

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are expected to host 90% of HIV/AIDS patients by 2030, and, unfortunately, Pakistan is one of them.[6] There are approximately 0.2 million cases of HIV in Pakistan, according to the National AIDS Control Programme's official data, while registered HIV cases are only 53,718[7]. This increased prevalence of HIV is not an overnight surge, but a persistent rise over the last three decades. The government of Pakistan has failed to adopt policies to halt the spread of this contagious disease.

Carcinoma cervix stands at the top in malignancy-associated deaths in HIV-positive females.[4] In order to promote complete gynaecological assessment of females infected with HIV, and to assess the risk associated with cervical cancer, the Centre for Disease Control and Prevention (CDC) included this malignancy in the list of diseases that lead to diagnosis/suspicion of AIDS in the presence of immunosuppression.[4] Literature shows that in patients presenting with HIV having CD4+ cell count ≤200, the chances of developing human papillomavirus (HPV)-associated cervical lesions, recurrent episodes of malignancy and consequently grave outcomes are dramatically increased compared to HIV-negative females. Therefore, screening and re-examination of lesions in HIV-positive females is strongly recommended.[4]

The implementation of cytology-based programmes is considered the main reason for the declining number of cervical cancers in the developed countries. The Bethesda reporting system of cervical cytology was established almost 30 years ago, and has evolved over the years to improve reporting and interpretation of cervical cytopathology.[8] The latest Bethesda system (2014), in addition to defining guidelines for reporting of endometrial cells in females aged <45 years, management of atypical squamous cells in which high-grade squamous intraepithelial lesion cannot be ruled out on cytology (ASC-H), assessment of adequacy in special conditions and statistics related to the recent HPV genotyping and use of immunocytochemical stains, also incorporated a whole new Chapter 12, emphasising risk assessment based on various risk factors along with cytology having a pivotal role. The basic idea behind was "similar management for similar risk." [8]

In Pakistan, though cervical cancer is the second leading malignancy reported in the females aged 15-44 years, with 5,008 new cervical cancer cases diagnosed in 2020,[9] the data regarding its cytomorphological screening and evaluation is still hard to find[4]. The latest published study from Pakistan reported Papanicolaou (Pap) smear cytology results in line with Bethesda 2001 classification[10]. In various Asian countries, including Pakistan, studies showed poor knowledge, attitude and practice (KAP) of participants towards cervical cancer screening. Comprehensive approach to cervical cancer prevention and control can be adopted only by increasing KAP of cervical cancer screening. In this regard healthcare providers (HCPs) have a leading role[11].

Despite vulnerability of HIV-positive females to recurrent cervico-vaginal infections with resultant dysplastic changes, to our knowledge, no local data related to cervical screening under Bethesda guidelines[8] (2014) is available in Pakistan. The current study was planned to fill the gap by probing cervical cancer screening practices in Pakistan women positive for HIV, and to determine cervical cytological changes in them.

Subjects and Methods

The serial cross-sectional study was conducted at the Jinnah Hospital and Services Hospital, Lahore, Pakistan, from April 2019 to October 2020, and comprised female patients registered with the Punjab AIDS control programme (PACP), and presented to the Special HIV/AIDS Clinic running concurrently at the two hospitals.

After approval from the ethics review committee of the University of Health Sciences (UHS), Lahore, the sample size was calculated using the formula[12] n_0=(Z^2×P (1-P))/d^2 where z = standard normal deviation set at 95% confidence level, P = percentage of HIV patient with abnormal cytology including atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) (12.1%)[13], d = acceptable standard error of the mean, N = total population of interest, n_0 = sample size. After one year, data was again collected from these patients regarding the status of the disease and clinical stability of the patients based on WHO guidelines according to which a patient is considered stable on antiretroviral therapy (ART) based on the following criteria: on ART for at least 1 year, no current illnesses, good understanding of lifelong adherence and evidence of treatment success with 2 consecutive viral load measurements <1,000 copies/ml[14]. Females of child-bearing age 15-45 years on ART for <2 year who presented at least 2 days after the menstrual cycle were included using convenience sampling technique, while those pregnant, using intra-uterine contraceptive devices (IUCDs) or having any concomitant/active debilitating disorder, like tuberculosis (TB), bleeding disorders, uncontrolled diabetes mellitus (DM) or hypertension (HTN, patients having taken antibiotics/steroids/anti-inflammatory therapy within the preceding 3 weeks and those with present or past history of any malignancy, including carcinoma of the cervix, were excluded. In

addition, inadequate/unpreserved/unlabelled smears were also excluded.[8]

Written informed consent was taken from all the patients, and their confidentiality was ensured at every step of the study.

