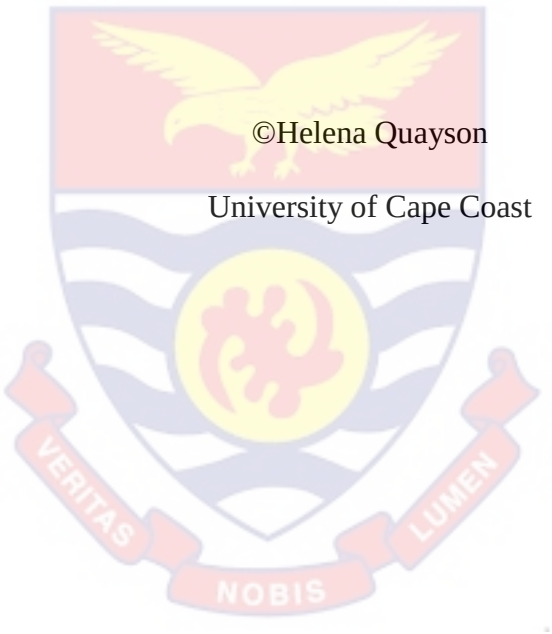
UNIVERSITY OF CAPE COAST

CIRCULATING MICRORNA IN PLASMA OF CERVICAL CANCER PATIENTS AT SOME SELECTED TEACHING HOSPITALS IN GHANA

HELENA QUAYSON

2024



UNIVERSITY OF CAPE COAST

CIRCULATING MICRORNA IN PLASMA OF CERVICAL CANCER PATIENTS AT SOME SELECTED TEACHING HOSPITALS IN GHANA

BY

HELENA QUAYSON

Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Infections and Immunity

JANUARY, 2024

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:

Supervisors' Declaration

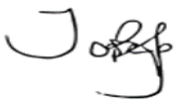
We hereby declare that the preparation and presentation of the thesis we supervised were in line with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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

Name:

Co-Supervisor's Signature: ..... Date: ……

Name:

Co-Supervisor's Signature: …  … Date: ……

Name: Prof. Joseph Humphrey Kofi Bonne

ABSTRACT

Circulating microRNAs (miRNAs) in plasma have emerged as promising biomarkers for various cancers, including cervical cancer. This study investigates the profile of circulating miRNAs in the plasma of cervical cancer patients and their potential role in diagnosis and prognosis. Plasma samples were collected from a cohort of 19 cervical cancer patients and 21 age-matched non-cancerous patients. We identified a panel of differentially expressed miRNAs associated with cervical cancer using quantitative real-time PCR. Notably, miR-27a, and miR-155, exhibited significant upregulation in the patient group compared to non-cancerous patients. Furthermore, receiver operating characteristic (ROC) analysis demonstrated the potential of these miRNAs as biomarkers, with area under the curve (AUC) values exceeding 0.7. Correlation analyses revealed that elevated levels of these miRNAs were associated with advanced clinical stages and poorer overall survival rates. Our findings suggest that circulating miRNAs in plasma could serve as non-invasive biomarkers for cervical cancer, aiding in early diagnosis and personalized treatment strategies. Further validation in larger cohorts is warranted to confirm their clinical utility.

KEY WORDS

MicroRNA

Cervical cancer

Plasma

HPV

Non-invasive biomarker

ACKNOWLEDGMENTS

To God be the glory, great things he has done. Glorious God, I thank you for your guidelines, sustenance, provisions how far you have brought me.

I want to start by expressing my sincere gratitude to my supervisors, Dr. Prince Amoah Barnie, Dr. Roland Osei Saahene, Prof. Joseph Humphrey Kofi Bonney, Rev. Dr. Benjamin Amoani, Dr. Daniel Sam and Dr. Gloria Francisca Nuer-Allornuvor for their enormous assistance, direction, and patience in completing this thesis.

Secondly, I appreciate the immense support of individuals and organizations throughout my study.

I appreciate the tremendous financial support, prayers, and encouragement from my family, friends, and everyone else. I ask that God provide every one of you with unmerited favor and an abundance of grace.

DEDICATION

I dedicate this thesis to the Almighty God, my children, Juanita Baaba Ofori, Eldad Myles Paa Kwamena Ofori and my beloved husband, Dr. Emmanuel Ofori.

TABLE OF CONTENTS

CHAPTER TWO: LITERATURE REVIEW

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CC	Cervical cancer
HPV	Human papilloma virus
CIN	Cervical Intraepithelial Neoplasia
WHO	World Health Organization
SCC	Squamous Cell Carcinoma
VIA	Visual Inspection with Acetic Acid
CA125	Carbohydrate antigen 125
MIRNA	Micro RNA
nt	Nucleotide
tsmiRs	Tumour-Suppressor miRNAs
OncomiRs	Oncogenic MiRNAs
UTR	Untranslated regions
MRNA	Messenger Ribonucleic Acid
HIV	Human immunodeficiency virus
CEA	Carcino embryonic antigen
STD	Sexual Transmitted Disease
IARC	International Agency for Research on Cancer
RISC	RNA-induced Silencing Complex
LnCRNARs	Long non- Coding RNAs
CircRNAs	Circular RNAs
CeRNAs	Competitive endogenous RNAs
PTEN	Phosphatase and Tensin Homeolog
PDCD4	Programmed Cell Death 4
EMT	Epithelial- mesenchymal transition

ZEBI	Zebibyte
SMAD2	Suppressor of Mothers against Decapentaplegic
MDR1	Multi drug resistance protein1
BCL2	B -Cell lymphoma 2
ICC	Invasive Cervical Cancer
TCGA	The Cancer Genome Atlas
qRT-PCR	quantitative Reverse Transcription -Polymerase Chain Reaction
LKB1	Liver Kinase 1
PAK1	Activated Kinase 1
CDC42	Cell division Control Protein 42 homolog
LSIL	Low–grade squamous intraepithelial lesion
FIGO	International Federation of Gynaecology and Obstetrics
STAT3	Signal transducer and activator of transcription 3
NF-B	Nuclear Factor Kappa B
CART	Combination antiretroviral therapy
CCTH	Cape Coast Teaching Hospital
KATH	Komfo Anokye Teaching Hospital
EDTA	Ethylenediaminetetraacetic acid
PCR	Polymerase Chain Reaction
CDNA	Complementary DNA
SD	Standard Deviation
IQR	Interquartile range
ROC	Receiver Operation Characteristic Cur

CHAPTER ONE

INTRODUCTION

This chapter describes the background, problem statement, hypothesis, objective and specific objectives, research questions, significance of the study, and study organization.

1.0 Background of the study

Human papillomavirus is a group of more than 200 related viruses, of which over 40 could infect the genital area, mouth, and throat. Certain kinds of Human papillomavirus have been categorized as high-risk because they are linked to several cancers, such as oropharyngeal, cervical, and anal cancers (Borroso et al., 2022). Usually, sexual contact is how the Human papillomavirus can be spread. Although the Human papillomavirus might raise the risk of certain cancers, warts are a common sign. The Human papillomavirus could be spread mainly through close skin-to-skin contact. Though most sexually active people may contract Human papillomavirus at some point, many infections remain asymptomatic and disappear independently (Saintibert, J. 2024). Prolonged exposure to high-risk Human papillomavirus strains is the main cause of cervical cancer risk. Additional risk factors include using oral contraceptives for an extended period, smoking, and having several sexual partners (World Health Organization, 2021). Persistent Human papillomavirus infections can cause precancerous alterations and cancer, but most infections are cleared by the immune system (Saintibert, J. 2024). Though high-risk Human papillomavirus strains can be detected in cervical cells by Human papillomavirus DNA testing, this test is particularly advised for women 30 years of age and older, frequently in conjunction with

Pap smears, to improve cervical cancer screening (World Health Organization, 2021). The presence of high-risk HPV is indicated by a positive Human papillomavirus DNA test, which may call for additional diagnostic testing (World Health Organization, 2021). Samples utilized for Human papillomavirus DNA testing include self-collected vaginal swabs, cervical swabs, and liquid-based cytology samples, etc.

According to the World Health Organization, 2021, there were 604, 000 cervical cancer diagnoses with an estimated number of 342,000 deaths worldwide among women. This life-threatening illness is mostly caused by the human papillomavirus (HPV). The main risk factors for cervical carcinogenesis are long-lasting, high-risk HPV types 16 and 18 infections. However, invasive illness only occurs in a tiny proportion of women with morphologically expressed HPV infection, showing that other factors were probably connected with the occurrence of cervical cancer. Due to improper detection at the earliest stages of the disease, the mortality rate for cervical cancer is greater in developing nations (Sankaranarayanan et al.,2001).

With regards to histology, cervical cancer can be categorized into two, namely: Squamous cell carcinoma, which results from intraepithelial neoplasia of the cervical epithelium and accounts for about 80% of cases of cervical cancer, and 5–20 percent of cases are adenocarcinomas, which result from glandular dysplasia and intraepithelial adenocarcinomas (Müller,2013). Cervical intraepithelial neoplasia (CIN) can be divided into two types: CIN1 (low-grade CIN), and CIN2–3 (high-grade CIN). High-grade CIN could occur two to three years after a high risk of HPV infection, which may culminate in cervical cancer after 10 years (Nagamitsu et al., 2016). However, cervical

cancer could also be staged using the International Federation of Gynaecology and Obstetrics (FIGO) system. The four main stages are 1, 2, 3 and 4, with substages of 1A, 1A1, 1A2, 1B, 1B1, 1B2, 2A, 2A1, 2A2, 2B1, 3A, 3B, 3C, 3C1, 3C2, 4A, and 4B which account for the different features of cervical cancer (Lee, S. I., & Atri, M. 2019).

A study by Etzioni et al. (2003) proposed that a wide array of therapeutic techniques would be readily available, and the diseased condition would be cured if cervical cancer is detected early before it progresses to an invasive condition. However, the disease exhibits symptoms and signs which include vaginal bleeding with or without discharge and pelvic pain, typically in its advanced stage. Pap smear, colposcopy, and the Visual Inspection with Acetic Acid (VIA) test are a few examples of diagnostic procedures. The Pap smear test's sensitivity, specificity, positive predictive value, and negative predictive value were evaluated, and they were discovered to have corresponding values of 54.8%, 81.70%, 47.20%, and 85.80%. (Honarvar, Kermani, & Robati, 2021). Even though the invasiveness of these procedures limits their efficiency, colposcopy, and random biopsies may be able to detect some early stages of cervical cancer. Though Cervical intraepithelial neoplasia (CIN) has been asymptomatic and difficult to detect with palpation or physical examination, using these techniques to diagnose the condition may not always be successful (Jia et al., 2015). To monitor and forecast cervical cancer, however, the two most common serum tumor indicators were squamous cell carcinoma (SCC) antigen and carbohydrate antigen 125 (CA125). Neither one of these biomarkers, however, was specific to this cancer. In light of this, for early detection of malignancies, it is relevant

to take into account more precise, accurate, and non-invasive biomarkers to lower global morbidity and mortality. Available treatments could be used to cure patients with cervical cancer when detected in its early stages (WHO, 2020). This necessitates another dimension of research involving the use of microRNA. The non-coding ribonucleic acids known as microRNAs, which are about 19–24 nucleotide (nt) in length and are primarily dysregulated in cancer, have proven to be particularly helpful as tissue-based markers for cancer classification (Sorensen et al.,2013; Li et al.,2010).

MicroRNAs target the transcripts of proto-oncogenes or tumor suppressors in a variety of tumor forms (Wang et al.,2008). Tumor-suppressor microRNAs (tsmiRs) and oncogenic microRNAs (oncomiRs) are two different categories of microRNAs (Svoronos et al.,2016). OncomiRs are frequently displayed, which promotes the growth of tumors and maintains their phenotypic characteristics. The tsmiRs, however, suppress carcinogenesis by regulating apoptosis, invasion, and other cancer-related processes in cells. In the majority of human malignancies, these tsmiRs are often downregulated (Ali et al., 2020). Previous research by (Pereira et al.,2010; Hu et al., 2010) has highlighted the various tissue microRNA expression profiles in cervical cancer with accurate prognosis and diagnosis. Although microRNA appears to be helpful in diagnosis, its global patterns of expression in patients with cervical cancer plasma have not yet been discovered (Jia et al., 2015). Given that microRNA may be found in the majority of bodily fluids, including plasma, this emphasizes the necessity for more investigation into microRNAs' potential as biomarkers. In addition, this diagnostic procedure is less invasive and cost-effective (Yang et al., 2013).

