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REVIEW ARTICLE

A PRAGMATIC APPROACH FOR ANTENATAL SCREENING OF GENITAL HERPES IN PREGNANT FEMALES AT RESOURCE CONSTRAINED CLINICAL SETTINGS: A NARRATIVE REVIEW

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ABSTRACT

It is estimated that around 67% of the world's population under the age of 50 years is anticipated to acquire genital herpes simplex virus (HSV) infection; the reported incidence, however, fluctuates considerably among different regions. Pregnant women, due to suppressed immunity, are more prone to herpes virus infection and thus bear an increased risk for dissemination to the fetus. Data regarding the screening of pregnant females for HSV utilizing sensitive but affordable laboratory techniques is scarce in developing countries. The current narrative review briefly emphasizes the value of screening of pregnant females for infectious viral diseases such as Herpes simplex virus 1 & 2 despite the constraints in clinical and laboratory resources in developing countries. Online databases of relevant published articles up to December 2022 were searched using PubMed, Google Scholar and Web of Science. The data exhibits that with the advancement and introduction of new laboratory techniques worldwide, cytological methods using liquid-based cytology (LBC), cell blocks, immunohistochemistry (IHC), immunofluorescence (IF), and serum analysis through Enzyme-Linked Immunoassays (ELISA) may be the first line diagnostic modalities for herpes virus in developing countries. Although polymerase chain reaction (PCR) is the gold standard but IF is a rapid, cost-effective, and easy-to-perform technique even in the moderate-level laboratory settings of remote areas thus offering a sensitive and specific test for HSV detection to the general population. Early and effective diagnosis of cervicovaginal infections especially subclinical HSV infections in pregnant females may lead to reduced morbidity and mortality by integrated health service delivery involving advanced laboratory techniques and promotion of preventive educational strategies.

KEY WORDS: Pregnancy; Genital herpes; Early diagnosis; Liquid-based cytology; Cell block; Immunofluorescence; Immunohistochemistry.

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INTRODUCTION

In developing countries, abortions triggered by TORCH (Toxoplasmosis, Rubella virus, Cytomegalovirus, Herpes s*implex* Virus) infections have the highest (10-15%) incidence. If the disease remains untreated, it can result in retarded fetal growth which would lead to still birth and/or premature birth.[1] Neonatal chorio-retinitis, microcephaly, and skin lesions has been associated with a primary outbreak occurring in the first trimester of pregnancy. Herpes s*implex* virus (HSV) has been coupled with rise in probability of spontaneous abortion.[2] In developing countries with diagnostic and therapeutic advances, reduction in the disease burden and morbidity has been seen over the years. In contrast, the underdeveloped countries where facilities are restrained, and lack of awareness regarding genital herpes prevails, long-term disabilities and even deaths have been reported in the neonates. The primary source of transmission of herpes simplex virus type 1(HSV-1) is oral-to-oral contact or acquaintance with the HSV-1 virus in sores, saliva, and surfaces in or around the

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mouth. Herpes *simplex* type 2 virus (HSV-2) is mainly sexually transmitted, through contact with genital surfaces, mucosal surfaces and blisters. However, the recent data reports that now HSV-1 can also cause genital herpes through oral-genital contact. Transmission of HSV by women with asymptomatic viral shedding is of more significance, since neonates mostly develop infection without being recognized.

Nevertheless, just before the start of labor, all women should endure thorough examination and probing to assess for occurrence of prodromal symptoms or herpetic lesions. If herpes infection is suspected, a Caesarean delivery is proposed to inhibit HSV spread to the infant.[3]

According to World Health Organization (WHO), globally around 3.7 billion people under the age of 50 years (67%) suffer from HSV-1 infection and approximately 491 million people aged 15-49 years (13%) get infected by HSV-2 annually.[4] HSV-2 and syphilis co-infections can raise the risk of Human immunodeficiency virus (HIV) acquisition to three-fold or more. Sexually transmitted infections (STIs) can cause life-threatening reproductive health outcomes beside the immediate impact of the infection itself (e.g., infertility or mother-to-child transmission).[5] Factors that may be implicated in communication of the disease comprises of the mode of maternal infection (primary or recurrent), the existence of trans-placental maternal neutralizing antibodies, the interval of rupture of membranes before delivery, the application of fetal scalp electrodes and the approach to the delivery. The consequences are greatest when a woman gets a new infection (primary genital herpes) in the third trimester, specifically within 6 weeks of delivery, as viral shedding may continue, and the baby is expected to deliver prior to the development of protective maternal antibodies. Disseminated herpes is more frequently seen in preterm infants and appears as an outcome of primary infection in the mother.[6] Increased rates of preterm birth are caused by genital HSV-2 infection because of intra-vaginal bacterial infection. The peculiar manifestation of estrogen and progesterone receptor in the cervical epithelium is also caused by viral infection.[7]