At recruitment, all patients were interviewed in-depth using a questionnaire designed in line with CDC guidelines for complete personal and sexual history and any significant history for risk assessment of HIV and cervical cancer.[15] The questionnaire was translated into Urdu and the questions were well-explained to the patients ensuring that the validity and reliability of the translated version remain intact. Moreover, a pilot study was conducted including 10 patients. The validity of the questionnaire was assessed by using Cronbach's alpha and the value of test was 0.765. All patients were interviewed, maintaining strict confidentiality. Husbands were allowed to sit with them in instances when females were reluctant to answer in isolation. For the clinical staging of HIV, the WHO clinical staging criteria were used.[5]

Quantitative and qualitative HIV assay was performed through HIV rapid test (Catalogue number: 7D23-47; Determine HIV-1/2, Abbott) and enzyme-linked immunosorbent assay (ELISA) (Catalogue number: 9E25-01; Murex HIV-1.2.0, DiaSorin Diagnostics),[16] respectively. The current CD4+ cell count was recorded through flow cytometry (Catalogue No.342413, BD TritestTM, Becton, Dickinson and Company BD Biosciences) as per the manufacturers' instructions. Values of the previous CD4+ count, recorded at least 06 months earlier, and the data of current viral load was retrieved from patient's clinical record at the clinic.

The patients were advised to attend the Special Clinic for cervical cytological sampling at least 2-3 days after the last menstrual cycle with abstinence from sexual intercourse for at least 48 hours. Endocervical smears were taken by an experienced gynaecologist using BD SurePathTM 10 mL collection vial for liquid-based cytology (LBC). Cytological material, taken under direct vision, was smeared on a sterile glass slide, admixed with one drop of sterile physiological saline (wet mount) and examined immediately under low and medium magnification for the presence of Trichomonas (T.) vaginalis, identified by its characteristic jerky movements.[17] The brush material was then smeared on at least two glass slides to prepare conventional Pap smears and final brush sample was preserved in labelled BD SurePathTM vial after the brush tip was sniped off in the vial for LBC. Pellets were formed, and a portion of it was then applied to slide in a monolayer.[8] Manual LBC (MLBC) was performed and the prepared slides were then stained by the recommended procedure of Pap staining at the Morbid Anatomy and Histopathology Department of UHS, and screened according to the updated Bethesda guidelines[8]. The smears were categorised microscopically as negative for intraepithelial lesion or malignancy (NILM), which included no cell changes, inflammatory changes, organisms, atrophic changes and reactive changes; ASCUS; ASC-H; LSIL; HSIL; and squamous cell carcinoma (SCC).[8]

For patients with clinical suspicion of herpes simplex virus (HSV), immunofluorescence with HSV1 and HSV2 (catalogue number: SimulFluor® HSV1/HSV2 DFA Kit #3293, Light Diagnostics, EMD Millipore Corporation, USA) was carried out on cervical smears.

Patients with abnormal Pap smears, indicated by ASC-H or suspicion of LSIL, were referred for colposcopic biopsy to the Departments of Obstetrics and Gynaecology at Jinnah and Services hospitals after appropriate counselling and consent. Biopsy specimens were fixed in 10% formalin. Detailed gross examination of the labelled specimens was carried out, according to the College of American Pathologists (CAP) guidelines and the histopathological findings were recorded. The lesions were staged according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system[18] Representative tissue sections were taken and processed to form the paraffin-embedded tissue blocks for histological examination.[19]

Data was analysed using SPSS 25. Mean ±standard deviation (SD) values were used for quantitative variables, while frequencies and percentages were used for qualitative variables. Chi-square, Fischer Exact and independent t test were used as needed, and Cox proportional hazards regression was done to compare CD4+ cell counts at baseline and one year later, as well as to examine the association of explanatory variables on disease outcome of HIV/AIDS. $P<0.05$ was considered significant.

Results

There were 150 women with mean age 32.08±7.13 years (range: 21-45 years). Age at marriage/sexual activity was 17.33±4.73 years in 15(10%) subjects. Sociodemographic and clinical characteristics of the subjects were noted in detail (Table 1).

