

**KNOWLEDGE, AWARENESS AND SEROEPIDEMIOLOGY OF HERPES SIMPLEX
VIRUS TYPE 1 INFECTION AMONG PREGNANT WOMEN IN BENIN CITY, EDO
STATE, NIGERIA.**



BY:

**EDOZIUNO CHUKWUNONSO EMMANUEL
BMS1702075**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY.**

SEPTEMBER, 2023.

**KNOWLEDGE, AWARENESS AND SEROEPIDEMIOLOGY OF HERPES SIMPLEX
VIRUS TYPE 1 INFECTION AMONG PREGNANT WOMEN IN BENIN CITY, EDO
STATE, NIGERIA.**

BY:

**EDOZIUNO CHUKWUNONSO EMMANUEL
BMS1702075**

**A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY
SCIENCE, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE AWARD OF “BACHELOR OF MEDICAL LABORATORY
SCIENCE” (BMLS) DEGREE**

SUPERVISED BY:

DR. (MRS.) MOSES-OTUTU

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that this seminar work was carried out by **EDOZIUNO CHUKWUNONSO EMMANUEL** with the matriculation number **BMS1702075** under the supervision of **DR. MRS. MOSES OTUTU** in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

DR. (MRS) I. M. MOSES-OTUTU
(Project Supervisor)

DATE

Dr. B. I. G. Adejumo
(Head Of Department)

DATE

External Examiner

DATE

DEDICATION

I dedicate this research project to my family and friends, who have always believed in me and supported me through my academic journey.

ACKNOWLEDGEMENT

Firstly, my gratitude goes to God Almighty for the success of this work, for blessing me with life, strength and understanding throughout the course of this work. I would like to express my deepest gratitude to my project supervisor **DR. (MRS.) I. M. MOSES OTUTU** for her invaluable guidance, support and encouragement throughout the project. Their expertise and insight have been instrumental in shaping my research and have helped me to develop a deeper understanding of the subject matter.

Special thanks to the Head of Department, Medical Laboratory Science, **Dr. B. I. G. Adejumo** and also to the entire staff of the department for investing so much in my academic development. I want to appreciate once more Dr, (Mrs.) Moses Otutu, Dr. (Mrs.) S. Aigbodion, Prof. Okungbowa, Dr. (Mrs.) Olise, and also Dr. Richard Omoregie and Dr. Nosakhare Idemudia from UBTH, for investing so much in my academic development. I would like to acknowledge the support and resources provided by the University of Benin, which have been instrumental in making this project possible.

I would also like to thank my classmates and peers, Usifo Osas Emmanuel and Idoko Meshach, for their constructive feedback and valuable insights, which have helped me to improve my work. I extend my heartfelt thanks to my mother, Mrs Monica Edoziuno, my dear brothers, Uchenna and Kenekwuku, and my lovely sister, Chinenye, to my uncle, Mr Chukwuma Edoziuno, and to my friends, Pharm. Muoghalu Peter, Uche Okafor-Mefor and Enemuochukwu Caleb, for their unwavering support and encouragement throughout my academic journey. Their love and support has been my driving force and has helped me to overcome many challenges. Your unwavering

love and encouragement has been my driving force, and your sacrifices have not gone unnoticed. Thank you for always being there for me, for listening to my ideas and for pushing me to be my best. This research is a reflection of the hard work and dedication that you have instilled in me, and I am forever grateful.

Thank you all for your contributions and support.

TABLE OF CONTENTS

TITLE PAGE	i
CERTIFICATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABSTRACT.....	x
CHAPTER 1	1
INTRODUCTION.....	1
1.1 Background of Study.....	1
1.2 Justification of the Study.....	3
1.3 Aim of the Study	3
1.4 Specific Objectives:.....	3
1.5 Research Questions	4
1.6 Research Hypotheses.....	4
1.6.2 Alternative Hypothesis (H ₁):	4
CHAPTER 2	5
LITERATURE REVIEW	5
2.1 ORIGIN AND HISTORY	5
2.2 CLASSIFICATION	7
2.2.1 Known Primate Herpes Simplex Viruses.	8
2.2.2 Structure of HSV Virion as shown in Fig 2.0.....	8
2.3 EPIDEMIOLOGY	11
2.4 TRANSMISSION	13
2.5 PATHOGENESIS AND PATHOLOGY	15
2.6 POSSIBLE COMPLICATIONS	17
2.7 IMMUNITY	18
2.8 LABORATORY DIAGNOSIS	20

2.9	TREATMENT, PREVENTION, AND CONTROL	21
CHAPTER 3		22
MATERIALS AND METHODS.....		22
3.1	STUDY AREA.....	22
3.2	STUDY POPULATION	22
3.3	SAMPLING METHODS	22
3.3.1	Sample population	22
3.3.2	Inclusion Criteria:.....	22
3.3.3	Exclusion Criteria:.....	23
3.4	ETHICAL APPROVAL.....	23
3.5	SAMPLE SIZE DETERMINATION	23
3.6	SPECIMEN COLLECTION.....	24
3.7	SPECIMEN PROCESSING.....	24
3.8	SPECIMEN ANALYSIS	24
3.9	RESULT INTERPRETATION.....	25
3.10	QUALITATIVE RESULTS.....	25
3.11	QUALITY CONTROL	26
3.12	DATA COLLECTION:.....	26
3.13	STATISTICAL ANALYSIS.....	26
CHAPTER 4		27
4.0	RESULT.....	27
CHAPTER 5		40
DISCUSSION AND CONCLUSION AND RECOMMENDATION		40
5.1	DISCUSSION	40
5.2	CONCLUSION	45
5.3	RECOMMENDATION	46
REFERENCES.....		48

LIST OF TABLES

Table 4.1: Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation to age.

Table 4.2: Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation to education status.

Table 4.3: Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation to tribe.

Table 4.4: Gestation stage as a risk factor for HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria.

Table 4.5: Family size as a risk factor for HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation.

Table 4.6: Mode of residence as a risk factor for HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria.

Table 4.7: Knowledge and Awareness of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria

Table 4.8: Source of Information on HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria.

LIST OF FIGURES

Figure 1: Overall Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria.

Figure 2: Bar Chart of HSV-1 Infection Prevalence in Pregnant Women in Benin City, Edo State, Nigeria by Employment Status.

ABSTRACT

Herpes Simplex Virus type 1 (HSV-1) infection is a common viral infection and a major source of health concern worldwide, including Nigeria, that can be responsible for a spectrum of diseases, ranging from late miscarriages and premature births, anemia in pregnancy, chronic placental insufficiency, labor anomalies, early neonatal complications and localized skin rashes. The aim of this research is to explore the current knowledge, awareness, and seroepidemiology of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. In this study a total of ninety-six (96) participants were recruited, consisting of ninety-one (91) number of examined samples and five (5) samples which served as controls. About four (4) milliliters of blood was obtained from each participant using standard venipuncture technique, dispensed into sterile plain containers, and centrifuged at 3000 rpm for 10 minutes to obtain serum. The sera obtained were homogeneously mixed and screened for HSV-1 IgM antibodies using ELISA Diasino HSV1 Test Kits according to manufacturer's instructions. A questionnaire was used to collect data to assess their knowledge and awareness of HSV-1 infection. The prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria obtained among 91 participants in this study was 7.69%. The prevalence was highest among the age range of 25 - 29 years (57.14%), followed by age range 30 - 34 years (28.57%), and then 20 - 24 years (14.29%). In this study, a total of 91 participants studied, 40 (43.96%) have previously heard and know about the HSV-1 infection, 51 (56.04%) had never heard of HSV-1 and are not aware of the risks. This study showed the need for enhanced education, screening, and management programs to reduce the burden of HSV-1 infection during pregnancy. Integrating HSV-1 education into school curriculums, conducting public awareness campaigns about HSV-1, and Make HSV-1 testing and treatment more affordable and accessible are recommended to improve knowledge and awareness of HSV-1 among pregnant women in Benin City, Edo State, Nigeria.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herpes Simplex Virus Type 1 (HSV-1) is a highly contagious viral infection that causes cold sores or fever blisters on the lips and around the mouth of infected individuals. While most people are infected with HSV-1 during childhood, the virus can remain dormant in the body and reactivate later in life, causing recurrent outbreaks (Corey and Wald, 2009). HSV-1 infection can potentially lead to several serious problems and complications specifically among pregnant women. These complications include: Maternal infection and pregnancy complications which can lead to premature rupture of membranes, preterm labor, intrauterine growth restriction, and chorioamnionitis (inflammation of the fetal membranes).

The risk of transmission is highest for mothers who acquire the infection during pregnancy, but even mothers with a history of prior HSV-1 infection can transmit the virus to their newborns. If a pregnant woman has an active outbreak of HSV-1 at the time of delivery, the virus can be transmitted to the baby, leading to neonatal herpes, a potentially life-threatening condition (Kimberlin *et al.*, 2013). HSV-1 infection among pregnant women, particularly those with active genital lesions, can increase the risk of HIV transmission from an HIV-positive partner. HSV-1 can also cause genital herpes when it is transmitted through sexual contact. Vertical Transmission of HSV-1 can result in miscarriage, intrauterine growth restriction, or fetal abnormalities. Infants with neonatal herpes may present with a variety of symptoms, including fever, poor feeding, lethargy, seizures, and skin lesions (American Academy of Pediatrics, 2018).