Most microRNAs are known to regulate their target mRNA through diverse physiological and pathological mechanisms and have been discovered to be inappropriately expressed in a variety of human cancers (Sohel, 2016). It is well known that microRNA binds to the 3'-UTR of target mRNA to impede translation of the target mRNA (O'Brien, Hayder, Zayed, & Peng, 2018). This could also result in target mRNA degradation. MicroRNAs have the potential to control about one-third of human genes, which can have up to 1,000 targets (O'Brien, Hayder, Zayed, & Peng, 2018). About 50% of microRNAs located in the genomic region are closely linked with cancer (Miao et al., 2020; Nagamitsu et al., 2016). These serve as sufficient reasons to research the different expression profiles of microRNA for specific cancers (Miao et al., 2020).

1.1 Problem Statement

Cervical cancer is a major worldwide health concern, especially in areas with limited resources. Traditional screening methods like Pap smears and HPV tests are crucial for early diagnosis, but they are usually associated with a variety of disadvantages, including discomfort when sampling, invasiveness, and logistical challenges. These factors have led to reduced screening uptake and delayed diagnosis, contributing to higher mortality rates. There is therefore a critical need for non-invasive, trustworthy biomarkers that can improve early identification, monitor treatment responses, and risk-based patient stratification because existing cervical cancer screening techniques, such as Pap smears and HPV testing, might overlook precancerous lesions or early-stage malignancies (Sharma et al., 2019). Recent research has identified microRNAs (miRNAs) which are short, non-coding RNA molecules that

regulate gene expression and are involved in cancer progression. miRNAs serve as potential non-invasive biomarkers, offering a promising alternative for the early detection of cervical cancer and monitoring of treatment in diagnosed patients. According to research, miRNAs, like miR-21 and miR-145, are dysregulated in cervical cancer and may be used as non-invasive indicators for early observation (Gonzalez et al., 2017; Zhang et al., 2018).

This study determined the expression patterns of circulating plasma microRNAs and their potential use as diagnostic and prognostic biomarkers in cervical cancer.

1.2 Aim

The general aim of this study was to assess the expression patterns of microRNA that are differently expressed in cervical cancer patients on or not on therapy and non-cancerous patients.

1.3 Specific objectives

The study aims to accomplish the following

1. To identify the expression patterns of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a that are differently expressed among cervical cancer patients on therapy, cervical cancer patients not on therapy and non-cancerous patients.
2. To identify the expression patterns of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a that are differently expressed in cervical cancer patients based on the FIGO stages of cervical cancer.

3. To identify the effect of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a that are differently expressed in cervical cancer patients on therapy.
4. To determine the diagnostic performance of the expression pattern of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a that are differently expressed in cervical cancer patients, not on therapy and non-cancerous patients using the Receiver Operating Characteristic (ROC) curve.

1.4 Research questions

1. What microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a are differentially expressed among cervical cancer patients on therapy, cervical cancer patients not on therapy and non-cancerous patients that could serve as diagnostic, prognostic or treatment marker?
2. What microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a are differentially expressed between cervical cancer patients based on the FIGO stages of cervical cancer?
3. What is the effect of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a that are differentially expressed among cervical cancer patients on therapy?
4. What is the diagnostic performance of the expression patterns of microRNAs such as miR-146a, miR-29a, miR-29b, miR-34a, miR-233, miR-155, and miR-27a among cervical cancer patients not on

therapy and non-cancerous patients using the Receiver Operating Characteristic (ROC) curve?

1.5 Hypothesis

H_1: There is a significant difference among microRNAs that are differently expressed in cervical cancer patients on or not on therapy and healthy individuals.

H_0: There is no significant difference among microRNAs that are differently expressed in cervical cancer patients on or not on therapy and healthy individuals.

1.6 Significance of the study

Worldwide, cervical cancer is a serious health risk for women, hence efficient and reliable screening methods are required. Traditional techniques, such as HPV testing and Pap smears, are crucial for early detection but several drawbacks have reduced their efficacy. Many women find pelvic exams and cervical swabbing, which are traditional screening methods, to be frightening and uncomfortable, and this may deter them from regularly participating in screening programs (Schiffman et al., 2016). Women may find it challenging to obtain timely screenings in low-resource environments due to limited access to healthcare facilities and trained specialists in sample taking. The problem is further complicated by the requirement for clinical facilities and qualified personnel (World Health Organization, 2021).

Traditional screening methods may again yield false results, leading to unnecessary anxiety, additional testing, or missed diagnoses. This unreliability can erode trust in the screening process (Sharma et al., 2019). In contrast to these standard screening methods, research has shown that microRNAs

(miRNAs) from blood could be used as viable alternative biomarkers. Specific miRNAs are differentially expressed in cervical cancer patients compared to healthy individuals, indicating their potential for early detection (Zhang et al., 2018). Early identification can significantly improve treatment outcomes. Utilizing miRNA-based testing from blood could reduce the costs linked to traditional screening methods, as non-invasive testing may require fewer resources and infrastructure (Sharma et al., 2019). Studies have demonstrated that miRNAs such as miR-21 and miR-145 are promising candidates for cervical cancer biomarkers, showing significant differences in expression levels between cancerous and non-cancerous tissues (Gonzalez et al., 2017). Implementing miRNA testing from blood could enhance accessibility to cervical cancer screening, particularly in underserved populations, thereby reducing disparities in healthcare access (World Health Organization, 2021). In 2016, Peng and Croce conducted a study in which they discovered that microRNA from blood was vital for the advancement, progression, and therapeutic targets of cervical cancer.

The findings of this research would provide preliminary data that could serve as a push to further advocate for the use of microRNAs from plasma in early diagnosis and prognosis in cervical cancer patients.

1.7 Delimitation

The research is a cross-sectional study and the main areas of attention were Cape Coast in Ghana's Central Region and Kumasi in Ghana's Ashanti Region. Samples were obtained from the Obstetrics and Gynaecology Unit of Cape Coast Teaching Hospital and the Oncology Department of the Komfo Anokye Teaching Hospital. The total number of samples obtained was 40

using a convenient sampling technique, thus, samples were taken from available participants that were willing to partake in the study after answering the well-structured questionnaire. Socio-demographics of the participants were collected.

1.8 Limitation

Two selected teaching hospitals were used for the study's sample collection and because these hospitals were referral centers, only data available was used. Even though the sample size used was within the acceptable range, it was relatively small and may affect the generalization of the result in certain instances. Data was collected from females between the ages of 24 to 60 years. The study design was cross-sectional rather than longitudinal study. The duration was based on the timelines given to complete the academic exercise, however, the number of participants that enrolled in the study was limited because we used available samples at each selected teaching hospital within the time frame.

1.9 Definition of terms

Human Papillomavirus (HPV) Infection: HPV infection is a sexually transmitted infection caused by the human papillomavirus, some high-risk types of HPV can lead to cervical cancer and other anogenital cancers, as well as oropharyngeal cancers.

Cervical cancer: A specific form of cancer that affects the uterine cervix.

HIV: The human immunodeficiency virus is an infection that damages the body's immune system.

MicroRNA: This is a 21–23 nucleotide, single-stranded, tiny non-coding RNA molecule.

1.12 Organization of the Study

Five (5) chapters make up the study's framework.

CHAPTER ONE: As a result, the first chapter describes the background of the entire study, knowledge gaps, the purpose of the investigation, and the research issues that had to be addressed.

CHAPTER TWO: This chapter reviews the available literature. therefore, it includes information on the epidemiological traits of cervical cancer, theories of microRNA as well as the biogenesis of those traits. Regarding this work, many comparable research and reports that have been published locally and internationally are reviewed.

CHAPTER THREE: This comprises the numerous tools, supplies, and reagents employed during the study. An overview of the research methodology was provided, including a discussion of the study design, study location, sample size, data collection, sample collection, target population, inclusion and exclusion criteria, laboratory procedures, analysis, and ethical clearance.

CHAPTER FOUR: This comprises the discussion of the major findings as well as the study's results. The explanation of the findings, with a focus on how the data compares or diverges from previously collected data. The study's goals were discussed in the discussions.

CHAPTER FIVE: The background and conclusions of the study's findings were summarized in the last chapter, which also addresses the study's goals and objectives. Based on the investigation, a conclusion was drawn, along with some suggestions.

1.10 Chapter Summary

In conclusion, this chapter provides an overview of the study's background, with a focus on essential terms including HPV infection, cervical cancer, HIV, and microRNA. Additionally, the main objectives of the study are outlined, along with the precise queries it aims to address. The study's goals, including knowledge gaps, contradictions, and the use of study data, are further explained in this chapter.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter discusses articles and research on the causes of cervical cancer and the patterns of microRNA expression in patient plasma. The literature review in Chapter 2 examines the thematic areas of the research objectives. These include the Genome of HPV, the Global HPV infection rate, HPV infection rates in West Africa, HPV infection rates in Africa, Classification of HPV, Risk factors of HPV, Specimens used for HPV testing, WHO Recommendations for HPV DNA screening, the Genome of cervical cancer, the Global cervical cancer infection rate, cervical cancer infection rates in West Africa, cervical cancer infection rates in Africa, Classification of cervical cancer, Risk factors of cervical cancer, diagnosis for cervical cancer, WHO Recommendations for cervical cancer screening, the epidemiology of HPV and cervical cancer, theories of microRNA, functions of microRNA in cervical cancer, microRNA expression patterns in cervical cancer patients on therapy, and those not on therapy, and patterns of microRNA expression in patients with or without HIV who have cervical cancer.

Although Human papillomavirus (HPV), may infect a range of anatomical sites, including the neck, anogenital organs, oral cavity, and pulmonary cavities, the majority of studies and publications have found that over 95% of instances of cervical cancer are caused by the most prevalent etiologic pathogen, HPV (Katsenos & Becker,2011; Pierce et al.,2016). Certain human papillomavirus (HPV) genotypes increase the likelihood of malignant lesions developing. On the other hand, viruses are not the only

factor in this disease's development. Other behavioral factors can influence the development of cancer including prolonged use of hormonal birth control, smoking, initial sexual encounters at an early age with unknown status of partners, family history, having numerous partners, and decreased immunity (Song et al.,2015). It has been known for the past 30 years that persistent high-risk HPV infection results in intraepithelial alterations at the squamous-columnar junction that eventually leads to cancer (Castellsague, 2008).

In 2018, 6.6 percent of all cancer diagnoses and 7.5 percent of all cancer deaths among female patients were attributable to cervical cancer, the fourth most common disease among women worldwide (Bray, 2018). Cervical cancer is one of the leading causes of death among fertile women (Parkin et al., 2008). Though cervical cancer is non-specific, and asymptomatic in its early stages, it has a high mortality rate, and very challenging to identify it early (Kyrgiou et al., 2010). Early detection of cervical cancer is crucial, and there are various effective treatments available (Du et al., 2020). The two most often utilized diagnostics for detecting cervical cancer at the moment are the colposcopy and the Papanicolaou test (Pap smear). The specificity for pap smear screening was 98%, while its sensitivity was just 51% (Wright et al., 2004).

Furthermore, cervical cancer in situ and adenocarcinoma are difficult to detect with a Pap smear screening. Cervical cancer biopsies and colposcopies may be able to detect certain early-stage cervical cancers, but invasive patients may delay treatment, increasing costs and risks (Jia et al., 2015). Since serum tumor biomarkers may be analyzed using non-invasive blood samples, cervical cancer is commonly identified and tracked using these

methods. Squamous cell carcinoma antigen (SCC Ag), CA19-9, and carcinoembryonic antigen are some of these indicators (CEA) (Tsikouras et al., 2016). However, none of them have the sensitivity and specificity required to find cervical cancer in its earliest stages (Tsikouras et al., 2016). Moreover, according to current studies, microRNAs may prove to be incredibly sensitive and specific markers for the non-invasive screening of cervical cancer (Pereira et al., 2010; Hu et al., 2010). It has been discovered that microRNA dysregulation frequently happens in cancer (Bartel et al., 2004; Lee et al., 2018).

In healthy and pathological circumstances, microRNAs have a deleterious effect on the transcriptional and post-transcriptional phases of gene expression. MicroRNAs have changed over time to become species-widely conserved (Ambros, 2008; Hrdlickova et al., 2014). Research has shown that microRNAs may have an impact on up to 60% of protein-coding genes, earning them the title of "master modulators" of the human genome (Lewis et al., 2005; Lim et al., 2005). A microRNA may typically aim at 200 mRNAs; however, it is also possible for several microRNAs to target the same mRNA (Krek et al., 2005).