Foul smelly vaginal discharge and intense vulval itching was seen in 24(16%4) patients. of them, 18(12%) patients showed strawberry cervix showing small red dots. The

Table-1: Clinical and demographic characteristics (N=150).

Clinicodemographic variables	N (%)
Marital status	
Married	126 (84%)
Unmarried	12 (8%)
Widow/Separated	12 (8%)
Socio economic Status*	
Poor	99(66.7%)
Middle	51 (33.3%)
Parity	
Multiparous	51(33 %)
Para 3	30 (19 %)
Para 2	42(28.6 %)
Para 1	6 (4.8 %)
Nulliparous	21(14.3 %)
Educational Status	
Uneducated	87 (57 %)
Primary education	30 (19 %)
Secondary education	21(4.3 %)
Higher secondary/above	18 (8.3 %)
Religion	
Muslims	108 (76.2%)
Non-Muslims	36 (23.8%)
Past Medical History	
Significant	117(78%)
Insignificant	33(22%)
Surgical history	
Significant	66 (43 %)
Insignificant	84 (57 %)
Vaginal discharge	
Yes	3 (2 %)
No	147 (98 %)
WHO HIV Stage	
I	99 (66 %)
II	42 (28.6%)
III	0
IV	9 (4.8%)
Current CD4+ count	
$< 500/mm^3$	63 (43%)
$\geq 500/mm^3$	87 (57%)
Viral Load	
Detectable	57 (38%)
Not detectable	93 (62%)
Antiretroviral therapy	
Yes	147 (98%)
No	3 (2%)

WHO: World Health Organisation, HIV: Human immunodeficiency virus, CD: Cluster of differentiation. *World Bank defines poor as "a person is considered poor if living on $ 1.90 per day". (Based on information about basic needs collected from 15 low income countries) (https://www.worldbank.org/en/topic/poverty)

wet smears of all of these 18(12%) patients showed T. vaginalis, and 9(4%) of them gave positive fungal staining (Figure 1).

Spouse history for HIV/AIDS was significantly positive in 114(76%) females, while 6(4%) had HIV-positive children

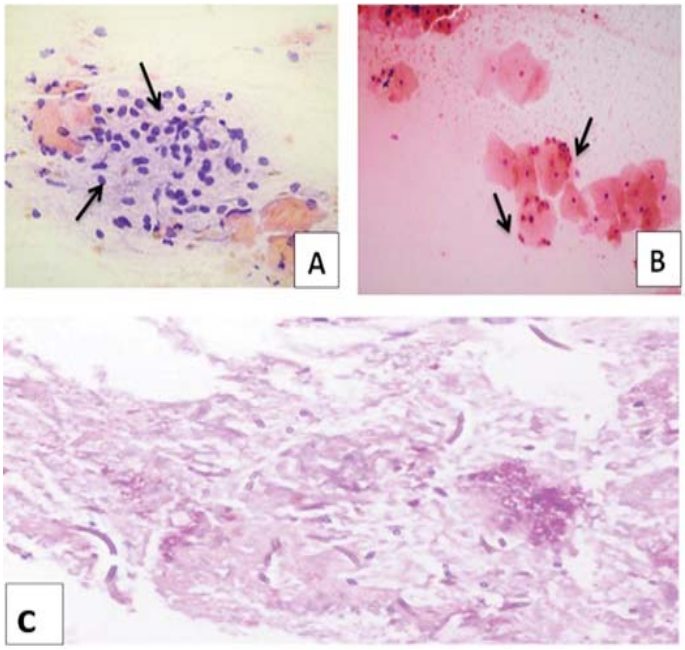

Figure-1: (A) Photomicrograph showing "follicular cervicitis" comprising looser aggregate of small lymphocytes (arrow) in Pap smear (100x; Pap stain). (B): "Cannon ball" or "puss ball "formation (arrows) associated with trichomonas (T.) vaginalis infection (100x; Pap stain). (C): Periodic acid-Schiff (PAS) stain of pap smear showing fungal hyphae (100x; PAS stain).

because of maternal transmission. When inquired about the mode of HIV acquisition, 132(89.5%) females reported transmission from their partners through the sexual route, 9(6%) also reported intravenous (IV) abuse of addictive drugs, and 18(12%) reported a history of repeated blood transfusions or frequent IV drug administration from local dispensaries for medical reasons.

