HIGH RISK HUMAN PAPILLOMAVIRUS DEOXYRIBONUCEIC ACID TYPING, RISK FACTORS AND SUITABILITY OF AVAILABLE HUMAN PAPILLOMAVIRUS VACCINE FOR PREMENOPAUSAL WOMEN IN IMO STATE, NIGERIA

BY

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20154989378

A DISSERTATION SUBMITTED TO THE POST GRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE (DOCTOR OF PHILOSOPHY) Ph.D DISEASE PREVENTION AND CONTROL

APRIL, 2025

CERTIFICATION

This is to certify that this work "High Risk HPV Typing and Available HPV Vaccine Suitability for Women in Imo State, Nigeria'' was carried out by Emily, Akuabia Nzeribe, Reg. number (20184142078) in partial fulfillment for the award of the degree of Doctor of Philosophy (PhD) in Public Health in the Department of Public Health of the Federal University of Technology, Owerri.

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ACKNOWLEDGEMENTS

My special gratitude to Prof O.C Abanobi of blessed memory, my research lecturer and mentor who inspired me to take on this topic despite all odds, to Prof I.N.S. Dozie, Prof. (Mrs.) S.N.O. Ibe, Prof. (Mrs.) E. A. Nwoke, my project supervisors, for painstakingly supervising, reviewing and correcting this work and Dr U.M. Chukwuocha for being my guide over the years. I sincerely appreciate Dr C.C. Iwuala the current HOD Public Health for his great organizational prowess. I also wish to appreciate the former HODs during this programme in the person of Rev. Sr. Prof E.T. Oparaocha, Prof. (Mrs.) E. A. Nwoke, Prof. (Mrs.) S.N.O. Ibe and Prof U M Chukwuocha who midwifed this project.

I appreciate the former PG coordinator Late Dr (Mrs.) C. O. A. Amadi and the current PG Coordinator, Dr. (Mrs.) W. U. Dozie, Prof. Agwu N. Amadi, Dr. Raymond. O. Chimezie (Fulbright Scholar from USA), Dr. G. U. Ekeleme and Mr. Greg Iwuoha for their guidance in the journey this far. My gratitude goes to the Dean, Postgraduate School Prof. Nwosu, former dean Prof B. O. Esonu, and Associate Deans for successfully piloting the affairs of the school. I will not fail to appreciate the former Dean, School of Postgraduate Studies in the person of Prof. (Mrs.) Nnenna Oti, who has been elevated to the position of the Vice Chancellor, Federal University of Technology, Owerri.

I wish to also appreciate the staff of NIMR, Lagos especially Prof Oliver Ezechi, Dr Chika Onwuamah and all others who made valuable input to the executing of this project, also Prof. Jona Musa from University of Jos, Plateau State, Dr. Ifeanyi Amaechi from NCDC for professional activities.

I owe a deep sense of gratitude to my husband, Chief (Hon.) Engr. Chime Nzeribe and our children Dr. (Mrs.) Adaobi Nzeribe-Nwankwo, Mr. Benjamin Uzoukwu Nzeribe, Engr. Obidi Constantine Nzeribe and Miss Nneoma Victoria Nzeribe and my grandchildren Ugonna, Ebube and Ifeoma, for bearing with me during all my days of studying and preparing the thesis. Most of all my gratitude goes to almighty God, the author and finisher of my faith who sustained me throughout the field work and writing of the thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Given the wide variety of high-risk human papillomavirus (hr-HPV) types capable of causing cervical cancer and the limited coverage provided by existing vaccines, there is a need to determine the specific hr-HPV types that commonly infect women, cause premalignant cervical lesions, and potentially lead to cervical cancer among women in Imo State, South East Nigeria. Therefore, the objective of this study was to identify the high-risk HPV DNA types prevalent among women in Imo State and assess the suitability of currently available HPV vaccines in preventing these infections. This study was designed as a population-based, cross-sectional, observational, and analytical study conducted in Imo State, South East Nigeria. A total of 257 premenopausal women aged 30 to 49 years, who had ever been sexually active, were selected using a multistage sampling technique. Data were collected through an interviewer-administered questionnaire. Visual inspection methods (VIA/VILI) and high-risk HPV screening were performed using the AmpFire HPV genotyping assay. Ethical approval was obtained from the Institutional Ethical Review Board of the Federal Medical Centre, Owerri (now Federal University Teaching Hospital, Owerri). Data analysis involved both descriptive and inferential statistical methods. Descriptive analysis included the construction of frequency distribution tables and charts, while inferential analysis involved Chi-square tests and logistic regression. The prevalence of hr-HPV infection in this study was 43.6%. The genotypes identified were: HPV 51 (16.7%), HPV 59 (12.2%), HPV 18 (10.1%), HPV 52 (8.6%), HPV 16 (8.6%), HPV 68 (8.6%), HPV 53 (7.4%), HPV 31 (7.0%), HPV 56 and HPV 39 (4.7% each), HPV 35 (3.1%), HPV 58 (2.3%), and HPV 66 (1.6%) in descending order. Over half of the infected women harbored HPV types not covered by any of the currently available vaccines in Nigeria. The odds of being infected with non-vaccine-targeted HPV types were significantly high (OR = 2.594; 95% CI: 1.744–3.858; $p < 0.0001$). Among women with abnormal cervical lesions, 52.7% were infected with HPV types not covered by the nonavalent vaccine ($p = 0.000$). The frequency of abnormal VIA/VILI results among hr-HPV-positive women was highest for HPV 16 (72.7%), followed by HPV 51 (55.8%), HPV 53 (52.6%), HPV 56 (50.0%), HPV 68 (45.5%), HPV 59 (41.9%), HPV 18 (38.5%), HPV 52 (36.4%), HPV 31, 39, and 58 (33.3% each), and HPV 35 (25.0%). The findings suggest that the existing vaccines may not provide optimal protection for women in this region. A more extensive study focusing on women with abnormal cervical lesions is recommended. Additionally, the development and deployment of vaccines with broader genotype coverage are necessary, particularly for sub-Saharan Africa.

Keywords: high-risk HPV, HPV vaccines, bivalent vaccine, nonavalent vaccine, VIA, VILI, vaccine-targeted hr-HPV, suitability, Imo State, Premenopausal women.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cervical cancer remains a major cause of mortality among women in Nigeria and most low- and middle-income countries (LMICs), primarily because many women present at advanced stages of the disease. This contrasts with developed countries, where the adoption of the Papanicolaou (Pap) test as a screening method has reduced cervical cancer deaths by over 60–70% (Fuentes & Garcia, 2016). In Imo State, South East Nigeria, awareness and uptake of cervical cancer screening remain low (Ezem, 2007; Umeh & Ezedinachi, 2015). A tertiary hospital-based study in Imo State reported that 54% of gynecological malignancies were due to cervical cancer. Risk factors identified in that study included multiparity, early age at first pregnancy, early marriage and coitarche, marital instability, and multiple sexual partners (Anolue, Ojiyi, Dike, Okeudo & Ejikeme, 2014).

Screening has not been effective in low-resource countries due to the substantial demand for skilled manpower, as well as material and technical resources (Momenimovahed & Salehiniya, 2017). Human Papillomavirus Deoxyribonucleic Acid (HPV DNA) testing has been advocated as a valuable tool for early detection and treatment of cervical cancer (Burd, 2003; Ronco et al., 2014). A large pooled analysis of randomized controlled trials in Europe showed that HPV DNA testing is significantly more effective than cytology (Pap smear) in detecting high-grade cervical intraepithelial neoplasia (CIN2/3) and preventing invasive cervical cancer through earlier diagnosis and longer protective effect (Ronco et al., 2014;). HPV has been firmly established as the etiological agent in cervical cancer, being present in approximately 99.7% of cervical cancer specimens (Brebi, 2017). A positive HPV DNA test indicates a significantly increased risk of developing premalignant changes, especially when caused by high-risk HPV (hrHPV) types.

High-risk HPV refers to specific types of the virus that are associated with cervical and other cancers, such as anal, vaginal, penile, and oropharyngeal cancers. In contrast, low-risk HPV types are primarily responsible for genital and skin warts. Infection with hrHPV often progresses

from a sexually transmitted infection to cervical cellular changes, and subsequently to premalignant lesions.

A combination of vaccination and screening methods—including Visual Inspection with Acetic Acid (VIA), the Pap test, or HPV DNA testing—has been described by the World Health Organization as the most effective strategy ("best buy") for preventing cervical cancer (WHO, 2019). Unfortunately, women in LMICs, where approximately 85% of the global burden exists, often lack access to these preventive measures.

The identification of HPV as the causative agent of cervical cancer has led to the development of preventive vaccines (Cutts, 2007; Schiller & Lowy, 2018). These vaccines have been designed to target the most common hrHPV types, particularly HPV 16 and 18, which are responsible for nearly 70% of cervical cancer cases (Burd, 2003). Some vaccines also include low-risk types (HPV 6 and 11). The most recent addition is the nonavalent vaccine, which provides broader protection against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 (Joura et al., 2015).

HPV DNA typing, which detects specific HPV genotypes from cervical cells, enables the identification of the exact viral types and associated risk. Given the wide diversity of hrHPV types and the limited genotype coverage of existing vaccines, there is a need to determine which types most commonly infect women, lead to premalignant lesions, and potentially progress to cervical cancer in Imo State, South East Nigeria.

The findings of this study aim to assess the suitability of currently available vaccines in preventing prevalent hrHPV infections in this population. In this context, *suitability* refers to the appropriateness or effectiveness of a vaccine for protecting a specific population. For the purpose of this study, the HPV types targeted by available vaccines will serve as the independent variable, while the high-risk HPV DNA types identified among women will be the dependent variable.

1.2 Problem Statement

The appropriate and timely application of cervical cancer prevention strategies—whether primary, secondary, or tertiary—has the potential to completely avert the onset of the disease. While HPV vaccination represents a key primary prevention tool, it is currently still at the pilot

stage in Nigeria, and national immunization with the HPV vaccine is only just being rolled out (Bruni, 2018).

For such vaccination programs to be effective, it is crucial that the available vaccines are suitable for protecting against the prevalent high-risk HPV (hrHPV) types in the target population. Given ongoing concerns about the affordability and accessibility of HPV vaccines in low-resource settings, it is essential to evaluate whether the vaccines currently available in Nigeria offer adequate protection for women in Imo State, South East Nigeria.

A major challenge lies in the fact that some geographical regions have a predominance of hrHPV types that are not covered by the existing bivalent, quadrivalent, or nonavalent vaccines. As such, vaccination may not provide optimal protection in these settings. Additionally, there is limited local data on the specific hrHPV genotypes associated with abnormal cervical lesions in Imo State. Without this knowledge, policymakers may be unable to make informed decisions regarding effective vaccine implementation and cervical cancer prevention strategies.

Understanding the distribution and prevalence of high-risk HPV types among women in Imo State is therefore imperative. This information will be critical in assessing the suitability of existing HPV vaccines for this population and will support evidence-based planning and policy development for vaccine deployment and cervical cancer control in the region.

1.3 Objectives

Main Objective

The general objective of this study was to determine the high-risk human papillomavirus (hrHPV) DNA types present among women in Imo State, South East Nigeria, and assess the suitability of available HPV vaccines in preventing the prevalent hrHPV DNA types.

Specific Objectives

i. To determine the prevalence of high-risk HPV infections among women in Imo State, South East Nigeria.

ii. To assess the relationship between the prevalence of high-risk HPV infection and the socio-demographic, behavioral, and reproductive characteristics of women in the study population.

iii. To evaluate the prevalence of genetic strains (genotypes) of high-risk HPV infection among women in Imo State, South East Nigeria.

iv. To determine the proportion of study participants with abnormal cervical lesions in Imo State, South East Nigeria.

v. To determine the distribution of prevalent hrHPV genotypes among women with abnormal cervical lesions compared to those with normal cervices in Imo State, South East Nigeria.

vi. To correlate the prevalent hrHPV types among women in Imo State, South East Nigeria, with the high-risk HPV types targeted by the currently available vaccines.

vii. To correlate the prevalent hrHPV types found in women with abnormal cervical lesions in Imo State, South East Nigeria, with the high-risk HPV types covered by existing HPV vaccines.

1.4 Hypothesis

The following research hypotheses were addressed.

ia) Null hypothesis: There is no relationship between the prevalence of high risk HPV infection and the socio-demographic, behavioral and reproductive characteristics of study women in Imo State, South East Nigeria.

ib) Alternate hypothesis: There is a relationship between the prevalence of high risk HPV infection and the socio-demographic, behavioral and reproductive characteristics of study women in Imo State, South East Nigeria.

iia) Null hypothesis: There is no significant difference between the prevalent hr-HPV types and those covered by available HPV vaccines in Imo State, South East Nigeria.

iib) Alternate hypothesis: There is a significant difference between the prevalent hr-HPV types and those covered by available HPV vaccines in Imo State, South East Nigeria.

iiia) Null hypothesis: There is no relationship between HPV DNA types and the occurrence of abnormal cervical changes in women in Imo State, South East Nigeria using visual methods ofcervical cancer screening.

iiib) Alternate hypothesis: There is a relationship between HPV DNA types and the occurrence of abnormal cervical changes in women in Imo State, South East Nigeria using visual methods of cervical cancer screening.

1.5 Research Questions

This study answered the following research questions:

i. What is the prevalence of hr-HPV infections among women in Imo State, South East Nigeria?
ii. What is the relationship between the occurrence of high risk HPV infection and the socio-demographic, behavioral and reproductive characteristics of study women in Imo State, South East Nigeria?
iii. What is the prevalence of genetic strains of high-risk HPV infection among women in Imo State, South East Nigeria?
iv. What is the proportion of study women with abnormal cervical lesions in Imo State, South East Nigeria?
v. What is the distribution of the prevalent hr-HPV genotypes among women with abnormal cervical lesions and those with normal cervices in Imo State, South East Nigeria?
vi. What is the correlation between the prevalent hrHPV types among women in Imo State, South East Nigeria, and the high-risk HPV types targeted by the currently available vaccines?
vii. What is the correlation between the prevalent hr-HPV types among study women with abnormal lesions with the available HPV vaccines in Imo State, South East Nigeria?

1.6 Justification of the Study

Cervical cancer remains a significant public health burden in Nigeria and across sub-Saharan Africa, with the highest incidence and mortality rates occurring in low- and middle-income countries (LMICs). (Dzinamarira *et al*., 2023). Despite the availability of HPV vaccines globally, their suitability and effectiveness in specific populations depend on the match between vaccine-targeted HPV types and the types most prevalent in the target population.

This study is justified by the need to generate population-based, molecular-level evidence on the specific high-risk HPV DNA types that are circulating among premenopausal women in Imo

State. The study provided an opportunity to determine whether the currently available HPV vaccines (bivalent, quadrivalent, and nonavalent) offer adequate coverage against the locally prevalent high-risk HPV types, especially among women with abnormal cervical lesions.

The study also contributed to a better understanding of the sociodemographic, behavioral and reproductive factors associated with high-risk HPV infections and their relationship with abnormal cervical lesions, using both molecular techniques and visual inspection methods (VIA/VILI). The integration of HPV DNA typing with sociodemographic and behavioral data provides a comprehensive view of HPV epidemiology and the potential effectiveness of vaccine-based interventions in this population.

Findings from this study will benefit multiple stakeholders: Women in the community were directly engaged, educated, screened, and some received follow-up care—thereby increasing awareness, early detection, and prevention. Healthcare providers will gain critical insights into the most prevalent high-risk HPV genotypes, enabling them to provide more informed counseling and make better clinical decisions. Policy makers and public health planners will be equipped with local data needed to allocate resources efficiently and adapt immunization strategies that reflect the HPV genotype distribution in Imo State. Researchers and scientific communities will benefit from a strengthened evidence base and can build on this work to explore vaccine development, cost-effectiveness analysis, and expanded prevention strategies. The government and donor agencies will be better positioned to advocate for or support broader vaccine coverage, improved cervical cancer screening programs, and have stronger political commitment for cervical cancer control.

Overall, this study provides a foundation for guiding cervical cancer prevention efforts, enhancing vaccine policy formulation, and promoting equitable health outcomes for women in Imo State and similar settings across Nigeria and sub-Saharan Africa.

CHAPTER TWO

LITERATURE REVIEW

2.1. Conceptual Framework

The conceptual framework for this study is grounded in the interrelationship between virological, reproductive health, behavioral, and public health dimensions of HPV infection, particularly focusing on high-risk HPV (hrHPV) types, associated risk factors, and the extent to which current vaccines provide coverage against locally circulating genotypes in premenopausal women aged 30 to 49 years. It is based on three primary theoretical foundations of the epidemiological triad model, health belief model and molecular typing and vaccine matching paradigm.

In the **Epidemiological Triad Model** (Agent–Host–Environment), the *agent* refers to the high-risk HPV genotypes, the *host* comprises premenopausal women aged 30–49 years in Imo State, and the *environment* includes factors such as sexual behavior, socio-demographic characteristics, and access to healthcare.

The **Health Belief Model** (HBM) is used to explain the acceptance and uptake of HPV vaccination and screening. Its key components include perceived susceptibility, perceived severity, perceived benefits and barriers, and cues to action.

The **Molecular Typing and Vaccine Matching Paradigm** assesses the molecular prevalence of high-risk HPV genotypes and evaluates the extent to which available vaccines (bivalent, quadrivalent, or nonavalent) align with the circulating strains.