In biological systems, among the many cellular processes and functions that microRNAs play a significant part in controlling are angiogenesis, cell cycle regulation, differentiation, apoptosis, DNA repair, and stress response (Chen, 2010). MicroRNAs have later become crucial disease regulators, including those of cancer when alterations in their expression take place (Farazi et al.,2011). Genetic and epigenetic processes influencing

microRNA expression include DNA amplifications or deletions that add or remove microRNA locus regions (Jiménez-Wences et al.,2014).

2.1 Genome of HPV

HPV has a circular double-stranded DNA genome that is approximately 7,000 to 8,000 base pairs in length (Tsakogiannis., et al2017). The genome is divided into **early (E)** and **late (L)** regions and these are; **Early Genes**:**E1**: Involved in viral DNA replication, **E2**: Regulates the transcription of other early genes, **E4**: Plays a role in the viral life cycle and may facilitate cell lysis, **E5**: Enhances the ability of the virus to evade immune detection, and **E6 and E7**: Critical for oncogenicity, as they interfere with tumor suppressor proteins (e.g., E6 binds to p53, and E7 binds to Rb). **Late Genes**: **L1**: Codes for the major capsid protein, forming the viral shell, and **L2**: Codes for the minor capsid protein. High-risk HPV types (e.g., HPV 16 and HPV 18) possess genetic characteristics that allow them to disrupt normal cellular processes, leading to carcinogenesis (Bouvard et al., 2009; Dutra et al., 2012).

2.2 Global HPV Infection Rates

HPV is one of the most prevalent sexually transmitted infections (Johnson, A., & Jackson, J. B. 2021). Estimates suggest that about **11-12%** of sexually active individuals globally are infected with HPV at any given time (Forman et al.,2012). By age 50, up to **80%** of sexually active women may have been infected with at least one type of HPV (Smith et al.,2008). High-risk HPV types, particularly **HPV 16** and **HPV 18**, are responsible for approximately **70%** of cervical cancer cases worldwide. Low-risk types, such as **HPV 6** and **HPV 11**, are primarily associated with benign conditions like genital warts (Garland, S. M. 2002). Higher prevalence rates are observed in

sub-Saharan Africa and Latin America, while lower rates are noted in some parts of Asia and North America. The overall prevalence of high-risk HPV types varies significantly by region, with studies indicating rates of around **30-40%** in certain populations (Smith et al.,2008). HPV vaccination programs have shown reduced infection rates among vaccinated cohorts, particularly younger women (Orumaa et al.,2020).

2.3 HPV Infection Rates in Africa

HPV prevalence in sub-Saharan Africa is notably high, with studies indicating rates of approximately **25-50%** among sexually active women (Ginindza et al., 2017). High-risk HPV types are found in about **30-40%** of women, with HPV 16 and HPV 18 being the most common among those with cervical cancer (Kulkarni et al.,2011). Africa has one of the highest rates of cervical cancer globally, with an estimated incidence of **11.9 per 100,000 women (**Denny et al., 2014). The burden of HPV-related diseases is significant, contributing to the high mortality rates from cervical cancer in the region (Vaccarella, S., & Bray, F. 2015). The prevalence of HPV can vary widely between countries and regions within Africa. Factors such as access to healthcare, screening programs, and vaccination uptake play critical roles. Efforts to implement HPV vaccination programs are ongoing, with increasing coverage expected to reduce HPV prevalence in the future (Bruni et al.,2021).

2.4 HPV Infection Rates in West Africa

HPV infection rates in West Africa vary widely, with studies reporting prevalence rates between **20% and 50%** among sexually active women (Smith et al., 2008). High-risk HPV types, particularly HPV 16 and 18, are commonly detected and are associated with cervical cancer (Ramakrishnan et

al., 2015). Specific countries such as Ghana and Nigeria report higher prevalence rates, often exceeding **30%** among women attending gynecological clinics (Konadu et al.,2019). Variability is influenced by factors such as access to healthcare, awareness of HPV, and cultural practices (Btoush et al.,2022). High-risk HPV types are responsible for over **90%** of cervical cancer cases, making the understanding of HPV prevalence crucial for public health strategies (Sammarco et al.,2020). Efforts to implement HPV vaccination programs are ongoing, aimed at reducing future infection rates and the incidence of cervical cancer (Oliveira, C. R., & Niccolai, L. M. 2021).

2.5 HPV Infection Rates in Ghana

HPV prevalence among sexually active women in Ghana has been reported to be around **30% to 50%**, with high-risk types contributing significantly to cervical cancer cases (Adams et al.,2019). A study indicated that high-risk HPV types were found in approximately **37%** of women tested (Trottier et al., 2006). Ghana has a notable incidence of cervical cancer, with estimates suggesting an incidence rate of about **25 per 100,000 women (**Appiah, M. T. 2023). The country has initiated programs to improve screening and vaccination to combat cervical cancer. Efforts to increase HPV vaccination coverage among young girls are underway, aimed at reducing future infection rates and cervical cancer risk.

2.6 Classification of HPV

HPV is classified into more than 200 types, which are generally grouped into two main categories based on their oncogenic potential:

1. **Low-Risk HPV Types**: Associated with benign conditions like genital warts and low-grade cervical lesions.

Common Low-Risk Types: **HPV 6**: Often linked to genital warts.

HPV 11: Also associated with genital warts.

2. **High-Risk HPV Types**: Linked to the development of cervical cancer and other anogenital cancers.

   Common High-Risk Types: **HPV 16**: Responsible for a significant proportion of cervical cancer cases.

   - **HPV 18**: Another major contributor to cervical cancer.
   - Other notable high-risk types include **HPV 31, 33, 45, 52, and 58**.

2.7 Risk Factors for HPV Infection

1. **Sexual Activity**: Having multiple sexual partners increases the risk of exposure to HPV.
   - Early initiation of sexual activity can also heighten the risk.
2. **Weakened Immune System**: Individuals with weakened immune systems (e.g., due to HIV infection or immunosuppressive treatments) are at a higher risk of persistent HPV infection.
3. **History of Sexually Transmitted Infections (STIs)**: A history of STIs can increase susceptibility to HPV.
4. **Lack of HPV Vaccination**: Not receiving the HPV vaccine increases the risk of contracting high-risk HPV types.
5. **Smoking**: Tobacco use is associated with a higher risk of developing cervical cancer in HPV-infected individuals.

2.8 Specimens Used for HPV DNA Testing

HPV DNA testing is crucial for detecting high-risk virus types, especially in cervical cancer screening. The types of specimens commonly used include:

1. **Cervical Swabs**

   The most common specimen type for HPV DNA testing is a cervical swab, which can be collected during a Pap smear. This method allows for the collection of cells from the cervix, which are then tested for HPV DNA (American College of Obstetricians and Gynecologists, 2016).

2. **Liquid-based Cytology Samples**

   In liquid-based cytology, cervical cells are collected and suspended in a liquid medium. This specimen can be used for both Pap and HPV DNA testing, offering improved sensitivity and easier processing (Centers for Disease Control and Prevention, 2022).

3. **Self-collected Vaginal Swabs**

   Research has shown that self-collected vaginal swabs can be effective for HPV DNA testing. This method increases accessibility and comfort for women, especially in screening programs (World Health Organization, 2021).

4. **Endocervical Curettage**

   In certain cases, endocervical curettage (a procedure that involves scraping cells from the inside of the cervical canal) may be used to collect specimens for HPV DNA testing. This method is less common but can be helpful in specific clinical situations (National Cancer Institute, 2023).

2.9 WHO Recommendations for HPV DNA Screening

1. **Screening Age**: Women should begin HPV DNA screening at age 30.
2. **Frequency**: HPV DNA testing is recommended every 5 years.

3. **Method**: HPV DNA testing is preferred over other screening methods (such as Pap smear) due to its higher sensitivity for detecting cervical precancer and cancer.
4. **Follow-Up**: Women with positive HPV DNA results should be referred for further evaluation and management, including colposcopy.
5. **Integration with Vaccination**: The WHO emphasizes the importance of integrating HPV vaccination programs with screening efforts to maximize cervical cancer prevention.

2.10 Genomic profile of Cervical Cancer

Cervical cancer is characterized by genetic alterations, including mutations, chromosomal rearrangements, amplifications, and deletions (Balasubramaniam et al., 2019). Key oncogenes affected include **CCND1** (cyclin D1) and **MYC**, while tumor suppressor genes like **TP53** (p53) and **RB1** (retinoblastoma) are frequently altered (Xing, D., & Fadare, O.2021). High-risk HPV types, particularly **HPV 16** and **HPV 18**, integrate their DNA into the host genome (Liu et al.,2015). This integration can disrupt normal cellular processes and lead to oncogenesis (Pett, M., & Coleman, N. 2007). The expression of HPV oncogenes E6 and E7 is critical in inactivating tumor suppressor proteins, leading to uncontrolled cell proliferation (Almeida et al.,2019). Whole-genome sequencing has identified distinct molecular signatures in cervical cancers that may guide therapeutic approaches (Cancer Genome Atlas Research Network et al., 2017). These signatures can include alterations in pathways related to cell cycle regulation, apoptosis, and DNA repair (Dixon, K., & Kopras, E. 2004). Understanding the genomic landscape

of cervical cancer is crucial for developing targeted therapies and personalized medicine strategies (Hu, Z., & Ma, D. 2018).

2.11 Global Cervical Cancer Rates

Cervical cancer is the fourth most common cancer among women worldwide, with an estimated **604,000 new cases** diagnosed in 2020 (Schubert et al.,2023). The global age-standardized incidence rate is approximately **9.2 per 100,000 women (**Zhang et al.,2019). Cervical cancer accounts for about **342,000 deaths** annually, making it a significant cause of cancer-related mortality among women (Hosono, S. 2024). The global mortality rate is approximately **7.5 per 100,000 women (**Sopik et al.,2015). The highest incidence rates are observed in sub-Saharan Africa, which can exceed **43.1 per 100,000 women (**Jedy-Agba et al.,2020). Regions with effective screening and vaccination programs, such as North America and Western Europe, have much lower rates (Chan et al.,2019). The majority of cervical cancer cases (over **70%**) are associated with persistent infection with high-risk HPV types, particularly HPV 16 and HPV 18 (Chan et al.,2019).

2.12 Cervical Cancer Rates in Africa

Cervical cancer is a leading cause of cancer among women in Africa, with an estimated incidence rate of about **43.1 per 100,000 women** across the continent (Jedy-Agba et al.,2020). Certain countries, particularly in sub-Saharan Africa, report significantly higher rates, exceeding **50 per 100,000 women** in some regions (De Vuyst et al.,2013). Cervical cancer results in approximately **66,000 deaths annually** in Africa, making it a major public health concern (Afroj et al.,2017). The mortality rate varies widely, reflecting healthcare access, screening, and treatment disparities (Baciu et al.,2017).

Sub-Saharan Africa bears the brunt of the burden, with high-risk factors including limited access to screening programs and HPV vaccination (Murewanhema et al.,2024). Countries such as Malawi, Zambia, and Tanzania report particularly high incidence and mortality rates. The majority of cervical cancer cases (over **90%**) in Africa are linked to persistent infections with high-risk HPV types (De Martel et al.,2017).

2.13 Cervical Cancer Rates in West Africa and Ghana

Cervical cancer is a significant health issue in West Africa, with incidence rates ranging from **20 to 13.1 per 100,000 women** in various countries (Arbyn et al.,2020). Countries like Ghana, Nigeria, and Sierra Leone report some of the highest rates in the region. Cervical cancer is one of the leading cancers affecting women in Ghana, with an estimated incidence rate of about **29.3 per 100,000 women (**Abotchie, P. N., & Shokar, N. K. 2009). The country has one of the highest rates of cervical cancer in West Africa. Cervical cancer accounts for a significant number of cancer-related deaths among women in Ghana, with approximately **18.6 per 100,000 deaths** annually attributed to the disease (Krings et al.,2019). The region experiences high mortality rates associated with cervical cancer, with an estimated **341,831 deaths** annually attributed to the disease (Bogdanova et al.,2022). Limited access to screening and treatment contributes to these high mortality figures (Binka et al.,2019). The prevalence of high-risk HPV types is a major contributor, with studies showing that **over 90%** of cervical cancer cases are linked to HPV infections (De Martel et al.,2017). Other factors include lack of awareness, cultural barriers, and inadequate healthcare infrastructure (Petersen et al.,2022). Efforts are ongoing to improve HPV vaccination and cervical

cancer screening programs, but coverage remains low compared to global standards (Wirtz et al.,2022).