Overall, 12(8%) patients reported sexual relations with multiple partners, and all these 12(8%) women affirmed having >3 male partners in a week other than their husbands. Among these women, 1(0.66%) had MRSA infection. Another 1(0.66%) had severe herpetic eruptions on the body, including genitals, and was later clinically diagnosed with HSV infection. The patient was then referred for HSV serology, and HSV immunofluorescence was carried out on the smear specimen that was HSV2-positive.

Further, 51(33.3%) patients were diagnosed for <1 year, while 99(66%) reported a duration of >1 year since diagnosis, with a mean duration of 6.57+2.75 months. All 150(100%) patients were screened for hepatitis B and C as well as TB on their routine visits to clinical centres.

The majority of the women 135(90%) had never been screened for cervical cancer.

Table-2: Cytopathological characteristics.

Cytopathological findings	n= (%)
Culture Findings:	
Gram +ve Cocci	33(22%)
Gram –ve Rods	33(22%)
Normal Vaginal Flora	33(22%)
MRSA	9 (6%)
ESBLP (Gram –ve Rods)	15(10%)
No Pathogen Seen	12 (8%)
Gram +ve Rods	15(10%)
Trichomonas vaginalis on Wet microscopy:	
Seen	30 (19%)
Not seen	120 (81%)
Bethesda Category: Conventional smear	
NILM	144 (95.2 %)
ASCUS	6 (4.8 %)
Liquid based cytology (Sure path)	
NILM	135 (90%)
ASCUS	12 (8 %)
ASC-H	3 (2 %)

ASC-H: Atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion, ASCUS: Atypical squamous cells of undetermined significance, ESBLP: Extended-spectrum beta-lactamase, MRSA: Methicillin-resistant staphylococcus aureus, NILM: Negative for intraepithelial lesion or malignancy.

The overall mean current CD4+ count was 388.78±164.33 cells/mm^3 while the corresponding value for six months earlier was 368.33±153.61 cells/mm^3. CD4+ cell counts were >500/mm^3 in 63(42.86%) patients. Viral load was detectable in 57(38%) patients.

WHO clinical stages showed significant association with the viral load, with the majority of cases with detectable viral load being in stage II and above (p=0.000).

T. vaginalis was seen on wet microscopy in 30(19%) patients. Cytological examination of both conventional and LBC smears showed NILM as the most frequent

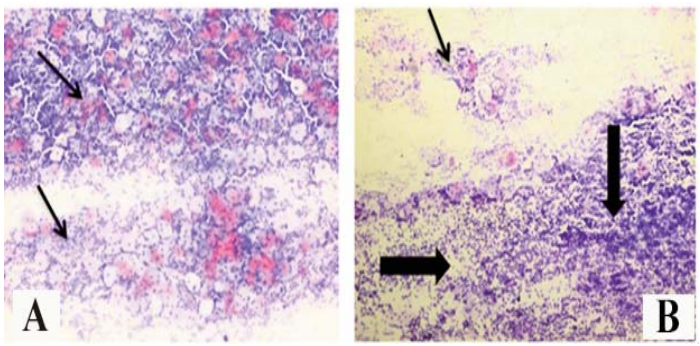

Figure-2: (A) Photomicrograph showing dense inflammatory background in Bethesda category; negative for intraepithelial lesion or malignancy (NILM) (thin arrow) (40x; Pap stain). (B): Photomicrograph showing superficial squamous cells (thin arrow) against exudative background (thick arrow). The case demonstrated methicillin-resistant staphylococcus aureus (MRSA) infection on culture (40x; Pap).

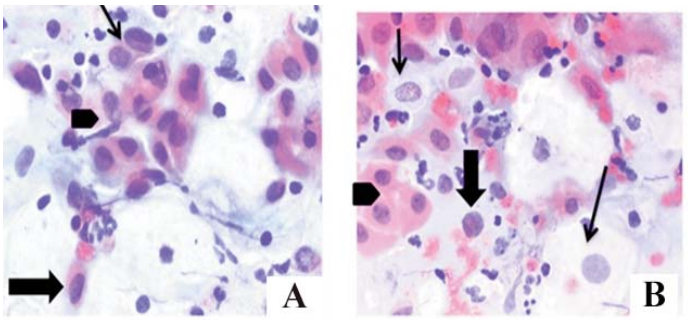

Figure-3: (A) Photomicrograph showing atypical squamous cells of undetermined significance (ASCUS) category. There was high N/C ratio ((thin arrow), mild nuclear pleomorphism (thick arrow) and variable chromatin pattern (arrow head) (400X; Pap stain). (B): Photomicrograph showing ASCUS with uniform vesicular nuclear pattern (thin arrow). Few bare nuclei (thick arrow) and metaplastic cells (arrowhead) are also seen (100x; Pap stain).