HSV-1 is promptly spreading among men and women who are involved in sexual activities in settings with elevated HSV-2 sero-prevalence. Co-infection of HSV-2 with Human papilloma virus (HPV) and HIV affects the local immune responses which raises the likelihood of progression of synchronous HIV and HPV-associated lesions.[8] An approximately 300 million women globally have an HPV infection which is one of the leading causes of cervical cancer. HSV, especially in combination with HIV infection, can negatively influence pregnancy rates and leads to an increased risk of miscarriage, abortion, and infertility.[9]

HSV-2 is also considered as an important risk factor for bacterial vaginosis (BV); the odds of acquiring HSV-2 infection increases twice in women with bacterial vaginosis. Pharmacologic HSV-2 inhibition may decrease the incidence and unfavorable outcomes associated with bacterial vaginosis.[10] Healthy vaginal microbiota is essential for preserving vaginal health and inhibiting local infections. Vaginal microbiota consistent with BV or intermediate flora is considerably linked with an increased risk for numerous viral STIs acquisition. Hence understanding and future research should concentrate on whether intervention should be recommended for the women with an altered microbiota.[11]

Pregnant females are by far the second largest group of adults affected with disseminated HSV infection due immunosuppressive state. Both HSV-1 and HSV-2 can cause neonatal disease. The transmission of Herpes simplex virus to the neonate may occur during delivery through infected genital tract, ruptured amniotic membranes, intrauterine or postnatal HSV exposures. During delivery, neonatal herpes may be manifested as the localized forms of the disease (dermatologic, ocular or neurologic) whereas recurrent herpes remains undiagnosed as it is usually asymptomatic.[12]

It is reported that approximately 70% of the infants get affected by disseminated neonatal herpes thus facing worst prognosis (mortality around 30%) whereas 17% of the infants suffer from long-term neurological consequences. It is reported that almost 50% of the untreated neonates with central nervous system (CNS) symptoms will eventually die.[2]

The contagious cycle of HSV-1 is thought-provoking comprising of a primary oro-labial infection infecting non-keratinized stratified squamous mucosa such as labial and buccal mucosa. After the primary infection, the virus ascends to the sensory nerve axons and creates chronic, latent infection in trigeminal ganglia. Different triggering factors such as physical or emotional stress, malaise, ultraviolet radiations, tissue injury, microbial co-infection, and hormonal imbalances results in viral reactivation regardless of the host cell-mediated and humoral immunity which then develops recurrent lesions at the location of primary infection.[13] To ascertain and sustain latency, both innate and acquired immune responses like inhibition of major histocompatibility complex expression) play vital role.[14]

The viral structure of HSV-1 and HSV-2 is quite identical comprising of >80% similar amino acids. In addition, both HSV types have the capability of infecting oral or genital mucosa. The capacity of the virus to be acquired and transmitted without manifesting any symptoms, permits it to propagate effectively throughout the affected population.[15]

The primary, initial or recurrent phases of HSV-1 or HSV-2 infestation even remain unnoticed in most of the people with no or mild symptoms.[3]

The clinical presentation of genital HSV differs upon whether the infection is primary, non-primary first episode, or recurrent. Clinical outcomes are comparable in pregnant and non-pregnant women. Primary genital infection exhibits agonizing genital ulcers, pruritus, dysuria, fever, tender inguinal lymphadenopathy, and headache. Recurrent infections include moderate prodromal symptoms, such as pruritus, burning, or pain without localized non-tender lesions. There are no systemic findings however, the interval of lesions and viral shedding is quicker as compared to a primary episode.[16]

The vertical transmission of HSV infection is intra-uterine (5%), perinatal (85%) and postnatal (10%). Most of the pregnant females who have established primary HSV-1 or HSV-2 infection near term are a major source of maternal-fetal transmission. Neonatal HSV-1 infection accounts for 30–50% of all stated HSV-1 types[17]. Two most important approaches to minimize the possibility of vertical transmission of HSV-1 or HSV-2 infection comprises of either decrease the possibility of relapse at labor and initiating suppressive antiviral therapy at 36 weeks or plan for cesarean delivery. However, no intervention has completely eradicate the possibility of neonatal herpes.[16]