2.14 Classification of Cervical Cancer

Cervical cancer can be classified based on histological type and tumor behavior:

1. **Histological Types**:
    - **Squamous Cell Carcinoma (SCC)**:
        - The most prevalent type, making up about 70-90% of cervical cancer cases. It arises from the squamous epithelial cells of the cervix.
    - **Adenocarcinoma**:
        - Accounts for approximately 10-25% of cervical cancers, originating from the epithelial cells. Subtypes include endocervical adenocarcinoma and clear cell carcinoma.
    - **Other Rare Types**:
        - Include neuroendocrine carcinoma, small cell carcinoma, and adenosquamous carcinoma, which are less common but may be more aggressive.
2. **Staging**:
    - Cervical cancer is also classified by the FIGO (International Federation of Gynecology and Obstetrics) staging system, which includes:
        - **Stage I**: Invasive cancer confined to the cervix.

- **Stage II**: Cancer that has spread beyond the cervix but not to the pelvic wall or lower third of the vagina.
- **Stage III**: Cancer that has spread to the pelvic wall, the lower third of the vagina, or causes hydronephrosis.
- **Stage IV**: Cancer that has spread to distant organs.

2.15 Risk Factors for Developing Cervical Cancer

1. **Long-term Use of Oral Contraceptives**: Extended use (five or more years) of birth control pills has been associated with an increased risk of cervical cancer.
2. **Multiple Pregnancies**: Women who have had multiple full-term pregnancies may have a higher risk.
3. **Age**: The risk of cervical cancer increases with age, particularly in women over 30.
4. **Low Socioeconomic Status**: Limited access to healthcare services can lead to lower screening rates and delayed diagnosis.
5. **Genetic Factors**: A family history of cervical cancer may increase an individual's risk.
6. **Co-infection with HIV**: HIV-positive Women have a significantly higher risk of developing cervical cancer due to immunosuppression.

2.16 Diagnosis of Cervical Cancer

1. **Screening Tests**:
    - **Pap Smear (Pap Test)**: Detects precancerous changes in cervical cells. It is recommended for women starting at age 21.

- **HPV Testing**: Identifies high-risk HPV types that can lead to cervical cancer. It can be done alone or with a Pap smear (co-testing) for women aged 30 and older.

2. **Diagnostic Procedures**:
   - **Colposcopy**: If abnormal cells are detected, a colposcopy may be performed, which allows the doctor to examine the cervix more closely using a magnifying device.
   - **Biopsy**: During colposcopy, a biopsy may be taken to determine if cancerous cells are present.
3. **Imaging Tests**:
   - **Pelvic MRI or CT Scan**: These imaging tests may be used to assess the extent of cancer if diagnosed.
4. **Histological Examination**:
   - A tissue sample is analyzed to confirm the diagnosis and determine the type and grade of cancer.

2.17 WHO Recommendations for Cervical Cancer Screening

1. **Screening Age**:
   - **Initiation**: Women should start screening at age 30.
   - **Frequency**:
     - Every 5 years with HPV testing.
     - Every 3 years with Pap smear (cervical cytology) for women aged 30-49.
     - Women aged 50 and older can continue screening until age 70.

2. **Screening Methods**:
    - **HPV Testing**: Recommended as the primary method due to its higher sensitivity for detecting precancerous lesions.
    - **Pap Smear (Cytology)**: it can be used, but less sensitive compared to HPV testing.
    - **Visual Inspection with Acetic Acid (VIA)**: An alternative method for low-resource settings.
3. **Follow-Up**:
    - Women with abnormal screening results should be referred for further evaluation and management according to local guidelines.
4. **HPV Vaccination**:
    - The WHO highlights the importance of HPV vaccination as a preventive measure to reduce the incidence of cervical cancer.

2.18 Epidemiology of HPV and cervical cancer

Human papillomavirus (HPV), a common sexually transmitted illness, is associated with cervical cancer and other diseases. In this response, a general overview of the HPV and cervical cancer epidemiology are provided.

Overview of HPV prevalence: HPV is the most prevalent sexually transmitted disease (STD) globally, affecting both men and women. By geography and population, HPV infection rates differ globally. According to the World Health Organization (WHO), 2014, approximately 1 in 10 women worldwide have an HPV infection at any given time. The majority of HPV cases occur in sexually active people in their late teens and early twenties. According to studies by Akpabla, G. S., & Sun, K. (2021), there were over

570,000 new cases of cervical cancer discovered worldwide in 2018 with HPV infection being responsible for nearly 90% of these cases. The prevalence of the human papillomavirus was higher in low- and middle-income countries. For example, a study by Gu et al. (2022) reported that the age-standardized HPV prevalence among women aged 15–64 years varied from 3.2 to 19.3 percent in Australia/New Zealand and sub–Saharan Africa.

HPV types and cervical cancer risk: Significant risk factors are the emergence of cervical carcinoma and HPV infection. Certain HPV strains, especially high-risk variations like HPV-16 and HPV-18, are the primary causes of cervical cancer cases (Ramakrishnan et al., 2015). Cervical cancer risk is significantly increased by ongoing exposure to high-risk HPV strains (Walboomers et al.,1999). It was estimated that HPV infection causes 99 percent of cases of cervical cancer. Due to its high frequency and mortality in low- and middle-income nations, access to screening and care was restricted, making cervical cancer a serious global health concern.

According to the International Agency for Research on Cancer (IARC), cervical cancer caused 604,127 new cases and 341,831 fatalities worldwide in 2020. Sub-Saharan Africa, Latin America, and the Caribbean have the greatest incidence and mortality rates for cervical cancer, which differ greatly by region. Numerous studies have shown that sexual activity poses a significant risk for HPV infection and cervical cancer growth (Faridi et al.,2011). Some of these risk factors include high-risk sexual conduct, several sexual partners, and first sexual contact at an early age (Brinton et al.,1987).

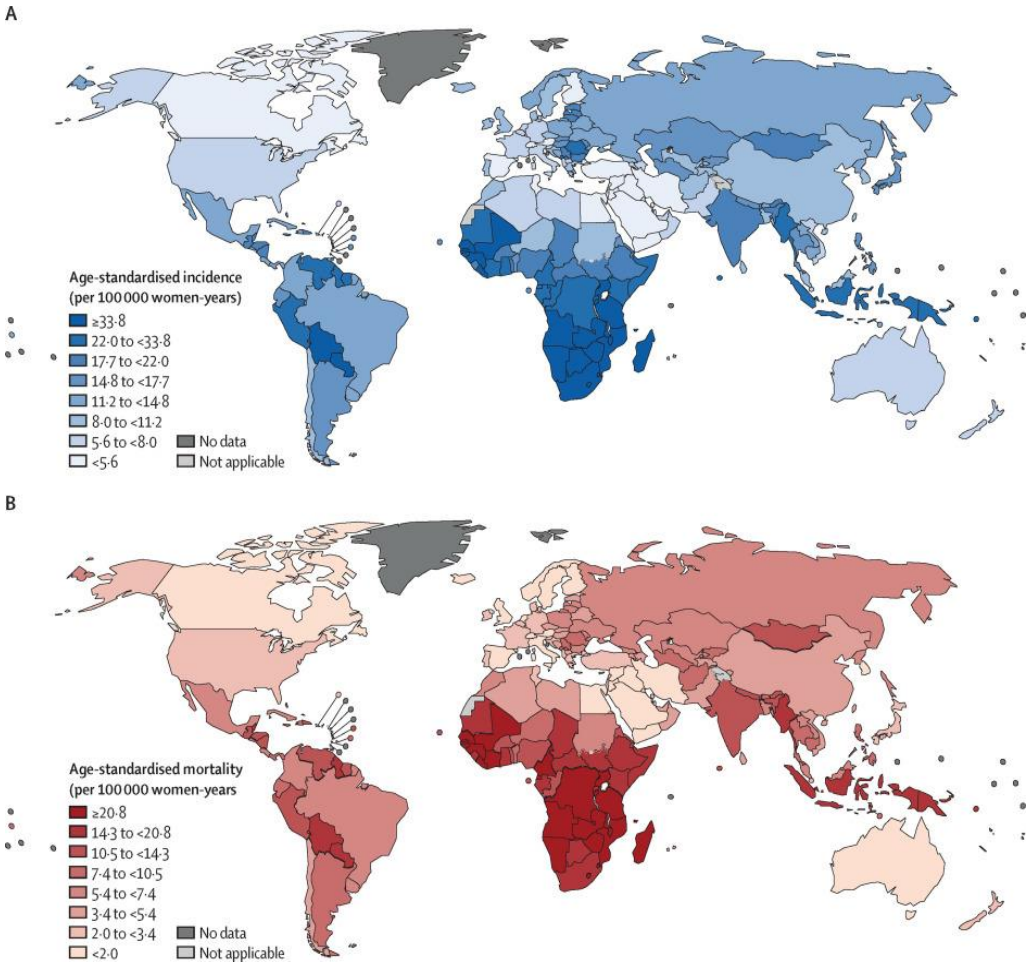


Figure 2.1: Age-standardized incidence (A) and mortality rates (B) of cervical cancer by country in 2020
Source: (Singh et al.,2023)

To stop HPV infection and the consequent development of cervical cancer, HPV vaccines and screenings have been developed. High-risk HPV strains like HPV-16 and HPV-18 are being warded off with these immunizations (Paavonen et al.,2009). Adolescents can prevent HPV infection and problems associated with it by being vaccinated against it regularly (Marek et al.,2011). Precancerous alterations or early-stage cervical cancer can only be found with cervical cancer screening, including HPV tests and Pap smears, which enables prompt intervention and treatment (Lea, J. S., & Lin, K. Y., 2012). Cervical cancer, genital warts, and other HPV-associated malignancies have significantly decreased in frequency as a result of HPV

vaccination (Lowy, 2016). In populations that have received immunizations, studies have shown a much lower prevalence of HPV infection and related illnesses (Markowitz et al.,2010). The HPV vaccine does not only protect those who receive it directly, but it also boosts herd immunity (Garnett, 2005). By reducing the circulation of vaccine-type HPV strains in the population, the vaccine indirectly protects unvaccinated individuals, resulting in a decline in HPV transmission and related diseases (Gray, P. G. 2022). Long-term efficacy and durability, a study has demonstrated that HPV vaccination provides long-term protection against HPV infection and associated diseases (De et al.,2014). A follow-up study has shown that sustained vaccine efficacy and durability could protect up to 10 years after vaccination (De Vincenzo et al.,2014). Host factors like alcoholism, smoking, genetics, reproductive factors like having many full-term pregnancies, giving birth at a young age, and hormonal effects typical of chronic hormonal oral contraceptive use, as well as reproductive variables, make women more likely to develop cancer (Deligeoroglou et al.,2013; Obiri Yeboah et al., 2017).

Additionally, cervical cancer develops at a higher incidence among women who have HIV (Ochodo, 2010), due to reduced immunity, the difficulty most of these victims have in accessing healthcare, and their ignorance of cervical cancer and HPV (Larbie, 2023).

2.19 Biogenesis of microRNA

MicroRNAs (miRNAs), small RNA molecules with lengths of 21–25 nucleotides, play a role in gene regulation post-transcription. They are produced as a result of the transcription of genomic DNA and are involved in a variety of biological processes, including cell differentiation, development,

and disease pathogenesis (Sonkoly, E., & Pivarcsi, A. 2009). The generation of mature microRNAs involves several steps, starting with transcription as the initial stage (Finnegan, E. F., & Pasquinelli, A. E. 2013).

Canonical pathways; These pathways, which include transcription by RNA polymerase II, nuclear processing and transcription by Drosha and DGCR8, cytoplasmic processing by transcription by Dicer in the nucleus, and further processing by Dicer in the cytoplasm, explain the sequential biogenesis process of microRNAs. The mature microRNA then integrates into the RNA-induced silencing complex (RISC) (Lewis et al.,2005).