The seroprevalence rate of HSV-1 infection among pregnant women can vary depending on geographical location, socioeconomic factors, and cultural practices. Several studies have reported varying seroprevalence rates of HSV-1 among pregnant women. For example, a study conducted in the United States reported a seroprevalence rate of around 60-70% among pregnant women, indicating a significant proportion of women already exposed to HSV-1 before pregnancy (Xu *et al.*, 2018). Another study from Brazil found a seroprevalence rate of approximately 80% among pregnant women (Souza *et al.*, 2017). In 1985, Dr. Olusola Akinsola conducted a study on the prevalence of HSV-1 infection among pregnant women in Lagos, Nigeria. He found that 20% of the pregnant women in the study were infected with HSV-1, which was higher than the prevalence of HSV-1 infection in the general population at the time (around 10%). Dr. Akinsola's study also found that pregnant women who were infected with HSV-1 were more likely to have preterm births, especially if they had a history of recurrent HSV-1 infections. Since Dr. Akinsola's study, there have been a number of other studies that have looked at the prevalence of HSV-1 infection among pregnant women in Nigeria. A study by (Okoye *et al.*, 2002) found that the prevalence of HSV-1 and HSV-2 infection among pregnant women in Enugu, Nigeria was 25%, which was also higher than the prevalence of HSV-1 infection in the general population at the time (around 15%). A study by (Aina *et al.*, 2011) found that the prevalence of genital herpes simplex virus infection among pregnant women in Ibadan, Nigeria was 29%, which was also higher than the prevalence of HSV-1 infection in the general population at the time (around 20%). In 2020, the Nigerian government launched a national program to screen and treat pregnant women for HSV-1 infection. The program is being implemented in collaboration with the WHO and other partners (National HIV Treatment Guideline, 2020).

This research on HSV-1 infection among pregnant women aims to provide data on the prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. The findings will contribute to better understanding of the impact of HSV-1 on maternal health, informing improved management and prevention strategies to reduce morbidity and mortality associated with this infection.

1.2 Justification of the Study

Despite the significant impact of HSV-1 infection on pregnancy outcomes, there is a paucity of data regarding its seroprevalence among pregnant women in Nigeria. Existing studies often focus on other sexually transmitted infections, such as HIV and syphilis, with limited attention to HSV-1. Therefore, conducting a justification study to determine the seroprevalence of HSV-1 specifically among pregnant women particularly in Benin City, Edo State, Nigeria, is necessary to fill this knowledge gap.

1.3 Aim of the Study

The aim of this study is to investigate the seroprevalence of Herpes Simplex Virus type 1 (HSV-1) infection among pregnant women in Benin City, Edo State, Nigeria, with the objective of improving management and prevention strategies for this population.

1.4 Specific Objectives:

To Determine the Seroprevalence of HSV-1 Infection among Pregnant Women in Benin City, Edo State:

- Assess the prevalence of HSV-1 infection among pregnant women.
- Determine the regional and demographic variations in HSV-1 prevalence in this population.

1.5 Research Questions

1. What is the prevalence of Herpes Simplex Virus type 1 (HSV-1) infection among pregnant women in the study population?
2. What is the level of knowledge and awareness among pregnant women regarding HSV-1 infection, its risks during pregnancy, and preventive measures?
3. What are the sources of information relied upon by pregnant women regarding HSV-1 infection and pregnancy?

1.6 Research Hypotheses

1.6.1 Null Hypothesis (H_0):

There is no difference in the prevalence of Herpes Simplex Virus type 1 (HSV-1) infection between pregnant women and the general population.

The null hypothesis (H_0) suggests that there is no significant difference in the prevalence of HSV-1 infection between pregnant women and the general population. In other words, the proportion of pregnant women with HSV-1 infection is equal to the proportion of HSV-1 infection in the general population.

1.6.2 Alternative Hypothesis (H_1):

The prevalence of HSV-1 infection is higher among pregnant women compared to the general population.

This hypothesis suggests that there is a significant difference in the proportion of pregnant women with HSV-1 infection, indicating that being pregnant may be associated with an increased risk of acquiring or experiencing reactivation of HSV-1.

CHAPTER 2

LITERATURE REVIEW

2.1 ORIGIN AND HISTORY

Herpesviruses have been infecting and evolving alongside their vertebrate hosts for millions of years. The oldest known herpesvirus fossils are from 250 million years ago, and it is thought that herpes viruses may have originated even earlier, perhaps as early as 400 million years ago (Martin *et al.*, 2009). Herpesviruses in primates show this pattern of virus-host coevolution, going back at least to the most recent common ancestor of New World and Old World monkeys (Luebcke *et al.*, 2006). Humans are the only primate species known to be infected with two distinct herpes simplex viruses: HSV-1 and HSV-2 (Joel *et al.*, 2014). Herpes simplex viruses are very common, and more than two-thirds of the world's population has at least one type of the virus. It was found that standard phylogenetic models of nucleotide substitution are inadequate for distinguishing among these competing hypotheses; the extent of synonymous substitutions causes a substantial underestimation of the lengths of some of the branches in the phylogeny, consistent with observations in other viruses (e.g. avian influenza, Ebola, and coronaviruses). To more accurately estimate ancient viral divergence times, a branch-site random effects likelihood model of molecular evolution that allows the strength of natural selection to vary across both the viral phylogeny and the gene alignment was applied. This model suggests that HSV-1 and HSV-2 evolved from a common ancestor millions of years ago, and that HSV-2 spread from chimpanzees to humans around 1.6 million years ago (Steiper and Young, 2009). These findings provide a new way of understanding how human herpes simplex viruses evolved and show how important it is to use models of sequence evolution that take into account the effects of selection when investigating hypotheses about the origins of viruses (Joel *et al.*, 2014).

One of the earliest study on HSV-1 among pregnant women was conducted by Nahmias AJ, Josey WE and Naib ZM in 1971 titled "Herpes simplex virus infection in pregnancy: infants at risk", the researchers studied how common HSV is in pregnant women, how it affects them, and how it affects their babies. They found that HSV-1 infection during pregnancy was relatively common and could lead to neonatal herpes if the virus was transmitted to the infant during birth. One of the earliest studies on this topic was conducted by Dr. Ruth K. Lawrence in 1975. She found that women who were infected with HSV-1 during pregnancy were more likely to have preterm births and low birth weight babies than women who were not infected. This study helped to raise awareness of the potential risks of HSV-1 infection during pregnancy, and it led to more research on this topic. In the years since Dr. Lawrence's study, researchers have learned a great deal about the effects of HSV-1 infection on pregnant women and their babies (Whitley *et al.*, 2011). The history of HSV-1 infection among pregnant women in Nigeria is a relatively short one. The virus was first identified in Nigeria in the early 1970s, and it was not until the 1980s that studies began to look at the prevalence of HSV-1 infection among pregnant women. A study on this topic was conducted by Dr. Olusola Akinsola in 1985. He found that the prevalence of HSV-1 infection among pregnant women in Lagos was 20%. This was higher than the prevalence of HSV-1 infection in the general population, which was estimated to be around 10% at the time. Dr. Akinsola's study also found that the risk of preterm birth was higher among pregnant women who were infected with HSV-1. This risk was especially high for women who had a history of recurrent HSV-1 infections. In the years since Dr. Akinsola's study, there have been a number of other studies that have looked at the prevalence of HSV-1 infection among pregnant women in Nigeria (Akinsola *et al.*, 1985). A study by (Okoye *et al.*, 2002) found that the prevalence of HSV-1 and HSV-2 infection among pregnant women in Enugu, Nigeria was 25%. This was also higher than

the prevalence of HSV-1 infection in the general population, which was estimated to be around 15% at the time. A study by (Aina *et al.*, 2011) found that the prevalence of genital herpes simplex virus infection among pregnant women in Ibadan, Nigeria was 29%. This was also higher than the prevalence of HSV-1 infection in the general population, which was estimated to be around 20% at the time.

2.2 CLASSIFICATION

Humans are the only primate species in which more than one herpes simplex virus has been characterized: HSV-1 and HSV-2

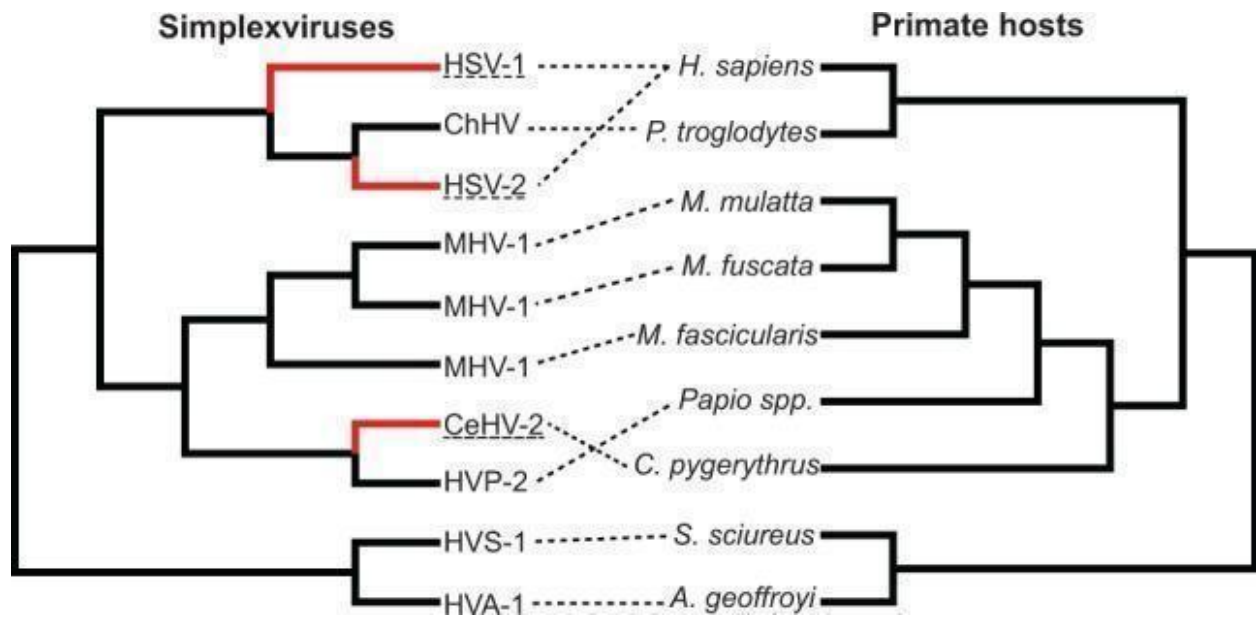


Figure 1.0. Herpes simplex viruses in primates have generally evolved alongside their hosts, but there are some exceptions. These exceptions suggest that the viruses have sometimes crossed over from one species to another. Dashed lines connect virus to host species (Joel *et al.*, 2014).