In Pakistan, a local study from Peshawar reported a much lower frequency (1.1%) of HSV-IgM antibodies on serology in pregnant females[18] whereas a high seropositivity (99.4%) of immunoglobulin – G (IgG) against HSV-1 and HSV-2 was seen among pregnant women of Port Harcourt, Nigeria.[19] A study by Ghazi et al. perceived a high seropositivity of HSV-1 (90.9%) and HSV-2 (27.1%) IgG antibodies in pregnant women of Saudi Arabia.[20] These high prevalence rates of HSV-1 and HSV-2 were detected for pregnant women in their first and third trimester.

Over the decades, the role of cervical cytology has been pivotal in the diagnosis of cervical infections and carcinoma.

Liquid-based cytology (LBC) was presented in mid-1990s as an alternative technique to process cervical samples.[21] The ThinPrep® Pap test was the first of this latest methodology to receive approval from the United States Food and Drug Administration (FDA) for usage in cervical cancer screening. This test offers clinicians a more sensitive and specific methodology for detection of cervical dysplasia. Successively, the SurePath® Pap test was authorized by the FDA which is principally a result of the progress in overall clinical benefit and versatility of LBC in contrast with conventional Pap tests.[22]

Conventional cytology and LBC methods, both, have the similar technique of sampling from the cervix (i.e. cells are scraped off with a brush or analogous device from the histological transition zone) [23]. In LBC, the method of transferring of cells from brush to slide varies from conventional cytology. The cells are immediately smeared on a slide in conventional cytology (Pap smears), while in LBC, the brush is initially washed into a container with a preservative fluid and then carried to a laboratory where a homogenous layer of cells is prepared on the slide after centrifugation. The technique of cell transport (which contrasts between SurePath and ThinPrep) results in a more precise interpretation of the whole sample as compared to conventional cytology.[24]

Manual Liquid Based Cytology (MLBC) permits cells to be held in a monolayer and consequently improves specimen adequacy and recognition of precursor lesions. However, this method is restricted to laboratory, accessibility of equipment, preservatives, and polymer. Identification of nuclear changes against a clear background is facilitated by polymer solution which enables to construct a thin monolayer of cells.[25] MLBC enhances the efficacy of screening of cervical cancer in mass population by expanding the identification of histologically confirmed neoplastic and pre-neoplastic diseases. Another advantage of MLBC is that it can be utilized for ancillary studies like cell block preparation, immunocytochemistry, and HPV testing. The preparation of cell blocks from residual tissue fluids and fine-needle aspirations can play an integral part in formulating smears for a more definitive cytopathologic diagnosis. Initially LBC was used only for gynecologic cytology but with the passage of time this technique has shown its worth for non-gynecologic cytology as well.[26,27]

Globally, Pap test is still being employed as the principal laboratory tool for prevention of cervical cancer in the routine screening programs. However, the conventional Pap smear faces certain issues regarding the quality and adequacy of the smear, and interpretation of the results (increased false-negative results). In addition, the higher cost and trained cytotechnicians needed for LBC methods have made the conventional pap smear still a method of choice for cervical cancer screening in the developing countries.

Cell blocks (CB) prepared by Plasma Thrombin method is a simple, cost effective and user-friendly method for the lab personnel and is increasingly becoming popular choice in resource limited settings. The pellet prepared from centrifuged cell suspension is enmeshed into a clot by adding equal proportions of thrombin and plasma. The clot is positioned into a labeled cassette and then processed routinely in the histopathology laboratory like paraffin embedded blocks method.[28] The diagnostic accuracy of MLBC smears is enhanced by cell blocks prepared from the additional samples.

Histologic evaluation of the cell blocks facilitates in the description of certain lesions which may be obscured in the cytology smears. Microscopic analysis of the LBC and CB samples of HSV infected patients shows a distinctive morphology revealing dark,

intra-nuclear inclusions surrounded by a clear halo. Multinucleated cells with nuclear molding and chromatin margination presents clearance or "stained glass appearance" of the nuclei.[29] Large multinucleated cells seen on smear or cell block preparation may be confused with certain other cells such as reactive endocervical cells, syncytiotrophoblastic infiltrate, low-grade squamous intraepithelial cells, neoplastic cells (such as carcinosarcoma, choriocarcinoma), and radiation or chemotherapy induced changes.[30] Hence, for HSV detection, a combination of more sensitive methods like antigenic detection and serological assays can be utilized for definite diagnosis.