Bethesda category, and within the category, a variety of non-neoplastic changes were found associated with protective and reactive responses, representing chronic cervicitis (Table 2). Canon ball formation comprising neutrophil aggregation on the surface of superficial squamous cells was typically seen in 9(6%) T. vaginalis-positive cases (Figure 2).

The detection rate of squamous epithelial lesions was higher in LBC preparations compared to conventional smears, and 6(4%) cases that were finally labelled as ASCUS, and 3(2%) as ASC-H on LBC, were initially diagnosed as NILM on conventional Pap smear (Table 2, Figure 3).

Table-3: Comparison of clinical and demographic variables with Bethesda categories..

Clinico-demographic variables	Bethesda Categories n (%) NILM	ASCUS	ASC-H	*P* value (Chi-square test)
S/E status				
Poor	87 (58%)	9 (6%)	3(2%)	0.279
Middle class				
Younger age at marriage/sexual				
activity			3 (2%)	
Yes	0	12 (8%)		0.002*
No	135 (90%)	0		
Parity				
P0	21(14%)	0	3 (2%)	
P1	3	0		0.347
P2	36(24%)	6 (4%)		
P3	24(16%)	6 (4%)		
MP	51 (34%)	0		
Smoking				
Yes	0	0	3(2%)	
No	135 (96%)	12 (8%)		

S/E: Socio-economic, ASC-H: Atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion, ASCUS: Atypical squamous cells of undetermined significance, MP: Multiparity, MRSA: Methicillin-resistant staphylococcus aureus, NILM: Negative for intraepithelial lesion or malignancy.

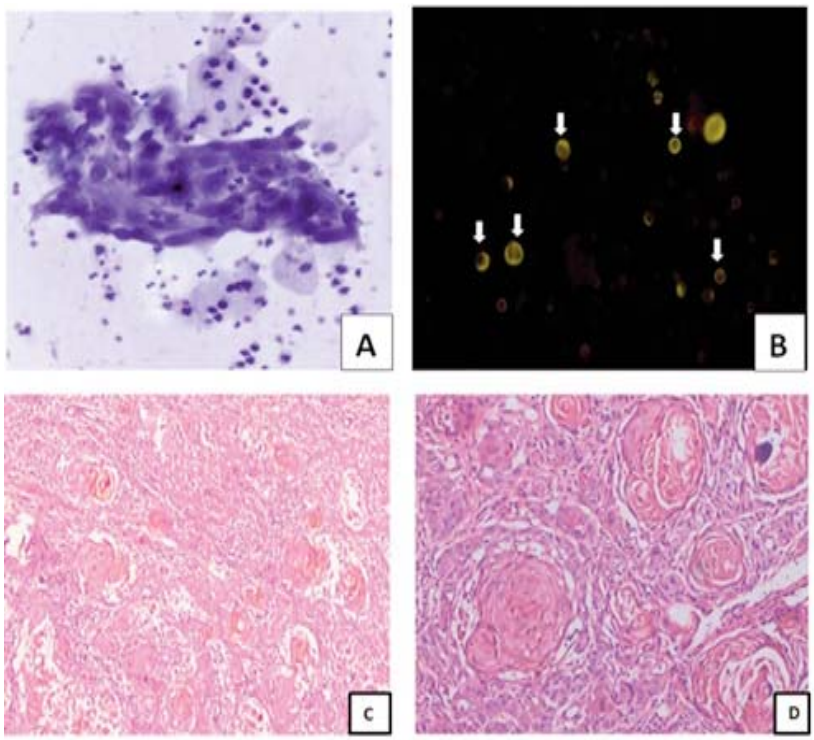

Figure-4: Photomicrograph showing (A) high-grade squamous intraepithelial lesion (HSIL; 20X) on cytology; (B) Herpes simplex virus2 (HSV2)-positive infected cells (arrow) with yellow-golden fluorescence on liquid-based cytology (LBC) smear (IF; 40x); (C-D) Well-differentiated squamous cell carcinoma of cervix on colposcopic biopsy (Haemotoxylin and Eosin [H&E], 10x)

