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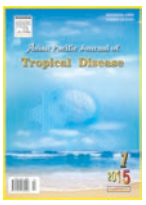

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Validity of genito-urinary discharges, genital ulcers and genital rashes as indicators of seroincident HSV-2 infection

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ABSTRACT

Objective: To evaluate the validity of vaginal discharges, urethral discharges, genital rashes, and painful genital ulcers as indicators of early detection of incident herpes simplex virus type 2 (HSV-2) infection among pregnant women in Benin metropolis.

Methods: Participants were antenatal clinic attendees of University of Benin Teaching Hospital and Central Hospital, Benin. Baseline sociodemographic, obstetric and HSV-2 serological data were collected. The HSV-2-seronegative returned for a repeat HSV-2 antibody assay before delivery date. Data on incidence of genital rashes, abnormal vaginal discharges, painful genital ulcers and urethral discharges were collected.

Results: The sensitivities of abnormal vaginal discharges, genital rashes, urethral discharges and painful genital ulcers were 82.3%, 70.6%, 41.2% and 28.6% respectively; while their positive-predictive values were 53.8%, 60.0%, 58.3% and 66.7% respective. All the symptoms had >95% specificities and 95% negative-predictive values for seroincident HSV-2 infection.

Conclusions: Abnormal vaginal discharge, genital rashes, urethral discharges and genital ulcers are valid indicators of seroincident HSV-2 infection and could be useful in formulation of screening tools in resource-limited settings.

1. Introduction

Herpes simplex virus type 2 (HSV-2) belongs to the Herpesviridae family, Alphaherpesvirinae sub-family and *Simplexvirus* genus[1]. The virus has a wide host range, including animals, but the reservoirs of the human diseases are humans, only. It is known to be the principal causative agent of genital herpes, which is transmissible principally by sexual contact[1-3]. The transmission of this virus in Nigeria seems very efficient, as shown by its high prevalence among pregnant women in Benin[4]. The high transmission efficiency is fuelled by its synergy with HIV infection in the sub-Saharan African region[5,6].

First-episode HSV-2 infection of pregnant women (whether primary or non-primary infection) not only causes genital herpes, but also places the woman at risk of severe disseminated HSV-2 disease[2]. The fetus could also be at the risk of spontaneous abortion, intrauterine fetal death and congenital malformations[7]; and the newborn, at the risk of neonatal herpes[1,2,7]. While the infection is incurable, effective interventions are available for preventing these obstetric complications[8-12]. Sitution of such interventions depends on early recognition of the incident infection, and this can be a challenge.

Primary genital herpes is known to be asymptomatic in most cases[13]. The few symptomatic cases usually present as mild genital vesico-ulcerative lesions, which tend to resolve so spontaneously and rapidly that patients hardly recall the episodes[1,14]. Other associated features of the primary disease, such as genito-urinary discharges, are usually either overlooked or unnoticed by physicians and patients[2,11,15]. Consequently, obstetric complications tend to be the sole pointer to the prior existence of the infection[12,13,16]. It is imperative that improved case detection through routine type-specific HSV antibody screening, as practiced in New Zealand and Australia, will contribute to preventing these complications[17,18].

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But resource-limitations make this an unfeasible option in Nigeria and other resource-poor settings. There's, thus, a near-total dependence on clinical features in the detection of cases in the health-care centres located in these settings. Even before deciding to visit the health-care centers, recognition of the symptoms of the disease by the patient is critical to initiation of this health-seeking behavior, which usually marks the beginning of the case detection process. But the predominant incidence of clinically inapparent infections hampers this important phase of case detection. The clinically inapparent nature of these infections may be due to inaccurate definition of the symptoms perculiar to the region, and this could be related to the paucity of local studies on this subject in such regions. Excessive focus on genital ulceration as the classical presenting feature seems partly responsible for the categorization of most cases as asymptomatic[14]. This is because of the low prevalence of this symptom in these settings[13,16]. It has also been observed among pregnant women in Nigeria that ulcerations in the genital area are not quite noticed or taken as disease episodes[14]. In a cross-sectional study, only 8.4% of HSV-2 seropositive participants could recall episodes of ulcerative features[14]. They tended to better recognize and associate the term genital rashes with disease events[14]. Clear definition of the symptoms (especially, patient-recognizable symptoms) of incident HSV-2 infection in these local settings is imperative. In support of this imperative is the finding that studied definition and communication of the clinical features of HSV-2 infection among a group of USA women lead to clinical recognition of cases previously classified as asymptomatic[19]. But this prospect in Nigeria is partly hampered by the lack of local research data on this subject. Furthermore, the near-100% seroprevalence of HSV-1 infection potentially modulates the clinical features of HSV-2 infection, such that typical symptoms may become modified or absent; and other associated features may become more apparent[3,8,14]. While genital ulcers have been shown to be uncommon among the HSV-2-infected, some patient-recognizable symptoms have been shown to be more significantly associated with HSV-2-seropositivity[14]. These symptoms include genital rashes (rashes of any morphology occurring on any aspect of the external genitalia), urethral discharge and abnormal vaginal discharge.

