UNIVERSITY OF CAPE COAST

CIRCULATORY INFLAMMATORY CYTOKINES AND MICROBIOME DIVERSITY AMONG ADULT FEMALES AT RISK OF CERVICAL CANCER

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: …… Date: ……..

Name: Loretta Betty Blay Mensah

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: …….. Date: ….....

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ABSTRACT

Cervical cancer continues to be a global burden, especially in LMICs. Currently, the role of dysbiosis in immune modulation that favours carcinogenesis is been highlighted. The main purpose of this study was to assess the association between circulatory inflammatory cytokines (IL-4, IL-6, IL-10, TNF-α and INF-γ) and cervico-vaginal microbiome diversity among adult females in a rural population. A total of 157 women of 21- 80 years old were recruited and closed-ended questionnaires were used to obtain data on awareness and knowledge of HPV infection and cervical cancer. Conventional Pap smear test, culture and ELISA were employed for cytology, bacteria isolation and cytokines estimation respectively. Mean age of the participants was 41.2 (1.1) years. Among the participants 36% (56/157) and 10% (16/157) were aware and had knowledge of Cervical cancer and HPV infection respectively. The primary sources of information were mainly broadcasting media (68.0%) and health care workers/facilities (53.0%). Participants with LSIL+ were 14/157 (8.9%). The significant infection among LSIL+ were Bacterial vaginosis and Candida ($p<0.05$). The significant bacteria isolate among LSIL+ were *Staphylococcus aureus* (22/101, 21.8%), *Escherichia coli* (32/101, 31.7%) and *Citrobacter spp.* (16/101. 15.8%). Finally, IL-10 concentrations increased among participants with dysbiosis and LSIL+ (RTI vs LSIL+RTI vs HSIL+RTI) [9.98(1.85) vs 13.61(3.648) vs 15.11(4.70) vs 9.22 (3.91) pg/nl respectively, $p>0.05$]. In conclusion, knowledge and awareness of cervical cancer and HPV infection among the adult women in rural communities is limited and dysbiosis possibly influenced immune suppression thus favouring the microenvironment for tumorigenesis.

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I thank my family and friends, all and sundry for your relevant support in finances, prayers and encouragement. I pray for increasing blessings from the Almighty God for you all.

DEDICATION

I dedicate this work to myself, for not giving up and soaring through the various challenges.

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LIST OF ABBREVIATIONS

HPV	Human Papillomavirus
STIs	Sexually Transmitted Infections
RTI	Reproductive Tract infections
TH1	T Helper 1 Cell sub-type
TH2	T Helper 2 Cell sub-type
Hr-HPV	High Risk Human Papillomavirus
CIN	Cervical Intraepithelial Neoplasia
SIL	Squamous Intraepithelial Lesion
LSIL	Low grade Squamous Intraepithelial Lesion
HSIL	High grade Squamous Intraepithelial Lesion
NIL	No Intraepithelial lesions
NSG	No significant growth
ASCUS	Atypical Squamous Cells of Undetermined Significance.
IL	Interleukin
IFN	Interferon
TNF	Tumour Necrotic Factor
DNA	Deoxyribonueleic Acid
WHO	World Health Organisatio
LMICs	Low-to-middle income countries

CHAPTER ONE

INTRODUCTION

Chapter Introduction

The risk of acquiring sexually transmitted infections (STIs) increases with active sexual life. The risk further increases with predisposing lifestyle behaviours, such as having multiple sexual partners. Human Papillomavirus (HPV) infection is among the most common STIs, which upon persistent thriving on the host's epithelium of the cervix, occasionally leads to the development of cervical cancer. Many factors have been postulated to increase a woman's risk of developing cervical cancer, but the immunological evidence available now points to alteration in the cell mediated immunity, where there is an imbalance between the Th1 and Th2 cell lineages. This study looked at the possible effect of cervico-vaginal microbiome diversity on the cytokine profiles in persons at risk of HPV infection.

Background

Human Papillomavirus (HPV) infection is the most common sexually transmitted infection among sexually active persons; both males and females (Palefsky, 2010; Palefsky, Holly, Ralston, & Jay, 1998). It is considered that the risk of sexually active persons, especially women, getting infected within their lifetime is nearly 80%. Persistent infection of high-risk HPV (hr-HPV) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 has been implicated as the predominant aetiological factor (>90%) in the development of cervical carcinoma (Schiffman, Castle, Jeronimo, Rodriguez, & Wacholder,

2007; Walboomers et al., 1999). However, other lifestyle behaviours and conditions, such as smoking, prolonged oral and/or hormonal contraceptive usage, coinfections, multiparity and immune-related diseases, put women at risk of developing this carcinoma (Deligeoroglou et al., 2013).

In developing countries, such as Ghana, cervical cancer accounts for 15% of cancers in females aged less than 65 years old (D. Song, Li, Li, & Dai, 2015). Hitherto in Ghana, cervical cancer is the second most common cancer diagnosed among women (Awua, Sackey, Osei, Asmah, & Wiredu, 2016) and the mortality rate, as a result of it, is more than three times the global cervical cancer mortality rate (Domfeh et al., 2008; Williams & Amoateng, 2012).

Cervical carcinoma does not emerge suddenly; it involves a multistep process in the alteration of the squamous epithelial layer of the cervix as a result of persistent HPV (commonly hr-HPV) infection, the development of different stages of cervical intraepithelial neoplasia (CIN) lesions and ultimately cervical cancer (D. Song et al., 2015). Currently, the immunological evidence available on the infection and carcinogenesis indicates alteration in cell mediated immunity (Scott, Nakagawa, & Moscicki, 2001). A number of studies have also implicated high cervico-vaginal microbiome diversity to cause immune modulation which affects the production of certain pro- and anti-inflammatory cytokines during persistent HPV infection; whiles in other studies too, peripheral parasites such as intestinal parasites were implicated (Chen et al., 2019; Mbulaiteye et al., 2013; Norenhag et al., 2019). The cytokines are necessary for the induction of many immune-related activities that include initiation of inflammation and recruiting of phagocytes, regulation of immune response

activities and stimulation of the proliferation of activated T and B cells (Kemp et al., 2010).

All around the world, one of the most common complaints of patients during physician visits is reproductive tract infections that are mostly associated with genital dysbiosis (Paavonen & Brunham, 2018). These irregularities are known to gradually cause local immunosuppression that increases the risk of developing squamous intraepithelial lesions and ultimately cancer (Audirac-Chalifour et al., 2016). Increased diversity of cervico-vaginal microbiomes has been proposed to inform the secretion of certain immunosuppressive cytokines such as IL-10 (Anahtar et al., 2015; Audirac-Chalifour et al., 2016; Kyrgiou, Mitra, & Moscicki, 2017; Norenhag et al., 2019). This was evident in the study by Anahtar et al. (2015), where specific bacteria species isolates from the cervico-vaginal region of healthy South African women were identified to modulate the local inflammatory immune response. The trend of local inflammatory cytokines expressions has been described to be similar to that of peripheral inflammatory cytokines among women with cervical lesions and cervical cancer. However, the concentrations of the former is usually more elevated than that of the latter (Ali, Ali, & Jubrael, 2012).

The expressions of cytokines in cervical dysplasia have widely been under study and the findings confirm TH1/TH2-type cytokines imbalance (Ali et al., 2012; Li et al., 2019; D. Song et al., 2015); however, no consistency of the specific cytokines have been established. TH1-type cytokines such as IL-2, TNF-α and IFN-γ (mostly anti-inflammatory cytokines) and TH2-type cytokines such as IL-4, IL-6, IL-10 and IL-13 (mostly pro-inflammatory cytokines) have been estimated in cervical dysplasia due to the various roles they play in eliciting

appropriate immune response (Ali et al., 2012; Li et al., 2019; Otani et al., 2019; D. Song et al., 2015). The TH1 type cytokines induce cell mediated immunity which helps in HPV clearance and regression of cervical dysplasia and the TH2 type cytokines have immune-inhibitory action on cell mediated immunity that favours the progression of cervical dysplasia to carcinoma (S.H. Song et al., 2008). IL-10 is an immunomodulatory cytokine for the regulation of immune response by also inhibiting IFN-γ and IL-12 (Ali et al., 2012), IL-4 inhibits cytotoxic activity and IFN-γ synthesis (Barros, de Oliveira, de Melo, Venuti, & de Freitas, 2018a), TNF-α possess apoptotic effect, anti-tumour properties and autocrine mechanisms to inhibit proliferations of affected cells (Landskron, De la Fuente, Thuwajit, Thuwajit, & Hermoso, 2014; Rotar et al., 2014) and IFN-γ enhances the HPV clearance (D. Song et al., 2015). IL-6 has both anti- and pro-inflammatory properties; however, it promotes angiogenesis of cervical cancer as a TH2 type cytokine by initiating trans-signaling with increasing expression vascular endothelial growth-factor (VEGF) via STAT3 pathway (Li et al., 2019; Wei et al., 2003).

Furthermore, awareness and knowledge of HPV infection as the predominant aetiological factor in the development of cervical cancer and the available vaccines for its prevention is usually lower among the rural population (Kadian et al., 2020; Mohammed et al., 2018; Reichheld, Mukherjee, Rahman, David, & Pricilla, 2020; Zahnd et al., 2018). This finding has been evident in the USA where some studies have demonstrated lower proportion of the rural population, as against that of the urban population, having better levels of knowledge of HPV infection, HPV vaccines and cervical cancer (Mohammed et al., 2018; Zahnd et al., 2018). Similarly, in Africa, the trend has been reported

by various studies in the region (Ebu, Mupepi, Siakwa, & Sampselle, 2014; Shabani, Moodley, & Naidoo, 2019).

Ghana, just like most African countries, has data gaps on the prevalence and awareness of cervical cancer. This study therefore targeted the awareness of HPV infection and cervical cancer, certain risk characteristics and the prevalence of squamous intraepithelial lesions among adult females in the rural settings of Ghana. The study further evaluated the relationship between the state of cervico-vaginal microbiomes and circulatory inflammatory cytokines in the development of cervical cancer among the study population.

Problem Statement

It has been noted that HPV infection is the most common viral sexually transmitted infection in both men and women (Palefsky, 2010; Palefsky et al., 1998). The burden of genital HPV infection and its progression into cervical cancer is at the peak in low- and middle- income countries, such as Ghana (Awua et al., 2016; Domfeh et al., 2008; D. Song et al., 2015).

Recently, some studies have been highlighting the possible involvement of cervico-vaginal microbiomes in persistent HPV infection and its progression to carcinoma of the cervix (Chen et al., 2019; Norenhag et al., 2019). Currently, bacterial-viral interactions in human host depend on two pathways; generation of microbiota bioproducts that could promote virus-host interactions and possible host gene expressions alterations influenced by bacteria-host interactions that stimulate tumorigenesis coupled with viral infections (Curty, de Carvalho, & Soares, 2019; Mitra et al., 2016). This implication could be associated with a possible dysregulation of Th1/Th2 immune responses in light

of increased diversity of cervico-vaginal microbiomes with less dominance of *Lactobacillus* sp.

Aside early age of sexual debut and multiple sexual partners, intravaginal practices such as douching, increases the risk of reproductive tract infections including STIs. (F. N.-A. McCarthy, Nii-Trebi, Musah, & Asmah, 2015; Ziba, Yakong, Asore, Frederickson, & Flynch, 2019). In Ghana, douching is done for various reasons including cleansing, tightening of vaginal muscles to increase sexual pleasure as well as therapeutic purposes; and these alters the composition of the microbiota leading to less dominance of *Lactobacillus sp* and causing imbalance of the local pH (F. N.-A. McCarthy et al., 2015; Ziba et al., 2019).

Currently in Ghana, there is scarcity of data on the awareness of HPV infection and its ultimate sequela, cervical cancer, especially in the rural settings. This study therefore looked at the degree of awareness of HPV infection and cervical cancer, risk behaviours of adult females and identified the women at risk of hr-HPV infection and cervical cancer. Also, the study looked at the association between cervico-vaginal microbiomes and certain circulatory inflammatory cytokines- IL-4, -6, -10, TNF-α and INF-γ.

Aim and Objectives

Aim

To assess the association between circulatory inflammatory cytokines (IL-4, IL-6, IL-10, TNF-α and INF-γ) and cervico-vaginal microbiome diversity among adult females at risk of Cervical cancer.

Objectives

The study was undertaken to:

1. assess the socio-demographic and socioeconomic characteristics, knowledge of HPV infection and Cervical cancer, awareness of the availability of vaccines and also the risk factors among a rural population.
2. screen participants and identify the proportion of participants with squamous intraepithelial lesions.
3. isolate and identify various cervico-vaginal microbiomes among participants.
4. estimate and compare the levels of IL-4, IL-6, IL-10, TNF-α and INF-ɣ circulatory cytokines among the participants.

Hypothesis

There is no relation between the circulatory inflammatory cytokines' expressions and cervico-vaginal microbiome diversity among women at risk of cervical cancer.

Significance of the Study

Human Papillomavirus (HPV) infection continues to be the most prevalent among sexually transmitted infections recorded, especially in the developing part of the world (Joel M. Palefsky, 2010; Joel M Palefsky et al., 1998; WHO, 2018). This infection, through a series of physiological alterations

in the squamous epithelial layer of the uterine cervix, causes the development of carcinoma. Various mechanisms of its pathogenesis have been proposed and proven. They include: the insidious noncytopathic replication of hr-HPV, making them exhibit less clinically significant manifestation; inhibition of interferon (IFN) synthesis through E6 and E7 oncoproteins, leading to interference in IFN signaling pathways; induction of regulatory T cell (Treg) infiltration and interleukin (IL)-10 or transforming growth factor β (TGF-β) production, expression of low levels of MHC class I, resulting in impaired CTL function and lastly, induction of ineffective CD4 and CD8 T lymphocytes accumulation in stage II/III CINs.

Although, numerous factors have been suggested to affect the persistence of HPV infection and the development of cervical cancer, the one factor that seem to be gaining recognition is the high diversity of cervico-vaginal microbiomes and its possible involvement in the determination of inflammatory cytokines locally and in circulation too.

The findings of this study add to the scientific knowledge on the association between dysbiosis and the development of cancer. They also provide further knowledge on possible immune modulation in the presence of squamous intraepithelial lesion and pathogenic microbiomes. This will ultimately serve as a pedestal in biomedical needs application in the development of better therapeutic options.

Delimitations

This was a cross-sectional study that involved sampling from the Akyemansa Sub-district in the Eastern Region of Ghana. The district is made up

of 96 communities and has a total population of 97, 374. However, only four (4) of the communities: Abenase; Adjobue; Akyemansa; and Mukyia were conveniently selected. Only women of 20 years of age and above and have lived in the selected communities for at least 3 months were recruited. Pregnant women, women who had undergone total hysterectomy or chemotherapy or menstruating on the day of sampling were, however, excluded.

Other variables included in the study were the educational level, occupational, marital and financial statuses of participants and certain risky behavioural characteristics.

Limitations

Cervical specimens were obtained for Pap smear examination and molecular identification and typing of HPV among the participants. However, the latter could not be achieved and will be captured in further studies. Also, among all the communities visited in the Akyemansa District, comparable number of respondents were recruited from the different participating communities. This observation was informed by the availability, accessibility and resource potentials of the health facilities in the respective communities.

Definition of Terms

HPV infection : A sexually transmitted infection caused by a Human Papilloma virus (HPV). It is a self-limiting infection, but occasionally leads to genital warts and cancer.

Cervical cancer: A type of cancer that affects the cervix of the uterus

Vaginal microbiomes: Describes micro-organisms that live in and on multicellular layers of the vagina and its surroundings. The micro-organisms can be pathogenic or commensals.

Pap Smear test: A cervical screening test that involves swabbing the cervix for cells, smearing it on a glass slide and fixing it, staining it by the Papanicoloau technique and examining it under microscope.

Cytokines: A group of small proteins, secreted by certain cells, that are involved in chemical signaling either on self-producing cells or other effector cells.

Organisation of the Study

The study is composed in a five-chapter thesis.

CHAPTER ONE: This includes background of the study, knowledge gaps, purpose of the study and research questions that needed to be addressed. Likely keywords encountered are Human Papillomavirus (HPV), microbiomes/microbiota, cell mediated immunity and cytokines.

CHAPTER TWO: This chapter looks at the epidemiological characteristics, global prevalence and all other relevant characteristics of HPV infection and cervical cancer. Various similar studies and reports published locally and around the world concerning this work are reviewed. Also, research knowledge gaps observed are emphasised.

CHAPTER THREE: The various materials and equipment, reagents and methods (for sampling and laboratory analysis) used to conduct the study are highlighted. There is a brief description of the study design, area and target population. Also, information on how data obtained is processed and analysed is stated.

CHAPTER FOUR: The results obtained are presented in tables and/or figures. This is followed by discussion of the results with emphasis on congruence or discrepancies of the data with previously existing data. The discussions are carefully made to address the objectives of the study.

CHAPTER FIVE: This final chapter summarises the background and findings of the result with concern on the purpose and objectives of the study. Finally, further recommendations are made based on certain important as well as lacking findings.

Chapter Summary

HPV infection, being the most common viral sexually transmitted infection, is implicated in the development of cervical cancer. Persistence of high-risk HPV infection leads to the development of the cancer. Recently, evolving evidence implicates the increased cervico-vaginal microbiomes diversity, dysbiosis, in TH1/TH2 imbalance. Thus, the study looked at the impact of cervico-vaginal microbiomes on circulating cytokines among women at risk of cervical cancer.

CHAPTER TWO

LITERATURE REVIEW

Chapter Introduction

This section considers the epidemiology of cervical cancer and HPV infection, natural history of HPV infection and its effect on the host's immune system, screening and diagnostic tools for the detection of the virus and diagnosis of the cancer and the roles of cervico-vaginal microbiomes and circulatory cytokines in the development of cervical intraepithelial lesions.

Introduction

Various studies and reports have concluded that Human Papillomavirus (HPV) is the most common etiologic agent (over 95%) of cervical cancer, even though HPV infects different anatomic sites, including, but not limited to, oral and respiratory cavities, and neck and anogenital organs (Katsenos & Becker, 2011; Pierce Campbell et al., 2016). The high-risk HPV genotypes have the potential of causing the development of malignant lesions. However, the mere presence of the virus does not lead to development of this pathology. Other behavioural factors- prolong hormonal contraceptives use, smoking, early age at first sex, family history and multiple sex partners and compromised immunity promote the carcinogenesis (D. Song et al., 2015).

Over the past 30 years, it has been established that persistent infection with high-risk HPV accounts for intraepithelial alteration of the squamo-columnar junction thus gradually leading to carcinoma (Castellsagué, 2008). Cervical cancer has been noted to be the predominant cancer among women of developing countries, and globally, it is the fourth (Arbyn et al., 2020).

Epidemiology of HPV and Cervical Cancer.