Table 2.1. Key Constructs and Relationships

The study is structured around the following core constructs:

Independent Variables	Intermediate Variables	Dependent Variables
• Sociodemographic factors (age, marital status, education, occupation, residence) • Behavioral factors (age at sexual debut, number of sexual partners, STI history) • Clinical factors (HIV status, parity, screening results)	• Molecularly identified high-risk HPV genotypes (HPV16, 18, 31, 35, 39, 45, 51, 52, 53, 56, 59, 66 and 68) • Available vaccine types (bivalent, quadrivalent, nonavalent)	• Prevalence of high-risk HPV infection • Distribution of genotype-specific HPV infections • Proportion of genotypes covered by current vaccines • Identification of associated risk factors

Conceptual Relationships

The framework proposes that individual-level (host) and environmental (contextual) factors influence the acquisition of high-risk HPV infections. Molecular analysis of HPV DNA enables typing of specific genotypes, which are then compared with available vaccines to determine suitability.

A simplified pathway in this framework is:

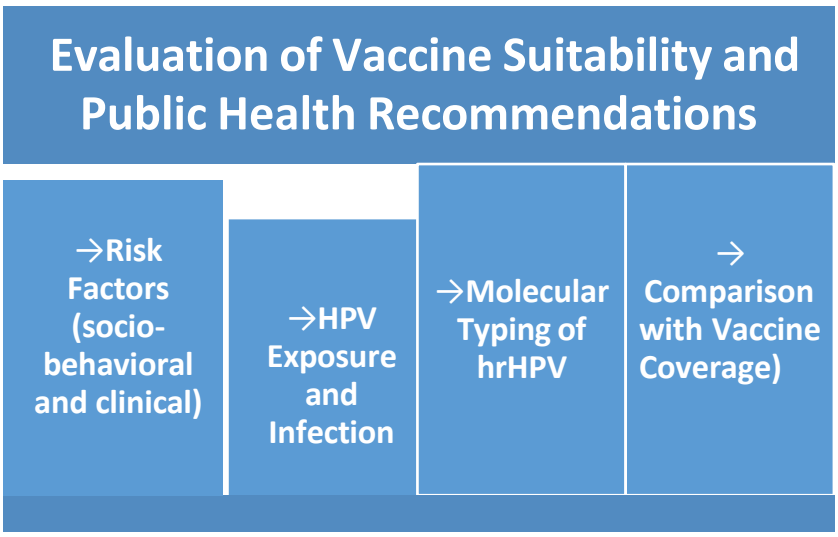

Fig. 2.1 Conceptual Relationships of study

Policy and Practice Relevance

This conceptual framework provides a logical structure for interpreting the study's findings in light of vaccine policy, cervical cancer prevention strategies, and public health interventions. If significant genotype mismatch is found between circulating strains and vaccine-included types, findings can support calls for the introduction of broader-spectrum vaccines and enhanced screening.

2.1.1 Overview of Human Papillomavirus and Cervical Cancer

Human papillomavirus (HPV) is a double-stranded DNA virus and the primary etiological agent in the development of cervical cancer. Globally, cervical cancer ranks as the fourth most common cancer among women, with an estimated 604,000 new cases and 342,000 deaths recorded in 2020 (World Health Organization [WHO], 2021). The burden is disproportionately higher in low- and middle-income countries (LMICs), particularly in sub-Saharan Africa, where over 90% of cervical cancer-related deaths occur due to limited access to early diagnosis and treatment services.

HPV is the most common sexually transmitted infection worldwide (Woodman, Collins, & Young, 2007). It has been firmly established as the causative agent of malignant and premalignant lesions of the cervix (Walboomers et al., 1999). A meta-analysis revealed that HPV DNA is present in approximately 99.7% of cervical cancer tissues (Brebi et al., 2017; Clifford, Smith, Aguado, & Franceschi, 2003).

2.1.2 High-Risk HPV Genotypes and Their Global Distribution

Over 200 HPV genotypes have been identified, with about 14 classified as high-risk (hrHPV) due to their oncogenic potential. Among these, HPV types 16 and 18 are responsible for approximately 70% of all invasive cervical cancer cases worldwide. Other prevalent high-risk genotypes include types 31, 33, 35, 45, 52, and 58. The distribution of these genotypes varies geographically, which affects vaccine efficacy and highlights the need for region-specific epidemiological data to guide public health strategies.

2.1.2.1 Risk Factors Associated with High-Risk HPV Infection

Multiple studies have identified several risk factors associated with hrHPV infection in Nigerian women. These include early coitarche, multiple sexual partners, early first pregnancy, long-term

use of hormonal contraceptives, and smoking. HIV-positive status remains a critical risk factor, increasing susceptibility to persistent hrHPV infections due to compromised immune function (Kabuga et al., 2021; Okunade et al., 2017).

Characteristics of persons with cervical cancer is indispensable in planning and making policy decisions in the prevention of cervical cancer as well as in research on the subject. A retrospective study in a tertiary cancer hospital in Mumbai found a median age of 54 years; over half were illiterate, and this was statistically significant. Only 13% presented with early disease and 77% had not received prior care (Jain, Ganesh, Bobdey, Sathwara & Saoba, 2017). A hospital-based study in Brazil on 99 women aged 40–54 years revealed coitarche between 15 and 18 years. These women had over five lifetime sexual partners and had never used condoms. Most were married, religious, and 70.7% were economically active professionals before diagnosis, but only 36% remained economically active afterward (Conde & Lemos, 2018). This aligns with findings in Northern Nigeria, where low socioeconomic status, early coitarche, multiple sexual partners, and high parity increased the risk of cervical cancer (Adewuyi, Shittu & Rafindadi, 2008). Existing studies have not described the socio-demographic characteristics of women with cervical cancer in Eastern Nigeria or Imo State. However, there is high awareness of screening methods among nurses, with limited uptake (Ezebialu, Ezenyeaku, Ikeako, Ubboe & Ojiyi, 2017).

2.1.3 Historical Perspectives of Human Papillomavirus (HPV).

Choi and Park (2015) provided a detailed historical background of HPV. In 1946, HPV particles were first visualized in human warts; the genome structure was characterized in 1965. Dr. Zur Hausen's work in the 1970s linked HPV to cervical cancer. By the 1980s, HPV types were identified in cervical cancer biopsies. In the 1990s, high-risk (hr) HPV types were recognized as the primary risk factor for cervical cancer. In 2002, the American Cancer Society included HPV genotyping in screening guidelines. In 2006, the FDA approved the bivalent HPV vaccine by GlaxoSmithKline, followed by the quadrivalent vaccine by Merck in 2008. Dr. Zur Hausen was awarded the Nobel Prize in 2014 for defining HPV's role in cervical cancer etiology. The 2015 ASCCP interim guidelines incorporated HPV genotyping for screening (ACS, 2014).

2.1.4 HPV Virology

HPV is a ubiquitous, heterogeneous virus in the Papillomaviridae family, found in both humans and animals. The alpha genus infects mucosal epithelia. Over 200 types exist, with 120 more under characterization via L1 gene sequencing (Burd, 2003). Around 30–40 types infect the anogenital region. HPV is a small (~50–55 nm), double-stranded DNA virus with a genome of ~8 kb housed in an icosahedral capsid of 72 capsomeres. The genome comprises three regions: early (E), late (L), and long control region (LCR). E6 and E7 oncoproteins play key roles in oncogenesis by impairing apoptosis and destabilizing tumor suppressors p53 and Rb, respectively. L1 and L2 proteins, expressed in the superficial epithelium, are highly immunogenic, making them viable vaccine targets. The LCR regulates viral replication and transcription.

2.1.5 HPV Risk Types

HPV is divided into low-risk types (e.g., 6, 11, 42, 44) and high-risk types (e.g., 16, 18, 31, 33, 45, 52, 58) based on carcinogenic potential. Low-risk types cause warts; high-risk types are linked to cervical and other anogenital cancers (CDC, 2005). The IARC classifies 12 hrHPV types as Group 1 carcinogens ie HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59 (ACS, 2014). Oncoproteins E6 and E7 inhibit apoptosis by targeting tumor suppressors (Brebi et al., 2017).

2.1.6. HPV Infectivity

High-risk HPV infection is necessary but not sufficient for cervical cancer development. Most HPV infections clear naturally; persistent infection occurs in less than 10% of women (ACS, 2014; Choi & Park, 2016; WHO, 2019). HPV is associated with cervical and other anogenital cancers, as well as some oropharyngeal cancers. It spreads via direct skin or mucosal contact during sexual activity. Women testing positive for HPV DNA have a higher risk of developing squamous intraepithelial neoplasia within two years (Castellsague et al., 2016). HPV types 16 and 18 cause about 70% of invasive cervical cancers (Herrero & Murillo, 2018). Progression risk is influenced by viral infectivity and host immune status (Clifford et al., 2003).

2.1.7 Mode of Transmission

HPV is primarily transmitted via skin-to-skin contact during sexual activity (Castellsague, 2008). Vertical transmission is rare (Castellsague, Drudis & Canadis, 2009). Penetrative sex is not required for transmission (WHO, 2019). Infection occurs when the virus accesses the basal epithelial cells through abrasions (Christensen, 2016). Most infections are asymptomatic and resolve within 12–24 months. However, about 15% persist, leading to premalignant or malignant lesions. Coinfections are common due to lack of cross-immunity (CDC, 2005).

2.1.8 Risk Factors of Cervical Cancer

Risk factors include early sexual debut, smoking, immunosuppression, multiple sexual partners, high parity, prior genital warts, abnormal Pap smears, oral contraceptive use, low socioeconomic status, STIs, and poor hygiene (Burd, 2003; ACS, 2020). Condom use is not fully protective. Modifiable risk factors include smoking and HPV exposure; non-modifiable factors include age and family history (Cancer.Net Editorial Board, 2019).

2.1.9 Natural History of HPV Infection

HPV infection is highest during cervical metaplasia, notably at puberty and post-childbirth. The transformation zone, where metaplasia occurs, is the site of cervical carcinogenesis. HPV infections are common between ages 18 and 30 and often asymptomatic. Persistent infection leads to cellular changes, then to high-grade CIN (CIN2/3), and eventually cancer if untreated. High-grade lesions may regress. Cervical cancer peaks in women over 35 who were persistently infected in youth (Burd, 2003). Immunosuppression promotes persistence. Nigerian women face elevated risks due to early coitarche and polygamy.

2.1.10. HPV Vaccination: Types and Coverage

Three prophylactic vaccines exist: bivalent (HPV 16, 18), quadrivalent (HPV 6, 11, 16, 18), and nonavalent (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58). Their efficacy depends on genotype match within the target population.

2.1.11 Suitability of Available HPV Vaccines in Nigeria

In Nigeria, types 16 and 18 are prevalent, but types like 35 and 58 are also common. Current vaccines may not offer full protection, necessitating continuous surveillance to inform vaccine policy and development.

2.1.12 Cervical Cancer Prevention Strategies

2.1.12.1 Primary Prevention: HPV Vaccination

As detailed in Section 2.3, prophylactic HPV vaccines targeting high-risk genotypes (e.g., bivalent, quadrivalent, nonavalent) represent a cornerstone of primary prevention. While global vaccination programs have reduced cervical cancer incidence in high-income countries, implementation in low-resource settings like Nigeria remains limited. Challenges include vaccine cost, logistical barriers, and genotype mismatches (e.g., nonavalent vaccine unavailability in Imo State). Region-specific HPV surveillance is critical to optimize vaccine efficacy and policy (see Section 2.7).

2.1.12.2 Secondary Prevention: Screening and Early Detection

Secondary prevention hinges on early detection of precancerous lesions through screening. Despite the proven efficacy of cytology-based methods, disparities persist in access and outcomes between high- and low-resource settings.

Cytology-Based Screening

Introduced by George Papanicolaou in 1941, the Pap smear revolutionized cervical cancer prevention by enabling early detection of dysplastic changes. The World Health Organization (WHO) recommends Pap testing every 3–5 years or HPV DNA testing every 5 years for women age 30, complemented by treatment of premalignant lesions. However, cytology-based screening faces systemic challenges in low-resource contexts: Low Sensitivity (50–60%)**,** sampling and interpretation errors contribute to false negatives, necessitating repeat screenings. High Costs and infrastructure demands, requiring of skilled cytotechnologists, laboratory infrastructure, and follow-up protocols, which are often unavailable in regions like Sub-Saharan Africa lead to loss to follow-up: Multiple visits for testing and treatment exacerbate dropout rates in settings with geographic and socioeconomic barriers (Tota et al., 2014; Hypolito, 2017). Liquid-Based Cytology (LBC)**,** while reducing sampling artifacts, remains impractical in resource-limited areas due to higher costs and technical requirements.

Visual Inspection Methods

In order to address cytology's limitations, visual inspection methods have gained traction in low-resource settings. Examples are Visual Inspection with Acetic Acid (VIA): Application of 3–5% acetic acid induces acetowhite changes in dysplastic epithelium. Though cost-effective and immediate, its subjectivity (operator-dependent accuracy) and moderate sensitivity (65–80%) limit reliability. Visual Inspection with Lugol's Iodine (VILI)**:** Non-uptake of iodine by abnormal cells produces yellow discoloration, offering complementary data to VIA. Automated Visual Evaluation (AVE)**:** Emerging AI-driven algorithms analyze cervical images with >90% accuracy, promising to enhance visual methods' precision (Sullivan, 2019). Despite their utility, visual methods require rigorous quality assurance and training to mitigate variability.

2.1.12.3 Barriers to Screening in Low-Resource Settings

In Nigeria and similar contexts, cervical screening uptake is hampered by health system deficits such as fragmented infrastructure, lack of national guidelines, and shortages of trained personnel. There are also socioeconomic factors like poverty, low health literacy, and cultural stigma surrounding gynecological exams as well as geographic inequities like rural populations facing long travel distances to screening centers thereby exacerbating attrition (Chirenje et al., 2001; Tsu, 2017).

2.1.12.4 Testing for HPV DNA

Over the past decade, the use of human papillomavirus (HPV) DNA testing has gained significant attention as a primary screening tool for cervical cancer. This method is highly reproducible and sensitive**,** with studies reporting sensitivity rates exceeding 90% for the detection of pre-malignant cervical lesions (Leinonen et al., 2009). The World Health Organization (WHO) has recommended HPV testing—with or without visual inspection with acetic acid (VIA) triage—for implementation in low-income countries as a strategic method for early detection (Committee Opinion, 2015).

A landmark study in rural India demonstrated that a single lifetime HPV DNA screening resulted in a 50% reduction in cervical cancer incidence and mortality (Sankaranarayanan et al., 2009). Similarly, HPV screening followed by cryotherapy led to a 77% reduction in the incidence of

pre-malignant cervical lesions (Denny et al., 2010). These findings underscore the potential of HPV DNA testing to drastically reduce cervical cancer burden.

Despite these benefits, HPV DNA screening in Nigeria has not achieved widespread implementation, primarily due to cost-related barriers. However, this challenge could be mitigated by leveraging the widespread availability of GeneXpert machines, which are already utilized in HIV and tuberculosis screening programs and approved by WHO for HPV testing (WHO, 2017). GeneXpert technology offers automated, quantitative detection and differentiation of high-risk HPV types, representing a feasible platform for HPV DNA testing in resource-limited settings.

Another challenge in HPV DNA testing is the infrastructure required for equipment and logistics, often necessitating multiple clinic visits. Innovations such as point-of-care HPV tests—which are cost-effective and clinically validated—could address these barriers. Additionally, the use of self-collected specimens has emerged as a promising alternative. These allow for increased privacy, confidentiality, and convenience, while offering detection rates comparable to physician-collected samples (Burd, 2016).

2.1.13 High-Risk HPV DNA Testing

HPV DNA testing is widely used for the detection and genotyping of high-risk (hrHPV) types. These assays are based on the presence of HPV DNA in nearly all cervical cancer specimens (WHO, 2005), and they are integral to several clinical applications. These include primary screening for women aged 30 years and older, triage of women with atypical squamous cells of undetermined significance (ASC-US), monitoring post-treatment for cervical intraepithelial neoplasia (CIN) grades 2 and above, and follow-up of discordant results between cytology and histopathology

Initially, HPV DNA assays could detect 13–14 high-risk types without distinguishing individual genotypes. However, recent advancements have led to the development of genotyping assays capable of detecting specific types such as HPV 16 and 18**,** which are responsible for the majority of cervical cancer cases globally.

Despite their high sensitivity, HPV DNA tests have relatively low specificity, which may lead to false positives (Gradíssimo & Burk, 2017). Nonetheless, their role in population-level screening

remains pivotal. Common examples of hrHPV DNA-based assays include Hybrid Capture 2 HPV DNA Test, Cervista HPV HR Test, Amplicor HPV Test, careHPV Test (HPV4A), ACE Screening CE assays, HPV/STD4 ACE Screening CE and AmpFire HPV High Risk Genotyping Assay (Poljak & Kocjan, 2010). An alternative to DNA-based assays is the E6/E7 mRNA assay**,** which uses reverse transcriptase polymerase chain reaction (RT-PCR) to detect expression of the E6 and E7 oncogenes—markers that are more directly associated with disease progression**.** Examples include Aptima HPV Assay, APTIMA HPV 16, 18/45 Genotype Assay (Hologic Gen-Probe Inc., San Diego, CA), both approved by the U.S. FDA in 2012 (Lapierre, 2019). These assays provide critical insights not just for diagnosis, but also for prognostic assessment and therapeutic decision-making in cervical cancer prevention and control.

2.1.14. High-Risk HPV DNA Testing Methods

A variety of high-risk (hr) HPV DNA detection methods have been developed and commercialized for the molecular diagnosis and genotyping of HPV in cervical specimens. These molecular methods, which include signal amplification, PCR-based assays, and isothermal amplification techniques, are integral to screening, diagnosis, and management of HPV-related cervical lesions.