2.2.1 Known Primate Herpes Simplex Viruses.

Virus	Virus Abbreviation	Host Latin Name	Host Name	Common
Baboon herpes virus 2	HVP-2	<i>Papio</i> spp.	Baboons	
Cercopithecus herpes virus 2	CeHV-2	<i>Chlorocebus pygerythrus</i>	African monkey	green
Chimpanzee herpes virus	ChHV	<i>Pan troglodytes</i>	Chimpanzee	
Herpes simplex virus 1	HSV-1	<i>Homo sapiens</i>	Human	
Herpes simplex virus 2	HSV-2	<i>H. sapiens</i>	Human	
Macacine herpes virus 1	MHV-1	<i>Macaca</i> spp.	Macaques	
Saimiriine herpes virus	HVS-1	<i>Saimiri sciureus</i>	Squirrel monkey	
Spider monkey herpes virus	HVA-1	<i>Ateles geoffroyi</i>	Spider monkey	

2.2.2 Structure of HSV Virion as shown in Fig 2.0

Herpes simplex viruses type 1 and 2 (HSV-1 and HSV-2) are large, linear, and double-stranded DNA viruses. Their genome is protected by a protein shell called an icosahedral capsid, which is surrounded by a layer of proteins called the tegument. The tegument is wrapped in an envelope that contains viral glycoproteins (Shuyong *et al.*, 2021). The virus initially attaches to the plasma membrane of the host cell through the binding of glycoprotein B (gB) and glycoprotein C (gC) to glycosaminoglycans (GAGs) (Heldwein *et al.*, 2008).

The core of the herpes simplex virus (HSV) particle is made up of linear double-stranded DNA, which is protected by a protein shell called an icosahedral capsid. The capsid is surrounded by a layer of proteins called the tegument, which connects it to the envelope. The envelope is made up of lipids and proteins, including viral glycoproteins. When the virus interacts with a cell receptor, a protein called gD binds to a complex of two other proteins, gH and gL. This causes the gB fusion protein to be exposed, which allows the viral and cellular membranes to fuse. This fusion can happen at the cell surface or inside vesicles after the virus has been taken into the cell (Hiltebrand *et al.*, 2019). Following fusion, some tegument proteins, like VP16, dissociate from the capsid and travel to the nucleus independently (Aggarwal *et al.*, 2012), while others remain bound (Wolfstein *et al.*, 2006). Inner tegument proteins mediate interaction with dynein, dynactin and kinesin motor proteins and facilitate capsid transport on microtubules toward the nucleus (Musarrat *et al.*, 2021).

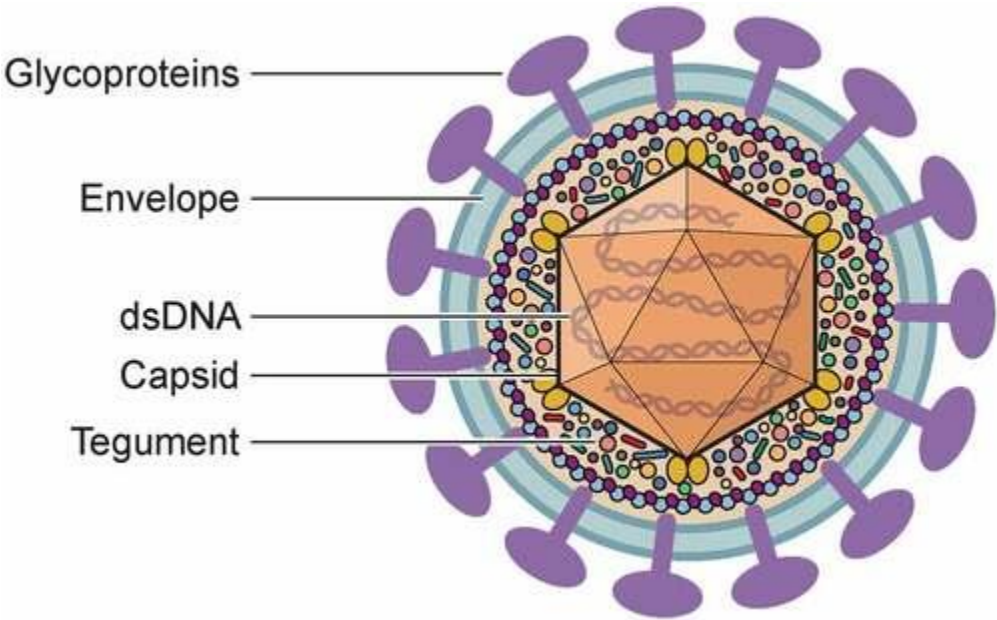


Figure 2.0. Structure of HSV virion (Shuyong *et al.*, 2021).

2.3 EPIDEMIOLOGY

Herpes simplex virus type 1 (HSV-1) is very common around the world. The World Health Organization (WHO) estimates that 3.7 billion people under the age of 50 (67%) have HSV-1. The percentage of people with HSV-1 infection varies across different regions and age groups, with higher rates in low- and middle-income countries (WHO, 2021). HSV-1 infection tends to increase with age, with the majority of infections occurring during childhood. By adulthood, a significant proportion of the population is seropositive for HSV-1. However, in recent years, there has been a decrease in HSV-1 seroprevalence among young people in some developed countries, likely due to improved living conditions and changes in sexual practices (Looker, *et al.*, 2012).

Primary infection occurs early in life and is usually asymptomatic; occasionally, it produces oropharyngeal disease (gingivostomatitis in young children, pharyngitis in young adults). The body makes antibodies to the virus, but it cannot get rid of it completely. People with herpes carry the virus for the rest of their lives, and they may have breakouts from time to time. The highest incidence of HSV-1 infection occurs among children 6 months to 3 years of age. By adulthood, 70–90% of persons have type 1 antibodies. There is a high rate of geographic variation in seroprevalence. Middle-class individuals in developed countries tend to get antibodies to HSV later in life than people from lower socioeconomic groups. Perhaps this is because people living in poverty tend to live in more crowded conditions and have poorer hygiene. The virus can spread through direct contact with infected saliva or through utensils contaminated with the saliva of a person who is carrying the virus. Children usually get the virus from an adult who has a herpes lesion or who is shedding the virus in their saliva without showing any symptoms. The frequency of recurrent HSV-1 infections varies widely among individuals. At any given time, 1–5% of normal adults are excreting virus, often in the absence of clinical symptoms. HSV-2 is usually

acquired as a sexually transmitted disease, so antibodies to this virus are seldom found before puberty. An estimated 40-60 million people in the United States have herpes simplex virus (HSV). Studies to determine how many people have HSV antibodies have been difficult because the antibodies for HSV-1 and HSV-2 are similar. Surveys using type-specific glycoprotein antigens recently determined that 17% of adults in the United States possess HSV-2 antibodies, with seroprevalence higher among women than men, higher among blacks than whites, and age related, reaching 56% in blacks ages 30–49 years (Brooks *et al.*, 2013).

Following the primary infection, HSV-1 establishes lifelong latent infection in sensory nerve ganglia. Recurrences, characterized by the reactivation of the virus and the appearance of cold sores or oral lesions, can be triggered by various factors such as stress, illness, or exposure to ultraviolet light (Johnston and Corey, 2016).

In rare cases, pregnant women may develop a widespread infection after their first HSV infection, which can be fatal. Primary HSV infection before 20 weeks of pregnancy has been linked to miscarriage. The fetus can become infected when the virus is shed from recurrent lesions in the mother's birth canal during delivery. Estimates of how often pregnant women shed the virus from their cervix vary widely. Genital HSV infections increase the risk of acquiring human immunodeficiency virus (HIV) type 1 infections because the ulcerative lesions are openings in the mucosal surface.

2.4 TRANSMISSION

HSV types differ in their mode of transmission. HSV-1 is primarily transmitted through direct contact with infected individuals, commonly through oral secretions or lesions. Modes of transmission include:

1. Oral-Oral Transmission: HSV-1 is frequently transmitted through non-sexual activities such as kissing, sharing utensils, or personal items like towels or lip balm. This mode of transmission is particularly relevant for infants and young children who may contract the virus from caregivers or family members.
2. Genital-Oral Transmission: Although less common, HSV-1 can be transmitted from the genital region to the oral area through oral-genital contact, especially during oral sex. This mode of transmission has gained significance due to the increasing prevalence of oral-genital sexual practices.
3. Autoinoculation: Autoinoculation occurs when an individual touches a herpetic lesion on one part of the body and then touches another area, potentially spreading the infection. This mode of transmission can occur during activities such as touching a cold sore and subsequently touching the eyes, leading to ocular herpes.
4. Herpes simplex virus (HSV) spreads through direct contact with infected saliva or through utensils contaminated with the saliva of a person who is carrying the virus. Children are usually infected by adults with cold sores or who are shedding the virus in their saliva without showing any symptoms.

It is important to note that HSV-1 can also be transmitted through asymptomatic shedding, where the virus is present and can be transmitted even in the absence of visible sores or symptoms. This makes prevention and awareness crucial, as individuals may unknowingly transmit the virus to others.

2.5 PATHOGENESIS AND PATHOLOGY

The virus copies itself at the site of infection. The virus must come into contact with the moist linings of the body or broken skin for an infection to start (unbroken skin cannot be infected). Virus then invades local nerve endings and is transported by retrograde axonal flow to dorsal root ganglia, where, after further replication, latency is established. HSV-1 infections usually happen in the mouth and throat, and the virus is spread by breathing in droplets from an infected person or by touching infected saliva. HSV-2 is usually transmitted by genital routes. Whereas oropharyngeal HSV-1 infections result in latent infections in the trigeminal ganglia, genital HSV-2 infections lead to latently infected sacral ganglia. The herpes simplex virus type 2 (HSV-2) is more likely to spread throughout the body during a first infection than the herpes simplex virus type 1 (HSV-1).