Considering the fact that regional factors could affect the epidemiology and clinical features of HSV-2 infection[5], there is a need to assess the validity of these patient-recognizable symptoms as indicators of incident HSV-2 infection in this environment. The findings will provide some evidence-base on which health-care centres in resource-poor settings will be able to formulate valid symptom-based screening instruments, essential for presumptive detection of cases before possible type-specific serodiagnosis and/or molecular diagnosis. Such validated symptom-based case definitions in terms recognizable by the average pregnant women will also make public health campaign communications effective. Recognition of the validated symptoms by members of the community will promote health-seeking behavioral response to the infection; and so enable initiation of the diagnostic process. This study is aimed at validating the above-named patient-recognizable symptoms for possible use in presumptive detection of incident HSV-2 infection in resource-poor communities.

2. Materials and methods

2.1. Study location, design and duration

The participants were drawn from the antenatal clinic of the two major tertiary hospitals in Benin metropolis: University of Benin Teaching Hospital and Central Hospital, Benin-City. This longitudinal study took place between November 2011 and September, 2012.

2.2. Sampling and data collection

The study was approved by the Ethical Committee of University of Benin Teaching Hospital, Benin. Informed consent was obtained from the participants. The consenting participants were consecutively recruited.

Sociodemographic, obstetric data and data on incidence of specific symptoms were collected using structured interviewer-administered questionnaire and hospital case records. Blood samples were obtained from the participants and the serum was used for glycoprotein G-based type-specific assay for HSV-2 IgG antibodies. The HSV-2 IgG-seronegative participants were asked to return for a repeat HSV-2 IgG and HSV-2 IgM assays on a later date to assess seroconversion. To this end, a consensus appointment date was selected for each follow-up participant. This appointment date was selected to fit, as well as possible into the usual ante-natal clinic appointment pattern, while being about 2 weeks to the expected date of delivery.

On return, further data was collected on the incidence of the following symptom types: genital rashes (rashes of any morphology occurring on the external genitalia), abnormal vaginal discharge, urethral discharge, and painful genital ulcerations.

2.2.1. Research assistants

These consisted of laboratory technicians attached to the antenatal clinic laboratories; and nurses attached to the antenatal clinics. They were trained on the baseline and follow-up protocols. They maintained adequate contact with the follow-up participants in order to encourage compliance with clinic appointments.

2.2.2. Laboratory procedures

Blood samples were collected in 5 mL plain vacutainer tubes and allowed to clot and sera separated by centrifugation at room temperature. Storage of serum was in cryovials at -20 ℃.

The HSV-2 IgG and IgM assays were by means of ELISA kits produced by Dia. Pro. Diagnostic Bioprobes, Milano, Italy. These are based on glycoprotein G-dependent ELISA technique. All specimens and kit reagents were brought to room temperature and gently mixed before the assays. Procedures were performed in accordance with instructions in the kit manuals[20,21]. Each batch of tests ran with both positive and negative controls and results were qualitative.

2.3. Data analysis

Seroconversion was defined as the detection of HSV-2 antibodies of either IgG or IgM isotype by HSV gG2-based type-specific ELISA assay more than 30 days after previous HSV-2 seronegative assay result[22].

Data collected was analyzed using the Excel 2007 and SPSS version 16 computer software Specificities, sensitivities and relative risks of genital rashes, urethral discharges, vaginal discharges and genital ulcers were calculated in accordance with known standards[23]. Fishers exact test and *Chi* square was used to test associations and statistical significance was ascribed based on P-values < 0.05.

3. Results

3.1. Characteristics of the participants

3.1.1. Baseline study population

Six hundred and seventy four participants were recruited. The ages of these participants were between 18 and 44 years (mean = 30.6 ± 5.2 years).

Most (85.2%) of the participants were married. They were either Christians or Muslims, in the ratio 4.7:1. Those who had good education were also in the majority. Only 6.5% did not achieve complete secondary education (Table 1).

Table 1
Sociodemographic characteristics of participants.