Non-Canonical Pathways: Non-canonical pathways propose alternative mechanisms of microRNA biogenesis, such as independent processing by Dicer, AGO2-mediated cleavage, or splicing of introns containing microRNA sequences. These pathways provide additional layers of complexity to microRNA regulation (Bartel 2009). However, in seed sequence theory, the microRNA's seed region (positions2-8) and the target mRNA's complementary base pairs are what significantly contribute to microRNA binding. This theory provides a basis for target prediction algorithms and has facilitated large-scale microRNA target identification (Ha et al.,2014). According to non-canonical targeting mechanisms, microRNAs may be able to detect and attach to target locations other than the 3' UTR region, including the coding sequence or the 5' UTR. These non-canonical interactions allow more microRNAs to have an impact on a gene (Xu et al.,2014).

MicroRNA transcription is the first step in the production of most long primary transcripts, or pri-microRNAs, by RNA polymerase II (Pol II). These distinct genes or the introns of protein-coding genes can both create these pri-

microRNAs (Lee et al.,2004). Specific transcription factors and promoter elements control how pri-microRNAs are transcribed. Transcriptional regulation has been deemed a crucial step in microRNA biogenesis by a number of studies, with disturbances resulting in changed of microRNA expression patterns (Kim, 2005).

Processing of pri-microRNAs: This occurs in the nucleus and aided by the activity of two essential enzymes; Drosha and DGCR 8, Nuclear RNase III enzyme. Drosha breaks down the pri-microRNA to release a precursor microRNA with a hairpin-like structure (Han et al., 2017). As a co-factor for Drosha, DGCR8, an RNA-binding protein with two strands, aids in the identification and processing of pri-microRNAs. This processing phase was strictly controlled and affects the stability and abundance of microRNAs (Griffiths-Jones,2004). After being processed in the nucleus, pre-microRNAs are transported to the cytoplasm where they can keep maturing Exportin-5, in conjunction with GTP-bound Ran, which recognizes and transports pre-microRNAs via the nuclear pore complex (Kim, 2005).

The export phase was crucial for microRNA processing and loading into the RNA-induced silencing complex (RISC) in the cytoplasm (Yi et al., 2003). The RNA-induced silencing complex (RISC) was loaded with strands after being chosen. To be included in the RISC, one of the guiding strands of microRNA duplex's two strands were preferred over the passenger strand (Hu et al.,2009). As a result of mRNA breakdown or translational repression, target mRNAs are directed by the guide strand to the RISC (van de Berg et al., 2008).

Furthermore, at various stages, microRNA biogenesis is tightly regulated. The regulating mechanisms for microRNA expression and activity include post-transcriptional changes, RNA-binding proteins, and signalling pathways (Palanisamy et al., 2012). Numerous illnesses, including cancer and neurological disorders, have been linked to dysregulation of microRNA biogenesis (Tan et al.,2015). Finally, the pri-microRNA was acted upon in the nucleus through a number of stages to produce precursor microRNAs (pre-miRNAs) (Shenouda, S. K., & Alahari, S. K.2009). The key enzyme involved in this step was called Drosha, which cleaves the pri-microRNA into a 60–70 nucleotide hairpin-like shape (Bernstein et al.,2001).

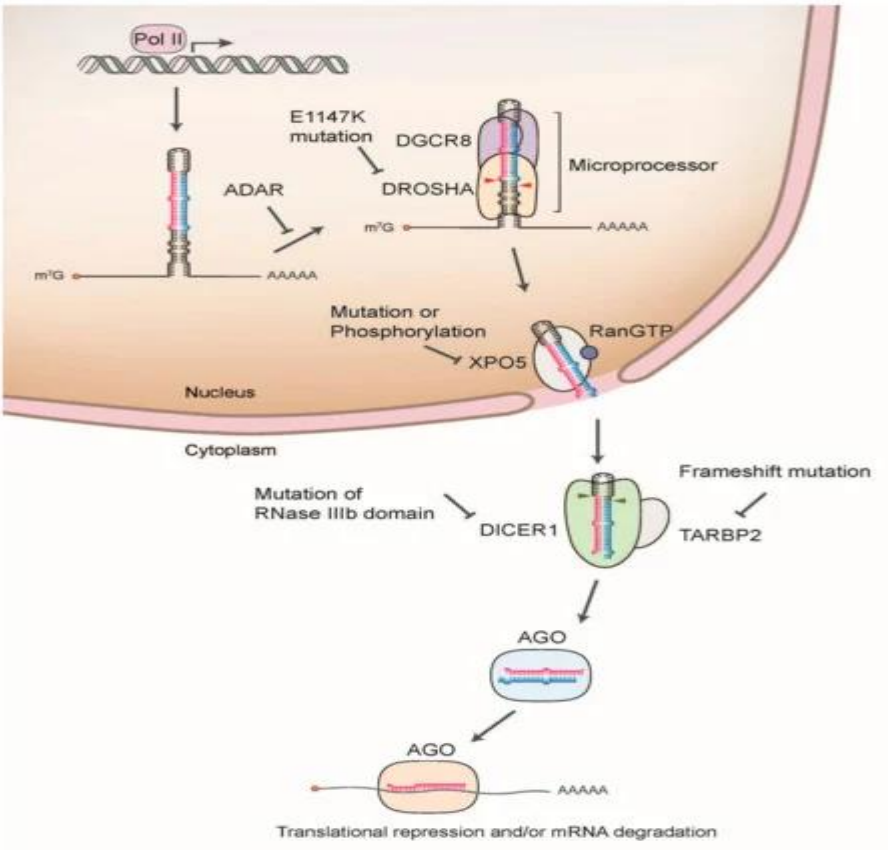


Figure 2.2: Schematic illustration of microRNA biogenesis dysregulation in cancer
Source: (Ali et al.,2020)

2.20 Theories of microRNA

The regulation of post-transcriptional gene activity was greatly influenced by microRNAs. They take part in numerous biological processes including cell division, immune response, disease, and cell proliferation (Tüfekci et al., 2014).

Theory of microRNA-mediated gene silencing; microRNAs target messenger RNAs (mRNAs), which they attach to their 3' untranslated region (UTR), resulting in their degradation or having their translation suppressed, which inhibits the target gene's expression proposed by Valinezhad et al. (2014). The RNA-induced silencing complex (RISC), which was created when microRNA binds to target mRNA sequences, mediates gene silencing (Lewis et al.,2005).

Theory of microRNA regulation of mRNA stability; the theory suggests that microRNAs can influence mRNA stability by promoting deadenylation and decay of target mRNAs (Fabian et al.,2010). It claims that specific mRNAs are targeted by the RNA-induced silencing complex (RISC) as a result of microRNAs, leading to mRNA instability (Mayya, 2021). According to this theory, additional RNA molecules, like long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), could interact with microRNAs in addition to their mRNA targets through shared microRNA response elements (MREs). These interactions result in a complex regulatory network where different RNA molecules compete for microRNA binding, affecting the expression of each other (Guil, S., & Esteller, M. 2015).

Moreover, the notion of microRNA-mediated translational repression suggests that microRNAs prevent target mRNAs from being translated while not affecting the stability of the target mRNAs (Cottrell et al., 2017). When

target mRNAs' 3' UTRs were bounded by microRNAs, restrictive protein complexes were drawn to the scene, leading to translational repression (Bartel, 2009). MicroRNAs can control gene expression at the transcriptional level in post-transcriptional regulation. This theory suggests that microRNAs can bind to promoter regions of target genes and either enhance or repress their transcription. This process involves interactions with transcription factors and chromatin-modifying complexes (Barrett, R. M., & Wood, M. A. 2008).

The theory of the microRNA sponge or decoy effect; suggests that certain RNA transcripts, known as competitive endogenous RNAs (ceRNAs) or microRNA sponges, have numerous microRNA binding sites and can sequester microRNAs away from their target mRNAs to modify gene expression (Bak, R. O., & Mikkelsen, J. G.2014). This mechanism was thought to have implications in fine-tuning microRNA-mediated regulation (Re et al.,2017). The microRNA sponge effect theory suggests that certain RNA molecules, such as pseudogenes or artificially engineered transcripts, can function as decoys for microRNAs. These "sponges" contain multiple binding sites for specific microRNAs, effectively sequestering and titrating them in opposition to their endogenous aims (Ebert et al.,2007).

2.21 Functions of some microRNA in cervical cancer patients

For cervical cancer to start and progress, microRNAs (miRNAs) are crucial. By attaching to messenger RNAs (mRNAs), microRNA can influence the expression of genes by preventing or deteriorating mRNA translation. Proto-oncogenes or tumor suppressor transcripts are the targets of microRNAs in several varieties of tumors (Wang et al., 2008). Tumor-suppressor microRNAs (tsmicroRs) and oncogenic microRNAs (OncomicroRNAs) are

two separate classes of microRNAs (Svoronos et al., 2016). OncomicroRNAs are typically overexpressed, which promotes tumor growth and preserves the tumor phenotype, whereas tsmicroRNAs prevent carcinogenesis by regulating apoptosis, apoptosis-related processes, cell invasion, and other cancer-related processes. Most human malignancies usually have a downregulation of these tsmicroRNAs (Ali et al., 2020).

The known relationship between microRNAs and cancer is strongly correlated with abnormal microRNA expression in a variety of cancer types (Lu et al.,2005). Any microRNA tumor profile has the potential to be a therapeutic, prognostic, and a diagnostic tool (Tricoli, J. V., & Jacobson, J. W., 2007). To use microRNAs for diagnosis or treatment, it is necessary to comprehend their oncogenic or tumor-suppressive functions as well as how their dysregulation affects tumor formation (Cho, 2012). OncomicroRNAs may considerably aid in the treatment of cancer through silencing or modulation of their expression and the focused overexpression of the tsmicroRNAs which could have therapeutic effects (Chen et al.,2017).

MiR-18a is an example that contributes to the transition of HPV-positive cervical cells into cancerous cells when miR-18a expression was upregulated by HPV E6 and E7 (Morgan et al., 2020). According to Li et al. (2023), it was discovered that miR-27a increased the production of TAB3 by attaching to its 3′UTR, which then caused the activation of NF-kB signaling. The functional promotion of cervical cancer malignant potential by miR-27a was demonstrated, and the study shows that miR-27a's elevation of TAB3 was directly responsible for the elevated malignant potential brought on by TAB3 overexpression (Li et al.,2023).

By blocking oncogenic pathways, several microRNAs restrict tumor growth in cervical cancer. For instance, miR-34a targets numerous oncogenes, including Notch1, c-Met, and E2F3, and its decreased expression promotes tumor invasion and growth (Wang et al.,2013). By focusing on several genes involved in tumor formation and metastasis, miR-145 works similarly to stop the spread of cervical cancer (Cao et al.,2020).

Li et al. (2011) proposed that miR-29b would promote cervical carcinogenesis by inhibiting YY1 and CDK6 since the E6 and E7 oncoproteins encoded by the HPV genome were able to bind to p53 and pRb, respectively, to suppress cellular proliferation and death. Numerous microRNAs can act as oncogenes throughout the oncogene activation process and promote the growth of cervical cancer. For instance, miR-21, a tumor suppressor gene targets numerous tumor suppressor genes including PTEN and PDCD4, it was frequently seen in increased concentrations in cervical cancer and was also known to enhance cell proliferation, metastasis, and invasion (Wang et al.,2015).

MiR-155 is a distinct microRNA that encourages cancer cells to migrate and multiply; it has been connected to a worse prognosis in cervical cancer patients (Kim et al.,2018). MicroRNAs can control the epithelial-mesenchymal transition (EMT), a crucial stage in the growth of cancer. According to Lei et al. (2012), the tumor suppressor miR-155, which has been connected with cervical cancer, inhibits cell division, migration, and invasion, halts EMT, and increases the chemosensitivity of cervical cells. MiR-155 mimics have the potential to partially correct the down-regulation of E-cadherin brought on by EGF (epidermal growth factor) therapy in cervical

cells. It specifically targets the 3' UTR, which has two binding sites, of the Smad2 gene, this microRNA was overexpressed, which causes SMAD2 to downregulate (Lie et al.,2012). MiR-155 also prevents EGF-induced EMT in cervical carcinoma by blocking the EMT. MiR-155 makes cervical cells more sensitive to the chemotherapy drug cisplatin (Lie et al.,2012).

