Herpes Simplex Virus Gene Variants among Asymptomatic Women in Ghana: a pilot study

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Abstract

Herpes simplex virus infections account for a large burden of disease worldwide. HSV-1 is traditionally considered to cause orofacial infections, whereas HSV-2 is known for genital infections. A number of studies have suggested an increase of genital herpes infections caused by HSV-1. As reporting of diseases caused by herpes simplex virus is not mandatory in Ghana, reliable statistics on the epidemiology of infections are not available. We took advantage of the Cervicare program in Ghana to screen for the presence of HSV variants 1 and 2 among a convenient subset of asymptomatic women presenting for cervical screening in Accra, Ghana (n = 94). Genetic markers for both HSV 1 and 2 were detected in cervical swabs. There was a preponderance of HSV-1 (12.8%) genital infections in our study sample: compared to HSV-2 (4.8%). HSV-1 and 2 co-infection was detected in 4.3% of study population. Among positive cases for HSV-1 DNA, 92% had confirmed seropositive HSV-1 status and 8% were borderline result. All positive HSV-2 DNA were confirmed seropositive HSV-2 status. We have successfully demonstrated the presence of herpes simplex virus type 1 and type 2 gene variants in genital swabs. Owing to the lack of epidemiological data on genital HSV-1 infection in Ghana, the role of sexual transmission for HSV-1 is unclear: the findings of our pilot study have important public health implications. A bigger surveillance study is recommended in Ghana to identify the etiology of genital herpes and estimate the true burden of asymptomatic herpes infection in the population.

1. Introduction

Herpes Simplex Virus (HSV) is a well-known pathogen which accounts for lifelong infections in humans. Globally, millions of people are infected with either one or both serotypes of the virus with 19.2 million new infections occurring yearly [1, 2]. Two types of viruses most commonly cause all clinical manifestations: HSV type 1 and HSV type 2. HSV type 1 typically causes orofacial infections, whereas HSV type 2 is better known for causing genital herpes and is the leading cause of genital ulcer disease worldwide [3, 4]. Clinical data suggests an increasing number of genital infections caused by HSV – 1, although HSV – 2 is a predominate agent [5]. The virus is capable of causing recurrent infections in the host due to its ability to go into a latency state [4, 6, 7, 8]. HSV also responsible for neonatal herpes infections, genital ulcer disease and increases the acquisition of other sexually transmitted infections such as Human Immunodeficiency Virus (HIV) and Human Papillomavirus (HPV) [1, 3]. Modern lifestyles such as oral sex and multiple sexual relationships contribute to the spread of the virus. In their review article, Looker *et al.* (2015), reported a high incidence of genital HSV-1 cases in America and European, and negligible incidence in Africa and South East Asia [9]. Although the prevalence of HSV infection in Ghana is also thought to be high [5, 9, 10], case reporting is not mandatory in Ghana. Consequently, statistics on the occurrence of infections are not available in Ghana, as well as in most African countries. The few available data on the prevalence of infection were generally achieved in isolated studies conducted in public health clinics on sexually transmitted diseases; and mostly based on serology [10, 11, 12].

Due to the lack of vaccines and medications to aid in the treatment and elimination of the virus [3, 8] it is important to develop sensitive methods to distinguish genital HSV-1 from HSV-2 in clinical settings to better predict symptoms and response to treatment [13]. The aim of this study was to optimize an assay for reporting the epidemiology of herpes simplex virus type 1 and type 2 gene variants in Ghana. .

2. Materials And Methods

2.1. Study Design and Settings

In 2000, the Ministry of Health (MOH) began to establish Cervicare Centers in selected regional and district facilities across the country to expand access to cervical screening services [14]. Two of such centers is situated at the Greater Accra Regional Hospital in Accra, the capital of Ghana and Kumasi South Hospital in Kumasi in the middle-belt of Ghana. Between October 2013 and March 2014, we undertook a cross-sectional study on the distribution of genital human papillomaviruses and seroprevalence of herpes infection among Ghanaian women presenting to this facility [10, 15]. In all, three hundred and eighty (380) women attending routine Cervicare Centers at two Regional Hospitals (Greater Accra Regional Hospital, Accra and Kumasi South Regional Hospital, Kumasi) were enrolled in the parent study. An opportunistic sub-set of asymptomatic non-pregnant females above the age of 20 who attended the Cervicare Centers for Papanicolaou (Pap) smear test or for visual inspection with acetic acid was randomly drawn for the present study. For the purposes of this pilot, cervical swabs from a total of 94 women attending Cervicare services for the first time at the Greater Accra Regional Hospital, Accra were randomly selected using computer-generated codes. In addition, the serological HSV status of these women were known.