Among the causes of human death in the world, cancers rank second, especially in persons aged 5 years and older (Ritchie & Roser, 2020). The burden of gynaecological cancers among the cancer population is quite significant with cervical cancer being among the most commonly diagnosed in developing countries, even though it is preventable (Sankaranarayanan & Ferlay, 2006).

According to the 2020 World cancer report by International Agency of Research on Cancer (IARC), cervical cancer reigns as the fourth (4^{th}) common cancer diagnosed and causes death among adult females in the world (IARC, 2020). Persistent infection with Human Papillomavirus (HPV), especially the high risk- HPV types, has been implicated to cause over 90% of cervical cancer cases (Katsenos & Becker, 2011; Pierce Campbell et al., 2016). Also, among all the infectious agents associated with certain carcinogenesis, HPV is the second predominant agent causing almost 700, 000 global cases of cancer in 2018 (de Martel, Georges, Bray, Ferlay, & Clifford, 2020).

Most sexually active persons have been infected with the HPV virus at least once a period in their lifetime (Bosch & de Sanjosé, 2007; Bosch et al., 1995; Bosch, Qiao, & Castellsagué, 2006); commonly after debut of sexual activities. Thus, HPV infection is regarded as the most common sexually transmitted infection (Castellsagué, 2008; Schiffman & Castle, 2003). This is strongly backed with evidence from a number of longitudinal studies which detected HPV DNA among female participants, who were earlier confirmed to be virgins, after they were engaged in their first sexual relationships during the study period (Bosch et al., 2006; Krüger Kjaer et al., 2001).

The main behavioural determinants evinced to be implicated with HPV infection are early age at first sex and multiple number of lifetime sex partners (Bosch & de Sanjosé, 2007; Bosch et al., 2006; Krüger Kjaer et al., 2001). Similarly, the circumcision status of male partners has also been implicated with risk of HPV infection and transmission among males and to their female partners respectively; thus, circumcised males have less risk of HPV infection and transmission (Castellsagué et al., 2002). With the different HPV types existing (low-risk and high-risk types), sero-conversion in an infected person has been identified to be dependent on the type. In this regard, HPV 16 VLP is reported to seroconvert among participants who test positive for it (Castellsagué et al., 2002).

There are over 200 HPV types that have been identified via genetic characterization; with about 40 types been transmitted via direct sexual contact (Burd, 2003; Cubie, 2013). With at least 14 types considered as oncogenic- high risk HPV (hr-HPV) types, the rest are noted to cause benign conditions such as cutaneous and anogenital warts (Manga, Adeola, & Yahaya, 2019). The most common oncogenic HPV types that infects sexually active persons are HPV 16 and HPV 18 genotypes (Manga et al., 2019; Zampronha et al., 2013).

Currently, cervical cancer remains the leading cancer among the various reported cases on HPV caused-carcinomas, especially in the developing world (Lowy & Schiller, 2012; Parkin & Bray, 2006). Host factors such as alcoholism and smoking, genetics, reproductive factors in terms of multiple numbers of full-term pregnancies and early age at first delivery, hormonal impacts usually stimulated by prolong use of hormonal oral contraceptives are suggested to predispose women to the carcinogenesis (Deligeoroglou et al., 2013; Obiri-

Yeboah et al., 2017). Women living with HIV also have an increasing rate of cervical cancer development (Anorlu, 2008; French et al., 2009), which is due compromised immunity and the reality that majority of such victims are faced with several health access barriers apart from having inadequate knowledge about HPV and cervical cancer (Wong et al., 2018).

With over 570, 000 new cases and over 300, 000 deaths of cervical cancer reported globally, increasing incidence have been associated with low- and middle-income countries (de Martel et al., 2020). Thus currently, the disease is most common in Africa, especially in the Sub-Saharan region, and the South-East Asia; with mortality rate dramatically higher than those of the other parts of the world such as North America, West Asia, New Zealand and Australia (IARC, 2020).

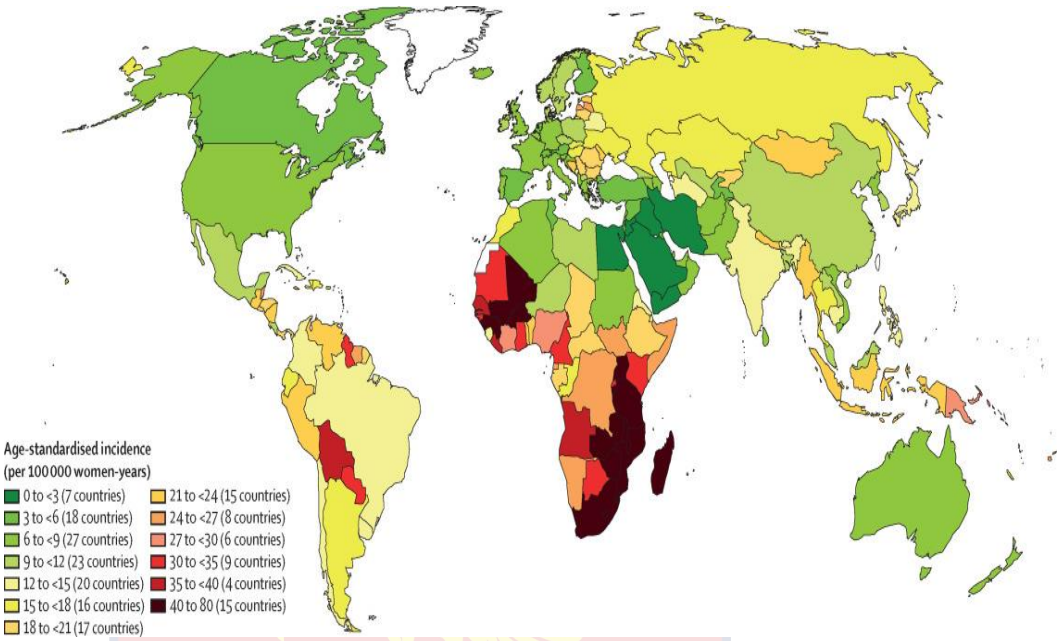

Figure 1: Global burden of cervical cancer in 2018

Source: (Arbyn et al., 2020)

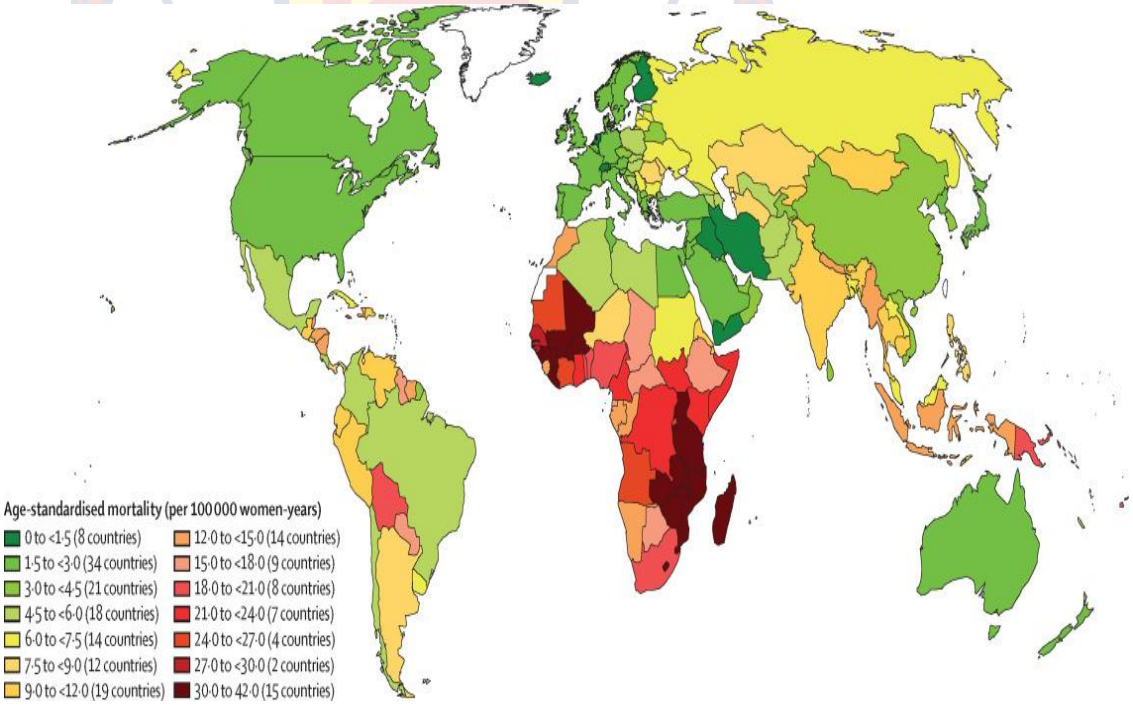

Figure 2: Global mortality rate of Cervical cancer in 2018

Source: (Arbyn et al., 2020)

In Africa, like most developing regions, most countries lack national screening policies for the detection of precursor lesions, monitoring and evaluation of cases (Denny & Anorlu, 2012); only some hand-picked nations like South Africa has a well instituted policy whereby women of age 30 and above have three (3) free screening opportunities in any testing public health facility with a period interval of 10 years (National Department of Health, 2017). The failures in the institution of such national policies have resulted in the shortfalls identified with the prevention and controlling of the disease in the burdened countries (Denny & Anorlu, 2012; Mboumba Bouassa, Prazuck, Lethu, Meye, & Bélec, 2017). In the Sub-Saharan region alone, over 75 000 new cases are recorded annually with about 50 000 deaths (Mboumba Bouassa et al., 2017). As at 2018, Malawi recorded the highest mortality rate (Arbyn et al., 2020).

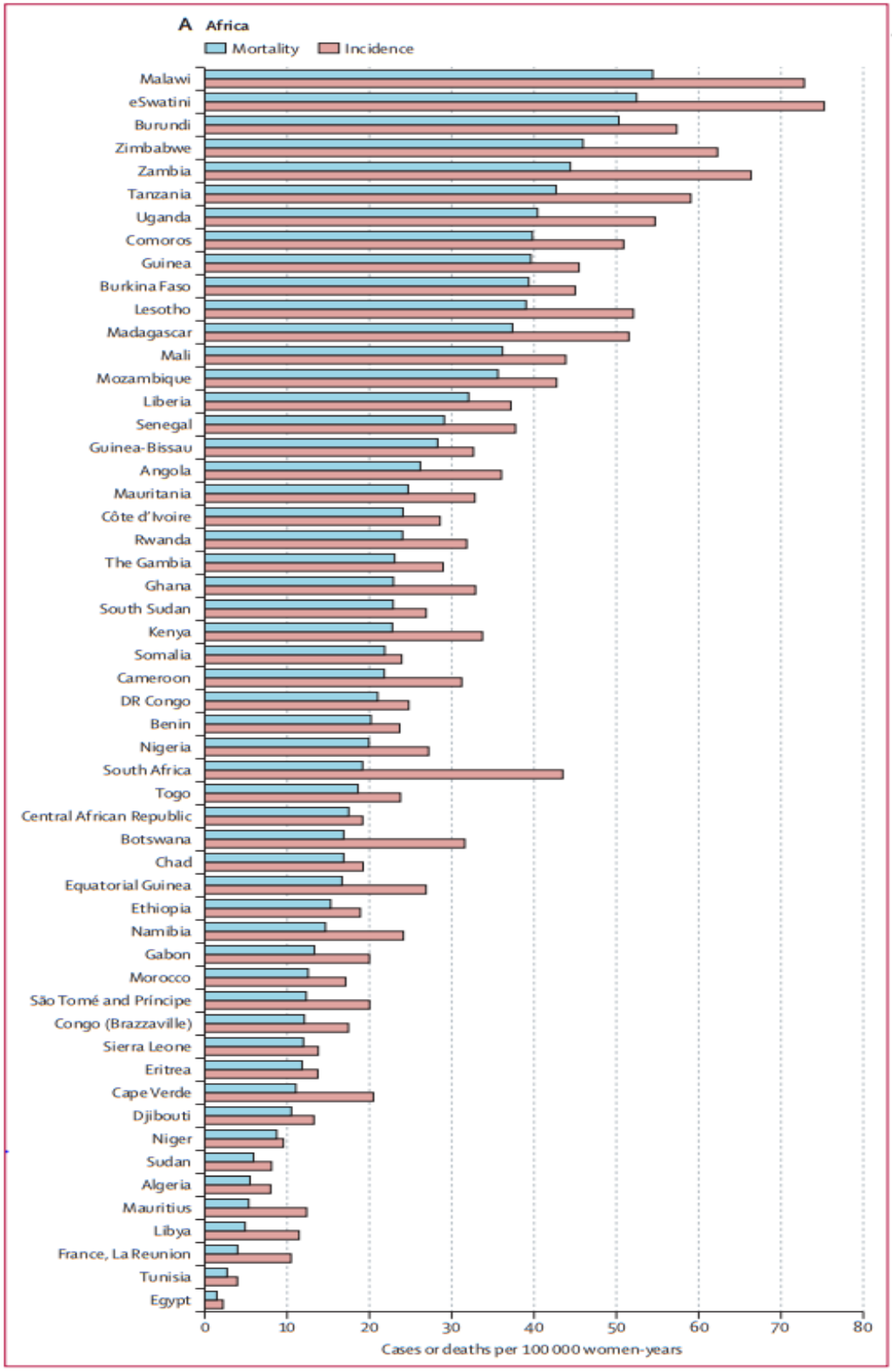

Figure 3: The Age-Standardised Mortality and Incidence rates of Cervical Cancer in Africa per 100 000 women.

Source: (Arbyn et al., 2020)

Overview on the Concept of Natural History of HPV Infection

Acceptable concepts available describes that the acquisition of HPV is via exposure to active sexual relations, not limiting it to penetrative sex but skin to skin contacts inclusive (Sabeena, Bhat, Kamath, & Arunkumar, 2017; Winer et al., 2003; World Health Organization, 2019). This has been evident with the detection of HPV in persons who had previously been identified not to be infected prior to the start of their active sex lives (Bosch & de Sanjosé, 2007; Bosch et al., 2006; Krüger Kjaer et al., 2001). It is suggested that women usually get initially infected with a specific HPV genotype within 1-3 years after sexual exposure and their possibility of been infected with other types of HPV occurs 6 months after initial infection (Malik, Hailpern, & Burk, 2009). Mostly, primary infection and re-infection are associated with involvement with new sexual partners. However, reactivation of earlier infection is possible in later life, usually after 45 years of age (Trottier et al., 2010).

After a successful HPV infection of the basal and metaplastic cells in the squamous epithelium and the squamo-columnal junction respectively, of the cervix, there is clearance of majority proportion (about 90%) of the virus within the first two (2) years of infection (Gravitt & Winer, 2017; D. Song et al., 2015). Among the population infected, only 10-15% of them experience infection persistence and a smaller proportion unfortunately exhibit detectable clinical complications of squamous intraepithelial lesions (D. Song et al., 2015; Westrich, Warren, & Pyeon, 2017). In most cases, a low grade squamous intraepithelial lesion (LSIL) resolves by the immune's intervention without treatment. In peculiar cases, other behavioural factors pre-dispose its

progression into high grade squamous intraepithelial lesions (HSIL) then finally invasive cancer (D. Song et al., 2015).

The early oncogenes, E6 and E7, are expressed by the virus to interrupt with the host's cells' function. The E6 oncogene inhibits the suppression of tumourigenesis via apoptosis by binding to and degrading Tumour protein 53 (p53) and E7 similarly inhibits the regulation of cell cycle by binding to Retinoblastoma protein (pRb) (Ault, 2006; Scott et al., 2001; D. Song et al., 2015; Westrich et al., 2017). Molecularly, the progression of HSIL into invasive carcinoma is facilitated by the integration of the HPV DNA into the host-cell chromosomes thereby initiating the proliferation of tumour cells (Ault, 2006; Scheurer, Tortolero-Luna, & Adler-Storthz, 2005).

Classification for squamous cell abnormalities, so far as cervical cytology is concerned, includes Atypical Squamous Cells of Undetermined Significance, Negative for squamous intraepithelial lesion, Low-Grade, and High-Grade Squamous Intraepithelial Lesion (Waxman, Chelmow, Darragh, Lawson, & Moscicki, 2012). At present, over 100 HPV subtypes have been proposed, of which about 35 are noted to reside in the genital tract of females (Bosch et al., 1995; Chan et al., 2002). The HPV have been categorized as a high-risk type (HPV 16 and -18) and a low-risk type (HPV 6 and -11) depending on their comparative risk of inducing cervical cancer (Munoz et al., 1992). The viruses are recognized for their ability to inhibit cell cycle repair through the coding of proteins which further interferes with cytoskeletal development. Moscicki, Grubbs Burt, Kanowitz, Darragh, and Shiboski (1999) in a study to elucidate the relevance of squamous metaplasia in the progression of a low grade squamous intraepithelial lesion suggested that, the activities of the HPV

including it replication and transcription may be supported by the pathogenesis of squamous metaplasia, that is, the presence of an active squamous metaplasia may be a determinant factor for the transformation of a low grade squamous intraepithelial lesion (Moscicki et al., 1999). Further, the persistence of the low-grade intraepithelial lesion has been recorded to be significantly associated with the high risk for a high grade squamous intraepithelial lesion (Moscicki et al., 2004). Considering the significance of high-risk HPV tenacity on the pathogenesis of cervical cancer, it is extremely essential to initiate related reviews on the myriad factors with potential role in influencing HPV induced cervical cancer (Godoy-Vitorino et al., 2018).

Overview on Immune Response to HPV Infection

The HPV-infection-induced cancer usually does not involve the release of pro-inflammatory or anti-inflammatory cytokines due to the absence of necrosis, cytolysis and or inflammation (Karim et al., 2011). The growth and replication of the infection takes place in intraepithelial cells earmarked for death by detachment. However, the immune system does not generate sufficient attack for the removal of the infection possibly because there exist no threat signals to induce the immune system (Roberts et al., 2007). Sasagawa, Takagi, and Makinoda (2012) extrapolated that, about 80% of all HPV-induced cervical cancer infections are eliminated by the cells of the innate immunity such as dendritic cells, Langerhans cells, macrophages, keratinocytes and natural killer cells through the stimulation of a pro-inflammatory process (Sasagawa et al., 2012). The innate host of the HPV infection occurring in the cervix is the keratinocytes (immune sentinels). These cells are characterized by the

expression of toll-like receptors (TLR), particularly TLR-1, TLR-2, TLR-4, TLR-5 and TLR-6 all representing cell surface receptors and TLR-3 and TLR-9 in endosomes. The activation of TLR-9 specifically results in a subsequent generation of type-1 cytokines (interferons) thereby activating the natural killer cells (Grandvaux, Servant, & Hiscott, 2002), responsible for the elimination of HPV infected cells (Koch, Steinle, Watzl, & Mandelboim, 2013). In other words, the reduction of the natural killer cells resulting from the down-regulation of the type-1 cytokines (interferons) will ultimately give way for the development of persistent HPV infection with subsequent progression into cervical cancer.