MiR-200a, miR-200b, miR-200c, miR-141, and miR-429 are members of the miR-200 family that target the transcriptional repressors of E-cadherin ZEB1 and ZEB2, which downregulate in cervical cancer (Li et al.,2010). MicroRNAs also played a part in chemoresistance by controlling how susceptible cervical cancer cells were to chemotherapy treatments. The specific targeting of PTEN and p21 by miR-21 and miR-106b, respectively, has been linked to increased chemoresistance by enhancing cell survival and lowering apoptosis (Wang et al.,2015). Nevertheless, other microRNAs, such as miR-143 and miR-145, specifically target Bcl-2 and MUC1 to increase cervical cancer cells' sensitivity to the chemotherapeutic agent cisplatin (Yao et al.,2012; Zhao et al.,2017).

Patients with cervical cancer may react differently to chemotherapy, in contrast to microRNAs. MiR-214, miR-21, and miR-34a are some examples of microRNAs that are implicated in controlling chemosensitivity (Hummel et al., 2010). Drug resistance was influenced by the dysregulation of the multidrug resistance protein 1 (MDR1) and Bcl-2 expression (Vaidya et al., 2020). MicroRNAs have the potential to be therapeutic targets for the treatment of cervical cancer since they can influence target gene expression and tumor cell activity (Doghish et al., 2023). This was made possible by changing microRNA expression with artificial microRNA mimics or by

blocking antagomirs (Wang, V., & Wu, W. 2009). Preliminary, research suggests that restoring tumor suppressor microRNAs or suppressing oncogenic microRNAs may be effective in treating cervical cancer (Zhao X, et al.,2016; Li et al., 2019).

Human Papillomavirus (HPV) regulation also states that HPV infection is a substantial cervical cancer danger sign. An HPV infection's ability to cause cancer was regulated by certain microRNAs. For example, miR-34a inhibits cell proliferation and induces death when it targets the HPV oncogenes E6 and E7. Cervical cancer and high-risk HPV infection are both associated with decreased miR-34a expression (Li et al.,2010). MiR-34a targets several oncogenes, including Notch1, c-Met, and E2F3, downregulate in cervical cancer, and its reduced expression aids in the development of tumors and invasion (Wang et al.,2013). A tumor suppressor microRNA called miR-34a controls cell cycle progression and apoptosis at a moderately high level in healthy cervical tissue (Li et al., 2010). When it comes to cervical cancer, its expression typically becomes downregulated, fostering the tumor's growth and spread (Chen, 2015).

Many microRNAs that have been identified as potential biomarkers for the diagnosis and prognosis of cervical cancer raise some questions about their predictive abilities (Kannappan et al., 2021). Extremely high levels of miR-200c expression in cervical cancer patients are associated with a lower chance of survival and a worse prognosis (Allouch et al.,2020). On the other hand, lymph node metastases, a later stage of the tumor, and a worse overall survival were associated with lower levels of miR-375 expression (Wei et al., 2021). Cervical cancer tissues express certain microRNAs, like miR-205, miR-375,

and miR-424, differently from healthy cells. These microRNAs may be used as prognostic and early-detection indicators for cervical cancer (Doghish et al., 2023). In biological fluids including blood and cervical samples, their expression levels can help in the prognosis of patients as well as the early diagnosis of cervical cancer (Li X, et al.,2018). Cervical cancer diagnostic and prognostic markers may be derived from microRNAs. For instance, it has been established that miR-29a suppresses DNMT3A and DNMT3B to regulate the p16 methylation pattern in cervical cancer (Wang et al., 2021).

Additionally, there was evidence that miR-29a regulates HSP47, which was necessary for the development of collagen molecules. Lower HSP47 levels inhibited cancer cell invasion and migration, demonstrating that the miR-29a-HSP47 pathway contributes to cervical SCC metastasis (Yamamoto et al., 2013). Due to the downregulation of miR-205 in tissues with cervical cancer, it was, therefore, possible to differentiate between high-grade cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (Dudea-Simon et al.,2022). Cervical cancer patients who have elevated miR-21 levels had a worse prognosis and lymph node metastases (Wang et al.,2015). The target miR-223 was under-expressed forkhead box protein O1 (FOXO1) seen in cervical carcinoma (CC) cells (Jiao et al.,2021). Cervical cancer cell proliferation was considerably slowed by miR-223 overexpression (Yin et al.,2016). Targeting Akt/mTOR/p70S6K, miR-223 reduces cervical cancer cell growth, proliferation, and colony formation through binding to hypoxia-inducible factor-1 (IGF-1R) (Jia et al.,2011). Numerous studies found that miR-223 performed a significant part in controlling cervical cancer, mostly

through targeting the disease-related genes and interacting with various signaling pathways (Goa et al.,2017).

Finally, the management of cervical cancer may employ microRNAs as therapeutic targets. Artificial microRNA mimics or inhibitors can be used to modify target gene expression affecting tumor cell behavior (antagomirs). For instance, preclinical studies for the therapy of cervical cancer have shown potential for restoring tumor suppressor microRNAs or suppressing oncogenic microRNAs (Yao et al.,2016; Zhao et al.,2017; Wang et al.,2015).

2.22 Expression patterns of microRNA in cervical cancer and non-cancerous patients

MicroRNAs (miRNAs) play a crucial role in regulating gene expression. Cell division, proliferation, and death are just a few of the biological processes that have been connected to microRNA dysregulation in terms of disease. MicroRNA expression profiles can differ between various tissues and people.

Microarray analysis was utilized by Martin et al. (2014) to examine the expression of microRNAs and discover the differences between healthy and cervical cancer tissue. A study by Pereira et al. (2010) proposed that some microRNAs, including miR-21, miR-143, miR-200a, and miR-375, were found to have differential expression patterns across the various stages of cervical carcinogenesis, according to a notion that looked at the expression of microRNAs in a variety of cervical tissues, such as a healthy cervix and lesions that are precancerous or cancerous.

However, Li et al. (2013) discovered that several dysregulated microRNAs, including miR-21, miR-143, miR-200c, and miR-203, were

found using next-generation sequencing and were investigated in the cervical carcinoma tissue, microRNA expression profile, and neighboring non-cancerous tissue. Sharma, & Gupta, (2020) noticed that when compared to healthy controls, miR-21, miR-205, and miR-214 expressions were consistently downregulated in cervical cancer patients, while miR-143, miR-145, miR-218, and miR-375 expressions were consistently upregulated. It was shown that CIN and ICC had reduced miR-29a expression levels (Li et al.,2011). Gong et al. (2019) hypothesized that miR-29a's methylation of the tumor suppressor SOCS1 could stop cervical cancer from progressing.

Furthermore, miR-29a might function as a tumor suppressor in the development of cervical cancer (Li et al.,2011). In the presence of invasive SCC, miR-21 overexpression and miR-29a downregulation distinguish tumors from healthy cervical tissue and create a molecular signature that can be used to detect cervical cancer (Bauelos-Villegas et al., 2021).

Using information from The Cancer Genome Atlas (TCGA), Li et al. (2019) performed an extensive analysis of the patterns of microRNA expression in cervical cancer and discovered there were many microRNAs whose expressions varied. These included variations in miR-9, miR-21, and miR-203 overexpression and downregulation between cervical cancer samples and samples from healthy individuals. Moreover, Cheng & Huang, (2021) found that there was a significant downregulation of the miR-200 family in cervical cancer, suggesting that the tumor suppressor activity of these genes may have an impact on the development of cervical cancer. To achieve this, the expression patterns of the miR-200 family—which includes miR-200a, miR-200b, miR-141, miR-200c, and miR-429 in healthy cervical tissues and

cervical cancer tissues were examined. MiR-125b, miR-199a, and miR-375 were downregulated when miR-21 was increased, and when Su et al. (2021) examined the expression levels of these molecules in the non-cancerous tissues surrounding cervical cancer tissues. This suggests that these molecules were involved in the progression of the disease.

Additionally, it has been discovered that miR-21 was upregulated in several malignancies, including cervical cancer. However, miR-21 expression levels in cervical tissue are either negligibly low or absent in healthy people. A study by Wang et al. (2016) demonstrated that miR-21 overexpression in cervical cancer tissues and cell lines was associated with tumor growth, poor prognosis, tumor formation, invasion, metastasis, and resistance to treatment. Poor patient survival has also been linked to elevated levels of miR-21 (Jin et al.,2020).

A tumor suppressor microRNA called miR-34a controls cell cycle progression and apoptosis at a moderately high level in healthy cervical tissue. Its expression was frequently downregulated in cervical cancer, promoting the growth and spread of the tumor (Chen, 2015). Cell proliferation, migration, and invasion are three biological activities that are regulated by miR-143, and it was moderately expressed in the cervical tissue of healthy people. The onset and spread of cervical cancer have been linked to its downregulation (Zhang et al.,2015).

MiR-145, which was quite abundant in the cervical tissue of healthy persons, is another tumor suppressor microRNA that was commonly downregulated in cervical cancer. Reduced expression of miR-145 has been linked to increased cell invasion and proliferation in cervical cancer (Shen et

al., 2020). Higher miR-155 levels were linked to advanced tumor stage, lymph node metastasis, and a lower likelihood of survival.

These elements may serve as a prognostic biomarker and encourage the growth of cervical cancer. Tissues associated with cervical cancer have been reported to express miR-155 more frequently (Yin et al.,2016). Wang et al. (2009) showed that miR-155 expressed in plasma, tumor tissues, and patients with cervical cancer receiving treatment had a worse prognosis than those in the control group and the expression in tissues was related to tumor stage.

Other research by Lv et al. (2018) found that individuals with adverse drug reactions had significantly downregulated levels of the miR-155 gene, and this downregulation was linked to a better reaction to the medication. Recent research has also revealed that miR-27a levels are consistently elevated in a wide range of cancer types, supporting the malignant properties of cancer cells (Wu et al.,2015; Su et al.,2019).

Furthermore, overexpressed miR-27a promoted cell invasion, migration, and mortality in ovarian cancer (Li et al.,2019). The miR-27a gene is upregulated by cervical carcinoma and Wei et al. (2020) discovered that miR-27a substantially reduced the cervical cancer cells' aggressive characteristics.

Low levels of miR-375 expression in cervical cancer patients were associated with lymph node metastases, tumor aggressivity, and a poor prognosis when compared to healthy cervical tissues (Li et al.,2019). As a cervical cancer diagnostic test that can forecast the disease, miR-203 may be utilized because it has been observed that the expression of the gene is lower

in cervical cancer cell lines, tissues, tumor invasion, growth, and lymph node metastases (Zhao et al.,2013). MiR-146a levels were noticeably higher in tissue samples from cervical cancer patients, according to Brase et al. (2011). Pereira et al. (2010) utilized a microRNA array study to compare tissues from cervical cancer and age-matched normal tissue, and they found that miR-146a levels were increased in cervical cancer tissues and that its overexpression boosts cell survival, while Sathyanarayanan et al. (2016) revealed that human cervical cancer cells' ability to invade, spread, and survive was suppressed by miR-146a.

miR-27a was shown to be downregulated when cervical cancer tissues and cancer cell lines were compared to healthy cervical tissue. According to Wang et al. (2008), miR-27a suppresses tumor growth in the process of cervical cancer formation. According to research by Fang et al. (2018), cervical cancer cells expressed less miR-27a compared to healthy cervix squamous epithelia or glandular epithelia, and higher levels of miR-27a inhibit tumor growth by reducing TGF-RI production and TGF-signalling.

Numerous investigations have found dysregulated microRNAs in the plasma of people who do not have cervical cancer. For instance, a study by Song et al. (2019) discovered that patients with cervical intraepithelial neoplasia (CIN), a precancerous disease, had considerably higher plasma levels of the microRNAs miR-21, miR-146a, miR-150, and miR-223, which could serve as cervical cancer biomarkers. Similar to this, Cai et al. (2018) revealed that plasma samples from individuals with high-risk human papillomavirus (HPV) infections had a panel of dysregulated microRNAs,

including miR-145, miR-150, and miR-34a, which were strongly related with the development of cervical cancer.

The diagnostic potential of plasma microRNAs in cervical cancer has been the subject of numerous studies. In a study by Zhou et al. (2020), it was discovered that the combination of plasma miR-20a, miR-93, and miR-106b had high diagnostic specificity for differentiating cervical cancer patients from healthy controls and that patients with cervical cancer showed a significant increase in their expression, suggesting that they might be useful as non-invasive diagnostic biomarkers. Another study by Peralta-Zaragoza et al. (2017) discovered that specific microRNAs, including miR-203, miR-205, and miR-200b, were markedly suppressed in the plasma of patients with high-grade cervical intraepithelial neoplasia (CIN) in comparison to healthy controls. These microRNAs might therefore function as cervical cancer indicators for early identification.