Thus, in the resource constrained settings, the use of cell block method through LBC sampling not only reduces the biopsy load but is also considered a quick and reliable method for detection of genital lesions.[31]

Direct fluorescent antibody assay (DFA) is a frequently used method for direct recognition of HSV in a clinical virology laboratory. Clinical samples from the representative lesions can be observed on a microscopic slide directly or after cyto-centrifugation before being fixed and stained with fluorescein-labelled type-specific monoclonal antibodies (isotype, immunoglobulin G2b K) or polyclonal rabbit anti-HSV-1 antibody. Slides prepared by cytospin method ensure the quality of the slide for precise interpretation. The presence of a characteristic pattern of apple-green fluorescence in the nucleus and cytoplasm of the basal and parabasal cells are appreciated in the HSV-infected cells under a fluorescence microscope.[32]

Immunohistochemistry (IHC): In viral infections, IHC is frequently used for verification of histological findings or when there is a clinical suspicion, but no viral cytopathological effect is usually found. IHC is a potent ancillary technique in which anti-HSV immunohistochemical stain recognizes explicitly the glycoproteins present in HSV- infected tissue. The antibody characterizes both HSV-1 and HSV-2 (does not distinct between the two types) and exhibits nuclear staining in infected cells either on paraffin embedded tissue blocks or the cell blocks prepared from LBC method [33].

MATERIALS AND METHODS

Study protocol: A narrative review methodology and analysis of published works were planned, carried out, and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search Strategy: In the current study, a search of published works was done using the online databases of PubMed, Scopus, Web of Science, and Cochrane Library for relevant publications up to December 2022. The following medical subject headings (MeSH) keywords were used in our search strategy: ''oral potentially malignant lesions'', "pregnancy", "cytology", ''genital herpes", "cell block", "immunofluorescence", "immunohistochemistry". The reference lists of the articles were also searched to identify missed studies. No restriction was applied on time of publication or language. To facilitate the screening process of studies from online databases, all search results were downloaded into an EndNote library (version X8).

Inclusion and exclusion criteria: Hospital and clinic-based reviews were included till December 2022. Pregnant females suffering from vaginal discharge, with or without genital lesions, ranging in age from 18-40 years were included. Studies related to postnatal or postpartum period were excluded. Moreover, studies referring only to clinical features were excluded.

Data Extraction: Various information was obtained from the shortlisted studies such as first author name, year of publication, the geographical region in which the study was conducted, duration of the study, sample size and age of the analyzed sample, the incidence rate of HSV, study design, sampling method, laboratory techniques used, any statistically significant results found, and conclusions made by the authors regarding the efficacy of different laboratory techniques in the diagnosis of HSV 1&2.

RESULT

Table-1 describes the various studies globally conducted on Immunofluorescence over the years (2004-2021), which shows the pivotal role of IF in definitive diagnosis of HSV in pregnant females (Table-1). IF plays a crucial role in resource strained clinical settings where molecular studies are not widely or routinely available.

DISCUSSION

Globally, HSV-1 and HSV-2 prevalence is on a rise especially in Asia within the last decade [43]. A study by Talaat et al.[44] documented the detection IgM antibodies of HSV-1 (4.4%) and HSV-2 (7.7%) among N=90 pregnant females. A systematic review by Arabsalmani et al.[45] reported the prevalence of HSV infestation among pregnant females to be 77%. Moreover, a study was conducted in Turkey which stated 73.8% seroprevalence of HSV in pregnant women with genital lesions through PCR method.[46]

A literature review was conducted by McCormack et al.[47] to evaluate the effect of HSV hepatitis on pregnant females at 30 weeks of gestational age. This study concluded that the mortality from HSV hepatitis reaches up to 40% for both mothers and neonates and with proper care during antenatal period, the possibility of having this grave manifestation of HSV can be significantly reduced. Guidelines from the French College of Gynecologists and Obstetricians (CNGOF) recommend appropriate screening strate-

Table-1 Various studies conducted on Immunofluorescence of HSV-1 & 2 over the years (2004-2021).

