CYTOKINES GENES PATTERN IN HUMAN PAPILLOMAVIRUS AND *Chlamydia trachomatis* CO-INFECTION AMONG WOMEN IN ILORIN, NIGERIA

BY

OMOARE, ADESUYI AYODEJI
B. Sc. (ILORIN), M. Sc. (ATBU)
01/55EJ072

BEING A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D.) IN MEDICAL MICROBIOLOGY (CLINICAL AND MOLECULAR VIROLOGY) IN THE DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY, FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF ILORIN, ILORIN

SUPERVISED BY:
DR. O. O. AGBEDE
PROF A. P. ABOYEJI

DECEMBER, 2019

CERTIFICATION

We certify that the study reported in this thesis titled "Cytokine Genes Pattern in Human Papillomavirus and *Chlamydia trachomatis* Co-infection among Women in Ilorin, Nigeria" was conducted under the supervision of Dr. O. O. Agbede, in the Department of Medical Microbiology and Parasitology of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin. The thesis has been read and accepted as meeting the requirement of the Department of Medical Microbiology and Parasitology and the University for the award of Ph.D. degree in Medical Microbiology and Parasitology (Medical and Molecular Virology).

…… ……..
Dr. O. O. Agbede **Date**
Supervisor

…… ……..
Prof. A. A. Akanbi II **Date**
Head of Department

…… ……..
Dr. A. Nyamngee **Date**
PG Coordinator

…… ……..
Dr. A. O. Oyewopo **Date**
Internal/ External Examiner

…… ……..
External Examiner **Date**

DEDICATION

This research work is dedicated to my paternal grandmother Late Mrs. Dorcas Omoare whose painful experience with cancer elicited my interest in cancer research and also to many other women who have either died of cancer or are at risk of it.

ACKNOWLEDGEMENTS

I give all glory to the Almighty God the Father of my Lord Jesus Christ who gave me direction, inspiration and spared my life throughout this doctoral program. For in Him I live, move and have my being. I will also like to appreciate my supervisor Dr. O. O. Agbede under whose mentorship, financial support and laboratory space this work was carried out. Our relationship began as two strangers, developed into a supervisor-supervisee relationship until it culminated to a father son relationship and so much have been learnt under his mentorship. Worthy of mention is Prof. (Mrs.) E. O. Olorundare whose counsel has been rewarding. I remember when I was looking out for a Virology supervisor and sought her counsel and her reply was "I know of an excellent virologist…" Her recommendation brought me into contact with Dr. O. O. Agbede. I will like to express my profound gratitude to the Head of Department of Medical Microbiology and Parasitology Prof. A. A. Akanbi II and all members of staff of the department; Prof. L. D. Edungbola, Prof. C. Nwabuisi, Dr. A. Fadeyi, Dr. A. Nyamngee, Dr. S. Suleiman, Dr. O. Adeyemi, Mr. Ode, Mr. P.A. Adebayo, Mrs. Taiwo and Mr. T.A. Sanni for their support and assistance throughout the program.

The journey towards this degree has been a tough one sandwiched with generous faith and plenty of assistance from many. My special appreciation goes to Dr. G. K. Olawepo and Prof. S. Adejumo who supported financially in the purchase of my doctoral form and others such as; Brigadier General and Mrs. M. Ajala, Dr. and Mrs. Elujide, Dr. and Mrs. Adeyemi and especially to Engr. S. E. Ugeh for their financial support throughout the program.

Furthermore, I appreciate the generous gift received from Pavarska Chellankanu and Jeevan Prakarsh of EziPrep Liquid Based Cytology India for the LBC kits. Other generous gifts received for this work includes; Human Papillomavirus positive controls from Prof. Anna-Lise Williamson, Director of the WHO HPV Labnet for the African Region located at the University of Cape Town South Africa. The *Chlamydia trachomatis* positive control was from

Prof. Dr. Georg Hacker Principal Investigator of the Chlamydia study group in the University of Freiburgh Germany and Dr. B. S. Oderinde for generous gifts of consumables and financial support for this work.

Also, I thank Dr. Femi Oladiji of the Department of Epidemiology and Community Health, University of Ilorin for his input in the preliminary study design of this work. The selfless contributions of Dr. (Mrs.) Okesina, Dr. (Mrs.) Akogun, Dr. (Mrs.) Ojibara and all members of staff of the Obstetrics and Gyneacology department of Sobi specialist Hospital Ilorin who participated in sample collection.

My deep gratitude goes to Prof. A. P. Aboyeji of the Department of Obstetrics and Gyneacology of the University of Ilorin Teaching Hospital for his fatherly guidance, input and prayers as well as introducing me to the Gyneacology and Family planning clinics of the University of Ilorin Teaching Hospital and ensure I received every possible support. I thank Matron Ilori and her team in the Family Planning Clinic of the University of Ilorin Teaching Hospital and Matron Ilori of the Family planning Clinic of the Children Specialist Hospital centre Igboro Ilorin Kwara state for their assistance in sample collection.

I am grateful to the pathologists who screened and interpreted the cytology slides; Dr. O. O. K. Ibrahim and Dr. A.A. Abdulmojid of the department of Histopathology of the University of Ilorin Teaching Hospital and all participants who gave their consents to participate in this study.

Many thanks to the Regional Overseer Mountain of Fire and Miracles Ministries South West 3 Regional headquarters Ilorin Pastor Samuel Oladejo and the Youth Pastor Dr. O. Oyewopo and Mrs. Modupe Durotoye for their spiritual, moral and financial support. In addition, i sincerely appreciate the kind suggestions of Miss Yetunde Kayode and Mr. Kehinde Aina and all members of the Agbede's Lab. Also, the moral support received from Dr. S. Oyedeji, Dr. O. D. Owolabi, Dr. C. O. Ogunkunle, and Dr. Olorunmaye towards this work.

I appreciate Dr. Ashaka Oluwaseyi for his assistance and ability to pay attention to details which turned out to be very beneficial and it has also been great working together under the same supervisor. Dr. D.A. Animasaun has been an epitome of support to this work. I remember running out of consumables several times during the course of this work and he was always ready to assist with materials and never hesitated to share in his wealth of experience to make this work a success.

To my parents Mr. Festus Omoare and late Mrs. Comfort Omoare who were burdened with the vision of giving qualitative education to their children and to my siblings Pharm. Afolabi Bukola, Engr. Adedayo, Engr. Adeola, Engr. Adeolu, Miss Ayobami, Miss Adewunmi and Mr. Caleb. Not forgetting my In-laws; Mr. Boniface Ayausi, Mr. Richard, Mr. Jechoniah, Miss Grace, Miss Gift and Miss Elizabeth for their patience, moral and financial support throughout this work.

All these writings will not be complete without the mention of my darling wife Mrs. Omoare Joy who has been an invaluable source of support throughout this journey. Your rich source of resilience, understanding, support and love kept me keeping on and to my lovely children; Breakthrough, Anuoluwapo and Jeremiah for the lonely times they endured during this work.

Words are not enough to express my gratitude to all who made the success of this work a reality. May the Almighty God grant you help in your time of need.

Omoare, Adesuyi Ayodeji

September, 2019.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ad	adenovirus
AP 1	Activator Protein-1
ATM	Ataxia telangiectasia, mutated
ATP	Adenosine triphosphate
ATR	ATM and Rad3-related
BER	Base excision repair,
CA	Chromosomal aberrations
CAC	Colitis associated cancer
Cak	CDK activating kinase
CDC	Center for Disease Control and Prevention
CDK	Cyclin dependent kinase
CDKC	Cyclin dependent kinase complex
CFSs	Chromosome Fragile Sites
CHK	Checkpoint kinase
CIN	Cervical Intraepithelial Neoplasia
CKI	Cyclin dependent kinase inhibitor
CLRs	C-type lectin receptors,
CRPV	Cottontail Rabbit Papillomavirus
CT	*Chlamydia trachomatis*
DCs	Dendritic Cells
DDR	DNA Damage Response
DFA	Direct fluorescent antibody test
DNA	Deoxyribo Nucleic Acid
ds	double-strand

DSBs	Double Strand Breaks
EB	Elementary body
EBV	Epstein-Barr virus
EIA	Enzyme- immunoassay
ELISA	Enzyme-linked immunosorbent assay
E2F	Transcription Factor
HLA	Human Leucocyte antigen
HPV	Human Papillomavirus
HRHPV	High Risk Human papillomavirus
HSIL	High squamous intra-epithelia lesion
IARC	The International Agency for Research on Cancer
ICTV	International Committee on the Taxonomy of Viruses
ICLs	Intrastrand Crosslinks
IFN-γ	Interferon gamma
IL	Interleukin
LBC	Liquid based cytology
LCs	Lymphatic cells
LCR	Long Control Region
LGA	Local Government Areas
LGV	Lymphogranuloma venereum
LMP	Latent membrane protein
LSIL	Low squamous intra-epithelia lesion
mAbs	Monoclonal antibodies
MCP	Monocyte chemotactic protein
MHC	Major Histocompatibility Complex

MMR	Mismatch repair
MOH	Ministry of Health
MOMP	Major Outer Membrane Proteins
MSM	Men who have sex with men
MMTV	Mouse Mammary Tumour Virus
NAAT	Nucleic acid amplification tests
NER	Nucleotide excision Repair
NF-κB	Nuclear Factor-kappa B
NKT	Natural Killer T
NOD	Nucleotide-binding oligomerization domain
ORFs	Open Reading Frames
PAMPs	Pathogen-associated molecular patterns
Pap	Papanicolaou
PB	Persistence body
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
PV	Papillomavirus
PMN	Polymorphonuclear leukocytes
pRB	Retinoblastoma protein
PRRs	Pattern recognition receptors
PyMT	Polyoma virus middle T
p21	protein of molecular weight 21
$p27^{kip1}$	protein of molecular weight 27
p53	protein of molecular weight 53

RB	Reticulate body
RONS	Reactive Oxygen and Nitrogen Species
rRNA	Ribosomal Ribonucleic acid
SIL	Squamous intra-epithelia lesions
STAT3	Signal Transducers and Activators of Transcription
STD	Sexually Transmited Diseases
STI	Sexually Transmitted Infection
SV	Simian Virus
TAg	T-antigen
TAMs	Tumor associated macrophages
TCR	T cell receptors
TGF-β	Tumour growth factor- beta
Th	T helper cells
TLR	toll-like receptor
TNF-α	Tumour necrosis factor-alpha
TREMs	Triggering Receptors Expressed on Myeloid cells
URR	Upper regulatory regions
UV	Ultra Violet
VEGF	Vascular endothelial growth factor
VLPs	Virus Like Particles
WHO	World Health Organization

ABSTRACT

Human Papillomavirus (HPV) is a common sexually transmitted pathogen. HPV infection is a mandatory but not sufficient cause of cervical cancer. However, some women infected with HPV ultimately develop the disease. Several co-factors including *Chlamydia trachomatis* (CT) infection and pro-inflammatory cytokines are believed to influence the clinical outcome of HPV infection. Cytokines genes are polymorphic in nature and can lead to individual variation in cytokines production. This study assessed the cytokines genes pattern in HPV and CT co-infected women in Ilorin, Nigeria. The objectives were to: (i) identify participants with abnormal changes in cells of the cervix; (ii) detect HPV DNA in the exfoliated cervical cells of participants; (iii) determine the prevalence of HPV and the proportion of high risk HPV (HRHPV) among participants; (iv) identify CT DNA and its prevalence in cervical samples of participants; (v) determine the prevalence of HPV and CT co-infection; (vi) profile the cytokines genes associated with co-infection; and (vii) assess the risk factors associated with HPV and CT co- infections.

Exfoliated cervical cells from 376 women were analysed by Papanicolaou test. Deoxyribonucleic acids (DNA) was extracted from the cells and amplified by Polymerase Chain Reaction (PCR) assays. MY09/11 and GP5+/6+ primers were used for amplification of HPV DNA by nested PCR; eighteen specific type primers for HRHPV DNA were amplified by nested multiplex PCR. Cryptic Plasmid primers were used to detect CT DNA by conventional PCR. Pro-inflammatory cytokines genes [Interferon gamma (IFN-γ) and Tumour Necrosis Factor alpha (TNF-α)] and anti-inflammatory cytokines genes [Interleukin-10 (IL-10) and Tumour Growth Factor-beta (TGF-β codons 10 and 25)] were amplified with Amplification Refractory Mutation System PCR. Statistical analysis of the data was done using chi-square, student t-test (t) and multiple regressions at $p< 0.05$.

The findings of this study were that:

(i) 19 (5.1%) women had abnormal cervical cytology out of which 13 (3.5%) had Low Squamous Intra-epithelial Lesion, 4 (1.1%) had Atypical Squamous Cell of Undetermined Significance and 2 (0.5%) had High Squamous Intra-epithelial Lesion;

(ii) the fragments corresponded in position to base pairs (bp) of HPV (150 and 450 bp) and HRHPV (~118-457 bp);

(iii) the prevalence of HPV was 81.4%, out of which 53.5% had HRHPV genotypes with HPV 82 being the most abundant (33.5%);

(iv) CT fragments appeared at 201 bp, with a prevalence of 4.5%;

(v) the prevalence of co-infection was 4.0%;

(vi) comparison of co-infection with single infection showed significant differences ($p< 0.05$) in IFN-γ, TNF-α and TGF-β10. Also, when HPV and CT single infections were compared, IFN-γ and TNF-α showed significant difference ($p< 0.05$);

(vii) HPV and CT individual infections were associated with risk factors such as age, parity, age at sexual debut, female genital cutting and contraceptive use.

The study concluded that there were significant variations in predominantly pro-inflammatory cytokine genes in relation to HPV and CT co-infected women in Ilorin. The study recommended that these gene patterns may be used as predictor of HPV disease.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Human papillomavirus (HPV) is a virus from the *Papillomaviridae* family that has been associated with various infections in man. Human Papillomaviruses establish productive infections only in keratinocytes or mucous membranes (Grce *et al., 2007*). Most HPV genotypes are asymptomatic in many individuals. Furthermore, some types can cause warts, while others lead to different cancers associated with the; cervix, vulva, vagina, penis, oropharynx and anus. The genome of HPV consists of a small Deoxyribonucleic Acid (DNA) virus, with approximately 8000 base pairs (Shaurer *et al*., 2005).

There have been evidences linking HPV to benign and malignant tumors of the upper respiratory tract. The International Agency for Research on Cancer (IARC) has found that most people with lung cancer develop antibodies to several high-risk forms of HPV compared to those who do not (WHO, 2007; IARC 2007). Furthermore, HPV has been linked with an increased risk of cardiovascular disease (Kuol and Fujise, 2011). Also, infections with certain HPV types specifically types 16 and 18 have been strongly associated with elevated risk of developing throat cancer (Gillison, 2004).

The HPV life cycle strictly follows the differentiation program of the host keratinocyte (Kajitani *et al*., 2012). Genital HPV is the most common sexually transmitted infection globally (WHO, 2019). Human Papillomaviruses include more than 240 related viruses, in which more than 40 HPV types can infect the genital areas of men and women, including; the skin of the penis, vulva, anus and the linings of the vagina, cervix, and rectum. These types can also infect the linings of the mouth and throat (Ni Sima *et al.,* 2007; Van Doorslaer, 2013).

HPV types are often referred to as wart-causing (low-risk) or cancer-causing (high-risk), based on their ability to put a person at risk of cancer (Liviu *et al*., 2010). In the lifetime of sexually active people, more than half of them are infected with one or more HPV types. Available data shows that, at any point in time, about fifty percent of women have genital HPV infections, whereas low percentages of adults have oral HPV infections (Gillison, 2004). Genital HPV infection is very common, and while a large number of HPV types can infect the cervical epithelium, HPV16 and HPV18 have been shown to be responsible for approximately 70 percent of all cases of cervical cancer worldwide (Ni Sima *et al*., 2007).

Other infections of the cervix that could affect the clinical course and outcome of HPV genital infection includes; *Treponema pallidum* that causes syphilis, *Neisseria gonorrhoea* the causative agent of gonorhea and *Chlamydia trachomatis* (CT) which causes Pelvic Inflammatory Disease (PID). Worldwide, *Chlamydia trachomatis* infections rank among the most common sexually transmitted infections (STI), and cause notable reproductive morbidity in women (Marrazo and Suchland, 2014). It is the most common cause of inflammations of urethra and cervix in men and women respectively with certain strains causing severe proctitis in men who have sex with men (MSM) (CDC, 2012).

Besides, most *Chlamydia trachomatis* infections cause neither symptoms nor signs especially in women. Unfortunately, the asymptomatic nature of chlamydial infections in the female reproductive tract facilitates the silent immune pathogenesis that causes scarring of the fallopian tubes, ovaries, endometrial lining, and occasionally the adjacent perineum, promoting future ectopic pregnancy and tubal infertility. For this reason, *C. trachomatis* is thought to be the most expensive to treat non-viral STI, with total lifetime direct medical costs estimated at over 103 billion naira (Marrazo and Suchland, 2014).

Moreover, certain oncogenic genital HPV types that are sexually transmitted have been shown to be a necessary but not sufficient cause of cervical cancer. These oncogenic HPVs

most often result in short term infections of the cervix, with a small proportion of exposed women progressing ultimately to cancer of the cervix (Madeleine *et al*., 2007). Since only few women exposed to sexually transmitted HPV come down with cervical cancer, other co-factors may be involved in addition to high risk HPV to promote the development of cervical neoplasia and cervical cancer. One such possible co-factor is *Chlamydia trachomatis*, which is highly prevalent among sexually active young women and can infect the cervix for long periods of time.

Risk factors for *C. trachomatis* infection are similar to those for genital HPV infections, and includes but not limited to; a history of multiple sexual partners, a recent new sexual partner, *coitarche* and sporadic use of barrier contraceptives (Handsfield *et al*., 1986; Koutsky *et al*., 1992). *Chlamydia trachomatis* often causes cervicitis, which is a chronic infection of the endocervical cells of the transformation zone. Such inflammation may predispose women to other Sexually Transmited Infection (STIs), including genital HPV infection, by damaging epithelial integrity. Studies have shown that a history of *C. trachomatis* infection may be associated with persistence of oncogenic HPV infections, (Silins *et al*., 2005; Samoff *et al*., 2005) and other studies have revealed that persistent HPV infections are necessary for progression to high-grade cervical intraepithelial neoplasia (CIN) and carcinoma. Thus, chronic cervical inflammation induced by *C. trachomatis* could increase the risk of transformation of cervical cells that are persistently infected with oncogenic types of HPV (Madaleine *et al*., 2007).

Some types (HPV6 and HPV11) cause genital warts but do not usually transform to cancer, while other types (predominantly HPV16 and HPV18) cause cervical infection, with a significant risk of progression to cancer in the subsequent decades. In approximately 10 percent of women, cervical HPV infection is not eradicated by host cytotoxic lymphocyte responses and the persistent infection results in low-grade dysplasia, commonly known as cervical

intraepithelial neoplasia (CIN1). In a small minority of women, high-grade dysplasia (CIN2 and CIN3) results, and this can progress to invasive cervical cancer.

Cancer is a disease primarily caused by changes in cells that progress through a series of somatic mutations in specific genes leading to uncontrolled cellular proliferation. It may also be due to exposure to a variety of chemical or physical agents, by random errors of genetic replication, or by errors in DNA repair processes. In cancer disease the body cells grow out of control and are usually named for the part of the body where the proliferation begins (Raymnd, 2007).

Cervical cancer in HPV infection most often develop decades after initiation of infection with an oncogenic (cancer-causing) HPV type (Dunne and Markowitz, 2006). Persistent HPV infection is detected in 99 percent of women with cervical cancer (Manga *et al.*, 2015). Approximately 15 HPV oncogenic types are responsible for most cases of cervical cancer: HPV16 is responsible for about 60 percent and HPV18 for about 10 percent of cervical cancers, with types HPV31, HPV33 and HPV45 being the next most likely causes (MOH, 2011).

Genes controlling cell cycle progression are refered to as the gatekeeper genes while genes responsible for DNA repair pathways are called caretaker genes. Mutations in these genes can uncouple cancer development (Deininger, 1999). After mutation, certain genes emerge as oncogenes by acquiring new functions leading to increased cell proliferation thereby inactivating Tumour suppressor genes (Morin *et al*., 2005).

The role of the immune system in the pathogenesis of cancer cannot be ignored as several lines of evidences suggest that cell-mediated immune responses are important in controlling both HPV infections and HPV-associated neoplasms (Wu and Kurman, 1997). Cell-mediated immunity is regulated by cytokines that are secreted by T helper cells. In general, T-helper cells can be classified as Th1 and or Th2 cells on the basis of the different

types of cytokines they secrete. The Th1 are known to be very important cells in inflammatory responses and have been linked with delayed-type hypersensitivity. This group of cells secrete interleukin (IL-2) and interferon gamma (IFN-γ). While the Th2 cells produce IL-4, IL-5, IL-10, and IL-13 (Wu and Kurman, 1997).

Cytokines play vital roles in regulating and modulating the activities of cellular immune interactions and are produced by several cells including; lymphocytes, monocytes, macrophages, neutrophils, fibroblasts and endothelial cells (Jason *et al.,* 2001; Hemmerle and Neri, 2013). It has been noticed that qualitative and quantitative impairment of the immune response, due to alterations in cytokine type production, could be the major factor resulting in the development of pathologic diseases (Mario *et al.,* 1998). Besides, cytokines play a central role in the inflammatory component between the host's stromal cells and the tumor cells during tumorigenesis such as the upregulation of pro-inflammatory cytokines IL-6 and IL-8 during persistent DNA Damage Response (DDR). The fact that certain cytokines display growth factor activity as well as the ability to produce reactive oxygen and nitrogen Species (RONS) suggests that they may promote genomic instability in chronic inflammatory conditions (Aivaliotis *et al.,* 2012).

The interest in the progression of cervical cells to cancer from the perspective of inflammation by considering cytokine quality which may further explain the HPV clinical course progression especially in the presence of a Sexually Transmitted Infection agent like *Chlamydia trachomatis* was the focus of this study.

1.2 Statement of the Problem

Human Papillomavirus infect people globally targeting different anatomical sites such as; oropharynx, penis, anus, vulva, cervix, causing severe cases of cancer particularly in cervical cancer. Cancer of the cervix is a pandemic problem with disproportionate global distribution (Wentzensen *et al.,* 2004). Global prevalence of high risk HPV infection reveals

that sub-Saharan Africa which includes Nigeria has the highest disease burden of cervical cancer with increasing incidence and lacks organized screening programs for early detection of precancerous lesions. The increasing rate of diagnosis of HRHPV among women in developing countries, coupled with various degrees of challenges in their health system sustains this status (Atara, 2012; Piras, 2011).

Sexually transmitted High risk HPV types have been shown to be a compulsory but not sufficient cause of cervical cancer. These HPVs most often result in transient infections of the cervix, and only a small proportion of exposed women eventually progress to cervical cancer (Madeleine, 2007), making it necessary to consider an important co-factor known as *C. trachomatis* which causes symptomatic and asymptomatic cervicitis among women. Besides, literature search did not reveal any molecular study on the co-infection of *C. trachomatis* and HPV in Nigeria.

Moreover, there is paucity of information on the effect of inflammation caused by infection with high risk HPV, *C. trachomatis* or both and its implication on cervical cancer progression. Furthermore, certain cytokines are known to enhance cell cycle progression, promote apoptosis, supports inflammation and invasiveness (Aivaliotis, 2012), but their roles in relation to HPV, CT, HPV and CT co-infection among women in Ilorin has not been elucidated.

This study bridged some of the gaps that exist in HPV research in Ilorin, Nigeria. To this end some important research questions central to the study, have been answered by the researcher which include;

1. What is the outcome of HPV and *C. trachomatis* co-infection among women?
2. What is the effect of pro-inflammatory and anti-inflammatory cytokine genes on HPV and CT Co-infections?

3. What is the effect of the inflammation due to *Chlamydia trachomatis*, on the clinical course of co- infection with a high risk Human papillomavirus?
4. What are the predictive indices that may show persistence of cervical lesions in an HPV and CT co-infected individual?

Based on the above research questions the research work tested the following hypotheses:

1. **H_o**: *Chlamydia trachomatis* co-infection with high risk Human papillomavirus does not produce any change in squamous epithelial cells of the cervix.
2. **H_o**: Certain pro-inflammatory and anti-inflammatory cytokine polymorphic gene does not increase susceptibility to HPV and CT co-infection.
3. **H_o**: Inflammation due to *Chlamydia trachomatis* in a co-infection with high risk Human papillomavirus does not result in any alteration in the lifecycle of the Human papillomavirus.
4. **H_o**: The presence of specific anti-inflammatory cytokines genes in *Chlamydia trachomatis* and high risk Human papillomavirus co-infection is not an indication for continuos inflammation

1.3 Justification

About 400 million cases of genital HPV infection occur worldwide. It is estimated that 311,000 women die of cervical cancer each year with over 570,000 new diagnosis annually, 85% of which occur in the developing world (WHO, 2019). To the best of my knowledge, there has been no molecular study that has reported HPV and *Chlamydia trachomatis* co-infection among women in Nigeria. Priority areas for study are those at high risk for cervical cancer which include sub-Saharan Africa where Nigeria occupies an important position, Eastern Europe and areas where social changes and or urbanization may increase HPV infection among young generations. Besides, cytology-based screening is difficult to

implement, in resource-poor settings and HPV detection has been proposed as the alternative primary screening test for cervical cancer in low resource areas (Feng *et al*., 2010).

The role of the cytokine-mediated mucosal immune response in the clearance of cervical HPV infection remains poorly defined globally, while data are completely lacking in Nigeria. The prevalence and distribution of HPV genotypes associated with *C. trachomatis* infection and its clinical persistence are poorly explored. Furthermore, the absence of a baseline study on the molecular prevalence of the co-infection of HPV and *C. trachomatis* in Ilorin makes this study imperative.

1.4 Purpose of the Study

This work aims to provide evidence for the role of inflammatory cytokines in high risk Human Papillomavirus and *Chlamydia trachomatis* co-infection among women in Ilorin.

The specific objectives of this work were to:

1. identify participants with abnormal changes in cells of the cervix
2. detect the presence of Human Papillomavirus DNA in the exfoliated cervical cells of participants with normal and abnormal changes in cell population of the cervix.
3. determine the prevalence of HPV and the proportion of high risk HPV (HRHPV) among participants
4. identify *Chlamydia trachomatis* DNA and its prevalence in exfoliated cervical cell samples of participants.
5. To determine the prevalence of Human Papillomavirus and *Chlamydia trachomatis* co-infection in the study population.
6. profile cytokine gene polymorphism of four different cytokines namely; two pro-inflammatory cytokines; Interferon gamma (IFN-γ) and Tumour Necrosis Factor alpha (TNF-α), and two anti-inflammatory cytokines; Interleukin-10 (IL-10), Tumour Growth

Factor-beta (TGF-β codon 10, TGF-β codon 25) of participants with HPV and *Chlamydia trachomatis* co-infection.

7. access the risk factors associated with Human Papillomavirus and *Chlamydia trachomatis* co-infection in the study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Human Papillomaviruses

2.1.1 History and Discovery of Papillomavirus

The identification of a virus as the causative agent of warts was first recognised in 1907. The 19th century was a significant period when knowledge about the infectious nature of warts was unravelled. Prior to that time, genital warts were usually considered to be a form of syphilis or gonorrhoea. The viral origin of human warts was demonstrated in the early 1900s when cell-free filtrates from lesions were shown to transmit the disease (Ciuffo, 1907).

Papillomaviridae is a family of viruses containing 16 genera. Virions are non-enveloped, with a diameter of about 55 nm. The icosahedral capsid has 72 capsomeres in a skewed (T = 7) arrangement. The genome is a circular double stranded (ds) DNA, 6800–8400 base pair (bp) in length, encoding 8–10 proteins (Brian, 2009). The word papilloma is a Latin word derived from two key words namely; *Papilla* meaning nipple and *oma* meaning tumour.

The papillomaviruses (PVs) consists of a group of epithelial-loving DNA viruses that induce benign lesions of the skin (warts) and mucous membranes (condylomas). Papillomavirus (PV) has been isolated from many mammalian host species and birds, but, yet to be identified in non-vertebrates (Lancaster and Olson 1982, Sunderberg, 1987). Hundreds of PV types exists, which are mainly species-specific, and many of them infect a given host species (Antonsson *et al*., 2003, and Chan *et al*., 1997). Papillomaviruses have often been classified primarily according to the host species they infect and the sites of diseases with which they are associated. Because PVs are species-specific in their host range, it has been impossible to study the biology of human papillomavirus (HPV) in animals (Knipe *et al*., 2007).

Richard Shope identified the first animal PV in the 1930s and also characterized the transmissible nature of cutaneous papillomas arising in wild cottontail rabbits in contrast to domestic rabbits which resisted transmission. Shope's work followed information from McKichan of Cherokee, Iowa about the disease (Shope and Hurst 1933). Shopes papillomavirus, later called the cottontail rabbit papillomavirus (CRPV), was the first DNA tumour virus identified. Peyton Rous had however discovered the first RNA tumour virus known as Rous sarcoma virus in 1911 which laid the foundation for other tumor-inducing viruses in rabbits, mice, cats and non-human primates. Rous work which showed that some cancers have infectious etiology, led to the discovery of oncogenes, and laid the foundation for the molecular mechanisms of carcinogenesis (Weiss and Vogts 2011). Shope's research also showed that, although systemic injection with papilloma suspensions did not produce detectable infection, it could induce serum-neutralizing antibodies and protect rabbits against high dose cutaneous viral challenge (Shope, 1937; Weiss and Vogts 2011). In addition to causing benign papillomas, some warts induced by CRPV were observed to undergo malignant progression and CRPV for years became an important model for viral tumorigenesis research (Rous and Beard 1935; Silverton and Berry, 1935; Syverton, 1952; Kreider, 1981). However, the discovery of the polyomaviruses in the late 1950s, replaced CRPV as a model due to its ability of replicating in cultured cells and inducing morphologic transformation in-vitro (Molecular Biology of Tumor Viruses 1981).

The International Agency on Research for Cancer (IARC) has carried out HPV prevalence surveys and worldwide estimates of HPV type specific prevalence rates in women from different regions presenting with or without cervical lesions (IARC, Clifford *et al*., 2006). Researches findings in this area have increased knowledge on risk associated HPV. According to Akarolo-Anthony *et al.,* 2013 and 2014, Human papillomavirus is the most common sexually transmitted infection and at least 50% of sexually active people will get HPV at some

time in their lives. These viruses are ubiquitous and most women in the world are probably infected with at least one type of HPV during their sexual life giving a point prevalence of 10.1%. Human papillomavirus infection is commonly found in the anogenital tract of men and women with and without clinical lesions (ICO, HPV 2014).

2.2 Classification of Human Papillomavirus

Papillomaviridae are a family of epithelial-loving, non-enveloped double stranded DNA viruses with a circular genome of around 8000 bp. The description of this family of viruses has been made majorly in mammals and has also been found in; birds turtles and snakes (Gondinez *et al*., 2013). This family was previously known under the name *Papovaviridae*. This was based on the possession of similar, non-enveloped capsids and the common circular double-stranded DNA genomes like the polyomaviridae but was later split into two families, namely; *Papillomaviridae* and *Polyomaviridae* after discovering that the two virus groups have different genome sizes, completely different genomic organizations, and no major nucleotide or amino acid sequence similarities.

The revised taxonomy was accepted by the International Committee on the Taxonomy of Viruses (ICTV) more than a decade ago (Bernard *et al*., 2010; de Villiers *et al*., 2004). Papillomaviruses are considered to have closely co-diverged with their mammalian hosts and related PVs are known to infect related host species, therefore, the origin of PVs which infect humans is generally regarded as a result of ancestral primate inheritance (Gottschling *et al*., 2011). Papillomaviruses consist of over 200 distinct types classified in 37 genera. Papillomaviruses may be the biggest and most successful family of vertebrate viruses with clear prospect of an astonishing evolutionary success (Van Doorslaer, 2013). The large group of human papillomaviruses (HPVs) can roughly be sub-divided into two tropism sub-groups, one comprising the types that infect the keratinized surface of the skin, causing common warts,

(so-called cutaneous HPV types) and the other comprising types that infect the mucosa of the mouth, throat, respiratory tract, and especially the anogenital tract (mucosal HPV types). Phylogenetically, cutaneous and mucosal HPVs form distinct trees, reflecting their sequence divergence (Snijders *et al*., 2010; Fernades *et al*., 2013). The classification of PV types is based predominantly on nucleotide sequence, similarities with some biological and medical properties. Papillomavirus isolates are traditionally described as ‘‘types’’. Types were described based on the isolation of complete genomes, with a yet larger number presumed to exist based on the detection of sub-genomic amplicons. Many of these HPV types have been shown to be ubiquitous and globally distributed (Bernard *et al*., 2010; de Villiers *et al*., 2004). Figure 1 below summarises the papillomaviridae family with distinct genera and types.

The phylogenetic tree of PVs consists of different genera, named using various Greek letter prefixes**.** Among these genera, the alpha-papillomavirus genus is of interest in this study, comprising of the Papillomaviruses of human and primates that are of medical importance, which affects the mucosal and cutaneous region resulting in the formation of lesions. More than 200 different human papillomavirus types exists and over one hundred have been formally described, with 18 HPV types classified as ‘‘high-risk’’ mainly associated with cancer (Prado *et al*., 2005).

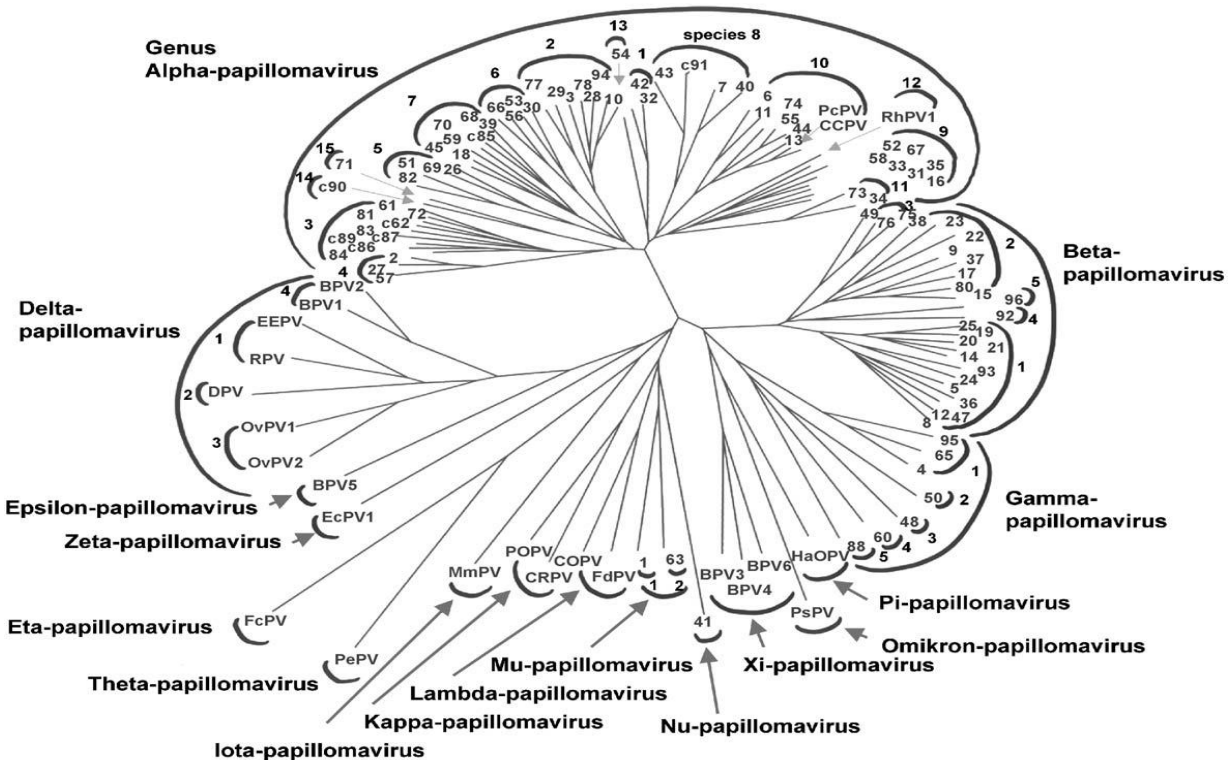

Figure 1: The Phylogenetic tree containing the sequences of 118 papillomavirus types, revealing different genera and types. (Source: de Villiers *et al*., 2004).

Papillomaviruses that infect non-human primates are found within the genera that include HPV, and some HPV are more closely related to non-human primate viruses and the HPV of medical importance are in the alpha genus group. Papillomaviruses have often been classified primarily according to the host species they infect and the sites or diseases with which they are associated. The current classification tries to provide logical designations to cover this wide range of homology: genus, species, type, sub-type and variant. The most diversed category is the genus. PVs are divided into 12 genera, each of which is designated by a letter of the Greek alphabet. Within a given genus, the L1 DNA of all members share more than 60% identity; conversely, they have less than 60% identity with members of other genera. A species is designated for those PV within a given genus that shares 60% to 70% identity. A viral type within a species has 71% to 89% identity with other types within the species. Within a type can exist sub-types, which share 90% to 98% identity, and variants, which have more than 98% identity, whereas relatively few sub-types exists (Bernerd *et al*., 2006; Chen *et al*., 2005).

Most alpha PVs primarily infects genital and non-genital mucosal surfaces and the external genitalia. This group of PV is often referred to collectively as the genital-mucosa types. The types that are associated with cervical cancer are often designated as high risk types (Munoz *et al*., 2003 and Schiffman *et al*., 2005). HPV-16, found most frequently in cervical cancer, is a member of species 9, whereas the next most common cancer-associated type, HPV-18, is a member of species 7. HPV6, which causes most cutaneous genital warts, is a species 10 member. Initially it was assumed that one human papillomavirus was responsible for all human warts, wherever they occurred and whatever their morphology. However in the 1980s, it became clear that there are over 100 different HPV serotypes, with predilections for infecting squamous epithelium at different sites, thereby causing the various types of warts for example, common, palmar, plantar or anogenital (MOH, 2011).

2.3 Human Papillomavirus Genome

Extensive research on HPV genomes shows that it contains approximately eight Open Reading Frames (ORFs) transcribed from a single DNA strand. The ORF are divided into three functional parts: the early (E) region that encodes proteins (E1–E7) vital for viral replication; the late (L) region that encodes the structural proteins (L1–L2) are required for virion assembly and a non-coding part referred to as the long control region (LCR), which contains *cis* elements esential for the replication and transcription of viral DNA. The E and L proteins are transcribed from the early promoter and late promoter respectively (Fehrmann and Laimins, 2003). The E1 and E2 proteins functions as origin replication recognition factors with E2 as the main regulator of viral gene transcription. E4, is associated with late stages of the life cycle and E5 is probably link with early and late phases. E6 and E7 facilitate stable maintenance of viral episomes and stimulate differentiating cells to re-enter the S phase. The L1 and L2 proteins are involved in assemble in capsomeres, during the generation of progeny virions (Fehrmann and Laimins, 2003). The genome of HPV 16 with its constitutive proteins is shown in figure 2.

Deoxyribonucleic Acid sequencing of many PV genomes has led to their phylogenetic organization, according to the comparative homology of the late proteins (L1), Open Reading Frame, which encodes the major structural viral protein and is the most highly conserved PV ORF (de Villiers *et al*., 2004). Similar phylogenetic relationships are also seen when homologies between other regions of the genome are compared, because PVs appear to have arisen primarily via point mutations scattered throughout the genome, rather than via recombination between PV (Chan *et al*., 1995). These similarities are consistent with the conclusion that PV have accompanied their host species during evolution and have evolved with them (Bernerd *et al*., 2006). Although all PV share a similar genetic organization, the L1 DNA sequence identity is just above 40% between the most divergent genomes. On the other hand, two very closely related isolates may differ by only a single nucleotide.

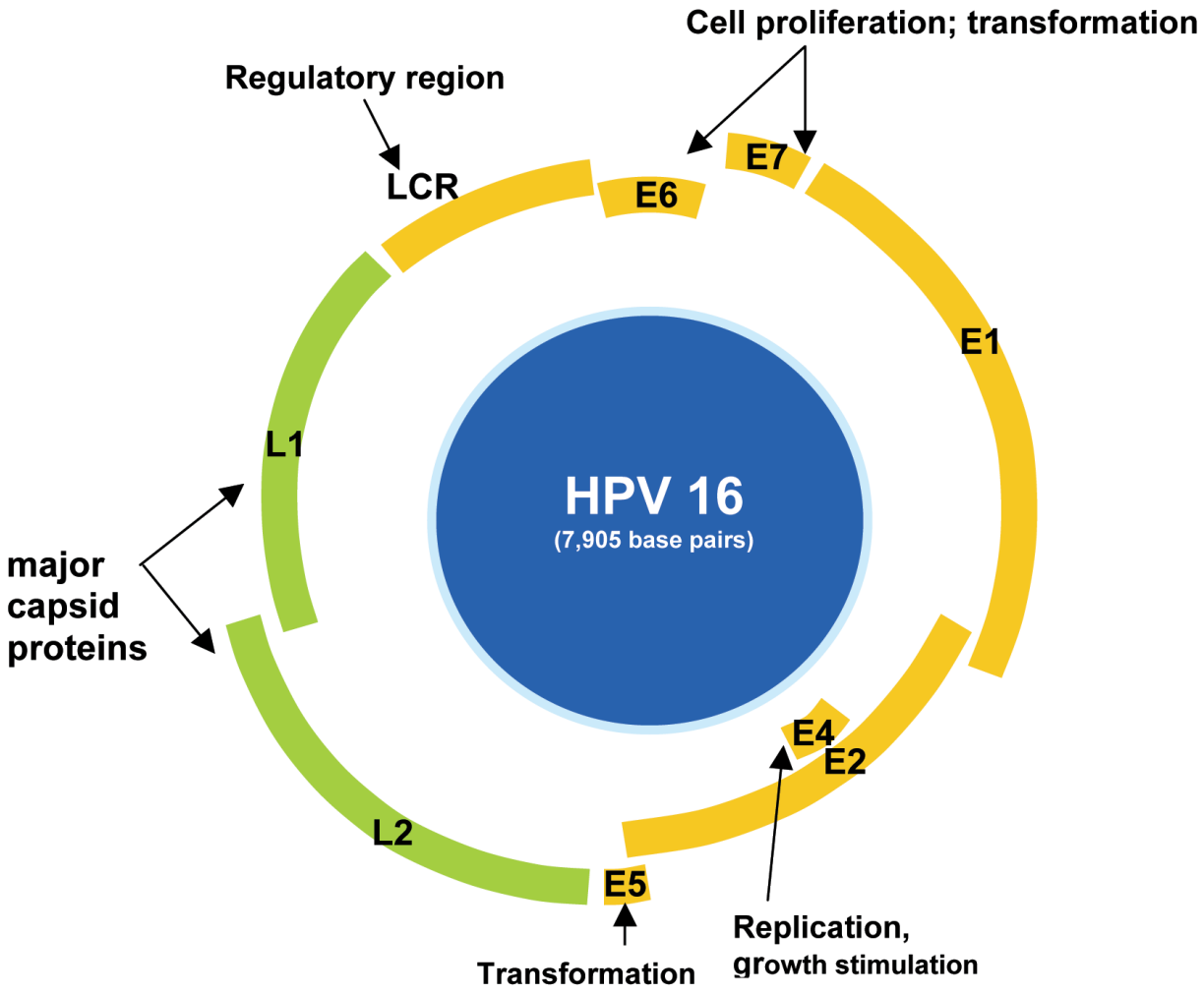

Figure 2: **High risk HPV 16 genome showing different regions of protein. Source: Villa, 2006.**

2.4 Viral Oncoproteins

The oncogenic potential of HPV forms the basis for classification into high and low risk groups. In the HPV genome organisation, the Early and Late genes are numbered according to size in a way that the higher the number, the smaller the corresponding open reading frame (Grce *et al*., 2012; Motoyoma *et al.,* 2004; Munger *et al.,* 2004).

2.4.1 E1 and E2 Proteins

Important proteins needed for the extrachromosomal DNA replication are encoded for by the E1 and E2 genes, these proteins are also neccessary for the completion of the viral life cycle. Secondly, E2 also encodes two proteins for transcription inhibition and increase both at the early region (Ward *et al*., 1989). A major hallmark of HPV-associated cervical carcinoma is loss of the expression of the viral E2 protein. However, other finding has revealed a lack of disruption of the E2 protein in the presence of cervical cancer (Knipe and David, 2007). An additional function of E2 is its ability to greatly stimulate E1 to initiate DNA replication and interacts with it to greatly enhance its ability to bind the replication origin. The early proteins 1 and 2 forms a complex known as the E1-E2 complex, which is a precursor to a larger multi-meric E1 complex, which after the removal of E2, can distort the replication origin and ultimately unwind the DNA. The E2 gene also serves as an auxiliary factor that fosters the assembly of the pre initiation complex at the origin, but itself plays no intrinsic role in viral DNA replication (Knipe and David, 2007).

2.4.2 E5 Protein

The HPV E5 protein is a very small protein of 83 amino acids. A major oncoprotein of bovine papillomavirus, it is a highly hydrophobic protein associated with Golgi apparatus, endoplasmic reticulum and nuclear membrane (Tsai and Chen, 2003). The protein may

contribute to early steps of cancer initiation. It is required for optimal growth due to their stimulatory growth effects. This is shown in the interaction with the 16 kD unit of vacuolar ATPase and this interaction prevents vacuolar acidification, which is supposed to allow better EGF receptor recycling and influence growth factor response and signalling (Knipe and David, 2007). It is worthy of note that E5 expression is generally lost in cervical carcinoma due to HPV integration and therefore, not necessary for maintenance of transformed phenotype but rather display weak transforming activity in vitro (McLaughlin-Drubin and Münger 2009; Venuti *et al*., 2011). Not all HPV genera code for an E5 protein, for example, the beta- HPV genus do not code for E5 unlike the alpha genus. The E5 ORF has shown expression during the early phases of the viral life cycle and refered to as the fourth ORFs on polycistronic transcripts (Venuti *et al*., 2011).

Early Protein 5 interacts with a number of cellular proteins and these interactions are deemed important for the biological activity of the protein in cell transformation and evasion of the immune response which include: reducing the expression of surface Human Leucocyte antigen (HLA) molecules, interacting directly with Bap31 protein which is also involved in vesicular trafficking and Major Histocompatibility Complex (MHC) molecule delivery to the cell surface and the over expression of gangliosides on the cell surface, which prevents immunological recognition by T-cells (Grce *et al*., 2012). The E5 protein has also been found to induce the degradation of Bax and thus has an anti-apoptotic role in viral infection (Oh *et al*., 2010).

2.4.3 E6 Protein

The E6 proteins are approximately 150 amino acids in size and contain four Cys-X-X-Cys motifs, which are presumed to be involved in binding zinc (Knipe and David, 2007). The high risk E6 protein has many functions and one of the widely studied HPV proteins found

interacting with many host cell proteins inspite of its limited size (Grce *et al*., 2012). An important function of E6 protein is inactivation and degradation of the human p53 tumor-suppressor protein (Scheffner *et al*., 1990). In a normal cell, p53 is activated upon DNA damage and either activates pathways leading to repair of damaged DNA or activates apoptosis or senescence (Reinhardt and Schumacher, 2012).

An important regulatory mechanism of the cell cycle occurs in G2-phase, when p53 plays a vital role during DNA replication. Usually, p53 is maintained at low concentrations by the MDM2-mediated degradation but when replication errors or other DNA damages occurs, the Checkpoint Kinases (CHK) CHK1 and CHK2 induce increased p53 activity by phosphorylation of various downstream molecules, including p53 itself. The p53 tetramer acts as a stress-induced transcription factor and induces the expression of p21, which inhibits several cyclin-cyclin dependent kinase (CDK) complexes and hinders the cell cycle, allowing correction of DNA errors or induction of apoptosis if the damage is too extensive (Ghittoni *et al*., 2010).

In a review by Munger and Howley in 2002, for the first time, the transforming properties of E6 were revealed when primary human fibroblasts was efficiently immortalized after the combination of E6 and E7 (Munger *et al*., 1989). The E6 proteins from both low and high risks HPV show similar transcriptional activation properties (Sedman *et al*., 1991). Besides it has been reported that the E6 proteins of the low risk HPV have little or no transformation activity in tested assays. It has become increasingly clear that many small DNA tumor viruses have evolved complex mechanisms to functionally inactivate the tumuor suppressor protein p53 which functions to stop the growth or altogether eliminate cells with ‘abnormal’ growth properties (Munger and Howley, 2002). The first transforming activity identified for the HRHPV E6 proteins was its ability to form complex with p53 (Werness *et al*., 1990), this property is lacking in LRHPV E6 proteins. Expression of the HRHPV E7 proteins

and their binding with pRB raise the levels of p53 within cells which, in turn, transcriptionally activates the expression of p21 genes and pro-apoptotic genes that can arrest the cell cycle or induce cell death (Jones *et al*., 1997). The half-life of p53 is dramatically decreased in E6-expressing cells, and E6 prevents the increase in p53 levels when cells are challenged with genotoxic agents (Hubbert *et al*; Scheffner *et al*., 1991). E6 also induces genomic instability, this is evidenced by the development of translocations and aneuploidy in culture (Reznikoff *et al*., 1994; White *et al*., 1994), maintenance of stable episomal replication during the viral life cycle (Park and Androphy 2002), and human mammary epithelial cells immortalization (Shamanin and Androphy, 2004). Apart from E6-p53 model, E6 has shown interactions with other proteins as; hADA3, GPS2, p150 (Sal-2), CBP and p300 all of which act on p53 expression or help in expression of p53 downstream targets (Kumar *et al*., 2002; Patel *et al*., 1999; Parroche *et al* 2011; Degenhardt and Silverstein 2001). Each of these protein-protein actions induces different effects in the HPV infected cell through either signal transduction or replication and transcription modulation (Grce *et al.*, 2012).

Furthermore, the E6 interferes with different apoptotic pathways by additional interactions with BAK, procaspase- 8, TIP60, DFF40, FADD,GADD34 and TNFR-1 proteins (Thomas and Banks 1998; Filippova *et al*., 2007; Filippova *et al*., 2004; Jong *et al*., 2012 ; Kazemi *et al*., 2004; Filippova *et al*., 2002; Jhas *et al*., 2010) and it also appears that E6 have some role in immune evasion as it interacts with IRF-3 and tyk-2 proteins both involved in interferon signalling (Lis *et al*., 1999; Ronco *et al*.,1998*).*

2.4.4 E7 Protein

The E7 protein is a small nuclear protein of about 100 amino acids; it binds zinc and is phosphorylated by casein kinase II (CK II) (Munger *et al*., 1992). Also, in HPV-16 E7 oncoprotein the amino acid shares sequence similarity with portions of the AdE1A proteins and

the SV40 Tag in a conserved regions crucial for the transforming activities in the three viral oncoproteins, shown to participate in the binding of a number of important cellular regulatory proteins, including the retinoblastoma tumor-suppressor gene pRB, and its pocket proteins p107 and p130 (Decaprio *et al*., 1988; Dyson *et al*., 1989; White *et al*., 1988).

The retinoblastoma protein (pRB) a member of a family of cellular protein which includes p107 and p130, homologous in their binding pockets for E7, AdE1A, and SV40 TAg. Its phosphorylation state is regulated through the cell cycle, being hypophosphorylated in G_0 and G_1 and phosphorylated during S, G_2, and M. The pRB becomes phosphorylated at multiple serine residues by cyclin-dependent kinases (CDKs) notably cyclin D1-CDK4 and cyclin D1-CDK6 complexes at the G1/S boundary and remains phosphorylated until late M, when it becomes hypophosphorylated again through the action of a specific phosphatase. HPV-16 E7, binds preferentially to the hypophosphorylated form of pRB, resulting in the functional inactivation of pRB through the release of transcription factors (E2F), thereby permitting progression of the cell into S-phase of the cell cycle (Boyer *et al*., 1996; Jones *et al*., 1997). Genetic studies indicate that complex formation between E7 and the pocket proteins, including pRB, is not sufficient to account for its immortalization and transforming functions, suggesting that likely additional cellular targets of E7 exist that are relevant to cellular transformation (Jewers *et al*., 1992). Other cellular targets of E7 includes; CDK inhibitors, $p27^{kip1}$, $p21^{cip1}$ and abrogates its inhibition of CDK as well as its inhibition of proliferating cell nuclear antigen (PCNA)-dependent DNA replication (Funk *et al*., 1997; Jones *et al*., 1997). E7 is known for its stable maintenance of HPV episomes in epithelial cells (Flores *et al*., 2000; Thomas *et al*., 1999), and some sequences in E7 are important for the functions in viral life cycle and cellular transformation (Longsworth and Laimins 2004; Thomas *et al*., 1999). Hence, the ability of E7 proteins to induce DNA replication through the release of E2F transcription factor complexes

and the inactivation of $p21^{CIP1}$ (Funk *et al*., 1997; Jones *et al*., 1997) and $p27^{KIP1}$ (Zerfass-Thomee *et al*., 1996) are essential components of the HPV replication strategy.

The HRHPV E7 proteins cause genomic instability in normal human cells, more specifically, HPV-16 E7 induces G1/S and mitotic cell cycle checkpoint defects and uncouples synthesis of centrosomes from the cell division cycle causing formation of abnormal multipolar mitoses resulting in chromosome missegregation and aneuploidy (White *et al*., 1994; Munger *et al*., 2004; Duesing *et al*., 2000). The two most important interactions of HRHPV E6 and E7 oncoproteins leading to cell transformation and immortalisation are the binding and degradation of the major tumor-suppressors p53 and pRb, respectively. In addition to many other protein-protein interactions of E6 and E7 oncoproteins, HPV was also found to modulate several important cell signalling pathways such as: mTORC signalling (Spangle and Munger, 2010) WNT signalling (Rampias *et al*., 2010), TGF-beta signalling (Iancu *et al*., 2010), HER/PTEN/Akt pathway (Stankiewicz *et al*., 2011), ERK-MAP pathway (Branca *et al*., 2004), ATM DNA Damage pathway (Moody and Laimins 2009) NOTCH-1 signalling (Talora *et al*., 2002), TGF-b signalling, calcium signalling, MAPK signalling (Perez-Plasensia *et al*., 2007) and insulin signalling (Lu *et al*., 2004).

2.5 Human Papillomavirus Integration

Integration is an important replication step for all retroviruses which requires an enzyme known as viral integrase. Human Papillomavirus DNA usually exists in episomal (extrachromosomal) in benign lesions, while in many cervical cancer cells or cell lines and HPV-transformed human keratinocytes in vitro, HPV DNA is integrated in the host genome. The virus can also exist in a mixed (episomal and integrated) form (Motoyoma *et al*., 2004). In normal HPV lifecycle, integration is unusual. This is different from retroviruses that encode integrase, thereby facilitating their integration into the host genome (Raybould *et al*., 2011).

Cervical carcinogenesis usually proceeds in two major steps namely; integration of HRHPV and "acquisition of an unstable host genome" (Pett *et al*., 2004). Reliable description of HRHPV integration into the human genome has been accepted as one of the crucial stages in malignant progression and serves as a potential biomarker that precedes invasive disease (Raybould *et al*., 2011). It is believed that DNA damage and repair pathways induce HPV integration since HPV is detected at fragile sites in the human genome (Raybould, 2013). Integration of HRHPV into host genome was once considered a late event in cervical carcinogenesis but recent report has challenged previous findings and revealed that it occurs early (Collins *et al*., 2009). Integration of HPV has, until recently, been a frequent but late event in cervical carcinogenesis. The temporal view has, however, been challenged lately as integrated forms of HPV have been isolated from normal and pre-neoplastic lesions (Otelinda, 2009). Many studies using HPV16 as a model have revealed that after viral integration, variable parts of the HPV genome are disrupted; for example, E2, E4 and E5 fragments of the ORFs are missing whereas the entire E1, E6 and E7 ORFs are integrated and retained (Raybould *et al*., 2011; Theelan *et al*., 2013). A study by Xue and colleagues (2012), reported that E2 ORF distruption and drastic transcripts reduction does not always follow viral DNA integration of the cellular genome. Human papillomavirus integration is yet to be fully understood, but believed to be supported by hypotheses such as; DNA instability, site of integration and transcriptional regulation of integrants.

2.6 Human Papillomavirus and Cancer

It was the pioneering work of Zur Hausen (a retiree of the German centre for cancer research and a virologist) who showed that papilloma virus is the most significant cause of cervical cancer, and beyond all odds maintained his position on the role of viruses in cancers (McIntyre, 2005). There were initial claim that Herpes Simplex Virus (HSV) infection was the

culprit in cervical cancer disease, this was the the accepted hypothesis that presumed that herpes simplex type 2 virus (HSV-2) was the tumour aetiology of cancer of the cervix and was experimentally supported (Rawls *et al*., 1969, Frenkel *et al*., 1972). However, Hausen consistently failed to find HSV-2 DNA in cervical cancer cells when applying the in situ hybridization technique at the laboratory of the Institute of Virology Würzburg Germany where he became increasingly sceptical about claims that cervical cancer, which was clearly sexually transmitted, was caused by the HSV. His hypothesis was that if tumour cells in man were transformed by a virus, then viral genetic information integration into the host genome must have occured. He was the first to connect HPV and cervical cancer over 30 years ago and since then, the isolation of type specific HPV DNA follows almost all cervical cancer biopsies. Zur Hausen's discoveries include; "detection of novel HPV types, isolation of types 16 and 18 in genomes, and expression of specific papilloma virus DNA genes integrated into the host cell genome". These findings have led to an understanding of cervical carcinogenesis, a characterization of the natural history of the human papilloma virus infection, and paved the way for the development of prophylactic vaccines (Karolinska Institute, 2014).