The adaptive immune system (second line of defense) is able to get rid of HPV infected cells through cytotoxic T lymphocytes targeting the E2 and E6 oncoprotein of HPV-16 (Sasagawa et al., 2012). Plasma cells resulting from the differentiation of B cells generate HPV antibodies capable of reacting with the antigens of HPV upon infection. The reaction is strengthened through an indirect binding of the B cells with cytokines (IL-4, IL-5 and IL-6) (Sasagawa et al., 2012). Stanley (2009) indicated that, majority of HPV-16 infected women expressed HPV-16, E4, E6, E7 and L1 antibodies in their sera and further studies reported similar antibodies in about 70% HPV infected patients. In HPV natural infection, averagely, only about 60% of infected women produce specific neutralizing antibodies against L1 protein of the virus (Mollers et al., 2013; Scherpenisse et al., 2013). Until recently, there was no certainty that these specific antibodies produced during primary natural infection offer similar protection in subsequent infections and cross-protection to phylogenetically related HPV genotypes (Ho et al., 2002; Malik et al., 2009; Palmroth et al., 2010;

Scherpenisse et al., 2013). These species-specific neutralizing antibodies produced usually inhibit the viral integration into the host genome by preventing internalization into the target cells (Hamsikova, Ludvikova, Stasikova, & Tachezy, 2013). Malik et al. (2009) and Ho et al. (2002) demonstrated that species-specific antibodies produced in natural infection reduces the risk of infection to the HPV type and phylogenetically related types. Further suggestion revealed that, possibly, antibodies to L2 protein are also produced in natural infection which may offer cross-protection to unrelated HPV types (Day, Gambhira, Roden, Lowy, & Schiller, 2008; Malik et al., 2009). Contrary, studies such as that of Viscidi et al. (2004) observed that species specific antibodies produced against HPV types have no association with reduced risks of incident infections with the HPV type and its related types. The reason for this finding could be due to wide duration of follow up after initial infection. It has been observed that follow up after more than 12 months reduces the seropositivity due to lack of persistence of antigen exposure which is likely to occur after "viral clearance" (Ho, Studentsov, Bierman, & Burk, 2004; Malik et al., 2009).

However, the levels of species-specific antibodies produced in natural infection are significantly lower (about seven folds) compared to that produced after vaccination. Interestingly, no standard antibody titers have been established to confer sufficient protection against HPV infections. Currently, most researchers rely on titres estimated from non-HPV infected women as controls to enable statistical association establishment. Also, as much as the specific neutralizing antibodies produced in natural infection have the tendency for significant protection, the avidity of the IgG antibodies is lower compared to that of the IgG antibodies post-vaccination (Scherpenisse et al., 2013). This likely

explains the better prevention of re-infection in specific HPV types and its related types in immunized women since humoral immune response to HPV infections has been found to delay on onset (Mollers et al., 2013; Pierce Campbell et al., 2016).

Knowledge, Awareness and Attitude towards HPV Infection and Cervical Cancer.

Globally, Human Papillomavirus (HPV) induced cervical cancer is extrapolated to cause about 275, 000 deaths with over 530, 000 new cases, therefore representing the fourth most prevalently detected cancers among females (Assoumou et al., 2015). Majorly, HPV vaccination plus screening remains the two most widely adopted preventive measures for HPV-induced cervical cancer (Kane, Sherris, Coursaget, Aguado, & Cutts, 2006). Controlled screening initiated in developed countries was effective in minimizing the prevalence of cervical cancer infection via early discovery and subsequent treatment (Chakkalakal et al., 2013; WHO, 2014). Yet, most developing countries are unable to commence regular screening for cervical cancer infection owing to myriad factors such as inadequate health workers, unavailability of health infrastructure as well as misplaced health policy priorities (Denny, Quinn, & Sankaranarayanan, 2006; Herrero et al., 2005; Munoz et al., 1992). Also, the establishment of regular and accessible HPV vaccination programmes, a procedure capable of easing the dangers of HPV infection among vulnerable population, have been hindered in almost all developing countriess due to high cost of the vaccines (Clendinen, Zhang, Warburton, & Light, 2016; Herlihy, Hutubessy, & Jit, 2016). The current prophylactic vaccines available for HPV

infection prevention are Cervarix (GlaxoSmithKline) – for HPV 16 and 18-, Gardasil and Gardasil-9 (**Merck** Sharp and Dohme) – for HPV genotypes 16, 18, 11, 6, 31, 33, 45, 52 and HPV 58- which are composed of synthetically made *Virus-like-particles* (VLP) of the L1 epitope. Gardasil-9 protects against all the nine genotypes while Gardasil protects against HPV genotypes 16, 18, 6 and HPV 11 (Angioli et al., 2016; Harper & DeMars, 2017).

Assessing and understanding the knowledge that a population has vis-à-vis cervical cancer and the HPV infection is very essential to organizing awareness schemes to bridge gaps towards the awareness of the infection and its ultimate sequelae, cervical cancer. Individual knowledge and understanding regarding cervical cancer and HPV play an influential role in their attitude and decision making against the acceptability of vaccines, as well as other relevant preventive methods (Baloch et al., 2017; Ning et al., 2019). It has been noted that one of the factors amidst the obvious ones affecting increased cervical cancer incidence and mortality rate in low- and middle-income countries is the lack of knowledge and awareness of the infection and cervical cancer (WHO, 2014).

A number of studies have demonstrated varied levels of awareness and knowledge of HPV infection and cervical cancer among women. Generally, it was observed that mostly adult women were more aware of cervical cancer than HPV infection and availability of HPV vaccines (Baloch et al., 2017; Bhuiyan, Sultana, Islam, Chowdhury, & Nahar, 2018; S. H. McCarthy et al., 2017; Ning et al., 2019; Touch & Oh, 2018). Factors that influence knowledge and awareness of cervical cancer and HPV infection includes economic strength of a nation, educational background, family history of any cancer, attitude and

cultural beliefs of women in a particular geographical area and regular pap testing (Assoumou et al., 2015; Baloch et al., 2017; Ebu et al., 2014; He & He, 2018; Ning et al., 2019; Obiri-Yeboah et al., 2017). Also, in relation to HPV vaccines, most women are willing to accept the available vaccines (He & He, 2018) whiles some women are concerned about the safety and efficacy of the vaccines (Ning et al., 2019). Interestingly, it has been reported that men associate HPV infection in their female partners as confirmation of infidelity (Fernandez et al., 2009)

To improve on knowledge and awareness of HPV infection and cervical cancer is a key factor towards the prevention of HPV infection and elimination of cervical cancer. This can widely be achieved through enhanced education strategized to meet the socio-demographic and socio-economic characteristics of a defined population. A number of studies have revealed gaps in knowledge and awareness of HPV infection and cervical cancer between the rural and urban population (Mohammed et al., 2018; Shabani et al., 2019). Thus, due to significant association among varied population and ethnic groups, Baloch et al. (2017) proposed that a community-based education is to be established to bridge the knowledge gabs at the community and individual level.

Association between Cervico-vaginal Microbiomes, HPV Infection and Cervical Cancer

The relationship between cervico-vaginal microbiome and immune modulation has become a very important direction of study; especially with its role in genital inflammation which directly affect the female reproductive health in terms of susceptibility to infections such as HPV and HIV (Anahtar et al.,

2015; Anahtar, Gootenberg, Mitchell, & Kwon, 2018; Gopinath & Iwasaki, 2015; Reimers et al., 2016). A balanced microbiome and a healthy genital impact whereby the microbes available have more co-operative relationship – mutualism and commensalism- is known as eubiosis whiles the opposite, which causes parasitism, is dysbiosis (Kalia, Singh, & Kaur, 2020). The occurrence of dysbiosis and microbiome diversity have been associated with recurring HPV infection and its progression into invasive cancer (Kyrgiou et al., 2017; Usyk et al., 2020). Now two proposals exist for bacterial-viral interactions in human host; generation of microbiota bioproducts that could promote virus-host interactions and possible host gene expression alteration influenced by bacteria-host interactions that stimulate tumorigenesis coupled with viral infections (Curty et al., 2019; Mitra et al., 2016).

In earlier studies, it has been demonstrated that HPV clearance is associated with eubiosis in terms of abundance of *Lactobacillus spp.*; and persisting viral infection is associated with high microbiome diversity with dybiosis whereby Bacterial vaginosis caused by *Gardenerella vaginalis* has been implicated (Gao, Weng, Gao, & Chen, 2013; Kwasniewski et al., 2018; Usyk et al., 2020).

Specific bacterial species have been understudied to identify their role in viral persistence and disease progression. Aside the implication of anaerobic bacteria-caused dysbiosis on poor prognosis of HPV infection, viral persistence and disease progression (Kwasniewski et al., 2018), other studies consider the presence of certain *Mycoplasma spp*. as a relevant risk for cervical dysplasia and carcinogenesis (Adebamowo et al., 2017; Klein et al., 2019; Klein et al., 2020a; Klein et al., 2020b) mainly via efficient methylation of Hr-HPV and cervical

somatic cells causing chromosomal alterations (Adebamowo et al., 2017). In sub-Saharan Africa, as the burden of HIV continues to increase it directly translates into the increased burden of HPV infection and HPV-induced cancers. In this regard, more common than in other regions, intracellular bacterial infections with Chlamydia and *Mycoplasma spp.*, especially *M. hominis*, are frequent suggesting the role of these microbes in cervicovaginal dysbiosis and its impact on the female reproductive health, in terms of disease prognosis (Adebamowo et al., 2017; Klein et al., 2019; Klein et al., 2020a; Klein et al., 2020b).

The synergistic influence of the cervical microbiota and HPV on the risk and development of cervical intraepithelial neoplasma reveals that, bacteria dysbiosis coupled with HPV infection may be considered as predetermining factors for cervical cancer (Oh et al., 2015).

Cytokines Profile of Women with HPV Infection and/or at varied grades of Squamous Intraepithelial Lesion

It is well-documented that the HPV plays a crucial role in the development and subsequent progression of cervical cancer (Giannini et al., 1998; WHO, 2014). The primary immunological defense against sexually transmitted microorganisms remains the vagina and the cervix. The presence of pathogens triggers for immune response resulting in the secretion of increased amount of the immune stimulating molecules (A. C. C. Campos, Murta, Michelin, & Reis, 2012). The innate and acquired immune response (immune environment of the cervix) exhibit a significant role in the fight and elimination of the HPV (Gutierrez-Xicotencatl et al., 2016). Thus, the development of HPV-

induced cervical cancer is perhaps the results of escape of the immune microenvironment of the cervico-vagina by the HPV virus. Previous studies have documented the relevant role exhibited by T cells in preventing HPV-induced cervical cancer (Barros et al., 2018b; Ram et al., 2018). The presence of HPV causes a TH1/TH2 cytokines imbalance in the levels of cytokines in the cervix; where the implication is due to an upsurge of levels of T helper-2 related cytokines - e.g., IL-4, IL-6, IL- 10 and IL-13- than T helper-1 related cytokines - e.g., IL-2, TNF α and IFN γ (Barros et al., 2018a; Li et al., 2019; Scott et al., 2001; Xu et al., 2009). Generally, it has been observed that, localized cytokines levels measured from cervical secretions tend to have similar trend with systemic cytokines levels; however, the former results in higher volume as compared to the latter (Ali et al., 2012; Mbulaiteye et al., 2013).

In Japan, Azar et al. (2004) estimated the levels of TNF-α, IFN-γ, IL-6 and IL-10 among women with and without cervical dysplasia using enzyme linked immunosorbent assay (ELISA). In the study, TNF-α was statistically increased among participants with HSIL as compared to participants with normal cytology or HPV negative. Similarly, IL-10 was statistically increased with participants with LSIL as compared to those with normal cytology and HPV negative. Additionally, it was observed that IL-6 and IL-10 had negative correlation with participants with normal cytology and LSIL; an increase in IL-10 suggested possible immune response inhibition in early cervical lesions. Also, increased levels of both TH1(especially TNF-α) and TH2 cytokines levels among participants with HSIL suggests impaired immune response in advanced stages of cervical lesions. Finally, it was concluded that, IL-10 and TNF-α in the cervical secretion may be an important indicator of the local immune response

as well as the phase or level of the cervical lesion induced by the HPV infection (Azar et al., 2004).

Again, in Costa Rica, Kemp et al. (2010) estimated the levels of 24 circulating cytokines among women with persistent HPV infection older than 45 years using multiplexed bead-based immunoarrays and ELISA. It was determined that the levels of IL-6, IL-8, TNF α, macrophage inflammatory protein-1α (MIP-1α), granulocyte macrophage colony-stimulating factor (GM-CSF), IL-1α and IL-1β were increased among participants with persistent HPV infection. However, the increased levels observed with IL-6, IL-8, TNF α and MIP-1α were of very high statistical significance (>10 high fold changes) (Kemp et al., 2010). In another study in Nigeria, Mbulaiteye et al. (2013) examined the systemic imbalance of TH1/TH2 cytokine levels in women in a rural setting. The circulating levels of different cytokines including TH1 related cytokines- IL-2, IL-12, TNF-α, IFN-γ; TH2 related cytokines - IL-4, IL-5, IL-6, IL-10 and IL-13; and other classes of cytokines such as innate/inflammation cytokines and cell development cytokines. With the analysis, levels of Eotaxin and TNF-α were significantly lower among women with HPV infection. Also, stratified by age, women of 35 years and older significantly had a reduced level of eotaxin and TNF-α (Mbulaiteye et al., 2013). In this study, no cytology was done to identify the role of cervical inflammation on the cytokine profile. Thence, Mbulaiteye et al. (2013) concluded that cervical HPV infection have no correlation with systemic cytokines imbalance in rural women in Nigeria, however, eotaxin and TNF-α have inverse association among women of 35years and older.

Ali et al. (2012) assessed immune response in women with different grades of cervical lesions caused by HPV infection and observed that IL-10 levels were significantly increased in cervical secretions and serum of women with HPV positive cervical lesions. Contrary, TNF-α showed no significant difference between participants with and without lesions. Ali et al. (2012) then concluded that increased levels of IL-10 than TNF-α demonstrates possible reduced tumour-specific immune responses to HPV infected lesions which provides more suitable microenvironment of the progression of the lesions into carcinoma.

To evaluate the cytokine profile of women living with HIV and having cervical dysplasia due to HPV infection, Li et al. (2019) utilized cytometric bead array to examine the levels of cytokines in the vaginal fluid whilst the performance of the cytokines score for the purposes of risk assessment and diagnosis were compared using the ThinPrep cytology tests. It was revealed that, increased levels of interleukin-6 were significantly associated with the severity of the HPV-induced cervical dysplasia and interleukin-2 was inversely related which favours the progression of cervical dysplasia to cervical cancer (Li et al., 2019).

Cervico-vaginal Microbiomes and Cytokines Profile at varied grades of Squamous Intraepithelial Lesion

A. C. C. Campos et al. (2012) compared the cytokine levels in endocervical secretions among women with HPV infections and other clinically significant cervico-vaginal microbiomes. In their findings, increased vaginal pH (>4.5) was associated with HPV infection and Bacterial vaginosis (BV) was

majorly (over 80%) caused by *Gardenerella vaginalis* and other anaerobic bacteria. Using ELISA, IL-2 and IL-12 cytokine levels were found to be elevated among participants with Bacterial vaginosis and HPV infection, IL-6 was elevated among participants with BV only and IL-2 and IFN-γ levels were also highly elevated among participants with HPV infection. The study observed that BV and HPV positive participants had a TH1 cytokines immune response (A. C. C. Campos et al., 2012).

Similarly, in Mexico, a study was done to evaluate the localised cytokine expressions- IL-4, IL-6, IL-10, TGF-β1, TNF-α and IFN-γ- at various stages of cervical lesions and with specific bacterial clusters (Audirac-Chalifour et al., 2016). In the study, cervical microbiota was determined using high throughput sequencing of 16S rDNA amplicons classified under community state types (CST) and after both alpha-diversity and beta-diversity mean difference analyses between and within histopathological diagnosis respectively. It was observed that there was a significant difference in microbiota's diversity among HPV-participants with no cervical lesion against women with squamous intraepithelial lesions and cervical cancer; and also, participants with cervical cancer had the most variation of microbiota within groups as compared to the control group. The predominant bacteria in women with normal cytology were *Lactobacillus spp*. (*L. crispatus* and *L. iners*), that for participants with SIL was *Sneathia spp*. and that for participants with Cervical Cancer was *Fusobacterium* spp. Finally with the cervical cytokines' estimation, there was higher median levels of IL-4 and TGF-β1mRNA in the CST dominated by *Fusobacterium spp* (Audirac-Chalifour et al., 2016). Audirac-Chalifour et al. (2016) suggested that the cervical microbiota could possibly be implicated in cervical cancer pathology.

Diagnostic Tools for Cervical HPV detection and Cervical Cancer diagnosis

The screening and diagnosis of Cervical cancer has over the years involved the use of the conventional staining protocol of fixed cervical smears following the Papanicolaou staining procedure (thus the test referred to as P ap Smear test) and or liquid based cytology; visual inspection with Acetic acid (widely used) or Lugol's iodine (VIA/VILI) and the detection of HPV and its types via various molecular techniques (Ferlay et al., 2018). The former is essential for the early detection of precancerous and cancerous squamous intraepithelial lesions whilst the later considers either the detection of HPV or the identification of the specific genotypes of HPV (hr-HPV types and lr-HPV types) (Ferlay et al., 2018; WHO, 2014).

The widely used Pap smear test is known for its limitation of low sensitivity (51%) especially on the diagnosis of Carcinoma Intraepithelial Neoplasia II (CIN2+) but a higher specificity (98%) which commonly demonstrates inadequate specimen sufficiency and misinterpretations of results (Boone, Erickson, & Huh, 2012; Nanda et al., 2000). Presently, the standardized technique for the identification of HPV involves the molecular detection of the nucleic acid (DNA or mRNA) of the HPV (Cuschieri, Whitley, & Cubie, 2004; Molijn, Kleter, Quint, & van Doorn, 2005; Tsikouras et al., 2016). The molecular detection of HPV nucleic acid, specifically DNA, makes use of different classes of molecular assays comprising non-amplified hybridization assays, southern transfer hybridization (STH) assay, signal amplified hybridization assay (hybrid capture assays), and target amplification assays

(polymerase chain reaction [PCR] and *in situ* PCR dot blot hybridization [DB] assay) (Tsikouras et al., 2016).

The use of DNA and RNA base assay (example, Cervista HPV High Risk test, Digene Hybrid Capture 2 High-Risk HPV DNA test, and the RNA-based Aptima® HPV assay) for the detection and identification of HPV induced infection has widely been approved (Tsikouras et al., 2016). These signal amplified hybridization assay tests are extremely sensitive and cost-effective technique for the detection of HPV; however, the method qualitatively detects targeted pooled HPV types, hence lacks specificity (Lie & Kristensen, 2008).