The study was approved by the Ghana Health Service Ethical Review Committee, Research and Development Division, Accra, Ghana (GHS-ERC:07/03/14) and from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences, University of Ghana (SBAHS-MLS./10572569/SA/2018–2019).

2.2. Data Collection and Analysis

During recruitment, all participants were fully informed about the purpose, procedures, risks, and benefits of participating in this study and required to fill and sign an informed consent and to respond to a questionnaire with the researcher's assistance. Information was obtained on socio-demographical, behavioral, clinical characteristics and knowledge of HSV infection. Data were stored using Microsoft Excel software after checking for completeness and consistency, and then exported to SPSS version 20 for analysis. Percentages were used to analyze qualitative variables. Association between HSV and demographical and behavioral factors were established by the Fisher's exact test. Statistical significance was set at a $p < 0.05$.

2.3. Sample Collection

Cytological specimens were collected from all participants by trained nurses, using the Pap Pak cytology kit (Medical Packaging Corporation, Camarillo, CA, USA) according to the manufacturer's instructions. The samples were stored in DNAgard (Biomatrica Co., San Diego, USA) for DNA preservation at room temperature and then sent to a laboratory where it was processed for DNA extraction.

2.4. DNA Extraction and PCR Analysis

The tubes containing the genital specimens were submitted to vigorous agitation before removal of the sterile swabs and were centrifuged at 300 g for 10 min. The supernatant was removed and the resulting pellet was processed for DNA extraction using commercial spin-column based QIAamp Mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions.

For HSV PCR analysis, the method described by Blankson *et al.*, (2019) was used [16]. In brief, the following primers were used in the work: for HSV-1: 5'-CGTACCTGCGGCTCGTGAAGT-3' (forward) and 5'-AGCAGGGTGCTCGTGTATGGGC-3' (reverse); for HSV-2: 5'-TGGTATCGCATGGGAGACAAT-3' (forward) and 5'-CTCCGTCCAGTCGTTTATCTTG-3'. A final PCR reaction mix of 25 µl was used containing 5X PCR buffer, 5mM MgCl₂, 200 µM of each of the four deoxyribonucleoside triphosphates (dNTP), 10 pmols of each primer and 1.25 units of Taq polymerase enzyme (New England Biolabs Inc., UK). Five microlitres (5 µl) of purified DNA extracts was used as template for the amplification reactions carried out on the MAXYGENE II Thermal Cycler (Corning Life Sciences Company, USA). The cycling parameters for PCR was as follows: 95° C for 5 minutes (initial denaturation), followed by 45 cycles of 95° C for 30sec (denaturation), 54° C for 30sec (annealing), 72° C for 30 sec (extension) and a single final elongation step of 72° C for 5 minutes.

2.5. Detection of PCR Products

The amplified PCR products were detected by agarose gel electrophoresis (2%) containing ethidium bromide. Ten microlitres of each sample was added to 2 µl of 6X gel loading dye (New England BioLabs Inc. USA) for the electrophoresis. Hundred base pair DNA molecular weight marker (New England Biolabs Inc., USA) was run alongside the PCR products. The gel was prepared and electrophoresed in 1X TAE buffer using a mini gel system at 100 volts for one hour. The gels were viewed in a benchtop UV illuminator (UVP, LLC, Upland, CA, USA) and photographed using Canon camera Sx230 HS.

3. Results

HSV Genotypic Prevalence

As shown in Table 1, cervical and vaginal swabs from ninety-four women were analyzed. Twelve women, representing 12.8% (95% CI: 6.8-21.2%) tested positive for genital HSV-1, whereas 4.3% (95% CI: 1.2-10.5%) were positive for genital HSV-2. Individuals with both variants of HSV infection occurring simultaneously represented 4.3% of women. Among twelve positive cases for HSV-1, eleven (92%) patients had confirmed seropositive HSV-1 status, and 8% (n=1) with borderline results. All PCR-positive cases for genital HSV-2 were seropositive HSV-2 as well (Table 2).

Associated Risk Factors

Prevalence of genital HSV infection (both types) was highest among women in age category 25 and 44 years, who are active (currently have work, private or government) and married or cohabiting (Table 1).