2.1.14.1 Hybrid Capture 2 HPV DNA Test

The Hybrid Capture 2 (HC2) assay, developed by Digene Corporation (now marketed by Qiagen), was approved by the U.S. Food and Drug Administration (FDA) in 1999 as a replacement for the initial HC1 format. It is widely used for the triage of women with atypical squamous cells of undetermined significance (ASC-US) cytology and for co-testing in women aged 30 years and above. The HC2 test employs an alkali-denaturation step, followed by hybridization with RNA probe cocktails specific for high-risk and low-risk HPV types. The resulting RNA-DNA hybrids are captured onto a microplate well coated with monoclonal antibodies and subsequently detected using a chemiluminescent signal. The output is semi-quantitative, expressed in relative light units (Poljak & Kocjan, 2010).

2.1.14.2 Cervista HPV HR Test

The Cervista HPV HR test (Hologic; Third Wave Technologies, Madison, WI) is based on Invader® chemistry—a signal amplification technique that allows detection of specific nucleic

acid sequences through a dual isothermal reaction system. The assay targets 14 hrHPV genotypes and categorizes them into three distinct probe sets: A5/A6 (HPV-51, -56, -66), A7 (HPV-18, -39, -45, -59, -68), and A9 (HPV-16, -31, -33, -35, -52, -58). Although it does not provide individual genotype results, it reports a positive or negative outcome for each probe group (Poljak & Kocjan, 2010).

2.1.14.3 Amplicor HPV Test

The Amplicor HPV Test (Roche Molecular Systems, Branchburg, NJ) is a qualitative PCR-based test first introduced in Europe in 2004. It detects the same 13 hrHPV genotypes as the HC2 assay. The test involves amplification of a 165 bp region of the L1 gene and co-amplification of a human β-globin control gene using biotin-labeled primers. Detection of amplified products is carried out on microwell plates via hybridization and enzyme-linked colorimetric detection, with final results expressed as positive or negative (Poljak & Kocjan, 2010).

2.1.14.4 Digene HPV Genotyping LQ Test

The Digene HPV Genotyping LQ Test (Qiagen) is a research-use-only (RUO) assay designed to identify 18 HPV genotypes, including HPV-16, -18, -26, -31, -33, -35, -39, -45, -51 to -53, -56, -58, -59, -66, -68, -73, and -82. It utilizes GP5+/GP6+ primers to amplify a 150 bp fragment of the L1 gene, followed by genotyping using type-specific bead-based hybridization on the LiquiChip 200 Workstation or Luminex IS system. The assay includes internal control primers for the human β-globin gene and is capable of detecting viral loads ranging from 5 to 10,000 copies per reaction (Poljak & Kocjan, 2010).

2.1.14.5 Atila AmpFire HPV High-Risk Genotyping Assay

The Atila AmpFire HPV High-Risk Genotyping Assay is an isothermal nucleic acid amplification-based test designed for the qualitative detection and genotyping of high-risk HPV types. The assay targets the E6/E7 regions of the viral genome and includes fluorescent probes specific to 15 hrHPV types: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, and -68. The assay is compatible with various specimen types, including dry cervical swabs, PreservCyt solution, BD SurePath preservative fluid, and formalin-fixed paraffin-embedded (FFPE) tissue. It includes internal controls and external positive and negative controls for assay validation. The test can be performed in most standard molecular laboratories using a

fluorescence real-time PCR instrument with FAM/HEX/ROX/CY5 detection channels (AmpFire HPV High Risk Genotyping, Version 4.0, March 2022). For the purposes of this study, the AmpFire HPV High-Risk Genotyping assay was used for testing dry cervical swab specimens.

2.1.15 Limitations of the Atila AmpFire HPV High-Risk Genotyping Assay

Despite its benefits, the Atila AmpFire assay has certain limitations. In low-prevalence populations, the positive predictive value may be reduced, and low viral loads or sampling errors can result in false negatives. Additionally, false positives may occur due to contamination from contraceptive products or improper specimen handling. The assay panel may not detect all oncogenic HPV types, posing the risk of missed infections. Cross-reactivity is also a potential concern; however, Atila Biosystems has evaluated possible cross-reactants, including bacteria, yeast, other viruses, and HPV types, to minimize this risk. The accuracy of results can be compromised by factors such as poor sample quality, suboptimal storage, or degraded DNA, all of which could lead to inconclusive outcomes (AmpFire HPV High Risk Genotyping, Version 4.0, March 2022).

2.1.16 Application of Data in Determining HPV Vaccine Suitability in Imo State

HPV testing and vaccination have been recognized as key strategies for cervical cancer prevention. While HPV screening identifies women already infected with hrHPV, vaccination provides primary prevention by reducing the risk of infection with specific oncogenic types. The currently available HPV vaccines include the bivalent vaccine which targets HPV types 16 and 18, the quadrivalent vaccine which covers types 6, 11, 16, and 18 and the the nonavalent vaccine which offers protection against types 6, 11, 16, 18, 31, 33, 45, 52, and 58.

At the time of this study, the nonavalent vaccine was not available in Imo State. The data generated, particularly on hrHPV genotypes identified among VIA/VILI-positive women, are crucial for informing public health decisions regarding vaccine selection. Knowledge of prevalent genotypes in the region can guide recommendations on the most appropriate and cost-effective vaccine to be introduced, considering the high cost of HPV vaccination and the limited resources available. Tailoring vaccine policy to the local genotype distribution will ensure better preventive outcomes and optimal resource utilization.

2.2 Theoretical Framework

This study is underpinned by three interrelated theoretical models that provide insight into the factors influencing high-risk human papillomavirus (hrHPV) infection, its persistence, and prevention through vaccination and screening.

a. The Epidemiological Triad Model

The Epidemiological Triad Model explains disease causation through the interaction of three key components: the **agent**, the **host**, and the **environment**.

In this study:

i. The **agent** is the high-risk human papillomavirus (hrHPV), particularly oncogenic genotypes associated with cervical cancer, such as HPV 16 18, 31, 35, 39, 45, 51, 52, 53, 56, 59, 66 and 68)

ii. The **host** is the premenopausal woman, whose susceptibility to hrHPV infection may be influenced by biological factors (e.g., immune status, HIV infection), demographic characteristics (e.g., age, education), and behavioral factors (e.g., sexual practices).

iii. The **environment** includes socio-cultural norms, access to sexual and reproductive health services, healthcare infrastructure, public awareness, and vaccination coverage within Imo State.

This model is instrumental in understanding how individual risk factors and environmental exposures interact to influence the acquisition and persistence of hrHPV infections, which are essential precursors to cervical carcinogenesis (Schiffman et al., 2007).

b. The Health Belief Model (HBM)

The Health Belief Model, developed by Rosenstock (1966), posits that an individual's health behavior is shaped by personal beliefs regarding disease risk and the effectiveness of preventive actions. In the context of HPV, the HBM helps explain the acceptance and uptake of HPV vaccination and cervical cancer screening among women. The key constructs include:

i. **Perceived Susceptibility** – The individual's belief about their likelihood of acquiring an hrHPV infection.

ii. **Perceived Severity** – Awareness of the potential consequences of persistent hrHPV infection, including the risk of cervical cancer.

iii. **Perceived Benefits** – Beliefs regarding the protective value of HPV vaccination and regular cervical screening.

iv. **Perceived Barriers** – Perceived obstacles to accessing HPV-related health services, including cost, cultural beliefs, misinformation, and stigma.

v. **Cues to Action** – External motivators such as public health campaigns, healthcare provider recommendations, or community awareness programs that prompt preventive behavior.

vi. **Self-Efficacy** – Confidence in one's ability to take appropriate preventive action, such as getting screened or vaccinated.

By applying the HBM, this study examines how health perceptions influence behavior toward HPV prevention among women in Imo State.

c. The Molecular Typing and Vaccine Matching Paradigm

This paradigm emphasizes the identification of circulating high-risk HPV (hrHPV) genotypes within a target population and assesses how well these genotypes align with the strains included in existing HPV vaccines—namely, the bivalent, quadrivalent, and nonavalent formulations. The core assumption is that optimal vaccine effectiveness is achieved when there is a close match between the vaccine-included genotypes and those prevalent in the population (Garland et al., 2016). In the context of this study, the paradigm is applied to evaluate whether current HPV vaccines provide adequate coverage for the genotypes most frequently detected among premenopausal women in Imo State. The findings aim to inform public health policy on vaccine selection and implementation strategies tailored to local epidemiological pattern.

2.3 Empirical studies

In Chile, Brebi et al. (2017) reported an 80.8% HPV positivity rate among women attending cervical cancer screening programmes. High-risk HPV was significantly associated with abnormal cervical lesions: 83.5% in low-grade squamous intraepithelial lesions (LSIL), 87.6% in high-grade SIL (HSIL), and 95.8% in squamous cervical cancer. HPV 16 was the most prevalent

genotype, particularly in younger women, whose infections might resolve spontaneously. Their clinic-based setting found HPV DNA in only 10.5% of women with normal cervical epithelium.

Global HPV prevalence in women with normal cervices has been estimated at 10.4% (95% CI: 10.2–10.7), with the highest rates in Africa (22.1%), Central America (20.4%), and North America (11.3%) (de Sanjosé, Diaz, & Castellsagué, 2007). Prevalence peaks were noted in women under 35 years and again at 45 years. In South Asia, another meta-analysis showed HPV prevalence of 94.6% in invasive cervical cancer, 86.5% in HSIL, 65.4% in LSIL, and 12% in women with normal cervices.

The International Agency for Research on Cancer (IARC) found HPV 16 and 18 in 75% of cervical cancer specimens, 41–67% of HSIL, and 16–32% of LSIL, with types 31, 33, 35, 45, 52, and 58 contributing an additional 20%. However, only vaccine-preventable HPV types were studied (IARC, 2010).

In a global study involving 10,575 histologically confirmed invasive cervical cancer cases across 38 countries, HPV DNA was detected in 85% of cases. Types 16 and 18 were responsible for 71% of these cases, and accounted for 94% of cervical adenocarcinomas, typically presenting at younger ages (de Sanjosé et al., 2007).

In Brazil, Fernandes et al. (2013) found an overall HPV prevalence of 65.2%, with HPV 16 being the most common, followed by HPV 58, especially in women with normal or mildly dysplastic cervices. In Italy, HPV 16 and 18 (covered by the bivalent vaccine) accounted for 22.7% of infections, while the 9-valent vaccine types represented 58.6%. HPV prevalence increased with lesion severity: NILM (14.5%), ASC-US (39.4%), LSIL (56.7%), and HSIL (60%) (Galati et al., 2017).

In African studies, HPV 35 was more prevalent than HPV 16 in Mozambique (Castellsagué et al., 2001), while HPV 52 dominated in Kenya (de Vuyst et al., 2003). In Senegal, HPV 16 and 35 were common (Xi et al., 2017). In Benin Republic, types 59, 35, 16, 18, and 45 were most frequent (Pira et al., 2011). In Chad, Bouassa et al. (2019) found a 15.8% hrHPV prevalence, with type 58 being the most prevalent. Only 20% of the population was protected by the 4-valent vaccine, while 50–70% of cases were potentially preventable by the 9-valent vaccine.

In a study spanning Nigeria, Ghana, and South Africa, 90.4% of invasive cervical cancer cases were HPV-positive, predominantly types 16 (51.2%), 18 (17.2%), 35 (8.7%), and 45 (7.4%) (Denny et al., 2014). A meta-analysis found that HPV 16's contribution to cervical cancer was lowest in West Africa (11.1%) compared to 32.3% in South Asia (Bruni et al., 2010).

2.3.1 Prevalence of High-Risk HPV in Nigeria

In Nigeria, the hrHPV prevalence is notably high. A systematic review and meta-analysis by Kabuga et al. (2021) reported a pooled prevalence of 25%, with types 16 and 18 accounting for 9% and 10% respectively. Among HIV-positive women, the prevalence was significantly higher at 71%, likely due to immune suppression and impaired viral clearance mechanisms.

2.3.2 Regional Variations in HPV Prevalence

Regional disparities in hrHPV prevalence have been documented across Nigeria's geopolitical zones. A recent meta-analysis reported an overall pooled prevalence of 42% in the general population, with the highest rates in the Northwest (73%) and the lowest in the Southeast (6%) (Kabuga et al., 2021). These disparities may be explained by differences in sexual behavior, cultural practices, and access to screening and healthcare services.

In Lagos (Southwest Nigeria), Okunade et al. (2017) found an hrHPV prevalence of 36.5%, with types 31 (25%), 35 (8%), and 16 (3.5%) being the most common. In contrast, Manga et al. (2015) in Northeast Nigeria reported 10 HPV genotypes, with types 18 and 16 responsible for 44.7% and 13.2% of infections, respectively. The study also identified types 31, 33, 35, 38, 45, 56, 58, 82, and KC5.

In Ife (Southwest), Fadahunsi et al. (2013) reported HPV 16 as the most prevalent, followed by 53, 18, and 52. In Ibadan, Thomas et al. (2004) found types 16 and 35 to be most common, followed by 31, 58, and 56. These findings raise concerns about the current vaccines' capacity to adequately protect Nigerian women.

The present study aims to determine the prevalent HPV genotypes among women in Imo State, Eastern Nigeria, and assess whether these types align with those covered by available vaccines. Furthermore, the study investigates whether HPV types in women with premalignant lesions differ from those in women with normal cervical cytology. These data are essential for determining vaccine suitability and tailoring public health interventions.

2.4. Gaps in Knowledge and Research Needs

Although numerous studies have explored the prevalence and risk factors associated with hrHPV infections in Nigeria, significant data gaps persist. One of the most pressing gaps is the lack of comprehensive genotype-specific data in distinct regions such as Imo State. There is an urgent need for region-specific studies employing validated molecular assays to determine the distribution of hrHPV genotypes and to assess the local effectiveness of currently available vaccines. Generating such evidence is critical for guiding public health policy and vaccine development, especially in regions with unique epidemiological patterns.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This is a population based, cross-sectional, observational, analytical epidemiological study. This is cross -sectional and observational because the variables such as detection of infecting high risk HPV types and the screening for pre malignant lesions of the cervix were done at a time and without any intervention. Denny and others in 2014 used a cross sectional, multi center and epidemiological study for 'sampling and HPV typing to look specifically at HPV type distribution in sub-Saharan African women with invasive cervical cancer (ICC), which is important to evaluate the potential impact of prophylactic vaccines in this region' (Denny *et al*., 2014).

Similarly, in Natal, North East Brazil, Fernandes and colleagues used a cross sectional study on women with either normal cervices or with abnormal cytology to determine HPV prevalence and genotype distribution (Fernandes *et al*., 2013) It is however analytical because we also established the association of the hr- HPV type prevalence (exposure) and the results from visual screening of the cervix (outcome) as well as used the data gathered to correlate with the HPV types that are vaccine preventable and determined HPV vaccine suitability in Imo State, Nigeria.

3.2 Study Area

This study was conducted in Imo State, located in South-East Nigeria. Imo State comprises 27 Local Government Areas (LGAs), with Owerri as the state capital. It is divided into three senatorial districts—Owerri, Okigwe, and Orlu—consisting of 9, 6, and 12 LGAs respectively. The state is bordered to the east by Abia State, to the west by the River Niger and Delta State, to the north by Anambra State, and to the south by Rivers State. It lies between latitudes 4°45′N and 7°15′N, and longitudes 6°50′E and 7°25′E, covering a total area of approximately 5,530 square kilometers (Imo State Government, 2010).

The majority of the population in Imo State are Christians, though there are also Muslims, traditional religious adherents, and atheists. The predominant ethnic group is Igbo, and the Igbo

language is widely spoken. People from other ethnic groups in Nigeria and beyond also reside in the state.

The primary occupations in Imo State include civil service, trading, and skilled and unskilled artisanal work. Most families are officially monogamous, although concubinage is commonly practiced. Imo State is also known for its numerous hotels and vibrant weekend and nightlife.

The healthcare infrastructure in the state includes two tertiary hospitals, several general hospitals, numerous private hospitals, pharmacies, chemist shops, maternity homes, and traditional birth attendant clinics. Cervical cancer screening is not yet widely practiced among women in the state, primarily due to limited awareness, denial, and low prioritization of the disease. While outreach programs that include cervical cancer screening occur intermittently, screening remains largely opportunistic—typically offered in gynecological clinics when a woman is perceived to be at risk.

A dedicated walk-in cervical and breast cancer screening unit is available at the Federal Medical Centre, Owerri (now the Federal University Teaching Hospital, Owerri). Screening methods employed include visual inspection, cytology, and, when abnormalities are detected, follow-up with colposcopy and electrosurgical interventions.

Fig.3.1: Map showing the senatorial zones and local government areas of Imo State. (Source: Adizua and Ohakwere –Eze, 2014)

3.3 Study population

Imo State is projected to have a population of approximately 5,408,800 in 2016, with a male-to-female ratio of 1.03:1, based on the 2006 population census conducted by the National Population Commission. The estimated female population in Imo State for 2016 was 2,650,290, based on projections from the same census. Considering that approximately 17% of women in Nigeria were between 30 and 49 years of age, and extrapolating this proportion to the estimated female population in Imo State for 2016, an estimated 459,550 women formed the study population (National Population Commission & National Bureau of Statistics, 2018). The study population consisted of premenopausal women aged 30 to 49 years who had ever been sexually active. This age group was selected because visual screening methods are most effective in women under the age of 50, when the squamocolumnar junction of the cervix remains visible, while HPV DNA testing is recommended primarily for women over 30 years of age (Bradley, Barone, Mahe, Lewis, & Luciani, 2005).

3.3.1 Inclusion criteria

The inclusion criteria were consenting women 30 to 49 years of age, residing in sampled communities, who have ever been sexually active, who were not pregnant, not bleeding and who had not had a hysterectomy.