2.7 Cervical Cancer and Human Papillomavirus Vaccine

Shope's work on 'immunization of rabbits to infectious papillomatosis' inspired several other workers on researching on the possibilities of reliable preventive vaccine against papillomavirus (Shope, 1937).

High incidence of cervical cancer exists in developing countries; The Caribbean and Latin America contribute about 83.9% of the cases and 81.2% of deaths for the total estimated cases. Persistent HRHPV infection is the biological cause of cervical cancer (Bosch and de Sanjose 2004; Ferlay *et al.,* 2004; Lewis, 2004; Stanley, 2010). A vaccine study group sponsored by GlaxoSmithKline reported a hundred percent efficacy in their trial against two

cervical cancer related HRHPV 16 and 18 in 2004 and concluded that "our data provide compelling evidence that the HPV 16 and 18 vaccine is highly -"efficacious" and "appeared to be safe and well tolerated". Also, at the 22nd International Papillomavirus Conference in Vancouver, in June 2005 a team from Merck Phermaceutical company reported that their vaccine had shown 90% protection against HPV 6, 11, 16 and 18 (Mclintre, 2005).

Vaccines against HPV are the the first group of vaccines developed to prevent cancers of viral origin (Baden *et al.,* 2007); they stimulate development of immune response preventing persistent infection and eventually genital cancer. Two classes of vaccines are currently available; prophylactic and therapeutic. Existing prophylactic vaccines are bivalent (Cervarix® and produced by GlaxoSmithKline) or quadrivalent (Gadarsil® and produced by Merck). Cervarix® confers protection against HRHPV 16 and 18, while Gadarsil® protects against types 6, 11, 16 and 18. Both vaccines work by inducing Immunoglobulin G (IgG) and L1 Virus-Like Particles (VLPs) are capable of inducing high levels of neutralising IgG. Available data shows that VLPs effectively prevent papillomavirus infections with a high level of safety (Denny, 2009; de Vinzenzo *et al.,* 2013). Besides, specific therapeutic vaccines aimed at established HPV infections and HPV-related disease are being developed (Hung *et al*., 2008, Bello *et al.,* 2011).

Significant progress has been made in high income countries where HPV vaccination programmes has been implemented; licensure and approval obtained in over 100 countries with 28 high resource nations with HPV vaccines included in their national immunization schedule (Bello *et al*., 2011). In contrast, the picture is different for developing countries like Nigeria where funding and cost of vaccine, religious and cultural mis-conceptions, government policies, education and communication difficulties have hindered vaccine administration, thus sustaining the burden of cancer in the region.

2.8 Human Papillomavirus Global Burden

The prevalence and type distribution of HPV related cervical infection varies greatly worldwide (Tornesello, 2007, Nweke, 2013). About 25–40% of women aged between 15–25 years have evidence of HPV infection (Sankaranarayanan, 2009). There are an estimated 400 million cases of genital HPV infection worldwide. About 311,000 women die of cervical related cancer each year with 570,000 new diagnosis every year, out of which 85% occur in resource poor countries (WHO, 2019).

Studies have shown a 10-fold variation of HPV infection between some areas in Spain and North Vietnam (Anh *et al*., 2003; de Sanjose´ *et al.*, 2003), where less than 2% prevalence was recorded among sexually active women aged 15–65 years. In Colombia (Molano *et al*., 2002) and Argentina (Matos *et al*., 2003), it was 15% or greater. Also, prevalence of HRHPV types in middle-aged women and the incidence of cervical cancer were strongly positively correlated (Franceschi *et al*., 2003). In a review by Forman and colleagues in 2012, global HPV prevalence was highest in Sub-saharan Africa. Women in Africa are relatively less likely to be infected with HPV 16 and relatively more likely to be infected with LRHPV types and high-risk types other than HPV 16.

2.8.1 Human Papillomavirus Burden in Developing Countries

Studies in Ibadan and Ile-Ife in Nigeria reported that HRHPV types, 16, 35 and 53 were most prevalent (Clifford *et al*., 2005, Thomas *et al*., 2004, Fadahunsi *et al.*, 2013). A similar study carried out in Benin Republic revealed that HPV-59 and HPV-35 were the commonest types, followed by other high-risk genotypes such as HPV-16, 18, 58, 45, 56, and low risk genotypes such as HPV-42, 6 and 11 (Piras *et al*., 2011). This shows that regional variation exist even in the West Africa sub-region. At present, more intensive studies on HPV are concentrated in high income countries where the burden of the disease is low, health facilities

are sophisticated and government willingness and people's acceptance are impressive. Priority areas for study are sub-Saharan Africa, Eastern Europe, and areas where social changes and or urbanization may increase HPV infection among young generations especially in China, Mongolia, and Turkey (WHO, 2005).

Estimates also show that more than 20% of women in Nigeria have cervical HPV infection at any given time (WHO/ICO, 2010). Cervical cancer in Nigeria ranks as the 2nd most frequent cancer among women ages 15 and 44 years and 51.4% of invasive cervical cancers are due to HPVs 16 or 18 (ICO, 2014). Although, a prevalence study in Abuja, Nigeria by Akorol-Anthony *et al.* in 2014, found out that HPV 35 was the most prevalent followed by HPV 56. On the contrary, HPV 16 and 18 were low in the population studied. This observation are likely to make the present vaccine intervention challenging.

2.9 Human Papillomavirus Detection

Considering the widespread transmission of HPV, the importance of HPV DNA detection and the use of related biomarkers, cannot be over emphasised. HPV testing for primary screening and triage is presently of widespread interest (Cuzick *et al*., 2013). There are no conventional cell cultures for HPV so the virus relies on a variety of immunological and molecular biology techniques. For accurate identification of HPV infection, diagnosis is done using molecular methods based on the detection of viral DNA in tissue biopsies or exfoliated cells at the site of infection, because HPV detection in serological assays have only limited accuracy such as; the late capsid proteins are only expressed in productive infections, the often expression of the E proteins in small amounts in infected tissues and the absence of sensitive and specific high quality antibodies against the viral proteins (Villa and Denny, 2006; Sahiner *et al.*, 2013).

Besides, most HPV detection assays rely on the assessment of viral nucleic acids, mostly DNA. There are currently two broad types subdivided into target amplification methods (nucleic acids amplification and nucleic acids hybridization) and signal amplification methods shown in figure 3 (Snidjers *et al.*, 2010**;** Abreu *et al*., 2012).

2.9.1 Signal-amplification Assays

The two approved signal amplification assays are; Digene® HPV test using Hybrid Capture® 2 (hc2) technology manufactured in USA by Digene Corporation and Cervista® HPV HR assay. The Hybrid Capture® 2 system is a non-radioactive signal amplification method that works on the hybridization of the target HPV-DNA to labelled RNA probes in solution (Bozetti *et al*., 2000). The assay detects 13 HRHPV types (-16,-18,-31,-33,-35,-39,-45,-51,-52,- 56,-58,-59 and -68) and 5 low risk types (-6, -11, -42, -43, and -44) it also distinguishes between HR and LR groups, but unable to genotype single HPV (Hwang and Shroyer, 2012). This is a notable limitation of the assay due to the fact that HPV genotyping in identifying single oncogenic HPV types is very important (Wright and Schiffmann, 2003) and to provide more information regarding risk stratification, persistence of infection and global variation in HRHPV distribution (Piras *et al.,* 2011; Frazer *et al*., 2011; Otero-Motta *et al.*, 2011; Forman *et al.*, 2012). On the other hand, Cervista® HPV (Hologic, Inc., Marlborough, MA, USA) assay detects the presence of 14 HRHPV types, consisting of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This assay also utilizes a signal-amplification method for the detection of specific nucleic acids which gives it an edge over the hc2 assay (Abreu *et al*., 2012).

2.9.2 Nucleic Acids Hybridization Assays

This method include: Southern blotting, in situ hybridization, and dot-blot hybridization which initially used radio-labelled nucleic acid hybridization assays. Although these techniques generated high quality information, the shortcoming of these direct-probe approaches include; low sensitivity, requires large amounts of purified DNA and the procedures are time-consuming (Villa and Deny 2006).

2.9.3 Nucleic Acids Amplification Assays

The nucleic acid amplification assays includes: Polymerase Chain Reaction (PCR), PapilloCheck®, PCR-RFLP (Restriction Fragment Length Polymorphism), Real time PCR, Abbott-Real-Time PCR, COBAS® 4800 HPV test, Microarray analysis, The Linear array®, HPV genome sequencing, CLART® human papillomavirus 2, INNO-LiPA, Clinical arrays® HPV, Microplate colorimetric hybridization assay (MCHA), and many more. Rebolj *et al* (2014) compared several HPV assays in primary screening and demonstrated substantial dis-agreement between the assays, particularly in primary screening of women aged 30 to 65 years. The benefits of nucleic acids amplification assays are: they have very high sensitivity, flexible and can permit multiplex analysis. The dis-advantages of this assay include: lower amplification signals of some HPV genotypes, contamination with previously amplified material which may result in false positives (Abreu *et al*., 2012). Most nucleic acid amplification assays use the PCR methods in Genotyping.

2.9.4 Polymerase Chain Reaction

Polymerase chain reaction was developed by Kary Mullis in the 1980s. The principle is based on DNA polymerase ability to synthesise new strands of DNA complementary to the template strand of interest (NCBI, 2015). This method requires a primer to which it can first

add the first nucleotide. This requirement makes it possible to target a specific region of template sequence of interest for amplification (NCBI, 2015). The detection of HPV by PCR requires specific primers designed for the conserved region of the viral genome. In most of the PCR-based HPV detection systems, a broad spectrum of HPV types is amplified by consensus primers, followed by detection with type-specific primers or probes. The consensus primers may be degenerate as in the MY09/11 (Gravitt *et al*., 1998) and CPI/II systems. Alternatively, they may contain mismatches that are accepted under low-stringency PCR conditions as in the GP5+/6+ system, they may contain inosine residues at ambiguous base positions such as in the IU/IWDO and SPF primers, or sets of overlapping primers as is the case in the PGMY and Amplicor systems (Brink *et al*., 2007).

The use of consensus primers is followed by specific typing, which can be done by using specific type primers in a multiplex or cocktail fashion. Traditionally, typing was done in most cases using reverse hybridization or Southern blotting or dot blotting technique, dot with type-specific oligonucleotides. Identification of specific HPV types in a biological specimen is preferentially done by PCR-based methods (Clifford *et al*., 2006). The figure below summarises the classification of different HPV tests.

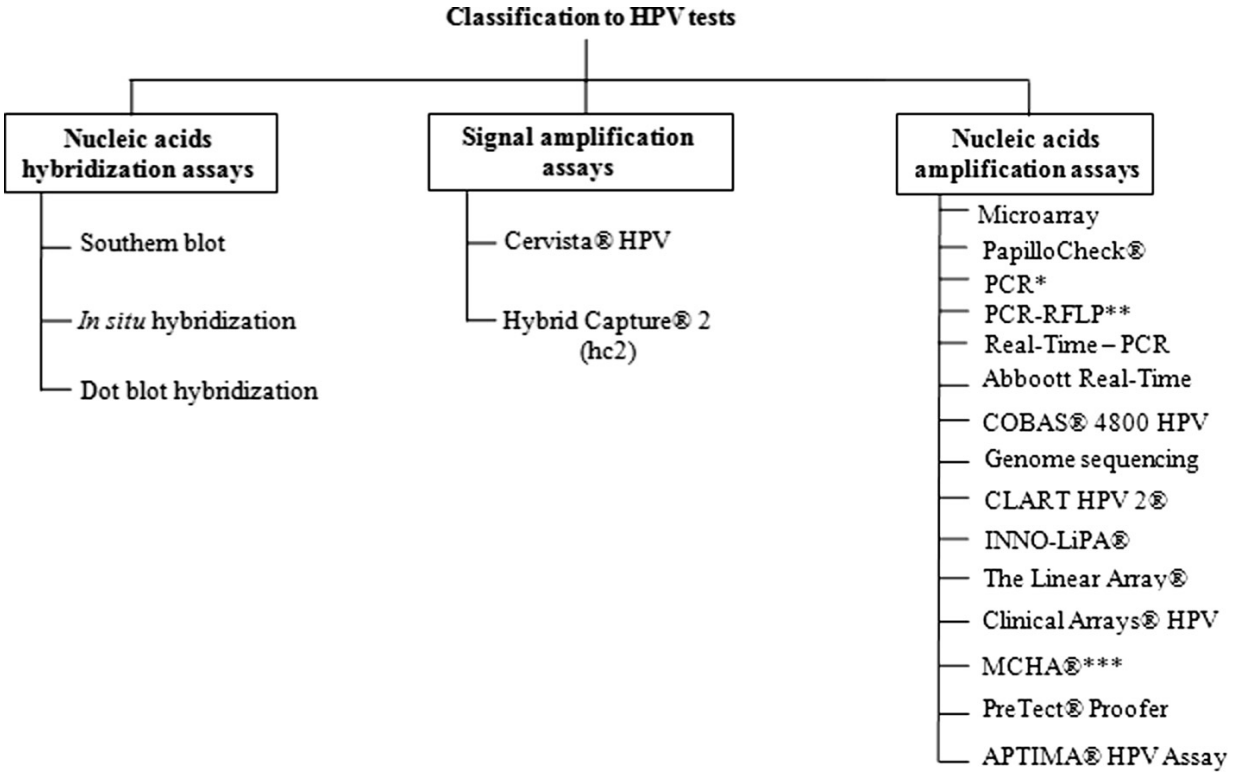

Figure 3: Schematic diagram of Classification for HPV tests, Source: Abreu *et al.*, 2012.

2.10 Chlamydia Biology

Chlamydia belongs to the order *Chlamydiales* which have been grouped into two genera namely; genus *Chlamydia* and genus *Chlamydophila* and nine species based on differences in phenotype 16S ribosomal Ribonucleic acid (rRNA) and 23S rRNA (Forbes *et al*., 2007). The genus *Chlamydia* consists: *C. trachomatis*, *C. suis* (affects only swine) and *C. muridarum* (affects only mice and hamsters). In addition, the genus *Chlamydophila* consist of six species which are; *C. pneumoniae, C. psittaci, C. abortus, C. pecorum C. felis* and *C. caviae*. However, *Chlamydiae* that infect humans are divided into three species namely; *Chlamydia trachomatis, Chlamydophila (Chlamydia) pneumoniae,* and *Chlamydophila (Chlamydia) psittaci* based on antigenic composition, intracellular inclusions, sulfonamide susceptibility, and disease production. A fourth species, *Chlamydia pecorum,* infects a variety of animals but is not known to infect humans (Brooks *et al.,* 2007).

All *Chlamydiae* are characterised by similar morphologic features, a common group antigen, and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The development cycle is unique and biphasic in which the organism exists in two distinctive forms (figure 5). They have an intra-cellular growth the replicative form called the reticulate body (RB), and an extra-cellular metabolically inert, ineffective form called the elementary body (EB) (Forbes *et al*., 2007). Once inside the host, the intracellular infectious cycle is repeated as bacteria pass from infected cell to neighbouring cell, metabolically inert, and frequently disguised in host derived material (Nancy *et al.,* 1989). Apart from the replicative cycle that is associated with acute chlamydial infection, multiple lines of evidence indicate that *Chlamydia* can also persist. It is believed that following an un-resolved infection, *Chlamydia* may persist in a viable but non-cultivable growth stage called a persistence body (PB) that result in a long-term stable relationship with the infected host cell commonly referred to as chronic infection (Byrne and Ojcius, 2004; Hogan *et al.,* 2004).

The *Chlamydiae* are gram-negative bacteria that lack mechanisms for metabolic energy production and cannot synthesize adenosine triphosphate (ATP), but have adopted specialised mechanisms to bring about satisfaction of their metabolic deficiencies. This defect restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. These properties earn them the name; obligate intracellular parasites**.** Chlamydia infections are the commonest bacterial Sexually Transmitted Infections (STI) in humans and with the highest cause of infectious blindness in children worldwide (Nancy *et al.*, 1989; Ryan and Ray, 2004; Brooks *et al.*, 2007). The fate of the bacteria is tissue destruction, followed by pathological changes, this usually occurs well before the host is immunologically aware of the invader.

2.11 Classification of Chlamydia

Chlamydiae were originally regarded as protozoa, or viruses, but were later found to posses properties that distinguished them as bacteria (Sarov and Becker, 1971). It was confirmed using rRNA analysis that Chlamydia was in fact eubacteria but with very little relatedness to other eubacterial orders (Weisburg *et al.*, 1986). The genus *Chlamydia* presently consists of four species, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae* and *Chlamydia pecorum*. Deoxy ribonucleic acid (DNA) homology between chlamydial species is reported to be less than 10% (Kingsbury and Weiss, 1968; Campbell *et al*., 1987; Fukushi *et al*., 1992), although structurally and biochemically they appear to be similar, with amino acid homology between analogous proteins being high.

Medically important Chlamydiae include; *C. trachomatis*, *C. pneumonia* and *C. psittaci*, Chlamydiae are arranged according to their pathogenic potential, host range, antigenic differences, and other methods. The three species that infect humans have been characterized in the table below.

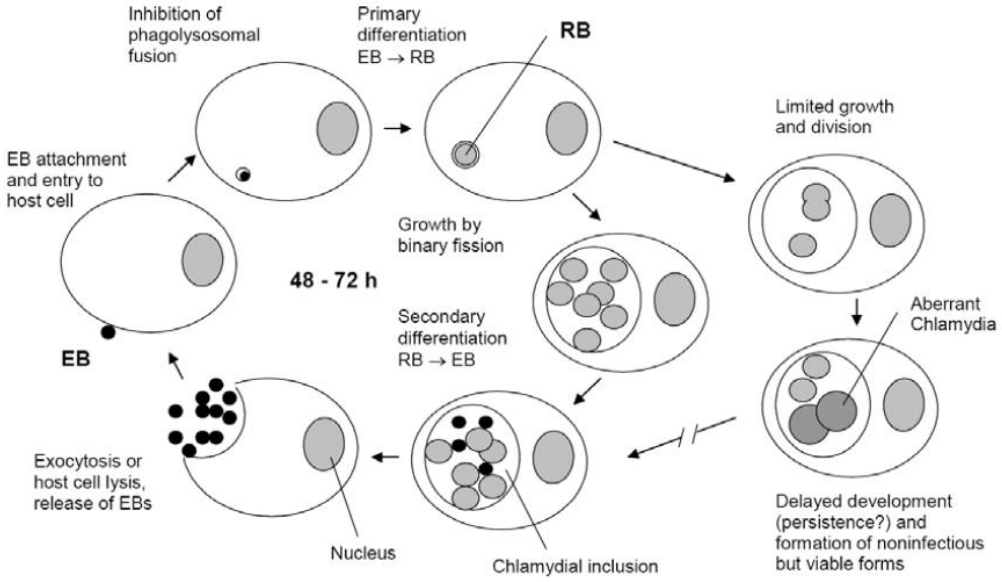

Figure 4: ***Chlamydial*** **developmental cycle, Source: Karinel, 2006.**

Table 1: The characteristics of medically important Chlamydia, Source: modified from Choroszy-Król *et al.* , 2012.

Species	*C. trachomatis*			*C. pneumoniae*	*C. psittaci*	*C. pecorum*
Biotype Serotype	Trachoma A, B, Ba, C, D, Da, E, F, G, H, I, Ia, J, K	LGV L1, L2, L2a L3	Mouse lack	TWAR TW-183,AR-37, AR-277, AR-388, AR-427, AR-231, LR-65	1, 2, 3–9 probably 1 i 2	no data no data
Natural host	Human			Human	animal	animal
Infection route	Contact			Inhalation	inhalation	inhalation
Place of infection	conjunctival and genitourinary epithelial cells			respiratory epithelium	respiratory epithelium	respiratory epithelium
Diseases	trachoma, venereal granuloma, infection of the urogenital system, inclusion conjunctivitis, reactive arthritis, conjunctivitis and pneumonia in children			Pneumonia	zoonoses	zoonoses
Number of serotypes	18			1	numerous	3
LPS antigen	(+)			(+)	(+)	(+)
EB shape	Round			Round	Pear-shaped	Pear-shaped
Antibiotic sensitivity	(+)			(–)	(–)	(–)

EB = Elementary Body, LPS = LipoPolySachararide, (+) = Present (-) = Absent

2.12 *Chlamydia trachomatis*

Chlamydia trachomatis is the most common bacterial STI worldwide (Walker *et al.*, 2012). *Chlamydia trachomatis* infection in 70 to 75% women is asymptomatic. Infection, can be transmitted during vaginal, oral, or anal sexual contact, and can be passed from the mother to her new-born during delivery, with subsequent risk of neonatal eye infection or pneumonia (Franceshi *et al*., 2007). The first description of CT inclusion bodies was in 1942, and Professor Feifan was the first to culture the organism in the yolk sacs of eggs (Tang *et al.*, 1957, 1958 and Darougar *et al.*, 1972). These bacteria infect man almost exclusively and cause various clinical syndromes (Forbes *et al*., 2007). Pelvic inflammatory disease (PID), a serious complication of CT infection, is a major cause of infertility in women *C. trachomatis* is sub-divided into three human biovars namely: serovars Ab, B, Ba, or C which causes trachoma (an infection of the eyes, which can lead to blindness), serovars D-K which cause urogenital tract disease (urethritis, PID, ectopic pregnancy, neonatal pneumonia, and neonatal conjunctivitis) and serovars L1, L2 and L3 which cause lymphogranuloma venereum (LGV) (Frendlund *et al*., 2004). Many CT strains have an extrachromosomal plasmid (Carlson *et al*., 2008). Chlamydia can exchange DNA between its different strains, thus the evolution of new strains is common (Harris *et al*., 2012).

2.13 Burden of *Chlamydia trachomatis*

Sexually transmitted infections (STIs) have a major impact on sexual and reproductive health worldwide. Although many pathogens are connected with STI with eight clearly linked to the greatest amount of morbidity. Three bacterial STIs implicated are currently curable namely; *Chlamydia trachomatis* (chlamydia), *Neisseria gonorrhoeae* (gonorrhea), and *Treponema pallidum* (syphilis), and one parasitic STI, *Trichomonas vaginalis* (trichomoniasis), are currently curable (Gottlieb *et al*., 2014). Among all the STI caused by bacteria, *Chlamydia*

trachomatis ranks the most common bacterial STI worldwide (Bilardi *et al*., 2010). The worldwide prevalence of CT among women age 15-49 years is between 3.7-4.7% with a total estimate of more than 100 million new cases globally. From these cases, the global regional incidences are: Africa- 8.3 million, Americas- 26.4 million, South East Asia- 7.2 million, Europe- 20.6 million, Eastern Mediterian Region- 3.2 million and Western Pacific Region- 40 million (Newman *et al*; 2015; WHO, 2012).

Chlamydia infections are usually latent with no clear-cut symptoms therefore diagnosis is mostly not done and treatment opportunity are missed, hence most infections caused by Chlamydia are usually mistaken for other STI (Okoror *et al*., 2007). Previous reports on the relative frequencies of CT infections in developing countries like Nigeria are sparse and infection could be higher in developing countries than expected (Esumeh *et al*., 2009). An Ibadan study on CT prevalence among women attending Sexually Transmitted Disease (STD) clinic was 26.7% (Darouga *et al.*, 1982). Also, the overall prevalence of CT antigen positivity in Abeokuta was 9.8%, (Ogiogwa *et al*., 2012). While in Benin City it was 47%, using indirect haemagglutinating (IHA) chlamydial antibodies (Azenabor and Eghafona 1997).

Furthermore, in an Owerri study by Enwuru and Umeh (2014) that determined the presence of CT among asymptomatic young males and females. The test was performed by detecting CT IgG in the serum of the patients using solid phase Enzyme Immuno Assay. The prevalence of CT among the studied population was 6.7%, while females had a prevalence rate of 8.7%. Furthermore, a study carried out in Kaduna and Kano found out a seroprevalence of 28% and 9.6% respectively (Ella *et al*., 2013; Nwankwo and Sadiq, 2014). In a recent study in Kaduna, Nigeria among sexually active female volunteers, a prevalence of 26% was obtained using PCR technique (Ige *et al.*, 2018). While an earlier similar study in Lagos revealed a prevalence of 27.7% (Adegbesan-Omilabu *et al*., 2014).

The prevalence rates of CT in Nigeria varies with several studies showing equally high prevalence in women of reproductive age (Darouga *et al.*, 1982; Amin *et al*., 1997), antenatal women (Aladesanmi *et al*., 1988) and individuals with STI in Nigeria (Darouga *et al.*, 1982; Tukur *et al*., 2006; Azenabor and Eghafona 1997; Omo-Agboja *et al*., 2007). Besides, owing to dynamic population characteristics and methods used for *Chlamydia* detection, wide variation in prevalence of *Chlamydia* infection exists as reported by Verkoyeen *et al*., (2002).

In many parts of Nigeria, CT is not routinely screened for, hence relative information about infection frequencies are based on laboratory reports and research findings (Adesiji *et al*., 2015). Apart from the work of Aboyeji and Nwabuisi in 2003 on STD prevalence among pregnant women in Ilorin which excluded *C. trachomatis*, literature search did not reveal any data on CT prevalence in Ilorin, Nigeria.

2.14 Diagnosis of *Chlamydia trachomatis*

Chlamydia species are readily identified and distinguished from other *Chlamydia* species using various tests. Traditionally, cell culture is used for sample suspected for Chlamydia. Culture method employing McCoy cells and a flourescein-conjugated monoclonal antibody for identification of intracellular inclusion was regarded as the gold standard. The demerit of the technique is that it is expensive and takes a longer time than other Chlamydia tests. Also, culture cannot be done rapidly in the field except in a laboratory. However, this technique has 100% specificity (Bauwens *et al*., 1993; Chernesky, 1999, 2005; Monif, 1998). Strains of CT are recognized by monoclonal antibodies (mAbs) to epitopes in the VS4 region of the major outer membrane protein (MOMP), (Ortiz *et al*., 2000). However, a major challenge with these mAbs is their cross-reacting ability with two other *Chlamydia* species, *C. suis* and *C. muridarum*. Enzyme-linked immunosorbent assay (ELISA, EIA) rapidly detects Chlamydia antigens by triggerring the immune system against *Chlamydia* infection. Direct fluorescent

antibody test (DFA) is another rapid test for *Chlamydia* antigens (Chernesky, 2005, 1999). The detection of Chlamydia DNA can be done using a probe test and Nucleic Acid Amplification Tests (NAAT). The probe test is a specific test less sensitive than Nucleic Acid Amplification Tests (NAAT). The NAAT detects the DNA of *Chlamydia* bacteria. These tests are very accurate and are unlikely to have false-negative results. PCR and Ligase Chain Reaction tests are examples of NAAT which are primarily suitable in symptomatic or high-risk, high prevalence asymptomatic populations and are now the new gold standard (Monif, 1998).

2.15 Burden of Chlamydia and Human Papillomavirus Co-Infection

Continuos infection with HRHPV types has been confirmed as a necessary cause of cervical cancer; however, sole infection with HPV is not sufficient to cause cervical cancer (Castellsague and Munoz, 2003; Koutsky *et al*., 1992). Other STIs such as CT and *Neisseria gonorrhea* have shown possibilities of increasing the risk of cervical cancer in women with HRHPV infection (Nobbenhuis *et al*., 1999).

Bacteria co-infection of CT in women with history of HPV infections is a potential factor that contributes to malignancies development and cervical cancer. There has been suggestions that previous CT infection is associated with a high risk of coming down with the disease (Calil *et al*., 2011).

Reports have revealed that genital Chlamydial infections influence the development of HPV-induced adenocarcinoma (Madeleine *et al*., 2007). Although controversies still exists on CT as a co-factor for HPV in cervical carcinogenesis. Some investigators proposed that CT infection affects acquisition of HPV and persistence, accelerate transformation to early precursor lesions, and increase likelihood of precursor lesions to cervical cancer (Miller and Em, 2011).

Besides, there have been mixed finding on CT and HPV in cervical carcinogenesis. In a study, CT has been reported to increase the risk of developing squamous cell carcinoma of the cervix (Madeleine *et al*., 2006; Smith *et al*., 2004). This association was demonstrated by PCR (Smith *et al*., 2004) as well as antibody titres (Verteramol *et al*., 2009) as measures of CT infection. Several studies have revealed higher prevalence of CT infection in HPV positive women when compared to HPV negative women (Gopalkrishna *et al*., 2000; Lehmann *et al*., 1999). However, other report did not find significant correlation in CT, HPV and CIN (Bhatla *et al*., 2013), and most data on HPV and CT association is from developed countries while fewer studies are from India and Brazil. In Nigeria, there are presently no published molecular studies on HPV and CT co-infection.

2.16 Immune Response and Cancer

A suggestion of an adoption of a new theory describing immune response and tumorigenesis is called immunoediting made by Dunn and his colleagues (Dunn *et al*., 2004). The theory, with three distinct stages are; elimination- a period in which the immune system successfully destroys precancerous and cancerous cells. Equilibrium-commemcement of evasion of cancer cells. Ecape-the cancer cells manage to successfully evade the surveillance system of the organism, resulting in aberrant cell proliferation and tumourigenesis (Dunn *et al*., 2004).

Recent advances in cancer biology research show a chronic indolent inflammation environment harbours potential tumour promoting mechanisms (Grivenikov *et al*., 2010). Hanahan and Weinberg work revealed that one of the crucial point in cancer is its ability to evade immunosurveillance and enhanced by inflammation propagated by the tumour (Hanahan and Weinberg, 2011). Compelling evidence supports the notion that the inflammatory micro-environment is essential for the survival of tumours (Grivenikov *et al*., 2011). There is proof of

several unresolved inflammatory reactions that follows persistent pathogen infection promoting human malignancies (Shacter and Weitzman, 2002). Pathogens contain specific patterns, called "pathogen-associated molecular patterns" (PAMPs), which are recognized by host receptors, named Pattern Recognition Receptors (PRRs), including Toll-like Receptors (TLRs), Nucleotide-binding Oligomerization Domain-like receptors (NOD-like) receptors, C-type Lectin Receptors (CLRs), and triggering receptors expressed on myeloid cells (TREMs) (Lin and Karin, 2007; Akira *et al*., 2006). The binding of PAMPs and PRRs results in inflammation-related cell activation which trigger host immune defense mechanisms against antigens (Lin and Karin, 2007).

2.17 The role of Inflammation in Carcinogenesis

Ferrero-Miliani *et al.,* (2007) defined inflammation as a complex biological response of vascular tissues to harmful stimuli. Aulus, a Roman medic in the first century, defined inflammation as: redness, swelling, heat and pain. Furthermore, Inflammation is a physiologic process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and or wounding (Philip, 2004). Rodolf Virchow postulated that micro-inflammation that results from irritation leads to the development of most chronic diseases, including cancer (Heidland *et al*., 2006). He also indicated that cancers tended to occur at sites of chronic inflammation (Balkwill and Mantovani, 2001). Philip *et al.,* (2004) have reported that acute inflammation contributed to the regression of cancer.

Contemporary approaches in cancer research have been influenced by the accumulating data unveiling the importance of inflammatory components in the tumour micro-environment. It is becoming more clearly evident that inflammation demonstrates a dualism effect on cancer development in close resemblance to a *yin-yang* (negative and positive) pattern. Inflammation may exhibit either a pro- or an anti-tumourigenic effect (Aivaliotis *et al*., 2012). Eiro and

Vizoso (2012) reported that estimated underlying infections and inflammatory reactions are linked to 15-20% of all cancer deaths. Furthermore, there are well known associations between inflammatory processes and cancer, such as bowel disease and colorectal cancer (Eaden *et al*., 2000; Van der Woude *et al*., 2004) viral hepatitis B and C or alcoholic liver cirrhosis and hepatocarcinoma (Matsuzaki *et al*., 2007), chronic reflux esophagitis resulting in Barrett's esophagus and esophageal carcinoma (Van der Woude *et al*., 2004), cervical infection by HPV and cervical cancer, prostatitis and prostate cancer, pancreatitis and pancreatic cancer, or gastric infection from *Helicobacter pylori*, which increases gastric cancer risk by 75% (Hussain *et al*., 2000; Kuper *et al*., 2000).

2.17.1 Progression of Inflammation

At the very early stage of inflammation, neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells pre-stationed in tissues (Coussens and Werb, 2002; Nathan, 2002). As the inflammation progresses, various types of leukocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signalling network involving a great number of growth factors, cytokines, and chemokines (Coussens and Werb, 2002; Nathan, 2002). All cells recruited to the inflammatory site contribute to tissue breakdown and are beneficial by strengthening and maintaining the defence against infection (Coussens and Werb, 2002). There are also mechanisms to prevent inflammation response from lasting too long (Maiuri *et al*., 2004). A shift from anti-bacterial tissue damage to tissue repair occurs, involving both pro-inflammatory and anti-inflammatory molecules (Maiuri *et al*., 2004). Prostaglandin E2 (Levy *et al*., 2002), transforming growth factor-h (Hodge-Dufour *et al*., 1998), and reactive oxygen and nitrogen intermediates (Nathan, 2002) are among those molecules with a dual role in both promoting and suppressing inflammation. The resolution of

inflammation also requires a rapid programmed clearance of inflammatory cells: neighbouring macrophages, dendritic cells, and backup phagocytes do this job by inducing apoptosis and conducting phagocytosis (Savil *et al*., 1989; Savil *et al*., 2000; Savil *et al*., 2002). The phagocytosis of apoptotic cells also promotes an anti-inflammatory response, such as enhancing the production of anti-inflammatory mediator transforming growth factor-h (Fadok *et al*., 1998; McDonald *et al*., 1999; Huynh *et al*., 2002).

However, if inflammation resolution is dysregulated, cellular response changes to the pattern of chronic inflammation. In chronic inflammation, the inflammatory foci are dominated by lymphocytes, plasma cells, and macrophages with varying morphology (Philip *et al.,* 2004). Macrophages and other inflammatory cells generate a great amount of growth factors, cytokines, and reactive oxygen and nitrogen species that may cause DNA damage (Coussens and Werb, 2002). If the macrophages are activated persistently, they may lead to continuous tissue damage (Macarthur *et al*., 2004). A micro-environment constituted by all the above elements inhabits the sustained cell proliferation induced by continued tissue damage, thus predisposes chronic inflammation to neoplasia (Balkwill and Mantovani, 2001).

2.17.2 Inflammation and Cytokine

Inflammation may exhibit either a pro- or an anti-tumorigenic effect. Cytokines possess a central role in the inflammatory component implicated in the interplay between the host's stromal cells and the tumour cells during tumorigenesis as well as cell-signaling protein molecules with effects on intercellular communication. The significant role of chronic inflammation and carcinogenesis process is now well understood (Grivennikov *et al*., 2010). Cytokines are a diverse group of signalling molecules that are produced as a result of infection, inflammation, injury, and cellular stress (Mairov *et al*., 2014). Advances in cancer biology research reveals that a chronic indolent inflammation environment harbours potential tumour

promoting mechanisms. It appears that inflammatory cells of the innate immunity usually display a tumour promoting role whereas cells of adaptive immunity appear to have a tumor suppressive effect. According Shacter and Weitzman (2002), evidence of several unresolved inflammatory reactions following persistent pathogen infection promotes human malignancies. The inflammatory mediated tumour promotion mechanism involves secretion of specific cytokines by both inflammatory and tumour cells as well as activation of transcription factors, such as; Nuclear Factor-kappa B (NF-κB), Signal Transducers and Activators of Transcription (STAT3), and Activator Protein-1 (AP 1). NF-κB and STAT3 expression can be detected in most cancers and these transcription factors activate genes responsible for cell survival, proliferation, angiogenesis, invasiveness, and production of cytokines (Aivaliotis *et al.*, 2012). Pro-inflammatory cytokines include IL-2, IL-6, IL-11, IL-15, IL-17, IL-23, TNF-α, and chemokine IL-8 while anti-inflammatory cytokines are IL-4, IL-10, IL-13, TGF-β and IFN-α. Pro-inflammatory cytokines, such as IL-1 and TNF-α which in turn stimulate IL-8, may also stimulate chemokines (Aivaliotis *et al.*, 2012).

Some cytokines play dual role in tumourigenesis by displaying Pro-tumourigenic and anti-tumorigenic character (Baker *et al.*, 2019). This shift in roles from pro-tumourigenic to anti tumorigenic may depend on the nature of inflammation (Multhoff *et al.*, 2012).

2.18 Inflammation and *Chlamydia trachomatis*

Mardh *et al.*, (1977) first reported *Chlamydia trachomatis* and its role in PID. This organism is now recognised as the commonest STI in high income countries (Hammerschlag, 1981). *Chlamydia* infections can ascend the reproductive tract resulting in PID and, consequently, lead to chronic pelvic pain, ectopic pregnancy, and infertility (Chernesky, 2005). Most complications associated with CT infection in women are avoidable by appropriate treatment. However, the asymptomatic nature of the infection often prevents the initiation of

treatment and the general screening cost is not effective. Recent clinical approach to CT infection is selective testing by identification of risk characteristics for chlamydial infections in women (Ravindran *et al*., 1998).

In a Norwegian study, it was observed that over 50% of the PID cases were caused by chlamydia, whereas Neisseria gonorrhoeae played a more subordinate role (Gjonnaess *et al*., 1982). A complicated CT infection often begins as a clinically benign condition which often remains undiagnosed which can progress to tubal damage and subsequently to infertility (Theunissen *et al*., 1994).

Infection with CT is accompanied by formation of reactive oxygen and nitrogen species with ability to damage DNA, proteins and cell membranes (Oshima *et al*., 1994; Rosin *et al*., 1994). This process and its influence can induce mutagenesis (Personnet, 1999). DNA damage caused by oxidative stress resulting from Chlamydia infection (Mayer *et al*., 1993), can be a mechanism for Chlamydia-induced carcinogenesis. CT also has shown anti-apoptotic activity (Fan *et al*., 1998). Consequence of chronic CT infection causes inflammation, necrosis, scarring and cervical hypertrophy. It has also been speculated that the immune system cells that are activated at Chlamydia infection sites may damage normal cells (Adegbesan-Omilabu *et al*., 2014).

According to Darville and Hiltke (2010), two hypotheses described for chlamydial pathogenesis include; the cellular and immunological paradigm. Under the cellular paradigm, pathogenesis is driven by inflammatory responses initiated by host epithelial cells, the primary targets of chlamydial infection (Stephens, 2003). It is worthy of note that both CT and HPV target epithelial cells. The infected epithelial cells secrete chemokines, and in turn recruit inflammatory leukocytes and cytokines, which induce and augment cellular inflammatory response (Stephens 2003; Rasmussen *et al*., 1997).

According to the immunological paradigm, pathogenesis is primarily the result of adaptive cellular immune responses directed at specific chlamydial antigens during repeat infection (Brunham and Peeling, 1994). CT specific adaptive T cell responses develop over time to help clear infection and are believed to induce complete tissue damage. Failure to clear infection, maintains inflammatory pathology during active chronic infection.

Clearly, interactions between inflammation leading to infection clearance and inflammation leading to pathology are an important consideration in understanding the natural history of CT infection (Gottlieb *et al*., 2010). Darville and Hiltke (2010) maintain that pathogenesis is dependent on ascension of CT from the cervix to the fallopian tubes. It seems the relationship between innate, adaptive response, and epithelial cell responses are crucial in chlamydia inflammation.

2.19 Cytokines

According to Blacks' Medical Dictionary (2005), cytokines are a family of protein molecules that carry signals locally between cells. Cytokines are released by antigens activated cells that act as enhancing mediators for immune response. These proteins include interleukins (produced by leucocytes), lymphokines (produced by lymphocytes), interferon, and tumour necrosis factor, one of whose many functions is killing tumour cells. Cytokines are a key component of inflammation with low molecular weight capable of triggering or inhibiting the differentiation, proliferation or function of immune cells (Roitt and Delves, 2001; Forbes *et al*., 2007). They also constitute a group of relatively small, secreted signalling molecules (generally containing about 160 amino acids) that control growth and differentiation of specific types of cells (Lodish *et al*., 2013). Cytokines are small non-structural proteins having molecular weights of approximately 8-40,000 d. They were originally called lymphokines and monokines based on early cellular sources and later as cytokine because of the later discovery of their

association with most nucleated cells (Dinarello, 2000). The structure of cytokines reveals that they all evolved from a common ancestral protein and have a similar tertiary structure consisting of four long conserved **α** helices folded together. They lack an amino acid sequence motif or a three dimensional structure. Likewise, the various cytokine receptors undoubtedly evolved from a single common ancestor due to their similar structures (Lodish *et al*., 2013; Dinarello, 2000).

Cytokines exhibit complex and antagonistic roles in the immune system either as pro or anti-inflammatory. Pro-inflammatory cytokines, such as IL-1β and TNF-α, induces inflammation as a consequence of infection or injury; In contrast, anti-inflammatory cytokines, like IL-10 and TGF-β, suppresses the activity and production of pro-inflammatory signals, blocking inflammation and host damage. Different cytokine combinations give distinct consequences, such as inflammation, proliferation and formation of new blood vessels. Imbalances in cytokine expression or signalling contribute to malignant transformation (Mairov *et al*. 2014).

Different cytokines are made by various cell types and generally act at a short range on neighbouring cells. Some cytokines of T-cell origin help B-cells to make antibodies, while others such as γ-interferon (IFN-γ) act as macrophage activating factors that switches on previously undermined microbicidal mechanisms of the macrophage bringing about the death of the intracellular microorganisms (Roitt *et al*., 2001).

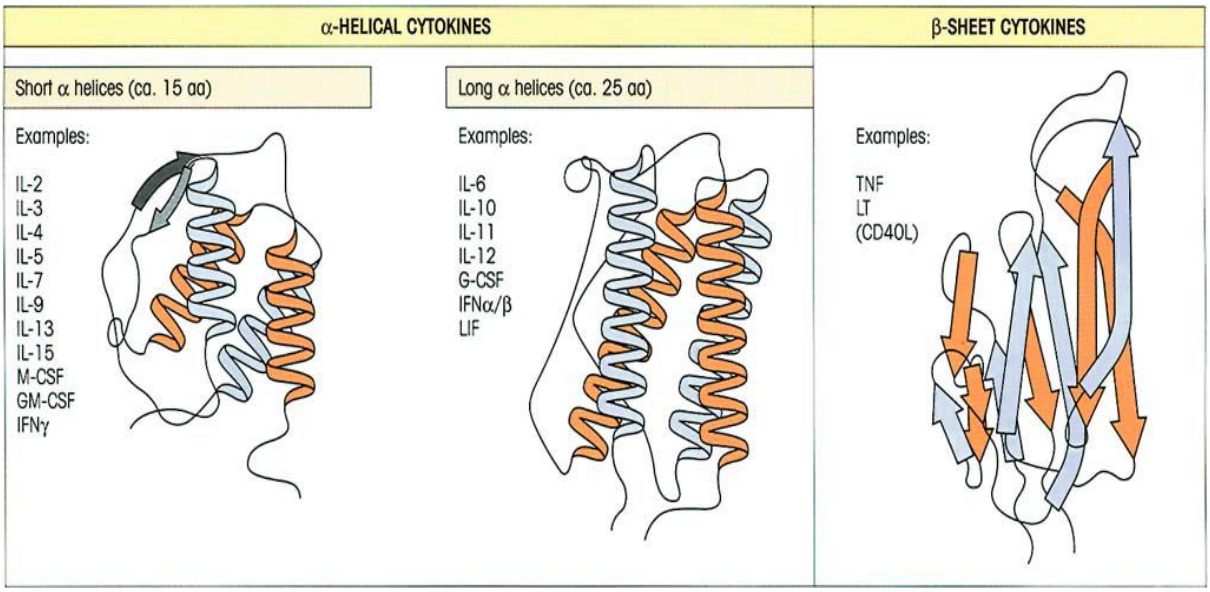

Figure 5: Stuctures of cytokine, Source: Roitt *et al*., 2001.

Cytokines are involved in the two arms of immunity namely; innate immunity (humoral) and adaptive (cellular immunity) respectively. The broad categories of humoral and cellular immunity can be distinguished by different characteristics. Adaptive T- and B-cell immune responses have unusual diversity, a slower kinetics of activation on primary exposure to an infectious agent, and rapid recall responses for classic memory and enhanced protection induced by vaccination. In contrast, the faster innate immune responses have conserved receptors that recognize unique molecular structures expressed by classes of infectious organisms but distinct from host determinants. Its responses depend on non-immune cells or cells of the innate immune system, such as monocytes or macrophages (monocytic cells), DC, natural killer (NK) cells, and polymorphonuclear leukocytes (PMN). It also mediates direct antimicrobial effects at the earliest times of primary infections, while slower adaptive immune responses are being expanded and activated, but innate immunity also delivers important immune-regulatory effects to shape downstream immune responses. Thus, the innate immune system act as a first line for sensing infection, to unleash protective defence mechanisms mediated through a high proportion of pre-existing cells, and to both direct and support other protective responses (Knipe and Howley, 2007).

2.19.1 Pro-Inflammatory Cytokines

Cytokines that distinctly promote inflammation are called pro-inflammatory cytokines majorly the IL-1, TNF and chemokines. Interferons exhibits antiviral activity. IFN-γ activates pathways leading to cytotoxic T cells. The ability of IFN-γ to augment TNF activity and induce Nitric Oxide (NO) establishes it as a pro-inflammatory cytokine (Dinarello, 2000). The network of pro-inflammatory events is mainly regulated by IL-1 and TNF. Interleukin-1 is an effective mediator of inflammation which influences the activities of many different cell types (Chizollni and Dayer, 1998).

2.19.2 Anti-Inflammatory Cytokines

Anti-Inflammatory cytokines are regulatory cytokine inhibitors that serve as immunomodulatory elements, with the ability to control injurious effects of sustained or excess inflammatory reactions. These anti-inflammatory mediators may provide insufficient control over pro-inflammatory activities in immune-mediated diseases or reimburse and inhibit the immune response, rendering the host at risk from systemic infection (Opal and Depalo, 2000). These anti-inflammatory cytokine (cytokine inhibitors) were discovered naturally in biological fluids and found to block IL-1 and TNF. They exhibit several mode of actions at different multiple level by inhibiting cytokine production and secretion (Chizollni and Dayer, 1998) Interestingly, all anti-inflammatory cytokine also have pro-inflammatory properties with the exception of Interleukin-1 receptor antigen (IL-1ra). Anti-inflammatory cytokines includes; IL-1ra (grouped into IL-1α, IL-1β), IL-4, IL-6, IL-10, IL-11, IL-13 and TGF-β (Opal and Depalo, 2000).

2.20 Human Papillomaviruses and Immune Evasion Strategy

In biological evolution, HPVs are termed successful infectious agents due to; their ability to induce persistent infections without frequent and serious complications to the host, the shedding of virions for transmission to other naive individuals. They reach a balanced state where the host usually is not seriously dis-advantaged by the HPV infection and the virus is not too limited in reproducing by the host's immune response. To achieve this lifestyle and to maintain a state of equilibrium, HPV must avoid the host's defence systems. Many factors contribute to evading immune pools, they include; absence of viral-induced cytolysis or necrosis, absence of inflammation, little or no release into the local milieu of pro-inflammatory cytokines, no blood-borne or viremic phase, only minimal amount of replicating virus exposed to immune defences. Other immune evading mechanisms are; infection is exclusively

intraepithelial, entry of the virus capsid is usually an activating signal for Dendritic Cells (DCs), but there is evidence that Lymphatic cells (LCs) are not activated by HPV capsid uptake. Most DNA viruses have mechanisms for inhibiting interferon synthesis and receptor signalling, by downregulating toll-like receptor (TLR)-9 and PV's are no exception. Despite HPV's ability to evade the host's immune system and down-regulate innate immunity, HPV infection is cleared naturally in about 90% of cases, indicating the important function of immunity in the resolution of cervical and anogenital HPV-associated diseases (Scott *et al*., 2001, Mariani and Venuti 2010, Frazer 2007, Eistein *et al.*, 2009, Stanley 2010).

Molina and colleagues in 2013 explained that, HPV can evade the immune response; mainly through E6 and E7 proteins action. They noted that viral mechanisms of immune evasion range from modulation of cytokines and chemo-attractant expression to alteration of antigen presentation, and down-regulation of IFN-pathways and adherence molecules. They further explained that evasion of the immune response by HPV is critical for a successful infection. Host defence is a partnership between innate immunity (phagocytes, soluble proteins for example; cytokines, complement and epithelial barriers) together with adaptive immunity (antibody, cytotoxic effector cells). The innate immune system detects the pathogen and acts as first line of defence, clearing up to an estimated 90 per cent microbial assaults alone (Stanley, 2009).

2.21 Human Papillomavirus and Immune Dynamics

Ambiguity still exists on the elements of the immune system that is useful in preventing or resolving HPV infections (Franceschi and Baussano, 2014). The incubation period of infectious HPV ranges from 3-4 weeks to months or years, the duration of this latent period may depend on the dose of virus received (Stanley, 2010). Importantly, a vast majority of infections by HPVs are asymptomatic and benign only a minority of infections (less than 10%)

become persistent and viral persistence rather than clearance is a crucial step that may precede cervical carcinogenesis (Mosciscki *et al.,* 2012; Murall *et al.,* 2019). Although, for reasons that are still not understood permissive viral growth commences, viral DNA can be detected and infectious virus is shed. This phase of active replication equally persists for a variable length of time but eventually the vast majority of infected individuals mount an effective immune response becoming DNA negative with subsequent sustained clinical remission from disease. An effective immunity usually consists of a cell mediated response to the early proteins, especially E2 and E6, necessary for lesion regression accompanied or followed by sero-conversion and antibody to the major capsid protein L1 (Stanley, 2010). Immune evasion by infectious HPV has been seen in approximately 20% of women who are at increased risk of cervical carcinoma (Mosciscki *et al.,* 2006).

The infectious cycle of HPV is without viraemia and an essential mechanism, very low levels of viral protein are expressed but crucially HPV is not cytolytic. Virus replication and assembly in HPV occurs in cells already destined for death, absence of inflammation and lack of danger signal to alert the immune system. The interferon response for HPV infection, a key antiviral defence mechanism (Pett, *et al.,* 2006), is actively suppressed with the E6 and E7 proteins of the high risk HPVs inhibiting the interferon receptor signalling pathways and the activation of the interferon response genes (Kanodia *et al.,* 2007). The E7 proteins downregulate TLR9 (Hassan *et al.,* 2007) and overall HPV effectively evades the innate immune response delaying the activation of adaptive immunity. Figure 6 summarises the natural course of genital HPV infection.

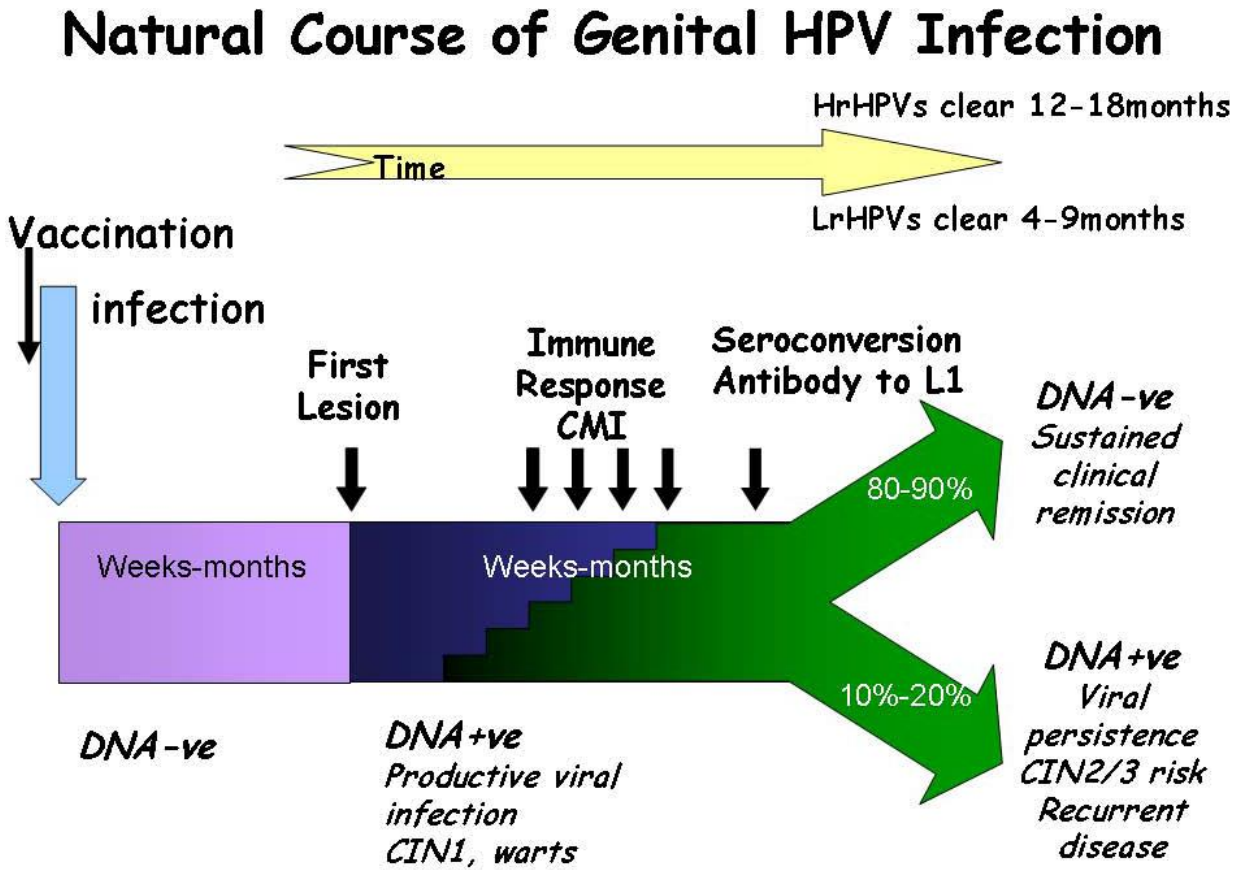

Figure 6: Natural course of genital HPV Infection. (Source: Stanley, 2010).

2.22 Cytokines and Human Papillomavirus

Human Papillomavirus that causes cervical and other types of cancer is of great interest to many scientists globally. A review by Motoyama and co-worker (2004), showed that the activities of E6 and E7 oncoproteins alongside other co-factors are crucial for the development of cervical cancer in women. The review also explained that the oncogenic effects of HPVs can be suppressed or reversed through; action of dexamethasone, viral oncogene antisense constructs, and cytokines.

Most HPV infections have been shown to clear within a few months or years; the 5–10% women with persistent infections remain at an increased risk for the development of CIN 3 and less commonly, cervical cancer (Scott *et al*., 2013). Based on current models for immune response using animal and in vitro studies, the exact mechanism associated with clearance of HPV infection remains unclear. Postulates that HPV persistence involves avoidance or repression of the innate and adaptive immune responses" in the presence of a tolerogenic local immune environment exists. In vitro studies of HPV infected keratinocytes show the rapid induction of important immune response cells, such as natural killer cells, accompanied by the production and release of different cytokines which aid in the recruitment and co-ordination of functions of cells essential to pathogen control (Boccardo *et al*., 2010). It is believed that cytokine activation occurs days to weeks after the establishment of an HPV infection and is subsequently reversed when immune success (HPV clearance) has been effectively communicated to the appropriate effector cells. This reduction in level of inflammatory mediators after viral clearance safeguard against toxic *sequelae* and harms normal tissues (O'Byrne and Dalgleish 2001; Balkwill and Montavani, 2001; Hawes and Kiviat 2002). Scott *et al* 2013 revealed that the role of the cytokine-mediated mucosal immune response in the clearance of cervical HPV infection remains poorly defined.

2.23 Cytokine Polymorphisms

The word polymorphism is an important aspect of genetic variation. It refers to the presence of two or more alternative forms of a distinct phenotype in the same population (Singh and Kulathinal, 2013). A common definition of genetic polymorphism is that the locus (genetic material) should contain two or more alleles, with the most common allele having a frequency of 99% or less. Polymorphism has been used extensively in areas such as; blood grouping, chromosome inversion, protein and DNA polymorphism. Other useful areas of application of Polymorphism, includes; population genetics, evolutionary genetics, systematics and molecular phylogeny, human genetics, agricultural genetics, and forensics (Singh and Kulathinal, 2013). Cytokine polymorphism is the genetic variation (allelic forms) that exists within different cytokines which may influence cytokine secretion or production. This is as a result of sequence differences discovered in promoters and coding regions of cytokines and their receptors (Gallagher *et al.,* 2003). Basal and cell-stimulated cytokine levels vary between individuals and this variation are influenced by both genetic and environmental factors. Genetic variation that results in altered structure or expression of a cytokine can have evident pathological consequences, capable of a number of debilitating outcomes such as; chronic diseases, increased risk of infection, and altered nature of acute disorders (Smith and Humphries, 2009).

The Pro-inflammatory and anti-inflammatory cytokines have both been implicated in various diseases due to their polymorphisms which may be linked with susceptibility to certain diseases or contribute to severity and progression. Inter-individual differences in cytokine profile appear to be partly due to allelic polymorphisms within regulatory regions of cytokine gene (Gallagher *et al.*, 2003; Bidwell et al, 1999).