The *in-situ* hybridization (ISH) technique for HPV detection unlike other robust molecular diagnostic methods allows for the detection of HPV specifically within the infected cells nuclei. This allows for both microscopic examination of the nuclei of the infected cells and physical evaluation of the virus through punctuate signals. The combination of PCR with *in situ* hybridization (PISH) has proven to be more powerful for HPV detection rate in cervical cancer and squamous intraepithelial lesion relative to the use of only *in situ* hybridization (ISH) method. The signal amplification method, initially employing hybridization procedure present with an incapability localization of HPV to a particular histologic area of interest (Venuti & Paolini, 2012).

The present challenge facing scientist and or healthcare workers in clinical medicine and cytopathology remains the early and precise detection of advanced squamous intraepithelial lesions and diagnosis of HPV-induced cervical cancer, considering the preponderance and mortality rate of the infection. There has been a present advancement in understanding the pathogenesis of the cervical cancer infection followed by a more precise way of

developing reliable diagnostic procedures using DNA microarrays (Dijkstra et al., 2014). Further experimental research has disclosed a number of genes expressed in patients with cervical cancer infection, representing a potential breakthrough in the detection and diagnosis of cervical cancer infection (Malinowski, 2005). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and HPV microarray technique were adopted by Hwang et al. (2003) to clinically ascertain the efficacy of HPV oligonucleotide microarray for the detection and diagnosis of HPV in cervical lesion. HPV DNA samples were obtained from 234 women exhibiting abnormal cytology in Pap smear. Findings from the study analysis revealed that, the detection of HPV DNA was successful with both PCR-RFLP and HPV microarray. The authors reported a strong correlation (kappa index > 0.65) for the detection of varied types of HPV (HPV-16,18,31,33,35,52,58). It was suggested that HPV microarray had a detection function akin to that of the already existing technique (PCR-RFLP) (Hwang et al., 2003). An antibody-based immunochemistry technique has been employed for the detection of cervical cancer. This was achieved through the use of DNA markers such as the family of minichromosome maintenance proteins (MCM) subtypes 2, 5,6 and 7 and an additional marker called the topoisomerase II-alpha. Cervical cancer infection from HPV results in the overexpression of the aforementioned protein markers *via* E2F transcription factor pathway. An antibody to the MCM class of proteins and topoisomerase II-alpha aids in the detection of HPV-infected cells and cervical cancer infection (Malinowski, 2005).

A cohort study comprising over 12000 women was performed to explore the performance features of different cervical diagnostic tool viz pap smear test,

Visual inspection with Acetic acid (VIA), visual inspection with iodine solution (VILI), HPV test, cervicography and colposcopy. The sensitivity level among the diagnostic technique was recorded in the order of increased sensitivity with colposcopy through to HPV testing, to VIA to VILI and finally to cervicography. Longatto-Filho et al. (2012) noted that, the combination of molecular test and pap smear test has proven efficient to advance the detection of high grade squamous intraepithelial lesion (Longatto-Filho et al., 2012). HPV DNA chip according to Park et al. (2004) may be as sensitive as other robust molecular diagnostic technique for the detection of HPV induced cervical cancer and additionally reveals the genotype of the HPV. The use of Matrix assisted laser desorption/ionization imaging coupled with mass spectrometry has also proven advantageous as a diagnostic tool for routine cervical cytology specimen (Schwamborn, Krieg, Uhlig, Ikenberg, & Wellmann, 2011).

CHAPTER THREE

RESEARCH METHODS

Chapter Introduction

This was a cross-sectional study, which employed convenient sampling technique to select participants. Cervical and blood specimens were taken for Pap smear, culture and cytokines estimation. Culture and identification of micro-organisms of significant growth were done, using aseptic techniques. Detailed procedure on the cytokine estimation is described. Finally, a brief description on the processing and analysis of the data obtained is given.

Study Design:

This was a cross-sectional study that assessed the expressions of circulatory inflammatory cytokines in participants with dysbiosis, who were predisposed to cervical cancer.

Study Site

This study was conducted in the Akyemansa Sub-district in the Eastern Region of Ghana. The district, which includes previously mining community-Adjobue, is located close to Akim Oda and New Abirem (which are great commercial and mining towns respectively). The district lies on longitude 1^0 10W and 1^0 0E. Over 60% of the population are within rural settlements and the main occupations of the inhabitants are farming and trading.

The Akyemansa Sub-district has a total population of 97,374, according to the 2010 Housing and Population Census. The female population is about 49, 370, representing 50.7% of the population. The district comprises 96

communities, but four (4) communities were conveniently selected—Abenase, Adjobue, Akyemansa, and Mukyia – for the study.

Ethical Clearance

Research approval was obtained from the Institutional Review Board of UCC (UCCIRB/CHAS/2019/30). Approval was also sought from the leaders of the communities prior to the start of the study. Informed consent was also sought from participants after thorough explanation of the study objectives to them. Participants who could not read or write were guided through the consent form and then endorsed by thumb printing in the presence of a neutral person. The anonymity of the participants was conserved.

Patients Selection and Sample Size

Population and Sampling

Female participants within the Akyemansa sub-district, specifically, Abenase, Adjobue, Akyekrom, and Mukyia communities, were considered for the study. Community-entry was done in each community by visiting the leaders of the respective communities and informing them of the aim of the study. Formal announcement was done in each community, with the help of the town-crier at their respective Information Centres. House-to-house visits were done to obtain data from available females on prior knowledge of HPV infection and cervical cancer and also to educate them on the infection and disease. Thus, all females were given the opportunity to take part in the study. Specific dates were scheduled for sample collection in each community.

Sample size calculation

Using the Cochran's formular and a prevalence of 7.51% from an earlier study done in Ghana (Obiri-Yeboah et al., 2017), the sample size was calculated. The calculated sample size was further increased by 40% to account for possible contingencies such as withdraw and non-responsiveness.

$$N = \frac{Z^2pq}{e^2} = 107 \text{ participants.}$$

where Z = 1.96; e= margin of error; p= proportion of affected population and q= 1-p.

After adjustment, N = 150 participants

Sampling technique

A total of 274 female participants were selected, however, 157 of them consented to be sampled thus were enrolled. Convenient sampling technique was employed to recruit the participants. House-to-house visits were done within the communities. Participants were educated on HPV infection and cervical cancer and given the opportunity to participate in the sample taking. Informed consent was obtained, afterwards, from participants, who agreed to take part in the study. Subsequently, each participant was assisted to answer the closed-ended questions on the questionnaire.

Inclusion criteria

Women of 20 years of age and above (≥20 years), who had history of active sexual life or were living in one, as at the time of the study, were included.

Exclusion criteria

Participants who have undergone or undergoing chemotherapy, those who had previous total abdominal hysterectomy, were menstruating on the day of sampling, or were pregnant were excluded from the study.

Sample and Data Collection Procedure.

Questionnaire

A closed ended questionnaire was used to obtain demographic and socio-economic data of participants; and other risky behavioural factors such as smoking and alcoholism. Also, data on awareness and knowledge of HPV infection and cervical cancer, and other relevant information were obtained.

Cervical swab collection

Three (3) specially trained midwives assisted in gynaecological examinations with speculum, during which cervical swabs were collected from the ecto and endocervix of participants, targeting the squamo-columnar junction using Ayre's spatula. Cervical smears were produced by smearing the sample from the spatula on a glass slide, then fixing it with absolute alcohol immediately. The slides were then packaged for cytological examination.

Also, two (2) High vaginal swab (HVS) samples (A and B) were also taken from each participant with the aid of cotton swabs. Both swabs were kept on ice and transported to the laboratory, with one swab (sample B) each kept in Peptone water as a transport medium. Sample B was then stored at 4-8 ^{o}C for seven (7) days. The other swabs were prepared for wet prep and gram staining.

Blood collection

Three (3) milliliters of venous blood samples were collected by a trained phlebotomist from participants into serum separation tubes (SST). The samples were then centrifuged at 400 rpm for 15 minutes after which the serum was aliquoted into 1.5 ml Eppendorf tubes and kept at -20 ^{o}C.

Laboratory Analysis

Cervical cytology

Cervical smears were prepared, from the cervical swabs, and stained in the Korle-Bu Teaching Hospital (KBTH) Histopathology laboratory following a standardized protocol for Papanicolaou (Pap) staining. Slides were read by a Consultant Cytopathologist using the Bethesda System (TBS) 2014 guidelines for SIL classification.

Briefly, the Papanicolaou staining protocol comprises the use of Haematoxylin and two (2) counterstains, Orange-Green (OG-6) and Eosin Azure (EA)-50. The fixed and uniquely labeled slides were put in 90% ethanol for 15 minutes to ensure absolute fixation. Then, the slides were put in 80% alcohol for 2 minutes followed by a dip in 60% alcohol too for 2 minutes. The slides were then dipped in two changes of distilled water five (5) times each. Afterwards, the slides were stained with Haematoxylin for 2 minutes after which they were rinsed in running tap water for bluing. The smears were dehydrated by introducing the slides to increasing percentages of alcohol for 2 minute each- 60 % ethanol, 2 changes of 80% ethanol and finally 95% ethanol. Next, the smears were introduced to the first (1st) counterstain, OG-6, for 90 seconds. The slides were then dipped into 95 % ethanol ten (10) times again. The smears were

subsequently introduced to the second (2^{nd}) counterstain, EA-50 for 150 seconds ($2\frac{1}{2}$ minutes). Afterwards, the slides were dipped 10 times into two (2) changes of 95 % ethanol, and then kept in 100% ethanol for 1 minute. Following this, the slides were cleared in two (2) changes of Xylene for 2 minutes each.

Finally, the slides were mounted with DPX (a mixture of Distyrene, Plasticizer and Xylene) and carefully cover slipped without trapping air bubbles with 22x50 mm glass cover slips.

Identification of yeast-like cells and clue cells.

Moderate normal saline (about 1 ml) was added to each one of the HVS samples and gently agitated to ensure mixing of the discharge with the normal saline. A drop each of the suspension was put on two microscope slides. A wet prep was done by putting a coverslip on one of the slides and mounting it on the light microscope with magnification of x400 and observed for the presence of budded yeast cells and motile trichomonas and clue cells. The other slide was heat fixed and gram stained. The stained smear was observed under oil immersion objective lens for the presence of clue cells, budded yeast cells and other bacteria.

The diagnosis of Candidiasis was established by the presence of greater than five (>5) budded yeast cells in the Wet prep and the Gram-stained smear. The diagnosis of Bacterial Vaginosis was also established using two out of the four standard clinical criteria - the presence of ‘clue cells’, vaginal discharge, pH >4.5 and positive amine test (A. C. C. Campos et al., 2012). In this study, two of the criteria were used- vaginal discharge and the presence of clue cells in wet prep, Gram-stained smears and Pap smears.

The other HVS samples were cultured on MacConkey, Blood and Chocolate agars and incubated aerobically at 37°C for 24 hours. The bacteria isolates were identified using various biochemical tests such Catalase, Coagulase, Triple Sugar Iron (TSI), Indole, Citrate and Urease tests.

Culture, Isolation and Identification

Preparation of Media for 85 plates.

MacConkey Agar: 88.40 g of the agar powder (Techno Pharmchem, India) was weighed with a beam balance (ADAM® PGW 453e) into two (2) 1 litre bottles containing 500 ml of distilled water each- 52 g in a 1 litre bottle and 36.40 g in the other bottle. The laboratory bottles are then filled to 1000 ml and 700 ml respectively and mixed well by swirling the bottle carefully. The bottles and media were then sterilised by autoclaving them in an autoclave at 121°C for 15 minutes. The media were allowed to cool to about 40°C until they were poured into sterile disposable Petri dishes.

5 % Sheep Blood Agar: 47.6 g of Nutrient Agar (Biomark Laboratories, India) was weighed with a beam balance (ADAM® PGW 453e) into two (2) 1 litre bottles containing 500 ml of distilled water each- 28 g in a 1 litre bottle and 19.60 g in the other bottle. The laboratory bottles are then filled to 1000 ml and 700 ml respectively and mixed well by swirling the bottle carefully. The bottles and media were then sterilised by autoclaving them in an autoclave at 121°C for 15 minutes. Then the bottles were kept in a water bath till the temperature fell to 50°C and 85 ml (5%) of the molten agar was sterilely taken from the total volume of the Nutrient agar base. Then a total of 85 ml of Sheep blood was added to the remaining Nutrient Agar base and gently swirled to ensure uniform

mixture. The media were allowed to cool to about 40°C until they were poured into sterile disposable Petri dishes.

Chocolate Agar: 47.6 g of Nutrient Agar (Biomark Laboratories, India) was weighed with a beam balance (ADAM® PGW 453e) into two (2) 1 litre bottles containing 500 ml of distilled water each- 28 g in a 1 litre bottle and 19.60 g in the other bottle. The laboratory bottles are then filled to 1000 ml and 700 ml respectively and mixed well by swirling the bottle carefully. The bottles and media were then sterilised by autoclaving them in an autoclave at 121°C for 15 minutes. Then the bottles were kept in a water bath till the temperature fell to 80°C and 85 ml (5%) of the molten agar was sterilely taken from the total volume of the Nutrient agar base. Then a total of 85 ml of sheep blood was added to the remaining Nutrient Agar base and gently swirled to ensure uniform mixture. The media were allowed to cool to about 40°C until they were poured into sterile disposable petri dishes.

Plating of Samples on Agar Plates.

Each agar plate was divided into two (2) and each part labeled with a sample's unique identification number. The corresponding swab stick was smeared on the respective agar portion in a circular motion in a space within a 1/4th portion of the agar plate. A sterilised inoculating loop (by flaming it in a Bunsen burner) was used to streak the inoculum in a parallel form. The loop was flamed again and then at the end of the parallel streaks; the streaks were extended in such a manner as not to cross into the other half of the agar plate to contaminate it. After the streaking, the inoculated agar plates were inverted and incubated aerobically at 37°C for 24 hours.

Identification of isolates

In addition to the type of haemolysis on the Blood Agar plate and other colony characteristics on the other agar plates, various biochemical tests were employed to identify the isolates. Initially, a smear is prepared from a pure colony and gram stained to identify the gram type using a Light Microscope with x1000 magnification. Depending on the gram type and shape of the bacteria, the necessary biochemical tests are performed. Basically, the biochemical tests done were Catalase, Coagulase, Triple Sugar Iron (TSI), Indole, Citrate and Urease tests were performed on them.

Gram Staining (Kit by BIO LAB DIAGNOSTICS, India)

A pure colony was picked with a flamed- sterilise loop and put on clean microscope glass slide. The colony is then emulsified with a drop of Normal Saline to prepare a smear. The smear was heat fixed and then covered with crystal violet stain for 60 seconds. The crystal violet was washed off with running tap water at a low pressure. The smear is then covered with Lugol's iodine for 60 seconds. The iodine is washed off running tap water at a low pressure. Afterwards, the smear was carefully and rapidly decolourised with Ethyl-Acetone for 10-15 seconds until the decolouriser was seen to be running from the slide clear. The smear was then counter-stained with Safranin for 120 seconds and then the stain was washed off. Finally, the smear was the air-dried and observed under the light microscope with the oil Immersion objective lens. The gram type of the isolates was defined as dark purple for Gram positive bacteria or Yeast cells and pale to dark red as Gram negative bacteria.

BIOCHEMICAL TESTS

Catalase test

This is used to identify bacteria which use the enzyme, Catalase, to catalyse the neutralisation of hydrogen peroxide (H_2O_2) into water and oxygen. Routinely, it is done to differentiate *Staphylococcocus spp*. from *Streptococcus spp.*

A slide test was done. With the use of a sterile wooden stick, about 10-15 colonies of the bacteria isolate were picked unto a clean slide. A drop of 3% H_2O_2 was added to it and it was observed for immediate bubbling in 10-15 seconds. A Catalase positive result was defined as the production of the immediate bubbles whiles a Catalase negative result was defined as the absence of bubbles or the production of scattered few bubbles.

Coagulase Test

The test identifies *S. aureus* which produces the enzyme, coagulase, to catalyse the clotting of plasma by converting fibrinogen to fibrin.

A slide test was done. On the two edges of a clean microscope slide, a drop of distilled water is put at each end. Using a sterile wooden stick, 15 to 20 colonies each were picked from the agar plates and emulsified in the distilled water drops at the 2 ends. A drop of EDTA anticoagulated human plasma free of HIV and Hepatitis B was added to one suspension at the end of the slide and uniformly mixed. The suspension was observed for clumping within 10 seconds and the observation was compared to the other suspension without the plasma. A positive Coagulase test was defined by observation of clumping within 10

seconds whiles no clumping within the stated time signified a negative coagulase test.

Triple Sugar Iron (TSI) Test

The TSI agar medium consist of three sugars- glucose, lactose and sucrose- and iron in the form of Ferrous Sulphate. The test identifies bacteria which utilize any or all of the sugars in its metabolism as well as produce H_2S gas. The agar in a tube is slanted to produce a slant and a bottom. The pure culture was inoculated into the TSI agar with a sterile straight inoculating needle by stabbing the center of the bottom through to almost the base of the tube. A zigzag streaking was done on the slant and the tube was loosely covered to allow for the production of the gas. The tubes were incubated at 35±2°C for 18-24 hours. And the results were read as follows;

Pink bottom and slant	-	No sugar fermentation
Yellow bottom and pink slant	-	Glucose fermentation
Yellow bottom and slant	-	Glucose, lactose and/or sucrose fermentation
Cracks in agar	-	H_2S gas

Indole Test

This test is also used to differentiate enterobacteria. A few colonies of bacteria are aseptically inoculated in Peptone broth. After 18- 24 hours of incubation at 37±2 °C, indole production is detected by the addition of Kovac's reagent. The reaction produces a red ring on the surface of the culture.

Urease Test

The test is usually used to differentiate enterobacteria. The Christensen's Urea agar (Techno Pharmachem, India), a medium that contains urea, was used for the test. With this test, a few bacteria colonies are aseptically picked with a loop and gently streaked on the slant of the agar in a bijoux bottle and incubated at 37±2 ^{o}C for 18-24 hours. Urease-producing bacteria will use the enzyme to hydrolyse the urea into ammonia and carbon dioxide. The release of the ammonia then makes the agar alkaline and the result is read as follows with the colour changes.

Pink colour - Urease Positive test

Creamish yellow/yellow - Urease negative test

Citrate Utilisation Test

This test is also used to differentiate enterobacteria. The test identifies bacteria that use citrate as their sole source of carbon. A Simmon's Citrate agar (Techno Pharmachem Haryana, India) slants in bijoux bottles were used. Aseptically, few colonies of the bacteria are picked and gently streaked in the slant and also stabbed in the bottom. The medium is then incubated at 37±2 ^{o}C for 18-24 hours. The result of the test is read as follows.