3.3.2 Exclusion criteria

Those excluded were

1) Women below 30 or above 49 years of age

2) Those who were pregnant

3) Those who were menopausal

4) Those who have had a hysterectomy for either oncology or non-oncology related conditions.

3.4 Sample Size

The planned sample size was 257 women between 30 and 49 years of age. The sample size calculation was done using a formula for representative sample for proportions in large populations in a cross sectional study (Cochran, 1963; Duru et al., 2018).

$$n = Z_{\alpha}^{2} \frac{[P(1-P)],}{d^2}$$

Where n is the sample size,

Where Z_{α} is the standard normal deviate set at 95% significance level which is 1.96 (from the probability at 95% confidence interval)

P is prevalence of HPV infection in a previous study which is 16.6% or 0.166 (Okoeguale et al., 2022).

d: The precision of the estimate/ the degree of accuracy set at 0.05

Sample size calculation/formula:

Therefore,

$$n = 1.96^2 \text{ x } \frac{[0.166\ (1-0.166)],}{0.05.^2}$$

= 3.84 x [0.166 x 0.834] /0.0025

= 0.5316 /0.0025

= 212.6 = 213

To account for attrition, 20% (n-44) was added to the calculated sample size which altogether suggested a sample size of 257 (ie. 213 + 44) being adequate to power the study.

3.5 Selection of Local Government Areas and Communities

A multi-stage sampling method was done. Firstly, Imo State was clustered into three existing geo-political zones (Orlu, Okigwe and Owerri). Then, a complete list of Local Governments areas (LGAs) in each zone was used as follows: 12 LGA's in Orlu, 6 in Okigwe and 9 in Owerri. Random sampling using ballotting was carried out to select one Local government from each zone. Ohaji Egbema was selected in Orlu zone, Ehime Mbano in Okigwe zone and Owerri municipal in Owerri zone.

Next was the selection of study communities. A list of communities in each of the selected LGAs was provided. Then simple random sampling technique using balloting was used to select the

community in each of the LGAs that participated in the study. Mgbirichi was selected from Ohaji Egbema LGA, Umunomo in Ehime Mbano and Aladinma in Owerri municipal.

Selection of participants at this point was done using purposive sampling method. A cervical cancer screening outreach was conducted in each of the communities selected. Consecutive consenting women were enrolled in the study. Purposive sampling was used at this stage because of the intimate nature of the study and need for privacy. Prior to this, advocacy visits to the stakeholders (traditional rulers, faith based leaders, women leaders etc.) was carried out to explain the benefits and justification for the screening exercise and also to obtain initial permission for the study.

Once permission had been obtained, a mass cervical cancer outreach for eligible women was organized. Lectures and health education sessions addressing cervical cancer awareness and prevention were conducted. Interactive sessions followed the lectures, and women who opted to participate in the study were further counseled individually to provide informed consent for screening. Screening was done first by collecting specimens for HPV DNA testing, followed by visual inspection using acetic acid or Lugol's iodine.

3.6. Collection of cervical specimen

Cervical specimen was collected by exposing the cervix using a sterile bivalve speculum. The well labelled dry cervical swab was used to take a smear from the cervical canal by rotating 360 degrees. The swab stick was carefully replaced in the casing and stored in a refrigerator. This was preserved at 2-8^{o}C for 2 weeks and for 3 months when frozen at -20^{o}C**. (**Centers for Disease Control and Prevention., **2003)**

A structured questionnaire was used an instruments in this study to address objective 2. The sections of the questionnaire included consent declaration, basic participant information, sociodemographic characteristics, social habits and / risk factors, reproductive history and knowledge, practice and attitude to cervical cancer prevention.

3.7 Interviewer administered questionnaires

An interviewer administered the questionnaire on the consenting volunteers / participants. Consent was obtained. Each consenting eligible woman was given a using study coded number based on the senatorial zone, local government, community and serial number. For example,

ZONE/LGA/COMMUNITY/participant serial number. For example, first participant in Okigwe zone, was from Umulomo in Ehime Mbano was recorded as OK/EM/UM/001. The basic information included the coded number, senatorial zone and local government of origin, phone number, email and date of interview and study participation, socio-demographic characteristics will include age, religion, education, marital status and occupation. The risk factors assessed were use of drugs, tobacco or alcohol in any form, the reproductive history which included age at coitarche, number of pregnancies and childbirth, number of sexual partners, type of marriage setting whether polygamous or monogamous, history of sexually transmitted diseases including HIV, use of contraception, etc. Finally, knowledge and use of cervical cancer prevention methods such as vaccination and screening were assessed and the participant was thanked.

3.8.1 Dry-based cervical swabs collection and storage

Consenting recruited eligible women were placed in dorsal position in a secured privacy assured environment, bimanual examination was done and a bivalve speculum was used to expose the cervix. Initial inspection of the cervix was carried out to determine if there are gross macroscopic lesions on the cervix. Thereafter, cervical samples were collected and stored in a refrigerator at temperature of 2 to 8 degrees centrigrade in waiting for HPV DNA/ viral studies. Few samples were stored for 3 months and where stored frozen at -20^{o}C when immediate laboratory work was not feasible.

3.8.2: Visual methods of screening (VIA/VILI) to determine presence of abnormal cervical lesions.

Following the collection of dry swab samples, the cervix was then painted with 5% acetic acid by dabbing it repeatedly or by spraying. This was to promote protein coagulation and mucous removal. The cervix was observed for 1-2 minutes for aceto-white areas. The results were noted as aceto-white positive or negative. Aceto white positive cervix were abnormal/ premalignant lesion.

Thereafter, Lugol's iodine was painted on the cervix. This changed the color of normal epithelium which contains glycogen to mahogany brown or black (iodo-positive). Immature metaplastic cells, inflammatory epithelium or regenerative zones appeared mustard yellow to light brown and was described as iodo-negative and therefore abnormal (Abdoulaye et al, 2017).

3.8.3 : hr-HPV screening using the AmpFire HPV high risk genotyping

The Atila AmpFire® HPV High-Risk Genotyping Assay is an isothermal nucleic acid amplification assay used for the qualitative genotyping of high-risk human papillomavirus (HPV) types. Type-specific primers and fluorescein-labeled probes targeting regions of the viral genome, including the E6/E7 regions, were used for amplification under isothermal conditions. The assay genotypes the following high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68. Cervical specimens collected using dry swabs were used for the assay.

3.8.4 a: Dry-based cervical swabs transportation

Endocervical and ectocervical cells were collected from the cervical canal using a dry swab. The sampled cervical cells were transported in frozen ice packs to the Infectious Disease and Molecular Epidemiology Laboratory, Department of Public Health, Federal University of Technology, Owerri, Imo State, Nigeria, for molecular analysis.

3.8.4 b: Sample inactivation and DNA extraction

Upon arrival of the gathered cervical swabs at the laboratory, they were sorted and placed in a Biosafety Cabinet Class 3 (Glove Box). The sample tube containing the dry swab were uncapped and the brush (swab stick) was placed into a 1 ml 1x lysis buffer (LB) and recapped for inactivation. The tube was then put in a vortex for 10-15 seconds and afterwards incubated at 95°C for 20 minutes. Following incubation, the tubes were allowed to cool down to room temperature before being spun in an Eppendorf Minispin for nucleic acid collection (DNA extraction). The vials were preserved frozen at -80°C (Copan Italia SpA, Brescia, Italy) for HPV DNA detection and genotyping.

3.8.4 c: Preparation of the four master-mixes using 4 primer mixes (N stands for unknown sample number)

Each of the master mix tubes (primer mix and reaction mix) were briefly vortexed for 15 sec. Equal volume of both the primer mix and the reaction mix were mixed-up in the Main Lab. as shown below:

A PM-1 master mix

HPVG4FRM (Reaction mix) (N+2) x 10= µL

HPVG4FPM-1 (primer mix 1) (N+2) x 10= µL

TOTAL (N+2) x20 = --- µL (N=Number of sample).

B PM-2 master mix

HPVG4FRM (Reaction mix) (N+2) x 10 = µL

HPVG4FPM-2 (Primer mix 2)(N+2) x 10 = µL

TOTAL (N+2) x20 = --- µL

C PM-3 master mix

HPVG4FRM (Reaction mix) (N+2) x 10 = µL

HPVG4FPM-1 (primer mix 3) (N+2) x 10 = µL

TOTAL (N+2) x20 = --- µL

D PM-4 master mix

HPVG4FRM (Reaction mix) (N+2) x 10 = µL

HPVG4FPM-2 (primer mix 4) (N+2) x 10 = µL

TOTAL (N+2) x20 = --- µL

3.8.4 d: Dispensing of the Master-mix into the MIC tubes

The prepared master mixes were transferred from the Main-lab to the Amplification Room.

Each of the four master mixes (20 µL) were dispensed into the MIC tubes (total tubes needed are 4N+8 or 48 well PCR plate) for every 10 specimens. A set of 4 tubes from 4 master mixes were used to genotype one specimen i.e A total of 48 wells were used for every 10 samples. Ten samples each were dispensed into each of the 40 wells, Non template control (NTC) and positive control (POS) were added to 4 wells each, respectively. This was done in the DEAD-AIR BOX 3.

3.8.4 e: Template Addition

Sequentially, 5 µL (total 20 µL needed) of heated specimen samples being the template containing the inactivated and extracted DNA were added into a set of MIC tubes that contained the 20 µL of the master mix. For the negative control reaction, 5 µL of negative control template were added into a set of 4 tubes and for the positive control reaction, 5 µL of positive control template were also added into another set of 4 tubes. This was done in the laminar flow hood, as described by the World Health Organization (2021).

All the tubes were capped with an optical-compatible film. Gently, the tubes were and briefly spinned to allow all the liquid settle to the bottom of the wells. The MIC tubes were placed into the sample holder in the compatible real-time PCR machine according to the template mapping and the lid closed to start the reaction run.

3.8.4 f: HPV detection and genotyping

Parallel detection and genotyping of HPV was carried out using the Atila AmpFire HPV High-risk genotyping assay which is an isothermal nucleic acid amplification assay for qualitative genotyping of high-risk types of human papillomavirus (HPV). High-risk HPV-specific primers and fluorescein probes amplified regions of viral genomic DNA including E6/E7 regions under isothermal conditions. The assay genotyped HPV 16,18,31,33,35,39,45,51,52,53,56,58,59,66,68. A multiplex Real-time assay amplified the HPV L1 gene for genotyping. Human housekeeping gene served as an endogenous internal control [IC] which can ensure the purification of DNA, verification of PCR reaction, and clarification of cell adequacy from each specimen. MIC Real-time PCR System (Bio-Rad) experiment setup was used for the detection of 15 types of HPV. The 15 HPV types detection and genotyping were done in four fluorescent channels (FAM, HEX, ROX, and CY5), each with individual parameters for target detection and validity; channel 1 HPV-31/-51/-39/-16, channel 2 HPV-35/-68/-18/-59, channel 3 HPV-33/-56/-66/-45 and channel 4 HPV-58/-53/-52. The Infectious Disease and Molecular Epidemiology laboratory of the Department of Public Health, Federal University of Technology, Owerri, was certified in 2022 by the Nigeria Centre for Disease Control (NCDC) according to the biological markers "HPV detection" and "HPV genotyping".

3.9. Method of Data Analysis

Statistical data analysis were performed in IBM-SPSS statistics version 25 (SPSS Inc. Chicago, Illinois USA). Initial data analysis involved descriptive techniques such as construction of frequency distribution table for categorical data. Others include construction of distributional charts such as pie charts and bar charts. Continuous data in the study were also described using summary statistics methods such and computations for mean and standard deviations. Inferential statistics was used to test hypotheses and make predictions about the sample data. This involved the use of chi-square tests, t-tests, and logistic regression analysis All test were performed at 5% level of significance and the probability value ($p>0.05$) and the 95% confidence interval were used to establish significance. Also, the odds ratio was used to account for size effects measures in the data.

3.10 Ethical considerations

Ethical approval had been obtained from the Institutional Ethical Review Board of Federal Medical Centre Owerri (now Federal University Teaching hospital, Owerri). Informed consent was obtained following detailed counseling. Only consenting participants were screened and with an opportunity to opt out at any point in the screening program.

The cost of the screening was sponsored by investigator and therefore free of cost to the participants with the exception of their time and transport fare if any.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Prevalence of High-Risk HPV in Imo State (Objective 1)

The overall prevalence of HPV among women aged 30–49 years in Imo State is shown in Figure 4.1**.** Out of the total study population, 145 women (56.4%) tested HPV-negative, while 112 women (43.6%) were found to be HPV-positive for any type of HPV. Thus, the overall prevalence of HPV in the study area was 43.6%**.**

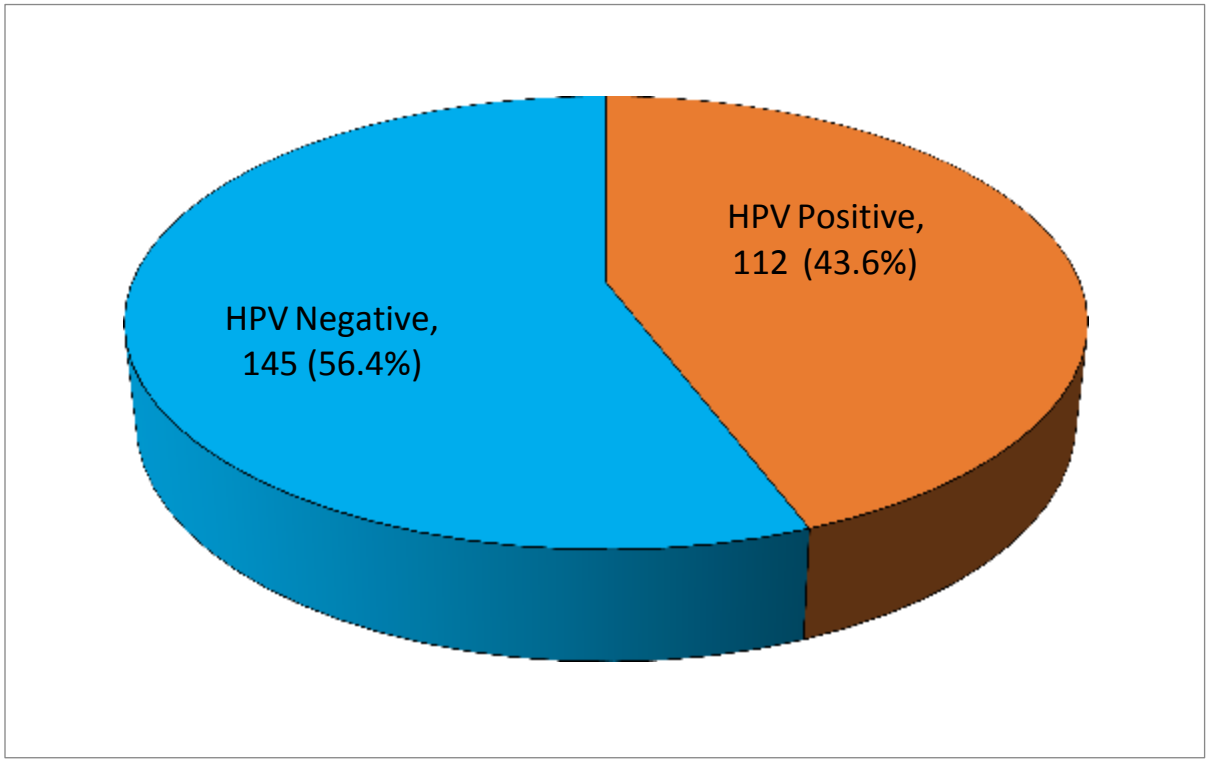

Figure 4.1: Pie chart showing the Prevalence of HPV in Imo State (obj.1)

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4.1.2 High-Risk HPV Infection in Relation to the Characteristics of the Study Group (Objective 2)

The association between high-risk HPV (hr-HPV) infection and socio-demographic characteristics of the study participants is presented in **Table 4.1**.

Occupation (**p = 0.015**, χ^2 = 19.060, d.f. = 8), ethnicity (**p = 0.035**), and place of residence (**p = 0.013**, χ^2 = 6.147, d.f. = 1) showed significant associations with hr-HPV infection. Higher infection rates were observed among students (66.7%), business women (58.6%), and housewives (55.6%). All four non-Igbo participants tested positive for hr-HPV. Women residing in rural areas showed a higher infection rate (53.8%) than their urban counterparts (37.8%).

Although **age** was not significantly associated with hr-HPV infection (**p = 0.496**), the highest prevalence was observed among women aged 30–34 years (52.9%), followed by those aged 40–44 years (42.9%) and 45–49 years (41.3%).

Widowed (66.7%) and single (51.6%) women had higher hr-HPV infection rates than married women (40.6%). Educational status also showed variation, with primary (50%) and secondary (51.5%) education levels having higher infection rates than tertiary education (42.5%). Among the predominantly Christian participants, the hr-HPV infection rate was 43.9%.

