



Genital Herpes: Synergy between Serology and Polymerase Chain Reaction in Laboratory Diagnosis among Pregnant Women Attending the Antenatal Clinic of Federal Medical Centre, Yenagoa Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author JEE designed the study and first draft of the manuscript. Authors ABA and JEE wrote the protocols. Authors ABA, MAA and JEE conducted the laboratory protocols and analysis of the study. Authors MAA and AOE managed the literature searches. Authors AOE and ABA performed and interpret the statistical analysis. Author ABA reviewed final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Herpes simplex type 2 (HSV-2) is the main cause of Genital Herpes (GH) infection worldwide with developing countries having larger proportions. Little is known about its diagnosis using the synergy techniques of Immunoglobulin G (IgG) and Polymerase chain reaction (PCR) for its accurate diagnosis. This study focuses on carrying out the synergy (concurrent) between serology and PCR techniques for diagnosing GH caused by HSV-2.

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Methods: A total of 388 pregnant women were sampled using a well-structured questionnaire, a self-collected vaginal swab for PCR shedding detection and 10 ml of blood collected for serological assay. Data were analyzed using statistical packages for Social sciences. P value were kept at <0.05.
Results: A total 85 (21.9%) of the 388 sampled women were positive for Herpes simplex type 2 by PCR shedding techniques. Majority of the samples 345 (88.9%) were positive to serology assay. 57 (16.5%) of all 345 seropositive respondent are also shedders of the virus by PCR assays, p <0.05. Conversely, 28 (65.1%) of all 43 seronegative patients were shedders of HSV-2.
Conclusion: The synergistic technique of Serology and PCR should be considered incorporated as detection techniques for accurate diagnosis as this will assist in prompt management of genital herpes infection.

Keywords: Genital herpes; HSV-2; PCR; serology; pregnant woman.

1. INTRODUCTION

Herpes Simplex type 2 (HSV-2) is documented to be the key course of genital herpes (GH) globally [1]. It is an infection associated with a DNA virus that belongs to the *Herpesviridae* family, subfamily *Alphaherpesvirinae*, and genus Herpes Simplex virus [2,3]. GH is among the commonly sexually transmitted disease in women of reproductive age [3,4]. The greater risk associated with GH involves psychological and physical impact on those infected and feasibly spread and acquisition of HIV to partner [3].

Though under reported in the developing countries due to limited documentation, the prevalence of HSV-2 is considered to be more prevalent in developing countries than in developed ones [5-7]. There is limited evidence in the use of synergy techniques using combination of serology and polymerase chain reaction (PCR) simultaneously. However, the combination of these diagnostics methods have been reported to assist the physician in making better decision on its prognosis and management [8-10].

This study focuses on the synergy between Serology assay using immunoglobulin G (IgG) and Polymerase chain reaction outcome variables for the accurate diagnosis and management of GH.

2. MATERIALS AND METHODS

2.1 Study Population

A cross-sectional descriptive and analytical study involving a cohort of pregnant women aged between 18-50years attending the antenatal clinic of Federal Medical Centre Yenagoa were

studied between the month of June through December 2019.

After obtaining Ethical clearance from the FMC ethical review committee and verbal consent from the patients, a written structured questionnaire was distributed to collect information from the subjects.

The inclusion criteria includes asymptomatic pregnancy and the individual must have attained the aged range between 18-50 years old.

2.2 Laboratory Methods

Aseptically, 10 mL of venous blood was collected into a sterile plain specimen bottle. Serologically, the screening for HSV-2 specific IgG was determined using HerpeSelect 2 (ELISA IgG kit, Focus Diagnostics, USA). Procedure was done in accordance with the manufacturer's techniques. Serum was separated and used immediately for serological assays, and where not possible, it was stored at 2-8⁰C for future use. Also, where the procedure could not be completed within 48 hours, they were frozen at -20⁰C. Samples were thawed and mixed prior to use. The pregnant women were instructed to self-collect vagina swab as described by McNicholl et al. [11], by inserting a sterile Dacron swab stick (Boen Healthcare Co., Ltd., Suzhou, China) 2cm into the vagina, rotate and given back. The swab tips were then broken off and inserted into a viral transport media containing phosphate buffered saline. These were stored in iced and transported to the Molecular Diagnostic Laboratory of the Bayelsa Medical University, Yenagoa for real time PCR determination in accordance with the procedure earlier described by Kessler et al. [12]. Briefly, HSV-2 DNA shedding were detected by amplifying HSV-2 gene using the primers gD gene (bp71) (forward primer 5'CGCCAAATACGCCTTAGCA-3';

reverse primer, 5'GAGGTTCTTCCCCGCGAAAT-3'), [13]. Extractions were performed under sterile conditions with commercial kits (Qiamp DNA mini and DNA Purification kits, Qiagen, UK) in accordance with the manufacturer's directions. The whole amplification was done using the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Netherlands). 50 µL extracted DNA was added to a master mix that consisted of forward primer (45 pmol/µL), 2.5 pmol/µL of reverse primer, 5 pmol/µL of Taqman probe, and 20 µL of isolated DNA. After incubation at 50°C for 2 mins and incubation at 95°C for 10 mins, the samples were amplified for 45 cycles at 95°C for 15 s and at 60°C for 1 min. The amplified genes were confirmed under fluorescence at the end of the extension step (performed at 60°C) of each cycle.