Bright blue colour - Positive Citrate test

Green or no change in colour - Negative Citrate test

Estimation of IL- 4, IL-6, IL-10, TNF-A and IFN-γ

As described by the Manufacturers protocol (Sunlong Biotech, China), a Sandwich ELISA test procedure was used to estimate the cytokines. Briefly,

antigens from diluted serum samples (dilution factor was 5) were coated into pre-specific antibody coated wells by passive adsorption and incubated for 30 minutes at 37°C. After the incubation, the wells were carefully washed five times with a washing buffer. Afterwards, 50 µL of a labelled antibody conjugated with enzyme (Horse-radish Peroxidase-conjugate) was added and incubated. The wells were carefully washed again five (5) times. Addition of 50 µL each of two (2) chromogens, Chromogen A and Chromogen B, to each well was done in the dark and mixed gently by shaking. The plate was incubated at 37°C for 15 minutes. Addition of 50 µL Stop Solution was done and it caused the colour change in the wells from blue to yellow. Finally, the optical density (OD) absorbances were read at 450 nm using a Microtiter Plate Reader (MULTISKAN EX by Thermo Electron Corporation) within 15 minutes after adding the Stop Solution.

To calculate the respective concentrations of the respective serum samples, the known concentrations of the respective cytokine standards and their corresponding optical densities were plotted on the y- and x- axes respectively using MS. Excel Worksheet. Standard Curve plots were obtained and linear plots were generated to estimate the equation for the lines ($y = mx + C$). Using the corresponding line equation for each plot, the concentrations of all the samples were determined.

Data Analysis

All data obtained were entered into MS Excel (version 2010) and then statistically analysed with STATA Software version 12 (STATA Corp, Texas USA) and GraphPad Prism 8 (GraphPad Software, San Diego- California, USA). The socio-demographic characteristics, risk behaviours and other relevant characteristics were described using Descriptive statistics.

The relationship between the parameters and groups were compared using Chi-square test or Fischer's Exact test for categorical parameters. Bivariate analysis was done for cytological abnormalities. The means of cytokine levels were analysed and compared using Two-way Analysis of Variance (ANOVA). All means were reported as mean ± Standard Deviation (SD). Statistical significance was considered when p-value is less than or equal to 0.05 (P value < 0.05).

Chapter Summary

This cross-sectional study recruited participants from the Akyemansa Sub-district in the Eastern region of Ghana. A convenient sampling technique was employed to select 274 female adults of 20 years and above. However, only 157 of the participants consented for their cervical and blood specimens to be taken thus they were enrolled.

Approval for the study was initially obtained from the UCC IRB board and a clearance identification number UCCIRB/CHAS/2019/30 was obtained. Also, approval was obtained from the community leaders prior to the start of the sample taking.

Cytological examination was done on the cervical smears following the Papanicoloau staining procedure. Also, wet prep examination and culture were also done to identify the presence of any parasite or micro-organism in the vagina. The concentration of the various cytokines, IL-4, IL-6, IL- 10, TNF-α and IFN-ɣ, were estimated using Sandwich ELISA. All the data obtained were entered into MS. Excel 2010 and then analysed with STATA Software, version 12 (STATA Corp, Texas USA).

CHAPTER FOUR

RESULTS AND DISCUSSION

Chapter Introduction

This chapter comprises of two sections, results and discussion. The former includes presentation of data obtained, in tables and figures, whiles the later relates the findings to previous knowledge available. Highlights are being made on key findings.

Results

Socio-demographic and economic characteristics of participants

A total of 157 participants were enrolled and the mean age was 41.2 (± 1.05) years. In Table 1, most of the participants were of the age 51 years and older (43, 27.4%) and they were chiefly from the Adjobue community. Most of the participants were married (81, 51.6%) and almost all were Christians (155, 98.7%). With regards to the educational status of participants, most of them have had formal education to the basic school level (115, 73.2%) (Tab. 2). Considering the employment status of the participants, most of them were engaged in trading (66, 42.0%) and this finding was statistically dependent on their community of residence. Despite the high proportion of economic activities (91.7%), most of the participants had their monthly income to be GH₵ 500.00 and less (141, 89.8%) (Tab. 2). All these showed no statistical significance except that for occupation as compared to the community of residence.

Table 1: Socio-demographic characteristics of respondents

		F (%)					
Variables		**Akyekrom (n=10)**	**Mukyia (n=33)**	**Abenase (n=23)**	**Adjobue (n=91)**	**N= 157**	**P-value**
Age/ years							0.435
Mean (SD)	41.2 (±1.05)						
21-30		3 (30.0)	11 (33.3)	9 (39.1)	16 (17.6)	39 (24.8)	
31-40		3 (30.0)	8 (24.2)	3 (13.0)	26 (28.6)	40 (25.5)	
41-50		2 (20.0)	8 (24.2)	4 (17.4)	21 (23.1)	35 (22.3)	
≥ 51		2 (20.0)	6 (18.2)	7 (30.4)	28 (30.7)	43 (27.4)	
Marital Status							0.738
Single		3 (30.0)	6 (18.2)	6 (26.1)	12 (13.2)	27 (17.2)	
Married		5 (50.0)	17 (51.5)	8 (34.8)	51 (56.0)	81 (51.6)	
Cohabiting		1 (10.0)	4 (12.1)	3 (13.0)	12 (13.2)	20 (12.7)	
Divorced		0 (0.0)	3 (9.1)	3 (13.0)	4 (4.4)	10 (6.4)	
Widowed		1 (10.0)	3 (9.1	3 (13.0)	12 (13.2)	19 (12.1)	
Religion							0.666
Christian		10 (100.0)	32 (97.0)	23 (100.0)	90 (99.1)	155 (98.7)	
Muslim		0 (0.0)	1 (3.0)	0 (0.0)	1 (0.9)	2 (1.3)	
Others		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Values are presented frequency,F, (Percentage proportion). The level of significance was established using Fischer's Exact test. Level of significance was established by P-value < 0.05. N implies total respondents from the Akyemansa Sub-district, n implies number of respondents from the respective communities.

Table 2: Socio-economic characteristics of respondents

Variables	F (%)				N= 157	P-value
	Akyekrom (n=10)	Mukyia (n=33)	Abenase (n=23)	Adjobue (n=91)		
Educational level						0.551
Tertiary	2 (20.0)	2 (6.1)	2 (8.7)	5 (5.5)	11 (7.0)	
SHS	1 (10.0)	2 (6.1)	2 (8.7)	5 (5.5)	10 (6.4)	
Basic Sch.	5 (50.0)	24 (72.7)	15 (65.2)	71 (78.0)	115 (73.2)	
No formal education	2 (20.0)	5 (15.2)	4 (17.4)	10 (11.0)	21 (13.4)	
Employment						0.000
Civil Servant	2 (20.0)	1 (3.0)	3 (13.0)	2 (2.2)	8 (5.1)	
Self employed	1 (10.0)	11 (33.3)	2 (8.7)	8 (8.8)	22 (14.0)	
Trading	0 (0.0)	14 (42.4)	11 (47.8)	41 (45.1)	66 (42.0)	
Farming	4 (40.0)	4 (12.1)	6 (26.1)	34 (37.4)	48 (30.6)	
No occupation	3 (30.0)	3 (9.1)	1 (4.3)	6 (6.6)	13 (8.3)	
Monthly income/ Gh₵						0.196
≤ 500	7 (70.0)	31 (93.9)	21 (91.3)	82 (90.1)	141 (89.8)	
600-1000	2 (20.0)	1 (3.0)	2 (8.7)	6 (6.6)	11(7.0)	
1100-1500	1 (10.0)	1 (3.0)	0 (1.5)	1 (1.1)	3 (1.9)	
>1500	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.2)	2 (1.3)	

Values are presented frequency,F, (Percentage proportion). The level of significance was established using Fischer's Exact test. Level of significance was established by P-value < 0.05. N implies total respondents from the Akyemansa Sub-district, n implies number of respondents from the respective communities.

Knowledge of HPV Infection and Cervical Cancer

In Fig. 4, majority of the participants (85, 54.0%) had no prior knowledge of HPV infection and/or cervical cancer and 56 (36%) had knowledge of only cervical cancer whiles 16 (10%) knew that HPV infection is majorly implicated as the aetiologic agent of cervical cancer. The primary source of their information was majorly from broadcasting media (TV and Radio stations, 68.0%) whiles rarely from schools and churches (2.3%) (Fig. 5). Factors that mainly influenced their acquisition of adequate knowledge and awareness of cervical cancer and HPV infection were educational level and marital status (Tab. 3). Also, 9 (5.73%) of the participants knew of either the Pap Smear test or Visual Inspection with Acetic Acid (VIA) or both as the most common screening tools used in Ghana (Appendix). In the past 5 years, only 2 (1.28%) of the participants had ever been screened for the presence of cervical lesions. Again, 29 (18.5%) of the respondents had prior knowledge of the transmission of HPV via sexual interactions. Finally, only 18 (11.46%) of the respondents knew of the existence of the available HPV vaccines, however, none had been vaccinated (Appendix).

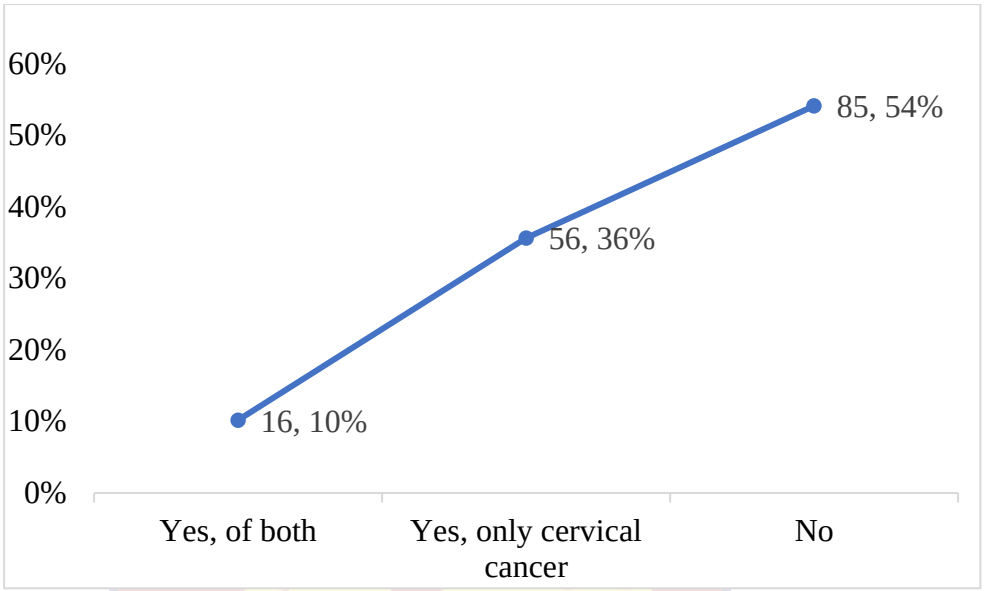

Figure 4: Proportion of respondents with knowledge of HPV infection and/or Cervical Cancer

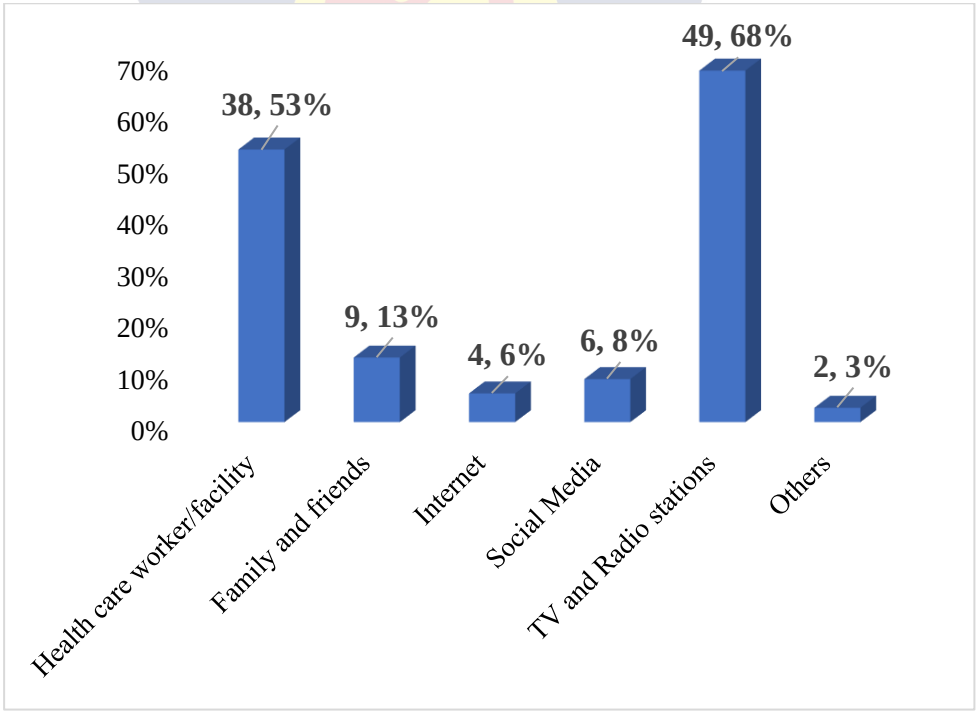

Figure 5: Distribution of sources of information on HPV infection and Cervical cancer

Table 3: Socio-demographic characteristics of participants and their level of knowledge/awareness of Cervical Cancer and HPV infection

VARIABLES	YES, n (%)	NO, n (%)	OR (95% CI)	P-VALUE
Age (years)				
21-30	18 (46.2)	21 (53.8)	1	
31-40	20 (50.0)	20 (50.0)	0.857 (0.354-2.074)	0.732
41-50	17 (48.6)	18 (51.4)	0.908 (0.364-2.264)	0.835
≥ 51	17 (39.5)	26 (60.5)	1.311 (0.545-3.153)	0.545
Marital status				
Single	16 (59.3)	11 (40.7)	1	
Married	42 (51.9)	39 (41.1)	1.351 (0.559-3.265)	0.505
Cohabiting	8 (40.0)	12 (60.0)	2.182 (0.671-7.092)	0.195
Divorced	2 (20.0)	8 (80.0)	5.818 (1.032-32.793)	0.046
Widowed	4 (21.1)	15 (78.9)	5.455 (1.423-20.910)	0.013
Educational level				
Tertiary	10 (90.9)	1 (9.1)	1	
SHS	4 (40.0)	6 (60.0)	15.000 (1.342-167.638)	0.028
Basic Sch.	57 (49.6)	58 (50.4)	10.175 (1.261-82.093)	0.029
No formal education	1 (4.8)	20 (95.2)	199.999 (11.296-3541.08)	0.000
Income				
≤ 500	61 (43.3)	80 (56.7)	1	
600-1000	8 (72.7)	3 (27.3)	0.286 (0.073-1.123)	0.073
1100-1500	2 (66.7)	1 (33.3)	0.381 (0.034-4.302)	0.435
>1500	1 (50.0)	1(50.0)	0.763 (0.047-12.436)	0.849

The level of significance was established with P value < 0.05. OR= Odds ratio CI= Confidence Interval. N=number of participants aware of Cervical cancer and HPV infection.

Characteristic Risk Behaviours of Participants.

From Table 4, most of the participants had their sexual debut at age 17 to 20 years (102, 65.0%) and there was no statistical difference when compared to their community of residence. Most of the participants (108, 68.8%) have had two and/or three sexual partners in their lifetime and the highest proportion (26, 78.8%) were identified from the Mukyia community. Similarly, most of the participants (85, 54.1%) have had one to four full term pregnancies. Fortunately, none of the participants had ever smoked and 44 (28.0%) reported of either taking alcohol daily or occasionally. This finding showed statistical significance when compared to their community of residence. Again, 53.3% (84) of the participants reported of ever taking in hormonal contraceptives. However, no statistical significance was recorded for these variables.

Table 4: Characteristic risk factors of Cervical cancer

Variables	F (%)					
	Akyekrom (n=10)	**Mukyia (n=33)**	**Abenase (n=23)**	**Adjobue (n=91)**	**N= 157**	**P-value**
Age at 1st sex/ years						0.634
Mean (SD) 18.3 (±0.2)						
≤ 16	1 (10.0)	11 (33.3)	6 (26.1)	17 (18.7)	35 (22.3)	
17-20	7 (70.0)	18 (54.5)	16 (69.6)	61 (67.0)	102 (65.0)	
21-24	2 (20.0)	3 (9.1)	1 (4.3)	11 (12.1)	17 (10.8)	
≥ 25	0 (0.0)	1 (3.0)	0 (0.0)	2 (2.2)	3 (1.9)	
Life time no. of sexual partners						0.163
1	4 (40.0)	4 (12.1)	1 (4.3)	13 (14.3)	22 (14.0)	
2-3	5 (50.0)	26 (78.8)	18 (78.3)	59 (64.8)	108 (68.8)	
≥4	1 (10.0)	3 (9.1)	4 (17.4)	19 (20.9)	27 (17.2)	
Alcohol						**0.008**
Daily	0 (0.0)	1 (3.0)	1 (4.3)	4 (4.4)	6 (3.8)	
Occasional	0 (0.0)	4 (12.2)	2 (8.7)	32 (35.2)	38 (24.2)	
Never	10 (100.0)	28 (84.8)	20 (87.0)	55 (60.4)	113 (72.0)	
Hormonal contraception						0.316
Ever[a]/Current	4 (40.0)	18 (54.5)	9 (48.5)	53 (56.0)	84 (53.5)	
Never	6 (60.0)	15 (45.5)	14 (51.5)	38 (44.0)	73 (46.5)	

Parity						0.074
Mean (SD)	4.2 (0.2)					
0		1 (10.0)	1 (3.0)	0 (0.0)	5 (5.5)	7 (4.5)
1-4		4 (40.0)	25 (75.8)	13 (56.5)	43 (47.3)	85 (54.1)
≥5		5 (50.0)	7 (21.2)	10 (43.5)	43 (47.3)	65 (41.4)

Values are presented as number, F, (Percentage proportion). The level of significance was established using Chi-square. P-value < 0.05 implies statistical significance. N implies total respondents from the Akyemansa Sub-district, n implies number of respondents from the respective communities, [a] implies ever taken contraceptives within previous 10 years.

Identification of Participants with Squamous Intraepithelial Lesions

With respect to the result from the Pap Smear test (Fig 6), 14 (8.9%) of the participants were identified to be having squamous intraepithelial lesions; 12 (7.6%) had low grade intraepithelial lesions (LSIL) and 2 (1.3%) had high grade squamous intraepithelial lesions (HSIL). None of the participants was identified to be having Atypical Squamous Cervicitis of Unidentified Significance (ASCUS) or cervicitis.