Research in Cytokine gene polymorphisms is important because it enhances understanding of aetiology and pathology of human disease, coupled with its advantage in

identifying potential markers of susceptibility, severity, and clinical outcome. It further identifies targets for therapeutic intervention and novel strategies to prevent disease or to improve existing preventions (Bidwell *et al.,* 1999). Research in the area of Cytokine gene polymorphisms has associated a number of this polymorphism to diseases. For example IL-10 has been linked with diseases such as Psoriasis, rheumatoid arthritis, type 1 autoimmune hepatitis, Epstein-Barr virus infection, multiple sclerosis, etcetera, TNF to Meliodosis, Multiple sclerosis, Myasthenia gravis, Primary biliary cirrhosis, Primary biliary cirrhosis, etcetera, IFNγ to Atopic asthma, Graft-versus-host disease, Hay fever, Insulin-dependent diabetes mellitus, Multiple sclerosis, Rejection of renal transplant, Systemic lupus erythematosus, etcetera. Also, TGFβ has been linked to Allergic athsma, allograft function, bone marrow transplantation, chronic hepatitis C virus, chronic obstructive pulmonary disease, etcetera (Bidwell *et al*.,1999 ; Haukim *et al*., 2001; Hollegaard and Bidwell, 2006).

2.24 Cytokines Polymorphisms and *Chlamydia trachomtis*

Infection of the genital tract by CT drives a network array of host immune responses involving arms of the immune system (Marks and Lycke, 2008). The innate immune response is the first line of defense against invading pathogens which has far reaching effect on the outcome and immunopathology of genital tract infections. This line of defence plays a vital role in reducing initial load of CT infection and promotes the induction of an adaptive immune response capable of specifically combating the infection (Marks and Lycke, 2008). Initiation of host defences against microbial invasion involves the quick activation of pro-inflammatory cytokines, such as; IL-6, IL-8, IFN-ɤ and TNF-α, whose production are required for the clearance of pathogens. Furthermore, secretion of chemokines by infected cells can recruit classical innate immunity cells such as natural killer (NK) cells and DCs, which are abundant in the genital mucosa (Du *et al*., 2018; Redgrove and McLaughlin, 2014). Chlamydial genital

infection activates pro-inflammatory responses characterized by production of cytokines by epithelial and other cells in the mucosa. The cytokine microenvironment promotes the development of Th1-dominated CD4+ T cell immunity. Natural Killer cells and neutrophils are the first immune cells that are recruited to the site of Chlamydial infection. It is believed that neutrophils acts to reduce direct chlamydial infection and limit spreading, with human neutrophils being able to effectively inactivate C. *trachomatis* in vitro (Redgrove and McLaughlin 2014).

2.25 Cancer and Cell Cycle

Cancer is frequently labelled a cell cycle disease. As such, it is not surprising that the deregulation of the cell cycle is one of the most frequent alterations during tumour development. Cancer originates from the abnormal expression or activation of positive regulators and functional suppression of negative regulators (Park and Lee, 2002).

Cell cycle transition is an ordered, tightly-regulated process that involves multiple checkpoints that assess extracellular growth signals, cell size, and DNA integrity. The somatic cell cycle is divided into four distinct phases. During two of these phases, the cells execute the basic events in cell division like generation of a single and faithful copy of its genetic material (synthetic or S phase) and partitioning of all the cellular components between the two identical daughter cells (mitosis or M phase). The two other phases of cell cycle represent gap periods (G1 and G2), during which the cells prepare themselves for the successful completion of the S and M phases, respectively. When the cells cease proliferation, due, either to specific antimitogenic signals or to the absence of proper mitogenic signalling, then they exit the cycle and enter a non-dividing, quiescent state, known as G0. In addition, the cell cycle may be arrested at the G1 or G2 checkpoints that assess cell size, extracellular growth signals, and DNA integrity. The molecular analysis of human tumours has shown that cell cycle regulators

are frequently mutated in human tumours, which underscores how important the maintenance of cell cycle commitment is in the prevention of human cancer (Park and Lee, 2002).

However, it has become clear between 1980s and 1990s, that dysregulation in the cell cycle machinery may actually be responsible for uncontrolled cell growth, characteristics of cancer. Both active oncogenes and mutations for lack of function in any tumour suppressor gene may lead to cancer. Intrinsic defects in cell cycle machinery may also cause cancer (Gupta, 2005).

2.26 Cell Cycle

Cell cycle is the ordered sequence of events that leads to cell division in which a eukaryotic cell duplicates its chromosomes and divides into two each containing chromosomes identical to those of the parental cell (Lodish *et al*., 2013). The cell cycle normally consists of four phases: G1 before DNA synthesis occurs; S when DNA replication occurs; G2 after DNA synthesis; and M when cell division occurs, yielding two daughter cells (Pasternak, 2005). Under certain conditions, cells exit the cell cycle during G, and remain in the G0 state as non-dividing cells. The figure below shows the phases of cell cycle and expression of the cyclins and their inhibitors.

Two main molecular processes take place during the cell cycle, with resting intervals in between: during the S phase of the cycle, each parental chromosome is duplicated to form two identical sister chromatids; in mitosis (M phase), the resulting sister chromatids are distributed to each daughter cell. Chromosome replication and segregation to daughter cells must occur in the proper order in every cell division. If a cell undergoes chromosome segregation before the replication of all chromosomes has been completed, at least one daughter cell will lose genetic information.

Likewise, if a second round of replication occurs in one region of a chromosome before cell division occurs, the genes encoded in that region are increased in number out of proportion to other genes, a phenomenon that often leads to an imbalance of gene expression that is incompatible with viability (Lodish *et al*., 2013).

2.26.1 Cell Cycle Progression and Checkpoints

The history of the factors involved in cell cycle regulation goes back many years. Definition of distinct phases of a division cycle, that is; G1, S, G2, and M, became established in the mid- to late 1950s when tritum labelling and cell synchronization techniques became available to score mitoses and to measure the time between one mitotic wave and another in cycling cells. In this way, the classic cell cycle of G1 to S to G2 to M was established (Ruddon, 2007).

Besides, between 1980 and 2000 it has been shown that there exists a central cell cycle control system, which regulates the orderly progress of events during the cell cycle. For instance, within the cell, there are devices which ensure that the cell is not driven into mitosis before DNA synthesis is completed or damaged DNA repaired, and it is not driven into anaphase before all chromosomes are aligned on the spindle (Gupta, 2005). It became clear from early studies of yeast mutants that certain genetically controlled factors played a key role in regulating the cell cycle. For this purpose, a control system exists, which is distinct from the machinery performing the essential processes (Gupta, 2005; Ruddon, 2007). For instance, while there is machinery involved in DNA synthesis, there is a separate and distinct control system which regulates the entry of the cell into S-phase and later into M-phase after DNA synthesis is completed. Such a distinction between a control system and the machinery involved in different processes has been recognised. In this control system there are checkpoints, where brakes and feedback signals can operate. The brakes can stop the cycle at

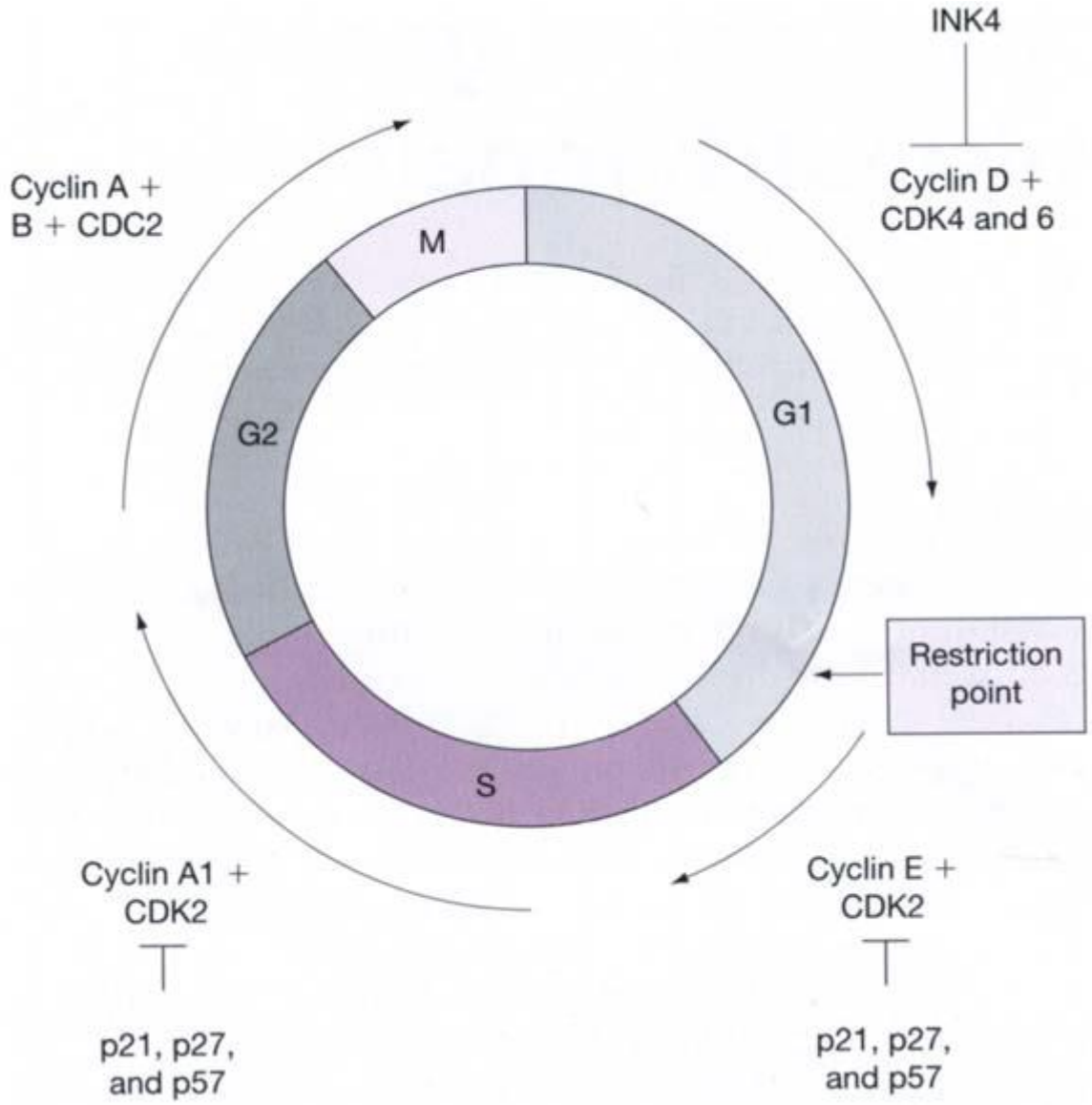

Figure 7: **Stages of the cell cycle and expression of the cyclins, CDKs and their inhibitors, the CKIs, Source: Mcdonald *et al*., 2005.**

checkpoints and feedback signals can delay progress of the control system itself, so that it will not be able to trigger the next event, before the previous one is completed (Gupta, 2005).

Cell cycle checkpoints are regulatory pathways that govern the order and timing of cell cycle transitions to ensure completion of one cellular event prior to commencement of another. The key regulators of the checkpoint pathways in the mammalian DNA damage response are the Ataxia telangiectasia, mutated (ATM) and ATM and Rad3-related (ATR) protein kinases. Both of these proteins belong to a structurally unique family of serine-threonine kinases characterized by a C-terminal catalytic motif containing a phosphatidylinositol 3-kinase domain (Abraham, 2001; Shiloh, 2001). Although ATM and ATR appear to phosphorylate many of the same cellular substrates (Kim *et al*., 1999) they generally respond to distinct types of DNA damage. ATM is the primary mediator of the response to DNA Double Strand Breaks (DSBs) that can arise by exposure to ionizing radiation (IR). ATR, on the other hand, plays only a back-up role in the DSB response, but directs the principle response to ultra violet (UV) damage and stalls in DNA replication.

2.26.2 Cyclins and Cyclin Dependent Kinases (Cdks)

Hartwell, Hunt, and Nurse won the 2001 Nobel Prize in Physiology or Medicine for their discovery of Cyclins and cyclin Dependent Kinases (Cdks) (Press Release, 2001). These Two key classes of regulatory molecules, cyclins and Cdks, determine a cell's progress through the cell cycle (Nigg, 1995). Cyclin dependent kinases (CDKs) are protein kinases involved in critical cellular processes, such as cell cycle, transcription and other major biological processes including neuronal differentiation and metabolism, whose activity requires association with specific cyclin subunits (Malumbres *et al*., 2009; Peyressatre, 2015).

Many of the genes encoding cyclins and Cdks are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that

incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially *Saccharomyces cerevisiae* (Spellman *et al*., 1998) as genetic nomenclature in yeast dubs many of these genes cell division cycle (*cdc*) followed by an identifying number, for example, *cdc25* or *cdc20*.

A cyclin-dependent kinase complex (CDKC, cyclin-CDK) is a protein complex formed by the association of an inactive catalytic subunit of a protein kinase, cyclin-dependent kinase (CDK), with a regulatory subunit, cyclin (Malumbres and Barbacid, 2005). Once cyclin-dependent kinases bind to cyclin, the formed complex is in an activated state. Substrate specificity of the activated complex is mainly established by the associated cyclin within the complex. Activity of CDKCs is controlled by phosphorylation of target proteins, as well as binding of inhibitory proteins (Lodish *et al*., 2013).

Cyclins form the regulatory subunits and CDK (kinase subunit; kinase bring about phoshorylation) the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and Cdks are inactive in the absence of a partner cyclin. When activated by a bound cyclin, Cdks perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK complexes (CDKC) determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals (Robbins and Cotran, 2004; Gupta, 2005). Both CDK and cyclin have one or more amino acid residues (threonine or tyrosine), which can be phosphorylated and dephosphorylated (revesible phosphorylation). A number of CDKs are now known and differ from one organism to the other. In mammals CDKs include; CDK1, CDK2, CDK3, CDK4 and CDK6. Similarly, three types of cyclin are known: mitotic cyclins (cyclin B) found during M phase, S-phase cyclins (cyclin A) found during S-phase and G1 cyclins (cyclins C, D, E and F) found in G1 phase (Gupta, 2005).

2.26.3 CDKs and Cyclins in Mammalian Cells

In mammalian cells, at least two and under special situation (cell growth) more than two CDKs are used. CDK1 (cdc2 protein) complexed with cyclin B is used for passage through mitosis or M-phase and CDK2 complexed with cyclin A is needed to activate the DNA replication machinery. CDK4 and CDK6, complexed with cyclin D, on the other hand are involved in cell growth (particularly after exit from a quiescent or G0 phase). The activation of CDK1-cyclin B complex also needs phosphatase cdc25 in late G2 to bring about dephosphorylation needed for activation of Cdk1. Furthermore, the CDK2-cyclin A complex needs to be phosphorylated at threonine-160 for full activation. Binding of p27 with the complex CDK2-cyclin A physically blocks the active site. Some non-cyclin proteins play major role in cell cycle regulation in mammalian cell. A product of tumour suppressor gene known as p53 is a strong activator of transcription and responds to DNA damage or to conditions unfavourable for DNA replication; its turn it activates a number of genes including the gene p21; p21 is a CDK-cyclin inhibitor, and therefore arrests the progression of cell cycle, therefore permitting time for DNA repair (Gupta, 2005).

2.26.4 Cdk Inhibitors

The CDK activity is also regulated due to binding of inhibitors called cyclin dependent kinase inhibitors (CKI). Several growth inhibiting signals including TGF-β act through these cyclin dependent kinase inhibitors; Sic1, Far1, Rum1, p21, p27, p57, p16, p15, p18, p19. The protein p21 is a universal CDK inhibitor and binds with CDK2, CDK4 and CDK6, suggesting that it inhibits progression through all stages of G1/S. The protein p27 has a sequence that is partly related to p21 and binds promiscuosly to all CDK-cyclin complexes. Both p21 and p27 bind and render the CDK-cyclin complex incapable of being activated through phosphorylation by CDK activating kinase (cak) (Gupta 2005).

2.27 Cyclins and CDKs in Cancer

Cyclin D1 is one of the eight or more cyclins known in mammals. The overproduction or production of cyclin D1 at the wrong time would stimulate inappropriate cell divisions by keeping the corresponding CDKs (CDK4, CDK6) on, when it should be turned off. In 1991 the gene for cyclin D1 was found to be on and its amplification and over-expression were shown to cause a variety of cancers namely: breast, oesophagus, B cell lymphoma, and etcetera. Similarly, cyclin E and A were found to be overexpressed in cancer cell lines. Some CDKs like CDK4 have also been found to be amplified in some cancer cell (Gupta, 2005).

2.28 Cell Cycle Inhibitors and Cancer

Loss of activity or function of some cell cycle inhibitors may also lead to cancer. These inhibitors include: p53; which blocks the activity of CDK2, and other CDKs, an inhibitor blocking specifically the Cdk4 and Inhibitor mediating TGF-β's inhibitory effects (Gupta, 2005).

2.29 Tumour Suppressors and Cancer

Two important tumour suppressor genes are: p53 and pRb gene. Protein p53 has actually been considered to be very important and has been described as the 'guardian of the genome' or a 'watchman', so that conditions like; DNA damage, hypoxia, oncogene activation and virus infection are sensed by p53, which in its turn either arrests the cell cycle, or cause cancer. One of the commonest mutated (inactivated) gene in most of all human cancer is p53 (Gupta, 2005).

The Rb protein is a tumour suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumour progression. It has been shown that Rb protein is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth. The

retinoblastoma family includes three members, Rb/p105, p107 and Rb2/p130, collectively referred to as ‘pocket proteins’. The pRb also represses gene transcription, required for transition from G1 to S phase, by directly binding to the transactivation domain of E2F and by binding to the promoter of these genes as a complex with E2F. Furthermore, pRb represses transcription also by remodelling chromatin structure through interaction with proteins such as hBRM, BRG1, HDAC1 and SUV39H1, which are involved in nucleosome remodelling, histone acetylation/ deacetylation and methylation, respectively. Loss of pRb functions may induce cell cycle deregulation and so lead to a malignant phenotype (Giacinti and Gordano, 2006).

2.30 DNA Damage

All cells have elaborate mechanisms to maintain their genomes. The maintenance of genome integrity and fidelity is essential for the proper function and survival of all organisms. This task is particularly daunting due to constant assault on the DNA by genotoxic agents: endogenous (attack by reactive oxygen species, replicative errors) and exogenous (ultraviolent radiation from the sun, x-rays, gamma rays, plant toxins, mutagenic chemicals, and viruses) nucleotide misincorporation during DNA replication, and the intrinsic biochemical instability of the DNA itself (Lindahl, 1993). DNA can be damaged during replication, by reactive metabolic bye products as well as environmental mutagens. Responding to and repairing DNA damage is critical for cell viability and disease prevention (Cimprich and Cortez, 2008).

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 10,000 to 1,000,000 molecular lesions per cell per day (Lodish *et al*., 2004). While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions in critical genes (such as tumour suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the

likelihood of tumour formation and contribute to tumour heterogeneity. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene the affected DNA encodes. Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damages in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including DSBs and DNA crosslinkages (interstrand crosslinks or ICLs) (Acharya, 1971; Bjorkstein *et al*., 1971). However, failure to repair DNA lesions may result in blockages of transcription and replication, mutagenesis, and or cellular cytotoxicity (Friedberg *et al*., 1995). In humans, DNA damage has been shown to be involved in a variety of genetically inherited disorders, in aging (Finkel and Holbrook, 2000) and in carcinogenesis (Hoeijmakers, 2001; Peltomaki, 2001).

In order to preserve genomic stability, eukaryotic cells harbour highly flexible and integrated signalling systems that facilitate the sensing of DNA damage and subsequent responses to promote repair or cell death. Concomitantly, such signalling may modulate cell cycle progression to support the process of repair. The process of cell cycle arrest as a consequence of DNA damage signalling is known as the ‘checkpoint response’. Checkpoint responses are intrinsically linked to the cell cycle and as such some of the first experiments that supported the concept of checkpoints in response to DNA damage were performed in cycling eukaryotic cell systems such as yeasts and cells derived from mammalian hosts (Garner and Constanzo, 2009).

2.31 DNA Repair

DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome (Lodish *et al*., 2004). The DNA repair ability of

a cell is vital to the integrity of its genome and thus to the normal functionality of that organism (Browner *et al*., 2004). Repair of damage to DNA requires complex biological mechanisms that are tightly regulated and integrated. Damage that is not corrected prior to replication may be cytotoxic and mutagenic, making DNA damage responses during the cell cycle of particular interest (Mjelle *et al*., 2015). Some proteins have DNA repair as their sole or main function. However, a number of DNA repair proteins also have additional functions, for example in adaptive immunity (Daniel and Nussenzweig, 2013), transcription (Fong *et al*., 2013) and in replication (Sancar *et al*., 2004).

Cell cycle Progression processes is normally monitored by distinct checkpoints in G1/S, intra-S and G2/M. These checkpoints control the progression through the various phases of the cell cycle. Although the checkpoints are distinct, they all respond to lesions in DNA and share several proteins (Houtgraaf *et al*., 2006). Checkpoint activation and recruitment of DNA damage response proteins depend on the type of lesion (Cimprich and Cortez, 2008).

Many mechanisms are involved in DNA repair which are; Mismatch repair (MMR), Base excision repair (BER), Double strand break (DSB), Nucleotide excision Repair (NER), and Intrastrand Crosslinks (ICLs) (Mjelle *et al*., 2015).

Cells respond to DNA damage in many ways, which include activating specific repair pathways, inducing transcription and undergoing apoptosis. A common response in all eukaryotes is to arrest the cell cycle by activating a checkpoint. In most organisms, checkpoint signalling targets CDK activity, thereby holding the cell in interphase to allow for repair of the damaged DNA.The CDKs must be inhibited when DNA damage occurs to prevent cell-cycle progression and to allow for repair (Duursman and Cimprich, 2010). Recent studies in multiple organisms have revealed that, besides their established but essentially passive role as targets of checkpoint signalling, CDKs act positively, to regulate the choice of repair pathway activated in response to a DSB (Wholdbold and Fisher, 2009).

In response to DNA damage eukaryotic cells activate cell cycle checkpoints - complex kinase signaling networks that prevent further progression through the cell cycle. Prior to mitosis, cells progress through G1/S, intra-S and G2/M cell cycle checkpoints (Abraham, 2001; Bartek and Lukas, 2003; Harper and Elledge, 2007). Checkpoint signaling is activated in response to incomplete DNA replication due to stalled replication forks, and damaged DNA induced by both internal and external sources. In parallel to implementing a cell cycle arrest, checkpoint signalling also mediates the recruitment of DNA repair pathways. If the extent of damage exceeds repair capacity, additional signalling cascades are activated to ensure elimination of these damaged cells. The DNA damage response has traditionally been divided into two major kinase branches.

2.32 Diagnosis of Cervical Cancer

2. 32.1 Papanicolaou Test Procedure

The Papanicolaou test (Pap test) known earlier as; Pap smear, cervical smear, or smear test is a method of cervical screening used to detect potentially pre-cancerous and cancerous processes in the endocervical canal (transformation zone) of the cervix. Georgios Nikolaou Papanicolaou (1883 – 1962) was a Greek pioneer in cytopathology and early cancer detection, and was the inventor of the Pap test (Papanicolaou, 1940).

The Pap test procedure is a non-invasive method, performed by opening the vaginal canal with a speculum, then collecting cells from the outer opening of the cervix of the uterus and the endocervix. The cells are usually collected with cytological brush or extended end spatula and consequently examined under a microscope to look for abnormalities. The test aims to detect potentially pre-cancerous changes (called cervical intraepithelial neoplasia (CIN) or cervical dysplasia). The test remains an effective, widely used method for early detection of

pre-cancer and cervical cancer. The test may also detect infections and abnormalities in the endocervix and endometrium.

Pap test is currently divided into two which are; the conventional Pap test and the liquid based cytology (LBC). The choice of method varies from country to country. For example, U.K changed to the LBC in 2008 (NHS, accessed 28/07/2016).

2.32.2 Conventional Pap Test

In a conventional Pap smear, samples are usually collected using a spatula and then smeared directly onto a microscope slide after collection which is then immersed in 95% alcohol and then observed under a microscope after pap staining (Chinaka *et al*., 2014).

2.32.3 Liquid Based Cytology

In the liquid based cytology, the sample of (epithelial) cells is taken from the Transitional Zone; the squamo-columnar junction of the cervix, between the ecto and endocervix. Liquid-based cytology uses an arrow-shaped brush, rather than the conventional spatula. The cells taken are not transferred directly to a microscope slide, but the sample is deposited into a small bottle of preservative liquid. At the laboratory, the liquid is treated to remove other elements such as mucus before a layer of cells is placed on a slide. The technique allows more accurate results (NHS Cervical screening Programme, 2015). In most developed countries of the world the LBC has replaced the conventional Pap test (Nicoleta Simeon *et al*., 2014).

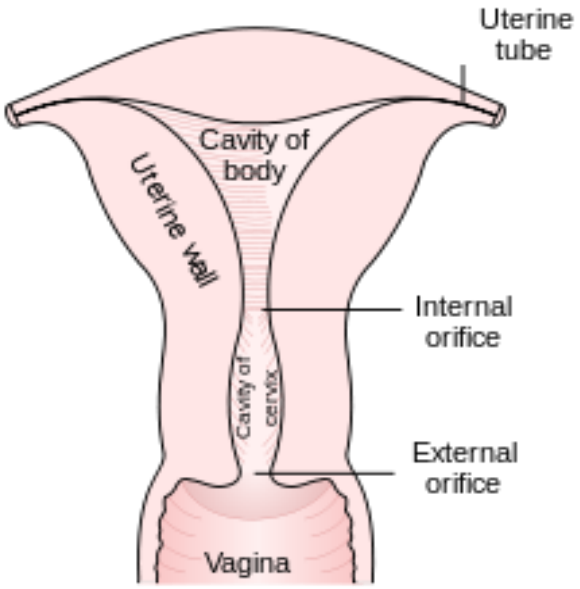

Figure 8: **Shows structure of a human cervix, source: modified from Moore and Dalley, 1999.**

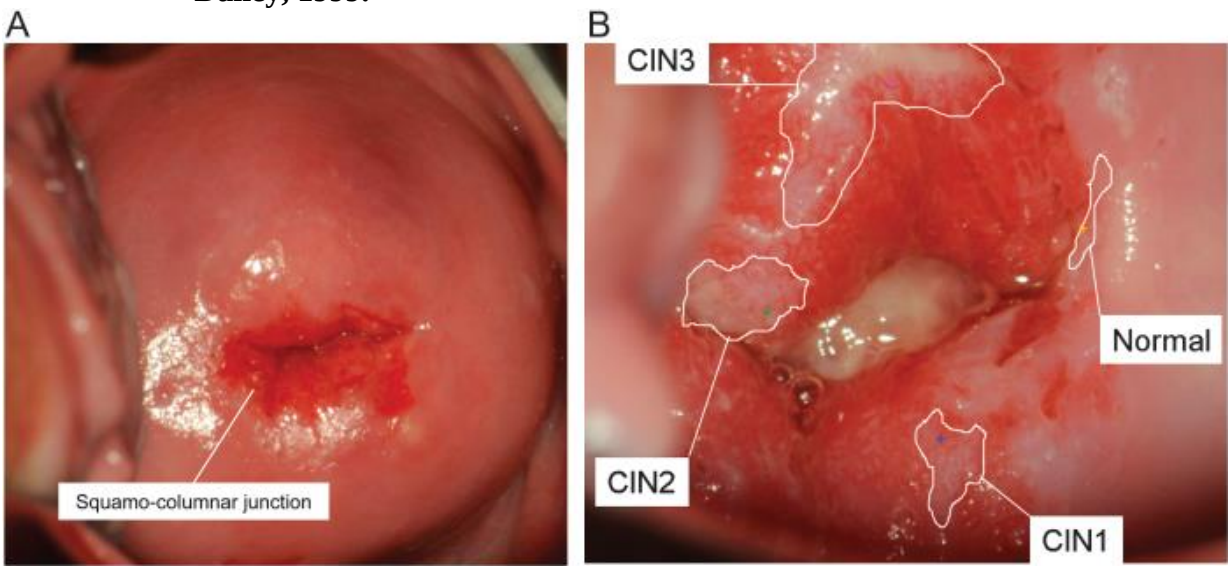

Figure 9: **A and B; shows the cervical transformation zone using a colposcope,**

Source: **Schiffman *et al.*, 2011.**

Transport media for LBC approved for use include SurePath (TripPath Imaging, Inc., Burlinghton NC) and ThinPrep (Cytc Corp, Marlborough, M.A). These media are very expensive and are used alongside specialized equipment which is not available in developing countries like Nigeria.

However, the EziPrep® (EziPrep, India) liquid based cytology is a welcome alternative to SurePath and ThinPrep. It is the world's first economical liquid based cytology and will be appropriate for resource poor countries.

Population-based screening using Pap Test and treatment programmes for pre-malignant lesions of the cervix has significantly reduced the morbidity and mortality associated with cervical cancer with global disparities across racial/ ethnic groups (Adams *et al*., 2013, Eric *et al* 2005, and Grce *et al* 2011).

2.33 Cytological Classification of Smears

Pap test results are classified based on the Bethesda system of classification. This system of classification uses terms and abbreviations which includes; Atypical Squamous Cells of Undetermined Significance (ASC-US), Low-grade Squamous Intraepithelial Lesion (LSIL), Atypical Squamous Cells, cannot exclude High-grade squamous intraepithelial lesion (ASC-H), High-grade Squamous Intraepithelial Lesion (HSIL), Atypical Glandular Cells not Otherwise Specified (AGC-NOS). The figures below compare the two different Pap test methods for various types of smears.

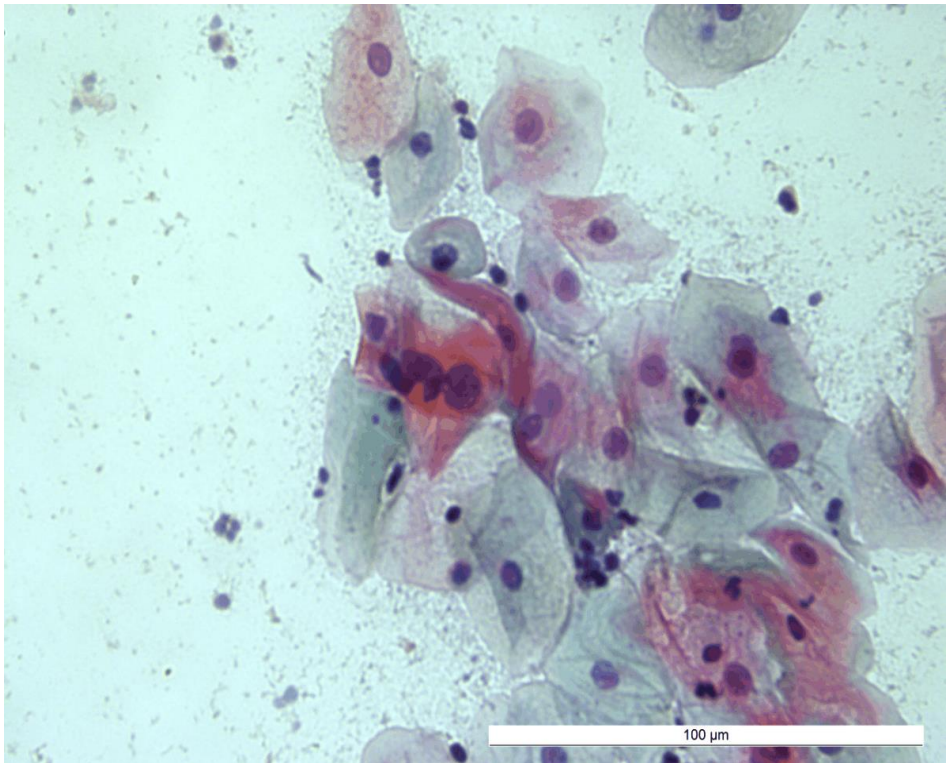

Figure 10: **ASC-US (conventional cytology, Pap stain, ×40).**
Source: **Nicoleta Simion et al., 2014**

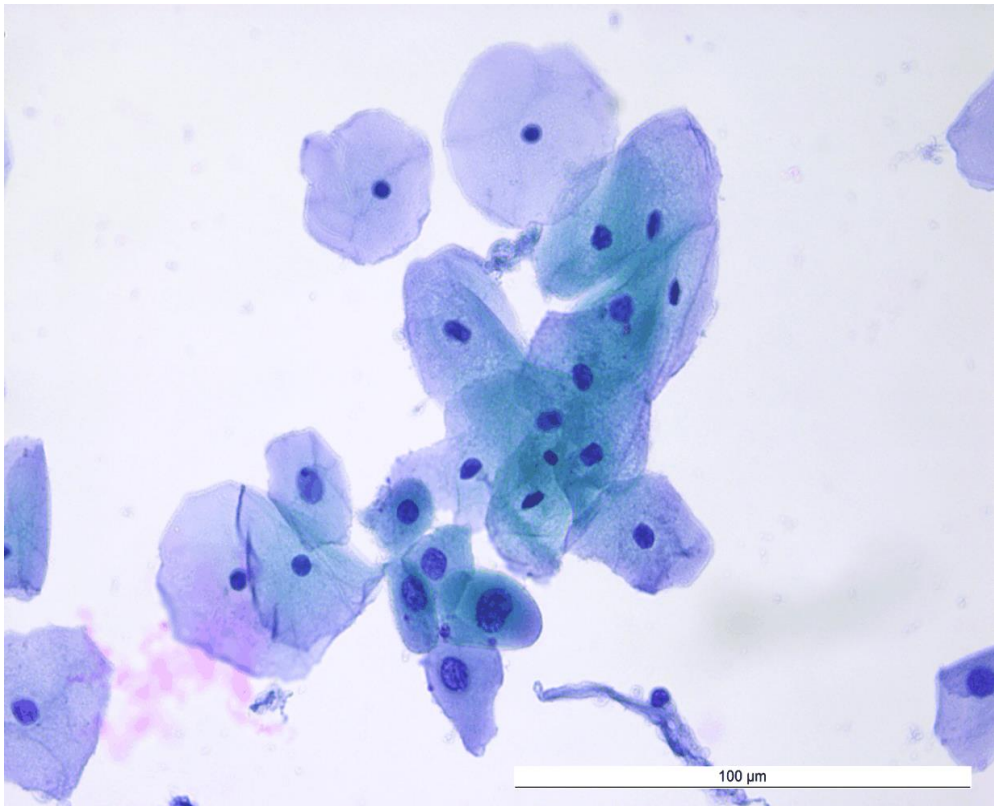

Figure 11: **ASC-US (LBC, Pap stain, ×40)**
Source: **Nicoleta Simion et al., 2014**

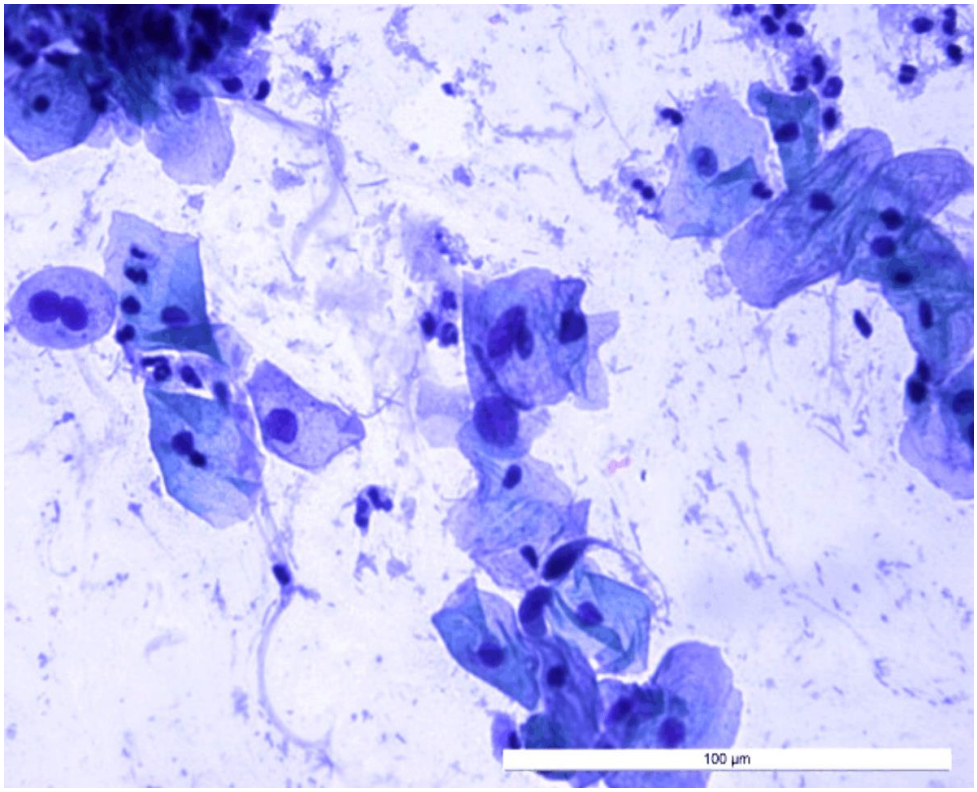

Figure 12: **LSIL (conventional cytology, Pap stain, ×40)**
Source: **Nicoleta Simion et al., 2014**

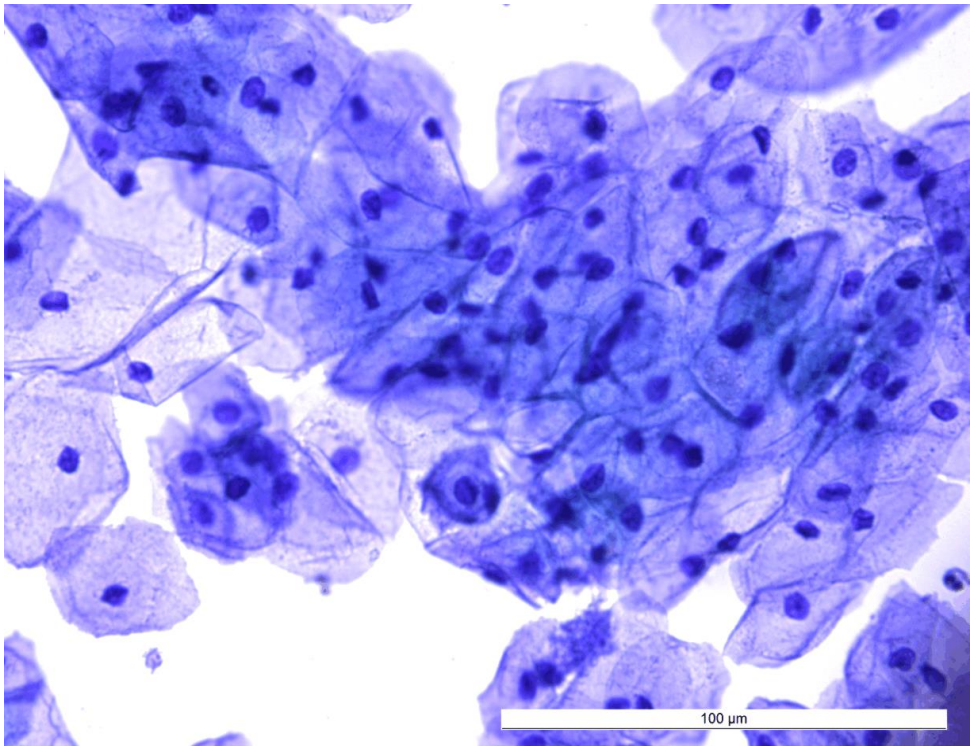

Figure 13: **LSIL (LBC, Pap stain, ×40)**
Source: **Nicoleta Simion et al., 2014**

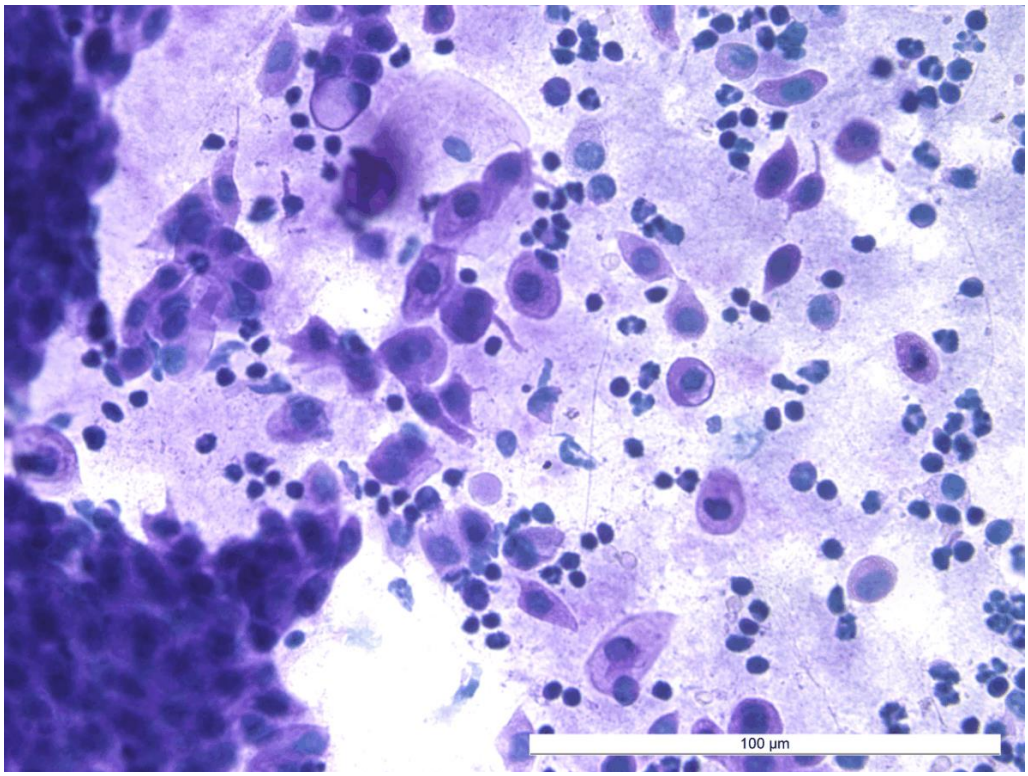

Figure 14: **HSIL (conventional cytology, Pap stain, ×40)**

Source: **Nicoleta Simion et al., 2014**

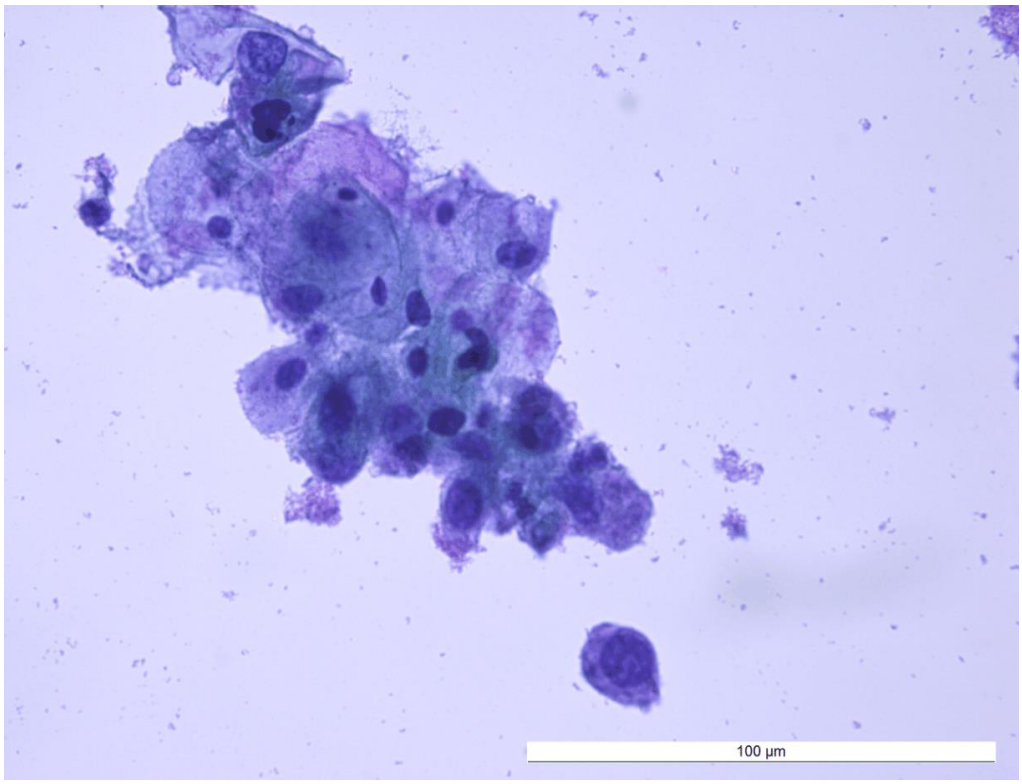

Figure 15: **HSIL (LBC, Pap stain, ×40)**

Source: **Nicoleta Simion et al., 2014**

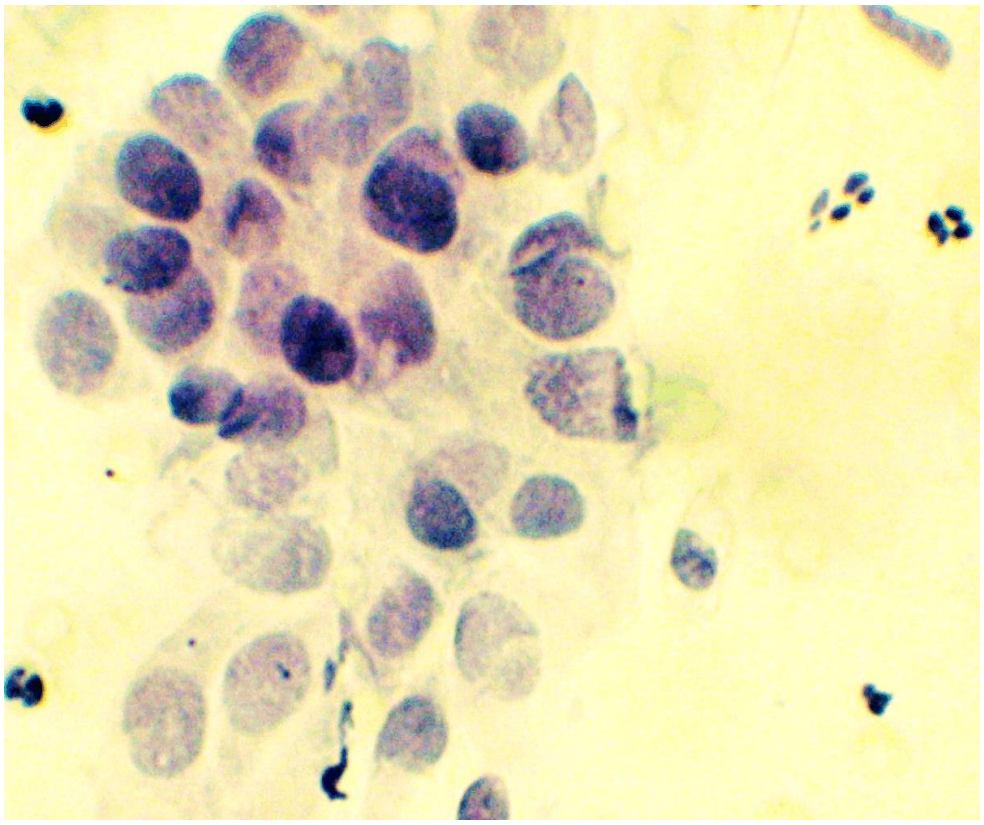

Figure 16: **AGC-NOS (conventional cytology, Pap stain×40)**
Source: **Nicoleta Simion et al., 2014**

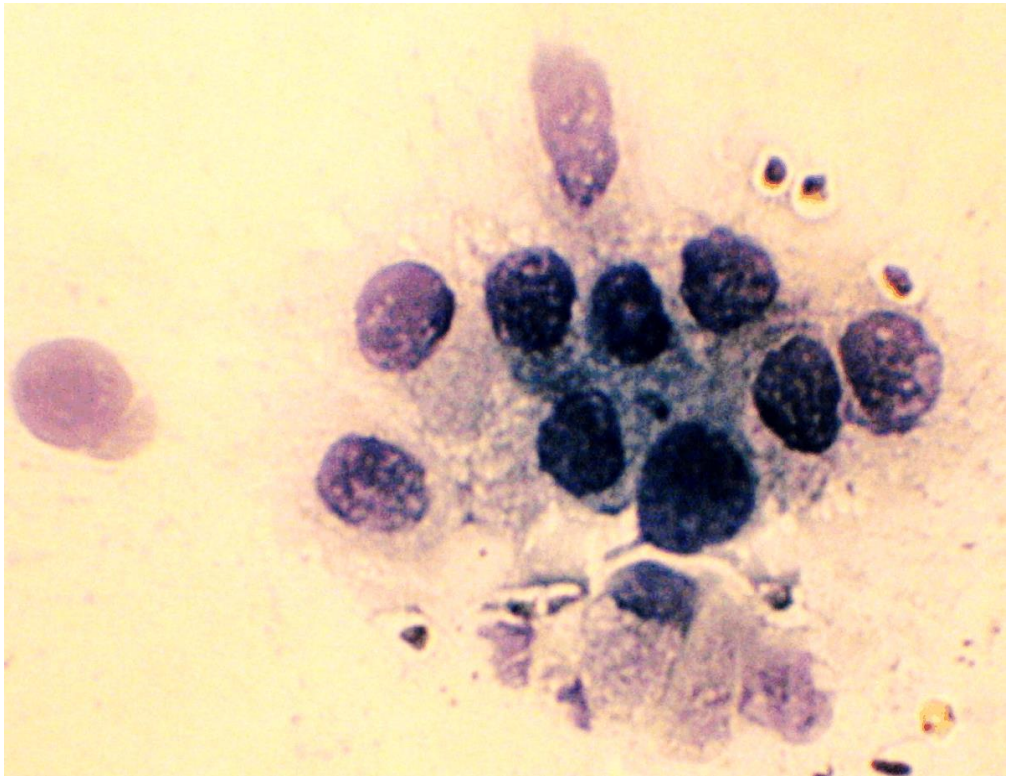

Figure 17: **AGC-NOS (LBC, Pap stain, ×40).**
Source: **Nicoleta Simion et al., 2014**

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Study Design

This study is a hospital based, cross sectional study that carried out cervical screening for women at the Family planning and Gynaecology clinics. The Family planning clinic focused on participants who visited for family planning related matters. On the other hand, the Gynaecology clinics focused on participants who visited for gynaecological related complaints.

3.2 Study Site

This study was carried out in Ilorin Metropolis the capital of Kwara state, one of the 36 states in Nigeria, created on 27 May 1964. Historically, it is believed that Ilorin was founded around 1600 to 1700 AD. It is geopolitically in the North central zone of Nigeria located on the latitude 8.5º North and longitude 4.55º East. There are a total of 16 Local Government areas (LGAs) in Kwara state with 3 LGA located within Ilorin metropolis namely; Ilorin-West, Ilorin-East and Ilorin-South LGA respectively. According to the National Population Census (NPC, 2006), Ilorin has a population of 814,192 out of 2,371,089 Kwara State population with an annual growth rate of 2.3% and a total land mass of 400 km². Ilorin has a number of hospitals which render Family planning and Gynaecological services. The hospitals used for this study include: Gynaecology clinic of Sobi Specialist Hospital located in Alagbado area in Ilorin East-LGA- a predominantly Muslim community with low socio-economic status, Family Planning clinic of Children's Hospital Centre Igboro; a hospital which is also located in Ilorin East-LGA with similar socio-economic status as that of Alagbado and the University of Ilorin Teaching hospital (UITH); a 600-bed hospital, located at Oke-Ose in Ilorin East-Local Government Area of Kwara state. The UITH is a tertiary hospital which serves an urban, semi-urban or rural community and caters for patients from Kwara State and parts of Oyo, Osun,

Kogi, Niger, Ekiti and Ondo States. It predominantly plays the role of a teaching hospital and serves as a referral centre for all other hospitals within Kwara state and other neighbouring states. This study was carried out in the Maternity section of the hospital specifically in the Gynaecology and Family planning clinics respectively. The choices of the clinics used in this study were based on easy assessibilty by patients within the community as well as the presence of facitilities supporting routine Pap test procedure.

3.3 Study Population

The study population included women (out patients) of ages 18-65 who attended family planning and Gynaecology clinics and also consented to participate in the study.

3.4 Sample Size Determination

The formula used for estimating proportions, a modified Fisher's formula was used in the determination of the sample size for this study (Araoye, 2003).

$$n = \frac{z^2pq}{d^2}$$

Where,

z = confidence limits of the survey result, 95% (1.96)

p= proportion of persons in a population expected to be HPV positive

q= (1-p) = proportion of persons in the population not HPV positive

d= [precision for α] = 0.05

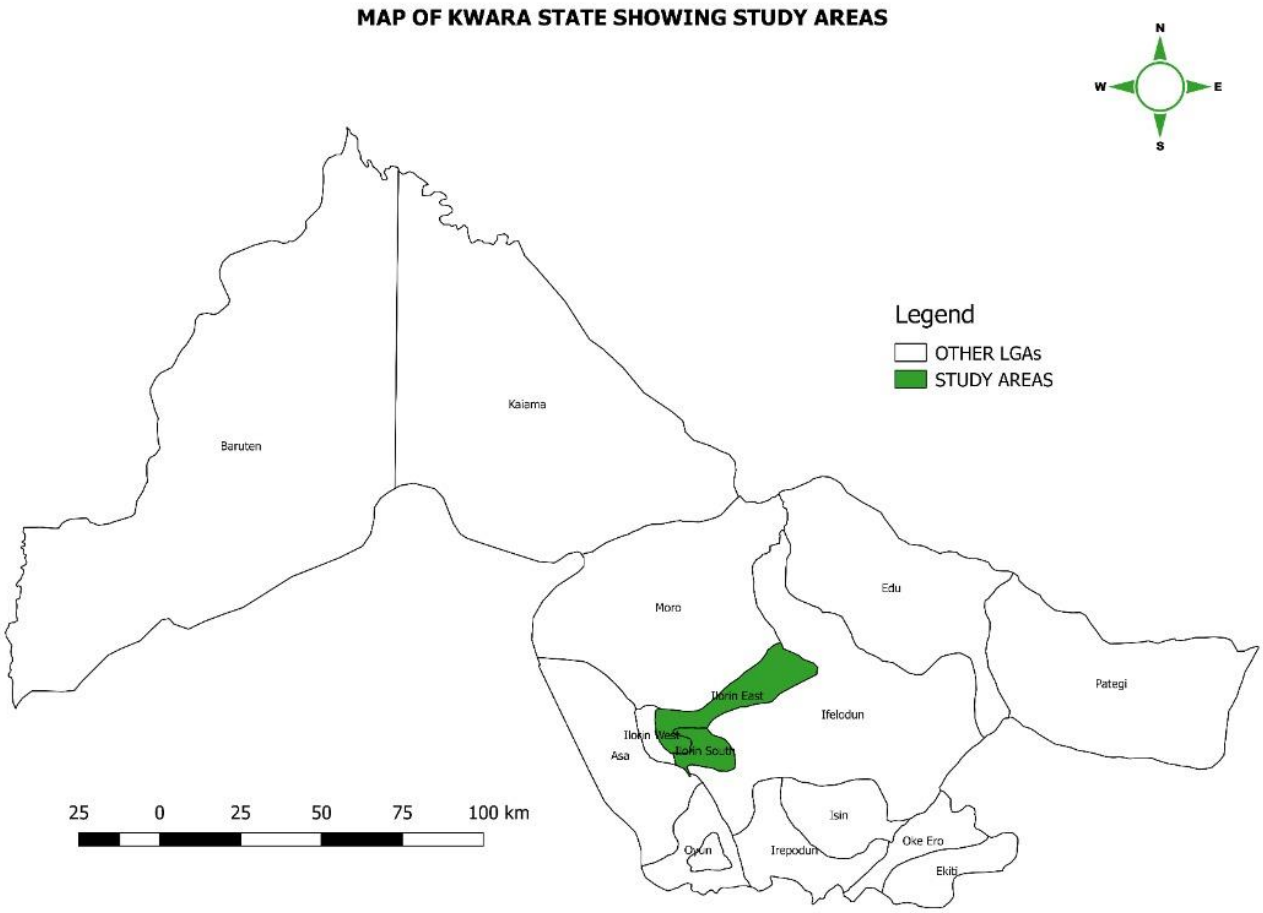

Figure 18: The Map of Kwara showing study area, Source: Designed with Quantum G eographical Information System (QGIS) Version 2.18.13.

The prevalence from the study carried out in Ibadan by Thomas *et al.*, 2004 was used to calculate the sample size for the HPV.

Where the prevalence =26.3%

p= 0.263

$$n = \frac{(1.96)^2 \times 0.263 \times 1-0.263}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.263 \times 0.737}{0.0025}$$

= 297.8

=298

The minimum sample size for HPV is 298. For the purpose of this study, a total of 376 participants were recruited from the Family planning (well) and gynaecology (non-well) clinics. One hundred and eighty-six (186) participants from the gynaecology clinic and 186 participants from the family planning clinic were recruited.

3.5 Inclusion Criteria

This study included the following categories of women:

1. Women between ages 18 and 65
2. Women attending Family planning clinic
3. Women attending Gynaecological clinic
4. Women who consent to participate in the study

3.6 Exclusion Criteria

The study excluded the following categories of women:

1. Pregnant women (Obstetric cases)

2. Post-Partum within the last 6 months
3. Women on the usual monthly menstrual cycle
4. Women bleeding due to gynaecological cause or complications
5. Women who have received partial or complete HPV Vaccines
6. Women who are physically or mentally unable to undergo an interview
7. Women younger than 18 years or older than 65 years
8. Women who had undergone trachelectomy or cervicectomy
9. Virgins

3.7 Sampling Technique

The method of consecutive sampling a non-probability method was used to select consenting participants from both the well and non-well clinics (Lunsford and Lunsford, 1995).