Earlier age of first intercourse was associated with higher HSV-2 positivity (Table 3). This was absent for HSV-1. Genital HSV-1 and HSV-2 prevalence was unrelated to increased number of sexual partners. However, the prevalence of both HSV strains was associated with condom use.

4. Discussion

The present study is the first study to report on the PCR detection of genital HSV-1 infection among women of reproductive age in Ghana. In this study, a higher prevalence of genital HSV-1 compared with genital HSV-2 was found. Single and multiple herpes simplex virus type 1 and type 2 gene variants were observed in the present study among asymptomatic women in Ghana.

In the past decade, a number of studies documented an increase in the frequency of genital HSV-1 compared with genital HSV-2 infection [17, 18, 19, 20, 21, 22, 23]. In contrast to what has been found in Africa [9, 24].

A reason for this increased genital HSV-1 infection may be the practice of oral-genital sex. Limiting sexual activity to oral-genital contact could be attractive to lovers for a number of reasons such as maintaining virginity and to avoid unwanted pregnancies and the erroneously belief that oral-genital sex does not transmit infectious diseases [13, 25].

Genital-oral contact with a partner who has oral-labial herpes, which is almost always caused by HSV-1, leads to an increased risk of HSV-1 acquisition [26]. Occurrence of primary genital HSV-1 infection late in pregnancy increases the risk for transmitting herpes infection to the newborn [27]. Therefore, persons who test negative for HSV-1 must be counseled, especially HSV-1- seronegative pregnant women.

Number of studies reported the low prevalence of genital HSV-1 infection due to the low frequency of oral sex by nonwhites [27, 28]. Another reason for explanation for the low prevalence of genital HSV-1 in Africa can be due to endemicity leading to high acquisition of this infection during childhood [9]. A detailed assessment of HSV-1 epidemiology in Africa conducted by Harfouche *et al.* demonstrated that HSV-1 is universally prevalent, with nearly every person acquiring the infection by age of 15 years. The low detection in Africa is probably best explained by most persons reaching sexual debut already infected with HSV-1 and subsequent development of natural protective antibodies against genital acquisition [24].

Alternatively, the low prevalence of genital HSV-1 could reflect limited or nonexistent surveillance of genital herpes infection in most African countries [29]. The deployment of sensitive assays in Ghana for routine characterization of herpes simplex virus type 1 and type 2 gene variants will lead to better appreciation of the changing molecular epidemiology of the virus. Moreover, it is important to distinguish HSV-1 from HSV-2 for prediction of clinical symptoms and response to treatment [13]. Genital HSV-1

infections are characterized by higher symptomatic shadings [30], higher frequency of transmission to a new partner [31, 32], longer recurrence intervals, and higher rates of clinical recurrence [31]. Patients presenting with symptomatic genital HSV-2 can be anticipated to have significantly more morbidity than patients with genital HSV-1 infections [31, 32, 33].

Presence of genital HSV in the clinical specimens of asymptomatic women, raise a main concern on the possible increase on transmission of herpes infection [9]. Routine serological screening to identify unrecognized herpes infected individuals, even among pregnant women, are not readily available in Ghana. To prevent genital herpes infection transmission and reduce symptoms and shadings, the identification of asymptomatic individuals, followed by counseling and treatment are an important strategy. At the very least, screening on HSV infection should be considered among pregnant women, since the transmission of genital herpes infection from mother to newborn may be highly efficient and lead to adverse pregnancy outcome [34, 35].

5. Conclusion

The data from our pilot study represents a first attempt to identify genital herpes infection among asymptomatic women. The study also revealed that genital HSV-2 was higher among participants who had their first sexual relations in earlier age. Multiple sexual partners was not a risk for genital HSV-1 and HSV-2 infection, but not use of condom led to increase risk of genital herpes infection acquisition. The findings has important public health implications. The lack of data on prevalence of genital HSV-1 infection, remains the role of HSV-1 in sexual transmission unclear. It is a need to perform a surveillance in Ghana to identify the etiology of genital herpes and identify the true prevalence of asymptomatic herpes infection in population.

Abbreviations

HIV
Human Immunodeficiency Virus
HPV
Human Papillomavirus
HSV
Herpes Simplex Virus
MOH
Ministry of Health
Pap
Papanicolaou
PCR
Polymerase Chain Reaction.