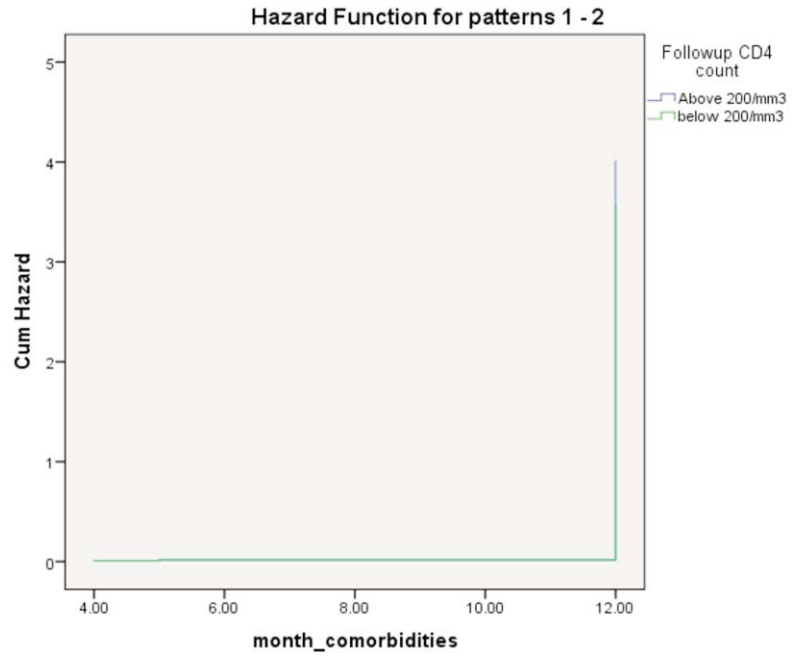

Figure-5: Survival/clinical stability pattern of HIV/AIDS patients keeping CD4+ count (baseline and after one year) as a predictor of stability.
HIV: Human immunodeficiency virus, AIDS: Acquired immunodeficiency syndrome, CD: Cluster of differentiation.

The LBC preparation of ASC-H showed scattered cells having size equal to that of metaplastic cells, but nuclei were 1.5-2.5 times larger with high nuclear to cytoplasmic ratio approximating to that of HSIL. Nuclear membranes were irregular and chromatin pattern was variable. Colposcopy of these cases was performed, and histological findings were consistent with invasive SCC, well-differentiated, FIGO stage-I in 2(1.33%) cases, while high-grade dysplasia was reported in 1(0.6%) case. The patient who clinically presented with herpetic eruptions and was HSV2-positive on immunofluorescence showed high-grade dysplasia on biopsy (Figure 4).

Bethesda category ASCUS was significantly associated with younger age at marriage/sexual activity (p=0.002), while previous and current CD4+ cell counts had no association with Bethesda categories (p>0.05) (Table 3).

After one year from the baseline, 84(56%) patients remained clinically stable, and in 70(46.6%) of them, CD4+ cell count was >200/mm^3. There were 51(34%) patients who developed one or another illness, including DM 17(11.33%), HTN 14(9.33%), oral thrush 7(4.66%), hepatitis B 7(4.66%) and hepatitis C 6(4%). Of these, 42(28%) had a CD4+ cell count <200/mm^3, representing severe immunosuppression, while the remaining 9(6%) cases suffered from moderate immunosuppression having CD4+ cell count between 200/mm^3 and-349/mm^3. There were 15(10%) dropout cases, while 33(22%) patients were referred to their nearby medical centres after collecting information for the treatment of their co-morbidities.

The unadjusted Cox model indicated a significant association between CD4+ cell count <200/mm^3 and the risk of developing any kind of illness, with a hazard ratio (HR) of 24.18 (95% confidence interval [CI]: 11.40-51.27, p<0.001). After adjusting the duration of the disease, the difference in the risk of developing co-morbidities in patients with a CD4+ cell count <200/mm^3 and those with a CD4+ cell count >200/mm^3 remained statistically

significant, with HR 24.28 (95% CI: 11.48-51.38, p<0.001) (Figure 5).