3.8 Sample Collection

Samples of exfoliated cervical cells were obtained from each participant with cytological brush and smeared on a microscopic glass slide before placing in cytopots containing 95% ethanol. The brush was detached into Eziprep preservative solution by Gynaecologists or qualified trained Nurses performing routine Pap test in each of the health facilities and transported in cold chain to the Molecular virology laboratory of the University of Ilorin Central Research laboratories where cytology and molecular analysis were carried out. Deoxyribonucleic acid (DNA) extraction was done and aliquots for Human papillomavirus, *Chlamydia trachomatis* and cytokine analysis were done. Consent were gotten from each participant (Appendix I), and biodata was collected via oral interviews using well-structured pre-tested questionnaires (Appendix II).

3.9 Cytology

The Liquid Based Cytology (LBC) medium (Eziprep) and Conventional Cytology methods were used simultaneously. Exfoliated cervical cells in the Eziprep vials were agitated with a vortex mixer, 3ml of separator solution was added into an empty tube followed by 7 ml of sample, and the mixture was centrifuged at 600 g for 10 minutes followed by the discarding of the supernatant. The pellets were vortexed and re-suspended in 1 or 2 drops of distilled water and fixed on a labelled frosted edge microscopic slide in duplicate before staining (EziPrep Manual 2016). The staining of the LBC slides was done alongside the slides prepared for convetional cytology by washing with 96% alcohol, 80% alcohol, 70% alcohol, 50% alcohol, and with distilled water respectively, and Stained in Harris' hematoxylin solution for 3 min. It was then rinsed under running tap water for 3 min, Washed with 70% alcohol, 80% alcohol, and 96% alcohol. The slides were stained in Orange G solution for 3 min, washed with 96% alcohol, stained in Eosin Azura 50 (EA 50), for 3 min, dehydrated with 96% alcohol, and further dehydrated in absolute alcohol for 5 min, and also dehydrated in xylene (Merck, 2013). The slides were air-dried and sent to a cytopathologist for examination. All women participating in this study were screened for the presence of abnormal cervical cytology by certified cytologists or pathologists. Pap test results were based on the Bethesda system of classification 2001 (Bethesda, 2001).

3.10 DNA Extraction from Exfoliated Cervical cells

Extraction of genomic DNA was performed before aliquots were taken for HPV, CT and cytokine detection by specific primers. FavorPrep™ Genomic DNA Extraction Mini Kit Favorgen (FABGK001-2, Taiwan) was used according to the manufacturer's instructions: The Liquid Based medium (EziPrep) containing cervical samples were vortexed. A 200 μL Eziprep

was transfered to a microcentrifuge tube, where 20 µL Proteinase K and 200 Ml FABG Buffer were added to the sample. It was mixed thoroughly by pulse-vortexing.

Incubation was done at 60 ºC for 15 minutes to lyse the sample. During incubation, the sample was vortexed every 3-5 minutes. The tube was briefly spun to remove drops from the inside of the lid. Also, 200 µL ethanol (96-100%) was added to the sample. Furthermore, the reaction was mixed thoroughly by pulse vortexing for 10 seconds. This was followed by briefly spinning the tube to remove drops from the inside of the lid. Thereafter, a FABG Mini Column was placed to a collection tube. The mixture (including any precipitate) was carefully transferred to the FABG mini column and centrifuged at 6,000 x g for 1 minute. After centrifugation, FABG mini column was placed into a new collection tube. A 400 µL W1 Buffer was added to the FABG Mini Column and centrifuged at full speed (18,000 x g) for 30 seconds before discarding the flow-through. In continuation, 750 µL Wash Buffer was added to the FABG Mini Column and centrifuged at full speed for 30 seconds before discarding the flow-through. A full speed additional centrifugation at 3 minutes was done to dry the column. Finally, the FABG Mini Column was placed into an elution tube and 200µL of heated elution buffer was added to the membrane centre of FABG mini column. The FABG mini column was allowed to stand for 3 minutes before centrifuging at full speed for 1 minute to elute total DNA. The total eluted DNA was stored at -20 ºC.

Qualitative and quantitative analysis of extracted DNA was done using agarose gel electrophoresis and spectrophotometric methods.

3.10.1 Qualitative Analysis of Extracted DNA

The quality of the genomic DNA was examined by gel electrophoresis using 0.7% agarose gel. Two micro-litre of each DNA sample was mixed with 1 µL of 1X DNA loading dye (1X loading dye consists of 4.16 mg bromophenol blue, 4.16 mg xylene cyanol and 0.66

g sucrose in 1ml water) and was loaded in the gel. Electric current was applied at 20 volts until DNA moved into the gel and was raised to 50 volt for rest of the run. Electrophoresis was stopped when the dye had travelled nearly two-third distance of the gel. Gel was visualized by a Gel doc system (InGenius 3 manufactured by SYGENE) under UV light and picture was captured using the gel doc camera.

3.11 HPV DNA Detection

HPV DNA detection was carried out on the samples at the Molecular Virology Laboratory of the University of Ilorin Central Research Laboratories (CRL). Primers used for HPV detection are shown in Table 3.

HPV DNA positive controls of 50 International Unit (IU) per 5 µL (appendix) were a generous gift from Professor Anna-Lise Williamson (Head of WHO/HPV Labnet laboratory for the Africa Region) of the Institute of Infectious Disease and Molecular Medicine and Division of Medical Virology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, South Africa. The HPV DNA negative control was sourced locally from the Virology laboratory in the CRL of the University of Ilorin, using Double Distilled De-ionized water (DDD).

Table 2: List of Primers used for HPV DNA detection

Primer Name	Forward Sequence	Reverse Sequence
MY09/11	5’-CGTCCMAARGGAWACTGATC-3'	5'-GCMCAGGGWCATAAYAATGG-3'
GP5+/6+	5’- TTT GTT ACT GTG GTA GATACT AC-3'	5'- GAA AAA TAA ACT GTA AAT CAT ATTC-3'
β-globin	5'-GAAGAGCCAAGGACAGGTAC-3'	5'-CAACTTCATCCACGTTCACC-3'
HPV_16	5'-CAC AGT TAT GCA CAG AGC TGC-3'	5'-CAT ATA TTC ATG CAA TGT AGG TGT A-3'
HPV_18	5'-CAC TTC ACT GCA AGA CATAGA-3'	5'-GTT GTG AAA TCG TCG TTT TTC A-3'
HPV_31	5'-GAA ATT GCA TGA ACT AAG CTC G-3'	5'-CAC ATA TAC CTT TGT TTG TCA A-3'
HPV_59	5'-CAA AGG GGA ACT GCA AGA AAG-3'	5'-TAT AAC AGC GTA TCA GCA GC-3'
HPV_53	5'-AAA TAC ACA GGA GCG ACC AC-3'	5'-TAC CTC TGA AGC TGT CAA TGC-3'
HPV_45	5'-GTG GAA AAG TGC ATT ACA GG-3'	5'-ACC TCT GTG CGT TCC AAT GT-3'
HPV_33	5'-ACT ATA CAC AAC ATT GAA CTA-3'	5'-GTT TTT ACA CGT CAC AGT GCA-3'
HPV_58	5'-GTA AAG TGT GCT TAC GAT TGC-3'	5'-GTT GTT ACA GGT TAC ACT TGT-3'
HPV_52	5'-TAA GGC TGC AGT GTG TGC AG-3'	5'-CTA ATA GTT ATT TCA CTT AAT GGT-3'
HPV_56	5'-GTG TGC AGA GTA TGT TTA TTG-3'	5'-TTT CTG TCA CAA TGC AAT TGC-3'
HPV_35	5'-CAA CGA GGT AGA AGA AAG CAT C-3'	5'-CCG ACC TGT CCA CCG TCC ACC G-3'
HPV_26	5'-CGA AAT TGA CCT ACG CTG CTA CG-3'	5'-TGG CAC ACC AAG GAC ACG TCT TC-3'
HPV_68	5'-GCA GAA GGC AAC TAC AAC GG-3'	5'-GTT TAC TGG TCC AGC AGT GG-3'
HPV_39	5'-GAC GAC CAC TAC AGC AAA CC-3'	5'-TTA TGA AAT CTT CGT TTG CT-3'
HPV_51	5'-GAG TAT AGA CGT TAT AGC AGG-3'	5'-TTT CGT TAC GTT GTC GTG TAC G-3'
HPV_66	5'-TTC AGT GTA TGG GGC AAC AT-3'	5'-AAA CAT GAC CCG GTC CAT GC-3'
HPV_73	5'-CTT ACA TGT TAC GAG TCA TTG GAC-3'	5'-GTT TCT GGA ACA GTT GGG GCA C-3'
HPV_82	5'-GCT ACG AGC AAT TTG ACA GCT CAG-3'	5'-CAT TGC CGA TGT TAG TTG GTC GCA-3'

3.12 First Round of HPV PCR Amplification

A 450 base pair (bp) fragment from the L1 region of HPV-DNA was amplified by PCR using the consensus primer MY09/MY11 with amplification of the cellular β-globin gene as an internal control as described by Wanderlei-Silva (Wanderlei-Silva *et al*., 2005). Briefly, Polymerase Chain Reaction (PCR) was performed in a 25 µL volume, with the following components and final concentrations: Amplitaq gold master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase), 0.8 µl each of primers MY09/MY11, 0.8 µL each of primers GH20/PC04 and 1.5 µL of DNA template. PCR reactions were subjected to 40 cycles of amplification on a thermal cycler using the following program: denaturation at 95 ºC for 9 min, annealing at 50 ºC for 1 min 30 sec, extension at 72 ºC for 2 min, and final extension at 72 ºC for 5 min. Amplified products were separated by electrophoresis in 1.5% agarose gels, stained with SYBR safe (New England Biolabs U.K) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

3.13 HPV Amplification by Nested PCR

A second round of amplification of the PCR products of the first round amplification was done by nested PCR using the GP5+/6+ primers that target the 150 bp fragment of the L1 region of HPV-DNA with amplification of the cellular β-globin gene as an internal control was carried out. Briefly, Polymerase Chain Reaction (PCR) was performed in a 25 µL volume, with the following components and final concentrations: Amplitaq gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase), 0.25 µL each of primers GP5+/6+ and 2.0 µL of amplicons from first round of PCR. The reactions was subjected to 40 cycles of amplification using the following program: initial denaturation at 95 ºC for 9 min, denaturation at 94 ºC for 30 sec., annealing at 45 ºC for 2 min, extension at 72 ºC for 1 min, 30 sec and final extension at 72 ºC for 4 min. Amplified products

were separated by electrophoresis in 1.5% agarose gels, stained with SYBR safe (New England Biolabs U.K) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

3.14 HPV Genotyping by Nested Multiplex PCR

Positive HPV DNA samples were genotyped by Nested Multiplex PCR technique using different high risk HPV type primers (Table 2). Optimization was carried out and high risk HPV type primers were grouped in two or three in cocktail fashion based on common annealing conditions and amplicon sizes (Table 3). The Nested Multiplex PCR was performed in a 25 μL volume, with the following components and final concentrations: 12.5 μL Amplitaq gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase), 0.6 μL HPV primers set in each cocktail (Table 3) and 2.0 μL of amplicons from the second round of PCR amplification (nested). The reactions were subjected to 40 cycles of amplification using the following program: initial denaturation at 95 ºC for 5 min, denaturation at 95 ºC for 30 sec., annealing at 46, 48, 50, and 54ºC respectively depending on the cocktail multiplex for 1 min, extension at 72 ºC for 45 sec and final extension at 72ºC for 7 min. Amplified products was separated by electrophoresis in 1.5% agarose gels, stained with SYBR safe (New England Biolabs, U.K) and photographed under UV light and Gel doc (Ingenius 3 by SYGENE).

Table 3: HPV Types by nested multiplex showing annealing (T°C) and amplicon sizes

Cocktail	HPV Types	Amplicon Size (bp)	Annealing (T°C)
1	45	151	
	26	239	**46**
	18	322	
2	66	172	
	51	223	**46**
	58	274	
3	56	181	
	73	221	**54**
	35	358	
4	53	118	
	16	457	**48**
5	59	215	
	31	263	**50**
6	52	229	
	39	280	**48**
	33	398	
7	82	240	
	68	333	**54**

3.15 Agarose Gel Electrophoresis for HPV Positive Samples

A 1.5% (w/v) agarose gel was prepared with SYBR safe (New England Biolabs, U.K), 1.5 g agarose powder was added to 100ml of 1X TBE. Agarose was melted in microwave with intermittent mixing. A 2.5 µL volume of SYBR safe (i.e. ratio 1:40,000) was added. Molten agarose was allowed to cool and poured into gel cast with comb and allowed to set for 10-20 minutes before removing cast. The cast was placed with gel into gel tank and comb was removed carefully. The tank was flooded with appropriate volume of 1X TBE buffer to tank. In a new tube (600 µL Eppendorf), gel-loading dye was added to PCR product accordingly in this case, for 5 µL PCR product, 1.0 µL loading dye (6x) was added. The entire volume of dye-loaded PCR product aliquot was dispensed into each well of the gel. Into one well of the gel, a 100bp size DNA-ladder was loaded followed by a positive HPV control and in the third well, a negative HPV control was dispensed. The DNA was separated by electrophoresis at 100 V and 400 A for 30 minutes. Care was taken not to run the dye off the gel. After the separation, the gel was placed on a UV transilluminator, viewed and pictures were taken with the aid of the gel doc system (Ingenius 3 by SYGENE).

3.16 Chlamydia Detection

Chlamydia trachomatis detection was done by PCR on the samples using the consensus primers that detect the MOMP and plasmid of the *Chlamydia trachomatis*, with modifications on primers and PCR cycles (Jalal *et al.*, 2006).

Chlamydia trachomatis positive control was a generous gift from Prof. Dr. Alexander Georg Häcker. Medical Director (Chlamydial study group) Centre for Microbiology and Hygiene Institute of Microbiology and Hygiene, University of Freiburg, Germany. The DNA was from HeLa 229 cells, infected with *C. trachomatis* L2 for 30 h with a Multiplication of Infection (MOI) of 1, and concentration 146.09 ng/uL.

Table 4: ***Chlamydia trachomatis*** **primers**

Primer	**Forward Sequence**	**Reverse Sequence**
MOMP	5'-AACTCAAAACCCTCTCATTCTCAA-3'	5'-AAACGTTCGTCCCAGGAAGAAGCC-3'
Cryptic Plasmid	5'-GCAAGATATCGAGTATGCGTTGTTAGG-3'	5'-TTCATTGTACTCATTAAACGAGCGG-3'
β-globin	5'-GAAGAGCCAAGGACAGGTAC-3'	5'-CAACTTCATCCACGTTCACC-3'

The CT DNA negative control was sourced locally from the Virology laboratory in the CRL of the University of Ilorin, using Double Distilled De-ionized water (DDD).

A 201 bp fragment from the L1 region of Chlamydia-DNA was amplified by PCR using the consensus primers with amplification of the cellular β-globin gene as an internal control. Briefly, PCR amplification reactions were performed in 25 µL volumes, with the following components and final concentrations: Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase), 1.2 µL of Major Outer Membrane Protein primers or Cryptic plasmid primers, 1.2 µL of β-globin (GH20/PC04) primers and 1.5 µL DNA template. PCR reactions were subjected to 40 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95ºC for 5 min, denaturation 1 min, annealing at 60 ºC for 1 min, extension at 72 ºC for 1 min, and final extension at 72 ºC for 5 min. Amplified products were separated by electrophoresis in 1.5% agarose gels, stained with SYBR safe and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

3.17 Agarose Gel Electrophoresis for Chlamydia detection

A 1.5% (w/v) agarose gel was prepared with SYBR safe, 0.6 g agarose powder was added to 100ml of 1X TBE. Agarose was melted in microwave with intermittent mixing. A 2.5 µL SYBR safe (i.e. ratio 1:40,000) was added. Molten agarose was allowed to cool and poured into gel cast with comb and allowed to set for 10-20 minutes before removing cast. The cast was placed with gel into gel tank and comb was removed carefully. The tank was flooded with appropriate volume of 1X TBE buffer. In a new tube (600 µL Eppendorf), gel-loading dye was added to PCR product accordingly in this case, for 5 µL PCR product, 1.0 µL loading dye (6x) was added. The entire volume of dye-loaded PCR product aliquot was dispensed into each well of the gel. Into one well of the gel, a 100 bp size DNA-ladder was loaded followed by a

positive *Chlamydia trachomatis* control and in the third well, a negative *Chlamydia trachomatis* control was dispensed. The DNA was separated by electrophoresis at 100 V, 400 A for 50 minutes. Care was taken not to run the dye off the gel. After the separation the gel was placed on a UV transilluminator and viewed before image was captured by the Gel doc-camera(Ingenius 3, SYGENE).

3.18 Qualitative analysis of Cytokine gene Polymorphisms by Amplification Refractory Mutation System PCR (ARMS-PCR)

3.18.1 IL-10

The amplification refractory mutation system PCR (ARMS-PCR) method was used for Short Nucleotide Polymorphism (SNP) genotyping of IL-10, as described by Perrey *et al*., 1999 and modified by da Silver *et al*., 2015. Briefly, two reactions containing an antisense generic primer and a sense allele-specific primer were performed for each polymorphism. The primers used in this study were antisense generic primer 5'-CAGTGCCAACTGA GAATTTGG-3' and two sense allele-specific primers namely; 1 (G) 5'-CTA CTA AGG CTT CTT TGG GAG-3' and 2 (A) 50-ACTACTAAGGCTTCTTTGG GAA-3' for the SNP at location-1082 (G/A). All PCR reactions had a final volume of 25µL, containing 2µL of DNA, Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase, 10 pmol of each primer. PCR reactions were subjected to 35 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95 ºC for 3 min, denaturation 40 sec, annealing at 60 ºC for 40 sec, extension at 72 ºC for 1 min, and final extension at 72 ºC for 7 min. The size of the fragment was 258 bp for the -1082. Human growth hormone was used as an internal control to confirm the success of the reaction, with primers 1 (5'-GCCTTCCCAACCATTCCCTTA-3') and 2 (5'-TCACGGATTTCTGTTGTGT TTC-3') generating a 429bp product. Amplified products was

separated by electrophoresis in 2% agarose gels, stained with SYBR safe (New England Biolabs) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

3.18.2 TNF-α

Briefly, two reactions containing an antisense generic primer and a sense allele-specific primer were performed for each polymorphism. The primers used in this study were antisense generic primer 5'- TCT CGG TTT CTT CTC CAT CG -3' and two sense allele-specific primers namely; 1 (G) 5'- ATA GGT TTT GAG GGG CAT GG -3' and 2 (A) 5- AAT AGG TTT TGA GGG GCA TGA -3' for the SNP at location-308 (G/A). All PCR reactions had a final volume of 25 µL, containing 2µL of DNA, Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase) and 10 pmol of each primer. PCR reactions were subjected to 35 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95ºC for 3 min, denaturation 40 sec, annealing at 60 ºC for 40 sec, extension at 72 ºC for 1 min, and final extension at 72 ºC for 7 min. The size of the fragment was 184 bp for the -308. Human growth hormone was used as an internal control to confirm the success of the reaction, with primers 1 (5'-GCCTTCCCAACCATTCCCTTA-3') and 2 (5'-TCACGGATTTCTGTTGTGT TTC-3') generating a 429 bp product. Amplified products was separated by electrophoreses in 2% agarose gels, stained with SYBR safe and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

3.18.3 IFN- γ

Interferon gamma gene polymorphism has been described (Pravica *et al.*, 2000). Briefly, two reactions containing an antisense generic primer and a sense allele-specific primer were performed for each polymorphism. The primers used in this study were antisense generic

primer 5'- TCA ACA AAG CTG ATA CTC CA -3' and two sense allele-specific primers namely; 1 (T) 5'- TTC TTA CAA CAC AAA ATC AAA TCT -3' and 2 (A) 5'- TTC TTA CAA CAC AAA ATC AAA TCA -3' for the SNP at location +874 (T/A). All PCR reactions had final volumes of 25 µL, containing 2 µL of DNA, Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase) and 10 pmol of each primer. PCR reactions was subjected to 35 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95 ºC for 3 min, denaturation 40 sec, annealing at 55 ºC for 40 sec, extension at 72 ºC for 1 min, and final extension at 72 ºC for 7 min. Human growth hormone was used as an internal control to confirm the success of the reaction, with primers 1 (5'-GCCTTCCCAACCATTCCCTTA-3') and 2 (5'-TCACGGATTTCTGTTGTGT TTC-3') generating a 429 bp product. Amplified products was separated by electrophoreses in 2% agarose gels, stained with SYBR safe (New England Biolabs) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

.

3.18.4 TGF-β codon 10

Briefly; two reactions containing a sense generic primer and two anti-sense allele-specific primers were performed for each polymorphism. The primers used in this study were sense generic primer 5'- TCC GTG GGA TAC TGA GAC AC -3' and two anti-sense allele-specific primers namely; 1 (C) 5'- GCA GCG GTA GCA GCA GCG -3' and 2 (T) 5'- AGC AGC GGT AGC AGC AGC A -3' for the SNP at codon 10 (C/T). All PCR reactions had final volumes of 25 µL, containing 2 µL of DNA, Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase) and 10 pmol of each primer. PCR reactions was subjected to 35 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95ºC for 3 min, denaturation 40 sec, annealing at 55 ºC for 40 sec, extension at 72 ºC for 1 min, and final extension at 72 ºC for 7

min. The size of the fragment was 241 bp. Human growth hormone was used as an internal control to confirm the success of the reaction, with primers 1 (5'-GCCTTCCCAACCATTCCCTTA-3') and 2 (5'-TCACGGATTTCTGTTGTGT TTC-3') generating a 429 bp product. Amplified products was separated by electrophoreses in 2% agarose gels, stained with SYBR safe (New England Biolabs) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

.

3.18.5 TGF-β codon 25

Briefly; two reactions containing an anti-sense generic primer and two sense allele-specific primers were performed for each polymorphism. The primers used in this study were anti-sense generic primer 5'- GGC TCC GGT TCT GCA CTC -3' and two sense allele-specific primers namely; 1 (G) 5'- GTG CTG ACG CCT GGC CG -3' and 2 (C) 5'- GTG CTG ACG CCT GGC CC-3' for the SNP at codon 25 (G/C). All PCR reactions had final volumes of 25 µL, containing 2 µL of DNA, Amplitaq Gold 360® master mix (1X Taq buffer, deoxiribonucleoside triphosphates (dNTPs), $MgCl_2$ and 2U *Taq* DNA polymerase) and 10 pmol of each primer. PCR reactions was subjected to 35 cycles of amplification on a thermal cycler using the following program: initial denaturation at 95 ºC for 3 min, denaturation 40 sec, annealing at 55 ºC for 40 sec, extension at 72 ºC for 1 min, and final extension at 72 ºC for 7 min. The size of the fragment was 233 bp. Human growth hormone was used as an internal control to confirm the success of the reaction, with primers 1 (5'-GCCTTCCCAACCATTCCCTTA-3') and 2 (5'-TCACGGATTTCTGTTGTGT TTC-3') generating a 429 bp product. Amplified products was separated by electrophoreses in 2% agarose gels, stained with SYBR safe (New England Biolabs) and photographed under UV light and Gel doc (Ingenius 3, SYGENE).

Table 5: Primer Sequence for Selected Pro and Anti Inflammatory Cytokines

Primer Name	Generic	First Allele	Second Allele
IL-10	CAG TGC CAA CTG AGA ATT TGG	CTA CTA AGG CTT CTT TGG GAG	ACT ACT AAG GCT TCT TTG GGA A
TNF-α	TCT CGG TTT CTT CTC CAT CG	ATA GGT TTT GAG GGG CAT GG	AAT AGG TTT TGA GGG GCA TGA
TGF-B1C10	TCC GTG GGA TAC TGA GAC AC	GCA GCG GTA GCA GCA GCG	AGC AGC GGT AGC AGC AGC A
TGF-B125	GGC TCC GGT TCT GCA CTC	GTG CTG ACG CCT GGC CG	GTG CTG ACG CCT GGC CC
IFN-ϒ	TCA ACA AAG CTG ATA CTC CA	TTC TTA CAA CAC AAA ATC AAA TCT	TTC TTA CAA CAC AAA ATC AAA TCA
Internal Control		GCC TTC CCA ACC ATT CCC TTA	TCA CGG ATT TCT GTT GTG TTT C

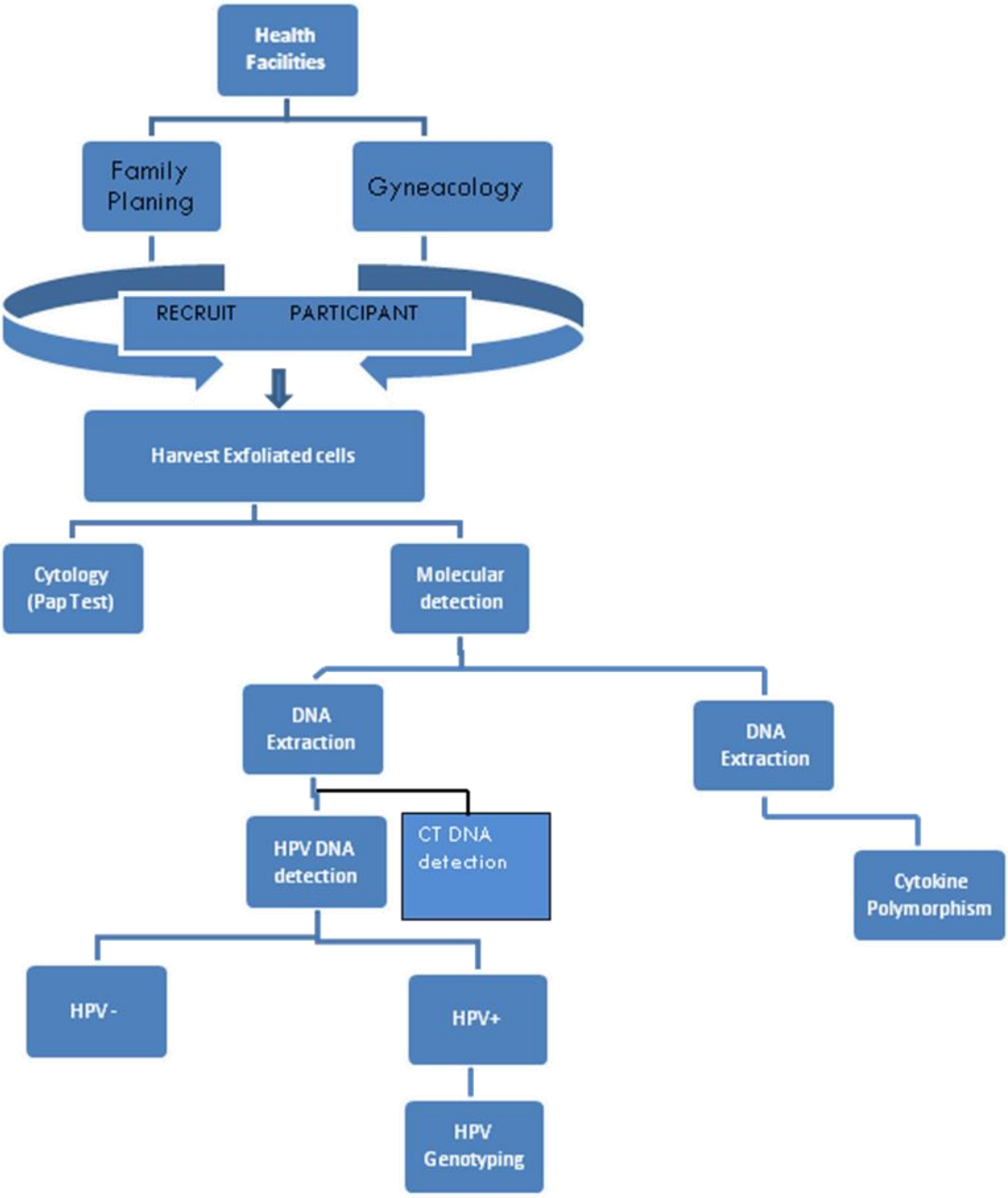

Figure 19: Study flow chart

3.19 Ethical Approval

All women received information on the study design and provided written consents (Appendix I). Approvals were obtained from the University of Ilorin Ethical Review Committee (Appendix III), UITH Ethical Review Committee (Appendix IV) and the Kwara State Ministry of Health, Ilorin (Appendix V).

3.20 Statistical Analysis

Data collection from administered structured pre-tested questionnaire were analysed using softwares: Epi info version 7.14 (2014) by Centre for Disease Control and Prevention (CDC), United States of America, Graph Pad and Statistical Packages for Social Sciences (SPSS), to calculate mean and standard deviation, chi-square test and student t-test which was used to test for statistical significance of the difference for discreet and continuous variables respectively. Log-binomial regression, multiple regression and linear regression models was used to examine the association between potential correlates, Odd ratios (ORs) for HPV and *Chlamydia trachomatis* positivity and co-infection of HPV and *Chlamydia trachomatis* respectively. Results have been presented in tables and charts. Statistical significance was determined at the probability level of $p<0.005$.

CHAPTER FOUR

4.0 RESULTS

4.1 Pap Test findings of the study population

Table 6 shows a summary of the cytology results of the participating women at different age group. A large number of the participants presented with normal cytology which is negative for intra-epithelial neoplasia and malignancy (94.4%). A small proportion of the women presented with abnormality of different grades. The highest case of abnormality was seen in age group 39-45 (6.2%).

Table 7 shows the distribution of abnormal cytology among different age groups. A total of 19 women had abnormal cytology presentation the highest type of abnormality was in Low Squamous Intra-epithelial Neoplasia (LSIL) 68.4% with ages 39-45 recording the highest number of women (5 women). No case of LSIL was recorded in ages 18-24 and 60-66. Atypical Squamous cell of undetermined significance (ASCUS) had only four cases (21.1%) with one case each in ages; 18-24, 32-38, 46-52 and 60-66. High Squamous Intra-epithelia Lesion (HSIL) had the lowest frequency of occurrence of only two women (10.5%), one each in ages 32-38 and 46-52.

Table 6: A Summary of Cytological Result of Participants in this Study

Age Group (years)	CYTOLOGY		
	Normal (n=357) N (%)	**Abnormal (n=19) N(%)**	**Total N (%)**
18-24	16(94.1)	1(5.9)	17(100)
25-31	79(97.5)	2(2.5)	81(100)
32-38	93(95.9)	4(4.1)	97(100)
39-45	76(93.8)	5(6.2)	81(100)
46-52	63(94.0)	4(6.0)	67(100)
53-59	19(90.5)	2(9.5)	21(100)
60-66	9(90.0)	1(10.0)	10(100)
Total	**357(94.9)**	**19(5.1)**	**376(100)**

A summary of the cytology result among the study population with most women Negative for Intra-epithelia Neoplasia or Malignancy (94.9%) and a small proportion (5.1%) presenting with abnormality. N(%)=Number of cases and percentage, n=sub total of specific class.

Table 7: Distribution of Abnormal Cytology among different age group

AGE GROUP (YEARS)	LSIL	ASCUS	HSIL	TOTAL
18-24	0(0.0)	1(100)	0(0.0)	1(100)
25-31	2(100)	0(0.0)	0(0.0)	2(100)
32-38	2(50.0)	1(25.0)	1(25.0)	4(100)
39-45	5(100)	0(0.0)	0(0.0)	5(100)
46-52	2(50.0)	1(25.0)	1(25.0)	4(100)
53-59	2(100)	0(0.0)	0(0.0)	2(100)
60-66	0(0.0)	1(100)	0(0.0)	1(100)
Total	**13(3.5)**	**4(1.1)**	**2(0.5)**	**19(5.1)**

This Table shows the distribution of abnormal cytology among the different age groups with the highest case of LSIL occurring among age group 39-42. In ASCUS, ages 18-24, 32-38 and 60-66 had one case each while HSIL had the lowest frequency of one each in ages 32-38 and 46-52.

4.2 Selected Photomicrographs of Normal and Abnormal Smears by Pap Test

Figure 20 is a representative photomicrograph of women whose exfoliated cells were collected and prepared by LBC method and later stained by Pap Test and viewed under a magnification lens of X40 objective. The cells in image **a** appear as normal and are said to be Negative for intra-epithelial Lesion or Malignancy (NILM), Image **b** shows changes in the squamous cells whose significance are not very clear and therefore refered to as Atypical Squamous Cell of Undetermined Significance (ASCUS). Image **c** reveals abnormal squamous cells gradually forming into mild lesions capable of further degeneration. This lesion is known as Low Squamous Intra-epithelial Lesion (LSIL). If the cells of LSIL fail to resolve, it can further degenerate into a High Squamous Intra-Epithelial Lesion (HSIL) shown in image **d**.

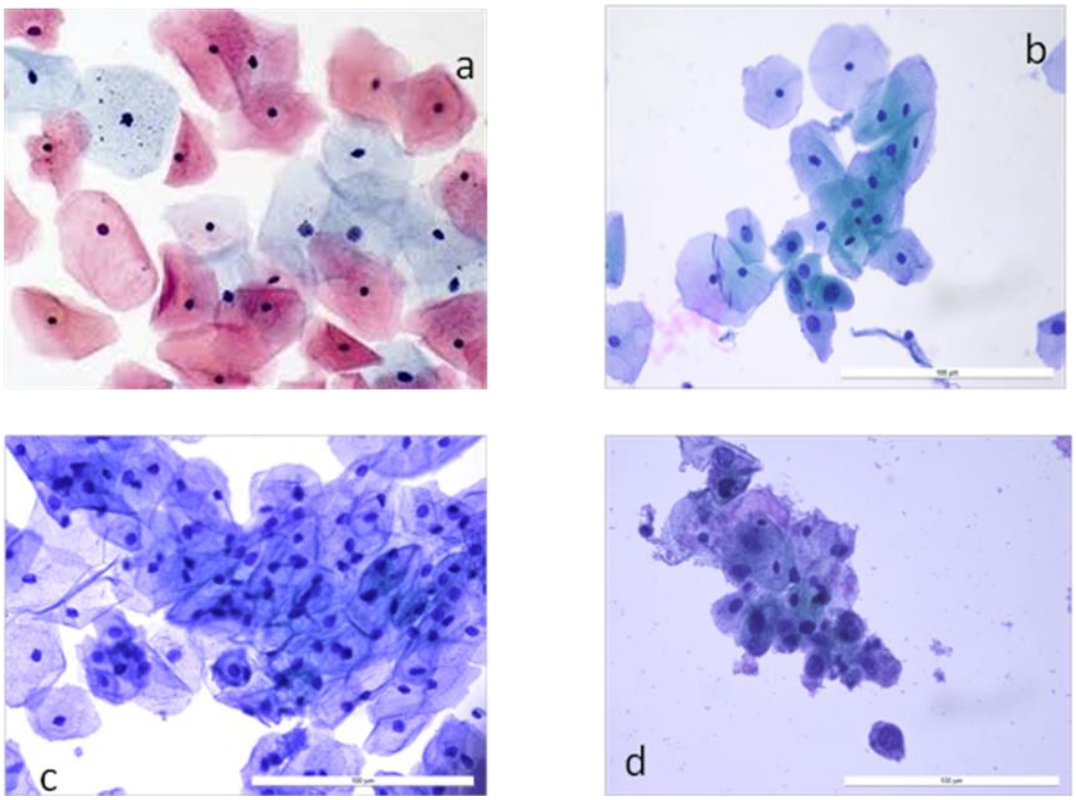

Figure 20: The Photomicrograph of squamous epithelial cells

Representing different possible stages that may progress to carcinoma. NILM (*LBC, Pap stain x 40*), is shown in image **a** with cells appearing normal with nucleus surrounded by cytoplasm. **b** shows image of changes in the squamous cells with very few cells showing bloated nucleus with no clear cut significance reffered to as ASCUS (*LBC, Pap stain x 40*), **c** reveals changes in the squamous cells with prominent numbers of enlarged nucleus reffered to as LSIL (*LBC, Pap stain x 40*), **d** shows cellular changes with increased number of enlarged nucleus and shrinked or disappearing cytoplasm which is refered to as HSIL (*LBC, Pap stain x 40*).

4.3 Agarose Gel Electrophoresis Results of HPV DNA Positive Samples

Figure 21 shows the representative Gel Picture for HPV DNA detection resolved on a 1.5% agarose gel and viewed under imaging and gel documentation system (Ingenius 3). Bands that correspond to the base pair size (bp) of the Positive Control p (450 bp) using the molecular ruler were considered positive for HPV DNA while all other non corresponding bands were regarded as negative.

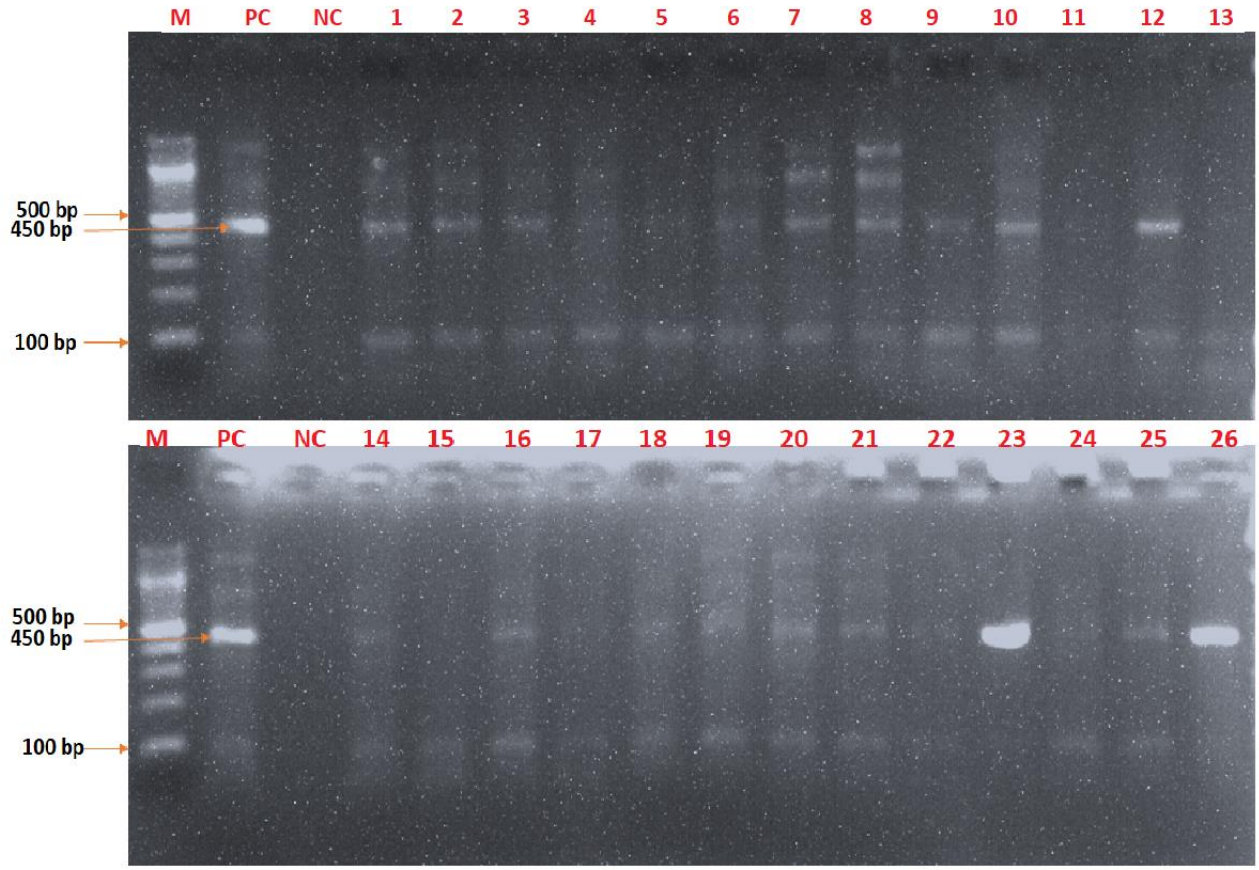

Figure 21: Representative gel picture of HPV DNA positive samples.

A 100 bp ladder denoted as M was used as the molecular ruler followed by a positive (PC) and Negative control (NC). Samples 1,2,3,7,8,10,16,20,21,23,25 and 26 (including all other samples) which had 450 bp fragments corresponding to the PC were all considered positive for HPV DNA while the rest were regarded as negative.

4.4 Prevalence of HPV among Participants in Ilorin

Table 8: Prevalence of Human Papillomavirus among participants of different age group in this study. The Prevalence of HPV among the participants is 81.4% in 306 cases. A total of 18.6% were negative in 70 cases. The age group with the highest occurrence of HPV infection are women in the age group 32-38. All women in age group 60-66 (10) had HPV infection (100%).

Table 8: Prevalence of Human Papillomavirus among participants of different age group in this study

Age Group (Years)	HPV Positive (n=306) N(%)	HPV Negative (n=70) N(%)	Total	*P*-Value
18-24	13(76.5)	4(23.5)	**17(100)**	
25-31	65(80.2)	16(19.8)	**81(100)**	
32-38	82(84.5)	15(15.5)	**97(100)**	
39-45	63(75.9)	20(24.1)	**83(100)**	
46-52	56(83.6)	11(16.4)	**67(100)**	
53-59	17(81.0)	4(19.0)	**21(100)**	
60-66	10(100)	0(0.0)	**10(100)**	
Total	**306(81.4)**	**70(18.6)**	**376(100)**	0.044

The table reveals HPV prevalence in the study where n=total possible outcomes, N=the number of participants in each group and %= is the percentage per number in a each group. The prevalence was generally high with no significant association between HPV infection and age group in years.

4.4b Prevalence of HPV among Participants in Ilorin

Figure 22: Prevalence of Human Papillomavirus among participants of different age group in this study is represented on a line graph. The graph shows multiple peaks of infection with distinction in age groups 32-38 and 60-66 which represents the modal peak.

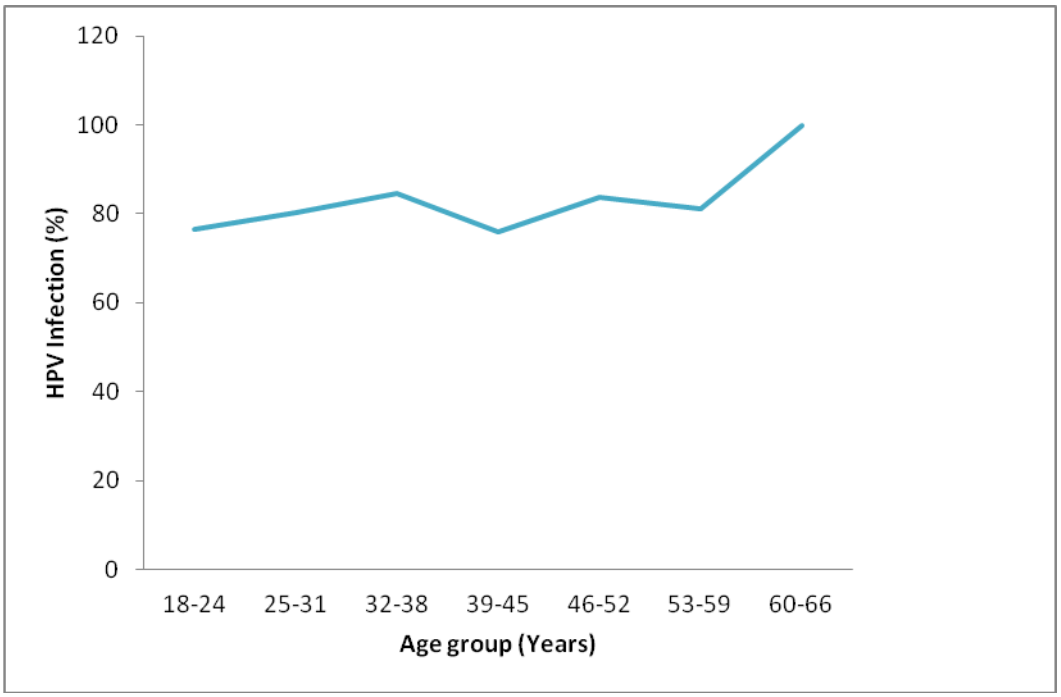

Figure 22: Line graph of HPV prevalence by age groups.

The HPV infection prevalence is shown to peak at multiple age group with the highest peaks in 60-66 and 32-38 years.

4.5 HPV genotyping By Nested Multiplex PCR method

The Agarose Gel Electrophoresis picture is a representation of HPV genotyping of multiple types in a single assay for distinct base pairs with similar PCR conditions pooled together in a cocktail fashion. Figure 23 represents the genotyping of HPV types 45, 26 and 32 with positive bands corresponding to 151, 239 and 322 repectively

Figure 24 on the other hand, represents the genotyping of 82 and 68 with positive bands corresponding to base pairs 240 and 333 respectively.

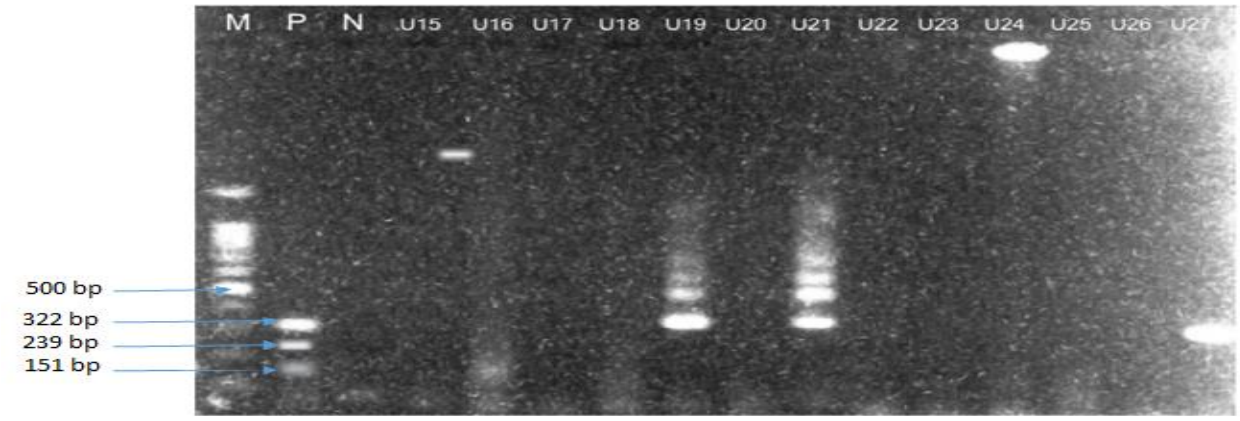

Figure 23: Representative gel picture of the nested Multiplex PCR for the detection of 3 high risk HPV types

Namely; 45(151 bp), 26 (239 bp) and 18(322 bp) repectively A 100 bp ladder denoted (M) was used followed by positive controls (P) and Negative control (N). Samples U4 had multiple high risk HPV (45&26) infections, wheras, U9, U19 and U21 were considered positive for high risk type 18.

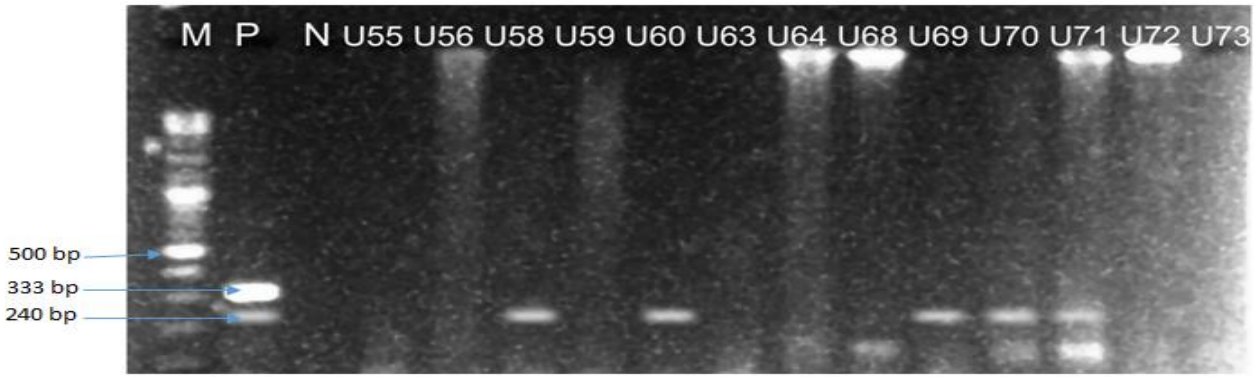

Figure 24: Representative gel image of the nested Multiplex PCR for the detection of 2 high risk HPV types

Namely; 82(240 bp), 68 (333 bp) repectively. A 100 bp ladder denoted (M) was used followed by positive controls (P) and Negative control (N). Samples U58, U60,U69-U71 all had high risk HPV single infection for type 82. No amplified DNA product in the gel correspond to base pair 333 (type 68).

4.6 Prevalence of High Risk Human Papillomavirus

Table 9: Shows the prevalence of high risk Human Papillomavirus among participants of different age group in the study. A total of 201 women (53.5%) were positive for one or more of the 18 high risk types. A total of 175 women 46.5% were negative for the 18 known high risk types. In relation to age groups, age group 32-38 years had the highest prevalence of high risk HPV infection while the lowest was in age group 60-66 years.

Figure 25 is a line graph representing high risk HPV in different age groups the highest peak was in age group 32-38 while the lowest peak was in age group 53-59 years.

Figure 26 is a pie chart showing the distribution of HPV Genotypes among women in Ilorin. A total of 18 high risk genotypes and a group of untyped genotype are represented in the pie chart. HPV type 82 had the highest proportion of 126 (33.5%), while HPV 52 had the lowest proportion 1 (0.3%).

Table 9: Prevalence of high risk Human Papillomavirus among participants of different age group in the study

Age Group (Years)	HRHPV Positive	HRHPV Negative	Total
	N(%)	N(%)	
18-24	8(47.1)	9(52.9)	**17(100)**
25-31	46(56.8)	35(53.2)	**81(100)**
32-38	57(58.8)	40(41.2)	**97(100)**
39-45	45(54.2)	38(45.8)	**83(100)**
46-52	34(50.7)	33(49.3)	**67(100)**
53-59	7(33.3)	14(66.7)	**21(100)**
60-66	4(40.0)	6(60.0)	**10(100)**
Total	**201(53.5)**	**175(46.5)**	**376(100)**

Table 9 represents high risk HPV prevalence. HRHPV=High risk HPV, N=Sub total, %=percentage.

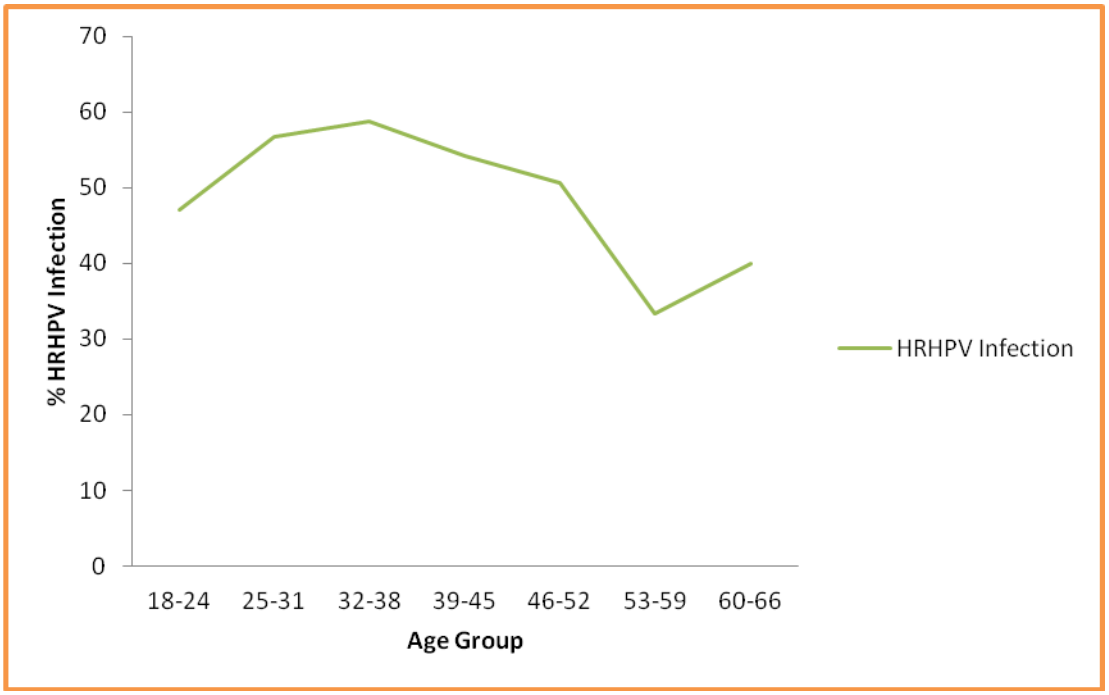

Figure 25: Prevalence of HRHPV Infection by Age Group

This shows a rise from age group 18-24 to 25-31 with modal peak in age group 32-38 followed by a dip at 46-52 years then a further rise between 53-59 years.

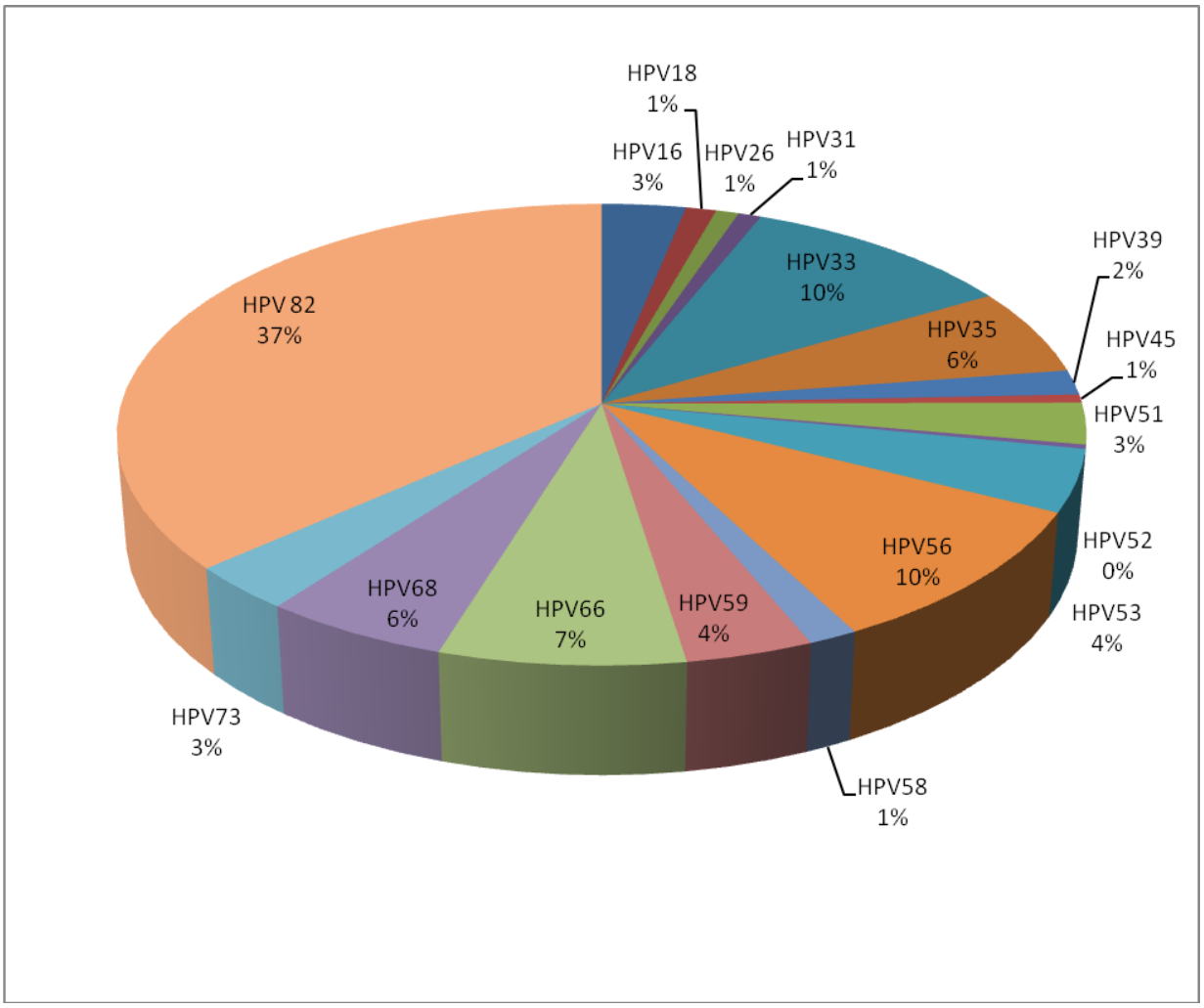

Figure 26: Distribution of HRHPV Genotypes among women in Ilorin.

The highest frequency is in genotype 82 (37%) represented by a large orange slice of the chart followed by HPV 33 and 56 with 10% slice each.

4.7 Prevalence of Single, Multiple and Untyped HPV among the participants

Figure 27 shows a multiple bar graph of age specific prevalence of high-risk; Multiple, single and untyped HPV infection among women in Ilorin. Age group 32-38 has the highest frequency of occurrence of the single, multiple high risk types and untyped HPV infection.The lowest frequency of occurrence for all categories of HPV infection in the study was among the geriatric (age group 60-66) years.