2.3 Data Entry and Analysis

Questionnaire responses and results from both serology and PCR were entered into Microsoft excel package 2010 and analyzed using a statistical software package 'IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA) [14]. Chi square was used to determine the association between HSV-2 viral shedding and sero prevalence. Level of confidence was set at 95%.

3. RESULTS

Three hundred and eighty-eight (388) asymptomatic pregnant women with mean age of 32 (range of 20 to 44) years were screened for HSV-2 (Table 1).

As shown in the table, age group of 30 - 34 years were the majority 137(35.3%), this is closely followed by age brackets of 25-29 years with frequency of 135(34.5%). Most of the sampled women were urban dwellers 221(63.5%) and are literates with tertiary education 245(63.1%). Majority of the subjects are married 351(91.2%).

Table 2 shows the PCR outcome. As shown, 85 (21.9%) positive cases of HSV-2 shedding were observed. However, majority 345(88.9%) of the pregnant women were positive to serological assays (Table 3).

Table 4 illustrates the sero-prevalence of HSV-2 (IgG) in relation to its shedding. As shown, 57(16.5%) of the 345 seropositive subjects are shedders of the virus when assayed by the PCR, while 28(65.1%) of 43 sero-negative subjects were shedders of HSV-2. Thus, there is significant difference between these relationships ($p < 0.05$).

Table 1. Sociodemographic characteristic of patients studied

Characteristics	Total Number (%) of Patients
Age	
20-24	16 (4.1)
25-29	135 (34.5)
30-34	137 (35.3)
35-39	67 (17.2)
40-44	36 (9.3)
Education	
Primary School	21 (5.4)
Secondary School	122 (31.4)
Tertiary	245 (63.1)
Place of Residence (location)	
Rural Area	57 (16.4)
Semi Urban Area	70 (20.1)
Urban Area	221 (63.5)
Marital Status	
Single	28 (7.2)
Married	351 (91.2)
Separated	6 (1.6)
Occupation	
Public Servant	98 (25.2)
Private Employment	104 (26.8)
Trading/Business	105(27.1)
Unemployed	76 (19.6)

Table 2. Seroprevalence of HSV-2

HSV 2 ELISA	Frequency Number (%)	95% C.I
Positive	345 (88.9)	79.891-87.5627
Negative	43 (11.1)	12.437-20.1099
TOTAL	388 (100.0)	

Table 3. Results of HSV-2 PCR

HSV 2 PCR	Frequency Number (%)	95% CI
Positive	85(21.9)	16.0689-24.4122
Negative	303 (78.1)	75.5878-83.9311
TOTAL	388 (100.0)	

Table 4. HSV-2 Sero-prevalence in comparison to its shedding

ELISA Result	Result of Real Time PCR		Total
	No. Positive (%)	No. Negative (%)	
Seronegative	28 (65.1)	15 (34.9)	43
Seropositive	57 (16.5)	288 (83.5)	345
Total	85 (21.9)	303 (79.1)	388

$$\chi^2=49.56 \quad P<0.05$$

4. DISCUSSION

We set out to investigate the reliability of a single diagnosis with synergic diagnosis of herpes simplex-2 infection amongst asymptomatic pregnant women attending FMC, Yenagoa in Bayelsa-State by employing the serological assay and real-time PCR assay. This study is vital because genital herpes has been considered to be lifetime infection that is associated with considerable morbidity to those infected and is also associated with severe outcomes, which includes neonatal herpes and increased risk for acquiring and transmission of human immunodeficiency virus (HIV) [15], it is however often a times under reported or neglected despite its significant contribution to sexually transmitted infections. One of such reasons maybe due to the clinical signs and symptoms of this infection that is often subtle, with most of its infection unidentified leading to it been undiagnosed [16,17]. However, studies have it that regardless of whether there are apparent clinical lesions, the infected individual continuous to shed the virus intermittently [18].