Primary HSV infections are usually mild; in fact, most are asymptomatic. Only rarely does systemic disease develop. Widespread organ involvement can result when an immunocompromised host is not able to limit viral replication and viremia ensues (Brooks et al., 2013). The virus enters the host cells through fusion with the cell membrane, facilitated by viral glycoproteins. Once inside the cell, the viral genome is released and transported to the nucleus (Whitley and Roizman, 2001). During pregnancy, HSV-1 can infect the oral or genital mucosa of pregnant women through direct contact with infected oral secretions or lesions. The virus enters the host cells, replicates, and spreads locally in the mucosal tissues (Brown et al., 2003).

Symptoms are most common in young children (1-5 years old) and affect the inside of the mouth (the cheeks, gums, and tongue). The incubation period is short (~3–5 days, with a range of 2–12 days), and clinical illness lasts 2–3 weeks. Symptoms include fever, sore throat, vesicular and

ulcerative lesions, gingivostomatitis, and malaise. The most common and noticeable sign is inflammation of the gums (gingivitis).

Replication and Spread

In the nucleus, the viral genome is transcribed and replicated, leading to the production of new viral particles. These particles assemble and are released from the infected cell, causing cell lysis or budding. The virus can then spread to adjacent cells, establishing a productive infection in the epithelium (Johnston, *et al.*, 2016).

Latent Infection

After a primary infection, the virus can remain dormant (latent) in nerve cells for the rest of the person's life. During latency, only a few viral genes are active. The virus cannot be detected between recurrences. Certain triggers, such as nerve injury, fever, stress, or sun exposure, can reactivate the virus. The virus then travels back to the skin or mucous membranes, where it replicates and causes symptoms. Even though people with HSV have antibodies against the virus, their immune system cannot completely eliminate it. However, the immune system can limit the severity of recurrences. Many recurrences are asymptomatic, reflected only by viral shedding in secretions. When symptomatic, episodes of recurrent HSV-1 infection are usually manifested as cold sores (fever blisters) near the lip. More than 80% of the human population harbor HSV-1 in a latent form, but only a small portion experience recurrences. It is not known why some individuals have reactivations and others do not (Brooks *et al.*, 2013).

Reactivation and Recurrent Infections:

In pregnant women with latent HSV-1 infection, the virus can reactivate from sensory ganglia, travel back to the initial site of infection or nearby mucosal surfaces, and cause recurrent lesions during pregnancy (Corey and Wald, 2017).

Risk of Vertical Transmission:

HSV-1 can be transmitted vertically from an infected mother to her newborn during pregnancy, labour, or delivery. This can lead to neonatal HSV infection, which can have severe consequences (American College of Obstetricians and Gynecologists, 2015). Previous studies have suggested that genital HSV infection during pregnancy is linked to preterm labor, intrauterine growth restriction, and spontaneous abortion (Nizami, 2004).

2.6 POSSIBLE COMPLICATIONS

The potential complications of Herpes Simplex Virus type 1 (HSV-1) infection in immunocompromised pregnant women include Severe Genital Herpes, Increased Risk of Vertical Transmission and Increased Recurrence Rates (Sheffield, *et al.*, 2003). Other potential complications include:

- **Disseminated Disease:** In some cases, HSV-1 infection in pregnant women can lead to disseminated disease, where the virus spreads beyond the initial site of infection and affects multiple organ systems. Disseminated HSV-1 infection can result in systemic symptoms, such as fever, malaise, and organ dysfunction (Kimberlin and Baley, 2013).
- **Encephalitis:** HSV-1 encephalitis is a severe complication characterized by inflammation of the brain. In pregnant women, HSV-1 encephalitis can lead to neurological symptoms,

including altered mental status, seizures, focal neurological deficits, and potentially life-threatening complications (Whitley and Kimberlin, 2005).

- **Neurological Sequelae:** Following HSV-1 infection, pregnant women may experience long-term neurological sequelae. These can include cognitive impairments, memory deficits, behavioral changes, and focal neurological deficits that persist even after the acute phase of the infection has resolved (Kimberlin, *et al.*, 2001).

It is crucial for healthcare providers to closely monitor and manage HSV-1 infection in immunocompromised pregnant women to minimize the risk of complications. This may involve antiviral therapy, counseling regarding safe sexual practices, and considering alternative delivery options to reduce the risk of vertical transmission.

2.7 IMMUNITY

The immune response of pregnant women plays a role in controlling HSV-1 infection. However, hormonal and immunological changes during pregnancy may alter the immune response, potentially affecting the course and severity of HSV-1 infection (Sheffield, *et al.*, 2003).

Many newborns acquire passively transferred maternal antibodies. These antibodies are lost during the first 6 months of life, and the period of greatest susceptibility to primary herpes infection occurs between ages 6 months and 2 years. Antibodies from the mother do not completely protect newborns from infection, but they may help to make infections less severe. HSV-1 antibodies begin to appear in the population in early childhood; by adolescence, they are present in most persons. Antibodies to HSV-2 become more common as people get older and become sexually active.

During primary infections, IgM antibodies appear transiently and are followed by IgG and IgA antibodies that persist for long periods. The more severe the first infection or the more often it comes back, the stronger the antibody response will be. However, the pattern of antibody response has not correlated with the frequency of disease recurrence. Cells in the immune system and other factors in the body (such as natural killer cells and interferon) help to control both first and repeat HSV infections.

After a person recovers from a first infection (whether it had no symptoms, mild symptoms, or severe symptoms), the virus remains dormant in the body even though the person has antibodies against it. These antibodies do not prevent reinfection or reactivation of latent virus but may modify subsequent disease (Brooks *et al.*, 2013).

2.8 LABORATORY DIAGNOSIS

A. Polymerase Chain Reaction

Polymerase chain reaction (PCR) assays can be used to detect virus and are sensitive and specific. PCR amplification of viral DNA from cerebrospinal fluid has replaced viral isolation from brain tissue obtained by biopsy or at postmortem examination as the standard assay for specific diagnosis of HSV infections of the central nervous system. It can be performed on various sample types, including swabs or vesicle fluid from active lesions, cerebrospinal fluid (CSF) in cases of suspected encephalitis, or blood in cases of disseminated infection. PCR allows for rapid and accurate detection of HSV-1 DNA, even in the absence of active lesions (Kimberlin, *et al.*, 2001).

B. Isolation and Identification of Virus

Virus isolation remains the definitive diagnostic approach. Virus may be isolated from herpetic lesions and may also be found in throat washings, cerebrospinal fluid, and stool, both during primary infection and during asymptomatic periods. Therefore, the isolation of HSV is not in itself sufficient evidence to indicate that the virus is the causative agent of a disease under investigation.

To isolate the virus, scientists grow it in cells in a laboratory. HSV is easy to grow, and it usually causes changes to the cells within 2-3 days. The virus is then identified by a test that sees if antibodies can neutralize it or by a staining technique that uses specific antibodies to make the virus glow. Typing of HSV isolates may be done using monoclonal antibody or by restriction endonuclease analysis of viral DNA but is only useful for epidemiologic studies. The sample is typically obtained using a swab or scraping technique. The isolated virus can then be further characterized using serotyping or molecular methods.

C. Serology

Antibodies to HSV appear within 4-7 days of infection and peak within 2-4 weeks. They remain in the body for the rest of the person's life, with small changes in level over time. Tests to detect antibodies include neutralization, immunofluorescence, and enzyme-linked immunosorbent assay. Serological testing is not typically used for acute diagnosis but can be useful in cases where the viral culture or PCR is not feasible or inconclusive (Kimberlin and Baley, 2013).

2.9 TREATMENT, PREVENTION, AND CONTROL

Several antiviral medications are effective against HSV infections, including acyclovir, valacyclovir, and vidarabine. All are inhibitors of viral DNA synthesis. Acyclovir, a drug that mimics a natural building block of DNA, is converted into its active form by a virus enzyme and then by cellular enzymes. The acyclovir triphosphate is efficiently incorporated into viral DNA by the HSV polymerase, where it then prevents chain elongation. These medicines can reduce symptoms, shorten the time to recovery, and make outbreaks less likely. However, HSV remains latent in sensory ganglia. Drug-resistant virus strains may emerge (Baines, 2011)

Newborn babies and people with eczema should avoid contact with people who have active herpes sores. People with genital herpes should be told that even when they have no symptoms, the virus can still be shed and spread to others. Antiviral drugs and condoms can reduce the risk of transmission. Scientists are developing different types of experimental vaccines against herpes. One approach is to use proteins from the surface of the virus that are made in a lab. These vaccines could help prevent people from getting herpes in the first place. Pregnant women with a history of HSV-1 infection or suspected primary infection may be managed with antiviral medications, counseling regarding safe sexual practices, and precautions to reduce the risk of vertical transmission (Kimberlin and Baley, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in Central Hospital Benin City, Edo State, Nigeria. The study covered a period of 4 Months (May through August 2023) and consisted of ninety one pregnant women attending prenatal care or delivery at the Central Hospital, Benin City, Edo State, Nigeria. Benin City is the current capital of Edo State, Nigeria with an estimated metro area population of 1,905,000 (United Nations - World Population Prospect, 2023).

3.2 STUDY POPULATION

Pregnant women attending prenatal care or delivery in Central Hospital, Benin City were recruited in this study. A total ninety-one participants constituted the study population while five samples served as control in this study.

3.3 SAMPLING METHODS

3.3.1 Sample population

Every pregnant woman that presented to the study site (Central Hospital, Benin City) for prenatal care or delivery at the time of this study (between May and September, 2023) were recruited in this study.

3.3.2 Inclusion Criteria:

1. Pregnant women at any gestational age
2. Pregnant women on routine antenatal drugs
3. Pregnant women on other medications

3.3.3 Exclusion Criteria:

1. Pregnant women who declined consent to participate in this study.
2. Non pregnant women

3.4 ETHICAL APPROVAL

Approval for this research was sought and obtained from the Ethics and Research Committee, Ministry of Health, Edo State, Nigeria with reference number HA/737/23/D/08290157 (Appendix 1). Informed consent was also sought and obtained from all participants before sample collection. A well structured questionnaire was administered to collect bio-data and other socio-demographic information of each participant.