Title	Authors, Location & year of study	No. of patients (N)	Results	Conclusions
Asymptomatic herpes simplex virus type 2 (HSV-2) infection among pregnant women in Turkey	Duran et al.[34] Turkey 2004	130 pregnant females	Of the 130 swabs samples, 22 (16.9%) were found to have the HSV-2 type-specific antigen by direct immunofluorescence assay (IFA) test.	The sensitivity and specificity of IFA were found to be 84.67 % and 96.6 % respectively. The seroprevalence of HSV-2 and genital HSV-2 infection was high among asymptomatic pregnant women in Turkey. HSV-2 type-specific antibody studies along with IFA should be used to detect HSV-2 in pregnant women (asymptomatic or subclinical genital HSV-2 infection).
Diagnosis of herpes simplex virus-1 keratitis: Comparison of Giemsa stain, immunofluorescence assay and polymerase chain reaction	Subhan et al.[35] India 2009	170 patients with HSV-1 keratitis	The sensitivity of PCR was 100%, and IFA had sensitivity of 85.7% whereas the specificity of PCR and IFA were found to be 67.9% and 85.3% respectively.	A combination of PCR and IFA should be adopted which has proven to the most suitable choice of tests for diagnosis of HSV-1 keratitis.
Direct immunofluorescence assay compared to cell culture for the diagnosis of mucocutaneous herpes simplex virus infections in children	Caviness et al.[36] USA 2010	499 Children	Of the 499 adequate specimens, overall HSV Direct Immunofloures-ence assay (DFA) test accuracy was: sensitivity 61%, specificity 99%	HSV DFA has a high specificity for HSV infection but it has a low diagnostic yield and is not as sensitive as HSV viral culture in pediatric patients.
Comparison of Simplexa HSV 1 & 2 PCR with Culture, Immunofluorescence, and Laboratory-Developed TaqMan PCR for Detection of Herpes Simplex Virus in Swab Specimens	Gitman et al.[37] USA 2013	171	Fifty-eight of 171 (33.9%) specimens tested were true positives (34 HSV-1 and 24 HSV-2) detected by DFA, viral culture and Polymerase chain reaction (PCR).	DFA showed sensitivity of 86% and specificity of 100% in the detection of HSV-2.
Laboratory diagnosis of genital herpes - direct immunofluorescence method	Majewska et al.[38] Poland, 2013	187 anogenital samples from females including 15 pregnant females	Seventy-three (73) samples were positive on IF. Almost 85% results were confirmed microscopically whereas 15% of samples (asymptomatic) stated positive IF in the absence of C	It is highly sensitive (97%) in women with clinically suspected infection. Elevated negative predictive value (99%) proves the clinical value of the direct IF.
Laboratory Diagnosis of Neonatal Herpes Simplex Virus Infections	Muller et al.[39] USA 2019	HSV infected neonates	Currently, for direct detection of HSV in clinical specimens, a widely used method in a clinical virology laboratory is the direct fluorescent antibody assay (DFA)	The strengths of DFA include brief testing time and relatively reduced cost price.
Detection of Herpes Simplex Virus Type 2 and Some Bacterial Vaginosis Isolated from Women in Hilla City/ Iraq	Hadi et al.[40] Iraq 2020	150 women with bacterial vaginosis	Immunofluorescence assay detected 55/150 (36.6%) of cases positive for HSV-2. Moreover, qPCR technique HSV-2 from blood samples by. It was found that, from 55(36.6%) samples of women with bacterial vaginosis (BV).	HSV-2 and BV are two of the most common infections of the female genital tract and are associated with an increased risk for acquiring HIV infection
A 20-year experience of ocular herpes virus detection using immunofluorescence and polymerase chain reaction	Satpathy et al.[41] India 2021	1863 suspected cases of herpetic keratitis	Almost 14.6% samples were found positive with IFA while 24.7% cases were positive on PCR. HSV-1 was detected in about 12 % cases with both IFA and PCR.	Diagnosis of suspected herpetic keratitis in patients using IFA has been routinely used for over 20 years. This technique has been proved to be a rapid diagnostic tool for the last 20 years. IFA being highly sensitive for the diagnosis of HSV, this single tool has been used for routine diagnosis of suspected herpetic keratitis patients.
Herpes simplex virus infection in pregnancy – An update	Hammad et al.[42] UK 2021	IF generates results faster than culture (<4 h), is inexpensive and has a sensitivity of about 95 %. Mother - child transmission of genital HSV infection can lead to serious morbidity and even mortality to the newborn. The classification of this disease and the stage of pregnancy, it is acquired is of high in order to adequately the infected women and plan the route of delivery. By adopting these measures, neonatal exposure to herpetic lesions will be prevented.		