Table 4.1: **Association between socio-demographic characteristics and high-risk HPV infection (Objective 2)**

Socio-demographic factors	Total	HPV: Positive	HPV: Negative	χ^2	P value
Age (Years)				2.387	0.496
30–34	51	27 (52.9%)	24 (47.1%)		
35–39	58	23 (39.7%)	35 (60.3%)		
40–44	56	24 (42.9%)	32 (57.1%)		
45–49	92	38 (41.3%)	54 (58.7%)		
Marital Status				5.693 (LR)	0.128
Single	31	16 (51.6%)	15 (48.4%)		
Married	202	82 (40.6%)	120 (59.4%)		
Separated	6	2 (33.3%)	4 (66.7%)		
Widowed	18	12 (66.7%)	6 (33.3%)		
Educational Level				9.044	0.060
Non-formal	2	2 (100%)	0 (0%)		
Primary	12	6 (50.0%)	6 (50.0%)		
Secondary	68	35 (51.5%)	33 (48.5%)		
Tertiary	146	62 (42.5%)	84 (57.5%)		
Others	29	7 (24.1%)	22 (75.9%)		
Occupation				19.060 (LR)	0.015
Applicant	6	2 (33.3%)	4 (66.7%)		
Housewife	9	5 (55.6%)	4 (44.4%)		
Student	8	4 (66.7%)	2 (33.3%)		
Civil Servant	146	57 (39.0%)	89 (61.0%)		
Business	58	34 (58.6%)	24 (41.4%)		
Farmer	22	6 (27.3%)	16 (72.7%)		
Artisan	2	2 (100%)	0 (0%)		
Apprentice	4	0 (0%)	4 (100%)		
Others	4	2 (50.0%)	2 (50.0%)		
Ethnicity				—	0.035 (F)
Igbo	253	108 (42.7%)	145 (57.3%)		
Others	4	4 (100%)	0 (0%)		
Religion				—	0.506 (F)
Christianity	255	112 (43.9%)	143 (56.1%)		
Others	2	0 (0.0%)	2 (100%)		
Place of Residence				6.147	0.013
Rural	93	50 (53.8%)	43 (46.2%)		
Urban	102	62 (37.8%)	102 (62.2%)		

4.1.2 b Behavioral Characteristics in relation to risk of hrHPV infection

In table 4.2, significant behavioral characteristics associating with HPV infection include use of habit forming drugs (P= 0.001, χ^2 = 13.79) and use of tobacco (P= 0.017, χ^2 = 8.117). Up to 36% of those that use habit forming drugs sometimes were HPV positive. Only two persons use tobacco always and both two tested HPV positive.

Alcohol intake was not found significant and just 2 persons indicated that they take alcohol always, of which half of them (50.0%) were found HPV positive.

Table 4.2: Behavioral Characteristics in relation to risk of hr-HPV infection

Behavioral Characteristics	HPV: POSITIVE		HPV: NEGATIVE		Total	χ^2	P
	Freq	%	Freq	%			
Use of Habit Forming Drugs							
SOMETIMES	9	36.0	16	64.0	25		
NEVER	93	41.9	129	58.1	222		
None response	10	100.0	0	0.0	10		
Total	112	43.6	145	56.4	257	13.79	0.001^{LR}
Alcohol Intake							
ALWAYS	2	50.0	2	50.0	4		
SOMETIMES	55	45.1	67	54.9	122		
NEVER	47	42.3	64	57.7	111		
None response	8	40.0	12	60.0	20		
Total	112	43.6	145	56.4	257	0.352	0.950
Use of Tobacco							
ALWAYS	2	100.0	0	0.0	2		
NEVER	88	40.2	131	59.8	219		
None response	22	61.1	14	38.9	36		
Total	112	43.6	145	56.4	257	8.117	0.017^{LR}

Note: LR = Likelihood Ratio Test, F =Fishers exact test. LR or F Test types are conducted if the assumptions of Chi-square (χ^2) test are not fulfilled.

4.1.2 c. Reproductive Characteristics in relation to risk of HPV infection

At 5% level, significant associating reproductive factors of HPV infection in this study include coitarche (P= 0.040, χ^2 = 8.336), number of pregnancies in the past (P= 0.008, χ^2 = 13.80), number of Living Children (P= 0.003, χ^2 = 16.02) and number of sexual partners (P= 0.008, χ^2 = 12.88). Others include: frequency of genital tract infections (P= 0.019, χ^2 = 11.80) and type of genital tract infection had (P= 0.001, χ^2 = 21.88) (Table 4.5c).

The table also shows that all the 4 women (100%) that had coitarche at below 13 years of age tested HPV positive compared to 39.1% and 42.1% found among the women that had it at 13 -18 years and above 18 years old respectively.

Those that have not had any past pregnancy recorded the largest rate of HPV (76.9%), followed by those with 1- 2 pregnancies (41.7%). Also HPV rate was highest among the women that do not have any child living (82.4%), followed by those that had just 1-2 number of living children (47.9%). Those having two to four sex partners had the largest HPV infection rate (63.6%).

Parity, Number of abortions and miscarriages had were not found significant in this study (P > 0.05) however, HPV infection rate is quite high among women that had abortions or miscarriages of up to 5 times and above (66.7%) and 3 - 4 times (44.8%).

Table 4.3: Reproductive Characteristics in relation to risk of HPV infection

Reproductive Characteristics	HPV: POSITIVE		HPV: NEGATIVE		Total	χ^2	P
	Freq	%	Freq	%			
Coitarche							
<13 Years	4	100.0	0	0.0	4		
13-18 Years	18	39.1	28	60.9	46		
> 18 Years	82	42.1	113	57.9	195		
None response	8	66.7	4	33.3	12		
Total	112	43.6	145	56.4	257	8.336	0.040
Number of Miscarriages/ Abortions Had							
None	53	43.8	68	56.2	121		
1-2	34	37.8	56	62.2	90		
3-4	13	44.8	16	55.2	29		
5 and above	4	66.7	2	33.3	6		
Non response	8	72.7	3	27.3	11		
Total	112	43.6	145	56.4	257	6.397	0.171^{LR}
Parity							
None	10	35.7	18	64.3	28		
1-2	29	51.8	27	48.2	56		
3-4	40	44.0	51	56.0	91		
5 and above	21	33.9	41	66.1	62		
Non response	12	60.0	8	40.0	20		
Total	112	43.6	145	56.4	257	8.813	0.146
Number of Living Children							
None	14	82.4	3	17.6	17		
1-2	35	47.9	38	52.1	73		
3-4	40	38.5	64	61.5	104		
5 and above	17	32.1	36	67.9	53		
Non response	6	60.0	4	40.0	10		
Total	112	43.6	145	56.4	257	16.017	0.003

LR = Likelihood Ratio Test, F =Fishers exact test. LR or F Test is conducted if the assumptions of Chi-square (χ^2) test are not fulfilled.

Table 4.3 continued

Reproductive Characteristics	HPV: POSITIVE Freq	%	HPV: NEGATIVE Freq		Total	χ^2	P
Number of Sexual Partners Had							
None	11	61.1	7	38.9	18		
One	75	38.9	118	61.1	193		
Two – Four	14	63.6	8	36.4	22		
Five and above	0	0.0	4	100.0	4		
None Response	12	60.0	8	40.0	20		
Total	112	43.6	145	56.4	257	12.88	0.012^{L}_{R}
Frequency of Genital Tract Infections							
NIL	3	25.0	9	75.0	12		
ONCE	28	43.8	36	56.3	64		
2-4 TIMES	38	43.2	50	56.8	88		
5 TIMES AND ABOVE	6	100.0	0	0.0	6		
None response	37	42.5	50	57.5	87		
Total	112	43.6	145	56.4	257	11.802	0.019^{L}_{R}
Type of Genital Tract Infection Had							
SYPHILIS	8	80.0	2	20.0	10		
GONORHOEA	2	100	0	0.0	2		
BACTERIA	20	51.3	19	48.7	39		
CHLAMYDIA	0	0.0	4	100	4		
CANDIDIASIS	33	34.4	63	65.6	96		
OTHERS	0	0.0	3	100	3		
None response	49	47.6	54	52.4	103		
Total	112	43.6	145	56.4	257	21.885	0.001^{L}_{R}
Tested for HIV in the Past							
Yes	91	45.3	110	54.7	201		
No	15	35.7	27	64.3	42		
Non response	6	42.9	8	57.1	14		
Total	112	43.6	145	56.4	257	1.294	0.524
HIV Test Result if Tested							
Positive	2	100.0	0	0.0	2		
Negative	94	42.5	127	57.5	221		
Do Not Know	6	66.7	3	33.3	9		
None response	10	40.0	15	60.0	25		
Total	112	43.6	145	56.4	257	4.769	0.190

LR = Likelihood Ratio Test, F =Fishers exact test. LR or F Test is conducted if the assumptions of Chi-square (χ^2) test are not fulfilled.

4.1.3 : Distribution for Different types of High Risk HPV infection among women in Imo State (obj. 3)

Table 4.4 represents the distribution for different types of high risk HPV infection among women in Imo State. The table indicates that 13 different HPV types were found positive among the study participants in Imo State. The positive HPV types include HPV16(22: 8.6%), HPV18(26 10.1%), HPV31 (18: 7.0%), HPV35(8: 3.1%), HPV39 (12: 4.7%), HPV 51(43: 16.7%), HPV52(22: 8.6%), HPV53(19: 7.4%), HPV56(12: 4.7%), HPV58 (6: 2.3%), HPV59(31: 12.2%), HPV66(4: 1.6%) and HPV68(22: 8.6%), HPV33 and HPV45 were not found positive in the study group. HPV 51 was the most common type infecting the study population (43, 16.7%) followed by HPV59 (31: 12.2%).

Table 4.4: Distribution for Types of HPV infection among women in Imo State

Sn	HPV TYPE	HPV: Positive Number (%)	HPV: Negative Number (%)
1	HPV 16	22 (8.6)	235 (91.4)
2	HPV 18	26 (10.1)	231 (89.9)
3	HPV 31	18 (7.0)	239 (93.0)
4	HPV 33	0 (0.0)	257 (100)
5	HPV 35	8 (3.1)	249 (96.9)
6	HPV 39	12 (4.7)	245 (95.3)
7	HPV 45	0 (0.0)	257 (100)
8	HPV 51	43 (16.7)	214 (83.3)
9	HPV 52	22 (8.6)	235 (91.4)
10	HPV 53	19 (7.4)	238 (92.6)
11	HPV56	12 (4.7)	245 (95.3)
12	HPV 58	6 (2.3)	251 (97.7)
13	HPV 59	31 (12.2)	226 (87.9)
14	HPV 66	4 (1.6)	253 (98.4)
15	HPV 68	22 (8.6)	235 (91.4)

The prevalence rate of occurrence of different types of high risk HPV infection among the study women in Imo state is shown in Figure 2. The figure shows that HPV51 was found as the most prevailing type of HPV in the study area at 16.7%, followed by HPV 59 and HPV18 at 12.2% and 10.1% respectively. Others include HPV 16, HPV 68 and HPV 52 at 8.6% each, HPV 53 and HPV31 were 7.4% and 7.0% respectively.

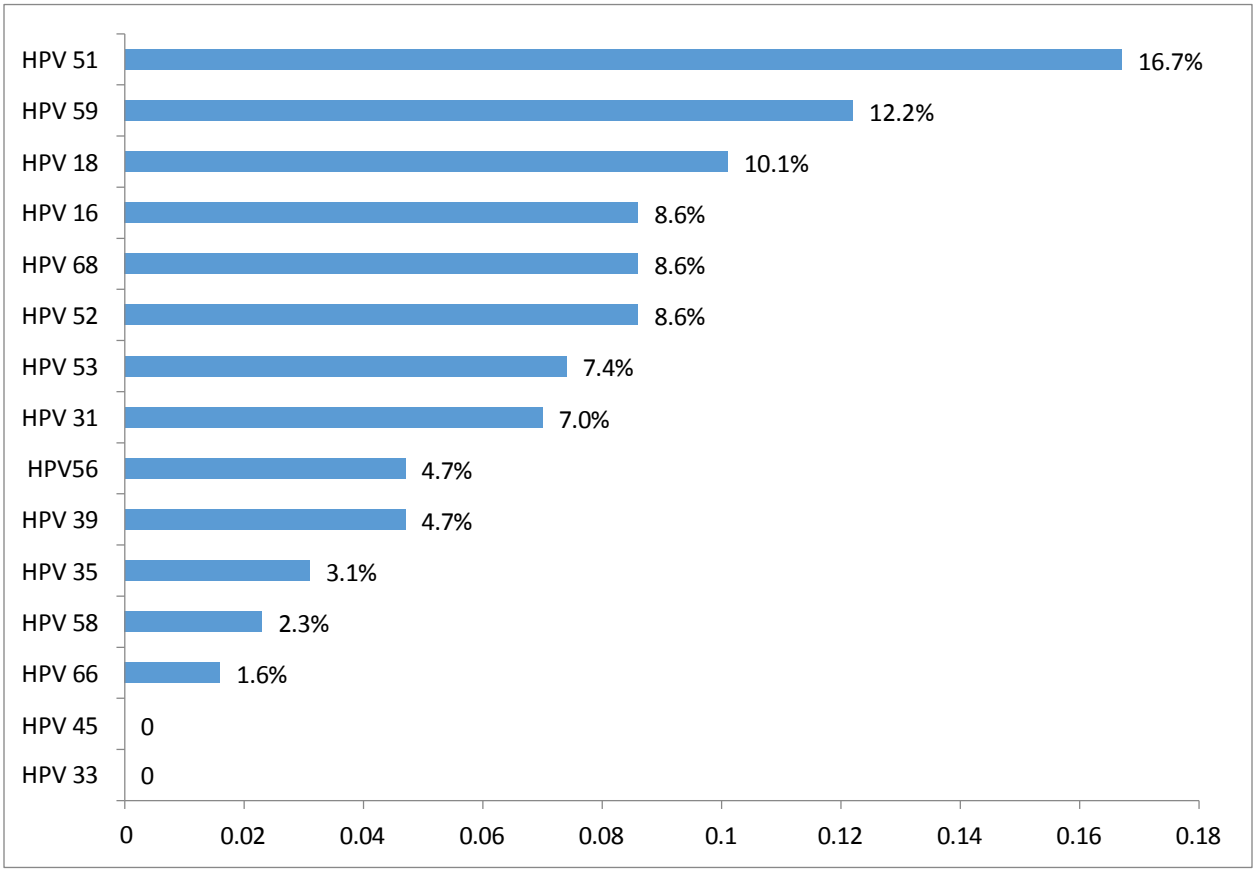

Figure 4.2: Prevalence for different types of High Risk HPV Infection in the study Area.

4.1.4. Association between bivalent vaccines targeted HPVs in women with normal and abnormal lesions (obj. 4&5)

4.1.4 a. Bivalent vaccine targeted HPV infection among women with abnormal lesions (obj. 4&5)

Table 4.5 shows the association between vaccines targeted HPVs in women with normal and abnormal lesions, it shows that 8 out of 28 women (27.8%) with abnormal lesions (screened with VIA) had bivalent targeted virus infection and could have been protected by the bivalent vaccine and the other 21 (72.4%) would not have been protected. The odds of having hr HPV that is covered by a bivalent vaccine was 1.91 (95% CI= 0.786, 4.618), but no significant relationship was established between the bivalent vaccine targeted HPV DNA types and the occurrence of abnormal cervical lesions among the study group using visual inspection with acetic acid methods of cervical cancer screening (p=0.149).

On the other hand, association between HPV DNA types and the occurrence of abnormal cervical lesions using VILI screening test was found significant (P=0.002). A total of 22 out of 74 women (29.7% of the women) with abnormal lesions would likely be protected with a bivalent vaccine while 52(70.3%) will require a wider coverage vaccine for protection. The odds of having hr HPV that is not covered by a bivalent vaccine is 2.8 times higher among women with abnormal lesion (OR= 2.80; 95% CI=1.452, 5.411).

Table 4.5: Association between bivalent vaccines targeted HPVs in women with normal and abnormal lesions (obj. 4&5)

Screening test	Bivalent targeted HPV		Total	□2	P	OR	95% CI	
	Presence number (%)	Absence number (%)					Lower	Upper
VIA								
Positive	8 (27.6)	21 (72.4)	29					
Negative	38 (16.7)	190 (83.3)	228					
Total	46 (17.9)	211 (82.1)	257	2.088	0.149	1.91	0.786	4.618
VILI								
Positive	22 (29.7)	52 (70.3)	74					
Negative	24 (13.1)	159 (86.9)	183					
Total	46 (17.9)	211 (82.1)	257	9.899	0.002	2.80	1.452	5.411

Chi-square value : χ^2 , Probability value: P, Odds Ratio: OR, 95% Confidence Interval: 95% CI

4.1.4 b. Non-bivalent vaccine targeted HPV infection among women with abnormal lesions

In table 4.5, using VIA screening technique, more than half of 29 women with abnormal lesions (15: 51.7 %) had infection with non- bivalent targeted HPV compared to 32% found among women with normal lesions. The odds of having a non-bivalent HPV infection in women with abnormal lesions is more than two times significantly higher (Odds ratio= 2.28, 95% CI= 1.043, 4.961, P= 0.035) than that of the women with normal lesions.

Using VILI as the screening method, out of the 74 VILI positive women, 39 (52.7%) had infection with non -bivalent targeted HPVs, against 26.8% for those that tested negative. Statistically significant association was found between non-bivalent targeted HPV and abnormal lesions with over three times odds of having non- bivalent HPVs among those found VILI positive (abnormal lesions) compared to the women with normal lesions (odds ratio = 3.05,95% CI 1.738, 5.343, P=0.000).