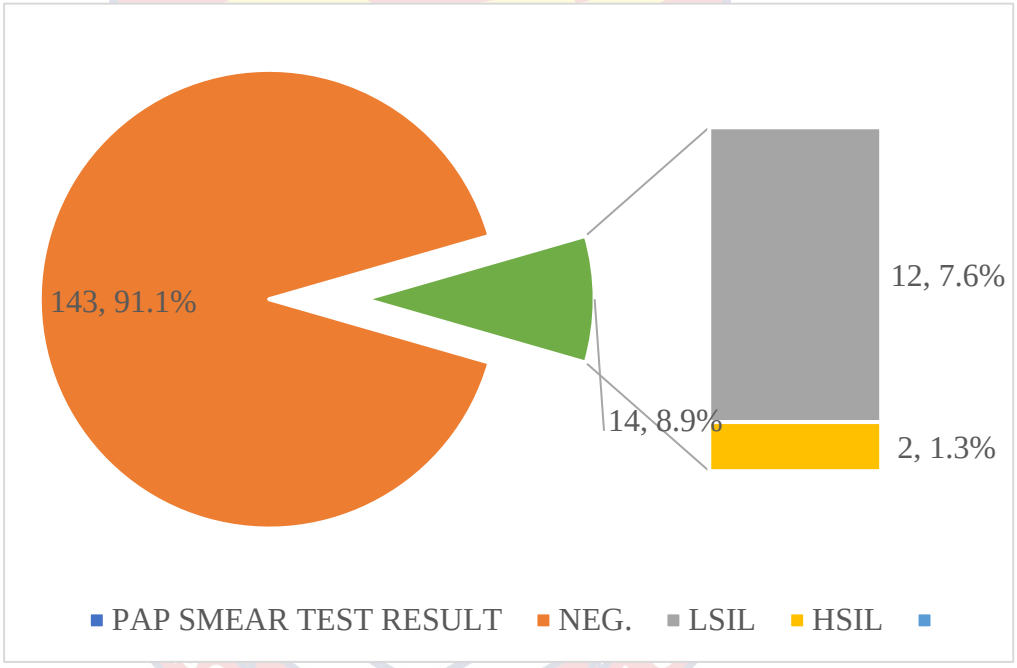

Figure 6: Proportion of participants with Epithelial Squamous cells abnormalities

Socio-demographic and Socio-economic characteristics of Participants with SIL

From Table 5, most of the participants with lesions (9) were from the Adjobue community-58.3% of the participants with LSIL and all participants (100 %) with HSIL. All the participants with lesions were of the age 46 years

and older and this showed statistical significance. Most of the participants with lesions were married (11, 78.6 %), had schooled to the junior high education level (8, 57.1 %), were traders (7, 50.0 %) and had their monthly income less the Gh₵ 600.00 (13, 92.9 %) (Tab. 6). None of the variables showed statistical significance.

Table 5: Socio- Demographic Characteristics of participants at risk of HPV infection

	PAP SMEAR RESULT N (%)				
VARIABLES	**NIL (n=143)**	**LSIL (n=12)**	**HSIL (n=2)**	**TOTAL N=157**	**P-VALUE**
Community					1.000
Adjobue	82 (57.3)	7 (58.3)	2 (100.0)	91 (58.0)	
Abenase	21 (14.7)	2 (16.7)	0 (0.0)	23 (14.6)	
Akyekrom	10 (7.0)	0 (0.0)	0 (0.0)	10 (6.4)	
Mukyia	30 (21.0)	3 (25.0)	0 (0.0)	33 (21.0)	
Age/ years					**0.001**
21-30	39 (27.3)	0 (0.0)	0 (0.0)	39 (24.8)	
31-40	40 (28.0)	0 (0.0)	0 (0.0)	40 (25.5)	
41-50	31 (21.7)	4 (33.3)	0 (0.0)	35 (22.3)	
≥ 51	33 (23.1)	8 (66.7)	2 (100.0)	43 (27.4)	
Marital Status					0.317
Single	27 (18.9)	0 (0.0)	0 (0.0)	27 (17.2)	
Married	70 (49.0)	9 (75.0)	2 (100.0)	81 (51.6)	
Cohabiting	17 (11.9)	3 (25.0)	0 (0.0)	20 (12.1)	
Divorced	10 (6.9)	0 (0.0)	0 (0.0)	10 (12.7)	
Widowed	19 (13.3)	0 (0.0)	0 (0.0)	19 (6.4)	

N= 157. The level of significance was established using Chi-square and Fischer's Exact tests. P value ≥ 0.05 implies no significant association between groups whiles P < 0.05 implies significant association between groups. NIL= Negative for Intraepithelial lesions;
LSIL= Low grade intraepithelial lesions; HSIL= High grade intraepithelial lesions.

Table 6: Socio-Economic Characteristics of participants at risk of HPV infection

VARIABLES	PAP SMEAR RESULT N (%)			TOTAL N=157	P-VALUE
	NIL (n=143)	LSIL (n=12)	HSIL (n=2)		
Educational level					0.783
Tertiary	11 (7.7)	0 (0.0)	0 (0.0)	11 (7.0)	
SHS	10 (7.0)	0 (0.0)	0 (0.0)	10 (6.4)	
JHS	73 (51.0)	6 (50.0)	2 (100.0)	81 (51.6)	
Primary	29 (20.3)	5 (41.7))	0 (0.0)	34 (21.7)	
No formal education	20 (14.0)	1 (8.3)	0 (0.0)	21 (13.4)	
Employment					0.891
Civil Servant	8 (5.6)	0 (0.0)	0 (0.0)	8 (5.1)	
Self employed	20 (14.0)	2 (16.6)	0 (0.0)	22 (14.0)	
Trading	59 (41.3)	5 (41.7)	2 (100.0)	66 (42.0)	
Farming/Fishing	43 (30.1)	5 (41.7)	0 (0.0)	48 (30.6)	
No occupation	13 (9.1)	0 (0.0)	0 (0.0)	13 (8.3)	
Monthly income/ GhȻ					0.488
≤ 500	128 (89.5)	11 (91.7)	2 (100.0)	141 (89.8)	
600-1000	11 (7.7)	0 (0.0)	0 (0.0)	11 (7.0)	
1100-1500	2 (1.4)	1 (8.3)	0 (0.0)	3 (1.9)	
>1500	2 (1.4)	0 (0.0)	0 (0.0)	2 (1.3)	

N= 157. The level of significance was established using Chi-square and Fischer's Exact tests. P value ≥ 0.05 implies no significant association between groups whiles P < 0.05 implies significant association between groups.
NIL= Negative for Intraepithelial lesions; LSIL= Low grade intraepithelial lesions; HSIL= High grade intraepithelial lesions

Characteristics risk behaviours of participants with SIL

Most of the participants with lesions had their sexual debut within the age bracket 17 to 20 years (9, 64.3%). Also, most of them (11, 78.6 %) have had two or three sexual partners in their lifetime. Again, 9 (64.3 %) of them had ever used a hormonal contraceptive and have had four or more (≥ 4) full term

pregnancies (Tab. 7). However, none of the variables showed statistical significance.

Table 7: Behavioural Characteristics of participants at risk of HPV infection and with abnormal cytology

VARIABLES	PAP SMEAR RESULT n (%)				
	NIL (n=143)	LSIL (n=12)	HSIL (n=2)	TOTAL N=157	P-VALUE
Age at 1st sex/ years					0.417
≤ 16	31 (21.7)	4 (33.3)	0 (0.0)	35 (22.3)	
17-20	93 (65.0)	8 (66.7)	1 (50.0)	102 (65.0)	
21-24	16 (11.2)	0 (0.0)	1 (50.0)	17 (10.8)	
≥ 25	3 (2.1)	0 (0.0)	0 (0.0)	3 (1.9)	
Life time no. of sexual partners					0.909
1	20 (14.0)	2 (16.7)	0 (0.0)	22 (14.0)	
2-3	97 (67.8)	9 (75.0)	2 (100.0)	108 (68.8)	
≥4	26 (18.2)	1 (8.3)	0 (0.0)	27 (17.2)	
Alcohol					0.380
Daily	6 (4.2)	0 (0.0)	0 (0.0)	6 (3.8)	
Occasional	36 (25.2)	1 (8.3)	1 (50.0)	38 (24.2)	
Never	101 (70.6)	11 (91.7)	1 (50.0)	113 (72.0)	
Hormonal contraception					0.548
Ever[a]/Current	75 (52.4)	7 (58.3)	2 (100.0)	84 (53.5)	
Never	68 (47.6)	5 (41.7)	0 (0.0)	73 (46.5)	
No. of full-term pregnancies					0.316
0	7 (4.9)	0 (0.0)	0 (0.0)	7 (4.5)	
1-3	80 (55.9)	5 (41.7)	0 (0.0)	85 (54.1)	
≥4	56 (39.2)	7 (58.3)	2 (100.0)	65 (41.4)	

N= 157. The level of significance was established using Chi-square and Fischer's Exact tests. P value ≥ 0.05 implies no significant association between groups whiles P < 0.05 implies significant association between groups. NIL= Negative for Intraepithelial lesions; LSIL= Low grade intraepithelial lesions; HSIL= High grade intraepithelial lesions. [a] implies ever taken contraceptives within previous 10 years.

Bivariate Analyses of LSIL+ among participants.

From Tab. 8, the main predictor of having squamous intraepithelial lesion is being over 46 years old (OR 11.16, 95% CI: 2.402-551.844). Other factors that influenced the development of squamous intraepithelial lesions but did not achieve statistical significance were early age of sexual debut (≤ 20 years), having two (2) or three (3) lifetime sexual partners, ever or currently using hormonal contraceptives and having a parity of four (4) and above (≥ 4).

Table 8: Bivariate analyses of LSIL+ among adult women in the Akyemansa Sub-district

VARIABLES	LSIL+ (N=14), n (%)	NIL (N= 143) n (%)	OR (95% CI)	P-VALUE
Age (years)				
≤ 46	12 (19.4)	50 (80.6)	1	**0.002**
> 46	2 (2.1)	93 (97.9)	11.16 (2.402-51.844)	
Education				
Non-formal	1 (4.8)	20 (95.2)	0.473 (0.586-3.818)	0.482
Formal	13 (9.6)	123 (90.4)	1	
Income				
≤ Gh₵ 1000.00	13 (8.6)	139 (91.4)	0.374 (0.039-3.599)	0.395
≥ Gh₵ 1100.00	1(20.0)	4 (80.0)	1	
Age at 1st sex				
≤ 20 years	13 (9.5)	124 (90.5)	1.992 (0.246-16.112)	0.518
> 20 years	1 (5.0)	19 (95.0)	1	
Life time no. of partners				
1	2 (9.1)	20 (90.9)	1	
2-3	11 (10.2)	97 (89.8)	1.134 (0.233-5.515)	0.876
≥ 4	1 (3.7)	26 (96.3)	0.385 (0.033-4.548)	0.448
Contraceptive Use				
Ever[a]/Current use	9 (10.7)	75 (89.3)	1.632 (0.521-5.110)	0.400
Never used	5 (6.8)	68 (93.2)	1	
Parity				
0-3	5 (5.4)	87 (94.6)	1	0.078
≥ 4	9 (13.8)	56 (86.2)	2.796 (0.891-8.776)	
Alcohol use				
Ever taken	2 (4.5)	42 (95.5)	1	0.244
Never	12 (10.6)	101 (89.4)	2.495 (0.535-11.634)	

The level of significance was established with P value < 0.05. LSIL+ = Low grade intraepithelial lesions and advanced pathologies. OR= Odds ratio CI= Confidence Interval. [a] implies ever taken contraceptives within previous 10 years

Serum Inflammatory Cytokines estimation of participants

From Tab. 9, participants with squamous intraepithelial lesions (SIL) have increased average IL-10 and TNF-α serum cytokine concentrations. However, only the average TNF-α concentration of participants with LSIL (48.73 ± 18.77 ng/L) showed statistical significance when compared to that of the NIL group ($p < 0.001$). Concentrations of IL-6 were also reduced among the participants with LSIL+.

Table 9: Mean concentrations of Serum inflammatory cytokines

	MEAN CONCENTRATION pg/ml		
SERUM CYTOKINES	**NIL (N=143)**	**LSIL (N= 12)**	**HSIL (N=2)**
IL-10	11.60 ± 3.519	13.61 ± 3.648^{ns}	15.11 ± 4.702^{ns}
IL-6^{U}	3.71 ± 2.018	3.50 ± 1.952^{ns}	2.06 ± 1.293^{ns}
IL-4	17.31 ± 6.518	19.82 ± 7.612^{ns}	16.27 ± 8.357^{ns}
IFN-γ	7.42 ± 2.556	8.55 ± 2.439^{ns}	6.62 ± 3.956^{ns}
TNF-α	38.95 ± 19.478	48.73 ± 18.77**	49.03 ± 0.423^{ns}

Concentration values are Mean ± SD. The level of significance was established using Two-Way Analysis of Variance (ANOVA) followed by Bonferroni's Multiple comparison test. Ns implies $P \geq 0.05$ for no significant relationship between any of the column variables- cases (LSIL and/or HSIL) and the control group (NIL)- within a row. ** represent $P < 0.001$ implying significant relationship between NIL and LSIL groups for TNF-α concentration only.
U= Unit of ng/L.

Description of Vaginal Microbiota

From Fig. 7, most of the organisms isolated were bacteria, 64.3% (101). Bacterial vaginosis was solely identified among 8.9% (14) of the participants. However, more than half of the participants with LSIL, 8 (66.7%), had both Candida and other bacterial infections; whiles all the participants with HSIL had bacterial vaginosis (Tab. 10). There was no statistical significance among the various categories of infection except that of bacterial vaginosis and Candida infection (Tab. 10).

Also, among all the significant bacteria growth isolates, *E. coli* (30.8%) was the most isolated organism from participants with no lesions whiles *Staphylococcus aureus* (60.0%) was the most isolated organism from the participants with low grade squamous intraepithelial lesions and advanced disease (Tab. 11). The least isolated microbe that was common between both participants with and without lesions was *Klebsiella* sp (10.0% and 4.4% respectively). Among all the bacteria isolated, only *Staphylococcus aureus, Citrobacter sp* and *Morganella morganii* showed significant statistical association between the two groups of participants (Tab. 11).

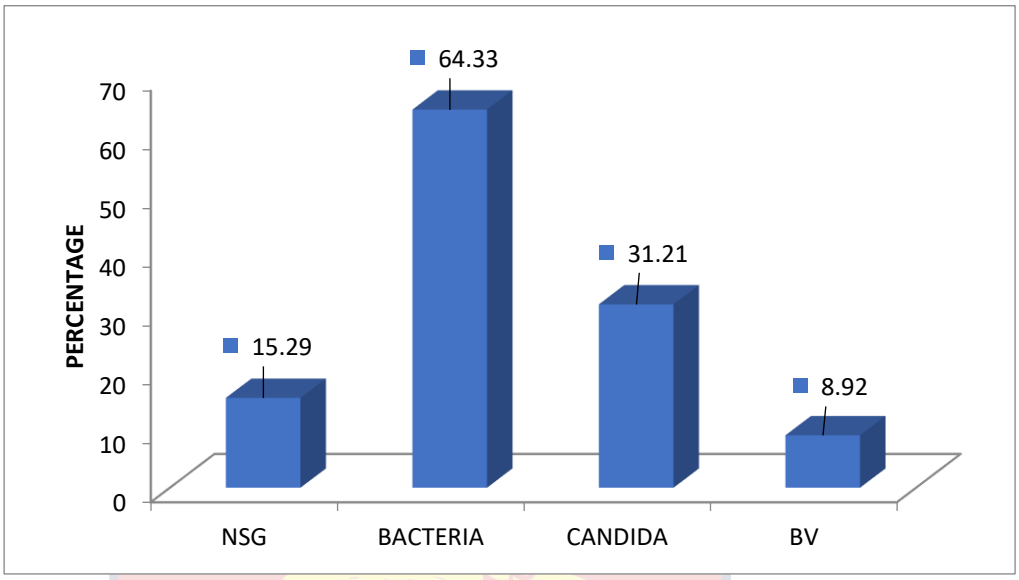

Figure 7: Percentage distribution of participants with Cervico-vaginal infection

Table 10: Proportion distribution of participants with SIL and Microbiome diversity

VAGINAL INFECTION	PAP SMEAR RESULT n (%) NIL (n=143)	LSIL (n=12)	HSIL (n=2)	TOTAL (n=157)	P-VALUE
NSG	24 (16.8)	0 (0.0)	0 (0.0)	24 (15.3)	0.437
Other Bacterial Infection	91 (63.6)	8 (66.7)	2 (100.0)	101 (64.3)	0.887
Candida Infection	40 (28.0)	8 (66.7)	1 (50.0)	49 (31.2)	0.009
Bacterial Vaginosis	8 (5.6)	4 (33.3)	2 (100.0)	14 (8.9)	0.000

N= 157. The level of significance was established using Fischer's exact tests. P value ≥ 0.05 implies no significant association between groups whiles P < 0.05 implies significant association between groups. NIL= Negative for squamous Intraepithelial lesions; LSIL= Low grade squamous intraepithelial lesions; HSIL= High grade squamous intraepithelial lesions. ≥ 0.05

Table 11: The proportion of isolated bacteria among participants with bacterial infection

OTHER ISOLATED BACTERIA	NIL, n (%) (N= 91)	LSIL+, n (%) (N=10)	TOTAL, n (%) (N=101)	P-VALUE
Lactobacillus spp.	12 (13.2)	0 (0.0)	12 (11.9)	0.603
Staph. Aureus	16 (17.6)	6(60.0)	22 (21.8)	**0.005**
Streptococcus spp	4 (4.4)	0 (0.0)	4 (4.0)	>0.999
E. coli	28 (30.8)	4 (40.0)	32 (31.7)	0.486
Pseudomonas	12 (13.2)	0 (0.0)	12 (11.9)	0.603
Citrobacter	12 (13.2)	4 (40.0)	16 (15.8)	**0.017**
Morganella morganii	4 (4.4)	4 (40.0)	8 (7.9)	**0.002**
Klebsiella	4 (4.4)	1 (10.0)	5 (5.0)	0.377

N= 157. The level of significance was established using Chi-square and Fischer's exact tests. P value ≥ 0.05 implies no significant association between groups whiles P < 0.05 implies significant association between groups. NIL= Negative for Intraepithelial lesions; NSG= No significant growth; LSIL+= Low grade Squamous intraepithelial lesions and advanced pathology.

Cytokines Concentration among Participants with Lesions and Microbiome Diversity

There was an increase in IL-10 which was parallel to the severity of the lesion, however, it was not statistically significant (Tab. 12). Participants with RTI and LSIL+ had reduced levels of TNF-α; only that of RTI was statistically significant (p <0.0001). Also, the levels of IL-4 and IFN-γ were numerically elevated among participants with LSIL+RTI when compared to that of the control group.