Abbreviations: HSV, Herpes simplex virus; IFA, Immunofluorescence assay; DSA, Direct immunoflouresence assay; BV, bacterial vaginosis; qPCR, Quantitative polymerase chain reaction.

gies as around 70-80% of pregnant women acquire HSV-1 in Europe. The reason for increased rates HSV infestation in pregnant females is presence of sexually transmitted diseases (STIs), multiple sexual partners and partner's history of herpes.[48] Another study concluded that 40% of all pregnant women in US are inclined to acquire primary HSV-1 or HSV-2 disease because of the demographic and behavioral factors including sexual activities and lower immune status.[49] A study conducted in Siena and Bari showed a higher prevalence of HSV-1 (91.2%) in pregnant females.[50]

According to a local study by Bukhari et al.[51] on 1000 married women, aged between 20 and 70 years, a total of 1.8% of conventional Papanicolaou smears detected positive herpes simplex virus infection on cytomorphology. Another local study from Khyber Pakhtunkhwa, Pakistan reported 47% sero-prevalence of HSV in N=145 pregnant females which is ten-fold higher than the other reported studies from Pakistan. The reason for such a high number in a developing country like Pakistan may be poor socio-economic conditions, lack of awareness, hygiene and compromised immune status [52].

A study conducted by Kulkarni et al. concluded better sensitivity and specificity of MLBC when compared with conventional pap smears for diagnosing neoplastic lesions with sensitivity of 67% vs. 50% and specificity of 84% vs. 71%.[31] According to another study, LBC and conventional cytological methods were consistent with all cytological criteria except for a significantly clearer background in LBC ($p = 0.045$) [53]. A study from Japan concluded that smears prepared by LBC technique showed slightly higher sensitivity as compared with conventional Pap smear (CPS), with no major variation in diagnostic efficacy between the two methods.[54] However, study by Rossi et al.[55] showed no variations in the sensitivity, specificity, or diagnostic accuracy between the CPS and LBC. The diagnostic yield of LBC has been reported much better than the simple smear method in non-gynecologic cytology. An analysis by Tyagi et al.[56] reported enhanced diagnostic accuracy of LBC in peritoneal washing samples. Similarly the combination of a conventional smear with LBC should be considered mandatory for a definite diagnosis as reported by Tripathy et al.[57]

Considering the diagnostic efficacy of cell block method over LBC and CPS, a study by Sadullahoglu et al.[58] concluded that cell block preparations resulted in 10 % rise in diagnostic sensitivity of bronchial aspiration specimens as compared to conventional cytology. The findings of another study suggested that cell block method must be combined with the immunohistochemical analysis for enhancing the diagnostic accuracy of cancerous cells in ovarian clear cell carcinoma.[59]

According to a review article by Dilnessa et al. [60] IF is broadly applied for the quick diagnosis of virus infections by detecting the virus antigen in clinical specimens and virus-specific antibodies (IgG or IgA or IgM). This study concluded that HSV direct fluorescence assay (DFA) sensitivity is 61% and specificity is 99%. Moreover, various other studies in the literature have compared different techniques for the detection of HSV infection and hence concluded that HSV type-specific antibody studies along with IFA should be used for initial screening, rapid accurate diagnosis and timely treatment in order to prevent immense morbidity and mortality caused by this deadly virus.[34-42]

CONCLUSION

Herpes simplex virus (HSV) infestation is one of the most widespread sub-clinically transmitted infections. Disseminated HSV infection is a frequent cause of mortality in Pakistan. However, unfortunately, no antenatal screening option for HSV is offered in any tertiary care hospital of Pakistan. For the initial detection of cervico-vaginal infections, cytological examination has been the pillar though its extensive application has not been feasible in Pakistan due to lack of awareness and reserves at public sector hospitals, situation being gravest for screening of pregnant females. Thus, advanced and sensitive detection techniques may assist to improve the diagnostic accuracy especially during subclinical course of HSV1/2 in pregnancy.

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CONFLICT OF INTEREST
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.