However, Takeuchi et al. (2020) used high-throughput sequencing and qRT-PCR to identify the microRNAs that were specifically expressed in cervical cancer and found that miR-21, miR-143, miR-145, miR-146a, miR-218, and miR-375 were significantly upregulated in cervical cancer tissues linked to nearby healthy tissues, whilst, in their meta-analysis of the microRNA expression patterns in cervical cancer patients from various publications, Pereira et al. (2017) discovered a large number of microRNAs, including miR-9, miR-21, miR-125b, miR-143, miR-145, miR-155, miR-34a, and miR-375, which were consistently dysregulated across various datasets. MiR-143, miR-145, miR-199a, miR-203, and miR-205 were found to be downregulated in cervical cancer tissues as compared to healthy tissues by

Pereira et al. (2010), who used microarray analysis to examine the expression of microRNAs in patients with cervical cancer.

Finally, Faraldi et al. (2018) found that miR-191, miR-484, and miR-1274a were reliable endogenous controls for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis in cervical tissues. Considering research by Wang et al. (2018), there was a connection between poor overall survival in cervical cancer patients and high plasma levels of the gene miR-192, a disease-predictive biomarker. Expression levels of miR-93, miR-98, and miR-197 in cervical carcinoma and healthy cervical tissue samples suggested they could act as diagnostic biomarkers for cervical carcinoma even though they were noticeably more active in cervical cancer tissues than in healthy cervical tissues (Hu, 2020). Low plasma levels of miR-203 have been linked by Wang et al. (2019) to an advanced tumor stage and a poor prognosis in cervical cancer patients. According to these results, miR-203 might be useful as a prognostic indicator for cervical cancer.

2.23 Expression patterns of microRNA in cervical cancer patients on therapy

A typical example of gynecological cancer in the world and a serious public health threat, cervical cancer is currently treated with surgery, radiation therapy, and chemotherapy. While various therapeutic options are available, the outcomes are still not satisfactory, especially for advanced cases. As a way to help with the diagnosis, prognosis, and monitoring of cervical cancer, it was necessary to find new treatment targets and biomarkers. While these treatments could be effective, more biomarkers are required for targeted and personalized therapies to enhance results for patients. MicroRNAs (miRNAs),

which show a crucial role in the control of genes, have been linked to the onset and spread of cancer. Using circulating microRNAs as markers for cancer diagnosis, prognosis, and therapeutic response, numerous studies have lately examined the detection, prognosis, and therapeutic response of cervical cancer.

Circulating microRNAs have been examined in numerous investigations of patients with cervical cancer receiving treatment. For example, Wang et al. (2018) discovered that cervical cancer patients taking chemotherapy had considerably lower expression patterns of multiple microRNAs (miR-141-3p, miR-200c-3p, and miR-429) than those who had not yet started treatment. Additionally, the study showed a connection between a poorer response to chemotherapy and lower expression of specific microRNAs (miR-200c-3p, miR-141-3p, and miR-429) (Wang et al.,2018).

Two chemotherapy medications frequently employed in the management of cervical cancer are Intex and Outex. Outex is a topoisomerase inhibitor that prevents DNA replication and repair, whereas Intex is a platinum-based medication that prevents DNA synthesis and triggers apoptosis. The processes underlying the reaction to these drugs, however, are not completely known.

Intex and outex therapy for cervical cancer patients has been the subject of several research examining changes in circulating microRNA levels. For instance, a study by Zhou et al. (2019) discovered that miR-146a levels were considerably increased in cervical cancer patients receiving outex treatment, and this overexpression was linked to a better prognosis. According to another study by Li et al. (2017), cervical cancer patients treated with Intex

had considerably advanced levels of the miR-106b gene, and this overexpression was linked to a worse outcome.

Furthermore, research by Lv et al. (2018) looked at the changes in circulating microRNA levels in cervical cancer patients receiving a combination of intex and outex. The study discovered that patients who reacted to the medication had much lower levels of miR-155, and this downregulation was linked to a stronger response to the therapy. MiR-155 enhances cervical cells' chemo-sensitivity to cisplatin therapy by inhibiting EMT (Lei et al.,2012). A study by Wang et al. (2008) reveals that tissues containing cervical carcinoma have greater expression of miR-155.

According to Ferrajoli et al. (2013), miR155 was overexpressed in monoclonal B-cell lymphocytosis patients' B cells compared to B cells from healthy people. MiR-155 down-regulated in cervical cancer cells resulting in apoptosis and a G1 phase cell cycle halt. It has been discovered that liver kinase B1 was the target gene of miR-155, a key tumor suppressor in several cancers (LKB1) (Lao et al.,2014). However, cervical cancer tissues have significantly lower levels of LKB1 mRNA and protein expression, whereas LKB1's luciferase activity and protein expression are increased when miR-155 expression is downregulated.

Consequently, miR-155 regulates LKB1 to encourage the proliferation of the cells of cervical cancer (Lao et al.,2014). Targets of the miR-200 family, including miR-200a, miR-200b, miR-200c, miR-141, and miR-429, were transcriptional repressors of E-cadherin ZEB1 and ZEB2, which were downregulated in cervical cancer (Panoutsopoulou et al.,2018). For the non-invasive exposure and follow-up of patients with cervical cancer undergoing

InTex therapy, circulating microRNA profiles are utilized as liquid biopsies "by Li et al (2021). The potential of microRNAs circulating as liquid biopsies for the non-invasive diagnosis and monitoring of cervical cancer patients during therapy was investigated using InTex, a novel inhibitor of the Wnt/-catenin signaling pathway (Li et al.,2021). The author discovered a panel of microRNAs whose expression levels significantly changed before and after receiving InTex treatment, indicating their potential as biomarkers for tracking therapeutic response (Li et al.,2021).

Circulating exosomal microRNAs may serve as predictors of treatment outcomes in cervical cancer patients receiving OutEx, according to He et al. (2020). For those receiving OutEx who have cervical cancer, an exosome-based therapy, a study sought to discover circulating exosomal microRNAs that might predict therapeutic response (Wu et al.,2021). The scientists discovered a group of exosomal microRNAs that were substantially related to therapeutic response, indicating that they could be utilized as biomarkers to track the effectiveness of treatment (Zen, K., & Zhang, C. Y., 2012).

Circulating microRNAs could serve as indicators for treatment response prediction in cervical cancer patients receiving InTex and OutEx combination therapy" (Wang et al.,2022). The potential use of circulating microRNAs as biomarkers to forecast therapeutic response for alternatives for treating cervical cancer with intex (intravenous chemotherapy and outex (oral chemotherapy has to be further investigated.

Zhu et al. (2021) studied serum samples from cervical cancer patients before and after treatment with Intex and proposed that miR-205-5p expression was noticeably greater in responders’ contrast to non-responders in

the cervical cancer serum samples. Furthermore, miR-205-5p was discovered to be linked to improved overall survival in these patients. In a different study, Lu et al. (2021) examined the microRNA expression in cervical cancer patients taking Outex and discovered that miR-21-5p was much more prevalent in responders compared to non-responders and was linked to a longer progression-free survival in these patients.

According to Jiang et al. (2021), oral chemotherapy using nedaplatin and paclitaxel was administered to 100 cervical cancer patients. Patients' plasma samples were taken both before and during treatment at various intervals, miR-222-3p expression levels were evaluated, among those with low plasma levels before treatment, and it was found to be improved in chemotherapeutic response with a longer overall life than those with high levels. Additionally, when the expression levels of miR-222-3p were compared to patients whose plasma levels did not change or rise during treatment, those who experienced a decrease had a better response and longer survival.

Jiang et al. (2021) concluded that plasma miR-222-3p may be used as a possible biomarker to evaluate the effectiveness and prognosis of oral chemotherapy for cervical cancer patients. According to Li et al. (2017), overexpression of miR-29b significantly inhibited both cervical cancer cells' ability to proliferate and progress through the cell cycle, and its pattern of expression was linked to the clinical stages of the disease. Additionally, miR-29b was involved in the regulation of tumor invasion.

Patients with cervical cancer getting treatment have been discovered to have many dysregulated microRNAs, and a link was found between the

expression of these microRNAs and the treatment's effectiveness. For instance, it has been shown that individuals with cervical cancer who do not react to cisplatin-based chemotherapy have elevated levels of the miR-21 gene (Liao et al.,2016). On the other side, radiation therapy resistance has been linked to miR-214's downregulation, suggesting how microRNAs may serve as biomarkers for gauging how well cervical cancer patients may respond to treatment (Tang et al.,2022).

Pedroza-Torres et al. (2016) have shown that the expression of miR-125b was strongly connected to chemoresistance and a poor prognosis in patients with cervical cancer. Similar to this, it has been found that miR-1271 overexpression might function as a biomarker to predict how cervical cancer patients would react to neoadjuvant chemotherapy (Pang et al.,2021).

Moreover, Cheng et al. (2021) indicated that high expression of miR-21 was linked to patients' chemo resistance to cisplatin-based chemotherapy. A similar finding was discovered in which miR-214 downregulation was linked to radiation therapy resistance (Tang et al.,2022). These results imply that microRNAs may be useful indicators for predicting cervical cancer patients' treatment responses.

It has also been established that there was a link between a patient's survival and the expression of specific microRNAs. For instance, a study found that individuals with cervical cancer following surgery and radiation had reduced expression of miR-143, which was connected to a low survival rate (Lu et al., 2016). Similarly, another study discovered that neoadjuvant treatment for cervical cancer patients who had high expression of miR-1271 was linked to better survival (Li et al.,2021). For example, miR-21 has

been demonstrated to promote cervical cancer cell proliferation, motility, and invasion, and its overexpression has been connected to chemotherapeutic resistance to cisplatin (Li et al.,2020; Bhattacharjee et al.,2022).

Moreover, patients with cervical cancer who have miR-214 downregulation have a higher risk of developing radiation resistance (Wang et al.,2018). However, Dinh et al. (2016) discovered a connection between the radiation dose used to treat non-small-cell lung cancer and the amount of miR-29a present in the blood. According to Chen et al. (2019), miR-29a may control the PAK1/LIMK signaling pathway, bind specifically to the 3′-UTR, and downregulate the expression of CDC42 to lessen cervical cancer cell proliferation, migration, and invasion while fostering cell death.

Furthermore, Chen et al. (2019) investigation revealed that high levels of the tumor suppressor miR-29a could prevent cervical cancer cells from growing and migrating as well as causing cell apoptosis. In tumors and LSILs with HPV, miR-29a has been demonstrated to be downregulated, and this has also been seen in cervical cancer cells (Yamamoto et al., 2013; Jia et al., 2015; Servín-González et al., 2015). According to a Sara et al. (2019) study, miR-29a levels in tumor samples were significantly lower than in the control groups. This finding raises the possibility that miR-29a functions as a tumor suppressor in the course of cervical cancer development.

Additionally, several microRNAs have been suggested as possible biomarkers for the efficacy of cervical cancer therapies and patient outcomes. The overexpression of miR-1271 has been determined to be a possible biomarker for predicting how cervical cancer patients would respond to neoadjuvant treatment (Li et al.,2021). Similarly, it has been found that

cervical cancer patients with miR-125b expression had a poor prognosis, chemoresistance, and low quality of life (Yang et al.,2016). Furthermore, several researchers have suggested that microRNAs could be used as cervical cancer treatment targets.

According to Kim et al. (2016), miR-34a/miR-34b/c overexpression reduces proliferation and triggers apoptosis in cervical cancer cells via controlling the expression of Bcl-2 and MMP-9, two genes essential for tumor growth and invasion. It also suggests its therapeutic target of miR-34a/miR-34b/c for the treatment of cervical cancer. However, research revealed that overexpressing of miR-34a reduces cervical cancer cell proliferation and increases sensitivity to cisplatin-based chemotherapy (Kim et al.,2016) According to recent research (Fujita et al., 2008; Wang, Yang, Li, & Han, 2015; Yu et al., 2015), several microRNAs, including miR-34a, can influence the response of chemotherapy with a range of tumor kinds, including cervical cancer.