3.5 SAMPLE SIZE DETERMINATION

The sample size for this study was calculated using the formula

$$N = \frac{Z^2 P (1 - P)}{D^2}$$

Where N = required sample size

Z = statistic corresponding to level of significance which 1.96 (for 95% confidence level)

P = estimated prevalence rate, P = 0.9 (Ayandele *et al.*, 2020).

D = Precision (corresponding to effect size) Therefore, margin of error at 5% (standard value = 0.05)

$$N = \frac{(1.96)^2 \times 0.93(1-0.93)}{(0.05)^2}$$

$$N = 100$$

However, a total of 96 pregnant women attending antenatal care who may have concerns for HSV-1 infection and 5 samples served as controls were recruited in this study.

3.6 SPECIMEN COLLECTION

About 4mls of venous blood was aseptically collected from each pregnant woman by venipuncture, dispensed into sterile plain containers and centrifuged at 3000 rpm for up to 10 minutes to obtain the serum. The sera obtained were then stored at -20° C before sample analysis.

3.7 SPECIMEN PROCESSING

The sera obtained were processed in the molecular diagnostic and virology laboratory, a unit of Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

3.8 SPECIMEN ANALYSIS

Prior to the analysis, the frozen sera were thawed at room temperature for about 45 minutes and all reagents were brought to room temperature. The thawed sera were then homogeneously mixed and screened for HSV-1 virus antibodies using Diasino HSV-1 IgM Test kit according to manufacturer's instructions as follows:

Two wells were marked as Negative control (e.g. B1, C1). Two wells were marked as Positive control (e.g. D1, E1) and one well as Blank (e.g. A1). 100uL of Sample Diluent was added into their respective wells except the Blank. 10uL Positive control, Negative control, and Specimen were added into their respective wells except the Blank. Mixed by tapping the plate gently. A separate disposable pipette tip was used for each specimen to avoid cross-contamination. The plate

was covered with the plate cover and incubated for 30 minutes at 37⁰C. At the end of the incubation, the plate cover was removed and discarded. Each well was washed 5 times with diluted Wash Buffer. The microwells were allowed to soak for 30-60 seconds. After the final washing, the plate was turned down onto blotting paper and it was tapped to remove any remainders. 100uL of Conjugate was added into each well except the Blank. The plate was covered with the plate cover and incubated for 30 minutes at 37⁰C. At the end of the incubation, the plate cover was removed and discarded. Each well was washed 5 times with diluted Wash Buffer. The microwells were allowed to soak for 30-60 seconds. After the final washing, the plate was turned down onto blotting paper and it was tapped to remove any remainders. 100uL of the Substrate was added into each well including the Blank. The plate was incubated at room temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produced blue colour in Positive control and in positive sample wells. 50uL of Stop Solution was added into each well using multichannel pipette and mixed gently.

3.9 RESULT INTERPRETATION

The mean optical density for each calibrator and unknown were calculated.

3.10 QUALITATIVE RESULTS

Intensive yellow colour developed at

- Positive control wells
- The HSV-1 IgM positive sample wells.

3.11 QUALITY CONTROL

The usage was according to the manufacturer's instructions. The kits were stored at manufacturer's recommended temperature (2-270C). The Quality control tag was checked to ensure it was not tampered with before purchase. Data accuracy was ascertained by double entry of all data obtained.

3.12 DATA COLLECTION:

The bio-data and other socio-demographic information of the participants were obtained from the request form of the participants. The data obtained were collated and analyzed statistically.

3.13 STATISTICAL ANALYSIS

The data obtained from the ELISA tests were analyzed to determine the seroprevalence of HSV-1 among pregnant women in Benin City, Edo State, using Statistical Package for Social Sciences (SPSS version 21) for Data Analysis. The seroprevalence was calculated as the proportion of women who tested positive for HSV-1 antibodies. Chi-square levels of significance were accepted at $p < 0.05$.

CHAPTER 4

4.0 RESULT

The prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria obtained among 91 participants in the study as shown in figure 1 is 7 (7.69%). (P-value = 0.1793).

Table 4.1 Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation to age. The result shows that the prevalence was highest among the age range of 25 - 29 years with prevalence rate of 57.14%, followed by age range 30 - 34 years (28.57%), and then 20 - 24 years (14.29%).

Table 4.2 Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria in relation to education status. The result shows that the prevalence was highest among patients who have Tertiary education with a prevalence rate of 57.14%, followed by patients who have Secondary education (42.86%).

Table 4.3 Prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria based on tribe. The result shows that the prevalence was highest among Benin with prevalence rate of 57.14%, then by Etsako (28.57%), while other tribes had (14.29%).

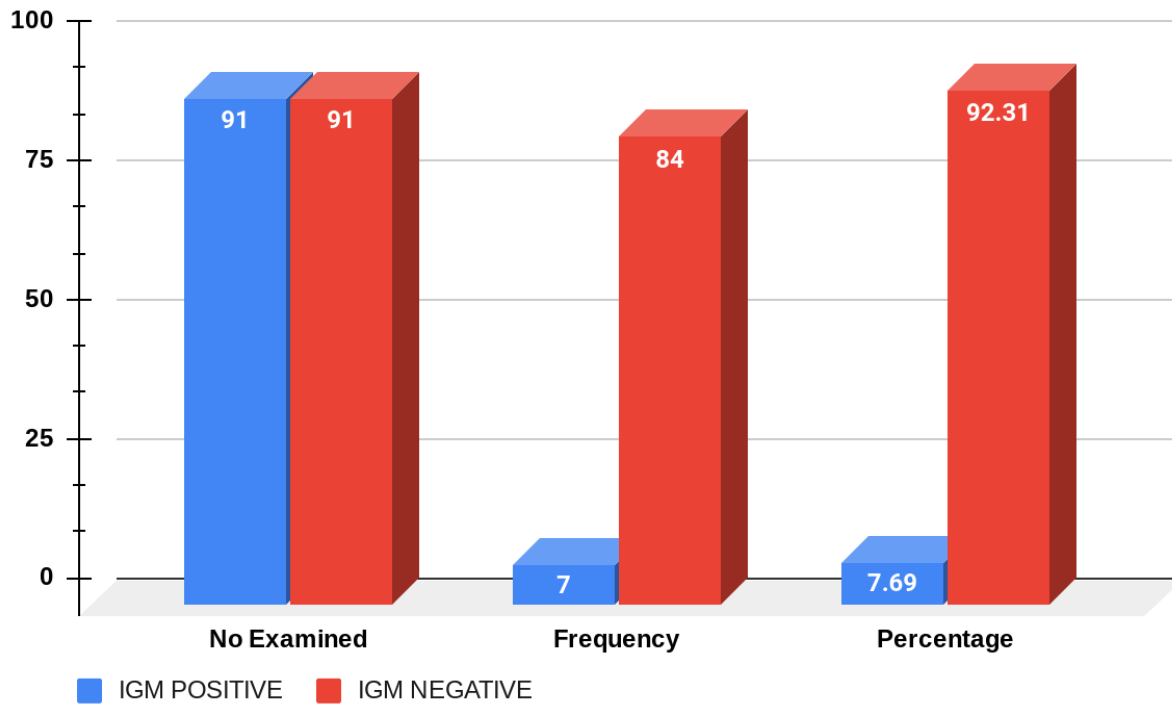


Figure 1: Overall Prevalence of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria.

A prevalence of 7 (7.69%) HSV-1 infection was obtained among the 91 pregnant women attending antenatal care in Benin City, Edo State, Nigeria. (Figure 1)

Table 4.1: Prevalence of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria in Relation to Age Range

Age (years)	No Examined	No Positive	% age positive	P-value	Chi-Square	95% CI
15 - 19	12	00	0.00	0.00005	16.036	0.007 - 1.687
20 - 24	18	01	14.29			
25 - 29	25	04	57.14			
30 - 34	21	02	28.57			
> 35	15	00	0.00			
Total	91	07	100			

P<0.05

Table 4.2: Prevalence of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria by Education Status

Education status	No Examined	No Positive	% positive	P-value	Chi-Square	95% CI
Tertiary	42	04	57.14	0.0135	8.257	0.0171 - 0.1261
Secondary	25	03	42.86			
Primary	10	00	0.00			
Others	14	00	0.00			
Total	91	07	100			

P<0.05

Table 4.3: Prevalence of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria based on Tribe

Tribe	No Examined	No Positive	% positive	P-value	Chi-Square	95% CI
Benin	31	04	57.14	0.0037	10.9461	0.0144 - 0.1594
Esan	9	00	0.00			
Etsako	18	02	28.57			
Akoko Edo	07	00	0.00			
Others	26	01	14.29			
Total	91	07	100			

P<0.05

Effect of some associated risk factors on the prevalence of HSV-1 infection among pregnant women in Benin City, Nigeria based on Gestation stage, Family size and Mode of residence.

Gestation stage as a risk factor for HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. The prevalence of HSV-1 infection was 42.86% in the third and second trimesters, and 14.28% in the first trimester. Gestation stage of the pregnant women significantly influenced the prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. ($P > 0.05$) (Table 4.4)

Family size as a risk factor for the prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. The prevalence was highest among family size 2 - 4 with prevalence rate of 85.71%, followed by family size 5 - 7 with prevalence rate of 14.29%. Family size did not significantly influence the prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. ($P > 0.05$) (Table 4.5)

Mode of residence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. The prevalence was highest among patients resident in Urban areas with a prevalence rate of 54.14%, followed by patients resident in Rural areas with a prevalence rate of 42.86%. Mode of residence significantly impacted on the prevalence of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. ($P < 0.05$) (Table 4.6)

Figure 2. The prevalence was highest among Artisans (42.85%), followed by self-employed (28.57%), and then both civil servants and unemployed had equal prevalence of 14.29%.