Declarations

Ethical Approval and consent to participate

The study was approved by the Ghana Health Service Ethical Review Committee, Research and Development Division, Accra, Ghana (GHS-ERC:07/03/14) and from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences, University of Ghana (SBAHS-MLS./10572569/SA/2018-2019). Voluntary written informed consent was sought from the participants. The study was conducted in an environment with no form of coercion and volunteers were adequately informed of the purpose, nature and procedures of the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

OD formulated the concept, carried out the implementation of the research, was involved in the recruitment of the participants and data collection, performed laboratory analysis, analyzed and interpreted the data, wrote the manuscript with input from all authors. RHA formulated the concept, directed the implementation of research, supervised findings of this work and contributed to the final version of the manuscript. EGAA carried out the implementation of the research, performed laboratory analysis, analyzed and interpreted the data and contributed to the final version of the manuscript. ETD formulated the concept, was involved in the recruitment of the participants and data collection, and contributed to the analyses of the results and was a major contributor in writing the manuscript. FAY supervised findings of this work, and contributed to the final version of the manuscript. AJKA supervised findings of this work, and contributed to the final version of the manuscript.

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Authors' Information

Not applicable.

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Tables

Table 1. HSV prevalence according to demographic and obstetric characteristics

Characteristics	Prevalence	
	DNA HSV-1, N (%)	DNA HSV-2, N (%)
<i>Positive</i>	12 (12.8)	4 (4.3)
<i>Negative</i>	82 (87.2)	90 (95.7)
<i>Age category (years)</i>		
25-44	9 (75)	4 (100)
45-64	3 (25)	0 (0)
<i>Education</i>		
Less than SHS	8 (67)	1 (25)
SHS & more	4 (33)	3 (75)
<i>Work status</i>		
Active	11 (92)	4 (100)
Not active	1 (8)	0 (0)
<i>Marital status</i>		
Single/ widowed/ divorced	2 (17)	0 (0)
Married/ cohabiting	10 (83)	4 (100)
<i>Number of pregnancies</i>		
Never pregnant	1 (8)	0 (0)
1	2 (17)	1 (25)
2	2 (17)	0 (0)
More than 3	7 (58)	3 (75)
<i>Age of first pregnancy, years</i>		
18-21	1 (8)	0 (0)
22-25	4 (34)	2 (25)
>26	3 (25)	2 (25)
Never pregnant	1 (8)	0 (0)
Do not remember	3 (25)	0 (0)

Table 2. Genotypic HSV prevalence among HSV sero-positive and HSV sero-negative participants

Parameters	DNA HSV-1		DNA HSV-2	
	Positive (n=12)	Negative (n=82)	Positive (n=4)	Negative (n=90)
<i>HSV-1 IgG positive, N (%)</i>	11 (92)	81 (99)	4 (100)	88 (98)
<i>HSV-1 IgG negative/borderline, N (%)</i>	1 (8)	1 (1)	0 (0)	2 (2)
<i>HSV-2 IgG positive, N (%)</i>	9 (75)	58 (71)	4 (100)	63 (70)
<i>HSV-2 IgG negative/borderline, N (%)</i>	3 (25)	24 (29)	0 (0)	27 (30)
<i>HSV-1⁺ IgG & HSV-2⁺ IgG, N (%)</i>	9 (75)	58 (71)	4 (100)	64 (71)

Table 3. Prevalence of HSV infection according to behavioral characteristics

Characteristics	Prevalence	
	DNA HSV-1, N (%)	DNA HSV-2, N (%)
<i>Age of coitache (years)</i>		
16-20	3 (25)	2 (50)
21-25	3 (25)	1 (25)
>26	3 (25)	1 (25)
Do not remember	3 (25)	0 (0)
<i>Number of sexual partners</i>		
1	7 (58)	2 (50)
2 - 10 ⁺	5 (42)	2 (50)
<i>Condom use</i>		
Yes	2 (17)	1 (25)
No	10 (83)	3 (75)
<i>Tobacco use</i>		
Yes	0 (0)	0 (0)
No	12 (100)	4 (100)
<i>Alcohol use</i>		
Yes	4 (33)	1 (25)
No	8 (67)	3 (75)
<i>Alcohol use frequency</i>		
Never indulge	8 (67)	0 (0)
Indulge occasionally	3 (25)	1 (25)
Indulge regularly	1 (8)	0 (0)