Serologically, 345(88.9%) of pregnant women attendees of the antenatal clinic were diagnosed seropositive. Thus an outcome, suggesting higher prevalence rate of GH (88.9%). On the other hand, only 57(16.5%) of these 345 seropositive patients were shedders of the virus when subjected to PCR assay. However, when the remaining 43 seronegative patients were

subjected to PCR assay, 28(65.1%) of them were shedders of herpes virus. Several evidences have been documented for recording higher seropositive results for herpes viruses without the shedding of the virus. Amongst these are:

- 1- That only few of these available tests are capable of differentiating between HSV-1 and HSV-2 [19,20].
- 2- There is no serological test that is able to differentiate between the oral and genital infection with HSV [21]. This is important to our studied location, for it being known endemic for malaria fever, majority of the habitants are positive for HSV-1 or oral HSV (locally referred to as after-malaria symptom). This indicates that there is need for both the serological and PCR assays been employed in synergy as laboratory diagnostics for accurate diagnosis and effective management of GH in our locality. Though, our locality been situated in a poor environment, the cost implication of this applications must also be put into consideration.
- 3- Seroreversion or waning of immune response to gG-2 occurs with time. and
- 4- The usefulness of any laboratory test for the diagnosis of HSV infection also relies mostly on the type of test conducted, the specimen obtained in terms of quality, the ability of the laboratory staff to perform the test accurately, and the interpretation of the test results [19].

Though, various investigators have previously studied the prevalence of HSV, to our knowledge, this will be the first comparative study employing both serological and PCR assays in our locality. The outcome of 88.9% prevalence rate in the serological assay is in agreement with earlier studies conducted in developing countries, Nigeria inclusive [5,7,22]. And the outcome is in sharp contrast to the lower prevalence rate reported from the developed countries [23,24].

The increased prevalence rate recorded when conducting serological assay doesn't necessary mean that there is an increase in or presence of shedding of the virus, however, some studies, particularly those in some developing countries have observed that the increase in serological assays leads to an increase in the shedding of the virus amongst those that were sero-positives [25,26]. Thus, underscoring the need for conducting a synergy assays using both serological and PCR. In particular, amongst pregnant women that are considered to have primary infection, reactivation and shedding of the virus more frequently due to decreased immune competence [27] which could lead to more transmission to the unborn child resulting in complications such as preterm labour, fetal growth retardation and still birth [28,29,25].

As revealed in this study, 21.9% [85 (57 seropositive; 28 seronegative)] are shedders of the virus, thus making it an equivalent prevalence rate of 22%. This is slightly higher than the 14% reported in the study conducted in Gabon by Ozouaki and Colleagues [30]. The status of been seronegative may be seen in patients with acute infection or in those at risk for acquiring the infection, while a seropositive status is seen in patients with latent or recurrent infections [31]. On the other hand, lower rates of shedding of the virus has been documented and this discrepancy has been attributed to [32]:

- i) Frequency of specimen collection and periodic changes in herpes viral shedding [25,33].
- ii) The type of test, the quality of the specimen obtained, the ability of the laboratory to perform the test accurately, and the interpretation of the test results by the requesting clinician [19].

It is of utmost importance to indicate that of all the 345 seropositives in the present study, only

57(16.5%) were shedders of herpes virus. This outcome is in consistent with earlier studies, [30,20,34,35] and the implication of this outcome is that immunoglobulin G (IgG) detects and act as a marker for past and not necessarily present infections, thus it has the ability to protect against the viral shedding since it stays in the body for a lifetime [32]. As such there is the need for synergy assays to detect the shedding of the virus is necessary or investigate if there are presence of genital lesions, thus the need for type-specific serology using immunoglobulin M (IgM) and direct virus testing which can help to establish if the episode is a new HSV infection or reactivation.

In an earlier study, in order to investigate the relationship between the ability of shedding HSV-2 virus among HIV positive patients, Augenbraun et al. [35] reported an increased in the shedding of HSV-2 virus amongst individuals that were HIV seropositive but were HSV-2 seronegative. The reason been that HIV seropositive individual are presumed to be immunocompromised which could lead to increase in the shedding of HSV-2. This reported outcome is comparable to the present study were 39.4% of patients were seronegative but were found to be shedders of the virus. In other words, this result underscores the significance of synergistic (combinatory) laboratory diagnosis of genital herpes using both type specific serology assays and PCR in order to enhance effective and specific management of genital herpes infection in our environment.

5. CONCLUSION

There is the need for laboratory confirmation of clinically suspected genital herpes diagnosis for this will help in the therapeutic management of ulcerative genital lesions, It is also important to considered the incorporation of combinatory (synergy) techniques of Serology and PCR as detection techniques among routine screening of pregnant women attending antenatal clinics for this will allow accurate diagnosis as well as assisting in the prompt management of genital herpes infections. Because both techniques will help to identify pregnant women that are at the risk of transmitting HSV infections.

6. LIMITATIONS

This study is limited in its scope by its failure to detect genital herpes caused by herpes simplex type 1 which also causes genital herpes in lower percentage of cases.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

After obtaining Ethical clearance from the FMC ethical review committee and verbal consent from the patients, a written structured questionnaire was distributed to collect information from the subjects.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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