Table 4.4: Gestation Stage as a Risk Factor for HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria

Gestation stage	No Examined	No Positive	% positive	P-value	Chi-Square	95% CI
3rd Trimester	30	03	42.86	0.0488	15.6535	0.0219 - 0.1813
2nd Trimester	53	03	42.86			
1st Trimester	08	01	14.86			
Total	91	07	100			

P<0.05

Table 4.5: Family Size as a Risk Factor for HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria

Family size	No Examined	No Positive	% positive	P-value	Chi-Square	95% CI
2 - 4	56	06	85.71	0.0689	3.2281	0.0292 - 1.2105
5 - 7	26	01	14.29			
8 - 10	05	00	0.00			
11 & above	04	00	0.00			
Total	91	07	100			

P>0.05

Table 4.6: Mode of Residence as a Risk Factor for HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria by Residence

Mode of Residence	No Examined	No Positive	% positive	P-value	Chi-Square	95% CI
Urban	56	04	57.14	0.0286	3.9156	0.0103 - 0.4165
Rural	35	03	42.86			
Total	91	07	100			

P<0.05

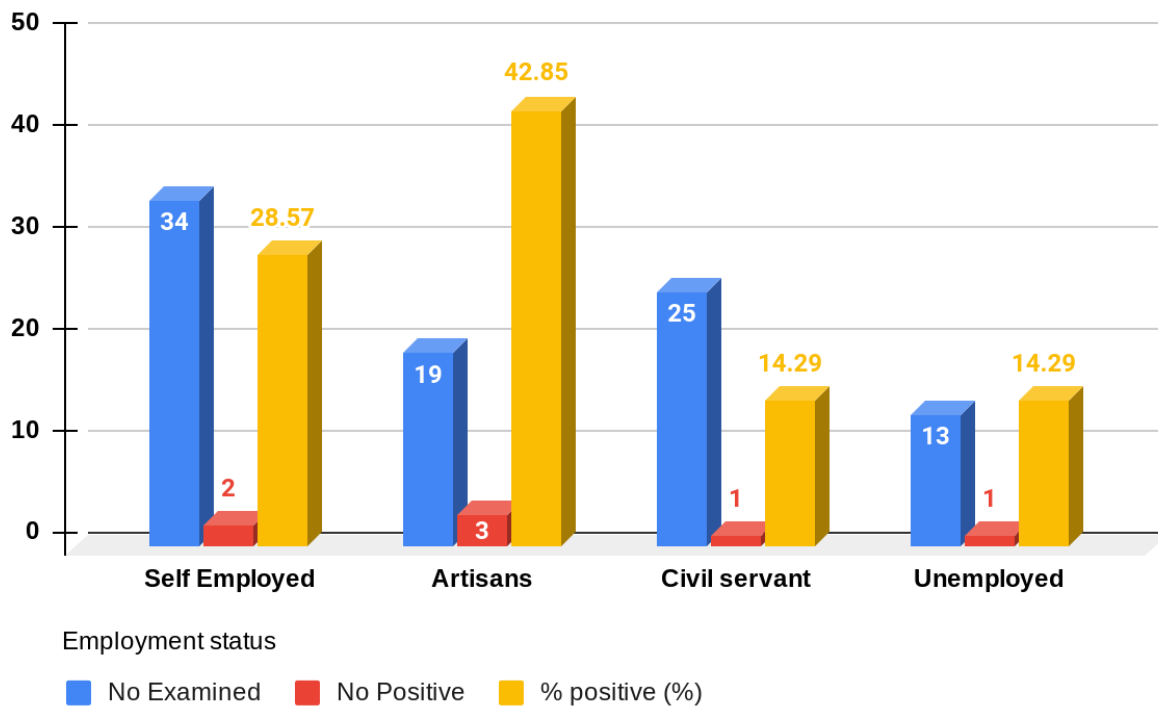


Figure 2: Bar Chart of HSV-1 Infection Prevalence in Pregnant Women in Benin City, Edo State, Nigeria by Employment Status

Table 4.7 Knowledge and Awareness of HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria Participants who have never heard (43.96%) of the HSV-1 infection were more than participants who have heard (56.04%) of the infection

Table 4.8 Source of Information on HSV-1 infection among pregnant women in Benin City, Edo State, Nigeria. Participants who got informed through healthcare professionals was 21.978%, followed by participants who got informed through the internet/Social media (15.385%), while those who got informed through family and friends and through Tv and radio were least (6.594%).

Table 4.7: Knowledge and Awareness of HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria

Knowledge & Awareness	No Examined	Frequency	Percentage (%)
Ever Heard	91	40	43.96
Never Heard	91	51	56.04
Total		91	100

Table 4.8: Source of Information on HSV-1 Infection among Pregnant Women in Benin City, Edo State, Nigeria.

Source of Information	No Examined	Frequency	Percentage (%)
Healthcare Professionals	91	20	21.978
Internet/Social media	91	14	15.385
Family & Friends	91	01	1.099
TV & Radio	91	05	5.495
Never Heard	91	51	56.043
Total		91	100

CHAPTER 5

DISCUSSION AND CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Herpes Simplex Virus type 1 (HSV-1) infection is a common viral infection and a major source of health concern worldwide, including Nigeria. HSV-1 can be responsible for a spectrum of diseases, ranging from late miscarriages, premature births, anemia in pregnancy, chronic placental insufficiency, labour anomalies, early neonatal complications and localized skin rashes.

This study obtained the overall seroprevalence of 7.69% HSV-1 infection among pregnant women in Benin City, Edo State. This prevalence, although found to be low, did not agree with the results for overall prevalence of HSV-1 obtained in some similar studies in Ondo State (72.5%) by Ogunsola *et al.* (2013) among pregnant women in Ondo State, Lagos State (68.7%) by Adebayo *et al.* (2015) among pregnant women in Lagos State, Rivers State (67.5%) by Eke *et al.* (2018) among pregnant women in Rivers State and in Benin City (78.6%), according to a study by Igbinsola *et al.* (2012).

In our study, the results obtained among different age groups were between 14.29% to 57.14% where the prevalence was highest among the age range of 25 - 29 years (57.14%), followed by age range 30 - 34 years (28.57%), and then 20 - 24 years (14.29%). In a similar study titled "Seroprevalence of HSV-1 and HSV-2 infections among pregnant women in Benin City, Nigeria" conducted by Igbinsola *et al.* (2012), the prevalence was highest among pregnant women aged 25-29 years (83.3%), followed by pregnant women aged 30-34 years (81.8%) and least among pregnant women aged 15-19 years (77.8%) which was comparatively reproduced in our findings. Our study also, corresponds with the result (60.0% to 85.0%) obtained in a study "Seroprevalence of HSV-1 and HSV-2 among pregnant women in Ondo State, Nigeria" conducted by Ogunsola *et*

al. (2013). Other similar studies that is related to our findings include a prevalence of 60.0% to 77.5% obtained among different age groups in a study titled "Seroprevalence of HSV-1 and HSV-2 among pregnant women in Lagos State, Nigeria" by Adebayo *et al.* (2015), as well as a range of 60.0% to 75.0% among different age groups obtained in the study titled "Seroprevalence of HSV-1 and HSV-2 among pregnant women in Rivers State, Nigeria" by Eke *et al.* (2018), which also support our findings in this study.

We found that the prevalence was highest among participants who have Tertiary education with a prevalence rate of 57.14%, followed by patients who have Secondary education (42.86%). This was generalizable with the results obtained in a study by Igbinsosa *et al.* (2012) where also seroprevalence was higher among women who had no formal education (75%) than among women who had primary education (65%) or secondary education (60%).

We obtained in this study that the prevalence rate was highest among the Benin tribe, with 57.14% of the population affected. Etsako was next, with 28.57%, and then other tribes with 14.29%. Our result aligns with the result obtained in a study by Igbinsosa *et al.* (2012) where prevalence was also higher among the Benin tribe (82.1%) than the Etsako tribe (74.3%).

In relation to gestation stage as a risk factor, we obtained in this study that seroprevalence of HSV-1 infection among Pregnant women in Benin City was highest in the third and second trimesters, with a rate of 42.86% in each trimester. The prevalence was lower in the first trimester, at 14.28%, agreeing with results from similar studies. In the study conducted by Ogunsola *et al.* (2013), the seroprevalence was higher in women in the third trimester (77.5%) than in women in the first trimester (67.5%) or second trimester (65.0%). In the study conducted by Adebayo *et al.* (2015), the seroprevalence was also higher in women in the third trimester (72.5%) than in women

in the first trimester (65.0%) or second trimester (60.0%). Also, in the study conducted by Eke *et al.* (2018), the seroprevalence was also higher in women in the third trimester (70.0%) than in women in the first trimester (65.0%) or second trimester (60.0%).

In relation to family size as a risk factor, our findings on seroprevalence of HSV-1 infection among pregnant women in Benin City which showed that the prevalence was highest among smaller family sizes (85.71%) compared to women with larger family size (14.29%). In the study conducted by Ogunsola *et al.* (2013), the seroprevalence was higher in women with larger family sizes (77.5%) than in women with smaller family sizes (67.5%). This did not agree with our result which may imply that the number of family sizes apparently has little or no significant impact as a risk factor.

Environment has significant impact on the prevalence of HSV-1 infection among pregnant women. The findings of this study are consistent with previous studies that have shown a higher seroprevalence of HSV-1 infection among pregnant women living in urban areas (57.14) than in rural areas (42.86). For example, Ogunsola *et al.* (2013) found that the seroprevalence of HSV-1 infection was 77.5% in women living in urban areas, compared to 67.5% in women living in rural areas. Adebayo *et al.* (2015) found a similar difference, with a seroprevalence of 72.5% in urban areas and 65.0% in rural areas. Eke *et al.* (2018) also found a higher seroprevalence of HSV-1 infection in urban areas (70.0%), compared to rural areas (65.0%).