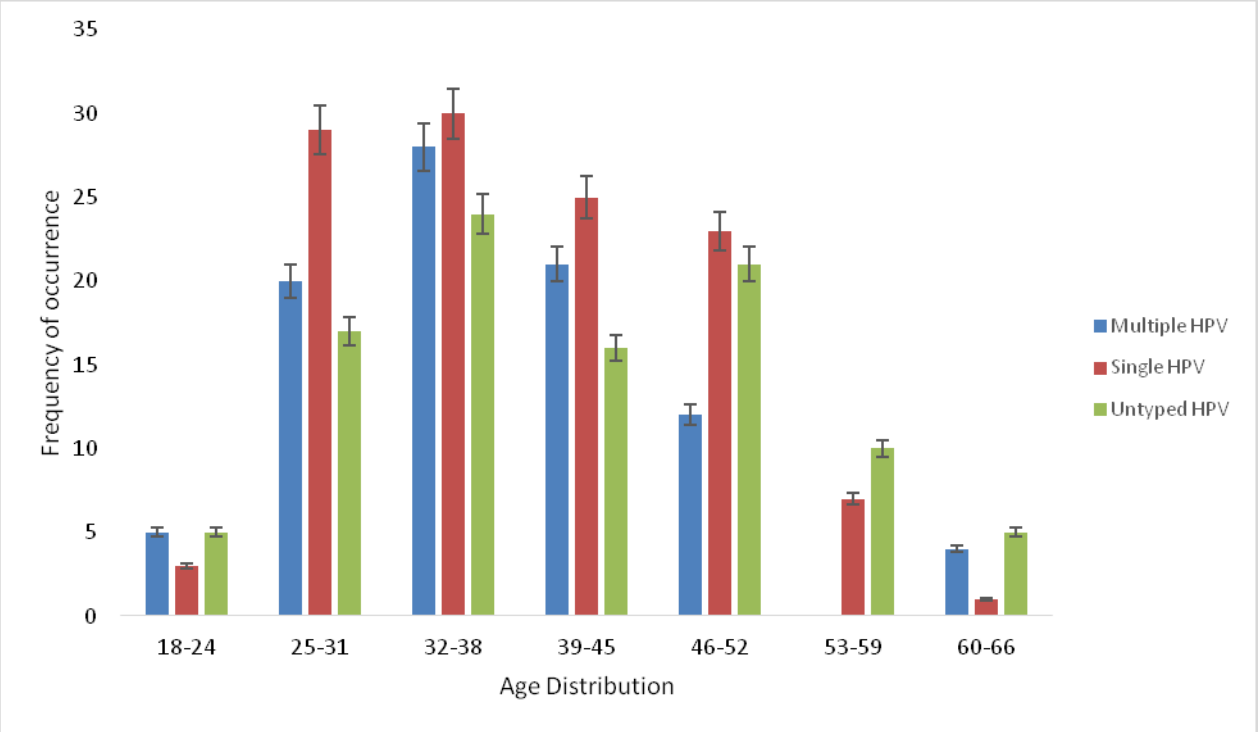

Figure 27: A multiple bar graph showing the Age specific prevalence of high-risk Multiple HPV infection, high risk single HPV infection and untyped HPV infection among women in Ilorin.

The multiple bars are represented by three different colours namely; blue (representing women with multiple HPV infections), brown (representing women with a single HPV infection) and green (representing women with untyped HPV infection which excludes the 18 HRHPV types).

4.8 Classification of High Risk HPV in Ilorin in Relation Available candidate vaccines

Figure 28: is a line graph showing Classification of infection with High risk HPV types based on available candidate vaccines. Bivalent vaccine peak corresponds to age group 39-45 with a frequency of 6 (7.4%), nonavalent vaccine peaks at age group 32-38 with a frequency of 20 (20.6%). Nonavalent vaccines excluding bivalent 16 and 18 peak corresponds to age group 32-38 with a frequency of 18 (18.6).

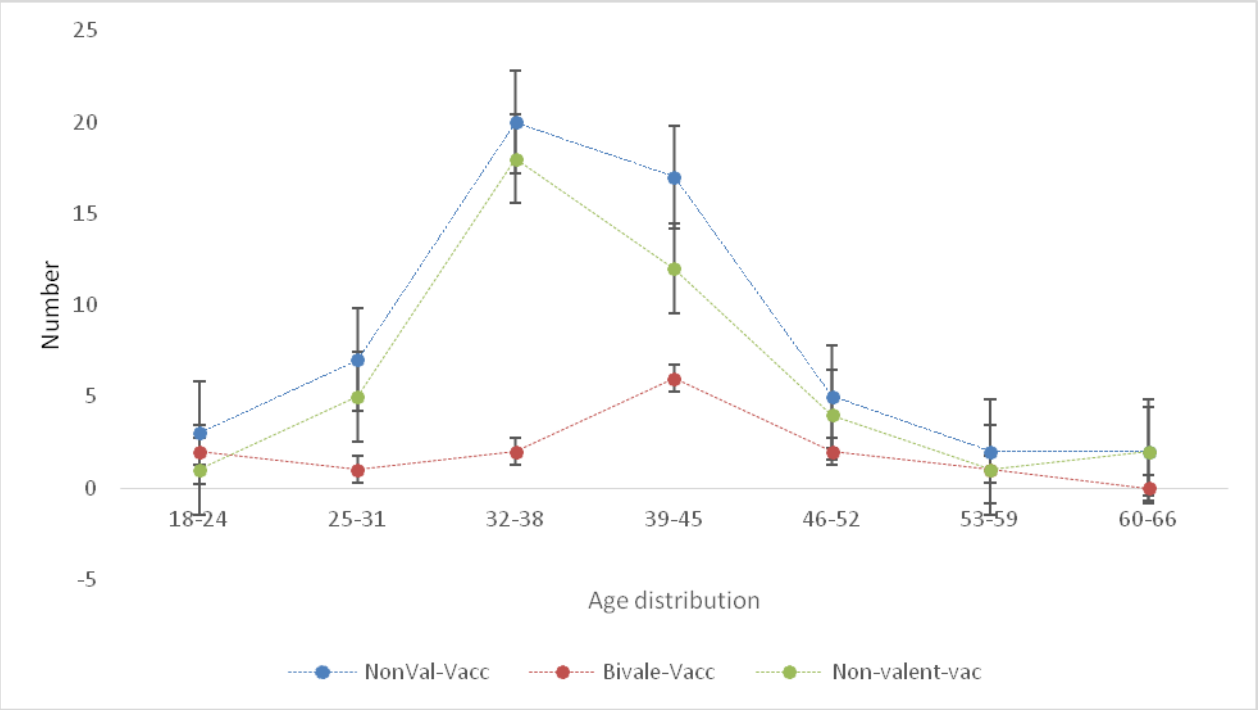

Figure 28: Line graph of the Classification of infection with High risk HPV types based on available candidate vaccines.

Blue line= Nonavalent vaccine preventable high risk type, Green line=Non valent vaccine preventable high risk type, Red line= Bivalent vaccine preventable high risk HPV.

4.9 Agarose Gel Electrophoresis of *Chlamydia trachomatis* positive samples

The Agarose Gel image of CT DNA is represented in figure 29 in a multiplex fashion using two different primers namely; Cryptic Plasmid and Major Outer Membrane Protein primers with 201 bp and 520 bp fragments respectively.

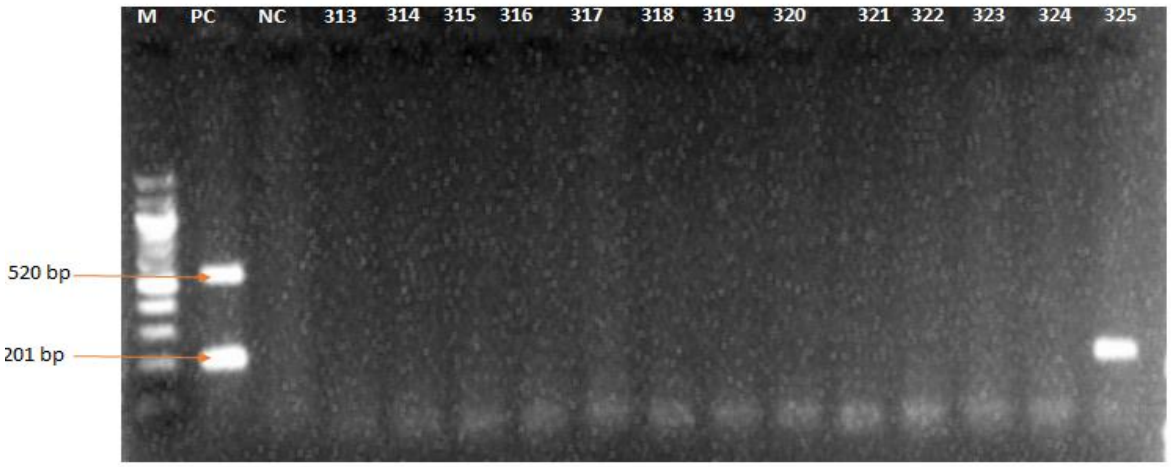

Figure 29: A representative gel picture of the multiplex run of CT DNA

Positive samples using Cryptic plasmid (201 bp) and the MOMP (520 bp) primers figure 29 shows A 100 bp ladder denoted as M was used as the molecular ruler followed by a positive (PC) and Negative control (NC). Sample 325 is positive for C.T DNA which has 201 bp fragments corresponding to the PC. The remaining lanes without bands were regarded as negative.

4.10 Prevalence of *Chlamydia trachomatis* Infection in Women in Ilorin

Table 10 shows the prevalence of *Chlamydia trachomatis* infection among participants of different age groups in the study. The prevalence of *Chlamydia trachomatis* was 4.5%. Age groups 46-52, 53-59 and 60-66 years had no case of detected *Chlamydia trachomatis* infection, while the highest occurrence in relation to age was found in age group 39-45 years with 5 cases.

Figure 30 is a line graph representing the CT infection among the women. Infection peaks at age 18-24 years droping steadily with a slight rise of CT infection in age 39-45 years which later drops in older participants of ages 46-66 years without CT case.

Table 10: Prevalence of *Chlamydia trachomatis* infections among participants of different age groups in the study.

AGE GROUP (YEARS)	CT Positive	CT Negative	Total	*P*-Value
	N(%)	N(%)		
18-24	2(11.8)	15(88.2)	**17(100)**	
25-31	6(7.4)	75(92.6)	**81(100)**	
32-38	4(4.1)	93(95.1)	**97(100)**	
39-45	5(6.0)	78(94.0)	**83(100)**	
46-52	0(0.0)	67(100)	**67(100)**	
53-59	0(0.0)	21(100)	**21(100)**	
60-66	0(0.0)	10(100.0)	**10(100)**	
Total	**17(4.5)**	**359(95.5)**	**376(100)**	0.187

Table 10 represents the prevalence of Chlamydia in the study where CT= *Chlamydia trachomatis*, N=number of women, %= percentage of women. Age group and CT Not statically significant at P<0.005.

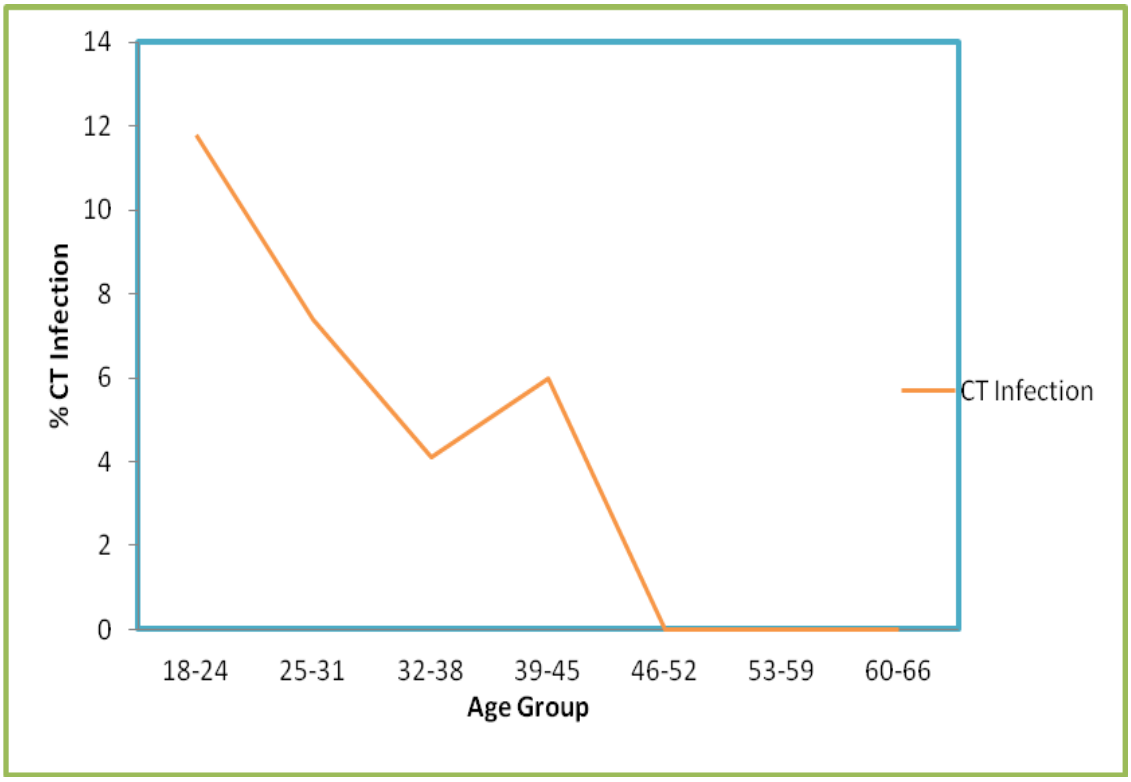

Figure 30: **A line graph of Percentage CT infection and Age group. The orange line represents CT infection among women in Ilorin.**

4.11 Age stratified Prevalence of Human Papillomavirus and *Chlamydia trachomatis* co-infection

The Age stratified prevalence of Human Papillomavirus and *Chlamydia trachomatis* co-infection among different age groups of the study population is shown in figure 31. A total of 15 women had HPV and CT co-infection with the highest number of cases in age groups; 25-31 (5 cases), 32-38 years (4 cases) and 39-45 years (5 cases). The lowest prevalence was in age group 18-24 years (1 case) and no case of co-infection was detected in age groups; 46-52, 53-59 and 60-66 years.

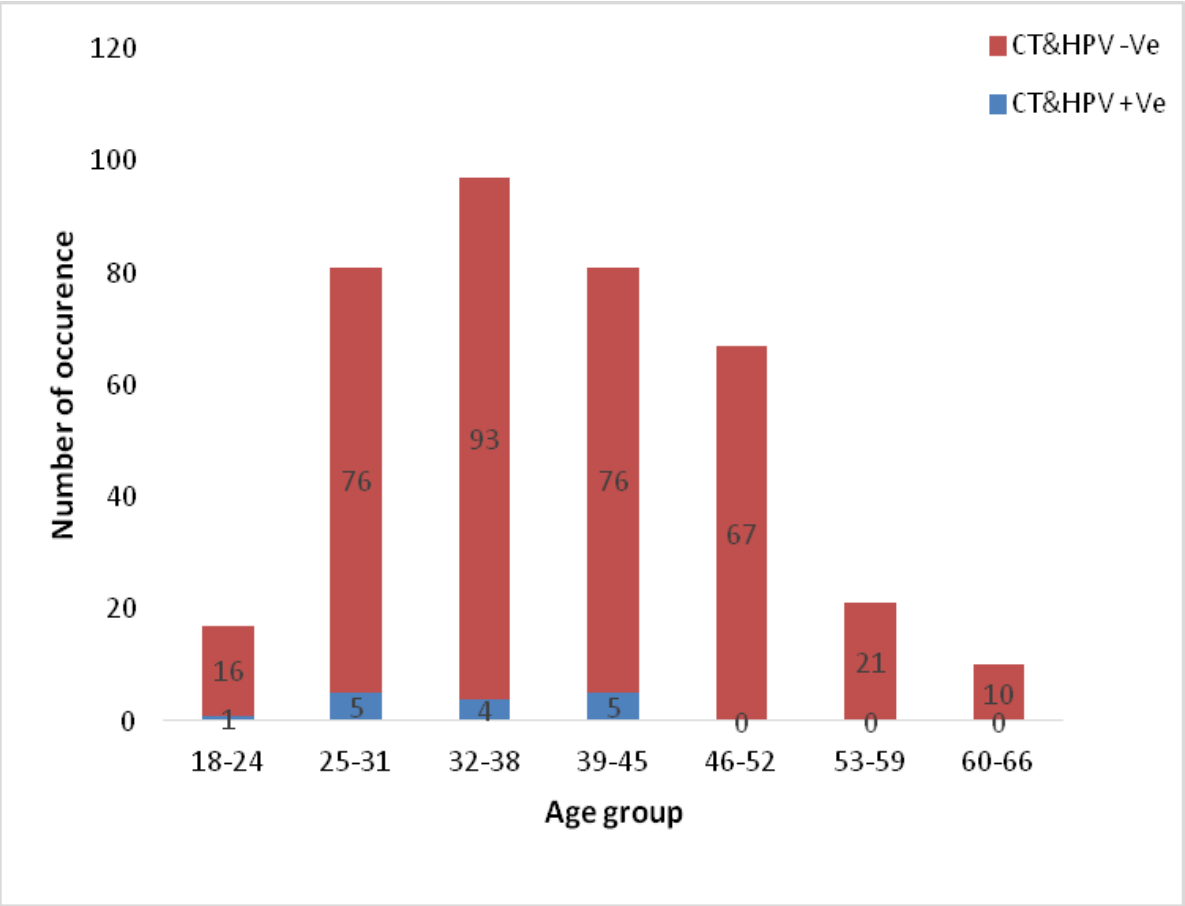

Figure 31: Human Papillomavirus and *Chlamydia trachomatis* co-infection among different age group of the study population.

CT and HPV-Ve: Number of cases of *Chlamydia trachomatis* and Human Papillomavirus independent infection in the study population; CT&HPV+Ve: Number of cases of *Chlamydia trachomatis* and Human Papillomavirus co-infection in the study population.

4.12 Distribution of Cytokine genes among women in Ilorin

Table 11 shows the distribution of cytokine genes profile for two anti-inflammatory cytokines (IL-10 and TGF-β codon 10 and 25 respectively) and two pro-inflammatory cytokines (IFN-γ and TNF-ɤ) in women with HPV and CT co-infection in this study. A total of fifteen women had co-infection with variation in cytokine genes transcribing for varied levels of cytokine production. TGF-β codon 10 and 25 showed significant difference at $P<0.05$ when compared with women who had HPV infection alone as well as women who had no infection in this study. The highest frequency in codon 10 was in CT (12), while CG (8) had the highest frequency with an intermediate level of cytokine production for codon 25. Also, IFN-γ and TNF-α were found to be significant in this population when their means were compared with HPV sole infected women and non-infected women. The highest frequency of cytokine production in IL-10 was in AG (10) but showed no significant difference when the mean was compared with HPV sole infection.

Table 11: Frequency of cytokine gene polymorphism in women with HPV and C.T Co-infection in the study population

CYTOKINE	GENOTYPE	YES N(%)	NO(%)	TOTAL
IL-10	AA	0(0.0)	15(100)	**15(100)**
	GG	5(33.3)	10(66.7)	**15(100)**
	AG	10(66.7)	5(33.3)	**15(100)**
TGF-β Codon 10	CC	1(6.7)	14(93.3)	**15(100)**
	TT	2(13.3)	13(86.7)	**15(100)**
	CT	12(80.0)	3(20.0)	**15(100)**
TGF-β Codon 25	CC	1(6.7)	14(93.3)	**15(100)**
	GG	5(33.3)	10(66.7)	**15(100)**
	CG	8(53.3)	7(46.7)	**15(100)**
IFN-γ	AA	3(20.0)	12(80.0)	**15(100)**
	TT	1(6.7)	14(93.3)	**15(100)**
	AT	11(73.3)	4(26.7)	**15(100)**
TNF-α	GG	2(13.3)	13(86.7)	**15(100)**
	AA	4(26.7)	11(73.3)	**15(100)**
	GA	9(60.0)	6(40.0)	**15(100)**

IL-10=Interleukin-10, with genotypes AA (low cytokine production), GG (High cytokine production) and AG (Intermediate cytokine production). TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively. TGF-β10 with genotypes; CC (low cytokine production), TT (High cytokine production) and CT (intermediate cytokine production) and TGF-β25 genotypes; GG (High cytokine production), CC (Low cytokine production) and CG (Intermediate cytokine production). IFN-γ=Interferon gamma with genotypes; AA (Low cytokine production), TT (High cytokine production) and AT (intermediate cytokine production). TNF-ɤ=Tumour necrosis factor alpha with genotypes; GG (Low cytokine production), AA (High cytokine production) and GA (Intermediate cytokine production).

4.13 The distribution of cytokine gene polymorphism in women with HPV infection alone in Ilorin

The distribution of cytokine gene polymorphism in women with HPV infection alone is shown in table 12. A total of 100 HPV infected women were profiled for two anti-inflammatory cytokines (IL-10 and TGF-β codon 10 and 25 respectively) and two pro-inflammatory cytokines (IFN-γ and TNF-ɤ). In the four cytokines and genes profiled, IL-10 had the highest frequency in genotype GG (73) with a potential for high cytokine production while genotype AG had the lowest frequency for low cytokine production. In the case of TGF-β codon 10, the highest frequency was observed in genotype CT (40) which has high cytokine production ability and genotypes TT and CT both have relatively low frequencies of 30 each. Codon 25 of the TGF-β has the highest frequency in CC (50) with a low cytokine production and lowest frequency in CG (10) with intermediate level of production. IFN-γ with genotypes AA, TT and AT has the highest frequency in TT (50) with a high level of cytokine production. TNF-ɤ with genotypes GG, AA and GA with the highest frequency in genotype AA. Significant difference was shown in TGF-β codon 10, IFN-γ and TNF-ɤ at $P<0.05$ level when the means of sole HPV positive infections was compared with sole *Chlamydia trachomatis* infection.

Table 12: Frequency of cytokine gene polymorphism in women with HPV infection alone in the study population

CYTOKINE	GENOTYPE	YES N(%)	NO(%)	TOTAL
IL-10	AA	17(17.0)	83(83.0)	**100(100)**
	GG	73(73.0)	27(27.0)	**100(100)**
	AG	10(10.0)	90(90.0)	**100(100)**
TGF-β Codon 10	CC	30(30.0)	70(70.0)	**100(100)**
	TT	30(30.0)	70(70.0)	**100(100)**
	CT	40(40.0)	60(60.0)	**100(100)**
TGF-β Codon 25	CC	50(50.0)	50(50.0)	**100(100)**
	GG	40(40.0)	60(60.0)	**100(100)**
	CG	10(10.0)	90(90.0)	**100(100)**
IFN-γ	AA	33(33.0)	67(67.0)	**100(100)**
	TT	50(50.0)	50(50.0)	**100(100)**
	AT	17(17.0)	83(83.0)	**100(100)**
TNF-α	GG	18(18.0)	82(82.0)	**100(100)**
	AA	40(40.0)	60(60.0)	**100(100)**
	GA	42(42.0)	58(58.0)	**100(100)**

IL-10=Interleukin-10, with genotypes AA (low cytokine production), GG (High cytokine production) and AG (Intermediate cytokine production). TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively. TGF-β10 with genotypes; CC (low cytokine production), TT (High cytokine production) and CT (intermediate cytokine production) and TGF-β25 genotypes; GG (High cytokine production), CC (Low cytokine production) and CG (Intermediate cytokine production). IFN-γ=Interferon gamma with genotypes; AA (Low cytokine production), TT (High cytokine production) and AT (intermediate cytokine production). TNF-ɤ=Tumour necrosis factor alpha with genotypes; GG (Low cytokine production), AA (High cytokine production) and GA (Intermediate cytokine production).

4.14 Distribution of Cytokine genes among women with CT infection alone in Ilorin

The distribution of the frequency of cytokine genes profile in women with CT infection alone is shown in table 13. Only two women had *Chlamydia trachomatis* infection without a concomitant HPV infection. Their genes profile revealed significance at $P<0.05$ for TGF-β codon 10, IFN-γ and TNF-ɤ when the means were compared for *Chlamydia trachomatis*, Human Papillomavirus and non-infected individuals.

Table 13: Frequency of cytokine gene polymorphism in women with CT infection alone in the study population

CYTOKINE	GENOTYPE	YES N(%)	NO(%)	TOTAL
IL-10	AA	0(0.0)	2(100)	**2(100)**
	GG	1(50.0)	1(50.0)	**2(100)**
	AG	1(50.0)	1(50.0)	**2(100)**
TGF-β Codon 10	CC	0(0.0)	2(100)	**2(100)**
	TT	2(100)	0(0.0)	**2(100)**
	CT	0(0.0)	2(100)	**2(100)**
TGF-β Codon 25	CC	0(0.0)	2(100)	**2(100)**
	GG	1(50.0)	1(50.0)	**2(100)**
	CG	1(50.0)	1(50.0)	**2(100)**
IFN-γ	AA	0(0.0)	2(100)	**2(100)**
	TT	0(0.0)	2(100)	**2(100)**
	AT	2(100)	0(0.0)	**2(100)**
TNF-α	GG	0(0.0)	2(100)	**2(100)**
	AA	1(50.0)	1(50.0)	**2(100)**
	GA	1(50.0)	1(50.0)	**2(100)**

IL-10=Interleukin-10, with genotypes AA (low cytokine production), GG (High cytokine production) and AG (Intermediate cytokine production). TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively. TGF-β10 with genotypes; CC (low cytokine production), TT (High cytokine production) and CT (intermediate cytokine production) and TGF-β25 genotypes; GG (High cytokine production), CC (Low cytokine production) and CG (Intermediate cytokine production). IFN-γ=Interferon gamma with genotypes; AA (Low cytokine production), TT (High cytokine production) and AT (intermediate cytokine production). TNF-ɤ=Tumour necrosis factor alpha with genotypes; GG (Low cytokine production), AA (High cytokine production) and GA (Intermediate cytokine production).

4.15 Distribution of Cytokine genes among women without infection in Ilorin

The Frequency distribution of cytokine gene polymorphisms in women without infection (apparently healthy women) in this study is shown in Table 14. A total of 70 women did not show HPV, CT infection or both. In IL-10, the highest frequency was found in AG (43) and lowest frequency in genotype AA (5). TT (31), GG (25) and GG (33) all had the highest frequencies in; TGF-β codon 10, TGF-β codon 25 and TNF-α respectively. The lowest frequency of genotype TT (14) was found in IFN-γ cytokine. Significant difference was found at $p<0.05$ level of significance when the means of TGF-β codon 10(CT versus no infection), TGF-β codon 25 (CT versus no infection, co-infection versus no infection), IFN-γ (no infection versus co-infection, no infection versus Chlamydia infection), and TNF-α (no infection versus co-infection) were compared.

Table 14: Frequency of cytokine gene polymorphism in women without infection in the study population

CYTOKINE	GENOTYPE	YES N(%)	NO(%)	TOTAL
IL-10	AA	5(7.1)	65(92.8)	**70(100)**
	GG	22(31.4)	48(68.6)	**70(100)**
	AG	43(61.4)	27(38.6)	**70(100)**
TGF-β Codon 10	CC	11(15.7)	59(84.3.0)	**70(100)**
	TT	31(12.5)	7(87.5)	**70(100)**
	CT	28(37.5)	5(62.5)	**70(100)**
TGF-β Codon 25	CC	24(34.3)	46(65.7)	**70(100)**
	GG	25(35.7)	45(64.3)	**70(100)**
	CG	21(30)	49(70)	**70(100)**
IFN-γ	AA	28(40)	42(60)	**70(100)**
	TT	14(20.0)	56(80.0)	**70(100)**
	AT	28(40.0)	42(60.0)	**70(100)**
TNF-α	GG	33(47.2)	37(52,8)	**70(100)**
	AA	19(27.1)	51(72.9)	**70(100)**
	GA	18(25.7)	52(74.3)	**70(100)**

IL-10=Interleukin-10, with genotypes AA (low cytokine production), GG (High cytokine production) and AG (Intermediate cytokine production). TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively. TGF-β10 with genotypes; CC (low cytokine production), TT (High cytokine production) and CT (intermediate cytokine production) and TGF-β25 genotypes; GG (High cytokine production), CC (Low cytokine production) and CG (Intermediate cytokine production). IFN-γ=Interferon gamma with genotypes; AA (Low cytokine production), TT (High cytokine production) and AT (intermediate cytokine production). TNF-ɤ=Tumour necrosis factor alpha with genotypes; GG (Low cytokine production), AA (High cytokine production) and GA (Intermediate cytokine production).

4.16 Cytokines genes polymorphism infection in women

Table 15 compared the genotypic association of some cytokines genes polymorphism in selected HPV infected women and apparently healthy participants. Significant association was found in TNF-α, IL-10, and IFN-γ at *P* 0.0001 respectively while TGFβ-25 was significant at *P* 0.0013. No significance was found in TGFβ-10.

Table 16 represents the association between *Chlamydia trachomatis* infection and controls and shows significant associations between CT infection IL-10, TGFβ-25, TGFβ-10 and IFN-γ. There was no significant association in TNF-α between having a CT infection and being healthy.

In Table 17 the association of IL-10, TGFβ-25, TGFβ-10, IFN-γ and TNF-α were all significant for Co-infection when compared with healthy controls.

Table 18 reveals the allele frequency distribution of Cytokine genes in HPV cases and Healthy Controls. The allele frequency was generally high with significant association ($P<0.005$) observed in the pro-inflammatory cytokines TNF-α and IFN-γ and some of the anti-inflammatory cytokine IL-10 and TGF-β10 with the exception of TGF-β25

Table 19 is a table of allele frequency distribution of Cytokine Polymorphisms in *Chlamydia trachomatis* cases and healthy Controls. Both pro and anti-inflammatory cytokines did not show significant association for alpha $P<0.005$.

Table 20 shows the allele Frequency Distribution of Cytokine Polymorphisms in HPV and CT co-infection and Healthy Controls. There was no significant association in the frequency of the allele in both pro and anti-inflammatory cytokines.

Table 15: Genotypic Frequency Distribution of Cytokine Polymorphisms in HPV cases and apparently Healthy Controls

SNPs	Genotype	Cases N(%) (HPV+)	Control N(%)	*P*-Value	X^2
TNF-α	AA	40	27		
	AG	42	26		
	GG	18	47		
				0.0001	19.23
IL-10					
	AA	17	7		
	GG	73	31		
	AG	10	61		
				0.0001	57.76
TGFβ-10	CC	30	16		
	TT	30	13		
	CT	40	38		
				0.0956	4.695
TGFβ-25	CC	50	34		
	GG	40	36		
	CG	10	30		
				0.0013	13.26
IFN-γ	AA	33	40		
	TT	50	20		
	AT	17	40		
				0.0001	22.81

SNP=Short nucleotide polymorphism, N=number, %=percentages, X^2=Pearson's chi square HPV+ =Human Papillomavirus positive, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively IFN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha. only TGFβ-10 did not show statistical significance when HPV and apparently healthy controls were compared at P<0.005.

Table 16: Genotypic Frequency Distribution of Cytokine Polymorphisms in CT cases and Apparently Healthy Controls

SNP	Genotype	Cases N(%) (CT+)	Control N(%)	*P*-Value	X^2
TNF-α	AA	18	27		
	AG	29	26		
	GG	53	47		
				0.3129	2.324
IL-10					
	AA	0	7		
	GG	35	31		
	AG	64	61		
				0.0258	7.314
TGFβ-10	CC	6	16		
	TT	24	13		
	CT	71	38		
				0.0034	11.39
TGFβ-25	CC	6	34		
	GG	35	36		
	CG	53	30		
				0.0001	25.86
IFN-γ	AA	18	40		
	TT	6	20		
	AT	77	40		
				0.0001	27.58

SNP=Short nucleotide polymorphism, N=number, %=percentages, X^2=Pearson's chi square CT+=*Chlamydia trachomatis* positive, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively FN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha. TNF-α did not show statistical significance when CT and apparently healthy controls were compared at P<0.005.

Table 17: Frequency Distribution of Cytokine Polymorphisms in HPV/CT cases and apparently Healthy Controls

SNP	Genotype	Cases N(%) (HPV+)	Control N(%)	*P*-Value	X^2
TNF-α	AA	27	27		
	AG	60	26		
	GG	13	47		
				0.0001	32.7
IL-10					
	AA	0	7		
	GG	33	31		
	AG	67	61		
				0.0255	7.339
TGFβ-10	CC	7	16		
	TT	13	13		
	CT	80	38		
				0.002	12.44
TGFβ-25	CC	7	34		
	GG	33	36		
	CG	53	30		
				0.0001	24.06
IFN-γ	AA	3	40		
	TT	1	20		
	AT	13	40		
				0.0001	22.56

SNP=Short nucleotide polymorphism, N=number, %=percentages, X^2=Pearson's chi square CT+=*Chlamydia trachomatis* positive, HPV+, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively FN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha.

Table 18: Allele Frequency Distribution of Cytokine Polymorphisms in HPV cases and Healthy Controls

SNPs	Allele	Cases (HPV+) N	Control N	*P*-Value
TNF-α	G	80	84	
	A	122	56	
				0.003
IL-10				
	A	44	53	
	G	156	87	
				0.002
TGFβ-10	C	100	50	
	T	100	90	
				0.010
TGFβ-25	C	110	69	
	G	90	71	
				0.321
IFN-γ	A	83	84	
	T	117	56	
				0.003

SNP=Single Nucleotide Polymorphism, N=number X^2=Pearson's chi square HPV+ =Human Papillomavirus positive, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively FN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha.

Table 19: Allele Frequency Distribution of Cytokine Polymorphisms in CT cases and Healthy Controls

SNP	Allele	Cases N (CT+)	Control N	*P*-Value
TNF-α	A	3	56	
	G	1	84	
				0.305
IL-10				
	A	1	53	
	G	3	87	
				1.000
TGFβ-10	C	0	50	
	T	4	90	
				0.298
TGFβ-25	C	1	69	
	G	3	71	
				0.620
IFN-γ	A	2	84	
	T	2	56	
				1.000

SNP=Single Nucleotide Polymorphism, N=number X^2=Pearson's chi square CT+=*Chlamydia trachomatis* positive, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively FN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha.

Table 20: Allele Frequency Distribution of Cytokine Polymorphisms in HPV+ and CT+ co-infection and Healthy Controls

SNP	Allele	Cases N(%) (HPV+)	Control N(%)	*P*-Value
TNF-α	A	17	56	
	G	13	84	
				0.1069
IL-10				
	A	10	53	
	G	20	87	
				0.682
TGFβ-10	C	16	50	
	T	16	90	
				0.157
TGFβ-25	C	10	69	
	G	18	71	
				0.217
IFN-γ	A	17	84	
	T	13	56	
				0.838

SNP=Single Nucleotide Polymorphism, N=number X^2=Pearson's chi square CT+=*Chlamydia trachomatis* positive, HPV+, IL-10=Interleukin-10, TGF-β Codons 10 and 25 =Tumour growth Factor Beta on the 10^{th} and 25^{th} codon respectively FN-γ=Interferon gamma, TNF-α=Tumour necrosis factor alpha.

4.17 Socio-demographic characteristics of the study population and HPV Infection

The socio-demographic characteristic of participants in this study is shown in Table 18. The mean and median ages of the participants are 38.9 and 38.0 repectively. A total of 24 participants formed the modal distribution at age 35. Majority of the women were married 274 (93.8%) with only a few single (3.6%) and only 10 divorcees participated in the study. The percentage of women who attended tertiary institution was 46.3 while only 5% of the participants had only informal education. Majority of the women were of the Yoruba extraction (91.8%) other etnic groups include Hausa and Igbo with 4% and 5% respectively. Acohol use among participants was very (low 2%)

Table 21: Socio-demographic characteristics of research population in relation to HPV Infection

Characteristics	HPV Positive (n =306)	HPV Negative (n =70)	*P*-Value
	N (%)	N (%)	
Age (years)			
18-24	13(4.2)	4(5.9)	
25-31	65(21.2)	16(23.5)	
32-38	82(26.8)	15(22.1)	
39-45	63(20.6)	8(26.5)	
46-52	56(18.3)	11(16.2)	
53-59	17(5.6)	4(5.9)	
60-66	10(3.3)	0(0.0)	
			0.044
Marital Status			
Single	11(3.6)	0(0.0)	
Married	287(93.8)	66(97.1)	
Divorced	8(2.6)	2(2.9)	
			0.282
Education			
Informal	15(5.0)	4(6.0)	
Primary	69(23.1)	19(28.4)	
Secondary	67(22.4)	18(26.9)	
Tertiary	148(49.5)	26(38.8)	
			0.473
Ethnicity			
Yoruba	278(91.1)	67(98.5)	
Hausa	4(1.3)	0(0.0)	
Igbo	5(1.6)	1(1.5)	
Others	18(5.9)	0(0.0)	
			0.155
Alcohol Use			
Yes	6(100)	0(0.0)	
No	290(81.5)	66(18.5)	
			0.243

The sociodemographic distribution of women in relation to HPV infection reveals the highest population in age group 32-38. Majority of the women were married and were from the Yoruba extraction. There was a very low percentage of alcohol use among theparticipants and a high number of women were exposed to formal education up to the tertiary level. HPV=Human Papillomavirus n=total number. N=number per case, %=percentages.

4.18 Sexual History and Behaviour of Participants in Relation to HPV

The sexual history and behaviour of participants in relation to infection with Human Papillomavirus is presented in Table 19. The lowest and highest ages at *coitarche* are 3 and 34 years respectively. While more women had their first child between the ages of 20-24 and also the highest prevalence of HPV infection of 39.4% was found in this age group. The development of female sexual characteristics expressed by *menarche* was common among participants at ages 13-15. All participants had at least one lifetime sexual partner and 10 was the highest number of life time partner observed in an individual. A steady decline in the number of participants is observed as the lifetime sexual partners increased. Besides, the use of condom was not common among the women (63.3%) compared with womenwho used condom (30.5%). Coital frequency ranged from once weekly (154 women) to more than four times weekly (6 women).

The history of reproductive health and Sexually Transmitted Infection of participants in relation to current HPV infection is shown in Table 20. Pap Test awareness and history of uptake among participants was very low (14%). Also, only few had history of genital warts infection (1.6%). Majority had not undergone pelvic examination (69%), however a good number of the participants already had female genital cuttings (64%). Previous diagnosis of STI was affirmed by 52 participants while 24% had history a history of abnormal vaginal discharge.

Table 22: Sexual History and Behaviour of Participants in relation to HPV Infection

Characteristics	HPV Positive (n = 306)	HPV Negative (n = 70)	*P*-Value
	N(%)	N(%)	
Age at Sexual debut (Coitarche)			
≤ 10	1(0.4)	2(3.2)	
11-15	20(7.0)	5(7.9)	
16-20	129(45.3)	36(57.1)	
21-25	103(36.1)	18(28.6)	
26-30	28(9.8)	2(3.2)	
31-35	4(1.4)	0(0.0)	0.004*
Age at onset of childbearing			
15-19	35(12.2)	9(13.6)	
20-24	110(39.4)	32(48.5)	
25-29	106(38.0)	20(30.3)	
30-34	26(9.3)	5(7.6)	
35-39	2(0.7)	0(0.0)	0.518
Menarche			
10-12	22(7.9)	5(7.5)	
13-15	142(50.9)	35(52.2)	
16-18	101(36.2)	25(37.3)	
19-21	14(5.0)	2(3.0)	0.861
Number of Lifetime sexual Partners			
1	166(57.2)	32(47.8)	
2	70(24.1)	23(34.3)	
3	41(14.1)	9(13.4)	
4	9(3.1)	1(1.5)	
≥5	4(1.4)	2(3.0)	0.184
Condom Use			
Yes	95(33.2)	20(29.9)	
No	191(66.8)	47(70.1)	0.635
Frequency of Sexual Intercourse weekly			
1	154(68.2)	45(70.3)	
2	83(29.4)	12(18.8)	
3	29(10.3)	5(7.8)	
≥4	6(2.1)	3(2.1)	0.262

The sexual history and behaviour relating to HPV infection in Table revealed the most frequent age of exposure to sexual intercourse as between ages16-20 when *menarche* in most participants had occurred, this corresponds to the age when the onset of child bearing begins to peak (ages 20-24) as at when condom use is less than 20%.

Table 23: History of Reproductive health and Sexually Transmitted Infection of Participants in relation to current HPV Infection

Characteristics	HPV Positive (n=306)	HPV Negative (n=70)	*P*-Value
	N (%)	N (%)	
Pap Test			
Yes	44(15.4)	9(15.8)	
No	242(84.6)	56(86.2)	0.847
Genital Warts			
Yes	5(1.7)	1(1.5)	
No	281(98.3)	64(98.5)	0.906
Pelvic Examination			
Yes	44(17.0)	12(21.1)	
No	215(83.0)	45(78.9)	0.467
Female Genital Cuttings			
Yes	190(65.3)	52(82.5)	
No	101(34.7)	11(17.5)	0.008*
Previous STI			
Yes	45(15.6)	7(11.1)	
No	244(84.4)	56(88.9)	0.278
Abnormal Vaginal Discharge			
Yes	46(16.1)	46(16.1)	
No	240(83.9)	61(92.4)	0.077

The history of reproductive health and STI of participants in relation to HPV infections shows a total of 53 women with previous Pap test history, many had no history of genital warts (345women), a total of 56 women had previous pelvic examinationsuspicious of possible of possible pelvic inflammatory disease. A female genital cutting is a common practice in the study area and many participants have been initiated. Few women had history of awareness of a previous infection with an STI as well as vaginal discharge. HPV infection was high irrespective of the outcome of reprodctive health and STI history.

4.19 Predisposing Risk Factors to Cancer in Relation to HPV Infection

The risk factors that can predispose participants to cancer in relation to HPV infection is shown in Table 24. Majority of the participating women are non-smokers (over 99%) with only one smoker (0.3%). More women fell in the 20-24 age brackets as the age at first pregnancy with fewer participants occupying 35-39 age brackets (0.7%). The use of hormonal contraceptive was 54.5% among the women and 4 was the most frequent parity in the study. A total of 7.6% of the women had a family history of cancer, while irregular menstruation was not common (32.4%) and post-coital bleeding was low (2.1%).

Figure 32 is a line graph showing the ranking of participants based on the number of lifetime sexual partners. A total of 198 women had only one lifetime sexual partners, 98 participants had only two lifetime sexual partners. The highest number of 10 sexual lifetime partners was recorded in only one participant.

Table 24: Risks factors predisposing to cancer among participants in relation to HPV infection

Characteristics	HPV Positive (n=306) N(%)	HPV Negative (n=70) N(%)	*P*-Value
Smoking			
Yes	1(100)	0(0.0)	
No	299(81.9)	66(18.1)	0.639
Age At First Pregnancy			
15-19	35(12.2)	9(13.6)	
20-24	110(39.4)	32(48.5)	
25-29	106(38.0)	20(30.3)	
30-34	26(9.3)	5(7.6)	
35-39	2(0.7)	0(0.0)	0.518
Use of Hormonal Contraceptives			
Yes	157(53.2)	48(71.6)	
No	138(46.8)	19(28.4)	0.013*
Parity			
0	16(5.2)	1(1.4)	
1	25(8.2)	1(1.4)	
2	40(13.1)	8(11.6)	
3	62(20.3)	21(30.4)	
4	94(30.8)	17(24.6)	
5	39(12.8)	11(15.9)	
6	19(6.2)	4(5.8)	
7	10(3.3)	6(8.7)	0.146
Family History of Cancer			
Yes	22(7.6)	5(7.5)	
No	268(96.4)	62(92.5)	0.195
Irregular Menstruation			
Yes	99(34.7)	23(35.4)	0.461
No	186(65.3)	42(64.6)	
Post-Coital Bleeding			
Yes	7(2.5)	1(1.5)	
No	278(97.5)	65(98.5)	0.323

The table of risk factors predisposing to cancer in relation to HPV reveals extreme low smoking among the women, low prevalence of women who had their first pregnancy at age 35-39, about half the study population were on hormonal contraceptives. A total of 16 women from the study had no children while 87.8% had no family history of cancer, irregular menstruation and post coital bleeding was not common among the participants.

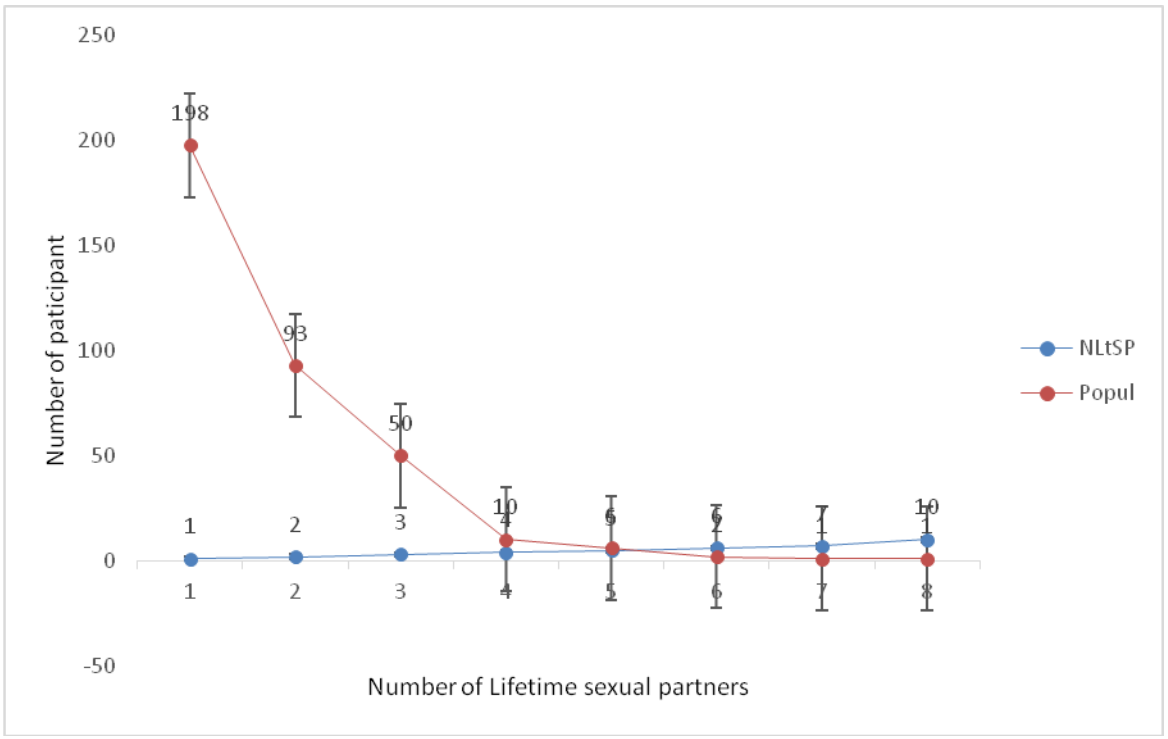

Figure 32: Ranking of participants based on number of lifetime sexual partners.

NLtSP=Number of Lifetime Sexual Partners represented by the blue line, the red line represents the population of the participants in the study.

4.20 Prevalence and Genotype Distribution of HPV in Relation to Cervical Cytology Status of Participants

Table 25 shows HPV Prevalence and genotype distribution in relation to cervical cytology status. A total of 281 HPV infected women were negative for Intra-epithelial Lesion or Malignancy (NILM/Normal), with 8 cases of Low Squamous Intra-epithelia Lesion (LSIL) and one case of Atypical Squamous Cell of Undeterminate Significance (ASCUS). NILM, LSIL, ASCUS, and HSIL were; 192, 8, 1 and 0 respectively in High-Risk HPV infected participants.Women with HPV genotype 82 had NILM, LSIL, ASCUS and HSIL as 118, 6, 2 and 0 cases respectively. In the untyped HPV NILM, LSIL, ASCUS and HSIL were; 92, 2, 2 and 2 respectively.

Table 25: HPV Prevalence and genotype distribution in relation to cervical cytology status

	Allcases (N=376)	**Normal Cytology(N=357)**	**Abnormal Cytology (N = 92)**			***P*-Value**
			LSIL (n = 13)	**ASCUS (n = 4)**	**HSIL (n = 2)**	
AHPV	306(81.4)	289(81.0)	8(61.5)	1(25.0)	0(0.0)	0.359
SHPV	118(31.4)	112 (31.4)	6(46.2)	0(0.0)	0(0.0)	
M HPV	90(23.9)	86(24.1)	2(15.4)	2(50.0)	0(0.0)	
UHPV	98(26.1)	92(25.8)	2(15.4)	2(50.0)	2(100)	0.004*
HR-HPV	201(53.5)	192(53.8)	8(61.5)	(25.0)	0(0.0)	
HPV16	11(2.9)	10(2.8)	1(7.7)	0(0.0)	0(0.0)	0.274
HPV18	4(1.1)	4(1.1)	0(0.0)	0(0.0)	0(0.0)	
HPV26	3(0.8)	3(0.8)	0(0.0)	0(0.0)	0(0.0)	
HPV31	3(0.8)	3(0.8)	0(0.0)	0(0.0)	0(0.0)	
HPV33	36(9.6)	35(9.8)	1(7.7)	0(0.0)	0(0.0)	0.586
HPV35	21(5.6)	20(5.6)	1(7.7)	0(0.0)	0(0.0)	
HPV39	6(1.6)	6(1.7)	0(0.0)	0(0.0)	0(0.0)	0.586
HPV45	2(0.5)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	
HPV51	10(2.7)	10(2.8)	0(0.0)	0(0.0)	0(0.0)	
HPV52	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	
HPV53	15(4.0)	15(4.2)	0(0.0)	0(0.0)	0(0.0)	
HPV56	34(9.0)	32(9.0)	1(7.7)	1(25.0)	0(0.0)	0.086
HPV58	5(1.3)	5(1.4)	0(0.0)	0(0.0)	0(0.0)	
HPV59	13(3.5)	13(3.6)	0(0.0)	0(0.0)	0(0.0)	
HPV66	25(6.6)	24(6.7)	1(7.7)	0(0.0)	0(0.0)	
HPV68	19(5.1)	17(4.8)	1(7.7)	1(25.0)	0(0.0)	0.268
HPV73	11(2.9)	10(2.8)	1(7.7)	0(0.0)	0(0.0)	
HPV 82	126 (33.5)	118(33.1)	6(46.2)	2(50.0)	0(0.0)	0.250

This table reveals the relationship between HPV genotype and cervical cytology. Correlation was significant at *P*=0.001 (*) for untyped HPV. HSIL=High Squamous Intra-epithelial Lesion, LSIL=Low Squamous Intra-epithelial Lesion, ASCUS=Atypical Squamous Cell of Undetermined Significance, n=no of cases, N=total number of cases, %=percentage

4.21 Frequency of HPV Infection and Female Genital Cutting

Table 23 shows HPV Prevalence and genotype distribution in relation to female genital cuttings. A total of 242 (64.4%) women had genital cuttings, in which all women infected with HPV were 190 (78.5%), untyped HPV 52(21.5%) and HPV 82 is 83 (34.2%). No female genital cutting was recorded in women with HPV type 45.

Table 26: HPV Prevalence and genotype distribution in relation to Female genital cuttings

	All cases (N=376)	Female Genital cuttings	Female Genital cuttings	*P*-Value
		Yes (N=242)	No (N=112)	
AHPV	306(81.4)	190(78.5)	101(90.2)	0.001*
SHPV	118(31.4)	77(31.8)	36(32.1)	0.990
M HPV	90(23.9)	60(24.8)	25(22.3)	0.590
UHPV	98(26.1)	52(21.5)	41(36.6)	0.003*
HR-HPV	201(53.5)	133(55.0)	58(51.8)	
HPV16	11(2.9)	7(2.9)	4(3.6)	0.541
HPV18	4(1.1)	2(0.8)	2(1.8)	0.961
HPV26	3(0.8)	1(0.4)	2(1.8)	0.345
HPV31	3(0.8)	2(0.8)	1(0.9)	0.736
HPV33	36(9.6)	25(10.3)	8(7.1)	0.243
HPV35	21(5.6)	16(6.6)	4(3.6)	0.191
HPV39	6(1.6)	3(1.2)	2(1.8)	0.436
HPV45	2(0.5)	0(0.0)	1(0.9)	0.450
HPV51	10(2.7)	6(2.5)	4(3.6)	0.349
HPV52	1(0.3)	1(0.4)	0(0.0)	0.389
HPV53	15(4.0)	11(4.5)	4(3.6)	0.840
HPV56	34(9.0)	23(9.5)	8(7.1)	0.730
HPV58	5(1.3)	3(1.2)	2(1.8)	0.533
HPV59	13(3.5)	8(3.3)	3(2.7)	0.949
HPV66	25(6.6)	20(8.3)	4(3.6)	0.327
HPV68	19(5.1)	14(5.8)	3(2.7)	0.172
HPV73	11(2.9)	7(2.9)	4(3.6)	0.450
HPV 82	126 (33.5)	83(34.3)	36(32.1)	0.213

Table 26 of HPV Prevalence and genotype distribution in relation to Female genital cuttings was significant at $p<0.05$ (*) and shows positive correlation.

4.22 Specificity of HPV DNA Detection using Consensus Primers

Table 24 reveals HPV DNA detection using the MY09/MY11 and GP5+/GP6+ primers. All positive HPV DNA detected by MY09/11and GP5+/6+ primers are 183(59.8) and 223(72.9) respectively. For High Risk HPV, detection by MY09/11and GP5+/6+ primers are 125(62.2) and 140 (69.7) respectively. Also, untyped HPV detected by MY09/11and GP5+/6+ primers are 54 (55.1) and 79(80.6) respectively.

Table 27: HPV DNA detection using the MY09/MY11 and GP5+/GP6+ primers

	All cases N=376	PRIMERS MY09/11	GP5+/6+	MY and GP+	EMY/GP+	*P*-Value
AHPV	306(100)	183(59.8)*	223(72.9)*	103(33.7)	302(98.7)	0.000*
HR-HPV	201(100)	125(62.2)*	140(69.7)	66(32.8)	199(99.0)	0.000*
UHPV	98(100)	54(55.1)	79(80.6)	35(35.7)	98(100)	0.700

This table shows HPV DNA detection using two consensus primers MY09/11 and GP5+/6+ X^2 was significant for AHPV,HR-HPV, UHPV. AHPV= Any HPV Infection, HR-HPV=High Risk HPV and UHPV=Untyped positive HPV Infection at $p<0.05$.

4.23 Socio-demographic characteristics of the study population and *Chlamydia trachomatis* Infection

Table 28 shows the Socio-demographic characteristics of the research population in relation to *Chlamydia trachomatis* Infection. A total of 17 positive cases of Chlamydia was detected among the women with highest frequency (6 cases) occurring among age group 25-31 (7.4%). This is followed by age group 39-45 with a proportion of 6.2% (5 cases). Age groups; 46-52, 53-59 and 60-66 had no case of *Chlamydia trachomatis* infection (0.0%).

Among the participants positive for *Chlamydia trachomatis*, only one was single (9.1%) and 4.5% (16 women) were married and no divorced woman tested positive to *Chlamydia trachomatis.* Most women who had tertiary level of education tested negative to Chlamydia (96.6). Chlamydia infection was predominant among people from Yoruba extraction (16 women). Only one woman who used alcohol tested positive for *Chlamydia trachomatis*.

Table 28: Socio-demographic characteristics of research population in relation to *Chlamydia trachomatis* Infection

Characteristics	CT Positive (n =17)	CT Negative (n =359)	*P*-Value
	N (%)	N (%)	
Age(Years)			
18-24	2(11.6)	15(88.2)	
25-31	6(7.4)	75(92.6)	
32-38	4(4.1)	93(95.9)	
39-45	5(6.2)	76(93.8)	
46-52	0(0.0)	67(100)	
53-59	0(0.0)	21(100)	
60-66	0(0.0)	10(100)	
			0.012*
Marital Status			
Single	1(9.1)	10(90.1)	
Married	16(4.5)	337(95.5)	
Divorced	0(0.0)	10(100)	
			0.319
Education			
Informal	0(0.0)	19(100)	
Primary	5(5.7)	83(94.3)	
Secondary	6(7.1)	79(92.6)	
Tertiary	6(3.4)	168(96.6)	
			0.749
Ethnicity			
Yoruba	16(4.6)	329(95.4)	
Hausa	0(0.0)	4(100)	
Igbo	0(0.0)	6(100)	
Others	0(0.0)	18(100)	
			0.266
Alcohol Use			
Yes	1(16.7)	5(83.3)	
No	16(4.5)	340(95.5)	0.163

The Socio-demographic characteristics of participants in relation to infection with *Chlamydia trachomatis* revealed a general low prevalence of 4.5% with the highest number of infection among women of ages 25-31. A very good numbers of the women were married and fell in the category of the infected and non-infected. Education of the women at both secondary and tertiary levels recorded the highest infection frequency. More Yorubas participated in this study and alcohol use was generally very low.

4.24 Sexual History and Behaviour of Participants in Relation to *Chlamydia trachomatis* Infection.

Table 29 shows the sexual history and behaviour of participants in relation to *Chlamydia trachomatis* infection. *Coitarche* for age group 16-20 had the highest frequency of occurrence of infection (11), followed by age group 11-15(4). *Chlamydia trachomatis* infection in women whose onset of pregnancy was in the age group 20-24(8), the infection was absent in age group 35-39.Women whose *menarche* fell in age group 13-15 had the highest occurrence of the bacteria infection (9/17) and majority of Chlamydia negative women also falls within this age group (94.9%). In the study population, more women had only two lifetime sexual partners either positive (8.9%) or negative (91.4%) for *Chlamydia trachomatis*. Weekly sexual intercourse once a week was common among the participants and more women (10) who had the infection did not use condom for sexual protection.