4.1.5 a: High Risk (hr) HPV types correlation with the vaccine targeted HPV types in order to determine HPV vaccine suitability in the prevention in study population (objective 6 and 7)

The index study identified HPV viruses were coded and classified into 2 major groups based on the coverage of existing vaccines. These groups were as follows:

a) High risk HPV covered by bivalent/quadrivalent vaccines (ie HPV 16 and 18) and the High risk HPV not covered by bivalent/quadrivalent vaccines (ie. non bivalent targeted HPV)(ie HPV 31,33,35,39,45,51,52,53,56,58,59,66,68)
b) High risk HPV covered by nonavalent vaccines(ie HPV 16, 18, 31, 33, 45, 52, 58), and the High risk HPV not covered by nonavalent vaccines (non-nonavalent HPV) (ie HPV,35,39,45,51,53,56,59,66,68)

4.1.5 b High risk HPV covered by bivalent/quadrivalent vaccines (ie HPV 16 and 18) and the High risk HPV not covered by bivalent/quadrivalent vaccines (ie. non bivalent targeted HPV)(ie HPV 31,33,35,39,45,51,52,53,56,58,59,66,68)

In table 4.6, a total of 46 women (17.9%) were infected with the bivalent targeted HPV. A total of 153 (59.7%) had absence of both the targeted and non -targeted HPVs. Only the 16 women (6.2%) who had solely bivalent targeted infections will be protected by the bivalent vaccine. There were 58 (22.6%) of the women who had HPV infections not preventable with the bivalent vaccines. Significant difference between the prevalent hr-HPV types and those covered by bivalent HPV vaccines in Imo state, South East Nigeria (p=0.000, odds of having the non-targeted HPV viruses = 4.95, 95% confidence interval= 2.511, 9.742) Therefore, there is a significant difference between the prevalent hr-HPV types and those covered by bivalent HPV vaccines in Imo state, South East Nigeria.

Table 4.6: Coverage of bivalent/ quadrivalent vaccines (obj. 6)

Vaccine target of HPV	Non bivalent targeted HPV		Total	□2	P	OR	95% CI	
	Presence Freq (%)	Absence Freq (%)					Lower	Upper
Bivalent targeted HPV								
YES	30 (11.7)	16 (6.2)	46 (17.9)					
NO	58 (22.6)	153 (59.7)	211(82.1)					
TOTAL	88 (34.2)	169 (65.8)	257 (100)	23.88	0.000	4.95	2.511	9.742

Chi-square value: χ^2, Probability value: P, Odds Ratio: OR, 95% Confidence Interval: 95% CI

High risk HPV covered by nonavalent vaccines(ie HPV 16, 18, 31, 33, 45, 52, 58), and the High risk HPV not covered by nonavalent vaccines (non-nonavalent HPV) (ie HPV,35,39,45,51,53,56,59,66,68) (obj. 6)

In table 4.7, for coverage of the nonavalent vaccines, a total of 66 women (25.7%) were infected with the nonavalent targeted HPV and 87 women (33.9%) had infection with non- nonavalent vaccine targeted HPVs. Only 25(7.7%) women had infection solely by the nonavalent targeted HPV and so will be protected by the nonavalent vaccine. Those infected with both nonavalent vaccine targeted and non nonavalent -targeted HPVs were 41 (16%). Thus they may still have potential for cervical cancer despite the use of the nonavalent vaccine.

A total of 46 (17.9%) had infection by the non- nonavalent targeted HPVs only and will not be protected by the vaccines. With odds ratio of 5.17 (95% CI = 2.843, 9.399), significant difference was found between hr-HPV types infection and coverage by nonavalent HPV vaccines in the present study ($P < 0.001$, $\chi^2 = 31.69$).

Table 4.7: Coverage of nonavalent vaccines ((obj. 6)

Vaccine target of HPV	Non nonavalent targeted HPV							
	Presence Freq (%)	Absence Freq (%)	TOTAL%	□2	P	OR	95% CI Lower	Upper
Nonavalent targeted HPV								
YES	41 (16.0)	25 (9.7)	66 (25.7)					
NO	46 (17.9)	145 (56.4)	191 (74.3)					
TOTAL	87 (33.9)	170 (66.1)	257 (100)	31.69	0.000	5.17	2.843	9.399

Table 4.8: Non-Bivalent vaccine targeted viruses and screening results (obj. 7)

Screening test	Non-Bivalent targeted HPV		Total	□2	P	OR	95% CI	
	Presence number (%)	Absence number (%)					Lower	Upper
VIA								
Positive	15 (51.7)	14 (48.3)	29					
Negative	73 (32.0)	155 (68.0)	228					
Total	88 (34.2)	169 (65.8)	257	4.437	0.035	2.28	1.043	4.961
VILI								
Positive	39 (52.7)	35 (43.3)	74					
Negative	49 (26.8)	134 (73.2)	183					
Total	88 (34.2)	169 (65.8)	257	15.93	0.000	3.05	1.738	5.343

Chi-square value: χ^2, Probability value: P, Odds Ratio: OR, 95% Confidence Interval: 95% CI

4.1.6. Association between nonavalent vaccines targeted HPVs in women with normal and abnormal cervical lesions

4.1.6 a Nonavalent targeted HPV virus infection in women with abnormal lesions

In table 4.8, among the 29 women who tested positive for VIA, 41.4% were infected with the HPVs targeted by the nonavalent vaccine, compared to 23.7% among those that tested negative. The result shows slight significant relationship (P= 0.040, □2 = 4.221). The odds of having nonavalent targetted virus was found to be approximately 2.3 times higher among those with abnormal cervical lesions than among those with normal cervices.

At VILI screening, 35.1% of the 74 VILI positive women had nonavalent targeted HPVs compared to 21.9% found among women that were test negative. Significant relationship was also established between nonavalent HPV DNA types and the occurrence of abnormal cervical lesions among women studied using visual inspection with Lugol iodine method of cervical cancer screening (P=0.027, 95% CI= 1.071, 3.501). For those that screened positive, the odds of having nonavalent HPV infection was found to be about 1.9 times higher compared to those that tested negative (OR=1.94)

Table 4.8: Association between nonavalent vaccines targeted HPVs in women with normal and abnormal cervical lesions

Result of screening test	Presence of nonavalent targeted HPV	Absence of nonavalent targeted HPV	Total	□2	P Value	OR	95% Lower	95% Upper
VIA								
Positive	12 (41.4%)	17 (58.6%)	29					
Negative	54 (23.7%)	174 (76.3%)	228					
Total	86 (25.7%)	191 (74.3%)	257	4.221	0.040	2.28	1.022	5.060
VILI								
Positive	26 (35.1%)	48(64.9%)	74					
Negative	40(21.9%)	143(78.1%)	183					
Total	66 (25.7%)	191(74.3%)	257	4.867	0.027	1.94	1.071	3.501

Chi-square value: χ^2, Probability value: P, Odds Ratio: OR, 95% Confidence Interval: 95% CI

4.1.7. Non nonavalent targeted HPV virus infection in women with abnormal lesions

Among the women with abnormal lesions using VIA for screening, 15 (51.1%) had infection with non nonavalent vaccine targeted HPVs and this was statistically significant (p=0.031). On the other hand, among the women who had abnormal lesions with VILI, 39 (52.7%) had infection by a non nonavalent targeted HPV and this was statistically significant (p=0.000) (table 4.9). There was therefore, a significant relationship between non nonavalent targeted HPV DNA types infection and the occurrence of abnormal cervical lesions among women in Imo state, South East, Nigeria using visual inspection with acetic acid and Lugol iodine method of cervical cancer screening

Table 4.9: Non nonavalent targeted HPV virus infection in women with abnormal lesions

Vaccine target of HPV	Non nonavalent targeted HPV		Total	□2	P	OR	95% CI	
	Present	Absent					lower	upper
Screening with VIA								
VIA positive	15 (51.7%)	14(48.3%)	29					
VIA negative	72 (31.6%)	156(68.4%)	228					
Total	87 (33.9%)	170(66.1%)	257	4.663	0.031	2.32	1.064	5.064
Screening with VILI								
VILI positive	39 (52.7%)	35(47.3%)	74					
VILI negative	48 (26.2%)	135(73.8%)	183					
Total	87 (33.9%)	170(66.1%)	257	16.49	0.000	3.13	1.785	5.502

Chi-square value: χ^2, Probability value: P, Odds Ratio: OR, 95% Confidence Interval: 95% CI

4.1.8. HPV DNA types and the occurrence of normal and abnormal cervical lesions among women in Imo state, South East, Nigeria using visual methods of cervical cancer screening.

Figure 4 contained a bar chart representing the HPV DNA types and the occurrence of normal and abnormal cervical lesions among the study group. On the figure, it can be observed that a total of 29 (11.3%) had abnormal lesions or aceto-white changes for the abnormal/premalignant lesions of the cervix screening using visual inspection with acetic acid (VIA), while 74 (28.8%) had abnormal lesions or areas of poor Lugol's iodine uptake using visual inspection with Lugol's Iodine (VILI).

At VIA screening, 21 of the 29 women (72.4%) who recorded abnormal lesions were HPV positive, while 91 (39.9%) among the normal cervices were HPV positive. At VILI screening, 47 out of 74 of the women that have abnormal lesions (63.5%) were HPV positives, but the rate was lower among women with normal cervices, where 35.5% of the women with normal cervices were of HPV positive.

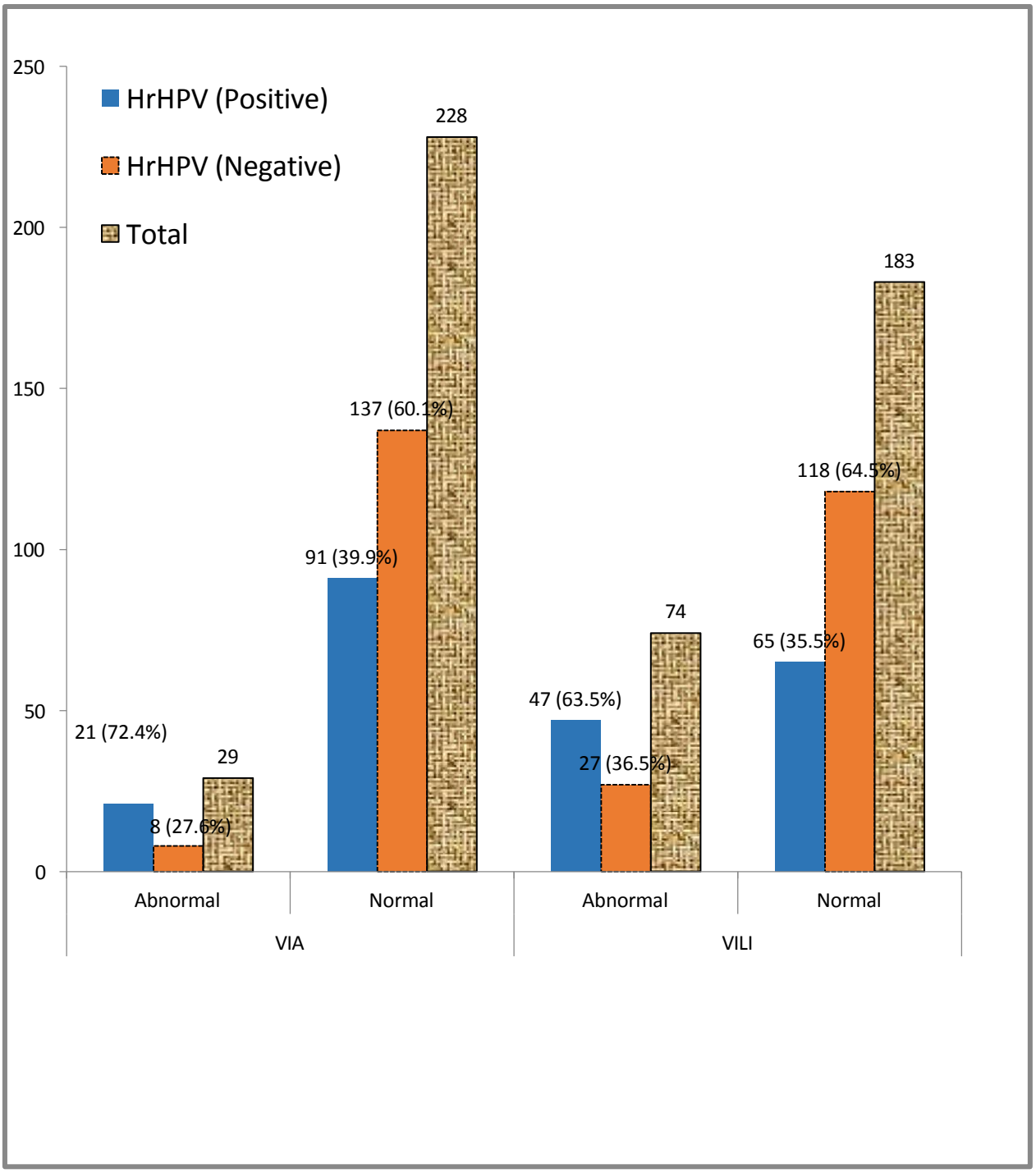

Figure 4.3: Bar chart for HPV DNA types and the occurrence of normal and abnormal cervical lesions among the study group

4.1.9. Relationship between HPV DNA types and the occurrence of abnormal cervical lesions using VIA screening (obj. 5)

Table 4.8 contained the result showing the relationship between HPV DNA types and the occurrence of abnormal cervical lesions among the study group. The result for the abnormal/premalignant lesions of the cervix screening using visual inspection with acetic acid (VIA) shows that significant association was found between the collective hr HPV infection and results from visual inspection with acetic acid, (p= 0.002, 95% conf. Int,=1.678, 9.305). Other significant relationships include association between individual HPV 16 (p= 0.002, 95% conf. Int,=1.231, 9.703), HPV 52 (p= 0.002, 95% conf. Int,=1.231, 9.703), HPV 59 (p= 0.040, 95% conf. Int,=1.046, 6.992) and HPV 68 (p= 0.002, 95% conf. Int,=1.231, 9.703).

Among the women who tested positive for HPV of any type, 21 (18.8%) had had abnormal lesions when screened with VIA compared to 8 (5.5%) found among women who were negative on HPV of any type. The odds for having abnormal lesion was found to be close to four times significantly higher for the women that tested positive on any HPV compared to those that tested negative (OR =3.95). For the women who were positive for HPV 16 type, 27.3% of them had abnormal lesions against 9.8% on women who had negative HPV 16. The odds for abnormal lesions was found to be approximately 3.5 times higher on those that tested HPV positive than on those that were negative (OR =3.46). Similar results were obtained for HPV 52 and HPV 68. On HPV 59, there were 22.6% abnormal lesions recorded among the women who tested positive compared to 9.7% abnormal lesions among women that were negative for HPV 59. The difference is accompanied with higher odds of 2.7 times higher for the HPV 59 positive group than then negative test group.

Other HPV types tested such as HPV 18, HPV31, HPV 33, HPV 35, HPV 39 HPV 45, HPV 51, HPV 53, HPV 56, HPV 58 and HPV66 were not found significant at VIA screening in this study (P > 5%). However, in almost all the HPV types, the rate for abnormal lesions were found higher among the women that were test positives. For instance in 15.4% of the women that were positive for HPV18 (HPV targeted by the bivalent vaccine) recorded abnormal lesions or acetowhite lesion compared to 10.8% found among women who were HPV 18 negative.

HPV 31 result also indicates abnormal lesions were more on women who were positive on the HPV type compared to the women that were negative. It showed that 22.2% of the women who

were HPV 31 positive have abnormal lesions while 10.5% of the women that tested negative in HPV 31 have abnormal lesion.

4.1.10: Relationship between HPV DNA types and the occurrence of abnormal cervical lesions using VILI for screening (obj. 6)

On table 4.10, the result for the VILI screening test was represented. It shows that significant association was found between the collective hr HPV results and visual inspection with Lugol's iodine VILI ($p < 0.0001$; 95% CI = 1.802, 5.543), as well as with HPV types 16 ($p < 0.0001$; 95% CI =3.042, 21.770), type 51($p < 0.0001$; 95% CI= 2.099, 8.179) and type 53 ($p < 0.022$; 95% CI = 1.174, 7.772). No evidence of significant association was found between other individual HPV types and visual inspection with Lugol's iodine VILI in this study ($p > 0.05$). For the significant HPV types, 42% of those that tested positive on HPV of any type have abnormal lesions compared to 18.6% of abnormal lesions among those that tested negative. As large as 72.7% who were HPV 16 positives showed abnormal lesions compared to 27.3% among those that tested negative for HPV 16.

Among the women that tested positive for HPV 51, a total of 55.8% have abnormal lesions compared to 23.4% that showed abnormal lesions among the women that were HPV 51 negative. Similar result was obtained for HPV 53 type where 52.6% of the type positive recorded abnormal lesions against 26.9% among the HPV 53 negative results. The odds for having abnormal lesions was found to be significantly higher on women with positive HPV test than on women with negative test (HPV of any type: 3.16 times higher; HPV 16: over eight times higher, HPV 51: over 4 times higher and HPV 53: more than three times higher).

For the other test types that were not found significant in this study, the frequency of having abnormal results with VILI among those who had hr HPV types infection was quite high with HPV 56 (50.0%), 68 (45.5%), 59 (41.9%), and 58 (33.3%).