Characteristics		Freequency	Percent (%)
Age group (years)	15-20	7	1.0
	21-25	98	14.5
	26-30	242	35.9
	31-35	225	33.4
	36-40	86	12.8
	41-45	16	2.4
Religions	Christianity	556	82.5
	Islam	118	17.5
Marital statuses	Married	574	85.2
	Single	53	7.8
	Divorced	22	3.3
	Widowed	25	3.7
Levels of education	Graduate and above	210	31.2
	Post-secondary	292	43.3
	Secondary completed	128	19.0
	Secondary uncompleted	14	2.1
	Primary completed	27	4.0
	Primary uncompleted	3	0.4

Most (85.3%) of the participants were recruited either in their second or in their third trimesters of pregnancy; while only 14.7% were first seen in their first trimesters. They were predominantly nullipara (43.5%). Twenty-eight percent (28.2%) of them were primipara; while a total of 27.6% of them were multi-para. Only five (0.7%) of the participants were grand multipara. (Table 2)

Table 2
Obstetric characteristics of participants.

Characteristics		Frequency	Percent (%)
Gestational ages on recruitment	1st trimester	99	14.7
	2nd trimester	268	39.8
	3rd trimester	307	45.5
Parity	Nullipara	293	43.5
	Primipara	190	28.2
	Para-2	123	18.2
	Para-3	43	6.4
	Para-4	20	3.0
	Para-5 or more	5	0.7

3.1.2. Follow-up study population

Three hundred and twelve (46.3%) of the participants were HSV-2 seropositive while 362 (56.7%) were HSV-2-seronegative and therefore susceptible to primary HSV-2 infection. Of the HSV-2 seronegative, 60 were lost to follow-up, while only 302 completed the follow-up, giving a response rate of 83.4%. The 302 participants who completed the follow-up protocol constituted the follow-up study population. The sociodemographic and obstetric characteristics of the follow-up study population was not significantly different from those of the baseline population.

Seventeen (5.6%) of the 302 participants became HSV-2 IgG seropositive, while 285 (94.4%) remained HSV-2 seronegative. None of the participants tested positive on HSV-2 IgM assay. The follow-up study population was therefore categorized into seroconversion and no seroconversion cohorts.

3.2. Incidence of the symptoms among the seroconversion cohort members

The commonest complaint made by the members of the seroconversion cohort was abnormal vaginal discharge; and this was followed by complaints of rashes occurring in the external genitalia, urethral discharge and painful genital ulcers, in descending order of incidence (Table 3). There was no asymptomatic case.

Table 3
Incidence of the symptoms among the seroconverted.

Symptoms	Frequency (n = 17)	Incidence (%)
Abnormal vaginal discharge	14	82.4
Genital rashes	12	70.6
Urethral discharge	7	41.2
Painful ulcers at external genitalia	2	11.8

3.3. Occurrence of symptoms among the 'seroconversion cohort' members relative to the 'no seroconversion cohort' members

The risk ratios of incidence of abnormal vaginal discharges, genital rashes, urethral discharges and painful genital ulcers were 106.2, 83.1, 39.2, and 37.9 respectively. All values in the 95% confidence intervals (95% *CI*) of these risk ratios were > 1.0 (Table 4).

All the assessed symptom types had specificity values of > 95% for seroincident HSV-2 infection. Abnormal vaginal discharge and genital rashes had the highest sensitivity scores of 82.3% and 70.6% respectively. The respective positive predictive values of abnormal vaginal discharges, genital rashes, urethral discharges and painful genital ulcers were 53.8%, 60.0%, 58.3% and 66.7%; while the negative predictive values were 98.9%, 98.2%, 96.6%, and 95.0% (Table 4).

Table 4
Comparision of the occurrence of the symptoms between the seroconversion and the no seroconversion cohorts.

	Symptoms	Sero-conversion (n = 17)	No sero-conversion (n = 285)	Total	Risk ratio (95% *CI*)	*P*-value	Sensitivity/Specificity (%)
Genital rashes	Yes	12	8	20	83.1 (23.6-292.4)	0.001	70.6/97.2
	No	5	277	282			
Painful genital ulcers	Yes	2	1	3	37.9 (3.2-441.4)	0.009	28.6/99.6
	No	15	284	299			
Urethral discharges	Yes	7	5	12	39.2 (10.6-145.2)	0.001	41.2/98.2
	No	10	280	290			
Abnormal vaginal discharges	Yes	14	12	26	106.2 (26.9-419.7)	0.001	82.3/95.8
	No	3	273	276			

4. Discussion

An HSV-2 seroconversion rate of 5.6% was found among the sub-population of pregnant women who successfully completed the follow-up protocol; and this value could be taken to represent the seroconversion rate of the entire study population, as there was no significant difference between the characteristics of the follow-up study population and the baseline study population.