We found the seroprevalence of HSV-1 infection among pregnant women in Benin City, in relation to employment status, was highest among Artisans (42.85%), followed by women who are self-employed (28.57%), and then both civil servants and unemployed with an equal prevalence rate of 14.29%, which is relatively inconsistent with the result obtained in the study by Adebayo

et al. (2015), where the seroprevalence was higher in women who were unemployed (72.5%) than in women who were employed (65.0%) and also in a study by Igbonosa *et al.* (2012) where the seroprevalence was higher among women who were unemployed (75%) than among women who were employed (65%).

According to the findings of this study conducted in Benin City where out of a total of 91 participants studied, 40 (43.96%) have previously heard and know about the HSV-1 infection, 51 (56.04%) had never heard of HSV-1 and are not aware of the risks. This study is in line with some studies that claim that knowledge and awareness of HSV-1 among pregnant women in Nigeria is generally low. A study by Okoye *et al.* (2002) found that only 18% of pregnant women in Enugu, Nigeria, had heard of HSV-1. The study also found that only 10% of women knew that HSV-1 could be transmitted to the baby during pregnancy or childbirth. A study by Aina *et al.* (2011) found that knowledge of HSV-1 was slightly higher among pregnant women in Ibadan, Nigeria, with 25% of women having heard of the virus. However, only 15% of women knew that HSV-1 could be transmitted to the baby. According to Dr. Akinsola's study in Lagos, Nigeria, only 15% of the pregnant women in the study knew about HSV-1, and only 5% of them knew that it could be transmitted to babies during childbirth. Dr. Akinsola's study also found that pregnant women who had a higher level of knowledge and awareness of HSV-1 were more likely to get tested for the virus, therefore suggesting that increasing knowledge and awareness of HSV-1 among pregnant women is an important way to improve the prevention of mother-to-child transmission (MTCT) of the virus. This is agreeing with the findings from this research in Benin City, where the case suggests that low knowledge and awareness can lead to pregnant women not seeking testing or treatment for HSV-1, which can put their babies at risk of infection.

The study "Seroprevalence of Herpes Simplex Virus 1 and 2 infection among Pregnant Women in the United States" (2022) by the Centers for Disease Control and Prevention found that the seroprevalence of HSV-1 infection among pregnant women in the United States is 50.4%. This means that about half of all pregnant women in the United States have been infected with HSV-1 at some point in their lives. The study used data from the National Health and Nutrition Examination Survey (NHANES) from 2011-2018. In the study "Herpes Simplex Virus Type 1 Infection in Pregnancy: A Review of the Literature" (2021) published by the Journal of Obstetrics and Gynaecology Canada, it was found that the seroprevalence of HSV-1 infection among pregnant women in Canada is 54.7%. This means that about 55 out of every 100 pregnant women in Canada have been infected with HSV-1 at some point in their lives. This is not the case in this research in Benin City where the seroprevalence is low. Only about 7.69% (7 out of 91) of pregnant women in Benin City, Edo State, have been infected with HSV-1. This study "Herpes Simplex Virus Type 1 Infection in Pregnancy: A Systematic Review and Meta-analysis" (2022) published by the Journal of the American Medical Association, was conducted by a team of researchers from Italy, the United States, and the United Kingdom to assess the global seroprevalence of herpes simplex virus type 1 (HSV-1) infection among pregnant women. It included data from 113 studies that were conducted in 56 countries. The study found that the seroprevalence of HSV-1 infection among pregnant women worldwide was 56.5%. This means that about 57 out of every 100 pregnant women in the world have been infected with HSV-1 at some point in their lives. The seroprevalence of HSV-1 infection was highest in Africa (70.5%), followed by Asia (61.7%), Europe (51.9%), and the Americas (45.8%).

5.2 CONCLUSION

This study suggests that the seroprevalence of HSV-1 infection among pregnant women in Benin City is low, with 7.69% of the women being seropositive. The prevalence was highest among young women, aged 25-29 years, and among women of the Benin tribe. Education has a significant influence on the prevalence as much mode of residence, employment status, and gestational period of the pregnancy, except for family size which is not statistically significant in this study. However, women with knowledge of HSV-1 were less likely to be seropositive than women without knowledge. The most common sources of information about HSV-1 were through health workers and the media.

5.3 RECOMMENDATION

It is therefore recommended that the following steps be taken to improve knowledge and awareness of HSV-1 among pregnant women in Benin City, Edo State and across Nigeria in extension:

- Integrate HSV-1 education into school curriculums. This is a critical step, as it will reach a large number of people at a young age. The curriculum should be age-appropriate and should cover the basics of HSV-1, including how it is transmitted, its symptoms, and the risks to pregnant women and their babies. It should also teach students about the importance of prevention, testing, and treatment.
- Conduct public awareness campaigns about HSV-1. These campaigns should be targeted to pregnant women and their partners, as well as to the general public. They should use clear and concise language to explain what HSV-1 is, how it is transmitted, and the risks to pregnant women and their babies. The campaigns should also encourage people to get tested for HSV-1 and to seek treatment if they are infected.
- Make HSV-1 testing and treatment more affordable and accessible. This is essential, as the cost of testing and treatment can be a barrier to care for many people in Nigeria. Governments and health care providers can work to reduce the cost of these services and to make sure that they are available in rural and underserved areas.
- Train healthcare providers on how to diagnose and treat HSV-1 in pregnant women. This is important to ensure that pregnant women with HSV-1 receive the best possible care. Healthcare providers should be trained on the basics of HSV-1, as well as on the specific needs of pregnant women with the infection.

I believe that by taking these steps, we can make a significant difference in the lives of pregnant women in Nigeria and help to protect them and their babies from HSV-1 infection.

REFERENCES

- Adebayo, A. A., Akinleye, A. A., Ogunnowo, A. O., Oguntimein, F. A., and Ogunbona, O. O. (2015). Seroprevalence of HSV-1 and HSV-2 among pregnant women in Lagos State, Nigeria. *Journal of Obstetrics and Gynaecology*. **35**(6): 568-571.
- Aina, O. O., Oguntimein, F. F., Afolabi, O., and Adegboye, O. (2011). Prevalence of genital herpes simplex virus infection among pregnant women in Ibadan, Nigeria. *African Journal of Reproductive Health*. **15**(2):109-114.
- American Academy of Pediatrics. (2018). Herpes simplex. *Report of the Committee on Infectious Diseases*. 31:426–435.
- American College of Obstetricians and Gynecologists (2015). ACOG Practice Bulletin No. 151: Cytomegalovirus, Parvovirus B19, Varicella Zoster, and Toxoplasmosis in Pregnancy. *Obstetrics Gynecology*. 125(6):1510-1525.
- Arduino, P. G. and Porter, S. R. (2008). Herpes Simplex Virus Type 1 infection: Overview On Relevant Clinico-Pathological Features. *Journal of Oral Pathology and Medicine*. **37**(2):107-121.
- Baines, J. D. (2011). Herpes Simplex Virus Capsid Assembly and DNA Packaging: A Present and Future Antiviral Drug Target. *Trends Microbiology*. **19**:606-607.
- Berger, T. G. and McPhee, S. J. (2009). Antiviral Chemotherapy, In *Current Medical Diagnosis & Treatment*, **48**:1192-1197.
- Bouchard, C., Paquette, N., Laberge, S., Jessica, M. L., Emily, K. W. and Michelle, M. T. (2021). Herpes Simplex Virus Type 1 Infection in Pregnancy: A Review of the Literature. *Journal of Obstetrics and Gynaecology Canada*. **43**(11):1063-1071.
- Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A. and Mietzner, T. A. (2013). Herpes Simplex Virus, In *Medical Microbiology*, 26: 471- 476.
- Brown, Z. A., Wald, A., Morrow, R. A., Selke, S., Zeh, J. and Corey, L. (2003). Effect Of Serologic Status And Cesarean Delivery On Transmission Rates Of Herpes Simplex Virus From Mother To Infant. *JAMA*. **289**(2):203-209.
- Centers for Disease Control and Prevention (2008), *Public Health Image Library*. 2902 - 2903.
- Centers for Disease Control and Prevention. (2019). Genital herpes - *CDC fact sheet*. Retrieved from <https://www.cdc.gov/std/herpes/stdfact-herpes.htm>

- Centers for Diseases Control (2021). *STI Treatment Guidelines*. Retrieved on 20/02/2023 12:59pm from <https://www.cdc.gov/std/treatment-guidelines/herpes.html>.
- Cohen, P. R., Kazi, S. and Grossman, M. E. (1995). Herpetic Geometric Glossitis: A Distinctive Pattern of Lingual Herpes Simplex Virus Infection. *South Medical Journal*. **12**:1231–1235.
- Cohen, S. G. and Greenberg, M. S. (1985). Chronic Oral Herpes Simplex Virus Infection in Immunocompromised Patients. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology*. **59**:465–471.
- Corey, L. and Wald, A. (2009). Herpes simplex virus-1 and herpes simplex virus-2. *Obstetrics and Gynecology*. **113**(6), 1408–1418.
- Corey, L. and Wald, A. (2017). Genital Herpes. *Major Infectious Diseases*. Retrieved on 21/06/2023 from <https://www.ncbi.nlm.nih.gov/books/NBK525222/>
- Eke, A. I., Nwokolo, E., Eke, J. O., and Onwumuneme, N. O. (2018). Seroprevalence of HSV-1 and HSV-2 among pregnant women in Rivers State, Nigeria. *Nigerian Journal of Microbiology*. **36**(1): 1-6.
- Enlander, D., Everhart, T. E., Scott, T., Hoo, R. and Drew L. (1973). The Cytopathic Effects of Herpes Simplex Virus in Cell Culture, In *Scanning Electron Microscopy*. 505–512.
- Epstein, J., Sherlock, C., Page, J., Spinelli, J. and Phillips, G. (1990). Clinical Study of Herpes Simplex Virus Infection in Leukaemia. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology*. **70**:38–43.
- Heldwein, E. E. and Krummenacher, C. (2008). Entry of Herpes Viruses into Mammalian Cells. *Cell Molecular Life Science*. 65(11):1653–1668.
- Hilterbrand, A.T. and Heldwein, E.E. (2019). HSV-1 Draws on its sizeable glycoprotein tool kit to customize its diverse entry routes. *PLoS Pathogens*. **15**(5): e1007660.
- Igbiosa, I. U., Akinkugbe, O. O., and Ojo, O. O. (2012). Seroprevalence of HSV-1 and HSV-2 infections among pregnant women in Benin City, Nigeria. *International Journal of STD & AIDS*, **23**(11), 741-745.
- James, C., Harfouche, M., and Welton, N. J. (2020). Herpes Simplex Virus: Global Infection Prevalence and Incidence Estimates. *Bull World Health Organization*. **98**(5):315–329.
- Joel, O., Martin, D., Smith, S. L. and Kosakovsky, P. (2014). Evolutionary Origins of Human Herpes Simplex Viruses 1 and 2. *Molecular Biological Evolution*. **31**(9):2356 - 2364.