Table 4.10: Relationship between HPV DNA types and the occurrence of abnormal cervical lesions using VILI for screening (obj. 5)

High Risk HPV Type	Total	VILI: Abnormal (+) Freq	%	VILI: Normal (-) Freq	%	Coef	P	OR	95% CI Lower	Upper
HPV of any Type										
Positive	112	47	42.0	65	58.0					
Negative	145	27	18.6	118	81.4	1.151	0.000	3.16	1.802	5.543
Total	257	74	28.8	183	71.2					
HPV 16										
Positive	22	16	72.7	6	27.3					
Negative	235	58	27.3	177	75.3	2.097	0.000	8.14	3.042	21.770
Total	257	74	28.8	183	71.2					
HPV 18										
Positive	26	10	38.5	16	61.5					
Negative	231	64	27.7	167	72.3	0.489	0.254	1.63	0.703	3.781
Total	257	74	28.8	183	71.2					
HPV 31										
Positive	18	6	33.3	12	66.7					
Negative	239	68	28.5	171	71.5	0.229	0.660	1.26	0.454	3.485
Total	257	74	28.8	183	71.2					
HPV 33										
Positive										
Negative	257	74	28.8	183	71.2	-	-	-	-	-
Total	257	74	28.8	183	71.2					
HPV 35										
Positive	8	2	25.0	6	75.0					
Negative	249	72	28.9	177	71.1	-0.199	0.810	0.82	0.162	4.156
Total	257	74	28.8	183	71.2					
HPV 39										
Positive	12	4	33.3	8	66.7					
Negative	245	70	28.6	175	71.4	0.223	0.723	1.25	0.365	4.284
Total	257	74	28.8	183	71.2					
HPV 45										
Positive	0	0	0.0	0	0					
Negative	257	74	28.8	183	71.2	-	-	-	-	-
Total	257	74	28.8	183	71.2					

Coef: regression coefficient, P: probability value, OR: odds ratio, 95% CI: 95% confidence interval

Table 4.13 continued

High Risk HPV Type	Total	VILI: Abnormal (+) Freq	%	VILI: Normal (-) Freq	%	Coef	P	OR	95% CI. Lower	Upper
HPV 51										
Positive	43	24	55.8	19	44.2					
Negative	214	50	23.4	164	76.6	1.421	0.000	4.14	2.099	8.179
Total	257	74	28.8	183	71.2					
HPV 52										
Positive	22	8	36.4	14	63.6					
Negative	235	66	28.1	169	71.9	0.381	0.414	1.46	0.587	3.650
Total	257	74	28.8	183	71.2					
HPV 53										
Positive	19	10	52.6	9	47.4					
Negative	238	64	26.9	174	73.1	0.106	0.022	3.02	1.174	7.772
Total	257	74	28.8	183	71.2					
HPV 56										
Positive	12	6	50.0	6	50.0					
Negative	245	68	27.8	177	72.2					
Total	257	74	28.8	183	71.2	0.957	0.108	2.60	0.811	8.350
HPV 58										
Positive	6	2	33.3	4	66.7					
Negative	251	72	28.7	179	71.3	0.218	0.804	1.24	0.223	6.937
Total	257	74	28.8	183	71.2					
HPV 59										
Positive	31	13	41.9	18	58.1					
Negative	226	61	27.0	165	73.0	0.670	0.089	1.95	0.903	4.225
Total	257	74	28.8	183	71.2					
HPV 66										
Positive	4	0	0.0	4	100					
Negative	253	74	29.2	179	70.8	-20.32	0.999	0.0	0.000	-
Total	257	74	28.8	183	71.2					
HPV 68										
Positive	22	10	45.5	12	54.5					
Negative	235	64	27.2	171	72.8	0.800	0.077	2.23	0.917	5.406
Total	257	74	28.8	183	71.2					

Coef: regression coefficient, P: probability value, OR: odds ratio, 95% CI: 95% confidence interval

4.2 Discussion

The need to prevent and possibly eliminate cervical cancer by 2030 cannot be overemphasized. To actualize this, the WHO has recommended the use of a combination of vaccination, screening and early detection and treatment of cervical cancer. Three vaccine types are currently available for prevention of cervical cancer. The bivalent vaccine which targets the high risk HPV 16 and 18 which has been adjudged to prevent about 70% of cervical cancer; the quadri or tetravalent types, targeting types 16, 18 and low risk types 6 and 11. There is also the nonavalent vaccine which is expected to protect against 9 HPV types including 6, 11, 16, 18, 31, 33, 45, 52 and 58. The latter vaccine is not readily available in Nigeria despite being approved by the FDA in the United States of America and the EMA in Europe.

High risk HPV types have been determined to cause cervical cancers according to the IARC and several molecular test methods have been developed to screen for these viruses. Eight out of the 15 HR HPVs are not targeted against by the available vaccines. Based on the recognizable geographical variation in the distribution of high risk HPV serotypes, this study sought to determine the prevalence of various high risk HPV viruses in Imo state, south east, Nigeria and determine the suitability of existing vaccines for preventing cervical cancer. The fifteen known serotypes that were associated with genital tract cancers (IARC)) were tested for in cervical swabs from 257 women in the population using the Ampfire, Atila biosystems. The burden detected in this study is quite remarkable as up to 43.6% of women in the study population had one or more HPV virus types, majority had multiple infection sometimes up to 6 HPV types in a person. Those with HPV types 16 and 18 commonly had co infections with either vaccine protected types and non-protected ones. Nigeria does not have a robust national screening program and the move by the government to procure 6 million HPV vaccine doses to be delivered to children 9-14 years is a very welcome development. However, knowledge of the particular gene types that infect the women in the state is necessary. This study found a divergent view from what is generally expected world wide – which is that HPV 16 and 18 were the most common and most important. Reports in the past have shown up to 99% involvement of HPV viruses in invasive cervical cancers (Okunade, 2020). It has also been found that there is less frequent occurrence of hrHPV infection in those with premalignant lesions and even lesser among those with normal cervices. Being a population based study, there was a mix of women who had normal cervices and abnormal lesions. Using VIA, twenty nine women (11.3%) tested

positive/ had abnormal cervical lesions (had acetowhite reaction) while with VILI seventy four women (28.8%) tested positive/ abnormal cervical lesions (were iodonegative).

This very high prevalence of HPV found in the index study could inform a potential future burden of cervical cancer in the study population. The rate found in the present study is higher than findings in other studies in Nigeria where the prevalence was 16.6% in Edo state, South South Nigeria, (Okoeguale et al., 2022), 26.3% overall in Ibadan South West Nigeria, (Thomas et al., 2004), 36.5% in LUTH Lagos Nigeria (Okunade, et al., 2017) and 34% in a study done in randomly selected clinics in the 6 geopolitical zones in Nigeria (Ohihoin et al.,2022). The HPV prevalence was however lower than the prevalence of 66.7% detected in ABUTH, Zaria and 76% detected in Kano, Nothern Nigeria (Auwal, Aminu, Atanda, Tukur, & Sarkinfada, 2013) as well as 73.11% in Spain (Paz-Zulueta et al., 2018). In the West African sub region, Ghana had the prevalence of HPV to be 10.7% while Cote d'Ivoire had 90.8%. (Seyoum et al., 2023). In a meta-analysis by Soheili and co-workers, 'a global prevalence of 11.7% was found in South Africa, 17.4% in East Africa, 33.6%, in Western Europe, 9.0%, Eastern Europe, 21.4% and Caribbean 35.4%'(Soheili et al., 2021). The findings above go on to support the suggestion that there is higher prevalence of high risk HPV infection in developing countries including Nigeria. (Kombe et al., 2020). This has been ascribed to the poor access to and use of health care, under nutrition and immune compromise as well as a near absent vaccination program. **(**Kombe, et al., 2020).

There is proven variability in HPV prevalence across the globe as well as in the distribution. In a study from 6 continents, HPV prevalence varied from 1.4% in Spain to 25·6% in Nigeria (Clifford, et al., 2003). Different studies even within countries seem to have divergent prevalence and distribution reports such as the extremes of 73.11% (Paz-Zulueta et al., 2018) and 1.4 % (Clifford, Smith, Aguado & Franceschi, 2003) in Spain. The difference in result could be actual but could also be related to the validity and scope of different laboratory methods of diagnosis used in the different studies and the diverse health status of the study participants at the time of testing. Many genotype assay methods such as the commercially available Hybrid Capture II (HC II, Digene), Abbott Real-Time HR-HPV and Roche Cobas 4800 HPV tests do not include rare HPV types (Al-Lawatia et al, 2020). The study in LUTH used PCR in assaying for the genotypes. The study at Ibadan used a general primer-mediated GP5 þ /6 þ -PCR and by

hybridization of PCR products in an enzyme immunoassay (EIA), used 2 probes to detect 36 HPV types and positive samples were further typed with reverse line blot hybridization (RLB). That, in the 6 geopolitical zones focused on studies that used COBAS PCR amplification of target DNA sequences using both HPV and β-globin. This study used the Atila AmpFire HPV High risk genotyping assay. Caution in making assumptions is that different studies screen for divergent pool of HPV viruses and this could affect the prevalence declared. Some tests screen for as many as 35 HPVs while others like ours screened for 15 viruses only and some others screened for fewer. This has a potential of giving divergent impressions on the prevalence values and types declared. It is of importance that the above and the heterogeneity of HPV infection distribution is considered when future provisions are made for screening and vaccine production. HPV16 and 18 prevalence in this study, is less than some other high risk HPV types and this calls for need to produce vaccines of wider coverage. Another important factor will be the type of study population studied whether those already diagnosed with cervical cancer or premalignant disease or those who are from the general population and not seeking health attention like ours (Seyoum et al., 2023).

Majority of the women in the study population were mostly educated, employed in one way or the other and were in relationships but these were not significantly associated with hr HPV infection. Although there was no significant relationship established between the age of participants and frequency of HPV infection in the present study, the rate was higher among the youngest age group compared to other groups. On the contrary, age has been a recurring risk factor in other studies in other climes such as in US women (Kahn, Lan & Kahn, 2007) and in China where there was a bimodal peak where the highest positive rate was among those who were above 60 years closely followed by those 56 to 60 and 51 to 55years (Hao, Wang, Liu, He & Jiang, 2020). In Russia, the younger group had more prevalence of hr HPV infection. Other associated risk factors in the Russia study like co-habiting, being nullipara, being a smoker, having more than three sexual partners and early coitarche did not seem to be significantly associated with HPV infection in this study (Babi et al., 2021). There was a reasonable level of awareness of cervical cancer and its relationship with HPV as the cause but very little on the knowledge and uptake of vaccines and screening as a preventive measure.

Occupation was found significant in this study with high rate of HPV infection found among students and business women. This is possible considering that most students are young, single sexually active, and thus have higher risk of HPV (Fagbule et al., 2020). High prevalence of HPV among students with high frequency of multiple infections and non-vaccine high-risk HPV genotypes have also been reported in northern Brazil (Vieira et al., 2015). Similarly business women are prone to engaging in sex with more than one partner as they always move about for business activities. Having more than one sex partner and also higher frequency of sexual involvements are both risk factors of HPV (Abulizi et al., 2017). Occupation was also found as a significant risk factor of HPV in a Chinese study, with taxi drivers having the highest risk (Yang, Zhong, Lv, & Yu, 2019), while none of the socio-demographic factors including occupation were found significant in rural Uyghur women in the Xinjiang province of northwestern China (Abulizi et al., 2017). Another significant socio-demographic factor in this study is area of residence which showed higher prevalence of HPV among rural women compared to women residing in the urban areas.

Reproductive characteristics like early coitarche, number of living children, the number of sexual partners, frequency and type of genital tract infections were found to be significantly associated with having HPV infection. This is similar to findings in a hospital based study in Uganda where age at coitarche, occupation, place of residence, marital status, alcohol intake in the past year, number of life time sexual partners were shown to be associated with hr HPV infection ($p<0.02$) (Nang et al., (2023).

We went further to determine the prevalent types of high risk HPV infection among the women in Imo State, South East Nigeria. The frequency of high risk HPV types detected were high in many of HPV types but HPV 33 and 45 were not detected in any of the women. HPV type 18 and 16 came a distant third and fifth respectively in order of frequency. This is quite a contrast with the finding in other parts of the world such as in China from 2016 to 2022 which gave the top ten genotype in descending order to be HPV16, HPV52, HPV58, HPV53, HPV39, HPV59, HPV66, HPV51, HPV18, and HPV56 and the least frequent genotypes were, in order HPV26, HPV45, and HPV82 (Zheng, Chen, Yang, Wang & Xu, 2023). According to Kombe Kombe and co-workers, the most common types reported by several studies were16, 18, 59, 45, 31, 33, 52, 58, 35, 39, 51, 56, and 53 in descending order of prevalence (Kombe Kombe et al., 2021). This

study also contrasted with a hospital based study in Ethiopia, where HPV16 (20.3%) was the commonest, followed by HPV35 (8.7%), HPV56 and HPV58 (each 5.8%), HPV18, HPV31 and HPV39 (each 4.4%), HPV45 (2.9%) and HPV59 and HPV68 (each 1.5%) were found among women with normal cytology. Among women enrolled in the national program for cervical cancer screening in Lazio Region, Italy, HPV 16 was the commonest (13.8%), followed by HPV 31 (12.9%), HPV 68 (10.6%), HPV 66 (8.0%), HPV 52 (7.5%), HPV 58 (7.5%), HPV 51 (7.3%), HPV 56 (6.7%), HPV 39 (5.3%), HPV 59 (4.8%), HPV 45 (4.2%), HPV 33 (4.2%), HPV 18 (4.0%),and HPV 35 (3.2%) (Cenci, Rossi & Pisani, 2023). Interestingly, the prevalent HPV types in our study and some others in developing world does not seem similar to that in the rest of the world where HPV 16 is the major contributor even among normal or undiagnosed women.

In LUTH Nigeria, HPV 31 (25.0%) was most common, followed by HPV 35 (8.0%) and HPV 16 (3.5%) (Okunade et al, 2017) and this differs from the report in other countries above by not having HPV16 as the lead but also differs from our study where HPV type 51 was the lead. A systematic review study of pooled prevalence of genital HPV infection in Nigeria found genotypes 31 (70.8%), 35 (69.9%) and 16 (52.9%) to be the most predominant HPV in circulation. The systematic review also gave HPV31, 51, 52, 35, 58, 16, 56, 18, 39 and 59 as the ten most common genotypes in women with atypical squamous cells of undefined significance (Emeribe et al, 2021). Like in this study and in some other studies in the rest of the country, HPV 16 is not the leading HPV and there is even a difference in the zones of Nigeria. This could be affected by the type of study population and the specific HPV types tested for. Just like in our study, HPV 18 is the commonest vaccine targeted HPV in most of the zones in Nigeria but not in the North West or South South. In this index study, the commonest vaccine targeted virus was HPV type 18. For the bivalent vaccines the commonest was HPV type 18 and with a frequency of 10.1%. However, the systematic review unlike this study had mostly nonavalent vaccine targeted HPVs as the common ones (Emeribe et al, 2021). Among the women with normal cervices (using VIA & VILI), 38 (16.7%) & 24 (13.1%) respectively were positive for HPV infection. The frequency of occurrence of the high risk viruses among the women with normal cervices when screened with VIA was HPV 51,59,52,18,68,31,53,39,35,16,56,66,58 in descending order. When screened with VILI, the frequency of hr HPV was HPV 51, 59, 52, 18, 68, 31, 53, 39, 16, 35, 56, 58 and 66 in descending order in women with normal cervices. HPV

types 33 and 45 were not detected at all. This is a sharp contrast to the findings in the meta-analysis between 1999 and 2019 in Nigeria where HPV 18, 16, 31, 35, 58, 52, 45, 51, 56 and 66 were the ten most common genotypes in females with normal cervical cytology in descending order (Isong, Emeribe & Nwofe, 2021). It is notable that that study did not find any report on normal cervices in the South East of Nigeria. The most frequent vaccine protected HPV was type 52 (which is nonavalent) then 18,31,35,16 and 58 in descending order. Presence of HPV infection in the absence of abnormal lesions could imply an early or new infection that is not yet persistent long enough to cause dysplasia. Secondly there may be false negatives from the screening test used.

Among the women with abnormal screening result using VIA, type 16, 51, 52 and 59 were the commonest, followed by 18,31,53,59, but those with abnormal result when screened with VILI had HPV 51 as the most common, followed by HPV 16, then 59,18,53,52,31,39,58 and 35. The frequency of HPV 16 is more prominent in this subset that has abnormal lesions. This buttresses the association between HPV infection and cervical dysplasia and cancer. Types 51 and 16 were significantly associated with abnormal cervical lesions. Among those with abnormal lesions using VILI, 27(18.6%) did not test positive to any of the HPV types tested for in this study.

There is a possibility that there may be other potential hr HPVs different from the 15 tested for especially among the probably high risk HPV subset like 81 and 82 that could be responsible for the abnormal lesions detected (Al-Lawatia et al, 2020). On another line, there may be a false positive result on the part of the visual method of screening used or there may be false negative from the HPV test method used. HPV 16 infection was significantly associated with abnormal lesions and this is supportive of the suitability of the vaccines in current use being able to reduce the incidence of cervical cancer. HPV type 66 on the other extreme was not associated with abnormal lesions but was rather seen only among those with normal cervices. This could imply a lower virulence of that virus and less capacity to lead to cervical cancer.

Coverage by the vaccines for the women in this study population will be poor as only 4.7% of the women studied had infection solely by HPV 16 and 18, in the bivalent vaccine which is currently in use in Nigeria and 11.5% had infection solely by the nonavalent targeted HPVs. Almost a quarter of the women tested (23.3%) were infected by non -bivalent-vaccine targeted viruses. This assumes that these cohort of women will not be protected by the existing bivalent

vaccine in the market. This proportion was reduced for women infected by viruses not targeted by the nonavalent vaccine to 17.1%. This supposes that introduction of the vaccines with wider coverage will be more useful in Nigeria.

The prevalence of the HPVs targeted in the bivalent vaccine was 17.1% in this study and was similar to 18.6 % found among South African youths in Soweto and Cape Town. For the nonavalent, this increased to 27.1% comparable to 38.5% in South Africa. (Mbulawa et al, 2018). However, in South Africa, type 16 was the most common, followed by HPV 58, HPV 51, HPV 66, HPV 18 and HPV 8.