Discussion

Most of the female patients (82%) in the current study were in the 2nd and 3rd decade of life, which was in concordance with the distinctive global demographic configuration of HIV prevalence.[20] Majority of the females (74%) belonged to lower socio-economic status, with only 28.6% being employed in different kinds of jobs, which is contrary to the results reported by Shiferaw et al.[21] who performed their study on Ethiopian HIV-positive females and showed a large majority (69.8%) to be economically independent. The patients of the current study had small jobs before their HIV diagnosis, but because of the disease-related health constraints and social barriers, they became unemployed and were totally dependent on their husband's income for health maintenance and treatment purposes. In the present study, most of the women (76%) had HIV-positive spouses, following the Asian trend of HIV transmission (concentrated epidemic). Price et al.[2] concluded that HIV infection was more common (15%) among patients having a HIV-positive partner, individuals with concurrent partners (50%) and having transactional sex (6%). However, Qadir et al.[22] reported a low incidence (3%) of HIV acquisition through HIV-positive partners.

The present study reported a small proportion (9.8%) of patients with a history of repeated blood transfusion. In 42.9% and 4.8% patients, a history of major or minor surgery and frequent IV drug administration were reported within the preceding five years, respectively. These findings were consistent with Ansari et al.[23] who predicted a high prevalence of HIV in Gujrat, Pakistan, in groups with high-risk behaviours. These facts are eye-opening as in local setup, limited health facilities are available to the huge majority of the population, adding more burden to regional health care centres.

There is a well-known link between cervical cancer and younger age at marriage/sexual activity, multiparity, obesity and smoking, as evidenced by information documented by CDC[24]. The current study also reported a significant association between younger age at marriage/sexual activity and the Bethesda category ASCUS (p=0.002). Though the category of ASCUS itself does not confer a high potential risk for malignancy, lack of screening, continued careless sexual practices and concomitant HIV-induced immunodeficiency may lead to rapid progression of atypia toward dysplastic changes.

In the current study, the majority (66.67%) of females presented in WHO clinical stage-I, and its association with Bethesda categories was not significant (p>0.05) In contrast, a study conducted in India by Chakravarty et al.[25] reported HIV clinical stage-III and IV (odds ratio [OR]=2.83) along with CD4+ count ≤350/µl (OR=2.84) being closely linked to cervical cytopathology.

Squamous lesions encompass the spectrum of HPV-associated intraepithelial abnormalities to high-grade lesions and eventually to invasive cancer[26]. It is quite evident that for cervical cancer screening, knowledge is essential, but in many developing countries there is a dearth of knowledge about cervical cancer screening and related services[27].

In Pakistan, the situation is even worse in HIV-positive females as cervical cancer screening is not documented in women living with HIV/AIDS by local studies. The current study evaluated awareness regarding risk factors, symptoms, mode of acquisition, cervical cancer screening, Pap test and barriers to screening among HIV positive patients. Unfortunately, the results were bothersome as 100% females were unaware of cervical cancer screening, Pap tests or HPV vaccination. Only 9.5% of females showed some awareness of cervical cancer. The collaboration between attending physicians and gynaecologists was totally missing as in 150(100%) cases, cervical screening/Pap test was never offered, and, in fact, HCPs never delivered any kind of related education. Similar to the current study, Razzaq et al.[28] stated that the reason for women's lack of knowledge about cervical cancer was total absence of information from HCPs owing to their ignorance of the severity of the condition. However, it was encouraging that after counselling and reassurance, all of the patients were willing to accept cervical cancer screening (if offered free of cost and in a suitable setting with strict privacy) for them and even for their female relatives.

Though conventional smears are cost-effective, LBC preparations are preferred as they have a low percentage of unsatisfactory results. The current study demonstrated similar findings as 9 cases that were initially labelled as NILM on conventional smear were later diagnosed as ASCUS 6 and ASC-H 3 on LBC preparations by the same group of pathologists.

The 3 females who presented with ASC-H had concomitant HSV2 infection on serology and were later diagnosed as invasive SCC and high-grade dysplasia on colposcopic biopsies. Okoye et al.[29] reported that squamous intraepithelial lesions progress to advanced lesions in the presence of co-infections, including HSV, in HIV-infected females. Similar link was established in 1 patient in the current study who had never been screened

for even HSV infection despite recurrent herpetic eruptions.