Table 12: Concentration of cytokines among participants with and without possible HPV infection and other vaginal infections.

	pg/ml			
CYTOKINES	**NIL+NSG (n=24)**	**RTI (n = 119)**	**LSIL+RTI (n=12)**	**HSIL+RTI (n=2)**
IL-10	9.22 ± 3.91	9.98 ± 1.85 ^{ns}	13.61 ± 3.648^{ns}	15.11 ± 4.70 ^{ns}
IL-6^{U}	3.75± 0.95	3.85 ± 2.64 ^{ns}	3.50 ± 1.952^{ns}	2.06 ± 1.29 ^{ns}
IL-4	18.68 ± 2.11	17.11 ± 4.88 ^{ns}	19.82 ± 7.612^{ns}	16.27 ± 8.36 ^{ns}
IFN-γ	6.52 ± 0.93	6.26 ± 1.78 ^{ns}	8.55 ± 2.439^{ns}	6.62 ± 3.96 ^{ns}
TNF-α	54.32 ± 9.50	31.99 ± 19.29****	48.73 ± 18.77$^{\#}$	49.03 ± 0.42

Concentration values are Mean ± SD. The level of significance was established using Two-Way Analysis of Variance (ANOVA) followed by Bonferroni's Multiple comparison test. Ns implies $P \geq 0.05$ for no significant relationship between any of the column variables- cases (LSIL and/or HSIL) and the control group (NIL)- within a row. * and $^{\#}$ represent $P < 0.05$ implying significant relationship between groups. * implies significant association between NIL+NSG and RTI groups for TNF-α concentrations. $^{\#}$ implies significant association between RTI and LSIL+RTI groups for TNF-α concentrations. U= Unit of ng/L

Discussion

Cervical cancer continues to be a global burden as it ranks as the fourth (4^{th}) diagnosed cancer among women (Arbyn et al., 2020). With over 550, 000 cases and over 300, 000 deaths reported globally in 2018-, low- and middle-income countries remain the most burdened as almost 85% of the cases are reported from such regions (Nartey et al., 2018; WHO, 2014). In Ghana, its estimated that every year over 3000 women between the ages of 15-44 years are diagnosed with cervical cancer and over 2000 of them die from the disease, making the mortality rate thrice higher than the global rate (ICO/IARC, 2019). Although the disease is preventable, its mortality rate continues to soar; as many nations, especially in the developing regions, lack established national policies for screening and preventing of cervical cancer; infrastructure and the necessary health resources including specialised human resource to aid in the diagnosis and management of the disease (Binka, Nyarko, Awusabo-Asare, & Doku, 2019; Nartey et al., 2018; Sancho-Garnier et al., 2013; Schiffman et al., 2011).

This study was conducted among a rural population in Ghana, specifically in four communities in the Akyemansa district in the Eastern Region of Ghana. A total of 157 women with mean age of 41.2 (±1.05) years (20-80 years), and where most of them were 51 years old and above (43, 27.4%), were enrolled to participate in the study. This signified that, averagely, women in the Akyemansa district were past the recommended age for the initial screening of cervical cancer and were approaching the recommended age for the second screening of cervical cancer (Gultekin, Ramirez, Broutet, & Hutubessy, 2020). However, there was no statistical difference among the number of participants within the specified age groups. Interestingly, this finding was in contrast with

a number of previous similar studies which had most of the participants to be 40 years of age and younger (Assoumou et al., 2015; Baloch et al., 2017; Kadian et al., 2020). This finding was due to the lack of access and absence of younger women who are more actively and daily engaged in various agricultural and economic activities to fend for their families; this directly affected the recruitment of younger women.

In this study, over 50% of the participants (81, 51.6%) were married and over 80% of the participants (130, 82.8%) had ever lived with a life partner. The reason for this finding is due to early age of marriage and the perception of marriage as prestigious and an indication of being responsible in a family and the society as a whole. In developing communities and countries, females tend to marry as early as 15 years whiles in developed countries like the U.S.A., it is in their mid-20s (Ahonsi et al., 2019; Bishop, 2017; Livingston, 2018; Sarfo, Salifu Yendork, & Naidoo, 2020; Sultana, Hossain, & Hoq, 2015). This finding was in concert with previous studies which also identified at least 50% of their participants to be married (Assoumou et al., 2015; Baloch et al., 2017; Kadian et al., 2020).

Again, most of the participants (115, 73.2%) had their formal education to the basic education level (primary or JHS) whiles very few of them (11, 7.0%) were graduates. This finding was due to burden of financial constraint, teenage pregnancies as well as early marriage. The finding was similar to that of S. A. Ahmed, Sabitu, Idris, and Ahmed (2013). However, a number of similar studies had contrary finding where most of the participants were graduates (Al Meer, Aseel, Al Khalaf, Al Kuwari, & Ismail, 2011; Assoumou et al., 2015; Baloch et

al., 2017; Kadian et al., 2020); this was due to difference in geographical locations and study population.

Trading and farming were the predominant occupations among the participants (42.0% and 30.6% respectively) as the communities were mainly engaged in agricultural activities. This was similar to that of S. A. Ahmed et al. (2013) and Durowade et al. (2012), but contrary to that of Assoumou et al. (2015) and Baloch et al. (2017) who had most of their participants to be employed (public and/or private, and self-employment). The disparity is due to difference in study population, geographical area and seasons.

With regards to the economic status of the participants, approximately 90% of them earned less than Gh₵ 600.00 monthly. Rural communities are noted for poor economy despite active engagement in agricultural activities. This is due to limited access to financial aids, limited investment into agricultural activities; no or poor access and use of technological methods and tools for work; inadequate infrastructure and poor occupational health and safety working conditions; climate change; and conflicts, low productivity and less profit from sales of raw products. This directly affect the population's standards of living and level of education (ILO, 2018; Islam & Mustaquim, 2014). This finding was similar to that of Baloch et al. (2017) and Assoumou et al. (2015).

Since the discovery of cervical cancer in the 4th century (Aviles, 2015), the disease knowledge and awareness continue to be limited in most part of the world especially in the developing regions (LaMontagne et al., 2011; Reichheld et al., 2020). A comparison between the awareness rate of cervical cancer and HPV infection in rural and urban settlements show no significant difference even though the awareness among rural populations is usually lower (Kadian et al.,

2020; Reichheld et al., 2020). In this study, the proportions of persons that had knowledge of cervical cancer and HPV infection were 36.0% (56) and 10.0% (16) respectively. These findings were consistent with that of earlier studies done in Elmina, a town along the coastal belt of Ghana; South Africa and Northeast China (Ebu et al., 2014; Hoque & Hoque, 2009; Ning et al., 2019; Shabani et al., 2019). On the contrary, previous studies done in Southwest China, Ethiopia, North Korea, Qatar and Nigeria observed more than 60% (>60%) of their participants to be aware and know of cervical cancer and HPV infection (Al Meer et al., 2011; Baloch et al., 2017; Mengesha, Messele, & Beletew, 2020; Owoeye & Ibrahim, 2013; Tran et al., 2011). The disparity is due to difference in study populations and availability of adequate information on the disease in the respective countries.

With regards to knowledge of the common screening tools for cervical cancer used in Ghana, only 9 (5.76%) of the participants knew of the Pap Smear test and Visual Inspection with Acetic Acid (VIA) and this contradicted the findings of Heena et al. (2019) and Owoeye and Ibrahim (2013) who reported over 50% (>50%) of the participants to know of at least one screening tool. The disparity is due to difference in study populations.

Also, in the past 5 years, only 2 (1.28%) of the participants had ever been screened for the presence of lesions. This finding disputed that reported in Saudi Arabia and North-East China where more than 25% (>25%) of the participants had ever been screened (Heena et al., 2019; Ning et al., 2019). Women in rural environments, and even urban and peri-urban towns, are mostly discouraged to patronise screening services due to cost and ease of access to screening services and facilities, cost of treatment options, possible misdiagnosis and inappropriate

conduct from healthcare personnel and superstitious belief on the aetiology of the disease (Binka et al., 2019). The disparity in the findings is due to difference in geographic locations.

Again, 29 (18.5%) of the respondents had prior knowledge of the possible transmission of HPV via sexual interactions. And, only 18 (11.46%) of the respondents knew of the existence of the available HPV vaccines, however, none had been vaccinated. These findings were identified to be similar to that of Shabani et al. (2019). The reason for this finding is inadequate education and knowledge about the disease in the communities.

Predictors that were significantly associated with the knowledge of cervical cancer and HPV infection were marital status and educational level of participants. Participants living without a life partner (divorced and widowed women), and those with low educational levels were identified to have limited or no prior knowledge and awareness of the infection and disease. Contrary, Ning et al. (2019) identified age to be a main predictor of good knowledge and awareness.

The primary sources of information were broadcasting media (TV and radio stations, 68.0%) and health workers/facilities (53.0%). Due to the perceived superstitious aetiology of the disease and possible stigmatisation, the women eschew confiding their personal and witnessed experiences of HPV infection and pre-cancerous and malignant lesions with family and friends. Contrary, in India, Kadian et al. (2020) identified their primary sources of information to be family, neighbours and friends. The disparity between the studies is due to the difference in attitude and beliefs of the study populations.

The mere existence of HPV infection does not entirely lead to the pathogenesis of Cervical cancer; certain risk factors predispose a woman into developing the pathology (D. Song et al., 2015). In this study, the mean age of sexual debut was 18.3 (± 0.2) years and 102 (65.0%) participants had their first sexual intercourse either from 17-20 years of age; however, this showed no statistical difference when compared to their communities of residence. This finding was similar to that of other studies (Durowade et al., 2012; Obiri-Yeboah et al., 2017).

Also, 108 of the participants (68.8%) have had either two (2) or three (3) life time sexual partners. HPV infection and its persistence has been strongly implicated with early age of sexual debut and multiple sexual partners (Bosch & de Sanjosé, 2007; Bosch et al., 2006; Krüger Kjaer et al., 2001). Previous studies reported similar findings (Ning et al., 2019; Obiri-Yeboah et al., 2017). Contrary, Durowade et al. (2012) reported of most of the participants having just one life time sexual partner.

Again, more than half of the population had ever taken hormonal contraceptives (84, 53.5%) and this finding was consistent with that of Sharma and Kapoor (2020). However, other studies identified most of their participants not to have ever taken any contraceptive (Durowade et al., 2012; Obiri-Yeboah et al., 2017). Finally, more than half of the participants had 1-4 full term pregnancies [85, 54.1%]; this finding was also similar to that of Sharma and Kapoor (2020) and Durowade et al. (2012). The disparity is associated with difference in attitude, practices and beliefs of the study populations.

For over five decades, cervical cytology has been the gold standard for the screening of cervical cancer; with Papanicoloaou smear (Pap smear) as the

preferred testing method (Boone et al., 2012). Apart from Pap Smear utilised as a screening tool for cervical cancer, pap-smear induced inflammation caused by trauma at the cervix induces more cell mediated immunity by upregulating inflammatory cytokines IL-12, TNF-α and IL-10 at the cervix; thus, a single ever patronised Pap Smear test in a woman's life is capable of reducing her risk to developing cervical cancer and also contribute to effective HPV clearance (Passmore, Morroni, Shapiro, Williamson, & Hoffman, 2007). Now, in this study, out of the 157 participants, 12 (7.6%) and 2 (1.3%) women were identified to have Low grade intraepithelial lesions (LSIL) and High-grade intraepithelial lesions (HSIL) respectively. None of the participants had Atypical Squamous cells of Undetermined Significance (ASCUS). This finding was similar to that of previous studies which found 7-11% of the participants to have abnormal cytology (Gaym et al., 2007; Wall et al., 2005).

Considering the socio-demographic and socio-economic characteristics of the participants with squamous intraepithelial lesions (SIL), 7 of the LSIL participants (58.3%) and all the participants with HSIL (n=2) were from the Adjobue community whiles 25% and 16.7% of the participants with LSIL were from Mukyia and Abenase communities respectively. Adjobue is a former mining community and as reported by a number of studies, women from such communities could be engaged in risky sexual behaviours that put them at increased risk (Botha, 2016; Erdiaw-kwasie, 2012; Weldegiorgis, Lawson, & Verbrugge, 2018) All the participants with squamous intraepithelial lesions (SIL) were 41 years old and above. Cervical cancer usually starts development from the transformation zone of the cervix- the junction where glandular and squamous cells of the covering of the cervix merge. As one ages and after

childbirth; this causes transformational changes which present suitable environment for the pathogenesis (Kashyap, Krishnan, Kaur, & Ghai, 2019). This finding was concurrent with the report by Plummer, Peto, and Franceschi (2012) as it was observed that among the unscreened population, the age-specific incidence rate of Cervical cancer remains at 45 years.

All the participants with SIL were living with a life partner, either married or cohabiting. In Sub-Sahara Africa where polygamy is widely accepted and two or more women could share a man, the risk of exposure and persistence of HPV, particularly hr-HPV infection, increases (Bayo et al., 2002; Domfeh et al., 2008).

Again, all the participants with SIL have had basic education (Primary and JHS levels of education) except a participant with LSIL. Contrary to this finding, women with no formal education were identified to be significantly diagnosed with cervical lesions (Saleem et al., 2019). Again, all the participants were economically engaged, however, all 8.3% of the LSIL participants earned less that GH₵ 600.00 monthly.

Assessing the risk behaviours of SIL participants, 93% of the them had their sexual debut before and at 20 years of age (≤ 20 years). HPV infection commonly affects a person soon after sexual debut thus it becomes proxy to claim the time of exposure to HPV from the age at first sex. Additionally, cervical cancer has been identified to be caused by strong early-stage carcinogens with incidence rates proportional to a power of time since first exposure to HPV (Plummer et al., 2012). This finding was similar to that of Louie et al. (2009).

The participants with SIL were identified to have had two (2) or more (≥ 2) lifetime sexual. Liu, Liu, Liu, Ye, and Chen (2015) suggested having multiple lifetime sexual partners as an independent risk factor for the angiogenesis of both malignant and non-malignant cancers. This finding was in concert with that of previous studies (Cooper et al., 2007; Liu et al., 2015).

In regards to alcohol consumption, 14.3% (2/14) of the participants with SIL occasionally took alcohol. As alcohol is listed as one of the Group 1 carcinogens (Haenel, 1989; Min, Lee, Lee, & Kim, 2013), its association with carcinogenesis has been defined that increased intake surges the risk of cancer in multiple organs including, not limiting it to the liver, breast, lungs and gastrointestinal tract (Min et al., 2013). Min et al. (2013) actually identified alcohol as an independent factor coupled with HPV infection in developing squamous intraepithelial lesions. Also, it has been evinced that alcohol drinkers coupling as second-hand non-active smokers have increased risk for HPV persistence (Seo et al., 2019).

Over 60% of the participants with SIL (64.3%) had ever used hormonal contraceptive within the past 10 years or was taking it as at the time of the study. The high patronage of hormonal contraceptive use was found to be related to increase education on family planning by the community health workers. Generally, the use of hormonal contraceptives, especially oral and injection formulas, increases the risk of carcinogenesis and severity of cervical cancer among women (McFarlane-Anderson, Bazuaye, Jackson, Smikle, & Fletcher, 2008). Current and prolong use of hormonal contraceptives (≥ 5 years), especially the oral formulas, have been estimated to increase the risk of developing cervical cancer, however, a ceasure in use of oral contraceptives for

after 10 years reverses the risk to that of women who have never used it (Sasieni, 2007; Smith et al., 2003).

Again, 64.3% of the SIL participants had 4 or more (≥ 4) full term pregnancies. The high parity is due to early age of sexual debut and early age of marriage, which are common practices in developing countries such as Ghana (Louie et al., 2009). As childbirth causes conformational changes in the transformation zone of the cervix (ACS, 2014; Kashyap et al., 2019), high parity has been associated with increasing the risk of developing cervical cancer (Muñoz et al., 2002).

The main predictor for the development of squamous intraepithelial lesions (SILs) was being of older age. Participants older than 46 years of age had increased odds as compared to participants of 46 years of age and below (11.16, 95% CI 2.4-51.8). The reasons for this finding are the possible reactivation of latent HPV infection which usually occurs between 45-50 years of age (Trottier et al., 2010), the insidious and slow progression of HPV infection to pre-cancerous and malignant lesions (IARC, 2020), the cause of conformational changes in the cervix due to aging and childbirth (Kashyap et al., 2019) and reduced or compromised immunity with aging (Fuentes, Feuntes, Alarcon, & Paloma, 2017; Montecino-Rodriguez, Berent-Maoz, & Dorshkind, 2013). In contrast, Parham et al. (2006) found the presence of hr-HPV as an independent predictor for the development of SIL. This disparity is due to the difference in study population.

Hitherto, as HPV infection is majorly implicated with the carcinogenesis of cervical cancer, appropriate immune response is needed both systemically and locally to help control the progression of the disease. This is achieved via

the release of cytokines which induce the production of other cytokines and actions to mediate the mount of immune-regulatory cytokine network (Bais et al., 2005; Beglin, Melar-New, & Laimins, 2009; Kemp et al., 2010). In this study, the concentrations of circulatory cytokines- IL-4, IL-6, IL-10, TNF-α and IFN-γ- were estimated using ELISA. It was observed that participants with LSIL+ had increased levels of IL-10 ($p \geq 0.05$) and TNF-α ($p < 0.001$) and reduced concentration levels of IL-6 ($p \geq 0.05$), and this was parallel to the severity of the lesion when compared to the NIL group. This signifies an anti-inflammatory immune response among the participants with LSIL+, especially those at the early stages of the lesion development gives a good prognosis of regressing lesion development. TNF-α is an anti-tumour cytokine and possess autocrine mechanisms which helps to inhibit the proliferation of affected cells (Landskron et al., 2014; Rotar et al., 2014). This finding was contrary to that of Li et al. (2019) who found elevated levels of IL-6 and decreased levels of IL-2. The difference in results is due to study population, geographic location and dietary differences.

Cervico-vaginal infections are one of the most common medical complaints physicians encounter in clinical medicine (O. Ahmed et al., 2016; Moradi, Tadris Hasani, Darvish, & Roozbeh, 2017). This usually occurs when there is dybiosis, that is increase in the diversity of the microbiome with reduced *Lactobacillus sp*. (Curty et al., 2019; Gao et al., 2013) High cervico-vaginal microbiome diversity, which has recently been classified as community state type (CST) IV based on 16S rRNA high-throughput sequencing (16S-HTS) data according to the bacterial species present (Ravel et al., 2011), has being in the limelight as potential factor for the acquisition and persistence of HPV infection

and subsequent progression into cervical dysplasia and cancer (Curty et al., 2019; Mitra et al., 2016). Although certain sexual practices leading to sexually transmistted infections (STIs) is widely associated with cervico-vaginal infections, behavioural practices that are common among women, especially in developing countries like Ghana, such as douching poses as possible cause for the infections (F. N.-A. McCarthy et al., 2015; Shaaban, Youssef, Khodry, & Mostafa, 2013; Ziba et al., 2019). Currently, the relationship between cervical lesions and cervico-vaginal infections is that, acquisition of one creates a conducive microenvironment for subsequent infections (Moradi et al., 2017). In this study, microbiological examination of cervico-vaginal samples revealed that participants with SIL significantly had Bacteria Vaginosis (BV) [42.9%] and *Candida spp*. infection (64.3%). Also, the predominat bacteria isolated from the participants with LSIL+ were *Staphylococcus aureus* (p=0.005), *Morganella morganii* (p=0.002) and *Citrobacter spp.* (p=0.017). Aside BV, CST IV has been implicated with Aerobic vaginitis where *S. aureus*, *E. coli*, Group B Streptococcus (GBS) are frequently isolated (Di Paola et al., 2017; Mulu et al., 2017; Mulu, Yimer, Zenebe, & Abera, 2015). BV caused by *Gardenerella vaginalis* is able to favour other genital tract infections acquistion by the secretion of an enzyme, sialidase, which breakdown the vaginal mucus by cleaving its glycoproteins thus breaking the barrier to inhibit bacteria-host interaction (Curty et al., 2019). The finding was concurrent with earlier studies that predominantly isolated *S. aureus* and *E. coli* (Mulu et al., 2017; Mulu et al., 2015).