Table 30 shows the History of Reproductive health and Sexually Transmitted Infection of Participants in relation to current *Chlamydia trachomatis* Infection. Most participants had never gone for Pap test (84.7%) with only 15.1% of the women with previous Pap test experience including those who tested positive for *Chlamydia trachomatis*. All women positive for *Chlamydia trachomatis* do not have previous infection with genital warts. Similarly, previous pelvic examination was few in women with the infection (8.9%), while the history of genital cuttings was (68.4%). Previous exposure with a sexually transmitted infection was not reported in any of those infected by *Chlamydia trachomatis*. Only 5.9% of the *Chlamydia trachomatis* positive women had history of abnormal vaginal discharge.

Table 29: Sexual History and Behaviour of Participants in relation to *Chlamydia trachomatis* infection

Characteristics	CT Positive Positive (n = 17) N(%)	CT Negative (n=359) N(%)	*P*-Value
Age at Sexual debut (Coitarche)			
≤ 10 (Years)	0(0.0)	3(100)	
11-15	4(16.0)	21(84.0)	
16-20	11(6.7)	154(93.3)	
21-25	1(0.8)	120(99.2)	
26-30	0(0.0)	30(100)	
31-35	0(0.0)	4(100)	0.008*
Age at onset of childbearing (Years)			
15-19	3(6.8)	41(93.2)	
20-24	8(5.8)	131(94.2)	
25-29	2(1.6)	194(98.4)	
30-34	3(9.7)	28(90.3)	
35-39	0(0.0)	2(100)	0.241
Menarche (years)			
10-12	1(3.7)	26(96.3)	
13-15	9(5.1)	168(94.9)	
16-18	7(5.6)	119(94.4)	
19-21	0(0.0)	16(100)	0.003*
Number of Lifetime sexual Partners			
1	4(2.0)	194(98.0)	
2	8(8.6)	85(94.1)	
3	4(8.0)	46(92.0)	
4	0(0.0)	10(100)	
≥5	0(0.0)	10(100)	0.048
Condom Use			
Yes	7(6.7)	108(32.1)	
No	10(58.8)	228(67.9)	0.552
Frequency of Sexual Intercourse weekly			
1	10(4.8)	199(95.2)	
2	4(4.2)	91(95.8)	
3	1(2.9)	33(97.1)	
≥4	0(0.0)	8(100)	0.667

The sexual history and behaviour of participants in relation to *Chlamydia trachomatis* infection revealed *coitarche* for both infected and un-infected participants is highest in age group 16-20. The most common age group at onset of childbearing is 25-29 among non-infected participants (98.4) but in the Chlamydia infected women age group 20-24 had the highest prevalence (5.8%). Age at *Menarche* was prevalent among women while at ages 13-15 irrespective of infection status. Most women had only one lifetime sexual partners (98.0%), with once weekly (95.2%) as the most frequent occurrence of sexual intercourse among participants and 63.3% did not use condom for protection.

Table 30: History of Reproductive health and Sexually Transmitted Infection of Participants in relation to current *Chlamydia trachomatis* Infection

Characteristics	C.T Positive (n=17)	C.T Negative (n=359)	*P*-Value
	N (%)	N (%)	
Pap Test			
Yes	2(3.8)	51(96.2)	
No	14(4.7)	284(95.3)	0.919
Genital Warts			
Yes	0(0.0)	6(100)	
No	16(4.6)	329(95.4)	0.590
Pelvic Examination			
Yes	5(8.9)	51(91.1)	
No	11(4.2)	249(95.8)	0.147
Female Genital Cuttings			
Yes	12(5.0)	230(95.0)	
No	5(4.5)	107(95.5)	0.840
Previous STI			
Yes	0(0.0)	52(100)	
No	15(5.0)	285(95.0)	0.921
Abnormal Vaginal Discharge			
Yes	3(5.9%)	48(94.1)	
No	13(4.3%)	288(95.7)	0.163

This table of History of Reproductive health and Sexually Transmitted Infection of Participants in relation to current *Chlamydia trachomatis* Infection reveals low uptake of previous Pap Test, reduced genital wart infection, low rate of past Pelvic examination, relatively high rate of female genital cuttings, with low previous history of Sexually Transmitted Infection and abnormal vaginal discharge.

4.25 Risks Factors Predisposing to Cancer among Participants in Relation to *Chlamydia trachomatis* Infection.

Table 31 shows the risks factors predisposing to cancer among participants in relation to *Chlamydia trachomatis* infection. None of the women positive for *Chlamydia trachomatis* were smokers (0.0%) and the proportion of non-smokers is 95.1%. The age group 20-24 witnessed the highest proportion of infection due to *Chlamydia trachomatis* (5.8%) of the age at first pregnancy and the prevalent age at first pregnancy was age 25-29 (98.5%). More than half of the participants infected by *Chlamydia trachomatis* (9 out of 17) used hormonal contraceptives. Generally the use of hormonal contraceptives was lower than those who did not use or used other method of family planning (62.4%). In this table, 4 was the highest frequency of parity (in 107 women), while majority of the infected women (6 of 17) had 3 children each. No infected woman had history of cancer in their family and family history of cancer was generally low (24 women). Irregular menstruation and post coital bleeding were 5.7% and 0.0% respectively in *Chlamydia trachomatis* infected individual

Table 31: Risks factors predisposing to cancer among participants in relation to *Chlamydia trachomatis* infection

Characteristics	CT Positive (n=17)	CT Negative (n=359)	P-Value
	N(%)	N(%)	
Smoking			
Yes	0(0.0)	1(0.3)	
No	17(4.7)	348(95.1)	0.826
Age At First Pregnancy			
15-19 (Years)	3(6.8)	41(93.2)	
20-24	8(5.8)	131(94.2)	
25-29	2(1.6)	194(98.4)	
30-34	3(9.7)	28(90.3)	
35-39	0(0.0)	2(100)	0.457
Use of Hormonal Contraceptives			
Yes	9(6.3)	135(93.8)	
No	8(3.4)	224(96.5)	0.345
Parity			
0	0(0.0)	1(100)	
1	2(8.0)	23(92.0)	
2	3(6.7)	42(93.3)	
3	6(7.2)	77(92.8)	
4	4(3.6)	107(96.4)	
5	2(2.0)	49(98.0)	
6	0(0.0)	23(100)	
7	0(0.0)	16(100)	0.034*
Family History of Cancer			
Yes	0(0.0)	24(100)	
No	16(4.8)	317(95.2)	0.824
Irregular Menstruation			
Yes	7(5.7)	115(94.3)	
No	9(3.9)	219(96.1)	0.446
Post-Coital Bleeding			
Yes	0(0.0)	8(100)	
No	16(4.7)	327(95.3)	0.533

This table of risk factors predisposing to cancer among the participants in relation to *Chlamydia trachomatis* infection reveals absence of smoking, Post-coital bleeding, family history of cancer among *Chlamydia trachomatis* infected women. Ages 35-39 recorded the lowest frequency of age at first pregnancy. Parity showed a frequency of 4 for majority of the participants and irregular menstruation was not a common occurrence among participants.

4.26 Infection Pattern in Human Papillomavirus and *Chlamydia trachomatis* among Participants

Figure 33 is a line graph showing the Infection pattern of Human Papillomavirus and *Chlamydia trachomatis* among participants in Ilorin in different age groups. HPV infection was more prevalent in age group 32-38 and lowest in age group 60-66. *Chlamydia trachomatis* infection was highest in age groups 25-31 and 39-45 and lowest in age groups 46-66. Human Papillomavirus and *Chlamydia trachomatis* co-infection had the highest frequencies in age groups 25-31 and 39-45 respectively. Lowest frequencies were in age groups 46-52, 53-59 and 60-66 years.

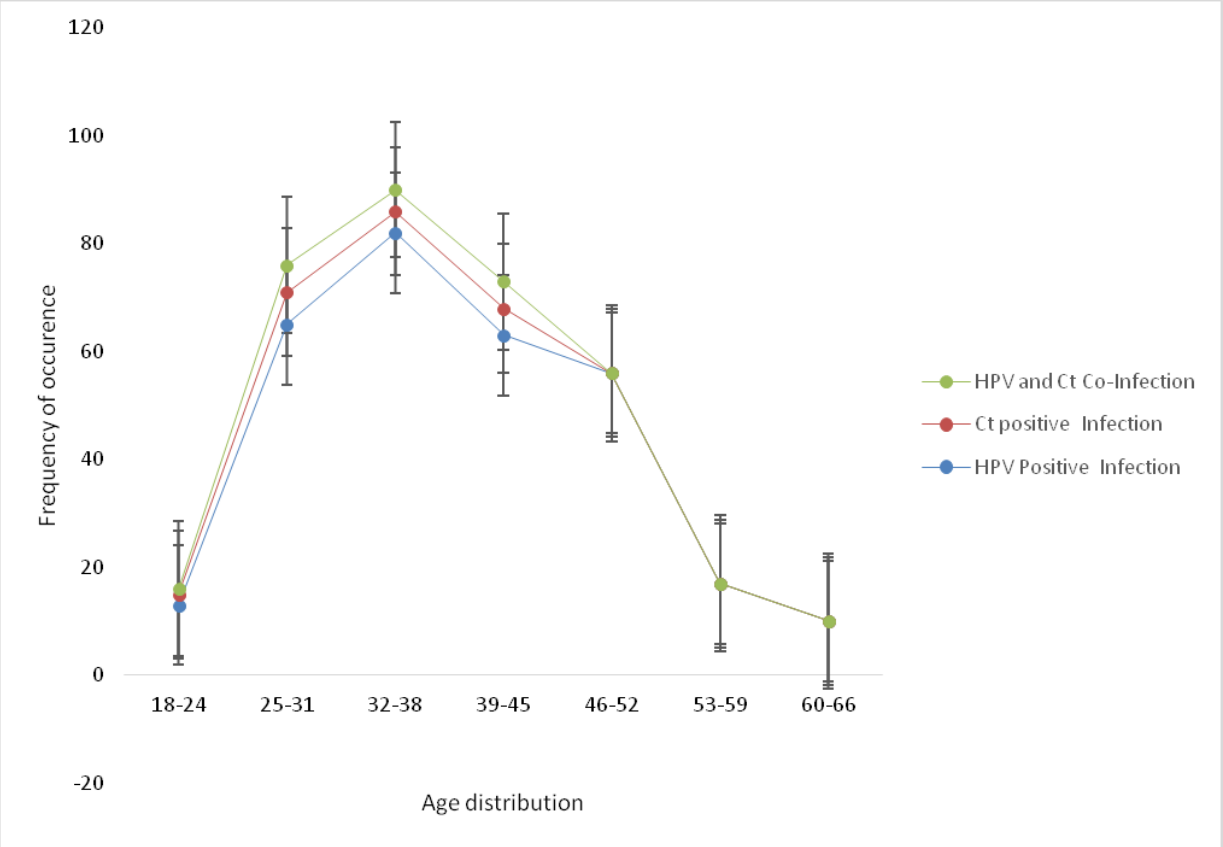

Figure 33: Infection pattern of Human Papillomavirus and *Chlamydia trachomatis* among participants in Ilorin in different age groups.

Green lines represents HPV and CT Co-infection, CT infection alone is represented with a red line while the blue line represents HPV Infection.

4.27 Distribution of HPV Genotypes and CT Co-Infection Among Women in Ilorin

Figure 34 represents the distribution of HPV genotypes and *Chlamydia trachomatis* co-infection among women in Ilorin Nigeria. A total of 17 (4.5%) women tested positive to *Chlamydia trachomatis*. A total of 81.4% of the participants tested positive to HPV DNA. HPV positive infection varied depending on types, with the highest frequency found in type 82 (33.5%). HPV and *Chlamydia trachomatis* co-infection was also highest among HPV 82 (7.9%), there were no co-infection in the following types; 16, 18, 26, 31, 33, 39, 45, 51, 52 and 58 respectively.

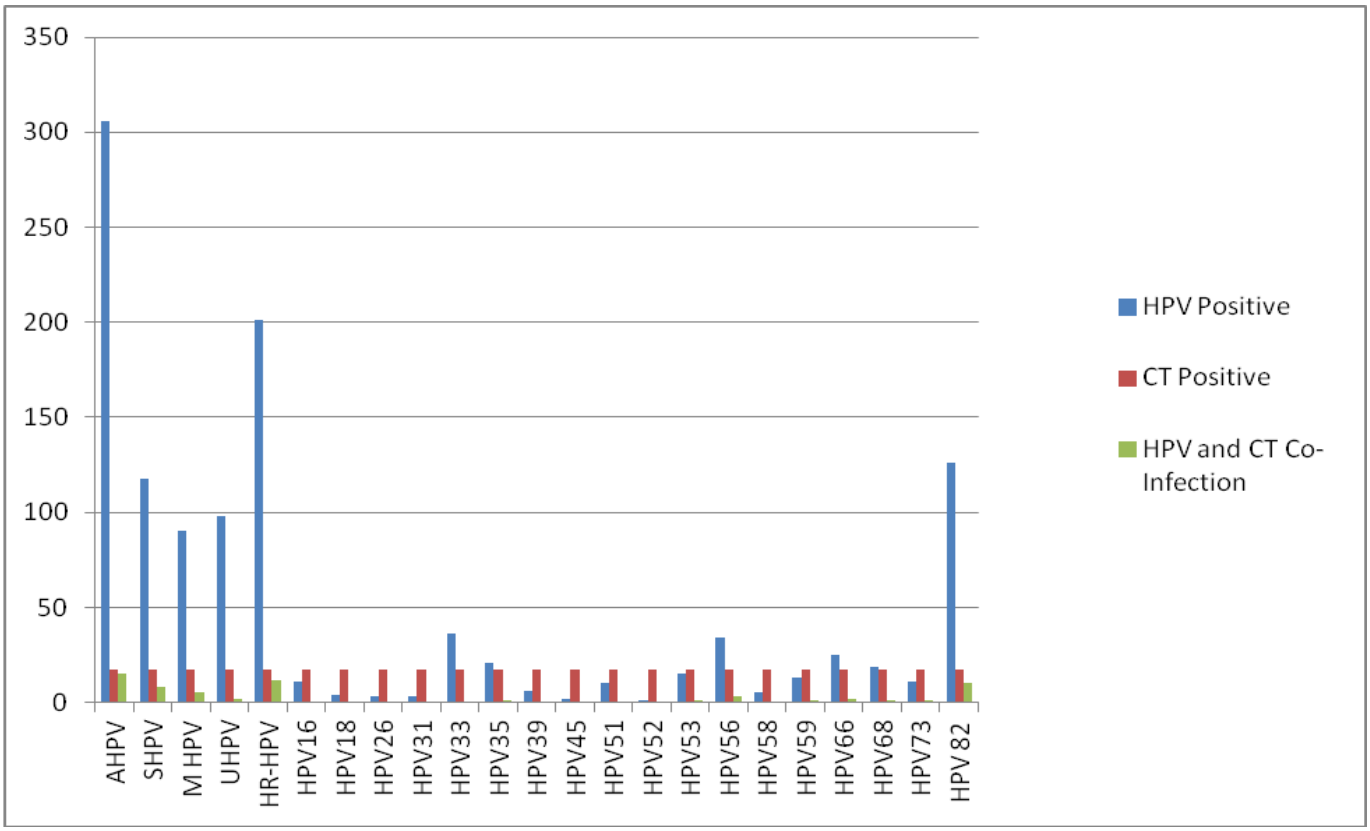

Figure 34: HPV genotypes and *Chlamydia trachomatis* co-infection among women in Ilorin Nigeria.

The blue bars represents HPV Infection, the red bars are CT infection while green bars are for Co-Infection.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Normal and Abnormal Smears

This study carried out opportunistic cervical screening in 376 participants from which only 53 (14.4%) (Table 6) participants had a history of Pap test. Participants were recruited at selected family planning and gyneacology clinics to identify normal and abnormal changes in the exfoliated cells of their cervix. The summary of the cytological findings are represented in Table 6 with a total prevalence of 5.1%, with ASCUS (1.1%), LSIL (3.5%) and HSIL (0.5%) respectively (Table 7). The result of this study was slightly lower than that of Thomas and co-workers (2012) findings of 7.6% abnormality and 1.5% HSIL, who carried out a community based study in Ibadan, Nigeria. The differences in these prevalence may be as a result of the numbers of women screened which was more in Ibadan (1020) compared to ours in Ilorin (376). Besides, the result of this study was higher than the works of Dursun and colleagues in 2009 followed by Azza and co-workers who independently recorded 1.8% each in turkey and Saudi Arabia respectively. The low prevalence in these studies may be due to socio-cultural and religious imperatives which might have curtailed promiscuity in these societies. Furthermore a higher prevalence of abnormality of 12.6% was recorded in Agogo a Ghanian community which was ascribed to lack of screening program and a lower level of education among this cohort. Although the lack of screening program in Agogo is similar to that of Ilorin, the lower prevalence in Ilorin compared to Agogo may be due to differences in socio-cultural practices in these population. Cervical cytological abnormalities among HIV positive Brazilian women is 23% (Lima *et al.,* 2014) and 56.3% (Lawal *et al.,* 2017) these high prevalence may be as a result of the immune-compromised status of participants in those studies contrary to this study that worked with apparently healthy outpatients. Generally, global prevalence of cervical cancer and cytological abnormalities is on the decline in high income countries with a

dis-proportionate increase in middle and low income countries. The increasing incidence of cervical carcinoma and cervical abnormalities in developing countries like Nigeria has been partly sustained by the absence of a national policy on cervical cytology, coupled with un-organized screening and detection of pre-malignant lesions. Strict adherence to the introduction of the Bethesda System (TBS) of classification of cytology will provide reliable record towards a national policy on cervical screening and research that is acceptable and verifiable (Sowemimo *et al.,* 2017; Nayar and Wilbur 2017).

5.2 Disparities in Human Papillomavirus Screenings

Many methods have been implored in the discovery of the aetiology of cervical cancer and HPV (Hausen, 2009; Kim, 2017). In this study, HPV DNA detection was used which is known to increase the sensitivity of cervical cancer screening compared to cytology (Salimovi´c-Beˇsi´c *et al.*, 2013). The amplification assay incorporates the PCR method with the advantage of high sensitivity and specificity using the consensus primers MY09/11 in a first round PCR followed by a nested PCR using GP5+/6+ consensus primers (Figure 23 and 24). The amplifications of a 450 bp and 150 bp fragments were taken as positives for MY09/11 and GP5+/6+ primer sets respectively. In this study, MY09/11 and GP5+/6+ Oligonucleotide showed varied detection of HPV (Table 27) based on AHPV, HRHPV and UHPV. Overall, more HPV were detected by GP5+/6+ (72.9%) compared to MY09/11 (59.8%). The use of MY09/11 was significant ($p< 0.05$) in the detection of HPVDNA and high risk HPV types, while the use of GP5+/6+ was also significant in the detection of HPV DNA and high risk HPV types with additional sensitivity in detecting unknown type HPV. This is slightly different from the work of Venceslau and colleagues (2014) who observed more HPV detection with GP5+/6+ than MY09/11 without significance difference which may be due to the sample size of their study (32 participants compared with 376 participants in our study. The use of both

primers in our study improved the detection of HPV DNA which is in agreement to the work of Camargo *et al.,* (2011).

The risks level of HPV is based on its association with invasive carcinoma. The detection of high risk HPV types in this study was by a nested multiplex PCR assay using primers synthesized from the E6, E7 oncoproteins region in the open reading frame of the HPV genome similar to Sotlar *et al*., 2004. The arrangement of the type specific nested multiplex PCR were done in seven different cocktails of four, three and two based on the closeness in sizes of the DNA base pair fragments (Table 3). DNA fragments corresponding to the high risk positive controls (Figure 24) in each cocktail were appropriately typed.

The global burden of HPV in women without cervical abnormality is put at 11-12% with sub- Saharan Africa accounting for 24% of the infection. Incidence of HPV related cervical cancer as at 2018 was 570,000 with an estimated death of 311,000 with more than 85% of this death occurring in low and middle income countries (Forman *et al*., 2012; WHO, 2019). In this study, HPV prevalence was 81.4% and supports the statement that HPV is presently the most common STI globally (CDC, accessed June 27th 2019). The prevalence from this study is higher than the studies of Thomas *et al.* Gage *et al.* Nejo *et al.* and Manga *et al*. who detected prevalence of 26.3% , 14.7% , 18.6% and 48.1% respectively in Ibadan, Irun, Saki-Ibadan and Gombe all in Nigeria. The higher prevalence in our study compared to others could be as a result of differences in the sensitivity and specificity of the method of detection used. For instance, Thomas *et al* (2004) utilized the conventional PCR method using the GP5+/6+ primers and then hybridized the PCR products in an Enzyme Immuno Assay. Gage *et al.* on the other hand used the MY09/11 consensus primers and the PCR products were typed by the dot blot hybridization technique using specific probes. In the case of Nejo *et al.,* a modified two sets of primers were used including the PGMY09/11 (modified MY09/11) and the degenerate GP-E6/E7 primers. In Gombe, Manga *et al*. used the nested PCR method and

then sequencing. In this study, conventional, nested and nested multiplex PCR methods were used in which first round MY09/11 primers was used in the conventional PCR followed by a nested PCR using the GP5+/6+ primers followed by specific typing of 18 HRHPV using type specific primers in a nested multiplex PCR. Besides, the socio-cultural behaviours of the population investigated, and a possibility of HPV transmission besides Sexual mode, especially by vertical transmission (Lee *et al.,* 2013; LaCour, 2012 and Park *et al.,* 2012) may have also accounted for the high prevalence. The study in Irun by Gage *et al.,* as well as that of Thomas *et al.,* were population based studies unlike our study, which is an hospital based study among out-patients. The prevalence from this study is consistent with the works of Levi *et al*., with a prevalence of 98% in Brazillian women, Ebrahim *et al*., who detected a prevalence of 76.3% among Young South African women and Diop-Ndiaye *et al.,* 79.8% who worked among Senagalese female sex workers.

In addition, the proportion of high risk HPV was 53.5% in this study and is higher than the works of Ezechi *et al.,* who recorded 19.6% prevalence in Ogun and Lagos, Okunade *et al.,* found out a prevalence of 36.5% in Lagos while Traore *et al.,* recorded 25.4% in a study in Burkina Faso. In this study, single and multiple high risk types infections were 31.4% and 23.9% respectively. HPV type 82 (33.5%) was the most predominant in single and multiple infections followed by HPV types; 33 (9.6%), 56 (9.0%), 66 (6.6%), 35 (5.6%) and 68 (5.1%) respectively. The prevalent high risk HPV types in this study (82, 33, 56, 66, 35 and 68) were different from the predominant genotypes in other parts of Nigeria. For instance, the work of Ezechi *et al.,* in Ogun and Lagos recorded high risks types 16 as the most predominant type followed by 35, 58 and 31 in decreasing order of prevalence. The study in Ibadan (Thomas *et al.,* 2004) showed higher prevalence in types 16, 31, 35 and 58 respectively while Okunade *et al.,* found out 31, 35 and 16 in decreasing order of predominance. Also, a high prevalence of genotypes 52 and 68 was found in Abuja among HIV negative women (Akarolo-Anthony *et*

al., 2013). In addition, Manga *et al.,* found out that the predominant high risk HPV types in Gombe were 18, 16, 31, 33 and 35 in decreasing order of prevalence. The result of this study further reveals variation in HPV type distribution in Nigeria and corroborates data from other parts of the world (Piras *et al.,* 2011). This will have implication on a national HPV vaccination policy on the currently available candidate prophylactic vaccine which does not confer a lifetime protection on vaccinee.

5.3 *Chlamydia trachomatis* Infection among Women in Ilorin

The gold standard for *Chlamydia trachomatis* detection was formerly thought to be culture method (Monif, 1998), which has been replaced by the Nucleic Acid Amplification technique (Moller *et al.,* 2011). PCR assay was used in this study with specific primer sets targeting the Major Outer Membrane Protein and the cryptic plasmid of CT (figure 29). The prevalence of CT in this study was 4.5% which corresponds to the global prevalence of *C. trachomatis* among sexually active women put at 3.7- 4.7% (Newman *et al*; 2015; WHO, 2012). It is also similar to the works of Tadele *et al.* (6.8%) in Ethiopia and Hussen *et al.,* (7.8%) but higher than the work of Adesiji *et al.,* (0.7%) who used a rapid kit test for the detection of CT. In contrast, the findings from this study is lower than the findings of; Ikeme et al. (29.4%) who studied seroprevalence of CT in women in Enugu, Arinze *et al.,* (30.2%) who worked among female undergraduate students in Port Harcourt using lateral flow assay and Adegbesan-Omilabu *et al.,* (27.7%) who did a cross sectional case control study of CT among women with normal and abnormal lesion using PCR. The disparities in those studies compared to this study may be due to the method of selecting participants for the studies, the type of assay used whether molecular or sero-prevalence and varied socio-cultural characteristics of the study population.

5.4 Co-infection of HPV and CT among Ilorin women

Persistence infection with high risk HPV types is now known to be a necessary but not sufficient cause of cervical cancer. This perspective is supported by the very few percentage of HPV infection that degenerates to disease. Other implicated factors such as infection with *Chlamydia trachomatis* which results in PID, a chronic type of inflammation can stimulate immunological response which may depend on the type of cytokine genes present in an individual with the potential of affecting the clinical course of HPV infection to HPV disease.

In this study the prevalence of HPV and CT co-infection was 4.0%, with a higher prevalence of CT in HPV positive samples when compared with CT in HPV negative samples. The CT co-infection was predominant in HPV 82, the most prevalent type among co-infected women. Both single and multiple infections were present in co-infected women without significant difference. The result of this work is similar to Ji Y *et al.,* who found a prevalence of 4.8% co-infection in a large cohort study among Chinese women and Ssedyabane *et al.,* who found out 8.6% prevalence in a pilot study among Ugandan women. The prevalence of this study was higher than that of Panetto *et al.,* which was 2.7% in young unvaccinated Italian women. In contrast, the prevalence of this study was lower than the work of Fowotade *et al.,* who recorded a prevalence of 11.1% in Ogun state Nigeria among Family Planning outpatients, de Castro-Sobrinho *et al.,* (15.1%) among Brazillian women and Fogue *et al.,* (23.3%) in Cameroonian women. The higher prevalence in these studies compared to our study may be as a result of population difference based on socio-cultural characteristics, the types of assay used and the sample size of the population. For instance, the work of Fowotade *et al.,* was limited to outpatients in the family planning clinic whereas this work considered outpatients in both family Planning and Gynaecological clinics. On the other hand, de Castro Sobrinho *et al.,* worked on HPV positive women who had CIN and had undergone cervical conisation in Brazil while Fogue *et al.,* studied women on routine gynaecology check up in Cameroon and used

ELISA and DNA detection technique for CT and HPV respectively. The use of ELISA technique for CT or HPV detection includes current and previous infection. Our study used molecular detection technique for both HPV DNA and CT DNA which is currently the gold standard and detects current infection.

5.5 Cytokines Genes Polymorphism in Ilorin Women

Variation exists in the response of individual to infection which is partly due to the quality or quantity of cytokines secreted and these secretions are controlled by unique genes in many forms called "cytokine polymorphisms" (Gallagher *et al.,* 2003). Apart from variations caused by cytokine genes, the environment also plays a crucial role in this variation (Smith and Humphries, 2009). These differences have influenced the pattern of cytokine genes between populations. Smith and Humphries in a review have explained that these variations lead to alterations in structure or expression of cytokines with pathological consequences in a number of infections. In this study, cytokine gene patterns for pro-inflammatory and anti-inflammatory cytokines (Table 11-20) among women in Ilorin as it relates to HPV and CT sole and co-infections were analysed.

IFN-γ (+874 A/T) Polymorphisms in HPV infection among Ilorin Women

It has been established that the IFN-γ gene is located on chromosome 12q24 spanning almost 5.4 kb with four exons and three intervening regions (Sun *et al.,* 2015). This study found out highest genotypic frequencies of IFN-γ TT in women with HPV infection. The TT genotype has a potential for high IFN-γ production unlike AA and AT with low cytokine production. There was significant association in IFN-γ at P=0.0001 and HPV infection this is similar to the work of Zheng *et al.,* who found out significant association between IFN-γ and HPV susceptibility among ruralwomen in China. Also this work agrees with Tamandani *et al.,*

on “the expression and polymorphism of IFN-γ gene” in Indian women who found out significant association between IFN-γ and HRHPV type 16. This work reveals that secretion of IFN-γ genotype favours HPV susceptibility.

IL-10 (-1082 G/A) Polymorphism in HPV Infection

This work focused on SNP of IL-10 at position -1082 G/A and found a significant association with HPV P=0.0001. The highest genotypic frequency was found in genotype GG which has a capability for high cytokine production and low frequency in AA and GAwhich had low and intermediate production respectively. The IL-10 G allele (Table 18) was most frequent in this work and agrees with the work of Matsumoto *et al.,* among women in Japan and inference that a possibility could exist for IL-10 gene polymorphism influence on immune response to HPV infection. Similarly, this work corroborates that of Chagas *et al.,* who associated IL-10 gene polymorphism with cervical lesion development in women with HPV infection. This work is not in tandem with the research of Barbesian *et al.,* who failed to find association between IL-10 and HPV infection. This may be due to the nature of study which was a case control study specifically in cervical cancer cases and healthy control, unlike this study that was unable to access any cervical cancer case.

TNF-α (-308 G/A) Polymorphism in HPV Infection

This work showed significant association between TNF-α and HPV when compared with apparently healthy individual P=0.0001. Deshpande *et al.,* explained that TNF-α is a pro-inflammatory cytokine usually released after an HPV infection with ability to up-regulate antigen presenting and processing pathways specifically in class 1 HLA’s. Besides, TNF-α has been the most widely studied cytokine and shown to be in association with autoimmune disease

and infection (Smith and Humphries, 2009). The result of this study was in contrast with the work of Barbesian *et al.,* who did not reveal any agreement between TNF-α and HPV infection.

TGF-β (Codons 10 and 25) Polymorphism in HPV Infection

In this study, there was no significant association in TGF-β10 *P*=0.0956 and HPV infection similar to the observation of Lima Juniour *et al.* who did not find a positive influence between HPV infection and TGF-β10 in a Brazilian women study which was also consistent with the work of Stanczuk *et al.* who inferenced that there was no statistical significance between TGF-β10 and women with invasive squamous cell carcinoma which normally have been infected cowith HPV. In contrast, TGF-β25 showed significant association with HPV infection *P*=0.0001 besides, allele frequency was high in HPV positive cases for C and T in TGF-β codon 10 and C and G in TGF-β codon 25 respectively. This work reveals that production of TGF-β10 does not affect HPV positive infection.

IFN-γ (+874 A/T) Polymorphisms in *Chlamydia trachomatis* Infection

IFN-γ has been shown to confer protection against *Chlamydia trachomatis* infection and insufficient production of IFN-γ invariably leads to CT persistence (Ohman *et al.,* 2011). This work revealed significant association when CT and apparently healthy controls were compared with IFN-γ (*P=0.0001)* which is in agreement with Ohman *et al.* who found a link between IFN-γ and CT induced lymphocyte proliferation responses. Similarly, this study was in agreement with Eleuterio *et al.* who recorded significant association between IFN-γ polymorphism and CT tubal infertility. This finding contradicts an earlier work by Ohman *et al.* in the year 2009 who did not find out significant association between IFN-γ polymorphism and CT tubal damage. The difference in this study could be attributed to the nature of study participants recruited for either study. For example, Ohman *et al.* recruited women with

confirmed cases of infertility, while this study focused on women attending the family planning clinic and gynaecology clinic without emphasis on infertility.

IL-10 (-1082 G/A) Polymorphism in *Chlamydia trachomatis* Infection

This work found significant association between CT infection and genotypic frequency of IL-10 polymorphism with low allelic frequency in comparison to apparently healthy controls (Table 19). The low frequency of allele was due to the very few cases (2 cases) of participants with sole CT infection. Allele frequency was not significant which was contrary to the earlier and later works of Ohman *et al.* who found significant association for IL-10 allele as risk factors for CT induced tubal damage.

TNF-α (-308 G/A) Polymorphism in *Chlamydia trachomatis* Infection

This work did not reveal any significant association between TNF-α and infection with CT. In contrast, Ohman *et al.* found out significant association in TNF-α allele as risk factors for CT induced tubal damage. This difference could be associated with the sample size of positive CT infected participants. A larger sample size could show different result.

Summarily, the genotypes with highest frequencies in an HPV infection include: GG of IL-10 (-1082 G/A), CT in TGFβ-10, TT in IFN-γ (+874 A/T) and GA in TNF-α (-308 G/A) all with the ability to induce the production of copious amount of the respective cytokines, except in genotype CC of TGFβ-25 which codes for an intermediate level of cytokine production. In the case of a co-infection of Human Papillomavirus and *Chlamydia trachomatis* infection, AG of IL-10 (-1082 G/A), CT in TGFβ-10, CG in TGFβ-25, AT in IFN-γ (+874 A/T) and GA in TNF-α (-308 G/A) had the highest genotypic frequencies. For apparently healthy individuals in this study, it was observed that the highest genotypic frequencies were in AG of IL-10 (-1082 G/A), TT in TGFβ-10, GG in TGFβ-25, AT and AA in IFN-γ (+874 A/T) and GG in TNF-α

(-308 G/A). This work showed significant association when HPV was compared with apparently healthy individual in the following cytokines; TNF-α (P=0.0001), IFN-γ (P=0.0001), IL-10 (P=0.0001), and TGFβ-25 (P=0.0001), but no significant association was found in TGFβ-10 (P=0.0956). Similarly, the comparison between CT and apparently healthy revealed significant association in IFN-γ (P=0.0001), IL-10 (P=0.0258), TGFβ-10 (P=0.0034) and TGFβ-25 (P=0.0001), but no significance association was found in TNF-α (P=0.3129). Also, when co-infection of HPV and CT were compared with apparently healthy controls significant association were shown in both pro and anti- inflammatory cytokines genes tested in this study such as; TNF-α (P=0.0001), IFN-γ (P=0.0001), IL-10 (P=0.0255), TGFβ-10 (P=0.002) and TGFβ-25 (P=0.0001).

Furthermore, themean comparison of co-infection with single infection showed significant association in IFN-γ, TNF-α and TGFβ-10. Also, when HPV and CT single infection were compared, IFN-γ and TNF-α showed significant difference. The evidence from this work substantiates that an interplay of different cytokine genes dominates in a Human Papillomavirus and *Chlamydia trachomatis* co-infection with potentialof a possible future debilitating effect under certain risk factors.

5.6 Major Risk Factors of HPV Infection in Ilorin

The high prevalence of HPV found in this study could be due to certain risk factors among women in Ilorin which may have predisposed the population to the risk of HPV infection. Our study did not reveal any significant association between age of participants and HPV (Table 8) but the highest number of participants were among age group 32-38 years of age which corresponds to the age at which most women seek for family planning intervention. This is in tandem with the work of Okunade *et al.,* 2016 who found out that women of age group 31-40 years make up over 60% of Family Planning attendees in a teaching hospital in

Lagos, Nigeria. The lowest participation with respect to age in our study was among women 60 years old and above. Smith *et al.,* in a global review of age-specific prevalence of HPV showed that HPV prevalence primarily decreases as women increase in age which peaks in age group younger or equal to 25 years, although variation exists from country to country. In our study, HPV prevalence by age peaked in the age groups; 32-38 years (middle age), 46-52 years (middle Age) and 60-66 years (old age) Figure 25. Thomas *et al.* also revealed a similar peak in HPV infections in both middle and old ages. Likewise, a study in Ife, Osun state by Fadahunsi *et al.,* in 2013 found out the similar trend. In contrast, some studies found out higher prevalence in women less than 25 years and lower prevalence in age group 65 years and older (Nejo *et al.,* 2018; Akarolo-Anthony *et al.,* 2014). The peak infection in middle age group 32-38 in this study corresponds to the early years in marriage for most participants and increased sexual activities. The drop in HPV infection in age group 39-45 years could be as a result of clearance of the virus, while a second slight peak in age 46-52 could be due to re-infection from spouses or other sexual partners. The peak of HPV infection at old age could be as a result of malnutrition or wane in immunity resulting in inability to clear the virus. Also HRHPV prevalence in different age group showed a modal curve in age group 31-38 and a rising peak in age group 60-66 years (Figure 25).

This study showed association between Age at first sexual debut (*coitarche*) and HPV infection (P=0.004). Most of the women in this study had their sexual initiation before age 20. Also *coitarche* was significantly associated with number of lifetime sexual partners ($p<0.01$). This is similar to the work of Khan *et al.* who found out association between early sexual activity and HPV infection among a mixed ethnicity (Whites, Hispanics, Blacks, Asian) in a University in United State. Similar work by Okunade *et al.* also significantly associated HRHPV, number of life time sexual partner and *coitarche* among women in Lagos Nigeria.

This study is in contrast with the work of Liu *et al.* who failed to record a significant association between *coitarche* and HPV infection among Chinese women.

Most of the women in this study were married (93.8%) while very few were single (3.6%) and divorced (2.6%). This shows that majority of the women who access family planning and Gyneacology clinic in Ilorin are predominantly married women. This study revealed significance association between HPV infection and marital status primarily in married women (p=0.045). A similar study by Akarolo-Anthony *et al.* also recorded higher prevalence of HPV infection among married women compared to unmarried women in Lagos Nigeria. On the other hand, a research in Oyo State Nigeria by Nejo *et al.* associated HPV infection among divorcees. The differences in these studies could be as a result of population size and socio-cultural behaviours of divorcees in both studies.

In addition, more women were in a monogamous relationship (72.5%) compared to 27.2% in polygamy. Marriage type in this study showed no significance association between HPV infection and polygamy. But significant association was seen between monogamy and HPV infection (p=0.001). This finding is at variance with studies of Nejo *et al.* Bayo *et al.* who found a significant association between Polygamy and HPV infection. The findings of this study may be due to partners multiple sexual relationships outside their monogamous marriage.

There was no significant association between parity and HPV infection, although the highest prevalence of HPV infection was among women with a total of 4 lifetime birth (30.8%) while the lowest HPV infection was in women with 10 lifetime birth (3.3%). The result of this study agrees with that of Thomas *et al.* and Miranda *et al.* but was in contrast with the works of Jensen *et al.* and Wang *et al*. who found out significant association between number of birth or pregnancy with HPV infection.

The use of contraceptive to control birth was significantly associated with HPV infection (*P*=0.013) in this study. This finding has been corroborated by the work of Marks *et*

al. and was in contrast with the study of Okunade *et al.* that failed to establish a relationship between contraceptive use and HPV infection.

In this study 68.6% of women had had female genital mutilation or cutting and the highest percentage of these women were in the age group 25-31 years (Table 23). There was significant association between HPV infection and FGC (*P*=0.008). Similarly, there was a significant relationship (*P*=0.03 at 0.001 level) between untyped HPV infection in this study and FGC. The practice of female circumcision has been a popular practice in Ilorin. Study by Aderibigbe *et al.,* in 2018 had revealed that 57% household practiced FGC either for religious reasons or for ease of childbirth. Other studies have shown that FGC is a common practice in Africa (Kouba *et al*., 1985; Bayo *et al.,* Costello, 2015; Akin-Tunde *et al.,* 2017) while the work of Okeke *et al.,* 2012 revealed an increase burden of FGC in Nigeria. Most of these studies did not consider HPV infection and its association with FGC. The research of Ostermann *et al.* revealed the significant possibility of HPV increasing the risks of FGC and susceptibility to cervical cancer. This study did not find significant association in possible risk factors such as; smoking, alcohol use, education, *menarche* and a family history of cancer.

5.7 Major Risk Factors Predisposing to Possible CT Infection

In this study, CT was significantly associated with age group (*P*=0.012) and the highest prevalence was in age group 25-31 (7.4%). This is similar to the work of Leon *et al.* who found out an inverse association of CT infection and age among men and women in Peru and Kučinskienė *et al.* who established age as a risk factor of CT infection in a review of infection among European women. On the other hand, age was not significantly associated with CT in the works of Dela *et al*. among Ghanaians and Huai *et al.* In this study, the prevalence of CT peaked at age group 25-31 years when sexual activity is presumed to be high which corroborates with earlier findings that the infection is usually high among young sexually

active women (Kučinskienė *et al.,* 2006) On the contrary, no current infection of CT was recorded among ages 46-60 years this may be as a result of the molecular assay used which excludes the possibility of a previous exposure to CT infection.

Chlamydia trachomatis infection in this study was further associated significantly with age of participants at sexual debut (*coitarche*) *P*=0.008 the result of this study is in tandem with the work of Huai *et al.,* 2018 who revealed significant association of CT in Chinese women who had their first sexual experience at age 20 or less. Ige *et al.* also corroborates the association of *coitarche* and CT infection among women in a Northern Nigeria Teaching Hospital. The result of this work was different from that of Adegbesan-Omilabu *et al.* who did not record significant association between CT infection and age at sexual debut. The disparity in these studies could be in the method of selection of participants. For example the work of Adegbesan-Omilabu *et al.* was a case control study that recruited women with confirmed cervical abnormality against controls while this present study considered women visiting family planning and Gynaecology clinics without consideration of their previous Pap test results.

There was also significant association between CT infection and Parity (*P*=0.034) this is in agreement with the work of Ige *et al.* among women in Northern Nigeria and in contrast with the work of Molano *et al.* and Borges *et al.* who did not record significant association in parity and CT among Colombian and Brazilian women respectively.

This study also found out significant association between age at first menstruation (*P*=0.003), age at first child (*P*=0.003) and CT infection using the multivariate logistic model. On the other hand, this study did not show any statistical significant association between the use of contraceptive, FGC, smoking, marital status, marriage type and education.

5.8 Possible Implicating Risk Factors in an HPV and CT Co-Infection in Ilorin

Human Papillomavirus has not been known to be solely responsible for the cause of invasive cervical carcinoma other factors especially STI have been implicated in a possible clinical course and ultimate development of cancer of the cervix (Ssedyabane *et al.,* 2019). The risk factors of HPV and *Chlamydia trachomatis* co- infection was considered in this study. This work found significant association between co-infection and age (*P*=0.029). Significant association also existed between co-infection and *coitarche* (*P*=0.024) which is similar to the report of a previous study by Fowotade *et al.* in Ogun state Nigeria. Significance was also associated with parity (*P*=0.035) only the work of Fowotade *et al.* to the best of my knowledge is currently published on HPV and CT co-infection in Nigeria even at that, the work was limited to serological study of HPV and CT and also considered a small sample size unlike this study that used molecular detection method which is the gold standard for HPV and CT on a fairly large population. Other worker like Samoff *et al.* was able to independently associate CT with HRHPV and cervical cancer. Although, this current research did not find significant association between CT and HRHPV, association exists between CT and HPV 82 (*P*=0.024). Besides, Ssedabane *et al.* found out significant association between HPV-CT co-infection and cervical abnormality but no such significant association was found in this study. The difference may be as a result of the method of selection of participants in both studies for instance, Ssedabane *et al*. worked in a cervical cancer clinic as well as a pathological referral clinic where cases for cervical abnormality were high. On the contrary, this study focused on Gynaecology and family planning clinics where most participants were apparently healthy and previous cytology status were not known. This study failed to find out significant association between co-infection and FGC, number of lifetime sexual partners, *menarche* and contraceptive use.

CONCLUSION

From the findings of this study the following conclusion were made;

1. The prevalence of cervical abnormality was low (5.1%) among women in Ilorin.
2. The use of multiple primers for this study increased the sensitivity of HPV DNA detection and reduced the possibility of false negative results.
3. Human Papillomavirus is a very common sexually transmitted infection among women in Ilorin with a prevalence of 81.4% of which HRHPV accounts for 53.5%.
4. *Chlamydia trachomatis* prevalence was low (4.5%) and common among younger people in this study. This low CT prevalence corresponds to global prevalence.
5. Human Papillomavirus and CT Co-infection prevalence was low (4.0%) in this study but the study established that a CT infection in a woman increase her susceptibility to HPV infection by about ninety percent (88.2%). This is because 88.2% women with CT infection also had HPV infection.
6. Gene polymorphisms from pro and anti-nflammatory cytokines produced varied association in Human Papillomavirus sole infection, *Chlamydia trachomatis* sole infection and co-infection
7. Pro-inflammatory cytokines genes of IFN-γ, TNF-α predominated significantly in Human Papillomavirus sole infection.
8. Anti-inflammatory cytokines genes of IL-10, TGFβ-10, TGFβ-25 predominated in *Chlamydia trachomatis* sole infection
9. Both pro and anti-inflammatory cytokines genes of IFN-γ, TNF-α and IL-0, TGFβ-10, TGFβ-25 were predominant in cases of co-infection.
10. Factors associated with the risks of Human Papillomavirus, *Chlamydia trachomatis* or co-infection includes; age, parity, *coitarche*, female genital cutting and contraceptive use.

RECOMMENDATIONS

Based on the results of this work, the following recommendations have been suggested;

1. Women in Ilorin should attend routine cervical screenings regularly and more awareness on Pap Test as well as a national policy on cervical screening should commence without delay.
2. Other auxillary test especially HPV DNA and CT should be done alongside Pap Test and to this effect, Liquid based Cytology method is most appropriate..
3. Molecular methods should be encouraged in the detection of HPV and CT infections due to its high sensitivity and specificity.
4. Prompt treatment of STI especially *Chlamydia trachomatis* should be done to avoid susceptibility to HPV and other debilitating complications like PID and cancer.
5. The practice of Female Genital cuttings should be discouraged and possibly abolished among women in Ilorin.
6. Cytokine gene patterns especially IFN-γ, TNF-α, IL-10 can be used as predictors of HPV and CT co-infection in women.
7. Further studies involving larger cohort of participants should be done using confirmed cervical carcinoma cases and quatitative as well as qualitative measurement of cytokines should be done simultaneosly.
8. As a result of individual variation in cytokine production coded and regulated by cytokine genes, it is important to consider cytokine therapy and individualized treatment of patient in response to persistent HPV infection.

CONTRIBUTIONS TO KNOWLEDGE

This study has contributed in the following ways:

1. This study found out that Human Papillomavirus is a very common Sexually Transmitted Infection, among women in Ilorin, with genotype 82 as the commonest high risk type.
2. Findings of Human papillomavirus and *C. trachomatis* co-infection among women with normal and abnormal changes in cell population of the cervix, will serve as a base line statistics for further research in Ilorin, Nigeria.
3. This work establishes a baseline prevalence for the molecular detection of HPV, *Chlamydia trachomatis* and HPV and *Chlamydia trachomatis*
4. This study establishes a relationship between Female genital cuttings and untyped HPV infection for the first time in Ilorin, Nigeria.
5. There is significant correlation between Untyped HPV and Abnormal cervical changes
6. There is significant correlation between HPV and CT Co-infection and pro and anti-Inflammatory cytokines genes of IFN-γ, TNF-α, IL-10, TGFβ codons 10 and 25

REFERENCES

Abbas, A.B., & Lichtman, A.H. (2009). "Ch.2 Innate Immunity". In Saunders (Elsevier). *Basic Immunology. Functions and disorders of the immune system* (3rd ed.). ISBN 978-1-4160-4688 2

Aboyeji, A.P. and Nwabuisi, C. (2003). Prevalence of sexually transmitted infection in young pregnant women in Ilorin, Nigeria. *Journal of Obstetrics and Gynaecology*. 23(6): 637-9.

Abraham, R.T. (2001). Cell cycle Checkpoint Signaling through the ATM and ATR kinases. *Genes Development* 15: 2177–2196. [PubMed: 11544175]

Abreu, A. L. P., Souza, R. P., Gimenes, F. & Marcia E L Consolaro, M. E. L. (2012). A review of methods for detecting human Papillomavirus infection. *Virology Journal* 9:262 http://www.virologyj.com/content/9/1/262.

Acharya, P.V. (1971). "The isolation and partial characterization of age-correlated oligo-deoxyribo-ribonucleotides with covalently linked aspartyl-glutamyl polypeptides.". *Johns Hopkins medical journal. Supplement* (1): 254–60. PMID 5055816.

Adam, Y., McIntyre, J. A. & de Bruyn, G. (2013). Incidence of cytological abnormalities within 24 months of a normal cervical smear in Soweto, South Africa. *South African Medical Journal.* 103(1):34-39. DOI:10.7196/SAMJ.6124

Adegbesan-Omilabu M. A., Kehinde Sharafadeen Okunade, Ayotunde Ayodeji Oluwole, Abidoye Gbadegesin, Sunday Aremu Omilabu (2014). *Chlamydia trachomatis* among women with normal and abnormal cervical smears in Lagos, Nigeria. *Int J Reprod Contracept Obstet Gynecol. ;3(3):501-506* www.ijrcog.org

Aderibigbe, S. A., Alatishe-Muhammad, B. W., Ameen, H. A., Salaudeen, A. G., Saka, M. J., Uthman, M. M. B., Bolarinwa, O. A., Akande, T.M. and Raji, H.O.(2018). Practice of female genital cutting amongst adults in Ilorin metropolis, North-Central Nigeria. *The Tropical Journal of Health Sciences* Vol 25 No 1.

Adesiji, Y. O., Iyere, S. I. & Ogah, I. J. (2015). Low Prevalence of *Chlamydia trachomatis* Infection in Women From Southern Nigeria. *NUJHS Vol.* 5, No.1, ISSN 2249-7110

Aggarwal, B.B., Vijayalekshmi, R.V. & Sung, B. (2009). Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clinical Cancer Research* 15:425–430. [PubMed: 19147746]

Aivaliotis, I. L., Pateras, I. S., Papaioannou, M., Glytsou, C., Kontzoglou, K., Johnson, E. O., & Zoumpourlis, V. (2012). How Do Cytokines Trigger Genomic Instability? *Journal of Biomedicine and Biotechnology*, 2012, 1–12. doi:10.1155/2012/536761

Akarolo-Anthony S. N., Al-Mujtaba, M., Famooto, A. O., Dareng, E. O., Olaniyan, O. B., Offiong, R., Wheeler, C. M. & Adebamowo, C. A. (2013). HIV associated high-risk HPV infection among Nigerian women *BMC Infectious Diseases* 13:521 http://www.biomedcentral.com/1471-2334/13/521

Akarolo-Anthony S. N., Famooto, A. O., Dareng, E. O., Olaniyan, O. B., Offiong, R., Wheeler, C. M. & Adebamowo, C. A. (2014). Age-specific prevalence of human papilloma virus infection among Nigerian women. *BMC Public Health*, 14:656 http://www.biomedcentral.com/1471-2458/14/656.

Akira, S., Uematsu, S. & Takeuchi, O. (2006). Pathogen recognition and innate immunity, *Cell*, vol. 124, no. 4, pp. 783–801.

Aladesanmi, A.F., Mumtaz, G. & Mabey, D.C. (1988). Prevalence of cervical chlamydial infection in antenatal clinics attendees in Lagos, Nigeria. *Genitourinary Medicine.* 65(2):130-2.

Alfonsi, G. A., Deblina Datta, S., Mickiewicz, T., Koutsky, L. A., Ghanem, K. G., Hagensee, M., ... Shlay, J. C. (2011). Prevalence of high-risk HPV Types and abnormal cervical cytology in American Indian/Alaska Native Women, 2003-2005. *Public Health Reports*, *126*(3), 330-337

Alfredo Amador-Molina, José Fernando Hernández-Valencia, Edmundo Lamoyi , Adriana Contreras-Paredes & Marcela Lizano (2013). Role of Innate Immunity against Human Papillomavirus (HPV) Infections and Effect of Adjuvants in Promoting Specific Immune Response. *Viruses 5*, 2624-2642; doi:10.3390/v5112624.

Amin, J.D., Zaria, L.T., El-Nafatty, A.U. & Mai, A.M. (1997). Genital trachomatis infection in women in a Nigerian hospital. *Genitourinary Medicine.* 73:146-7.

Ammirante, M., Luo. J-L., Grivennikov, S., Nedospasov, S. & Karin, M. B. (2010). cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature.* in press.

Anh, P.T., Hieu, N.T., Herrero, R., Vaccarella, S., Smith, J.S., Thuy, N.T., Nga, N.H., Duc, N.B., Ashley, R., Snijders, P.J., Meijer, C.J., Mun˜oz, N., Parkin, D.M. & Franceschi, S. (2003). Human papillomavirus infection among women in South and North Vietnam. *International Journal of Cancer* 104:213–220.

Anja, M. D. & Cimprich, K. A. (2010). Checkpoint recovery after DNA damage: a rolling stop for CDKs. *EMBOreports* Vol 11 No. 6.

Antonsson, A., Erfurt, C., Hazard, K., et al. (2003). Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents. *J Gen Virol* 84:1881-1886. DOI: 10.1099/vir.0.18836-0

Araoye, M.O. (2004) Sample Size Determination in Research Methodology with Statistics for Health and Social Sciences. Nathadex Publishers, Ilorin, 115-121.

Arinze, Au. H., Onyebuchi, N., & Isreal, J. (2014). Genital *Chlamydia trachomatis* infection among female undergraduate students of University of Port Harcourt, Nigeria. *Nigerian Medical Journal*, 55(1), 9. doi:10.4103/0300-1652.128147

Aspord, C., Pedroza-Gonzalez, A., Gallegos, M., Tindle, S., Burton, E.C., Su, D., Marches, F., Banchereau, J. & Palucka, A.K. (2007). Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. *Journal of Experimental Medicine* 204:1037–1047. [PubMed: 17438063]

Atara, N. (2012). Cervical Cancer in Sub Sahara Africa, Topics on Cervical Cancer with an Advocacy for Prevention, Dr. R. Rajamanickam (Ed.), ISBN: 978-953-51-0183-3, InTech, Available from: *http://www.intechopen.com/books/topics-on-cervical-cancer-with-an-advocacy-for-prevention/cervical-cancerin-sub-sahara-africa*

Azenabor, A. A. & Eghafona, N. O. (1997). Association of *Chlamydia trachomatis* antibodies with genital contact disease in women in Benin City, Nigeria. *Tropical Medicine and International Health* vol *2* NO. 4 pp *389*-392. doi/pdf/10.1111/j.1365-3156.1997.tb00156.x

Azza, A., E., M. (2015). Incidence and risk factors of abnormal cervical cytology in a university hospital - Saudi Arabia. *Saudi Arabia Journal of Health Sciences* http://doi.org/10.4103/2278-0521.157878

Bacteriology 167: 570-574.

Baden, L.R., Curfman, G.D., Morrissey, S. & Drazen, J.M. (2007). Human Papillomavirus Vaccine—Opportunity and Challenge. *New England Journal of Medicine* 356; 19: 1990-1.

Baker, K. J., Houston, A., & Brint, E. (2019). IL-1 Family Members in Cancer; Two Sides to Every Story. *Frontiers in immunology*, 10, 1197. doi:10.3389/fimmu.2019.01197

Bakkenist,C.J., Kastan, M.B, (2003). "DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation". *Nature* 421 (6922): 499–506. doi:10.1038/nature01368. PMID 12556884.

Baldeyron, C., Soria, G., Roche, D. Cook, A.J. & Almouzni, G. (2011). HP1alpha recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. *Journal of Cell Biology.* 193:81-95.

Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet*. 357:539–45.

Barbisan, G, Pérez, L. O., Contreras, A., Golijow, C.D. (2012). TNF-α and IL-10 promoter polymorphisms, HPV infection, and cervical cancer risk. *Tumour Biology.* 33(5):1549-56. doi: 10.1007/s13277-012-0408-1.

Bartek, J. & Lukas, J. (2003). Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* 3:421– 429. [PubMed: 12781359]

Bauwens J. E., Clark A. M., & Stamm W. E. (1993) Diagnosis of *Chlamydia trachomatis* Endocervical Infections by a Commercial Polymerase Chain Reaction Assay. *Journal Of Clinical Microbiology*, p 3023-3027 Vol. 31, No. 11

Bayo, S., Bosch, F. X., de Sanjosé, S., Muñoz, N., Combita, A. L., Coursaget, P., … Meijer, C. J. (2002). Risk factors of invasive cervical cancer in Mali. International Journal of Epidemiology, 31(1), 202–209. doi:10.1093/ije/31.1.202

Bello, F.A., Enabor, O.O. & Adewole, I.F. (2011). Human Papilloma Virus Vaccination for Control of Cervical Cancer: A Challenge for Developing Countries *African Journal of Reproductive Health 2011; 15[1]: 25-30.*

Berg, D.J., Davidson, N., Kuhn, R., Muller, W., Menon, S., Holland, G., Thompson-Snipes, L., Leach, M.W. & Rennick, D. (1996). Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *Journal of Clinical Investigation* 98: 1010–1020. [PubMed: 8770874]

Bergers, G., *et al.*, (2000). Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nature Cell Biol* 2:737–744. [PubMed: 11025665]

Bergonzini, V., Salata, C., Calistri, A., Parolin, C. & Palu, G. (2010).“View and review on viral oncology research,” *Infectious Agents and Cancer*, vol. 5, no. 1, p. 11. doi: 10.1186/1750-9378-5-11

Bernard, H.U., Calleja-Macias, I.E. & Dunn, S.T. (2006). Genome variation of human papillomavirus types: phylogenetic and medical implications. *International Journal of Cancer* 118:1071-1076. http://doi.org/10.1002/ijc.21655

Bernard, H-U., Burk,R. D., Chen, Z., van Doorslaer, K., Hausen, H. Z., & de Villiers, E-M. (2010). Classification of Papillomaviruses (PVs) Based on 189 PV Types and Proposal of Taxonomic Amendments. *Virology.* 25; 401(1): 70–79. doi:10.1016/j.virol.2010.02.002

Bhatla N., Kriti Puri, Elizabeth Joseph, Alka Kriplani, Venkateswaran K. Iyer and V. Sreenivas (2013). Association of *Chlamydia trachomatis* infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia - A pilot study. *Indian J Med Res* 137, pp 533-539

Bidwell, J., Keen, L., Gallagher, G., Kimberly, R., Huizinga, T., McDermott, M., … D’Alfonso, S. (1999). Cytokine gene polymorphism in human disease: on-line databases. Genes & Immunity, 1(1), 3–19. doi:10.1038/sj.gene.6363645

Bilardi, F.J., Fairley. C.K., Hopkins, C.A. et al. (2010). Experiences and outcomes of partner notification among men and women recently diagnosed with *Chlamydia* and

their views on innovative resources aimed at improving notification rates. *Sex Transm Dis* 37 (4):253-258.http:// doi: 10.1097/OLQ.0b013e3181d012e0

Bjorksten, J., Acharya, P.V., Ashman, S., & Wetlaufer, D.B. (1971). Gerogenic Fractions in the tritiated rat. *Journal of the American Geriatrics Society* 19 (7): 561–74. PMID 5106728.