Madan et al.[30] reported NILM as predominant finding seen in 83.2% females followed by ASCUS in 6.22%, while 3.4% cases were categorised as HSIL. Similarly, Mutuku et al.[31] showed abnormal cervical cytology in 5.1% of HIV-positive cases with the most predominant finding being HSIL (1.8%). Shin et al.[32] reported LSIL to be most predominant finding (6.5%), followed by HSIL (4.9%) in HIV-positive females. In the current study, no significant association was seen between abnormal cervical cytology, low CD4+ cell count (p=0.63) and viral load (p=0.52). These findings are similar to Madan et al.[30] However, De Lemos et al.[13] reported a very high frequency of abnormal cytology in patients with viral load >10,000 copies/ml, though statistically non-significant (->0.05 or p=1).

In the present study, all females were taking ART. This reflects satisfactory health education for HIV treatment at the HIV Special Clinics in leading tertiary care hospitals of Pakistan. Timely initiation of ART and strict compliance is associated with a decreased burden of abnormal cytology and subsequent progression to LSIL and HSIL or invasive cervical cancer[33]. However, the 1 patient who reported with ASC-H and concomitant HSV infection demonstrated poor compliance to ART with CD4+ count <200/mm^3 and was later diagnosed as high grade dysplasia on biopsy. This patient was counselled by the researchers and the treating gynaecologists for strict adherence to the therapeutic plan for cervical cancer.

In the current study, cervical infections, especially resistant bacterial infections, were reported as a co-factor for cervical abnormalities. Patients with T. vaginalis and fungal infection were referred for antimicrobial treatment, thus halting the progression to more complications. On culture, gram-ve cocci and gram-ve rods (other than ESBPL) were found to be the most predominant pathogens. Among 15 cases of ESBLP infection (gram-ve rods), 6 were reported as ASCUS, bearing no significant association to Bethesda categories (p=0.122). In 9 patients with MRSA infection, mild immunosuppression (CD4+ cell count <350/mm^3) was seen. Ako-Nai et al.[34] reported higher percentages of MRSA (20%) in their HIV population compared to MRSA-positive HIV seronegative women (10%). Kanyina et al.[35] stated that among 97.5% of the inflammatory smears, prevalence of cervical microbial infection was 32.8% with the most common being yeast (77.5%) and T. vaginalis (22%) (p≤ 0.05).

What was more disturbing was the fact that even in these HIV-positive patients, antimicrobial cover was offered for the first time after culture results. Curty et al.[36] showed an increased risk of HPV acquisition and cervical intraepithelial lesion (CIN) development in HIV-positive women which are associated with changes in microbiota, thus interfering with the clearance of high-risk HPV. Moreover, changes in microbiome composition could be used as a sensor for cervical alteration and risk for CIN progression, especially in HIV-positive women.

The present study had certain limitations. The sample size was small and all the subjects were from one province only, and from two health facilities in an urban centre. The liquid smears of HIV patients could not be processed further for molecular analysis of other common co-infecting viruses, like HPV or HSV. Also, because of financial constraints, samples could not be collected after one year to determine the pre- and post-ART cytological changes.

Conclusion

Cervical screening of HIV-positive patients revealed a high burden of subclinical or latent infections with associated atypical to high-grade CINs detected on pap smears. Lack of awareness among HIV-positive females regarding Pap test and inappropriate screening practices by the HCPs were found to be potential risk factors for the development of cervical cancer in this vulnerable population. Timely introduction of ART and health education regarding its compliance would decrease the risk of progression of the clinical stage and would thus improve overall survival. Unsatisfactory awareness, practices and negative attitudes among HIV-positive females regarding Pap smear screening and HPV vaccination were alarming.

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SI: Conception and Drafting, Pathological diagnosis and data accusation.
SZ: Co-supervisor, sampling and biopsies.
FR: Co-supervisor, Microbiological analysis.
MJ: Microbiological analysis, diagnosis and relating it with the cytological data.
HK: Monitoring antiretroviral therapy
SN: Technologist
RT: Co-supervisor for follow-up, Immunopathology
NN: Supervisor of the project, conception and study design, supervision of all pathological techniques, diagnosis and collaboration with clinical and immunopathological data, final approval.