Moving forward, apart from possible influence of cervico-vaginal microbiome on promoting the acquisition of other viral infections such as HIV,

it has been speculated to modulate immune response thus enhancing tumorigenesis (Curty et al., 2019). This study observed possible immune modulation by the microbiome which was evinced by reduced concentrations of TNF-α among the participants with RTI and those with RTI+LSIL+. However, IL-10 levels steadily increased among participants with dysbiosis and dysplasia, and it was parallel to the disease severity, however, no statistical significance was achieved. This finding exhibit immune suppression and a TH-2 like cytokines skewed immune response which gives a poor prognosis on the outcome of the lesion's progression. Again, the finding of this study was contrary to a number of similar studies previously done (Audirac-Chalifour et al., 2016; N. G. Campos, Tsu, Jeronimo, Mvundura, & Kim, 2017).

Chapter Summary

Most of the participants were 51 years of age and above. They were married, had at least basic education (Primary or JHS), were traders and farmers, and earned less than Gh₵ 600.00 (< Gh₵ 600.00) monthly. Only 39% of the participants had knowledge of cervical cancer and 10% of the participants knew of both cervical cancer and HPV infection, and 11.5% knew of the available HPV vaccines. The primary source of their information was the broadcasting media (TV and Radio stations).

After screening the participants with Pap smear, 7.6% and 1.3% of the participants had LSIL and HSIL respectively and the predictors for the lesion development was mainly old age with other factors such as early age of sexual debut, high parity, prolong use of hormonal contraceptives and multiple sexual partners.

Participants with lesions were identified to predominantly have Bacteria vaginosis and Candida infection whiles other bacteria isolates identified were *Staphylococcus aureus*, *E. coli* and *Morganella sp*. Circulatory cytokines estimation revealed immunosuppression (increased IL-10) among the participants with lesions partly influenced by the dysbiosis.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Chapter Introduction

This study was done to assess the knowledge of HPV infection and Cervical cancer, awareness of the availability of vaccines and also the risk factors among the rural population in the Akyemansa Sub-district of the Eastern region. And also, to assess the association between circulatory inflammatory cytokines (IL-4, -6, -10, TNF-α and INF-γ) and cervico-vaginal microbiome diversity among adult females at risk of Cervical cancer.

Summary

Awareness and knowledge of HPV infection and cervical cancer remains limited among both the rural and urban population; however, the proportion in the rural environment is usually lower. In this study, there was poor knowledge and awareness of Cervical cancer and HPV infection. Having good knowledge and awareness of HPV infection and cervical cancer was influenced by marital status and the educational levels of the participants. Most of the participants had multiparity and multiple lifetime sexual partners, had early age of sexual debut and prolong use of hormonal contraceptives, however, they showed no statistical significance. After screening with Pap smear, 8.9% of the population had precancerous lesions and the main predictor for the lesion development was old age; nevertheless, early age of sexual debut (≤20 years), multiple sexual partners (2-3 partners), current and prolong usage of hormonal contraceptives and high

parity (≥4) were found to also influence the development of the lesions (p >0.05).

Cervico-vaginal microbiota is largely dominated by *Lactobacillus sp.* whose bio-activities keep the region's pH low (< 4.5). Increased diversity of microbiome with the presence of certain bacteria such as *Gardenerella vaginalis* causes the pH imbalance thus making the reproductive environment conducive for the infection and growth of other opportunistic microbes. Recently, some studies have suggested that bacteria-virus-host interactions describes interesting pathways that may lead to modulation of immune response in women with pre-cancerous lesions and cervical cancer. To investigate the role of microbiome diversity on circulatory inflammatory cytokines, the levels of IL4, IL-6, IL-10, TNF-α and INF-γ were estimated.

In the study, comparing the cytokines levels among participants with NIL and those with lesions, TNF-α significantly increased among participants with lesions and these were parallel to the severity of the lesion, thus, signifying a TH1 cytokine-like geared immune response. Microbiological culture found the participants with lesions to predominantly have Bacterial vaginosis and Candida infection as well as other bacterial infections. This signified an improved anti-inflammatory immune response among participants with lesions. Furthermore, IL-10 average concentrations were numerically increased among all the cases and this finding was parallel to the severity of the lesion. Increased IL-10 suggests immunosuppression and TH2 cytokine-like immune response which creates favourable microenvironment for possible tumourigenesis.

Conclusions

Currently, this is the first study in Ghana to screen rural populations of risk of Cervical Cancer. The mean age of the participants was 41.2 (± 1.05) years and it signified that averagely the women in the Akyemansa district were past the recommended age for the first screening of cervical cancer, which is 35 years and were nearing the age for the second screening which is 45 years. Similar to the findings of other studies done in the country, awareness and knowledge of HPV infection, risk behaviours and Cervical cancer was low among the adult women population. The main source of information among the women was broadcasting media and the main predictors for good knowledge were marital status and educational level where women living without life partners and those of lower educational level had limited knowledge and awareness of cervical cancer and HPV infection.

About 9% of the participants had squamous intraepithelial lesions where 7.6% of them had LSIL and 1.3% had HSIL. The main predictor for development of squamous intraepithelial lesions was being of older age, especially being over 46 years of age.

Among the participants with LSIL+, Bacteria vaginosis and Candida infections were the predominant infections and the predominant bacteria isolates were *S. aureus*, *Citrobacter spp* and *Morganella spp*.

Comparing the cytokines levels among the participants with LSIL+, there was a good prognosis with a TH1-type immune response whereby elevated levels of TNF-α was observed. However, immunosuppression was observed with numerically elevated levels of IL-10 among the participants with dysplasia

and dysbiosis which provides a suitable microenvironment for the progression of cancer.

Recommendations

Based on the findings of the study, the following recommendations are suggested.

1. Extensive and wide coverage of peer-headed education on Cervical cancer, HPV infection and associated risk behaviours in the local dialects by Ministry of Health in collaboration with National Commission for Civic Education (NCCE), Ministry of Education and the religious bodies. This is to be done for target groups in the community, market places, schools and religious assemble points (churches, mosques, temples and others).
2. Ministry of Health to spearhead the establishment and implementation of policies and resources by the Government of Ghana for the screening and prevention of Cervical cancer. At least, free screening exercises should be done in Public Health facilities in deprived areas whiles a subsidy can be offered to women in urban and peri-urban towns at Public Health facilities for a specified number of screening times in a citizen's lifetime.
3. On the prevention of Cervical cancer, the government of Ghana should secure more funds to make at least HPV quadrivalent vaccine, Gardasil, available and accessible to girls, 9-14 years, in the community and schools.

4. For research purposes, Liquid based cytology test should be used to ensure a more reproducible and accurate cytology result. Thus, more funding should be made available for this and similar studies.
5. HPV identification and genotyping should be done to determine the virus-type distribution in the rural population.

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APPENDICES

APPENDIX A: Ethical Clearance reference for the study.

UNIVERSITY OF CAPE COAST

INSTITUTIONAL REVIEW BOARD SECRETARIAT

TEL: 0558093143 / 0508878309/ 0244287814 C/O Directorate of Research, Innovation and Consultancy
E-MAIL: irb@ucc.edu.gh
OUR REF: UCCIRB/A/2016/377
YOUR REF:
OMB NO: 0990-0279
IORG #: IORG0009096

3RD MAY, 2019

Ms. Loretta Bettey Blay Mensah
Department of Microbiology and Immunology
University of Cape Coast

Dear Ms. Mensah,

ETHICAL CLEARANCE – ID: (UCCIRB/CHAS/2019/30)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted **Provisional Approval** for the implementation of your research protocol titled **T Follicular helper cells and T Follicular regulatory cells involvement in B cell immunity in human papilloma virus (HPV) infection and its progression into cervical cancer**. This approval requires that you submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

Please note that any modification of the project must be submitted to the UCCIRB for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

Samuel Asiedu Owusu, PhD
UCCIRB Administrator

ADMINISTRATOR
ITUTIONAL REVIEW BOARD
NIVERSITY OF CAPE COAST
Date: 3rd May, 2019

APPENDIX B: Closed-Ended Questionnaire used to gather data on Socio-demographics, Socio-Economic, Risk Behaviours, and knowledge of HPV infection and Cervical Cancer.

UNIVERSITY OF CAPE COAST

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MICROBIOLOGY- SMS

QUESTIONNAIRE ON STUDY- *CIRCULATORY INFLAMMATORY CYTOKINES AND MICROBIOME DIVERSITY AMONG ADULT WOMEN AT RISK OF CERVICAL CANCER.*

INSTRUCTION: ***Please write the number of the most appropriate answer in the response code box provided.***

PARTICIPANT'S NAME: …….

CONTACT: ……

SECTION A: DEMOGRAPHIC DATA

DATE OF INTERVIEW:	**NAMEOF INTERVIEWER/SIGNATURE:**	**PARTICIPANT CODE:**
SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS		
S#	**QUESTIONS**	**OPTIONS**	**RESPONSE CODE**

1.	Gender	1. Male 2.Female	☐
2.	Age	1. 21-30 2. 31-40 3. 41-50 4. 51 and above	☐
3.	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed	☐
5.	Religion	1. Christianity 2. Islam 3. Traditional 4. Others	☐
SECTION B: SOCIO-ECONOMIC CHARACTERISTICS			
6.	Educational status	1. Tertiary 2. Senior High School 3. Junior High School 4. Primary School 5. No formal education	☐
7.	Occupational status	1. Civil servant 2. Self- employed 3. Trading 4. No occupational status	☐

8.	Level of monthly income	1. Gh₵ 100-500 2. Gh₵ 600-1000 3. Gh₵ 1100-1500 4. > Gh₵ 1500	☐
SECTION C; KNOWLEDGE OF HPV INFECTION AND CERVICAL CANCER.			
NUMBER	**QUESTIONS**	**OPTIONS**	**RESPONSE CODE**
1.	Have you heard of HPV infection before?	1. Yes 2. No	☐
2.	Have you heard of cervical cancer before?	1. Yes 2. No	☐
3.	Are you aware there are two types of HPV and the high-	1. Yes 2. No 3. I don't know	☐

	risk HPV causes cervical cancer?		
4.	Where did you hear of HPV infection and cervical cancer from?	1. Health care worker 2. Family and friends 3. Internet 4. Social Media 5. TV and Radio stations 6. Others …….	☐
5.	What are some of the screening tools you know are commonly used in Ghana?	1. Pap smear for cytology 2. VIA 3. Both 4. I don't know	☐
	Have you ever been screened of HPV infection?	1. Yes 2. No	☐

6.	If yes, how long has it been?	1. < 1 year 2. 1 year 3. 2 years 4. 3 years 5. > 3 years 6. N/A	☐
7.	Do you think you can get HPV through sexual contact	1. Yes 2. No 3. Not sure 4. I don't know	☐
8.	Are you aware there are HPV vaccines available that help to prevent Cervical cancer?	1. Yes 2. No	☐

SECTION D: RISK FACTORS FOR HPV INFECTION

NO	QUESTIONS	OPTIONS	RESPONSE CODE

1.	How often do you take in alcohol?	1. Always. 2. Most of times. 3. Sometimes. 4. Never.	☐
2.	Do you smoke?	1. Yes 2. No	☐
	If yes, how long have you been smoking?	1. < 1 year 2. 1 year 3. 2 years 4. 3 years 5. > 3 years 6. N/A	☐
3.	Do you take in contraceptives; oral or hormonal contraceptives?	1. Yes 2. No	☐
4.	If yes, how often?	1. Always. 2. Most of times. 3. Sometimes. 4. N/A	☐
5.	How many life-time	1. 1 2. 2 3. 3	☐

	sexual partners have you had?	4. >4	
6.	Do you have any chronic or autoimmune disease?	1. Yes 2. No	☐
7.	If yes, what is it?	1. Diabetes (Type I and II) 2. Rheumatoid Arthritis 3. Hypertension 4. N/A 5. Others. ……	☐
8.	Have you been pregnant before?	1. Yes 2. No	☐
9.	If yes, how many times?	1. 1 2. 2 3. 3 4. >4	☐

THANK YOU.

APPENDIX C

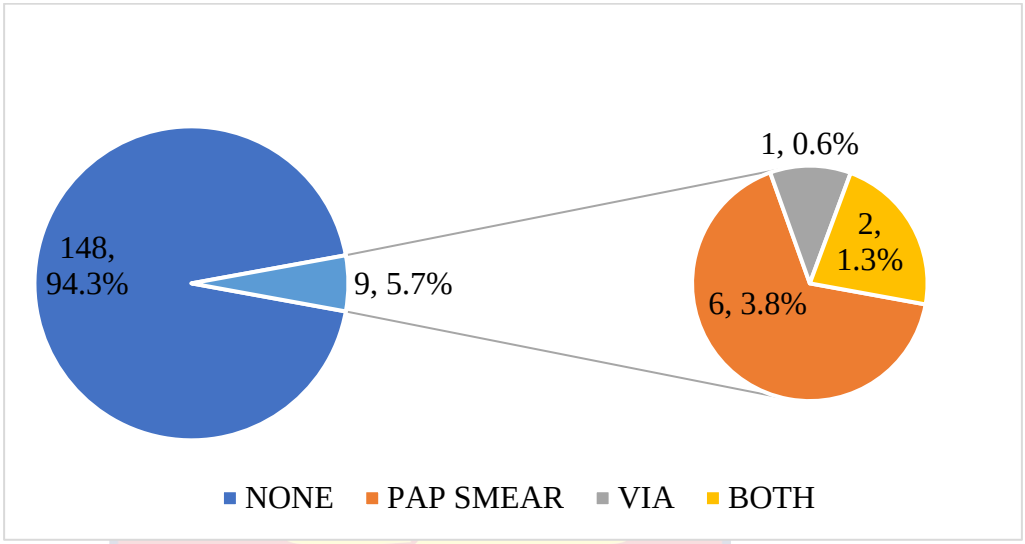

Figure A.1: Proportion of participants who knew of at least one of the common screening tools in Ghana

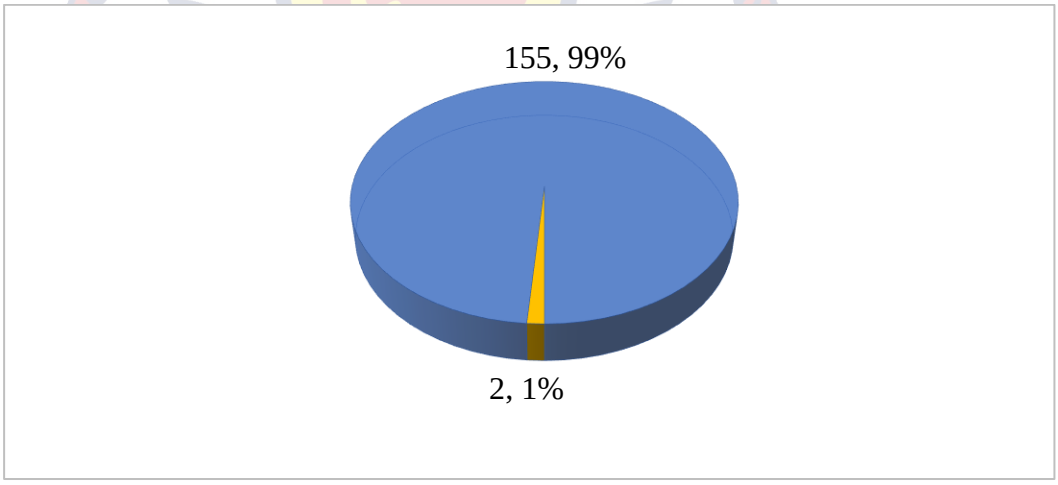

Figure A.2: Number of participants who had previously been screened in the past 5 years.

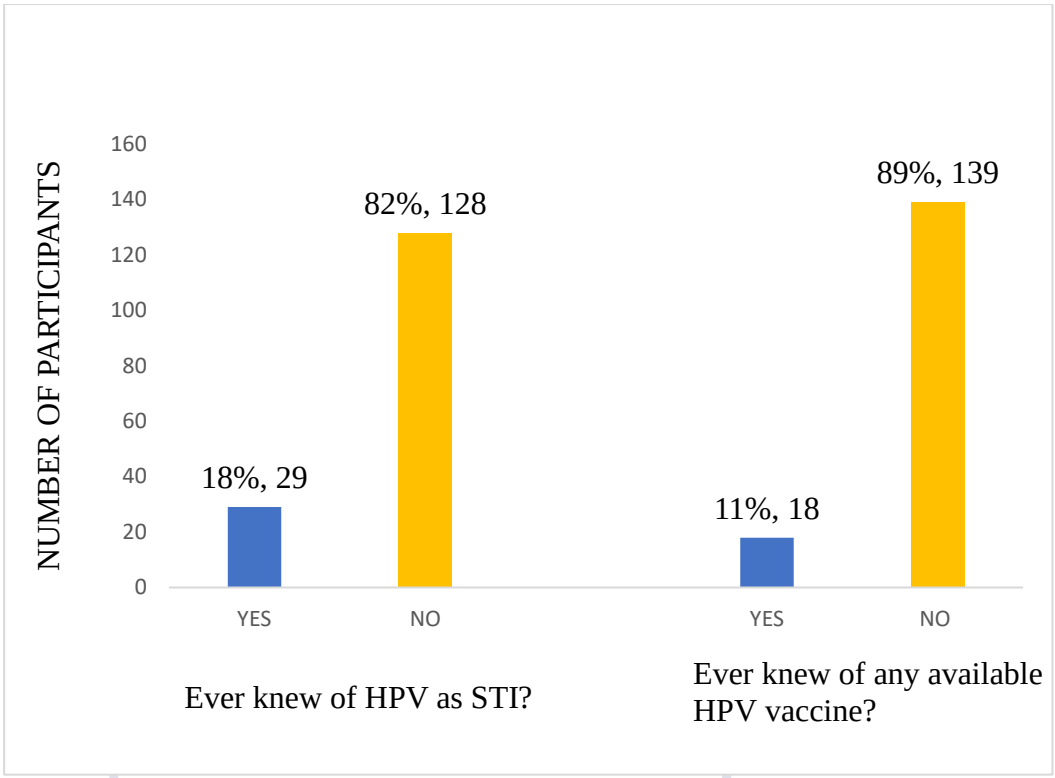

Figure A.3: Number of participants who ever knew HPV is an STI and of any HPV Vaccines available.