Boccardo, E., Lepique, A.P. & Villa, L.L. (2010). The role of inflammation in HPV carcinogenesis. *Carcinogenesis*. 31:1905–12. [PubMed: 20819779]

Bosch, F.X. & de Sanjose, S. (2003). Human papillomavirus and cervical cancer-burden and assessment of causality. *Journal of National Cancer Institute Monograph* 31:3–13 [chapter 1]. doi: 10.1093/oxfordjournals.jncimonographs.a003479

Botstein, D., Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol. Biol. Cell* 9 (12): 3273–97.

Boyer, S.N., Wazer, D.E. & Band, V. (1996). E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Research.* 56:4620-4624.

Bozzetti, M., Nonnenmacher, B., Mielzinska, I.I., Villa, L., Lorincz, A., Breitenbach, V.V., *et al*., (2000). Comparison between hybrid capture II and polymerase chain reaction results among women at low risk for cervical cancer. *Annals of Epidemiology.* 10: 466.

Branca, M., Ciotti, M., Santini, D., Bonito, L. Di, Benedetto, A., Giorgi, C., Paba, P., Favalli, C., Costa, S. & Agarossi, A. (2004). Activation of the Erk/Map kinase pathway in cervical intraepithelial neoplasia is related to grade of the lesion but not to high-risk human papillomavirus, virus clearance, or prognosis in cervical cancer. *American Journal of Clinical Pathology 122:* 902–911. http://doi: 10.1309/vqxf-t880-jxc7-qd2w

Brandie, D. T. & Catherine, L. H. (2011). Management of *Chlamydia trachomatis* Genital Tract Infection: Screening and Treatment Challenges. *Infection and Drug Resistance* 2011:4. http:// doi: 10.2147/IDR.S12715

Brian W.J. Mahy (2009). The Dictionary of Virology 4th edn. Elseevier Limited USA ISBN 978-0-12-373732-8.

Brigati, C., Noonan, D.M., Albini, A. & Benelli, R. (2002). Tumors and inflammatory infiltrates: friends or foes? *Clinical Experimental Metastasis* 19:247–258. [PubMed: 12067205]

Brink, A. A. T. P., Snijders, P. J. F. & Meijer, C . J. L. M. (2007). HPV detection methods. *Disease Markers* 23: 273–281 IOS Press

Brooks, G.F., Carroll, K.C., Butel, J.S & Morse, S.A. (2007). Jawetz, Melnick and Alderberg's Medical Microbiology 24th edn. The McGraw-Hill Companies, Inc.

Brown, P. O., Bowerman, B., Varmus, H. E., & Bishop, J. M. (1987). Correct integration of retroviral DNA in vitro. *Cell* 49:347–356. http:// DOI: 10.1016/0092-8674(87)90287-x

Browner, W.S., Kahn, A.J. Ziv, E., Reiner, A.P., Oshima, J., Cawthon, R.M., Hsueh, W.C., & Cummings, S.R. (2004). The genetics of human longevity. *American Journal of Medicine* 117 (11): 851–60. doi:10.1016/j.amjmed.2004.06.033. PMID 15589490.

Brunham, R. C., & Peeling, R.W. (1994) *Chlamydia trachomatis* antigens: role in immunity and pathogenesis. *Infect Agents Dis;* 3(5):218–233.

Byrne, G.I., & Ojcius, D.M. (2004). *Chlamydia* and apoptosis: life and death decisions of an intracellular pathogen, *Nature Review* 2: 802. http:// DOI: 10.1038/nrmicro1007

Calil, L. N., Igansi, C. N., Meurer, N. Edelweiss, M. I. A., & Bozzetti, M.C. (2011). *Chlamydia trachomatis* and Human papillomavirus Co-infection: Association with p16INK4a andKi67 expression in biopsies of patients with pre-neoplastic and neoplastic lesions. *Brazilian Journal of Infectious Disease.* 15 (2):126-131.

Camargo, M., Soto-De Leon, S., Sanchez, R., Munoz, M., Vega, E., Beltran, M., … Patarroyo, M. A. (2011). Detection by PCR of human papillomavirus in Colombia: Comparison of GP5+/6+ and MY09/11 primer sets. *Journal of Virological Methods*, 178(1-2), 68–74. doi:10.1016/j.jviromet.2011.08.014

Campbell, L., Kuo, C., & Grayston, J. (1987), Characterization of the new Chlamydia agent, TWAR, as a unique organism by restriction endonuclease analysis and DNA-DNA hybridization. *Journal of Clinical Microbiology* 25: 1911-1916.

Caribbean. Washington, DC: Pan American Health Organization.

Carlson, J. H., Whitmire, W. M., Crane, D. D. et al. (2008). The *Chlamydia trachomatis* Plasmid Is a Transcriptional Regulator of Chromosomal Genes and a Virulence Factor. *Infection and immunity* 76 (6): 2273–83. doi:10.1128/IAI.00102-08. PMC 2423098. PMID 18347045.

Castellsague, X., & Munoz, N. (2003). Co-factors in human papillomavirus carcinogenesis - role of parity, oral contraceptives and tobacco smoking. *Journal of National Cancer Institute Monograph*. 31:20-8.

Castellsague, X., Menendez, C., Loscertales, M. P., Kornegay, J. R., dos Santos, F., Gomez-Olive, F. X., *et al.,* (2001) Human papillomavirus genotypes in rural Mozambique. *Lancet* 358: 1429–1430.

Centers for Disease Control & Prevention (2012). Sexually Transmitted Disease Surveillance. [http://www.cdc.gov/std/stats12/surv2012.pdf]

Chagas, B. S., Gurgel, A. P. A. D., da Cruz,H. L. A., Amaral, C. M. M., Cardoso, M. V., Neto, J. D. C. S., *et al*. (2013). An interleukin-10 gene polymorphism associated with the development of cervical lesions in women infected with Human Papillomavirus and using oral contraceptives. *Infection, Genetics and Evolution* 19: 32–37.

Chan, S. Y., Bern ard, H. U., Ratterree, M., *et al.* (1997). Genomic diversity and evolution of papillomaviruses in rhesus monkeys. *Journal of Virology.* 71:4938-4943.

Chan, S. Y., Delius, H., Halpern, A. L., et al. (1995). Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *Journal of Virol* 69:3074-3083.

Chen, Z., Terai, M., Fu, L., *et al.* (2005). Diversifying selection in human papillomavirus type 16 lineages based on complete genome analyses. *Journal of Virol* 79: 7014-7023.

Cheng, S., Schmidt-Grimminger, D. C., Murant, T., *et al.*, (1995). Differentiation-dependent up-regulation of the human papillomavirus E7 gene reactivates cellular DNA replication in suprabasal differentiated keratinocytes. *Genes Dev* 9:2335-2349.

Chernesky, M. A. (1999). Nucleic acid tests for the diagnosis of sexually transmitted diseases *FEMS Immunology and Medical Microbiology* 24: 437- 446.

Chernesky, M. A. (2005). The laboratory diagnosis of *Chlamydia trachomatis* infection. *Can J Infect Dis Med Microbiol* 16: 39-44.

Chinaka, C. C., Abudullahi, M., & Mohammed, O. M. (2014). A Comparative Study on the Use of Liquid Based Cytology and Conventional Pap Smear in Cervical Screening. *Journal of Medicine and Medical Research* vol. 2(4): 40-50, ISSN: 2350-1502.

Chinaka, C. C., Abudullahi, M., Mohammed, O.M. (2014). A comparative study on the use of liquid based cytology and conventional Pap smear in cervical screening. *J Med Med Res* 2014; 2 : 40-50.

Chiruvella, K. K., Liang, Z., & Wilson, T. E. (2013). Repair of Double-strand breaks by End joining, Cold Spring Harb Perspect Biol, 5 a012757.

Chizollni, C., & Jean-Michel Dayer, (1998). Division of Immunology and Allergy University Hospital, Geneva Switzerland 1998 Cytokine Inhibitors Encyclopeadia of Immunology

Choroszy-Król, I., Frej-Mądrzak, M., Jama-Kmiecik, A., Bober, T., & Sarowska, J. (2012). Characteristics of the *Chlamydia trachomatis* species – Immunopathology and Infections *Advanced Clinical and Experimental Medicine*. 21, 6, 799–808 ISSN 1899–5276.

Cimprich, K. A. & Cortez, D. (2008). ATR: An Essential Regulator of Genome Integrity *Nature Review Molecular Cell Biology*. 9(8): 616–627.

Ciuffo G. (1907). Innesto positivo con filtrato di verruca volgare. Giorn Ital Mal Venereol 48 12-17.

Clifford, G., Franceschi, S., Diaz, M., Mu˜noz, N., & Villa, L .L. (2006). Chapter 3: HPV type-distribution in women with and without Cervical Neoplastic Diseases. *Vaccine* 24S3 (2006) S3/26–S3/34.

Condeelis, J., & Pollard, J. W. (2006). Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 124:263–266. [PubMed: 16439202]

Costello, S. (2015). Female genital mutilation/cutting: risk management and strategies for social workers and health care professionals. Risk Management and Healthcare Policy, 225. doi:10.2147/rmhp.s62091

Coussens, L. M., & Werb, Z. (2002). Inflammation and Cancer. *Nature* 420:860 – 7. http://doi: 10.1038/nature01322

Coussens, L. M., *et al.* (1999). Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 13: 1382–1397. [PubMed: 10364156]

Coussens, L.M., Tinkle, C.L., Hanahan, D., & Werb, Z. (2000). MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 103:481–490. [PubMed: 11081634]

Cuzick, J. L.C., Mesher, D., Austin, J., Ashdown-Barr, L., Ho, L., Terry, G., Liddle, S., Wright, C., Lyons, D., & Szarewski, A. (2013). Comparing the performance of six human papillomavirus tests in a screening population *British Journal of Cancer* 108,908-913 www.bjcancer.com | doi:10.1038/bjc.2013.22

Dall, K. L., Scarpini, C. G., Roberts, I., *et al.,* (2008). Characterization of naturally occurring HPV16 integration sites isolated from cervical keratinocytes under noncompetitive conditions. *Cancer Res* 68: 8249-59.

Daniel, J. A., & Nussenzweig, A. (2013). The AID-induced DNA damage response in chromatin. *Mol Cell*. 50: 309-321.

Darougar, S., Forsey, T. Osoba, A. O., Dines, R. J., Adelusi, B., & Coker, G. O. (1982). Chlamydial genital infection in Ibadan, Nigeria: A seroepidemiological survey *British Journal of Venereal Disease* 58: 366-9. http:// doi: 10.1136/sti.58.6.366

Darougar, S., Jones, B. R., Kinnison, J. R., Vaughan-Jackson, J.D., & Dunlop, E. M. (1972). "Chlamydial infection. Advances in the diagnostic isolation of Chlamydia, including TRIC agent, from the eye, genital tract, and rectum". *British Journal of Venereal Disease* 48 (6): 416–20. doi:10.1136/sti.48.6.416. PMC 1048360. PMID 4651177.

Darville, T., & Hiltke, T. (2010). Pathogenesis of genital tract disease due to *Chlamydia trachomatis. Journal of Infectious Disease* 201 (Suppl 2):S114–S125 (in this supplement). http:// doi: 10.1086/652397

de Castro-Sobrinho, J. M., Rabelo-Santos, S. H., Alves, R. R. F, Derchain, S., Sarian, L. O. Z., Pitta, D. R., Campos, E. A. and Zeferino, L. C. (2015). *Chlamydia trachomatis* Co-infection in HPV Positive women brings no additional Risk of High-grade Cervical Intraepithelial Neoplasia. *Journal of Cytology and Histology* S3:2 DOI: 10.4172/2157-7099.S3-023

de Gruijl, T. D., Bontkes, H. J., van den Muysenberg, A. J. C., van Oostveen, J. W., Stukart, M. J., Verheijen, R. H. M., *et al.* (1999). Differences in Cytokine mRNA Profiles Between Premalignant and Malignant Lesions of the Uterine Cervix. *European Journal of Cancer*, Vol. 35, No. 3, pp. 490-497.

de Lima Júnior, S. F., Tavares, M. M. F., de Macedo, J. L., de Oliveira, R. S., Heráclio, S. D., Maia, M. D.D., *et al.* (2016). Influence of IL-6, IL-8, and TGF-β1 gene polymorphisms on the risk of human papillomavirus-infection in women from Pernambuco, Brazil. *Mem Inst Oswaldo Cruz, Rio de Janeiro,* Vol. 111(11).

de Sanjose´, S., Almirall, R., Lloveras, B., Font, R., Diaz, M., Mun˜oz, N., Catala, I., Meijer, C.J., Snijders, P.J., Herrero, R., & Bosch, F.X. (2003). Cervical human papillomavirus infection in the female population in Barcelona, Spain. *Sexually Transmitted Diseases* 30: 788 –793

de Villiers, E. M., Fauquet, C., Broker, T. R., *et al.* (2004). Classification of papillomaviruses. *Virology* 324:17-27.

de Visser, K. E., Eichten, A. & Coussens, L. M. (2006). Paradoxical roles of the immune system during cancer development. *Nature Review Cancer* 6: 24–37. [PubMed: 16397525]

De Vuyst, H., Steyaert, S., Van Renterghem, L., Claeys, P., Muchiri, L., Sitati, S., Vansteelandt, S., Quint, W., Kleter, B., Van Marck, E., & Temmerman, M. (2003). Distribution of human papillomavirus in a family planning population in Nairobi, Kenya. *Sexually Transmitted Diseases* 30: 137–142.

Deans, A. J., & West, S. C. (2011). DNA interstrand crosslink repair and Cancer, *Nature Review Cancer*, 11 467-480.

DeCaprio, J. A., Ludlow, J. W., Figge, J., *et al.*, (1988). SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell* 54: 275-283. http:// doi: 10.1016/0092-8674(88)90559-4

Degenhardt, Y.Y. & Silverstein, S. J. (2001). Gps2, a Protein Partner forHuman PapillomavirusE6 Proteins. *Journal of Virology 75:* 151–160.

Deininger, P. (1999). Genetic instability in cancer: caretaker and gatekeeper genes. *The Ochsner journal*, 1(4), 206–209.

DeNardo, D.G., Barreto, J.B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N. & Coussens, L.M. (2009). CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* 16:91–102. [PubMed: 19647220]

Deshpande, A., Nolan, J. P., White, P. S., Valdez, Y. E., Hunt, W. C., Peyton, C. L., Wheeler, C. M. (2005). TNF-α Promoter Polymorphisms and Susceptibility to Human Papillomavirus 16–Associated Cervical Cancer *The Journal of Infectious Diseases*, Volume 191, Issue 6, 15 Pages 969–976, https://doi.org/10.1086/427826

DiCarlo, E., *et al.* ((2001). The intriguing role of polymorphonuclear neutrophils in antitumor reactions. *Blood* ; 97:339–345. [PubMed: 11154206]

Difilippantonio, M. J., *et al.* (2002). Evidence for replicative repair of DNA double-strand breaks leading to oncogenic translocation and gene amplification. *Journal of Experimental Medicine* 196 (4):469–80. [PubMed:12186839]

Dinarello, C. A., (2000). Impact of basic research on tomorrow's medicine: Pro-inflammatory cytokine *chest.* http:// doi.org/ 10.1378/chest.118.2.503

Diop-Ndiayea, H., Beitera, K., Gheitb, T., Ndoyea, A. S., Draméa, A., McKay Chopinb, S., Tommasinob, M., Boyea, C. S. B., Syllab, B. & Kanea, C. T. (2019). Human Papillomavirus infection in Senegalese female sex workers. *Papillomavirus Research* 7: 97–101

Do Carmo Vasconcelos de Carvalho, V., de Macêdo, J. L., de Lima, C. A. D., da Conceição Gomes de Lima, M., de Andrade Heráclio, S., Amorim, M., … de Souza, P. R. E. (2012). IFN-gamma and IL-12B polymorphisms in women with cervical intraepithellial neoplasia caused by human papillomavirus. Molecular Biology Reports, 39(7), 7627–7634. doi:10.1007/s11033-012-1597-9

Doorslaer, K. V. (2013). Evolution of the Papillomaviridae. *Virology*, 445:11–20.

Du K., Zhou M., Li Q., & Liu, X-Z. (2018). *Chlamydia trachomatis* inhibits the production of pro-inflammatory cytokines in human PBMCs through induction of IL-10 *Journal of Medical Microbiology* 67:240–248 DOI 10.1099/jmm.0.000672

Duensing, S. and Munger, K. (2002). The human papillomavirus type 16 E6 and E7 Oncoproteins independently induce numerical and structural chromosome instability. *Cancer Research;* 62:7075-7082.

Duensing, S., Lee, L.Y., Duensing , A. *et al.*, (2000). The Human papillomavirus type 16 E6 and E7 Oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proc Natl Acad Sci* U S A 97: 10002- 10007.

Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The three Es of cancer immunoediting," *Annual Review of Immunology*, vol. 22, pp. 329–360.

Dunne, E. F., & Markowitz, L. E. (2006). Emerging Infections: Genital Human Papillomavirus Infection. *Clinical Infectious Diseases*, 43(5), 624–629. doi:10.1086/505982

Dursun, P., Senger, S. S., Arslan, H., Kuşçu, E., & Ayhan, A. (2009). Human papillomavirus (HPV) prevalence and types among Turkish women at a gynecology outpatient unit. *BMC infectious diseases*, 9, 191. doi:10.1186/1471-2334-9-191

Eaden, J., Abrams, K., Ekbom, A., Jackson, E., & Mayberry, J. (2000). Colorectal cancer prevention in ulcerative colitis: a casecontrol study. *Aliment Pharmacol Therapy* 14: 145-153.

Ebrahim, S., Mndende, X. K., Kharsany, A. B. M., Mbulawa, Z. Z. A., Naranbhai, V., Frohlich, J., … Williamson, A.-L. (2016). High Burden of Human Papillomavirus (HPV) Infection among Young Women in KwaZulu-Natal, South Africa. *PLOS ONE*, 11(1), e0146603. doi:10.1371/journal.pone.0146603

Eichten, A., Rud, D. S., Grace, M., *et al.* (2004). Molecular pathways executing the atrophic sentinel • response in HPV-16 E7-expressing normal human diploid fibroblasts upon growth factor deprivation. *Virology* 319:81-93.

Einstein, M., Schiller, J. T., Viscidi, R. P., Strickler, H., Coursaget, P., Tan, T., Halsey, N. & Jenkins, D. (2009). Clinician's guide to human papillomavirus immunology: knowns and unknowns. *Lancet Infect Dis* 9:347-56.

Eiró, N., & Vizoso, F. J. (2009). Inflammation and cancer. *World Journal of Gastrointestinal Surgery* 4(3): 62-72 Available from: URL: http://www.wjgnet.com/1948-9366/full/v4/i3/62.htm doi: http://dx.doi.org/10.4240/wjgs.v4.i3.62

El.Mahalli, A. A. (2015). Incidence and risk factors of abnormal cervical cytology in a university hospital - Saudi Arabia. *Saudi Journal Health Sci* 4:104-10

Eleute´rio Jr, J., Teles, R. A., Iara M Linhares, I. M., Neil Normand, N., and Witkin, S. S. (2014). Interferon-gamma gene polymorphism influences the frequency of a *Chlamydia trachomatis* cervical infection in young women. *International Journal of STD & AIDS* 0(0) 1–5 DOI: 10.1177/0956462414563627

Ella, E. E., Shenge, H., & Ajoge, H. O. (2013). Prevalence of *Chlamydia trachomatis* among Female Students attending a Selected University Health Services in Zaria, Kaduna State, Nigeria. *The International Journal of Engineering and Science (IJES)* Vol. 2 Issue 11 pp 54-57.

Enwuru, C. P., & Umeh, S. I. (2014). Asymptomatic Carriage of *Chlamydia trachomatis* Among Young Adults in Owerri, South East Nigeria *IOSR Journal of Nursing and Health Science (IOSR-JNHS) Volume 3, Issue 2 Ver. I, PP 49-53 www.iosrjournals.org*

Erdman, S. E., Sohn, J. J., Rao, V. P., Nambiar, P. R., Ge, Z, Fox, J. G., & Schauer, D. B. (2005). CD4+CD25+ regulatory lymphocytes induce regression of intestinal

tumors in ApcMin/+ mice.*Cancer Research 65:* 3998– 4004. [PubMed: 15899788]

Eric, H., Green, Freund, K. M., Posner, M. A., & David, M. M. (2005). Pap Smear Rates Among Haitian Immigrant Women in Eastern Massachusetts. Public Health Reports / March–April 2005 / Volume 120 Association of Schools of Public Health.

Esumeh, F. I., Dennis E. Agbonlahora, D. E., & Okoror, L. E. (2009). Chlamydia activity in North East zone of Nigeria. *Eastern Journal of Medicine 14 10-16.*

Ezechi, O.C., Ostergren, P.O., Nwaokorie, F.O. et al. The burden, distribution and risk factors for cervical oncogenic human papilloma virus infection in HIV positive Nigerian women. Virol J 11, 5 (2014) doi:10.1186/1743-422X-11-5

Fadahunsi, O. O., Omoniyi-Esan, G. O., Banjo, A. A. F., Esimai, O. A., Osiagwu, D., *et al.* (2013) Prevalence of High Risk Oncogenic Human Papillomavirus Types in Cervical Smears of Women Attending Well Woman Clinic in Ile Ife, Nigeria. *Gynecology and Obstetric* 3: 185 doi:10.4172/2161-0932.1000185.

Fadok, V.A., Bratton D. L., Konowal, A., Freed, P. W., Westcott, J. Y., & Henson P.M. (1998). Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-h, PGE2 and PAF. *Journal of Clinical Investigation* 101: 890 – 8.

Fan, B., Lu, H., Hu, H., Shi, L., McClarty, G., Nance, D. *et al.* (1998). Inhibition of apoptosis in Chlamydia-infected cells: blockade of mitochondrial cytochrome C release and caspase activation. *Journal of Experimental Medicine* 187: 487-96.

Feller, L., Wood, N. H., Khammissa, R. A., & Lemmer, J. (2010). Human papillomavirus-mediate d carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. Part 2: Human papillomavirus associated oral and oropharyngeal squamous cell carcinoma. *Head & face medicine, 6,* 15. doi:10.1186/1746-160X-6-15

Feng, Q., Cherne, S., Winer, R. L., Popov, V., Zambrano, H., Yerovi, C., Hawes, S.E., Koutsky, L. A., & Kiviat, N. B. (2010). Evaluation of Transported Dry and Wet Cervical Exfoliated Samples for Detection of Human Papillomavirus Infection *Journal Of Clinical Microbiology*, P. 3068–3072. http:// doi.org/ 10.1128/JCM.00736-10

Ferlay, J., Bray, F., Pisani, P. and Parkin, D.M. (2004). GLOBOCAN 2002. Cancer incidence, mortality and prevalence worldwide. IARC Cancer Base No. 5 version 2.0. Lyon, France: IARC Press; 2004.

Fernandes, J. V., Galvão de Araújo, J. M., & de Medeiros Fernandes, T. (2013). Biology and natural history of human papillomavirus infection.*Open Access Journal of Clinical Trials* 5:1–12.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., Girardin, S. E., & Girardin, N. A. (2007). "Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation". *Clinical and Experimental Immunology.* 147 (2): 061127015327006—. doi:10.1111/j.1365-2249.2006.03261.x. PMC 1810472. PMID 17223962.

Filippova, M., Johnson, M. M., Bautista, M., Filippov, V., Fodor, N., Tungteakkhun, S. S., Williams, K., & Duerksen-Hughes, P.J. (2007). The Large and small isoforms of Human papillomavirus type 16 E6 bind to and differentially affect procaspase 8 stability and activity. *Journal of Virology 81:* 4116–4129.

Filippova, M., Parkhurst, L. & Duerksen-Hughes, P. J. (2004). The human papillomavirus 16 E6 protein binds to Fas-associated death domain and protects cells from Fas-triggered apoptosis. *J Biol Chem* 279: 25729–25744.

Filippova, M., Song, H., Connolly, J. L., Dermody, T. S. & Duerksen-Hughes, P .J. (2002). The human papillomavirus 16 E6 protein binds to tumor necrosis factor (TNF) R1 and protects cells from TNF-induced apoptosis. *Journal of Biological Chemistry 277:* 21730–21739.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*(6809), 239-247. https://doi.org/10.1038/35041687

Flores, E. R., Allen-Hoffmann, B. L., Lee, D, *et al*. (2000). The human papillomavirus type 16 E7 oncogene is required for the productive stage of the viral life cycle interaction of the papillomavirus transcription/replication factor, E2, and the viral capsid protein, L2. *Journal of Virology* 74:6622-6631.

Fong, Y. W., Cattoglio, C., & Tjian, R. (2013). The intertwined roles of transcription and repair proteins, *Mol Cell, 52* 291-302.

Forbes, B.A., D.F.Sahm, D. F., Weissfeld,A. S., & E.A. Trevino, E. A. (2007). Baileys and Scott Diagnostic Microbiology 12th edn. Mosby Inc.

Forman, D., de Martel, C., Lacey, C. J., Soerjomataram**a**, I., Lortet-Tieulent, J. Bruni, L., Vignat, *et al.* (2012). Global Burden of Human Papillomavirus and Related Diseases. *Vaccine 30S F12– F23*.

Fowotade, A., Osisanwo, D. A., & Bakare, R. A. (2018). Human papillomavirus infection among women attending family planning clinic in Nigeria: prevalence, correlates, and co-infection with *Chlamydia trachomatis*. *Journal of Immunoassay and Immunochemistry*, 1–13. doi:10.1080/15321819.2018.1493999

Franceschi, S., Clifford, G. M., Vaccarella, S., Shin, H. R., Sukvirach, S., Anh, P. T., *et al.* (2003). Geographic variation in HPV infection. In: Monsonego J (ed) Fifth International Multidisciplinary Congress Eurogin 2003. Paris, France, 13– 16 April. Bologna: Monduzzi Editore pp 35–40.

Franceschi, S., Smith, J.S., Herrero, A.V-D-B.R., Arslan, A., Anh, P-T-H., Bosch, F. X., Hieu, N-T., Matos, E., Posso, H., Qiao, Y-L., Shin, H-R., Sukvirach, S., Thomas, J.O., Snijders, P.J.F., MunOz, N., & Meijer, C.J.L.M. (2007). Cervical Infection with *Chlamydia trachomatis* and Neisseria gonorrhoeae in Women from Ten Areas in Four Continents a Cross-Sectional Study. *Sexually Transmitted Diseases* Vol. 34, No. 8, p.563–569.

Frazer I. H. (2006). HPV vaccines. *International Journal of Gynecology and Obstetric* 94(Suppl. 1): S81-S88

Frazer, I. (2007). Correlating Immunity with Protection for HPV infection. *International Journal of Infectious Disease*, (Supplement 2):S10-S16. https://doi.org/10.1016/S1201-9712(07)60016-2

Frazer, I. H., Leggatt, G. R., & Mattarollo, S. R. (2011). Prevention and treatment of Papillomavirus-related cancers through immunization. *Annual Rev Immunol*. 29:111–138. http:// doi.org/ 10.1146/annurev-immunol-031210-101308

Fredlund, H., Falk, L., Jurstrand, M., & Unemo, M. (2004). Molecular genetic methods for diagnosis and characterisation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: impact on epidemiological surveillance and interventions". *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* 112 (11–12): 771–84. doi:10.1111/j.1600-0463.2004.apm11211-1205.x. PMID 15638837.

Frenkel, N., Roizman, B., Cassai, E., & Nahmias, A. J. (1972). DNA Fragment of Herpes Simplex 2 and Its Transcription in Human Cervical Cancer Tissue. *PNAS,* 69: 3784-3789.

Fridlender, Z. G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., Worthen, G.S., & Albelda, S. M. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 16: 183–194. [PubMed: 19732719]

Friedberg, E. C. et al. (1995). DNA Repair and Mutagenesis, ASM Press, Washington, DC.

Fukushi, H., & Hirai, K. (1992). Proposal of Chlamydia pecorum sp. nov. for chlamydia strains derived from ruminants. *International. Journal of Systematic Bacteriology* 42: 306-308.

Gage, J. C., Ajenifuja, K. O., Wentzensen, N. A., Adepiti, A. C., Eklund, C., Reilly, M., … Schiffman, M. (2012). The age-specific prevalence of human papillomavirus and risk of cytologic abnormalities in rural Nigeria: implications for screen-and-treat strategies. *International journal of cancer,* 130(9), 2111–2117. doi:10.1002/ijc.26211

Gallagher G., Eskadale J., and Bidwell J.L. (2003) Chapter 2: Cytokine Genetics – Polymorphisms, Functional variations and Disease associations. The Cytokine

Handbook 4th Editon. Edited by Angus W. Thomson and Micheal T. Lotze. Elsevier Science Ltd. Pp22-55

Gallimore, A. M., & Simon, A. K. (2008). Positive and Negative Influences of Regulatory T cells on Tumour Immunity. *Oncogene* 27:5886–5893. [PubMed: 18836469]

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., Tosolini, M., Camus, M., Berger, A., Wind, P., *et al.* (2006). Type, density, and Location of immune cells within human colorectal tumors predict clinical outcome. *Science* 313: 19601964. [PubMed: 17008531]

Garner, E., & Costanzo, V. (2009). Studying the DNA damage response using *in vitro* model systems.http:// doi.org/ 10.1016/j.dnarep.2009.04.015

Gartel, A. L., & Tyner, A. L. (2002). The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. *Molecular Cancer Therapeutics* 1 (8): 639–49. PMID 12479224.

Ghittoni, R., Accardi, R., Hasan, U., Gheit, T., Sylla, B., & Tommasino, M. (2010). The Biological Properties of E6 and E7 Oncoproteins from Human Papillomaviruses. *Virus Genes 40:* 1–13.

Giacinti, C., & Giordano, A. (2006). RB and Cell Cycle Progression. *Oncogene* 25, 5220-522.

Gillison, M. L., Broutian, T., Pickard, R. K., et al. (2012). Prevalence of Oral HPV infection in the United States, 2009–2010. *JAMA*. 307(7):693–703.

Gjonnaess, H., Dalaker, K., Anestad, G., Mardh, P-A., Kvile, G., & Bergan, T. (1982). Pelvic Inflammatory Disease: Etiologic Studies with Emphasis on Chlamydial Infection. *Obstetrics and Gynecology* 59: 550-5.

Godínez, J. M., Heideman, D.A.M., Gheit, T., L., Alemany, L., Snijders, P. J. F., Tommasino, M., Meijer, C. J. L. M., de Sanjosé, S., Bosch, F. X., Bravo, I. G. (2013). Differential presence of Papillomavirus variants in cervical cancer: An analysis for HPV33, HPV45 and HPV58 Infection. 13:96–104. https://doi.org/10.1016/j.meegid.2012.09.011

Gomez-Roman, J.J., Echevarria, C., Salas, S., Gonza´ lez-Mora´ n MA, Perez-Mies, B., Garcı´ a-Higuera, I., Nicola´s Martı´nez, M., Val-Bernal, J.f. (2009). A Type-specific Study of Human Papillomavirus Prevalence in Cervicovaginal Samples in Three Different Spanish Regions. APMIS. 117:22–7.

Gopalkrishna, V., Aggarwal, N. & Malhotra, V. L. (2000). *Chlamydia trachomatis* and Human Papillomavirus Infection in Indian Women with Sexually Transmitted Diseases and Cervical Precancerous and Cancerous Lesions. *Clininical Microbiology Infection 6*: 88-93.

Gottlieb S.L., David H. Martin, Fujie Xu, Gerald I. Byrne, and Robert C. Brunham (2010). Summary: The Natural History and Immunobiology of *Chlamydia trachomatis*

Genital Infection and Implications for Chlamydia Control. *The Journal of Infectious Diseases*; 201(S2):S190–S204.

Gottlieba S. L., Nicola Lowb, Lori M. Newmana, Gail Bolanc, Mary Kambc, Nathalie Brouteta (2014). Toward global prevention of sexually transmitted infections (STIs):The need for STI vaccines *Vaccine* 321527–1535.

Gottschling, M., Go¨ker, M., Stamatakis, A., Bininda-Emonds, O. R. P., Ingo Nindl, I., & Ignacio G. Bravo, I. G. (2011). Quantifying the Phylodynamic Forces Driving Papillomavirus Evolution. *Molecular Biology and Evolution*. 28(7):2101–2113. 2011 https://doi.org/10.1093/molbev/msr030

Gravitt, P. E., Peyton, C. L., Apple, R. J., & Wheeler, C. M. (1998). Genotyping of 27 Human Papillomavirus Types by using L1 Consensus PCR Products by a Single-hybridization, Reverse Line Blot Detection Method, *Journal of Clininical Microbiology* 36:3020–3027.

Gravitt, P.E., Kamath, A.M., Gaffikin, L., Chirenje, Z.M., Womack, S. & Shah, K.V. (2002) Human Papillomavirus Genotype Prevalence in High-grade Squamous Intraepithelial Lesions and Colposcopically Normal Women from Zimbabwe. *Inernationalt Journal of Cancer* 100: 729–732.

Grce, M., Ivan S., & Nina M. G. (2012). The Transforming Properties of Human Papillomavirus Oncoproteins. *Periodicum biologorum, Vol 114*, No 4,479-487.

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer, *Cell*, vol. 140, no. 6, pp. 883–899.

Gupta, P.K. (2005). *Cytology Genetics Biotechnology and Biostatistics.* Rastogi Publications Shivaji Road, Meerut India pp.258-267.

Guven-Maiorov, E., Acuner-Ozbabacan, S. E., Keskin, O., Gursoy, A., & Nussinov, R. (2014). Structural pathways of cytokines may illuminate their roles in regulation of cancer development and immunotherapy. *Cancers*, *6*(2), 663–683. doi:10.3390/cancers6020663

Hagen, L., Kavli, B., Sousa, M. M., Torseth, K., Liabakk, N. B., Sundheim, O., Pena-Diaz, J., Otterlei, M., Horning, O. Jensen, O.N. Krokan, H.E. & Slupphaug, G. (2008). Cell Cycle-specific UNG2 Phosphorylations Regulate Protein Turnover, Activity and association with RPA. *EMBO Journal*, 27 51-61. http:// doi.org/ 10.1038/sj.emboj.7601958

Hammerschlag, M.R. (1981). Chlamydial pneumonia: Not for babies only? *JAMA* 245(13): 1346.

Han, J. & Ulevitch, R.J. (2005). Limiting inflammatory responses during activation of innate immunity," *Nature Immunology*, vol. 6, no. 12, pp. 1198–1205.

Hanada, T., Kobayashi, T., Chinen, T., Saeki, K., Takaki, H., Koga, K., Minoda, Y., Sanada, T., Yoshioka, T., Mimata, H., et al. (2006). IFN gamma-dependent, spontaneous development of colorectal carcinomas in SOCS1-deficient mice. *Journal of Experimental Medicine*; 203:1391–1397. [PubMed: 16717119]

Hanahan, D., & R. A. Weinberg,R. A. (2011). "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674.

Handlogten, K. S., Molitor, R. J., Roeker, L. E., Narla, N. P., Bachman, M. J., Quayson, S., *et al.* (2014). Cervical Cancer Screening in Ghana, West Africa: Prevalence of Abnormal Cytology and Challenges for Expanding Screening. *International Journal of Gynecological Pathology* 33:197–202, Lippincott Williams & Wilkins, Baltimore.

Handsfield, H. H., Jasman, L. L., Roberts, P. L., Hanson, V. W., Kothenbeutel, R. L., & Stamm, W. E. (1986). Criteria for selective screening for *Chlamydia trachomatis* infection in women attending family planning clinics. JAMA; 255:1730–1734. [PubMed: 3081742]

Hardeland, U., Kunz, C., Focke, F., Szadkowski, M., & Schar, P. (2007).Cell Cycle Regulation as a mechanism for functional separation of the apparently redundant uracil DNA glycosylases TDG and UNG2. *Nucleic acids research*, 35 3859-3867. http://doi.org/: 10.1093/nar/gkm337

Hariri, S., Unger, E.R., Sternberg, M., et al. (2011). Prevalence of Genital Human Papillomavirus among Females in the United States, the National Health and Nutrition Examination Survey, 2003–2006. *Journal of Infectious Diseases* 204(4):566–573.

Harper, J.W., & Elledge, S. J., (2007). The DNA damage response: ten years after. *Mol Cell* 28:739–745. [PubMed: 18082599]

Harris, S. R., Clarke, I. N., Seth-Smith, H. M. *et al.* (2012). "Whole-genome analysis of diverse *Chlamydia trachomatis* strains identifies phylogenetic relationships masked by current clinical typing". *Nat. Genet.* 44 (4): 413–9, S1. doi:10.1038/ng.2214. PMC 3378690. PMID 22406642.

Harvey, M. (2005). *Blacks' Medical Dictionary 41st edn.* A & C Black Publishers Limited London ISBN 0–7136–6146–1.

Hasan, U. A., Bates, E., Takeshita, F., Biliato, A., Accardi, R., Bouvard, V., *et al*. (2007): TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. *Journal of Immunology*, 178:3186-3197.

Hausen, H. Z. (2002). Papillomaviruses and Cancer: From Basic Studies To Clinical Application www.nature.com/reviews/cancer

Hawes, S.E., & Kiviat, N. B. (2002). Are Genital Infections and Inflammation co-factors in the Pathogenesis of Invasive Cervical Cancer? *Journal of National Cancer Institute.* 94:1592–3. [PubMed: 12419778]

Head and Face Medicine. (2010). 6:14 doi: 10.1186/1746-160X-6-14

Heidland, A., Klassen, A., Rutkowski, P., & Bahner U. (2006). The Contribution of Rudolf Virchow to the Concept of Inflammation: What is still of importance? *Journal of Nephrology* 19 Suppl 10: S102-S109.

Hodge-Dufour, J, Marino, M.W., Horton, M.R., et al. (1998). Inhibition of interferon G induced interleukin 12 production: a potential mechanism for the anti-inflammatory activities of tumor necrosis factor. *Proceedings of the National Academy of Science* U S A 95: 13806 – 11.

Hoeijmakers, J.H. (2001). Genome maintenance Mechanism for preventing cancer. *Nature* 411:366. http:// doi.org/ 10.1038/35077232

Hogan, R. J., Mathews, S. A., Mukhopadhyay, S., *et al.,* (2004). Chlamydial Persistence: Beyond Biphasic Paradigm, InfectImmun72:1843.

Houtgraaf, J.H., Versmissen, J., & van der Giessen W. J. (2006). A Concise Review of DNA Damage Checkpoints and Repair in Mammalian Cells, *Cardiovasc Revasc Med,* 7 165-172.

Huai, P., Li, F., Li, Z., Sun, L., Fu, X., Pan,Q., *et al.* (2018). Prevalence, risk factors, and medical costs of *Chlamydia trachomatis* infections in Shandong Province, China: a populationbased, cross-sectional study. *BMC Infectious Diseases*.18:534

Hubbert, N. L., Sedman, S. A., & Schiller, J. T. (1992). Human Papillomavirus type 16 E6 increases the degradation rate of p53 in human keratinocytes. *Journal of Virology* 66: 6237-6241.

Hung, C. F, Ma B., Monie A., Tsen S., & Wu, T. C. (2008). Therapeutic human papilloma virus vaccines: current clinical trials and future directions. *Expert Opin. Biol. Ther.* 8(4): 421-39.

Hussain, S. P., Amstad, P., Raja, K., Ambs, S., Nagashima, M, Bennett, W. P., Shields, P. G., Ham, A. J., Swenberg, J. A., Marrogi, A. J. & Harris, C. C. (2000). Increased p53 Mutation Load in Non-cancerous Colon Tissue from Ulcerative Colitis: a Cancer-prone Chronic Inflammatory Disease. *Cancer Res* 60: 3333-3337

Huynh, M-L. N., Fadok, V. A., & Henson, P. M. (2002). Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-h secretion and the resolution of inflammation. *Journal of Clinical Investigation.*109:41 – 50.

Hwang, S. J., & Shroyer, K. R. (2012). Biomarkers of cervical dysplasia and carcinoma. *Journal of Oncology* 507286. Epub. http://doi.org/ 10.1155/2012/507286

Iancu, I.V., Botezatu, A., Goia-Ruanu, C.D., Stãnescu, A., Huicã, I., Nistor, E., Anton, G., & Plea, A. (2010). TGF-beta Signalling Pathway Factors in HPV-induced Cervical Lesions. *Roum Arch Microbiol Immunol 69:* 113–118. ICO ON Human Papillomavirus and Related Diseases Report NIGERIA Version posted on www.hpvcentre.net in March 17th, 2014.

Jalal, H., Stephen, H., Al-Suwaine, A., Sonnex, C., & Carne, C. (2006). The superiority of polymerase chain reaction over an amplified enzyme immunoassay for the detection of genital chlamydial infections. *Sex Transm Infect* 2006;82: 37–40.

Jasin, M., & Rothstein, R. (2013). Repair of strand breaks by homologous recombination. *Cold Spring Harb Perspect Biol* . 5 a012740. http://doi.org/ 10.1101/cshperspect.a012740

Jeanne, M., & Robert, S. (2014). Recent advances in understanding and managing *Chlamydia trachomatis* infections F1000Prime Reports.6:120 http://doi.org/10.12703/P6-120).

Jensen, K. E., Schmiede, S., Norrild, B., Frederiksen, K., Iftner,T. & Kjaer,S. K. (2013). Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. Britisj Journal of cancer. 108:234-239. DOI:10.1038/bjc.2012.513

Jewers, R.J, Hildebrandt, P., Ludlow, J.W., *et al.* (1992). Regions of human papillomavirus type 16 E7 oncoprotein required for immortalization of human keratinocytes. *Journal of Virology*. 66:1329-1335.

Jha, S., Vande Pol S., Banerjeens, N. S., Dutta, A. B., Chow, L. T., & Dutta, A. (2010). Destabilization of TIP60 by Human Papillomavirus E6 Results in Attenuation of TIP60-dependent Transcriptional Regulation and Apoptotic Pathway. *Mol Cell 38:* 700–711. https://doi.org/10.1016/j.molcel.2010.05.020

Jones, D. L, Alani, R. M., Munger, K. (1997). The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21cip1 mediated inhibition of cdk2. *Genes Dev* 11: 2101-2111.

Jones, D. L., Thompson, D. A., Munger, K. (1997). Destabilization of the RB tumor suppressor protein and stabilization of p53 contribute to HPV type 16 E7-induced apoptosis. *Virology.* 239:97-107.

Jong, J. E., Jeong, K.W., Shinh, H., Hwang, L.R., Lee, D., & Seo, T. (2012). Human papillomavirus type 16 E6 protein inhibits DNA fragmentation via interactionwithDNA fragmentation factor 40. *Cancer Lett 324:* 109–117. https://doi.org/10.1016/j.canlet.2012.05.010

Jonjić, N., Peri, G., Bernasconi, S., Sciacca, F. L., Colotta, F., Pelicci, P., Lanfrancone, L., & Mantovani, A. (1992). Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. *J ournal of Experimental Medicine.* 176:1165–1174. [PubMed: 1383376]

Kahn, J. A., Rosenthal, S. L., Succop, P. A., Gloria Y. F. Ho, G. Y. & Burk, R. D. (2002) Mediators of the association between age of first sexual intercourse and subsequent Human Papillomavirus Infection. *Pediatrics*;109;e5F., DOI: 10.1542/peds.109.1.e5

Kajitani, N., Satsuka, A., Kawate, A., & Sakai, H. (2012). Productive Lifecycle of Human Papillomaviruses that Depends Upon Squamous Epithelial Differentiation. Frontiers in microbiology, 3, 152. doi:10.3389/fmicb.2012.00152

Kanodia, S., Fahey, L. M., & Kast, W. M. (2007): Mechanisms used by human papillomaviruses to escape the host immune response. *Currence Cancer Drug Targets*, 7:79-89.

Karin, M. (2006). Nuclear factor-kappaB in cancer development and progression. *Nature* 441:431–436. [PubMed: 16724054]

Karinen, L. (2006). Chronic chlamydial infection: impact on human reproductive health.Reproductive health research in the Northern Finland 1966 Birth Cohort (NFBC 1966) Acta Universitatis Ouluensis D 872. Oulu: University of Oulu. http://herkules.oulu.fi/isbn9514280229/isbn9514280229. Accessed on August–2015.

Kazemi, S, Papadopoulous, S., Li, S., Su, Q., Wang, S., Yoshimura, A., Matlashewski, G., Dever, T. E., & Koromilas, A. E. (2004). Control of alpha Subunit of eukaryotic translation initiation factor 2 (eIF2 alpha) phosphorylation by the human papillomavirus type 18 E6 oncoprotein: implications for eIF2 alpha-dependent gene expression and cell death. *Molecular Cell Biology 24:* 3415–3429.

Kessis, T. D., Slebos, R. J., Nelson, W. G., Kastan, M. B., Plunkett, B. S., Han, S. M., Lorincz A. T., Hedrick, L., & Cho, K. R. (1993). Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. Proc Natl Acad Sci U S A 1993; 90:3988-3992. *Proceedings of the National Academy of Science*. http:// doi.org/ 10.1073/pnas.90.9.3988

Kim, S.T., Lim, D. S., Canman, C. E., & Kastan, M. B. (1999). Substrate specificities and identification of putative substrates of ATM kinase family members *Journal of Biological Chemistry* 274:37538. http://doi.org/ 10.1074/jbc.274.53.37538

Kingsbury, D., & Weiss, E. (1968). Lack of deoxyribonucleic acid homology between species of the genus Chlamydia. *Journal of Bacteriology* 26: 1421-1423.

Knipe, D.M., & Howley, P. M. (2007). Fields Virology,5th edn. Lippincott Williams & Wilkins

Kohrt, H.E., Nouri, N., Nowels, K., Johnson, D., Holmes, S., & Lee, P. P. (2005). Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. *PLoS Med* 2:e284. [PubMed: 16124834]

Kouba, L. J., & Muasher, J. (1985). Female Circumcision in Africa: An Overview. African Studies Review, Vol. 28, No. 1 pp. 95-110: http://www.jstor.org/stable/524569 .Accessed: 19/06/2014

Koutsky, L.A., Holmes, K.K., Critchlow, C.W., Stevens, C.E., Paavonen, J., Beckmann, A.M., DeRouen, T.A., Galloway, D.A., Vernon, D. & Kiviat, N.B. (1992). A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to Papillomavirus infection. *New England Journal of Medicine.* 327:1272–1278. [PubMed: 1328880]

Kraus, I., Driesch, C., Vinokurova, S., Hovig, E., Schneider A., *et al.* (2008). The majority of viral cellular fusion transcripts in cervical carcinomas cotranscribe cellular sequences of known or predicted genes. *Cancer Research.* 68: 2514–2522.

Kreider, J.W. (1981). The Shope papilloma to carcinoma complex of rabbits: a model system of neoplastic progression and spontaneous regression. *Advances in Cancer Research*. 35:81-110.

Krokan, H. E., Bjoras, M. (2013). Base excision repair, Cold Spring Harb Perspect Biol, 5 a 012583.

Kučinskienė, V., Šutaitė, I., Valiukevičienė, S., Milašauskienė, Z., & Domeika, M. (2006). Prevalence and risk factors of genital *Chlamydia trachomatis* infection. *Medicina (Kaunas); 42(11) - http://medicina.kmu.lt*

Kumar, A., Zhao, Y., Meng, G., Zeng, M., Srinivasan, S., Delmolino, L. M., … Band, V. (2002). Human papillomavirus oncoprotein E6 inactivates the transcriptional coactivator human ADA3. Molecular and cellular biology, 22(16), 5801–5812. doi:10.1128/mcb.22.16.5801-5812.2002

Kuper, H., Adami, H.O., Trichopoulos, D. (2000). Infections as a major preventable cause of Human Cancer. *Journal of Internal Medicine*; 248: 171-183.

LaCour, D. E. (2012). Human Papillomavirus in Infants: Transmission, Prevalence, and Persistence. *Journal of Pediatrics and Adolesc Gynecology*; 25(2): 93–97. doi:10.1016/j.jpag.2011.03.001

Laghi, L., Bianchi, P., Miranda, E., Balladore, E., Pacetti, V., Grizzi, F., Allavena, P., Torri, V., Repici, A., Santoro, A., Mantovani, A., Roncalli, M., & Malesci, A. (2009). CD3+ Cells at the Invasive Margin of Deeply Invading (pT3-T4) Colorectal Cancer and risk of post-surgical Metastasis: a Longitudinal Study. *Lancet Oncol* 2009; 10:877–884. [PubMed: 19656725]

Langowski, J. L., Kastelein, R.A., & Oft, M. (2007). Swords into Plowshares: IL-23 repurposes tumor immune surveillance. *Trends in Immunology* 28: 207–212. [PubMed: 17395538]

Lawal, I., Agida, T. E., Offiong, R. A., Oluwole, P. O. (2017). Cervical Cytology among HIV Positive and HIV Negative Women in a Tertiary Hospital in North Central Nigeria: A Comparative Study. *Ann Med Health Sci Res.* 7: 308-311

Lee, S. M., Park, J. S., Norwitz, E. R., Koo, J. N., Oh, I. H., Park, J. W., … Song, Y. S. (2013). Risk of vertical transmission of human papillomavirus throughout pregnancy: a prospective study. PloS one, 8(6), e66368. doi:10.1371/journal.pone.0066368

Lehmann, M., Groh, A., Rödel, J., Nindl, I. & Straub, E. (1999). Detection of *Chlamydia trachomatis* DNA in cervical samples with regard to infection with Human Papillomavirus. *J Infect 38* : 12-7.

Lewis, M. J. (2004). A Situational Analysis of Cervical Cancer in Latin America and the

Li, S., Labrecque, S., Gauzzimc, M. C., Cuddihy, A. R., Wonga A. H., Pellegrini, S., Matlashewski, G. J., & Koromilas, A. E. (1999). The Human Papilloma virus (HPV)-18 E6 Oncoprotein Physically Associates with Tyk2 and Impairs Jak-STAT Activation by interferon- alpha. *Oncogene 18:* 5727–5737. http://doi: 10.1038/sj.onc.1202960

Lin, E. Y., Nguyen, A. V., Russell, R. G., Pollard, J. W., (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *Journal of Experimental Medicine* 193:727–740. [PubMed: 11257139]

Lin, W. W., & Karin, M. (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer," *Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1175–1183.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature* .362:709-715. https://doi.org/10.1038/362709a0

Liquid Based Cytology (LBC), NHS cervical screening programme (accessed 07/04/2015)

Liviu, F., Neil, H., Razia, W. Khammissa, A. G., & Johan. L. (2010). Department of Periodontology and Oral Medicine, University of Limpopo, Medunsa Campus, South Africa

Lobel, L. I., Murphy, J. E. and Goff, S. P. (1989). The palindromic LTR-LTR junction of Moloney murine leukemia virus is not an efficient substrate for proviral integration. *J. Virol.* 63:2629–2637.

Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL, Darnell J. (2004). Molecular Biology of the Cell, p963. WH Freeman: New York, NY. 5th ed.

Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Bretscher, A. Ploegh, H., Amon, A., & Scott, M. P. (2013). Molecular Cell Biology Seventh Edition W. H. Freeman and Company New York pp. 873-919. ISBN-13:978-1-4292-3413-9.

Longworth MS, Laimins LA. (2004).Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiol *Mol Biol Rev*68:362-372.

Lorusso G, Rüegg C. (2008). The tumor microenvironment and its contribution to tumor evolution toward metastasis. *Histochem Cell Biol* 130: 1091-1103.

Lu, H., Ouyang, W., & Huang, C. (2006). Inflammation, a Key Event in Cancer Development. *Molecular Cancer Research.* 2006; 4 (4).

Lu, Z., Hu, X., Li, Y., Zheng, L., Zhou, Y., Jiang, H., Ning, T., Basang, Z.,, Zhang, C., Ke, Y. (2004). Human papillomavirus 16 E6 oncoprotein interferenceswith insulin signaling pathway by binding to tuberin. *J Biol Chem 279:* 35664–35670.

Lunsford, B T. R., & Lunsford, R. (1995). Research Forum--The Research Sample, Part I: Sampling. *Journal of Prosthetist and Orthotists Vol.* 7 Num. 3 pp 105-112

Lynette D. (2009). Human Papillomavirus Infections: Epidemiology, Clinical Aspects and Vaccines. *The Open Infectious Diseases Journal, 3,* 135-142 1

Macarthur, M., Hold, G.L., & El-Omar, E.M. (2004). Inflammation and cancer. II. Role of chronic inflammation and cytokine polymorphisms in the pathogenesis of gastrointestinal malignancy. *American Journal of Physiol Gastrointest Liver Physiol* 286: G515 – 20.

Macdonald, F., Ford, C. H. J., & Casson, A. G. C. (2005). Molecular Biology of Cancer Second edition Taylor & Francis e-Library, ISBN 0-203-59747-8 pp.60-75

Mackenzie, I. C., Rous, P. (1941). The experimental disclosure of latent neoplastic changes in tarred skin. *Journal of Experimental Medicine* 73:391–415. [PubMed: 19871085]

Madeleine, M. M., Anttila, T., Schwartz, S. M., Saikku, P., Leinonen, M., Carter, J. J., … Daling, J. R. (2007). Risk of cervical cancer associated with *Chlamydia trachomatis* antibodies by histology, HPV type and HPV cofactors. *International journal of cancer*, *120*(3), 650–655. doi:10.1002/ijc.22325

Madeleine, M. M., Anttila, T., Schwartz, S.M., Saikku, P., Leinonen, M., Carter, J.J., *et al.* (2006). Risk of cervical cancer associated with *Chlamydia trachomatis* antibodies by histology, HPV type and HPV cofactors. *International Journal of Cancer*; *120* : 650-5.

Maiuri, M. C., Tajana, G., Iuvone, T., et al. (2004). Nuclear factor-κB regulates inflammatory cell apoptosis and phagocytosis in rat carrageenin-sponge implant model. *American Journal of Pathol*ology 165:115 – 26.

Malumbres, M., Harlow, E., Hunt, T. Tony Hunter, T. H., Lahti, J. M. Manning, G., Morgan, D. O., Li-Huei Tsai, L-H & Wolgemuth, D. J. (2009). Cyclin-dependent

kinases: a family portrait *National Cell Bioogyl.* ; 11(11): 1275–1276. doi:10.1038/ncb1109-1275.

Manga, M. M., Fowotade, A., Abdullahi, Y. M., El-nafaty, A. U., Adamu, D. B., Pindiga, H. U., *et al*. (2015). Epidemiological patterns of cervical human papillomavirus infection among women presenting for cervical cancer screening in North-Eastern Nigeria. *Infectious Agents and Cancer*, 10(1). doi:10.1186/s13027-015-0035-8

Mardh, P.A., Ripa, T., Svensson, L., Westrom, L. (1977). *Chlamydia trachomatis* infection in patients with acute salpingitis. *N. Engl J. Med* 296: 1377-9.

Mariani, L., Aldo Venuti, A. (2010). HPV vaccine: an overview of immune response, clinical protection, and new approaches for the future. *Journal of Translational Medicine* https://doi.org/10.1186/1479-5876-8-105/

Mario, C., Gene, M. S., & Enrico, C. (1998). Cytokine dysregulation in invasive cervical carcinoma and Other Human Neoplasias: Time to consider the TH1/TH2 Paradigm. *Journal of the National cancer institute, Vol. 90, No.4*

Markiewski, M. M. & Lambris, J. D. (2009). Is complement good or bad for cancer patients? A new perspective on an old dilemma," *Trends in Immunology*. 30(6):286-92. doi: 10.1016/j.it.2009.04.002. Epub 2009 May 8.