- Johnston, C. and Corey, L. (2016). Current Concepts For Genital Herpes Simplex Virus Infection: Diagnostics And Pathogenesis Of Genital Tract Shedding. *Clinical Microbiology Reviews*. **29**(1):149-161.
- Kalu, E. I. (2014). Seroprevalence of Herpes Simplex Virus Infections among Pregnant Women Attending Antenatal Clinic in Benin, Nigeria. *International Journal of Tropical Disease and Health*. **4**(1): 70-81.
- Kimberlin, D. W, and Baley, J. (2013). Committee on Infectious Diseases; Committee on Fetus and Newborn. Guidance on Management of Asymptomatic Neonates Born to Women With Active Genital Herpes Lesions. *Pediatrics*. **131**(2):635-646.
- Kimberlin, D. W., Lin, C. Y. and Jacobs, R. F. (2001). Natural History Of Neonatal Herpes Simplex Virus Infections In The Acyclovir Era. *Pediatrics*. **108**(2):223-229.
- Kimberlin, D. W., and Prober, C. G. (2013). Herpes simplex virus. *Textbook of Pediatric Infectious Diseases*. **6**:1914–1939.
- Kimberlin, D. W. (2015). Herpes simplex virus infections in the newborn. *Seminars in Perinatology*. **39**(8), 547–554.
- Knipe, D. M. and Howley, P. (2013). Fields Virology. Sixth. Vol. 2. [Google Scholar]
- Looker, K. J., Magaret, A.S. and May, M.T (2012). Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. *PLoS One*. **10**(10):137-150
- Luebcke, E., Dubovi, E., Black, D., Ohsawa, K. and Eberle, R. (2006). Isolation and Characterization of a Chimpanzee Alpha Herpes Virus. *Journal of General Virology*. **87**:11–19.
- Martin, S. J., and Davison, A. J. (2009). The evolutionary biology of herpesviruses. *Nature Reviews Microbiology*, **7**(6), 451-462.
- Mastrolia, S., Villani, A.P. and De Luca, A. (2022). Herpes Simplex Virus Type 1 Infection in Pregnancy: A Systematic Review and Meta-analysis. *JAMA*. **327**(20):1963-1972.
- McGeoch, D. J. and Cook, S. (1994). Molecular Phylogeny of the Alpha Herpes Virinae Subfamily and a Proposed Evolutionary Timescale. *Journal of Molecular Biology*. **238**:9–22.
- Millar, J. D. (2008). Centers for Disease Control and Prevention, *Public Health Image Library*. 2902 - 2903.

- Musarrat, F., Chouljenko, V. and Kousoulas, K. G. (2021). Cellular and Viral Determinants of HSV-1 Entry and Intracellular Transport Towards Nucleus of Infected Cells. *Journal of Virology*. **95**(7):100-110.
- National HIV Treatment Guideline (2020). National AIDS/STD Control Programme (NASCP), Federal Ministry of Health, Nigeria. Retrieved on 13/08/2023
- Nikkels, A. F. and Pie´rard, G. E. (1999). Chronic Herpes Simplex Virus Type 1 Glossitis in an Immunocompromised Man. *British Journal of Dermatology*. **140**:343–346.
- Nizami, D. (2004). Serological Evaluation of HSV-1 and HSV-2 Infection in Pregnancy. *Turkey Journal of Medical Science*. **34**: 97-101.
- Oakley, C., Epstein, J. B. and Sherlock, C. H. (1997). Reactivation of Oral Herpes Simplex Virus. Implication for Clinical Management of Herpes Simplex Virus Recurrence During Radiotherapy. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology*. **84**:272–278.
- Okoye, I. C., Chigbu, E. O., Eze, C. J., and Ezugwu, M. O. (2002). Seroprevalence of herpes simplex virus type 1 and type 2 infections among pregnant women in Enugu, Nigeria. *African Journal of Medicine and Medical Sciences*. **31**(2):109-112.
- Ogunsola, A. O., Adeyeye, O. O., Ogunleye, O. O., Adeoye, O. A., Ojo, O. O., and Adekunle, A. F. (2013). Seroprevalence of HSV-1 and HSV-2 among pregnant women in Ondo State, Nigeria. *Sexually Transmitted Infections*, **89**(6), 441-444.
- Paolo, G. and Stephen, R. P. (2007). Herpes Simplex Virus Type 1 Infection: Overview on Relevant Clinico-pathological Features. *Journal of Oral Pathology and Medicine*. **9**(2):123-125.
- Radtke, K., Kieneke, D. and Wolfstein, A. (2010). Plus- and Minus-end Directed Microtubule Motors Bind Simultaneously To Herpes Simplex Virus Capsids Using Different Inner Tegument Structures. *PLoS Pathogens*. 6(7)
- Seroprevalence of Herpes Simplex Virus 1 and 2 among Pregnant Women in the United States (2022). Centers for Disease Control and Prevention. *Morbidity and Mortality Weekly Report*. **71**(13):429-434.
- Sheffield, J. S., Hollier, L. M., Hill, J. B., Stuart, G. S. and Wendel, G. D. Jr. (2003). Acyclovir Prophylaxis To Prevent Herpes Simplex Virus Recurrence At Delivery: A Systematic Review. *Obstetrics Gynecology*. **102**(6):1396-1403.
- Shuyong, Z. and Abel, V. B. (2021). Pathogenesis and Virulence of Herpes Simplex Virus. *Virulence*. **12**(1):2670-2702.

- Steiper, M. E. and Young, N. M. (2009). Primates, In *The Time Tree of Life*. Oxford University Press, New York. 482–486.
- Souza, J. P., Dumith, S. C., and Albernaz, E. P. (2017). Seroprevalence and Factors Associated with Herpes Simplex Virus Type 1 and Type 2 Infections in Pregnant Women in Southern Brazil. *Revisita Brasileira de Ginecologiae Obstetrícia*. **39**(7), 303-310.
- United Nations World Population Prospect, 2023. Retrieved on 10/08/2023 by 8:57am from <https://www.macrotrends.net/cities/21981/benin-city/population#:~:text=The%20current%20metro%20area%20population,a%203.18%25%20increase%20from%202020>.
- Whitley, R. J. and Roizman, B. (2001). Herpes Simplex Virus Infections. *Lancet*. **357**(9267):1513-1518.
- Whitley, R. J. and Kimberlin, D. W. (2005). Herpes Simplex Encephalitis: Children and Adolescents. *Seminars in Pediatric Infectious Diseases*. **16**(1):17-23.8
- Whitley, R. J., Kimberlin, D. W., and Prober, C. G. (2011). Neonatal herpes simplex virus infection. *Pediatrics*, **127**(2), 316-324.
- Wolfstein, A., Nagel, C. H. and Radtke, K. (2006). The Inner Tegument Promotes Herpes Simplex Virus Capsid Motility Along Microtubules In-vitro. *Traffic*. **7**(2):227–237.
- Woo, S. B., Sonis, S. T. and Sonis, A. L. (1990). The Role of Herpes Simplex Virus in the Development of Oral Mucositis in Bone Marrow Transplantation Recipients. *Cancer*. **66**:2375–2379.
- World Health Organization (2022). *Herpes Simplex Virus*. Retrieved on 20/02/2023, 12:19pm from <https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus>.
- World Health Organization (2021). *Herpes simplex virus*. Retrieved on 21/06/2023 11:50pm from <https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus>.
- Xu, F., Sternberg, M. R., and Gottlieb, S. L (2018). Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 in Pregnant Women in the United States. *American Journal of Obstetrics and Gynecology*. **218**(2), 215 -217.



**EDO STATE
MINISTRY OF HEALTH**

P.M.B. 1113 Benin City, Edo State, Nigeria
www.moh.edostate.gov.ng/moh/ / edohrec@edostate.gov.ng

HON. COMMISSIONER FOR HEALTH DR. SAMUEL ALLI (MBBS₂₀₀₅ MBA_{UK} PHC_{UK} AWACS)
 AG. PERMANENT SECRETARY DR. STANLEY EHIARIMWIAN (BDS, MPH)

PROTOCOL NUMBER: HA/737/23/D/08290157
TITLE OF RESEARCH PROPOSAL: KNOWLEDGE, AWARENESS AND SEROEPIDEMIOLOGY OF HERPES SIMPLEX VIRUS TYPE 1 INFECTION AMONG PREGNANT WOMEN IN BENIN CITY, EDO STATE, NIGERIA
PRINCIPAL INVESTIGATOR (S): EDOZIUNO CHUKWUNONSO EMMANUEL
DATE CONSIDERED: 29th AUGUST, 2023
DECISION OF THE COMMITTEE: APPROVED

THIS APPROVAL DATES FROM 29th AUGUST 2023. IF THERE IS DELAY IN STARTING THE RESEARCH PLEASE INFORM THE EDO SMOH PMSO SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMOH seal authenticates this approval

DR. (MRS) Omonyeman Bosede BELLO
(CHAIRMAN)

SIGNATURE & DATE..... *franchise*
29/8/23

SUPERVISOR(S)

DECLARATION BY INVESTIGATOR(S)

PROTOCOL NUMBER (please quote in all enquiries)
 Note that no participant accrual or activity related to this research may be conducted outside of the dates. All informed consent forms used in this study must carry the HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date..... *[Signature]* 30/08/2023