Each of the seroconverted participants presented with one or more of the four symptom types, *viz* painful genital ulcers, genital rashes, abnormal vaginal discharge and urethral discharge. No seroincident HSV-2 infection was asymptomatic. This is contrary to known reports that first episode HSV-2 infections are asymptomatic or unrecognized in up to 90% of cases[2,13].

Relative to the no seroconverson cohort, all these symptoms were found to be significantly associated with seroincident HSV-2 infection. This result supports earlier finding, in a cross-sectional study, of significantly greater prevalence of these symptoms among those who were HSV-2 seropositive, relative to their HSV-2 seronegative counterparts[14].

Although painful genital ulcers is known to be a classical presenting feature of first episode HSV-2 infections[1], its frequency was the lowest, in this study. Its sensitivity of 28.6% was the lowest, although it had the highest specificity and positive-predictive value of 99.6% and 66.7% respectively. The clinical utility of this symptom is limited by the low sensitivity. While the near-100% specificity justifies its categorization as a classical symptom, the 66.7% predictive value for incident HSV-2 infection places a significant limitation on its diagnostic utility in this environment. The relative risk of occurrence of this symptom among the seroconversion cohort, although significant, included values close to 1 (3.2-441.4). This finding of low incidence of genital ulcers corroborates previous reports[14,24]. Moreover, Lymphogranuloma venereum, and not genital herpes, has been shown to be the major cause of genital ulcer disease in a group of Nigerian females[25]. The finding that only 11.8% of the follow-up participants had the typical painful genital ulcer symptoms implies that 88.2% of them would have passed as asymptomatic if genital rashes, urethral discharges and abnormal vaginal discharges were not emphasized in the study protocol. It follows, therefore, that dependence on the genital ulcer symptom will lead to most cases of incident HSV-2 infections passing undetected.

Abnormal vaginal discharge was the most frequent and most significant symptom among the 'seroconversion cohort', judging by the high relative risk, sensitivity and specificity values of 106.2, 82.3% and 95.8%, respectively. This result agrees with a study report from India in which abnormal vaginal discharge was the most frequent feature among the HSV-2-infected[25]. Moreso, previous history of urethritis of *Chlamydia* spp. and *Trichomonas* spp. origin, was significantly associated with HSV-2 infection as reported in Australia and India[26,27]. Moreover, the highly significant rating of vaginal discharge, as found in this study, is in keeping with the previously reported synergistic association between bacterial vaginosis and HSV-2 infection[26,28]. The 53.8% predictive value of this symptom for incident HSV-2 infection is indicative of the possible role of other genito-urinary diseases in producing this symptom[29]. The current practice in which HSV-2 infection is usually not a diagnostic consideration either in syndromic or aetiologic management of vaginal discharges should be reviewed, especially with respect to women of reproductive age.

Genital rashes was the most sensitive and most frequent symptom, after abnormal vaginal discharges, among the seroconversion cohort. Although incident HSV-2 infections are typically marked by vesicular lesions, the findings of this study suggests that public enlightenment campaigns should promote genital rashes as a defining symptom type in developing countries where low literacy level may not enable majority of patients to categorize cutaneous eruptions. Effective communication demands the use of such patient-recognizable symptoms in case definitions. The 97.2% specificity, 70.6% sensitivity and 98.0% negative-predictive values of this symptom were high enough to warrant its use in a screening tool. With its positive-predictive value of 60%, its combination with genitourinary discharges will increase the validity of the screening tool. This term genital rashes seems recommendable as a syndrome in sexually transmitted infectious disease management, especially in developing countries. In its utilization, HSV-2 infection should be a principal differential diagnosis. This is in keeping with a previous study conclusion that HSV-2 antibody assay should be performed on any patient presenting with uncertain genital symptoms[30].

It is instructive, from this study, that proper definition of the symptoms of incident HSV-2 infection and education of the public will improve case suspicion/recognition, which will in-turn persuade the patient to seek professional attention. All the symptoms had negative-predictive values of 95%. None of them had very high positive-predictive values, which implies that none of them should be used in isolation in making clinical impressions. A combination of the symptoms makes them useful for screening and public health campaigns so that opportunities for interventions would not be lost just because the patient could not find a reason to initiate health-seeking activity.

Since sexually transmitted infection control ought to be wholistic, the enhanced relevance and promotion of these typical sexually transmitted infection syndromes (vaginal discharge and urethral discharge) and 'genital rashes' in the public health control of HSV-2 infection will exert a ripple effect on the control of other sexually transmitted infections. Moreover, the combined validity parameters of abnormal vaginal discharge and genital rashes suggests that both symptoms could form the basis of a clinical screening tool for incident HSV-2 infection, especially in resource-limited settings.

Conflict of interest statement

We declare that we have no conflict of interest.

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