Marks E., Lycke N. (2008) Immunity Against *Chlamydia trachomatis*. In: Vajdy M. (eds) Immunity Against Mucosal Pathogens. Springer, Dordrecht

Martina Schmitz, M., Driesch, C., Lars Jansen,L., Runnebaum, I. B., & Durst, M. (2012). Non-Random Integration of the HPV Genome in Cervical Cancer *PLoS ONE* Volume 7 Issue 6 e39632 https://doi.org/10.1371/journal.pone.0039632

Matos, E., Loria, D., Amestoy, G.M., Herrera, L., Prince, M. A, Moreno J, Krunfly C, van den Brule, A. J., Meijer, C.J., Muñoz, N., Herrero, R.; (2003). Proyecto Concordia Collaborative Group. *Sexual Transission of Disease*. 2003 Aug; 30(8):593-9

Matsumoto K1, Oki A, Satoh T, Okada S, Minaguchi T, Onuki M, Ochi H, Nakao S, Sakurai M, Abe A, Hamada H, Yoshikawa H. (2010). Interleukin-10 -1082 gene polymorphism and susceptibility to cervical cancer among Japanese women.*Japanese journal of clinical oncology.* ;40(11):1113-6. doi: 10.1093/jjco/hyq094.

Matsuzaki, K., Murata, M., Yoshida, K., Sekimoto, G., Uemura, Y., Sakaida,. N, Kaibori, M., Kamiyama, Y., Nishizawa, M., Fujisawa, J., Okazaki, K., & Seki, T. (2007). Chronic inflammation associated with hepatitis C virus infection perturbs hepatic transforming growth factor beta signaling, promoting cirrhosis and hepatocellular carcinoma. *Hepatology*; 46: 48-57

Mayer, J. M., Woods, M.L., Vavrin, Z., & Hibbs, J.B. (1993). Gamma interferon-induced nitric oxide production reduces *Chlamydia trachomatis* infectivity in McCoy cells. *Infect and Immuity.* 61(2): 491–497.

McDonald PP, Fadok VA, Bratton D, Henson P.M. (1999). Transcriptional and translational regulation of inflammatory mediator production by endogenous TGF-h in macrophages that have ingested apoptotic cells. *J Immunol* 1999;163: 6164 – 72.

McIntyre, P. (2005). Finding the viral link: the story of Harald zur Hausen cancer world July-August 2005.

McLaughlin-Drubin, M. E., & Münger, K. (2009). Oncogenic Activities of Human Papillomaviruses. *Virus Researc*h. ;143(2):195-208. doi: 10.1016/j.virusres.2009.06.008. Epub 2009 Jun 18.

Mietz JA, Unger T, Huibregtse JM, *et al*. (1992). The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 oncoprotein. *EMBO J*; 11: 5013-5020.

Miller, W. C., Ko, E. M. (2011). Does the chlamydial infection increase the risk of cervical dysplasia? *Sex Transm Infect* 87: 366–367.

Ministry of Health. (2011). Immunisation Handbook.Wellington: Ministry of Health ISBN: 978-0-478-37458-2 (Online) http://www.moh.govt.nz

Missero C, Calautti E, Eckner R, et al. (1995). Involvement of the cell-cycle inhibitor Cip1/WAF1 and the E1A-associated p300 protein in terminal differentiation. Proc Natl Acad Sci U S A;92: 5451-5455.

Mjelle R., S.A. Hegre, P.A. Aas, G. Slupphaug, F. Drablos, P. Saetrom, H.E. Krokan, (2015). Cell cycle regulation of human DNA repair and chromatin remodeling genes, *DNA Repair.* http://dx.doi.org/10.1016/j.dnarep.03.007

Molano M, Posso H, Weiderpass E, van den Brule AJ, Ronderos M, Franceschi S, Meijer CJ, Arslan A, Mun˜oz N (2002). Prevalence and determinants of HPV infection among Colombian women with normal cytology. *Br J Cancer* 87: 324–333.

Molecular Biology of Tumor Viruses (1981). DNA Viruses, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Monif G. R.G. (1998). A New Gold Standard for the Detection of *Chlamydia trachomatis*? *Infectious Diseases in Obstetrics and Gynecology* 6:44-45 Wiley-Liss, Inc.

Moody, C. A., Laimins, L. A. (2009). Human Papillomaviruses Activate the ATM DNA Damage Pathway for Viral Genome Amplification upon Differentiation. *PLoS Pathog 5:* e1000605

Moore, K. L., & Dalley, A. F. (1999). *Clinically oriented anatomy*. Philadelphia: Lippincott Williams & Wilkins.

Morin PJ, Trent JM, Collins FS, Vogelstein B. (2005). Cancer genetics. In *Harrisons principles of internal medicine*. 16th edition. Edited by Kasper DL, Braunwald E, Fauci AS, Hauser SL, Lango DL, Jameson JL. New York: Graw-Hill; 447-453.

Moscicki, A. B., Schiffman, M., Kjaer, S., Villa, L.L. (2006). Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine;* 24 (Suppl 3):S3-42-S3/51.

Moscicki, A.-B., Schiffman, M., Burchell, A., Albero, G., Giuliano, A. R., Goodman, M. T., … Palefsky, J. (2012). Updating the Natural History of Human Papillomavirus and Anogenital Cancers. *Vaccine*, 30, F24–F33. doi:10.1016/j

Multhoff, G., Molls, M., & Radons, J. (2012). Chronic inflammation in cancer development. *Frontiers in immunology*, 2, 98. doi:10.3389/fimmu.2011.00098

Munger K, Baldwin A, Edwards KM, et al. (2004). Mechanisms of human papillomavirus-induced oncogenesis. *J Virol*;78:11451-11460.

Munger K, Scheffner M, Huibregtse J.M., et al.(1992). Interactions of HPV E6 and E7 with tumor suppressor gene products. *Cancer Surv*;12: 197-217.

Munger K, Werness BA, Dyson N, *et al*. (1989). Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J*;8: 4099-4105.

Munger, K., & Howley, P. M. (2002). Human papillomavirus Immortalization and Transformation functions *Virus Research* 89: 213-228.

Munoz N, Bosch FX, de Sanjose S, *et al.* (2004). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* :348:518-527.

Murall, C. L., Rahmoun, M., Selinger, C., Baldellou, M., Bernat, C., Bonneau, M., … Alizon, S. (2019). *Natural history, dynamics, and ecology of human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study. BMJ Open, 9(6), e025129.* doi:10.1136/bmjopen-2018-025129

Murdoch C, Muthana M, Coffelt S.B, Lewis CE. (2008). The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer*; 8:618–631. [PubMed: 18633355]

Nathan, C. (2002). Points of control in inflammation. *Nature.;*420:846 – 52.

Nayar, R., & Wilbur, D. C. (2017). The bethesda system for reporting cervical cytology: A historical perspective. Acta Cytologica, 61(4-5), 359-372. https://doi.org/10.1159/000477556

NCBI www.ncbi.org accessed 8th April, 2015

Nejo, Y. T., Olaleye, D. O., & Odaibo, G. N. (2018). Prevalence and Risk Factors for Genital Human Papillomavirus Infections Among Women in Southwest Nigeria. *Archives of basic and applied medicine,* 6(1), 105–112.

Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, *et al.* (2015) Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS ONE* 10(12): e0143304. doi:10.1371/journal. pone.0143304

Ni Sima, Shixuan Wang, Wei Wang, Debo Kong, Qian Xu, Xu Tian, Aiyue Luo , Jianfeng Zhou , Gang Xu , Li Meng , Yunping Lu , Ding Ma (2007) Antisense targeting human papillomavirus type 16 E6 and E7 genes contributes to apoptosis and senescence in SiHa cervical carcinoma cells. *Gynecologic Oncology* 106 299–304.

Nicoleta Simion, Irina-Draga Căruntu, Elena-Roxana Avădănei, Raluca Balan, Cornelia Amălinei (2014). Conventional cytology *versus* liquid based cytology incervical pathology: correspondences and inconsistencies in diagnosis, advantages and limits. *Rom J Morphol Embryol* 55(4):1331–1337

Nigg, E. A. (1995). "Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle". *BioEssays* 17 (6): 471–80.

Nobbenhuis, M. A., Walboomers, J. M., Helmerhorst, T. J., Rozendaal, L., Remmink, A.J., Risse, E. K. *et al*. (1999). Relation of human papillomavirus status to cervical lesions and consequences for cervical cancer screening: a prospective study. *Lancet.*; 354:20-5.

Nowicki, A., *et al*. (1996). Impaired tumor growth in colony-stimulating factor 1 (CSF-1)-deficient, macrophage-deficient op/op mouse: evidence for a role of CSF-1-dependent macrophages in formation of tumor stroma. Int J Cancer;65: 112–119. [PubMed: 8543387]

Nwankwo E. O., and Sadiq, M. N. (2014). Prevalence of *Chlamydia trachomatis* infection among patients attending infertility and sexually transmitted diseases clinic (STD) in Kano, North Western Nigeria. *African Health Sciences* Vol 14 Issue 3.

Nyarko, C.C., Unson, C., Nyarko, P.K. and Koduah, M., (2014). A *Chlamydia trachomatis* Prevalence In Ghana – A Study At A Municipal District In Western Ghana *International Journal Of Scientific & Technology Research Vol* 3, Issue 1,Issn 2277-8616.

O'Byrne, K. J., & Dalgleish, A. G. (2001) Chronic immune activation and inflammation as the cause of malignancy. *Br J Cancer*. 85:473–83. [PubMed: 11506482]

Odukogbe, A.-T. A., Afolabi, B. B., Bello, O. O., & Adeyanju, A. S. (2017). Female genital mutilation/cutting in Africa. Translational Andrology and Urology, 6(2), 138–148. doi:10.21037/tau.2016.12.01

Ogiogwa, I. O., Motayo, B. O., Okerentugba, P. O., Innocent-Adiele, H. C., Tafeng, Y., Onoh, C. C., Nwanze, J. C., Okonko, I. O. (2012). Detection of *Chlamydia trachomatis* Antigen among Attendees of a Fertility Clinic in Abeokuta, Ogun State, *Nigeria Researcher* ; 4(4)

Öhman, H., Tiitinen, A., Halttunen, M., Lehtinen, M., Paavonen, J., & Surcel, H-M. (2009) Cytokine polymorphisms and severity of tubal damage in women with *Chlamydia*-Associated infertility. *JID*:199 1359.

Ohman, H., Tiitinen, A., Halttunen, M., Paavonen, J., Surcel, H.-M. (2011). Cytokine gene polymorphism and *Chlamydia trachomatis*-specific immune responses. *Human Immunology* 72 (2011) 278–282.

Okeke, T., Anyaehie, U., & Ezenyeaku, C. (2012). An overview of female genital mutilation in Nigeria. Annals of medical and health sciences research, 2(1), 70–73. doi:10.4103/2141-9248.96942

Okunade, K. S., Nwogu, C. M., Oluwole, A. A., Anorlu, R. I. (2017). Prevalence and risk factors for genital high-risk human papillomavirus infection among women attending the out-patient clinics of a university teaching hospital in Lagos, Nigeria *The Pan African Medical Journal* ;28:227. doi:10.11604/pamj.2017.28.227.13979

Omo-Agboja LO, Okonofua FE, Oremu, SO, Larsen U, Bergstrom S. (2007). Association of *Chlamydia trachomatis* serology with tubal infertility in Nigeria women. *J Obstet & Gynaecol Res*.;33 (5):688-95.

Ortiz L, Angevine M, Kim SK, Watkins D, DeMars R (2000). "T-Cell Epitopes in Variable Segments of *Chlamydia trachomatis* Major Outer Membrane Protein Elicit Serovar-Specific Immune Responses in Infected Humans". *Infect. Immun.* 68 (3): 1719–23. doi:10.1128/IAI.68.3.1719-1723.2000. PMC 97337. PMID 10678996.

Oshima H, Bartsch H. (1994). Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutation Res.* 305:253-64.

Osterman, A. L., Winer, R.L., Gottlieb, G. S., Sy, M-P., Ba, S., Dembele, B., *et al*. (2018) Female genital mutilation and non-invasive cervical abnormalities and invasive cervical cancer in Senegal, West Africa: A retrospective study. *In Press*: doi: 10.1002/ijc.31829.

Otelinda de Morais (2009). Determination of viral load and integration status of HPV 16 in normal and LSIL exfoliated cervical cells Université de Montréal Département de microbiologie et immunologie Faculté de Médecine Montreal, QC, 20 pg v

Otero-Motta AP, Ordóñez JL, González-Celador R, Rivas B, Macías M, Macías MC, et al: (2011). Prevalence of human papillomavirus genotypes in cytologic

abnormalities from unvaccinated women living in north-western Spain. APMIS 119:204–215.

Otterlei M., E. Warbrick, T.A. Nagelhus, T. Haug, G. Slupphaug, M. Akbari, P.A. Aas, K. Steinsbekk, O. Bakke, H.E. Krokan, Post-replicative base excision repair in replication foci, EMBO J, 18 (1999) 3834- 3844.

Pahwa R, Jialal I. Chronic Inflammation.[Updated 2019 Jun 4] In: StatPearls[Internet]. Treasure Island (FL): StatPearls Publishing 2019, Jan-

Papaincolaou G.N. (1940). Introduction of Pap smear in early detection of cervical malignancies *Am J clin path*; 19:301-308.

Park R.B. and Androphy E.J. (2002). Genetic analysis of high-risk e6 in episomal maintenance of human papillomavirus genomes in primary human keratinocytes. *J Virol*;76:11359-11364.

Park, M-T. and Lee, S-J. (2003). Cell Cycle and Cancer *Journal of Biochemistry and Molecular Biology*, Vol. 36, No. 1, January, pp. 60-65

Parkin, D.M, Ferlay, J., Hamdi-Cherif, M., Sitas, F., Thomas, J.O., Wabinga, H. and Whelan S.L., (2003). Cancer in Africa: Epidemiology and Prevention. 4.3 Cervix Cancer. IARC Scientific Publications No 153. Lyon: IARC Press pp 268–276.

Parkin, D.M., Whelan S.L., Ferlay, J., Thomas, D.B. and Teppo, L. (2002). Cancer Incidence in Five Continents. IARC Scientific Publications No 155, Lyon: International Agency for Research on Cancer.

Parroche, P., Touka, M., Mansour. M., Bouvard, V., Thé- Pot, A., Accardi, R., Carreira, C., Roblot, G.G., Sylla, B.S., Hasanu, Tommasinom (2011). Human papillomavirus type 16 E6 inhibits p21(WAF1) transcription independently of p53 by inactivating p150(Sal2). *Virology 417:* 443–448

Parsonnet J. (1999). Helicobacter pylori and gastric adenocarcinoma. In: Parsonnet J, eds. Microbes and Malignancy. Infection as a Cause of Human Cancers. 1st ed. New York: Oxford University Press; 1999: 372-408.

Pasternak, J. J. (2005). An Introduction To Human Molecular Genetics *Mechanisms of Inherited Diseases* Second Edition John Wiley & Sons, Inc. pp.20. ISBN: 978-0-471-47426-5

Pateld, Huangsm, Baglia L A, Mccanced, J. (1999). The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300. *EMBO J 18:* 5061–5072.

Patrick Stordeur, Lionel F. Poulin, Ligia Craciun , Ling Zhou, Liliane Schandene, Aurore de Lavareille, Stanislas Goriely, Michel Goldman , (2002). Cytokine mRNA quantification by real-time PCR. *Journal of Immunological Methods* 259 55–64.

Peltomaki, P. (2001) Mutation Res. 488:77.

Perez-Plasenciac,Vazquez-Ortizg,Lopez-Romeror,Pina-Sanchez P,Morenoj, Salcedom (2007) Genome wide expression analysis in HPV16 Cervical Cancer: identification of altered metabolic pathways. *Inf Agents and Cancer 2:* 16

Perrey, C., Turner, S. J., Pravica, V., Howel, M., Hutchinson, I.V. (1999). ARMS-PCR technologies to determine IL-10, TNFalpha, TNF-beta and TGF-beta1 gene polymorphisms. *Transplant Immunol* 7:127.

Pett, M. R., Alazawi, W. O. F., Roberts, I., Dowen, S., Smith, D. I., Stanley, M. A., & Coleman, N. (2004). Acquisition of High-Level Chromosomal Instability Is Associated with Integration of Human Papillomavirus Type 16 in Cervical Keratinocytes. *Cancer Research* 64, 1359–1368.

Pett, M. R., Herdman, M. T., Palmer, R. D., Yeo, G. S., Shivji, M. K., & Stanley, M. A. *et al.* (2006): Selection of cervical keratinocytes containing integrated HPV16 associates with episome loss and an endogenous antiviral response. *Proceedings of National Academic of Science USA* 103:3822-3827.

Peyressatre M., Camille Prével, C. Morgan Pellerano, M. & Morris, M. C. (2015). Targeting Cyclin-Dependent Kinases in Human Cancers: From Small Molecules to Peptide Inhibitors *Cancers*, *7*, 179-237; doi:10.3390/cancers7010179

Phelps WC, Yee CL, Munger K, *et al.* (1998). The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. Cell ;53: 539-547.

Philip M, Rowley DA, Schreiber H. (2004). Inflammation as a tumor promoter in cancer induction. *Semin Cancer Biol* 14:433 – 9.

Pietenpol J.A., Stein R.W., Moran E, *et al*. (1990). TGFb1 inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains. *Cell* 61: 777-785.

Piras, F., Piga, M., De Montis, A., Zannou, A. R.F., Minerba, L., Perra, M. T., Murtas, D., Atzori, M., Pittau, M., Maxia, C., & Sirigu, P. (2011). Prevalence of Human Papillomavirus infection in women in Benin, West Africa. *Virology Journal* 8:514. http://www.virologyj.com/content/8/1/514

Prado, J. C., Calleja-Macias, I. E., Bernard, H. U., Kalantari, M., Macay, S. A., Allan, B., ... Villa, L. L. (2005). Worldwide genomic diversity of the human papillomaviruses-53, 56, and 66, a group of high-risk HPVs unrelated to HPV-16 and HPV-18. Virology, 340(1), 95-104. https://doi.org/10.1016/j.virol.2005.06.024

Pravica, V., Perrey, C., Stevens, A., Lee, J-H. & Hutchinson, I.V. (2000). A Single Nucleotide Polymorphism in the First Intron of the Human IFN-g Gene:

Absolute Correlation with a Polymorphic CA Microsatellite Marker of High IFN-g Production. *Human Immunology* 61, 863–866.

Press release". Nobelprize.org.

Rajeshwarkar R, Panse N, Dinshaw K.A. (2009). HPV screening for cervical cancer in rural India. *New England Journal of Med*icine 360:1385-1394.

Rampias T, Boutati E, Pectasides E, Sasakic,Kountourakis P, Weinberger P, Psyrri A. (2010). Activation of Wnt signaling pathway by human papillomavirus E6 and E7 oncogenes in HPV16-positive oropharyngeal squamous carcinoma cells. *Mol Cancer Res 8:* 433–443.

Rasmussen, S.J., Eckmann, L., Quayle, A.J, *et al.* (1997). Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. *J Clin Invest* 99(1):77–87.

Ravindran, J., Tan, Y.I. and Ngeow, Y.I.(1998). The Prevalence of *Chlamydia trachomatis* in Patients with Pelvic Inflammatory Disease *Med J Malaysia* Vol 53 No 1 16-21.

Rawls, W. E., Tompkins, W. A. F., and Melnick, J. L. (1969). The Association of Herpesvirus Type 2 and Carcinoma of the Uterine Cervix. *Am. J. Epidemiol*. 89: 547-554.

Raybould P., Alison Fiander and Sam Hibbitts (2011) Human Papillomavirus Integration and its Role in Cervical Malignant Progression. *The Open Clinical Cancer Journal,* Volume 5

Raybould R. (2013). Human Papillomavirus Integration: The Mechanism(s) Behind the High-Risk Associated with this Event and Cervical Disease Progression Institute of Cancer Research and Medical Genetics HPV Research Group School of Medicine Cardiff University 2013.

Raymond W. Ruddon (2007). Cancer Biology 4th ed. Oxford University Press, Inc. Madison Avenue, New York. pp. 143-145.

Rebolj, M., Preisler, S., Ejegod, D. M., Rygaard, C. Lynge, E., & Jesper Bond, J. (2014). Disagreement between Human Papillomavirus Assays: An Unexpected Challenge for the Choice of an Assay in Primary Cervical Screening. Volume 9 | Issue 1 | e86835 www.plosone.org

Redgrove, K. A. & McLaughlin, E. A. (2014). The role of the immune response in *Chlamydia trachomatis* infection of the male genital tract: a double-edged sword Redgrove. *frontiers in immunology Vol5;534pp1-22*

Reinhardt H. C., & Yaffe, M. B. (2009). Kinases that Control the Cell Cycle in Response to DNA Damage: Chk1, Chk2, and MK2 *Current Opinion in Cell Biol*ogy 21(2): 245–255. doi:10.1016/j.ceb.2009.01.018

Reinhardt, H. C., Schumacher, B. (2012). The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends Genet 28:* 128–136

Report of the Consultation on Human Papillomavirus Vaccines World Health Organization, Geneva, April 2005.

Reznikoff, C. A., Belair, C., Savelieva, E., *et al.* (1994).Long-term genome stability and minimal genotypic and phenotypic alterations in HPV-16 E7-, but not E6-immortalized human uroepithelial cells. *Genes Dev* 8: 2227-2240.

Roberts, S.J, Ng, B.Y., Filler, R.B., Lewis, J., Glusac, E.J., Hayday, A.C., Tigelaar, R.E., Girardi, M. (2007). Characterizing tumor-promoting T cells in chemically induced cutaneous carcinogenesis. *Proc Natl Acad Sci* U S A 104:6770–6775. [PubMed: 17412837]

Robin, A. W. and Peter K.V. (2011). 100 years of Rous sarcoma virus. *J. Exp. Med.* Vol. 208 No. 12 2351-2355 www.jem.org/cgi/doi/10.1084/jem.20112160

Roitt, I. M., & Delves, P. J. (2001). *Roitt's Essential Immunology tenth edn*. Blackwell Science Inc.,

Ronco L.V., Karpova, A.Y., Vidal, M., Howley, P.M. (1998). Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. *Genes Dev 12:* 2061–2072.

Rosin, M.P., Anwar, W.A., Ward, A.J. (1994). Inflammation, chromosomal instability, and cancer: the schistosomiasis model. *Cancer Res.* 54:1929-33.

Rous, P., and Beard, J.W. (1935). The progression to carcinoma of virus-induced rabbit papillomas (Shope). *Journal of Experimental Medicine*; 62:523-548.

Ryan, K.J., Ray, C.G. (editors) (2004). *Sherris Medical Microbiology* (4th ed. ed.). McGraw Hill. pp. 463–70. ISBN 0-8385-8529-9.

Salimovi´c-Be˘si´c, I., Anja Tomi´c-˘Ci˘ca, A., Smailji, A., & Huki´ca, M. (2013). Comparison of the detection of HPV-16, 18, 31, 33, and 45 by type-specific DNA- and E6/E7 mRNA-based assays of HPV DNApositive women with abnormal Pap smears *Journal of Virological Methods 194 : 222– 228.* http://doi.org/ *10.1016/j.jviromet.2013.08.036*

Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, Papp JR, Black CM, Unger E.R. (2005). Association of *Chlamydia trachomatis* with Persistence of High-Risk Types of Human Papillomavirus in a Cohort of Female Adolescents. *Am J Epidemiol*.162:668–675. [PubMed: 16120706]

Sancar A., Lindsey-Boltz L.A., Unsal-Kacmaz K., Linn S.(2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints, *Annu Rev Biochem, 73* 39-85.

Sankaranarayanan, R., Nene B.M., Shastri, S.S., Jayant, K., Muwonge, R., Budukh, A.M., Hingmire, S., Malvi, S.G., Thorat, R., Kothari, A., Chinoy, R., Kelkar, R., Kane, S., Desai, S., Keskar, V.R., Sarov,I ., and Becker, Y. (1971), " Deoxyribonucleic acid-dependanrti bonucleic acid polymerase activity in purified trachoma elementary bodies: effect of sodium chloride on ribonucleic acid transcription. "*Journal of Bacteriology* 107: 593-597.

Sankaranayaranan, R. (2006). Overview of cervical cancer in the Developing country.FIGO 26th Annual Report on the Result of treatment in Gynecological cancer. *Int. J Gynea Obstet*. 95 (Supp. I): S205-10.

Savill J, Dransfield I, Gregory C, Haslett C. A. (2002). Blast from the past: clearance of apoptotic cells regulates immune responses. *Nat Rev Immunol* 2:965 – 75.

Savill J, Fadok V.A. (2000). Corpse clearance defines the meaning of cell death. *Nature* 407: 784 – 8.

Savill, J., Wyllie, A.H., Henson, J.E., Walport, M.J., Henson, P.M., Haslett, C. (1989) Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J Clin Invest* 83:865 – 75.

Scharer, O.D. (2013). Nucleotide excision repair in eukaryotes, Cold Spring Harb Perspect Biol, 5 a012609.

Scheffner, M., Munger, K., Byrne, J.C., *et al.* (1991). The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci U S A 88:5523-5527.

Scheurer, M. E.; Torlorero-Luna, G.; Adler-Storthz, K. (2005). "Human papillomavirus infection: biology, epidemiology, and prevention". *International Journal of Gynecological Cancer* 15: 727–746.

Schiffman, M., Herrero, R., Desalle, R., *et al* .(2005). The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* 337:76-84.

Schiffman, M., Wentzensen, N., Wacholder, S., Kinney, W., Gage, J. C., & Castle, P. E. (2011). Human papillomavirus testing in the prevention of cervical cancer. *Journal of the National Cancer Institute*, *103*(5), 368–383. doi:10.1093/jnci/djq562

Schoppmann S, et al. (2002). Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol* 161:947–956. [PubMed: 12213723]

Scott Mark E., Yurii B. Shvetsov, Pamela J. Thompson, Brenda Y. Hernandez, Xuemei Zhu, Lynne R. Wilkens, Jeffrey Killeen, Dien D. Vo1, Anna-Barbara Moscicki, and Marc T. Goodman, (2013). Cervical Cytokines and Clearance of Incident

Human Papillomavirus Infection: Hawaii HPV Cohort Study *Int J Cancer*. 133(5): 1187–1196. doi:10.1002/ijc.28119.

Scott Mark, Mayumi Nakagawa, And Anna-Barbara Moscicki (2001). Cell-Mediated Immune Response to Human Papillomavirus Infection. *Clinical and Diagnostic Laboratory Immunology,* p. 209–220 Vol. 8, No. 2

Scott, M., Daniel P. Stites, and Anna-Barbara Moscicki (1999). Th1 Cytokine Patterns in Cervical Human Papillomavirus Infection *Clin. Diagn. Lab. Immunol.* Sept. 1999, p. 751–755 Vol. 6, No. 5

Sedman, S.A., Barbosa, M.S., Vass, W.C., *et al.* (1991). The full-length E6 protein of human papillomavirus type 16 has transforming and trans-activating activities and cooperates with E7 to immortalize keratinocytes in culture. *J Virol* 65:4860-4866.

Sergei I. Grivennikov, Florian R. Greten, and Michael Karin (2010). Immunity, Inflammation, and Cancer *Cell*. 140(6): 883–899. doi:10.1016/j.cell.2010.01.025.

Serwadda, D, Wawer, M.J., Shah, K.V., Sewankambo, N.K., Daniel, R., Li, C., Lorincz, A., Meehan, M.P., Wabwire-Mangen, F., Gray, R.H. (1999). Use of a hybrid capture assay of self-collected vaginal swabs in rural Uganda for detection of human papillomavirus. *J Infect Dis* 180: 1316–1319

Shacter, E. and Weitzman, S. A. (2002). "Chronic inflammation and cancer," *Oncology*, vol. 16, no. 2, pp. 217–230.

Shacter, E. and Weitzman, S. A. (2002). Chronic inflammation and cancer, *Oncology*, vol. 16, no. 2, pp. 217–230.

Shamanin, V.A., Androphy, E.J. (2004). Immortalization of human mammary epithelial cells is associated with inactivation of the p14ARF-p53 pathway. *Mol Cell Biol* 24:2144-2152.

Shankaran, V., Ikeda, H., Bruce, A.T., White, J.M., Swanson, P.E., Old, L.J., Schreiber, R.D. (2001). IFNg and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410: 1107–1111. [PubMed: 11323675]

Shiloh, Y. (2001) Curr. Opin. Genet. Dev. 11:71.

Shope, R.E. (1937). Immunization of rabbits to infectious papillomatosis. *J Exp Med* 65:219-231.

Shope, R.E., Hurst, E.W. (1933). Infectious papillomatosis of rabbits; with a note on the histopathology. *J Exp Med* 58: 607-624.

Sica, A., Allavena, P., Mantovani, A. (2008). Cancer related inflammation: the macrophage connection. *Cancer Lett* 267:204–215. [PubMed: 18448242]

Silins. I., Ryd, W., Strand, A., Wadell, G., Tornberg, S., Hansson, B.G., Wang, X., Arnheim, L., Dahl, V., Bremell, D., Persson, K., Dillner, J., *et al.* (2005). *Chlamydia trachomatis* infection and persistence of human papillomavirus. Int J Cancer. 116:110–115. [PubMed: 15756673]

Singh R.S, Kulathinal R.J (2013). Polymorphism. *Brenner's Encyclopedia of Genetics, 2nd edition,* Volume 5 pp 398-399 doi:10.1016/B978-0-12-374984-0.01189-X

Smith A. J. P., and Humphries A. J.P. (2009). Cytokine and cytokine receptor gene polymorphisms and their functionality

Smith, J.S, Bosetti, C., Munoz, N., Herrero, R., Bosch, F.X., Eluf-Neto, J., *et al*.(2004). *Chlamydia trachomatis* and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. *Int J Cancer 111*: 431-9.

Smyth, M. J., Dunn, G.P., Schreiber, R.D. (2006). Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol* 90:1–50. [PubMed: 16730260]

Sotlar, K., Diemer, D., Dethleffs, A., Hack, Y., Stubner, A., Vollmer, N., … Bültmann, B. (2004). Detection and typing of human papillomavirus by e6 nested multiplex PCR. Journal of clinical microbiology, 42(7), 3176–3184. doi:10.1128/JCM.42.7.3176-3184.2004

Soucek, L., Lawlor, E.R., Soto, D., Shchors, K., Swigart, L.B., Evan, G.I. (2007). Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat Med* 13: 1211–1218. [PubMed: 17906636]

Sowemimo, O. O., Ojo, O. O., Fasubaa, O. B. (2017). Cervical cancer screening and practice in low resource countries: Nigeria as a case study. *Tropical Journal of Obstetrics and Gynaecology* http://www.tjogonline.com/text.asp?2017/34/3/170/219072

Spangle, J.M., Münger, K. (2010). The human papillomavirus type 16 E6 oncoprotein activates mTORC1 signaling and increases protein synthesis. *J Virol 84:* 9398–94078.

Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Molecular Biology of Cell* 9 (12): 3273–97

Ssedyabane, F. Amnia, D. A., Mayanja, R., Omonigho, A., Ssuuna, C., Najjuma, J.N., & Freddie, B. (2019). HPV-Chlamydial Coinfection, Prevalence, andAssociation with Cervical Intraepithelial Lesions: A Pilot Study at Mbarara Regional Referral Hospital Hindawi *Journal of Cancer Epidemiology* Volume, Article ID 9092565, 7 pages https://doi.org/10.1155/2019/9092565

Stankiewicz E, Prowse D M, Ng M, Cuzick J, Mesher D, Hiscock F, Lu Y-J, Watkin N, Corbishley C, Lamw, Berneydm (2011). Alternative HER/PTEN/Akt Pathway Activation in HPV Positive and Negative Penile Carcinomas. *PLoS ONE 6:* e17517

Stanley M. (2010). Pathology and Epidemiology of HPV Infection in females. *Gynecol Oncol* 117 (Suppl 2):5–10.

Stanley, M. (2010). HPV - immune response to infection and vaccination. *Infect Agents Cancer* 5, 19 doi:10.1186/1750-9378-5-19

Stanley, M. A. (2009). Immune responses to human papilloma viruses. *Indian J Med Res* 130, September pp 266-276.

Sun, Y., Lu, Y., Pen, Q., Li, T., Xie, L., Deng, Y., & Qin, A. (2014). Interferon gamma +874 T/A polymorphism increases the risk of cervical cancer: evidence from a meta-analysis. *Tumor Biolgy*. DOI 10.1007/s13277-015-3100-4

Sundberg, J.P. (1987). Papillomavirus infections in animals. In: Syrjanen K, Gissmann LL, Koss LG, eds. Papillomaviruses and Human Disease. Berlin: Springer-Verlag; 40-103.

Swann, J.B., Smyth, M.J.(2007). Immune Surveillance of Tumors. *J Clin Invest*.117: 1137–1146. [PubMed: 17476343]

Syverton, J.T. (1952). The pathogenesis of the rabbit papilloma-to-carcinoma sequence. *Ann N Y Acad Sci* 54:1126-1140.

Syverton, J.T., Berry, G.P. (1935). Carcinoma in the cottontail rabbit following spontaneous virus papilloma (Shope). *Proceedings of the Society for Experimental Biology and Medicine* 33:399-400.

Talora, C., Sgroi, D.C., Crumc, P. and Dotto, G.P. (2002). Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. *Genes Dev 16:* 2252–2263.

Tang, F.F., Chang, H.L., Huang, Y.T. and Wang, K.C. (June 1957). Studies on the etiology of trachoma with special reference to isolation of the virus in chick embryo. *Chin Med J* 75 (6): 429–47. PMID 13461224

Tang, F.F., Huang, Y.T., Chang, H.L. and Wong, K.C. (1957). Isolation of trachoma virus in chick embryo. *J Hyg Epidemiol Microbiol Immunol*.1 (2): 109–20. PMID 13502539

Tang, F.F., Huang,Y.T., Chang, H.L. & Wong, K.C. (1958). Further Studies on the Isolation of the trachoma virus. *Acta Virol.* 2 (3): 164–70. PMID 13594716

Theunissen, J.J.H., Minderhoud-Bassie, Wagenvoort, W.J.H.T., Stolz, E. Michel, M.F. & Huikeshoven, F.J.M. (1994). *Chlamydia trachomatis*-specific Antibodies in Patients with Pelvic Inflammatory Disease: Comparison with Isolation in Tissue Culture or Detection with Polymerase Chain Reaction. *Genitourin Med 1994;70: 304-307.*

Thomas, Banks L. (1998). Inhibition of Bak-induced apoptosis by HPV-18 E6. *Oncogene 17:* 2943–2954.

Thomas, J. O. Herrero, R., Omigbodun, A. A., Ojemakinde, K., Ajayi I. O., Fawole, A., Oladepo, O., Smith, J.S., Arslan, A., Mun˜oz, N,. Snijders, P. J. F., Meijer, C. J. L. M., & Franceschi, S. (2004). Prevalence of papillomavirus infection in women in Ibadan, Nigeria: a population-based study. *British journal of cancer*. Vol 90(3). http://doi.org/ 10.1038/sj.bjc.6601515

Thomas, J.T., Hubert, W.G., Ruesch, M.N., *et al.* (1999). Human papillomavirus Type 31 Oncoproteins E6 and E7 are Required for the Maintenance of Episomes during the Viral Life Cycle in Normal Human Keratinocytes. *Proc Natl Acad Sci* U S A. 96: 8449-8454.

Thorland, E.C., Myers, S.L., Gostout, B.S. & Smith, D.I. (2003). Common Fragile Sites are Preferential Targets for HPV16 Integrations in Cervical Tumors. *Oncogene* 22: 1225-37.

Torisu, H., *et al.* (2000). Macrophage Infiltration Correlates With Tumor Stage And Angiogenesis in Human Malignant Melanoma: Possible Involvement of TNfα and IL-1α. *International Journal of Cancer* 85:182–188. [PubMed: 10629075]

Tornesello, M.L., Duraturo, M.L., Buonaguro, L., Vallefuoco, G., Piccoli, R., Palmieri, S. & Buonaguro, F.M. (2007). Prevalence of Human Papillomavirus Genotypes and Their Variants In High Risk West Africa Women Immigrants In South Italy. *Infect Agent Cancer* 2:1.

Tsai, T-C. and Chen, S-L. (2003). The biochemical and biological functions of human papillomavirus type 16 E5 protein. *Arch Virol 148:* 1445–1453.

Tsung, K., Dolan, J.P., Tsung, Y.L. & Norton, J.A. (2002). Macrophages as Effector cells in interleukin 12-induced T cell-dependent Tumor Rejection. *Cancer Res* 62:5069–5075. [PubMed: 12208763]

Van der Woude, C.J., Kleibeuker, J.H., Jansen, P.L. & Moshage, H. (2004). Chronic Inflammation, Apoptosis and Pre-Malignant Lesions in the Gastro-Intestinal Tract. *Apoptosis* 9: 123-130.

Venceslau, E. M., Bezerra, M. M., Lopes, A. C. M., Souza, E. V., Onofre, A. S. C., de Melo, C. M., Jeraldo, V. D. S., Onofre, F.B.D., (2014). HPV detection using primers MY09/MY11 and GP5+/GP6+ in patients with cytologic and/or colposcopic changes. *Journal of Bras Patol Med Lab* v. 50, n. 4, p. 280-285.

Venuti, A., Paolini, F., Nasir, L., Corteggio, Sante Roperto, A., Campo M. S. & Borzacchiello, G. (2011). Papillomavirus E5: the Smallest Oncoprotein with Many Functions. *Molecular Cancer* 10:140 http://www.molecular cancer.com/content/10/1/140

Verkoyeen, R.P., Peeter, M.F., Van Rijsoort-Vos J.H., van der Meijden, W.I. and Marton, J.W. (2002). Sensitivity and specificity of three new commercially available *Chlamydia trachomatis* tests. *Int J STD AIDS* 2:23-5.

Verteramol, R., Pierangela, A., Mancini, E., Calzolari, E., Bucci, M., Osborn, J., *et al*. (2009). Human Papillomaviruses and Genital Co-Infections in Gynaecological Outpatients. *BMC Infect Dis 9*: 16.

Villa, L. L. & Lynette D. (2006). Methods for Detection of HPV Infection and Its Clinical Utility. *International Journal of Gynecology and Obstetrics* 94 (Supplement 1), S71-S80.

Wahl, L.M. & Kleinman, H.K. (1998). Tumor-Associated Macrophages as Targets for Cancer Therapy. *Journal of National Cancer Institute* 90:1583–1584. [PubMed: 9811301]

Waldner, M.J. & Neurath, M.F. (2009). Colitis-associated Cancer: the role of T cells in Tumor Development. *Semin Immunopathol* 31: 249–256. [PubMed: 19495757]

Wanderlei-Silva, D. Mariana N., Rosa Silva Gonzaga, Luciana Silva Viana & Eduardo Ramalho Neto (2005). High Quality DNA from Human Papillomavirus (HPV) for PCR/RFLPs *Brazilian Archives of Biology and Technology* Vol.48, no.1: pp. 37-40

Wang, S. S., Zuna, R. E., Wentzensen, N., Dunn, N.S., Sherman, M. E., Gold, M. A., *et al.*, (2009) Human Papillomavirus Cofactors by Disease Progression and Human Papillomavirus Types in the Study to Understand Cervical Cancer Early Endpoints and Determinants. *Cancer Epidemiol Biomarkers Prev*;18(1).

Weisburg, W., Hatch, T., & Woese, C. (1986). Eubacterial Origin of Chlamydiae. *Journal of*

Wendy Theelen, Rogier J.N.T.M. Litjens, Svetlana Vinokurova, Annick Haesevoets, Martin Reijans, Guus Simons, *et al.* (2013). Human Papillomavirus Multiplex Ligation-Dependent Probe Amplification Assay for the Assessment of Viral Load, Integration, and Gain of Telomerase-Related Genes in Cervical Malignancies. *Human Pathology* 44, 2410–2418.

Wentzensen, N. and Doeberitz, M.V. K. (2007). Biomarkers in cervical cancer screening *Disease Markers 23* 315–330 315 IOS Press

Wentzensen, N., Vinokurova, S. & von Knebel Doeberitz, M. (2004). Systematic Review of Genomic Integration Sites of Human Papillomavirus Genomes in Epithelial Dysplasia and Invasive Cancer of the Female Lower Genital Tract. *Cancer Research* 64: 3878-84.

Werness, B.A, Levine, A.J, Howley, P.M. (1990). Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248:76-79.

White, A., Livanos, E.M. & Tlsty, T.D. (1994). Differential Disruption of Genomic Integrity and Cell Cycle Regulation in Normal Human Fibroblasts by the HPV Oncoproteins. *Genes Dev* 8:666-677.

Whyte, P., Buchkovich, K.J., Horowitz, J.M., *et al.* (1988). Association Between an Oncogene and an Anti-oncogene: the Adenovirus E1A proteins Bind to the Retinoblastoma gene product. *Nature* 334: 124-129.

Whyte, P., Williamson, N.M. & Harlow, E. (1989). Cellular Targets for Transformation by the Adenovirus E1A proteins. *Cell* 56:67-75.

Wohlbold, L. & Fisher, R. P. (2009). Behind the wheel and under the hood: Functions of Cyclindependent Kinases in Response to DNA damage DNA Repair (Amst). 2; 8(9): 1018–1024. doi:10.1016/j.dnarep. 04.009

World Health Organisation (2011). Prevalence and Incidence of Selected Sexually Transmitted Infections, *Chlamydia trachomatis, Neisseria gonorrhoeae,* syphilis and *Trichomonas vaginalis*: Methods and Results used by WHO to generate 2005 estimates.Geneva: World Health Organisation; http://www.who.int/reproductivehealth/publication/rtis.

World Health Organisation/International Cancer Organisation (2010). HPV information centre on HPV and cervical cancer (HPV information centre). Human papilloma viruss and related cancers in Nigeria. Summary report 2010. Available: www.who.int/hpvcentre.

World Health Organisation/International Cancer Organisation. Monographs on the Evaluation of Carcinogenic Risk to Humans. Volume 90 Human Papillomaviruses.

World Health Organization (2001). Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections: Overview and Estimates. Available from: http://whqlibdoc.who.int/hq/2001/WHO_HIV_ AIDS_2001.02.pdf. Accessed May 8, 2015.

World Health Organization (2012). Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections – 2008. Geneva: World Health Organization; http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/index.html.

World Health Organization (2019). Global incidence and prevalence of selected sexually transmitted infections. Geneva, Available at: http://www.who.int/reproductivehealth/publications/rtis/ stisestimates/en/

Wright, T.C. Jr, & Schiffman M. (2003). Adding a Test for Human Papillomavirus DNA to Crvical-cancer Screening. *New England Journal of Medicine.* 348:489–490.

Wu, T.-C. & Kurman, R. J. (1997). Analysis of Cytokine Profile in Patients with Human Papillomavirus- Associated Neoplasm. *Journal of the National cancer institute, Vol. 89, No.3.* Lancaster WD, Olson C. Animal papillomaviruses. *Microbiol Rev* 1982; 46:191-207.

Xue, W., Wang, Q., Li, Z., Lu, L., Zhu, J., He, X., Zhao, R., Yongyong Shi, Y., & Zou, H. (2016). The TNF-α-308G/A Polymorphism is Not Associated with Ocular *Chlamydia trachomatis* Infection in Han Chinese Children. *Ophthalmic Genetics*, DOI: 10.3109/13816810.2015.1028650. 1744-5094 (Online) http://www.tandfonline.com/loi/iopg20

Xulian Lu, Qiaoai Lin., Mao Lin, Ping Duan, Lulu Ye, Jun Chen, Xiangmin Chen, Lifang Zhang, Xiangyang Xue (2014). Multiple-Integrations of HPV16 Genome and Altered Transcription of Viral Oncogenes and Cellular Genes Are Associated with the Development of Cervical Cancer. *PLOS ONE* Volume 9 Issue 7 e97588 www.plosone.org.

Yang, C.R., Hsieh, S.L., Ho, F.M. & Lin, W.W. (2005). Decoy Receptor 3 Increases Monocyte Adhesion to Endothelial Cells via NF-nB-dependent Up-regulation of Intercellular adhesion molecule-1, VCAM-1, and IL-8 expression. *Journal of Immunology* 174: 1647 – 56.

Yang, W., Song, Y., Lu, Y.-L., Sun, J.-Z., & Wang, H.-W. (2013). Increased expression of programmed death (PD)-1 and its ligand PD-L1 correlates with impaired cell-mediated immunity in high-risk human papillomavirus-related cervical intraepithelial neoplasia. *Immunology*, 139(4), 513–522. doi:10.1111/imm.12101

Zamarron, B. F. & Chen, W. (2011). Dual Roles of Immune Cells and their Factors in Cancer Development and Progression," *International Journal of Biological Sciences*, vol. 7, no. 5, pp. 651–658.

Zerfass-Thome, K., Zwerschke, W., Mannhardt, B., *et al.* (1996). Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. *Oncogene* 13:2323-2330.

Zhang, Q-w, Song, J-y., Yu, J-h., Sun, M-z., Tang, S-y., Yang, S-z., *et al.*, (2018). Polymorphism of IFN-γ +874T/A associated with production of IFN-γ affects human papillomavirus susceptibility in rural women from Luohe, Henan, China. *OncoTargets and Therapy* :11 4339–4344.

Zhang, S., Kong, Y-L., Li, Y-L & Yin, Y-W. (2014) Interleukin-10 gene –1082 G/A polymorphism in cervical cancer and cervical intraepithelial neoplasia: Meta-analysis. *Journal of International Medical Research* Vol. 42(6) 1193–1201.

Zhu, C., *et al.* (2002). Unrepaired DNA breaks in p53-deficient Cells Lead to Oncogenic Gene Amplification Subsequent to translocations. *Cell* 109(7):811–21. [PubMed: 12110179]

Zuckerman, A. J., Banatvala, J. E. and Patisson J. R. (2000). *Principles and Practice of Clinical Virology* Fourth Edition John Wiley and Sons Limited.

APPENDIX I

CONSENT FORM

Serial no…….

Date ……..

I have been informed about the study, which is targeted at identifying the **Inflammatory Effect of *Chlamydia trachomatis* Co-Infection of the Cervix with Human Papillomavirus among women in Ilorin Nigeria.** The aim of the study and the need for taking part of my exfoliated cervical cells for Pap Test and the detection of Human Papillomavirus and *Chlamydia trachomatis* have been informed to me, and fully understood. I have also been informed about the confidentiality of the questionnaire. Additionally I have been told that my cooperation in following the study format and participation in the study is on voluntary bases and refusal to participate does not involve any penalty. Apart from this, I have been informed that the specimen will be used only for research purpose and I will benefit from the free laboratory investigations.

Therefore, with full understanding of the importance of the study, I agree voluntarily that part of my exfoliated cervical cells sample be collected for Pap test and other clinical investigations which can be used for further analysis.

The researcher will be at hand every time to answer question(s) you may have concerning the research topic. The researcher can be reached on 08062623328,adesuyiomoare@gmail.com.

Participant's signature/Thumb print and date Researcher's signature and date

APPENDIX II

STUDY QUESTIONNAIRE

A. Eligibility Screening (please confirm eligibility by crosschecking with the following checklist):

Above 18 years and not older than 65 years ( )

Not Pregnant or Recent Birth (6 months) ( )

Not on active menstruation ( )

Not Immunocompromised or on cancer drug ( )

Not recieved HPV Vaccines ( )

Had not undergone cervical biopsy ( )

B. BIO-DATA AND SOCIO-ECONOMIC HISTORY

Date…….

Sex……

Age……

Phone No……..

Occupation……

Address……

State of Origin……

1. Why are you in this clinic? Family planning ( ) Gynea ( ) Others (specify)…….
2. Education Status: informal ( ) Primary ( ) Secondary ( ) Tertiary ( )
3. Religion: Christianity ( ) Islam ( ) Others (specify)…….
4. Ethnicity: Yoruba ( ) Hausa ( ) Igbo ( ) Others (specify)……..

5. Marital status: Single ( ) Married ( ) Divorced/Widowed/Separated ( )
6. If married what kind of marriage? Monogamy ( ) Polygamy ( ) Polyandry ( )
7. How long have you been Married.....

C. REPRODUCTIVE HEALTH HISTORY

1. Have you undergone hysterectomy (removal of the uterus/womb)? Yes ( ) No ( )
2. Do you have any history of genital warts? Yes ( ) No ( )
3. What was your Age at first Menstruation (menarche)?
4. Do you have painful Menstruation? Yes ( ) No ( )
5. What was your Age at first Sexual Intercourse?.....
6. Have you ever been pregnant? Yes ( ) No ( )
7. If yes specify appropriate number of time (s)…….
8. What was your Age at onset of childbearing?.....
9. How many children do you have?.....
10. Have you ever experienced Miscarriage? Yes ( ) No ( )
11. If yes how many times……..
12. How often do you have sexual intercourse?
    i. Once a week ()
    ii. Twice a week ()
    iii. Thrice a week ( )
    iv. More than four times a week ( )
13. How many lifetime sexual partners do you have or have you had?.....
14. Do you use protection for sexual intercourse? Yes ( ) or No ( )
15. If yes how often?

I. Always Yes ( ) or No ( )

II. Occasionally Yes ( ) No ( )

16. What type (s) of protection do you use? a. Condom ( ) b. Microbicides ( ) c. spermicides ( ) d. Others (specify)……..
17. Are you on family planning? Yes ( ) or No ( )
18. If yes what type (specify)…….
19. Have you ever had Pap Test Yes ( ) or No ( )?
20. If yes when?.....
21. And how often do you go for Pap Test?.....
22. Have you had history of Pelvic examination? Yes ( ) No ( )
23. If Yes Explain……..
24. Have you experienced female genital cuttings/Circumcision? Yes ( ) No ( )
25. Have you had a history of instrumentation to your reproductive system Yes ( ) or No ( )?
26. If yes, explain……..

D. CANCER HISTORY

1. Have you previously heard/informed about cancer of the cervix? Yes ( ) or No ( )
2. If yes, how did you get to know?.....
3. Is there history of cancer in your family? Yes ( ) or No ( )
4. If yes, which of this family member have been diagnosed? Male ( ) or Female ( )
5. What is your relationship with the family member?.....
6. Which of the following is applicable to you?

i. Cigarette smoking Yes ( ) or No ( )

ii. Alcohol use Yes ( ) or No ( )

iii. Diagnosed of cancer Yes ( ) or No ( )

iv. Currently on cancer treatment Yes ( ) or No ( )

E. SEXUALLY TRANSMITED INFECTIONS HISTORY

1. Have you previously heard of Sexually Transmitted Infection (STI)? Yes ( ) or No ( )
2. Have you suffered from any form of sexually transmitted infection before? Yes ( ) or No ( )
3. If yes, which of the following?
   i. Gonorrhoea Yes ( ) No ( ) iv. Candidiasis
   ii. Syphilis Yes ( ) No ( )
   iii. Chlamydia Yes ( ) No ( )
4. Have you previously heard of Human Papillomavirus (HPV)? Yes ( ) or No ( )
5. If yes how did you get to know?.....
6. Have you ever been screened for Human Papillomavirus? Yes ( ) or No ( )
7. If yes what was the result? Positive ( ) or Negative ( )
8. If positive did you receive any treatment? Yes ( ) or No ( )
9. Have you ever received Human Papillomavirus immunization ? Yes ( ) or No ( )
10. If yes specify…….
11. Which of the following is applicable to you?

i. Painful Sexual intercourse Yes ( ) or No ( )

ii. Abnormal vaginal discharge Yes ( ) or No ( )

iii. Irregular menstruation Yes ( ) or No ( )

iv. Bleeding after Sexual intercourse Yes ( ) or No ( )

v. Post-menopausal bleeding Yes ( ) or No ( )

APPENDIX III

UNIVERSITY OF ILORIN, ILORIN, NIGERIA.

UNVERSITY ETHICAL REVIEW COMMITTEE

Vice-Chancellor: Prof. A.G. Ambali
DVM (ABU), M.V. Sc., Ph.D (Liverpool, UK),
MVCN, MCVSN, MNVMA,FCVSN
Registrar: Mr. E.D.Obafemi
B.A. (Hons), Cert, Public Information (Kaduna),
MNIPR

E-mail: P.M.B. 1515, Ilorin
uerc@unilorin.edu.ng
unilorin.uerc@gmail.com
Website: ethicalreview.unilorin.edu.ng
www.unilorin.edu.ng

Our Ref: UIL/UERC/01/55EJ072

Date: 8TH December, 2016

Protocol Identification Code: UERC/BMS/042
UERC Approval Number: UERC/ASN/2016/652

INFLAMMATORY EFFECT OF *CHLAMYDIA TRACHOMATIS* CO- INFECTION WIH HUMAN PAPILLOMAVIRUS AMONG WOMEN IN SELECTED HOSPITALS IN ILORIN, NIGERIA

Name of applicant/Principal Investigator: **OMOARE,** Adesuyi Ayodeji
Address of Applicant: Department of Medical Microbiological & Parasitology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin.

Type of Review: Full Committee Review
Date of Approval: 8/12/2016

Notice of Full Committee Approval

I am pleased to inform you that the research described in the submitted proposal has been reviewed by the University Ethical Review Committee (UERC) and given full Committee approval.

This approval dates from 8/12/2016 to 7/12/2019, and there should be no participant accrual or any activity related to this research to be conducted outside these dates.

You are requested to inform the committee at the commencement of the research to enable it appoints its representative who will ensure compliance with the approved protocol. If there is any delay in starting the research, please inform the UERC so that the dates of approval can be adjusted accordingly.

The UERC requires you to comply with all institutional guidelines and regulations and ensure that all adverse events are reported promptly to the UERC. No charges are allowed in the research without prior approval by the UERC. Please note that the UERC reserves the right to conduct monitoring/oversight visit to your research site without prior notification.

Thank You

Ismaila Isah
For: University Ethical Review Committee

UNIVERSITY ETHICAL REVIEW COMMITTEE
University of Ilorin, Ilorin, Nigeria

"....if it's not ethical, it's not scientific, if it's not scientific, it's not ethical"

APPENDIX IV

UNIVERSITY OF ILORIN TEACHING HOSPITAL

Chairman:

Chief Medical Director:
PROF. A.W.O. OLATINWO
MBBS. FWACS, MBA, AMNIM

Chairman Medical Advisory Committee:
PROF. M. O. BUHARI
MBBS. FWACP, MBA

Director of Administration:
MR. G. O. YUSUF
B. Sc. (HONS) Ibadan, PGDE, Cert Health Planning & MGT.

Old Jebba Road, Oke-Ose,
P.M.B. 1459, Ilorin,
Kwara State, Nigeria.

E-mails:
- unithilorin1980@yahoo.com
- info@uith.org

Telephone:
- 08055763942

UITH ERC Protocol Number: ERC PIN/2016/03/0406
UITH ERC Approval Number: ERC PAN/2016/04/1531

Our Ref: UITH/CAT/189/19 A/603 *Date: 25/04/2016*

INFLAMMATORY EFFECT OF CO-INFECTION OF THE CERVIX WITH CHLAMYDIA TRACHOMATIS AND HUMAN PAPILLOMA VIRUS AMONG WOMEN IN ILORIN NIGERIA

UITH Ethical Research Committee (ERC) assigned number: NHREC/02/05/2010
Name of Applicant/Principal Investigator: OMOARE ADESUYI AYODEJI
Address of Applicant: Dept. of Medical Microbiology & Parasitology, University of Ilorin, Ilorin.
Date of receipt of application: 04/03/2016
Type of Review: Full Committee Review
Date of full Committee Decision on the Research: 26/03/2016
Date of full Committee Approval: 25/04/2016

Notice of full Committee Approval

I am pleased to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed by the UITH Ethical Review Committee (ERC) and given full Committee approval.

This approval dates from 25/04/2016 to 24/04/2017. You are requested to inform the committee at the commencement of the research to enable it appoint its representative who will ensure compliance with the approved protocol. If there is delay in starting the research, please inform the ERC so that the dates of approval can be adjusted accordingly.
Note that no participant accrual or activity related to this research may be conducted outside these dates.

The UITH ERC requires you to comply with all the institutional guidelines and regulations and ensure that all adverse events are reported promptly to the ERC.
No changes are allowed in the research without prior approval by the ERC. Please note that the ERC reserves the right to conduct monitoring/oversight visit to your research site without prior notification.

Notwithstanding above, we will not be responsible for any misconduct on the part of the researcher in the course of carrying out the research.

Thank you.

PROF. (MRS) F.G. ADEPOJU MBBS, MSC, FMCOPH, FWASC, FICS, MNIM
Chairman, UITH Ethics Review Committee. (ERC)

APPENDIX V

MINISTRY OF HEALTH

OFFICE: P. M. B. 1386, FATE ROAD, ILORIN, KWARA STATE. 031-220349

Our Ref: MOH/KS/EHC/777/95 *Your Ref:* _____ *Date:* 3rd August, 2015

Omoare Adesuyi Ayodeji ,
Department of Medical Microbiology and Parasitology,
Faculty of Basic Medical Sciences
College of Heath Sciences
University of Ilorin.

APPROVAL TO CARRYOUT MEDICAL RESEARCH TITLED: INFLAMATORY EFFECT OF *Chlamydia trachomatis* CO-INFECTION OF THE CERVIX WITH HUMAN PAPILLOMAVIRUS AMONG WOMEN IN ILORIN.

Sequel to your request and the interest of the State Ministry of Health in Health related research activities to improve the health of the citizens.

I am directed to forward to you the approval of the Ministry of Health to carry out the dissertation as itemized in your Protocol. This approval dates from 3/08/2015 to 3/08/2016 except you seek for extension.

You are mandated to acknowledge the Ministry of Health in your presentations/ publications and deposit a final copy of your project to the Ministry of Health.

Best wishes in your research project.

F. O. P Oyinloye
Secretary Health Ethical Research Committee,
For: Honourable Commissioner.

CC:

- The CMD General Hospital, Ilorin.
- The CMD Sobi Specialist Hospital, Ilorin.
- The CMD Children Specialist Hospital, Centre Igboro, Ilorin.
- The officer In-charge Civil Service Hospital, Ilorin.