When the association with presence of abnormal cervical lesions was done, it was found that for results from VIA, HPV 16, 52, 59 and 68 were significantly associated (p value 0.019, 0.019, 0.040 and 0.019 respectively). Of these, only HPV 16 and 52 was vaccine protected. For VILI, HPV 16, 51 and 53 were significantly associated (p value 0.000, 0.000 and 0.022. (see table 4.13). HPV types 16 and 52 were the vaccine protected HPV types with significant association with having area of poor of Lugol iodine uptake on the cervix). Only HPV 16 was significantly associated with abnormal lesions with both screening methods employed. Multiplicity of infection was common in this study. One of the participants had contact bleeding and a biopsy was collected for histology which confirmed invasive non keratinizing squamous cell carcinoma of cervix. This participant had multiple infections with HPV 16,52,39,56 and 51. For her, the HPV vaccine will ordinarily have eliminated types 16 and 52 but not the others.

This study goes to affirm that women in sub Saharan Africa were more likely to have a higher overall prevalence of the hr HPVs but less likely to be infected predominantly by the vaccine targeted viruses than in other developed world. According to Dom–Chima and coworkers, the top five prevalent types found in a Nigerian cohort of women were HPV71 (17%), HPV82 (15%), HPV16 (16%), HPV6 (10%), and HPV 20 (7%) (Dom-Chima et al, 2023). In the 6 geopolitical zones of Nigeria, 3% and 4% respectively had infections with HPV types 16 and 18, while other high-risk groups contributed 27%. In the Gambia HPV-16, HPV -33 and HPV -58 were commonest. In referral hospitals in Eastern Uganda, 'HPV16 and 18 were the most prevalent genotypes preceded in the decreasing order by HPV 33, 52, 31 and 45'(Eilu et al, 2022). It is noteworthy that the Ugandan study was hospital based and not community based. Again, there is a subtle similarity in the HPV types detectable in developed world, in Longnan,

China, where HPV16, 58, 52 and 18 was associated with development of CINs (Zhao et al 2017). Another study in Shenzhen City, China found the commonest HPV type in patients with abnormal cervical lesions to be HPV16, followed by HPV52, HPV58, and HPV33 (Mai, Yang, Cheng, Wu& Wu. 2021) and likewise in Zhejiang Province, China, HPV 16, 52 and 58 were the main genotypes that caused cytological abnormalities (Yan et al, 2021) Similarly, in Italy, HPV16 was the commonest, then HPV 31, HPV 18 and HPV 56 (Carozzi et al., 2014).

Caution in making assumptions is that different studies screen for divergent pool of HPV viruses and this could affect the prevalence declared. Some tests screen for as many as 35 HPVs while others like ours screened for 15 viruses only and some others screened for fewer. This has a potential of giving divergent impressions on the prevalence values declared. It is of importance that the above and the heterogeneity of HPV infection distribution is considered when future provisions are made for screening and vaccine production. HPV16 and 18 prevalence in this study, is less than some other high risk HPV types and this calls for need to produce vaccines of wider coverage.

Abnormal lesions in this study was detected using visual methods of screening for cervical cancer namely VIA and VILI. These tests have the advantage of giving results on the spot and mostly used in developing countries. With VIA, 72.4% of the women who had abnormal lesions did not have infection by HPV16 or 18. While with VILI as the screening test was able to detect more women with abnormal lesions out of which 70.3% were not by bivalent targeted HPVs. This means that the abnormal lesions must have been caused by other HPVs other than hr HPV 16 and 18. The odds of having a non –bivalent targeted HPV infection in women with abnormal cervical lesion is more than two times significantly higher (Odds ratio= 2.28, 95% CI= 1.043, 4.961, P= 0.035). This supposes that these women would not be protected by the bivalent vaccine that is available. Reports on cross protection against HPV 31/33/45 infection by the bivalent vaccine are seen in the literature. These HPV types that are cross protected are also targeted by the nonavalent vaccine and may reduce the impact of non-availability of the nonavalent vaccine (Kamolratanakul & Pitisuttithum, 2021). The nonavalent vaccine has a wider coverage but 58.6% and 64.9% of women with acetowhite lesions and those with poor Lugol's iodine uptake respectively, were not infected by nonavalent targetted viruses (p= 0.040 and 0.027

respectively). This goes to even buttress the fact that more vaccines with even wider coverage will be necessary especially for sub Saharan Africa.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

There is a high prevalence (43.6%) of hr HPV infection among a population based study population of women 30 to 49 years in Imo state, south east Nigeria. This was significantly associated with rural place of residence. The high prevalence of HPV infection in this study implies the potential future burden of cervical cancer in the state if appropriate preventive measures are not adapted. HPV 51 which is not vaccine protected was the most common infection in the study population and was also significantly associated with abnormal cervical lesions determined using visual methods of cervical cancer screening. This was followed by HPV types 59, 18, 52, 16, 68, 53, 31, 56, 39, 35, 58 and 66. The prevalence of HPV types 16 and 18 which are in the available vaccines in the country was not the predominant HPV infection in this study as in the developed world. HPV type 16, however had more predominance in those with abnormal cervices and was significantly associated with abnormal cervical lesions. This implies a relevance in cervical cancer causation. The vaccines available will have some effect in prevention of cervical cancer in Imo state but a vaccine of wider coverage will be more protective while further awareness of the use of vaccination and screening is created in the prevention of cervical cancer in Imo state. There is a need for further studies to determine HPV typing focused on women with cervical cancer.

5.2. Recommendation

Further studies testing for hr-HPV on persons already diagnosed with cervical cancer will make determination of vaccine suitability more exact in Imo state, Nigeria. Secondly, a wider scope of hr-HPVs should be tested with newer test kits and assay types.

Research should be carried out to increase the range of vaccine targeted HPVs with the intention to accommodate the diversity of prevalent HPVs seen in different studies in different geographical locations of the world.

5.3. Contributions to Body of Knowledge

i. This is the first study that determined gene typing of high risk HPV in the South East of Nigeria to the best of my knowledge.

ii. The prevalence of high risk HPV in Imo state was 43.6% among reproductive age women between 30 and 49 years of age.

iii. Our study found out that HPV 16 and 18 were not the most common hr-HPVs infecting in the study population.

iv. Hr -HPV 51 which is not vaccine targeted was the most common genotype detected in our study population in Imo state, south East Nigeria.

v. The odds of being infected by non- targeted hr-HPV is 3 times higher than by the targeted hr-HPV in the study population.

vi. Hr-HPV 16 was more common among women with abnormal cervical lesions. Odds of having hr-HPV infection in women with abnormal VILI result was 8.14 times higher than in the normal VILI result.

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APPENDICES

Appendix I: Consent form

Section 1: Introduction

Dear respondent,

I am Emily Akuabia Nzeribe, a Ph.D student of the Department of Public Health, Federal University of Technology, Owerri. I am working on the research topic **'High risk HPV DNA typing and available HPV vaccine suitability for women in Imo state, Nigeria'** for my thesis. I request your consent to enroll you into this study. All findings and response will be treated with utmost confidentiality. You are free to choose whether to participate or not and there will be no consequences for refusal.

Sincerely,

Emily Akuabia Nzeribe

Section 2: Background information:

Human Papilloma Virus (HPV) has been established as a cause of cervical cancer. It is the most common sexually transmitted infection. Many sexually active women will have this infection at some point in their lifetime. There are various types of this virus and some have strongly been associated with progression to developing cervical cancer. Three types of vaccines exist for the primary prevention of cervical cancer. They are protective over some of the HPV types and not others. This study seeks to find out if the prevalent HPV types in Imo state are the same as those covered by existing vaccines and by extension the available HPV vaccine suitability for women in Imo state- Nigeria.

Eligible women will be consenting women who are 30 to 49 years of age, who have ever been sexually active and has not had a hysterectomy for any reason in the past. If you consent to participate in this study, we will appreciate if you fill out our questionnaire for the purposes of

the study. An interviewer will be available to assist you in the filling of the questionnaire. Should you wish to do it unattended, your wish will be obliged.

You will receive health education on cervical cancer prevention and screening for cervical cancer using HPV typing and visual methods. Your cervical specimen will be collected for the purposes of hrHPV DNA testing and typing. Visual inspection methods of cervical cancer screening will be used to determine presence or absence of pre cancer lesions on your cervix. The result will be received immediately for the visual method of screening but not for HPV DNA testing and typing.

The results will be discussed with you as soon as possible and if you have VIA positive result, you will be evaluated for treatment after counseling on the result findings and after obtaining consent for further treatment. If you are VIA positive, you will be offered ablative therapy as either cryotherapy or electrosurgical therapy. If you are HPV DNA positive but tested negative to VIA, you will be counseled on the need to have a repeat HPV DNA test within 4- 6 months and the self-collected specimen in the preservative sent to a designated facility for HPV DNA testing. Researchers will follow up such persons for further management. If you have HPV negative result, you will be informed of your result and on the need to have repeat screening done in 5 years.

Section C: CONSENT

Do you willingly give consent to participate in this study? Yes / no

Do you wish to be informed of your result after testing? Yes/ no

Is there someone else you want us to tell your result? Yes / no

If yes? Who? (Please give name and phone number) ----------

Appendix ii: Questionnaire

School of Postgraduate Studies

Federal University of Technology, Owerri

High risk HPV DNA typing and available HPV vaccine suitability for women in Imo state, Nigeria

Study coded number: ----------

SECTION 1: Basic information:

1) Senatorial region---------- 2) LGA----------3) Community----------

5) Phone number ----------/ email----------6) date ----------

7) Next of kin---------- 9) Questionnaire administered by----------

INSTRUCTIONS:

Dear respondent,

I am Emily Akuabia Nzeribe, a Ph.D student of the Department of Public Health, Federal University of Technology, Owerri. I am working on the research topic 'High risk HPV DNA typing and available HPV vaccine suitability for women in Imo state, Nigeria' for my thesis. I request your consent to enroll you into this study.

INSTRUCTIONS: Please circle the correct answer to the following questions.

Section 2: Socio-demographic characteristics

9) What is your age range? (years)

a)30 -34, b)35-39, c)40- 44, d)45- 49---------- Date of birth----/-----/---------- (day/month/year)

10) What is your marital status—a) Single (b) Married (c) Cohabiting (d) Separated (e) Divorced (f) Widowed g) others

11) What is your highest level of education attained? (a) Never attended school (b) Primary Education (c) Secondary Education (d) Tertiary education e) others

12) What level of Occupation do you have? (a) Unskilled (b) Semi-skilled (c) Skilled (d) Other (Please specify) -------

13) What is your ethnic group(a) Hausa (b) Igbo (c) Yoruba (d) Other (Please specify) …….

14) What is your religion? (a) Christianity (b) Islam (c) Traditional belief (d) Aetheist (e) others------ (please specify) ----------

Section 3: Social habits

15) Do you use drugs for social purposes? Yes/ no

16) If yes, what type of drug do you use? ----------

17) Do you take alcohol in any form? Yes /no

18) If yes, how many times a week? A) 1-4 times b) 5 -9 times c) 10 times and above

19) On the average how many bottles of alcohol or portions (for spirits) do you take at a time? (a) Less than 1 bottle (b) 1 to 3 bottles (c) 4 or more bottles

20) Do you smoke? Yes / No

21) Do you use tobacco in any other form? Yes /no.

22a) If yes, what form? a) chewable b) smoke c) pipe d) in drinks or food

Section 4: The reproductive history

22) At what age was your first sexual encounter? (a) less than 13years (b) 13 to 18 years (c) above 18 years

23) How many pregnancies have you had? (a) 0 (b) 1- 4(c) 5 and above

24) How many miscarriages/ abortions have you had? (a) 0 (b) 1-4 (c) 5 and above

25) How many pregnancies have you carried for up to 7 months? (a) 0 (b) 1-4 (c) 5 and above

26) How many living children do you have? (a) 0 (b) 1-4 (c) 5 and above

27) How many lifetime sexual partners have you had till date? (a) 1 (b) 2-5 (c) 6 -10 (d) 11 and above

28) Type of marriage setting (a) Only wife of husband (b) Have other co wives c) Spouse has concubines (d) any other ---------(please specify)

29) Have you ever had a Private part (genital Tract) infection? Yes/ no

30) If yes, please state the type if you know. ----------

31) How many times? a) 1 b) 2-4 times- c) 5 or more times

32) Have you been tested for HIV? a) Yes b) No

33) What was the result? Positive / negative

Section 5: Knowledge and practice on cervical cancer

34) Have you heard of cervical cancer? Yes/ no

35) If yes, what did you think was the cause? ----------

36) Have you heard of Human Papilloma Virus (HPV)? Yes /No

37) Can HPV infection be treated? Yes/no

38) Can HPV infection be prevented? Yes / No

39) Have you heard of HPV vaccine? Yes/No

40) Have you taken the vaccine? Yes /No

41) Do you know anyone who has taken the HPV vaccine? Yes/ No

42) Have you ever being screened for cervical cancer? Yes/ No

43) If yes to 42 above, what was the result? Normal/ abnormal

44) If yes to 42 above, where did you get screened?(a) hospital / clinic (b) Health Centre (c) outreach program (d) any other

Thank you for your time and cooperation.

SECTION 6: TEST RESULTS (TO B E FILLED BY RESEARCHER)

45) VIA SCREENING----- NORMAL/ ABNORMAL

46) VILI SCREENING --- NORMAL / ABORMAL

47) HPV RESULT -----Positive/Negative

48) High risk HPV types detected----------

Appendix iii: Synopsis

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

POSTGRADUATE SCHOOL

NAME OF STUDENT-: EMILY AKUABIA NZERIBE

REGISTERATION NUMBER: 20154989378

DEPARTMENT OF PUBLIC HEALTH

SCHOOL OF HEALTH TECHNOLOGY

DEGREE-IN –VIEW: DOCTOR OF PHILOSOPHY (Ph.D)

EXPECTED YEAR OF GRADUATION: 2024

TITLE OF THESIS: HIGH RISK-HUMAN PAPILLOMA-VIRUS DEOXYRIBONUCEIC-ACID TYPING AND AVAILABLE HUMAN PAPILLOMA-VIRUS VACCINE SUITABILITY FOR WOMEN IN IMO STATE

SYNOPSIS

INTRODUCTION:

The establishment of Human Papilloma Virus as the cause of cervical cancer has led to the emergence of vaccines against the virus. Vaccines have been developed against the most common HPV DNA types (16 &18) responsible for close to 70 percent of cervical cancer cases either alone or in combination with the low risk HPV types (6 &11). A recent entrant has been the nonavalent vaccine which has a wider coverage to include 16, 18, 31, 33, 45, 52, and 58 in addition to 6 and 11.

HPV typing, laboratory test in which DNA for HPV is searched for on cells scraped from the cervix makes it possible to know the exact types of HPV infection in a woman. A wide variety of high risk HPV types are capable of causing cervical cancer, but there is limited coverage of

existing vaccines. This, therefore created a need to determine the exact types that commonly infect women, cause premalignant cervical lesions and possibly cervical cancer among women in Imo state, South East Nigeria. The studied population were reproductive age women (30 to 49 years), and included those with and those without premalignant lesions of the cervix.

The prevalent hr HPV types were correlated with the vaccine targeted types in order to determine suitability of existing HPV vaccines for women in Imo state, South East Nigeria. Suitability implies the quality of being right or appropriate for a population, persons or situations. For the purposes of determining suitability of vaccine, the HPV types covered by available vaccines will be the independent variable whereas the high risk HPV DNA type that is present in the women will become the dependent variable.

METHODOLOGY: Two hundred and fifty seven consenting women selected from the three geo-political zones of Imo state following multistage sampling were studied. Following health education and counselling, a questionnaire was used to determine socio demographic variables. Specimen collection for HPV typing was done followed by screening for premalignant lesions using visual inspection methods. Thereafter, the cervical specimens underwent HPV gene typing using the Atila AmpFire HPV High risk genotyping assay, an isothermal nucleic acid amplification assay for qualitative genotyping of high risk types of human papilloma virus (HPV). The assay genotypes HPV 16,18,31,33,35,39,45,51,52,53,56,58,59,66,68.

Analysis was with IBM-SPSS statistics version 25 and involved descriptive analysis as well as inferential statistics at 5% level of significance, a probability value ($p>0.05$) and 95% confidence interval. The odds ratio was used to account for size effects measures in the data.

RESULTS

The prevalence of hr- HPV was 43.6% among women aged 30-49 years old in Imo state. HPV51 was the most prevalent type of HPV in the study area at 16.7%, followed by HPV 59 and HPV18 at 12.2% and 10.1% respectively. Others include HPV 16, HPV 68 and HPV 52 at 8.6% each, HPV 53 and HPV31 were 7.4% and 7.0% respectively. Occupation ethnicity , place of residence , use of habit forming drugs, use of tobacco, coitarche, number of pregnancies in the

past, number of living children , number of sexual partners, frequency of genital tract infections and type of genital tract infection were significantly associated with the HPV infection.

Only 16 women (6.2%) had solely bivalent vaccine targeted HPV infections and will be protected by the bivalent vaccine. There was a significant difference between the prevalent hr-HPV types and those targeted by bivalent HPV vaccines in Imo state, South East Nigeria.

The odds for having abnormal lesion was 4 times higher for the women that tested positive to any HPV compared to those that tested negative. There was significant association between hr HPV infection and premalignant changes on the cervix using both VIA and VILI and especially for HPV 16, 51, 52, 59, 68**.** Notably, HPV 51, 59 and 68 are not vaccine targeted but were associated with premalignant cervical changes.

CONCLUSION

Prevalence of HPV infection is high in Imo state. Existing vaccines will be effective in reducing cancer of the cervix due to HPV 16 and 18 but development of a new vaccine of wider coverage will be needed especially following more robust work focused on women who have been diagnosed of cervical cancer.

PROF. I. N.S DOZIE----------

(Supervisor) Date

PROF. S.N. IBE----------

(Supervisor) Date

DR C.C IWUALA----------

(Head of Department) Date

PROF (MRS) J.N. NWOSU ----------

(Dean of School) Date