The expression of tumor suppressive genes was also downregulated in cancer-derived cell lines and cervical tumors that included oncogenic HPVs, according to Wang et al. (2009)'s research on miR-34a in cervical cancer. The tumor suppressor p53 was weakened by viral protein E6, which also activates the miR-34a trans activator (Wang et al., 2009), whiles studies by Li et al. (2010), reveal that pri-miR-34a expression was less common in cervical cancers and precancerous lesions when HR-HPV E6 oncoprotein and the p53 pathway were in charge of suppressing miR-34a and the early onset incidence that contributed to the spread of cervical cancer.

2.24 Cervical cancer patients with or without therapy based on the International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer

The International Federation of Gynaecology and Obstetrics, or FIGO, system was used to stage cervical cancer. the four main stages are 1, 2, 3, 4, and substages, 1A, 1A1, 1A2, 1B, 1B1, 1B2, 2A, 2A1, 2A2, 2B, 3A, 3B, 3C, 31, 3C2, 4A, and 4B, account for different features of cervical cancer (Lee, S. I., & Atri, M. 2019).

The International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer, however, indicated that over half of all cervical cancer patients worldwide continue to receive a stage IB2 to IVA diagnosis, while, almost one-third of patients with cervical cancer in Korea had the disease in an advanced stage (Bartel, 2004).

Currently, platinum-based chemotherapy combined with radiation therapy is advised for patients with advanced FIGO stage IIB and greater than stage IIB (Bouyssou et al.,2014). Unfortunately, these patients' survival rates in the first five years were lower and their rate of recurrence was higher (Bartel, 2004). It is imperative to discover a novel biomarker that could correctly diagnose cervical cancer and improve clinical monitoring.

According to Wang et al. (2016), the expression level of miR-155 was markedly upregulated in cervical cancer tissues and elevated miR-155 levels could be linked to FIGO phases of cervical cancer (Fang et al.,2016). Cervical cancer was significantly aided in its development by miR-155's down-regulation. Thus, miR-155 could be used as a novel prognostic marker and a target for effective treatment in patients with cervical cancer (Fang et

al.,2016). Since the majority of studies did not specifically address the FIGO stages of cervical cancer, to create a trustworthy biomarker that might be applied to patients with cervical cancer detected in those stages for prognostic, therapeutic, or diagnostic purposes, more study is needed.

2.25 Expression patterns of microRNA in cervical cancer patients not on therapy

Despite the fact that individual microRNA genes do not produce proteins, they regulate the expression of cellular proteins by attaching to the 3′ UTR of the target mRNAs. Depending on how tightly the target mRNA was bounded by the 6 to 8 nucleotide seed sequence, each microRNA may target up to 100 mRNAs and either destroy them or suppress their translation (Bartel, 2004; Lim et al., 2005).

Additionally, some studies have also looked at how microRNAs are expressed and worked in cervical cancer patients who are not receiving treatment. In tissues with cervical cancer, miR-375 was downregulated, and its overexpression prevents cell proliferation and invasion (Gao et al., 2018). MiR-203 expression was downregulated in cervical cancer tissues, and its overexpression inhibits cell migration and proliferation (Song et al., 2018).

For example, it has been demonstrated that tissues linked to cervical cancer have higher levels of miR-205 and are highly sensitive and specific for detecting cervical cancer from healthy cells (Chen et al., 2016). As potential indicators for cervical cancer prognosis, miR-200a, miR-9, miR-143, and miR-145 were among the groups of microRNAs discovered (Yang et al., 2019). Additionally, it has been discovered that tissues with cervical cancer have elevated levels of miR-21 which was connected to a bad prognosis

(Wang et al., 2015). A further four microRNAs are putative predictors of the prognosis of cervical cancer: miR-218, miR-21, miR-27a, and miR-let-7i (Zheng et al., 2016).

In conclusion, microRNAs have a significant role in the development and spread of cervical cancer. Cervical cancer patients have a poor prognosis and several clinical characteristics linked to deregulation of microRNA expression. As biomarkers for cervical cancer detection and prognosis, microRNAs may be useful.

2.26 Expression patterns of microRNA in cervical cancer patients with HIV

Women with HIV (Human Immunodeficiency Virus) are at an increased risk of getting cervical cancer due to their increased vulnerability to the human papillomavirus (HPV), a significant contributing factor to cervical cancer. The expression of genes was closely regulated by microRNAs (miRNAs), whose dysregulation was known to occur in cancer. Even though there were no studies on circulating microRNA levels in HIV-positive patients with cervical cancer, However, several researchers have looked into the expression of microRNAs separately in people with HIV and people with cervical cancer.

The potential for microRNAs to serve as diagnostic and prognostic indicators in cervical cancer patients has been discovered. MicroRNAs have been shown to affect HIV replication and disease development in HIV-infected people, and miR-375 has been discovered as a possible diagnostic marker for cervical cancer (Arga et al.,2023). For instance, miR-155 has been

linked to persistent immunological activation and inflammation and has been reported to be elevated in HIV-infected people (Ojha et al.,2019).

Peralta-Zaragoza et al. (2016) conducted a study on the circulating microRNA profile in cervical cancer patients and many microRNAs' expressions were correlated with miR-21 and miR-375, which showed a significant difference when compared to patients with cervical cancer who did not have any symptoms. According to another study by Wu et al. (2014), the diagnosis of cervical cancer, miR-21 may be employed as a biomarker.

Although there aren't many studies looking at the microRNA profile in the plasma of cervical cancer patients who also have HIV, these studies give information on the possible role of microRNAs as biomarkers for HIV and cervical cancer patients. Future studies could build on these results by examining the microRNA profile in the plasma of cervical cancer patients with HIV and examining the potential of microRNAs as prognostic or diagnostic biomarkers in this population.

2.27 Chapter Summary

This chapter summarizes the research on comparable studies, including the genome of HPV, global HPV infection rate, HPV infection rates in West Africa, HPV infection rates in Africa, HPV infection rates in Ghana, classification of HPV, risk factors of HPV infection, specimens used for HPV DNA testing, WHO recommendations for HPV DNA screening, genome of cervical cancer, global cervical cancer rate, cervical cancer infection rates in West Africa, cervical cancer infection rates in Africa, cervical cancer infection rates in Ghana, classification of cervical cancer, risk factors of cervical cancer infection, diagnosis used for cervical cancer, WHO recommendations for

cervical cancer screening, HPV and cervical cancer epidemiology, biogenesis of microRNA, theories of microRNA, functions of some microRNA in cervical cancer patients, expression patterns of microRNA in cervical cancer patients and non-cancerous patients, expression patterns of microRNA in cervical cancer patients on therapy, cervical cancer patients with or without therapy based on international federation of gynecology and obstetrics stages, expression patterns of microRNA in cervical cancer patients not on therapy, and expression patterns of microRNA in cervical cancer patients with HIV.

CHAPTER THREE

METHODOLOGY

Chapter three describes the study design and sampling technique, study area and population, inclusion and exclusion criteria, sample size determination, data collection, laboratory procedures, analysis, and ethical clearance. In this cross-sectional study, individuals were chosen using a convenient sample technique. Some of the specific microRNAs were found in the plasma of individuals with cervical cancer and non-cancerous patients. Finally, a detailed summary of the handling and evaluation of the gathered data was presented with a detailed description.

3.0 Research design

The researcher used a cross-sectional design for the study. The cross-sectional research design was considered best for the study because, according to Cohen, Manion, and Morrison (2007), in a cross-sectional design, researchers gather data at a particular point in time to describe the nature of existing conditions or identify standards against which existing conditions can be compared. As Leedy and Omrod (2010) recommended, this method is suitable for making generalizations from a sample to a population so that inferences can be made about the population's characteristics, knowledge, practice, and experience. Cross-sectional design provides a more accurate and meaningful picture of an event. It allows researchers to compare many variables at the same time, and gather information about different age groups in a short period. Irrespective of the strengths of the cross-sectional survey mentioned above, Fraenkel, Wallen, and Hyun (2012) identified difficulty in ensuring that the questions to be answered are clear and misleading, getting respondents to

answer questions thoroughly and honestly is a setback, and getting a sufficient number of questionnaires completed and returned so that meaningful analysis can be made is also a setback for the descriptive survey.

Notwithstanding the difficulty and setbacks of the cross-sectional survey design indicated above, it was still deemed most appropriate and applicable for the study. It helped the researcher to gather accurate data on participants with cervical cancer and those without cervical cancer at both CCTH and KATH to answer the various research questions.

3.1 Study Area

Cape Coast Teaching Hospital (CCTH) in Cape Coast and Komfo Anokye Teaching Hospital (KATH) in Kumasi, both in Ghana served as the study locations. These two facilities were chosen because they met the requirements for the research purpose. The Cape Coast Teaching Hospital, a referral hospital with a 400-bed capacity, is located in Cape Coast. In the north, it is bordered by Abura Township; in the south, Pedu Estate/4th Ridge; in the east, Nkanfua; and in the west, Abura/Pedu Estate. Cape Coast Metropolis (including Cape Coast North Municipal, Cape Coast South Municipal) with 189,925 population (2021) – census 122.0 km², area 1,557/km², population density (2021) 1.0% annual population change from (2010 → 2021). The Komfo Anokye Teaching Hospital is located in the Ashanti Region of Ghana. It is located in the Kumasi District and the Subin sub-district. The teaching hospital is also close to Adum, known as the business hub of Kumasi. Also, one surrounding landmark of the Komfo Anokye Teaching Hospital is the Kumasi Kejetia.

3.2 Population

The current metro area population of Kumasi in 2023 is 3,768,000, a 3.8% increase from 2022. The population of cervical cancer cases in Kumasi was 108 stated in a study by (Amoako et al.,2019). However, the population of cervical cancer cases in Cape Coast in 2022 was 41 from records of CCTH. Females within the age group of 24 to 60 years from both Komfo Anokye Teaching Hospital and Cape Coast Teaching Hospital were recruited for the study.

3.2.1 Inclusion criteria

- Patients attending Komfo Anokye Teaching Hospital and Cape Coast Teaching Hospital who took part in the screening activities organized by both teaching hospitals and came out as negative for both HPV and cervical cancer were used as the non-cancerous patients, and those who turned out to be positive for cervical cancer patients were used for the study.
- The study considered individuals within the age group of 24 to 60, though according to WHO, screening of patients for both HPV and cervical cancer should start at age 30.
- The study only used patients who agreed to participate and gave their consent, the entire sampling process was assisted by a trained gynecologist from both Cape Coast Teaching Hospital and Komfo Anokye Teaching Hospital.

3.2.2 Exclusion criteria

- The study excluded cervical cancer patients with other comorbidities such as hepatitis B, HIV/AIDS, and syphilis, attending both Komfo

Anokye and Cape Coast Teaching Hospital after their samples were screened with rapid diagnostic test kits.

- Patients aged below 24 years and above 60 years were excluded.
- Patients from other teaching hospitals other than KATH and CCTH were excluded.

3.3 Sampling Procedures

3.3.1 Sample size determination

Choosing the appropriate number of observations or duplicates for a statistical sample is known as sample size determination. Convenient sampling was done, which is a non-probability sampling method where units are selected for inclusion in the sample because they are easier for the researcher to access.

Samples were selected based on:

- The availability of cervical cancer and non-cancerous patients at both Komfo Anokye Teaching Hospital and Cape Coast Teaching Hospital.
- The willingness of cervical cancer and non-cancerous patients at both Komfo Anokye Teaching Hospital and Cape Coast Teaching Hospital to participate in the study.
- If the health state of the cervical cancer and non-cancerous patients at both Komfo Anokye Teaching Hospital and Cape Coast Teaching Hospital was conducive enough for blood samples to be drawn.

Therefore, a total sample size of 19 cervical cancer patients was obtained from the two teaching hospitals; this included 9 cervical cancer patients who were on therapy from Komfo Anokye Teaching Hospital and 10 cervical cancer patients who were not on therapy from Cape Coast Teaching Hospital. In

addition, a total sample of 21 patients screened as negative for both HPV and cervical cancer, thus,11 samples from Komfo Anokye Teaching Hospital and 10 samples from Cape Coast Teaching Hospital were used, whose age range was similar to the age range of the cervical cancer patients (24-60years) were added to the study. Available samples collected were based on the inclusion and exclusion criteria.

3.3.2 Sampling technique

A total of 40 female participants who were sexually active and aged between 24 to 60 were recruited for this study. The participants were recruited using a convenient sampling technique. Venus blood was taken from each participant by a registered gynecologist. Available participants who accepted to participate in the study provided informed permission and were made aware of the study's purpose. Then, a questionnaire was given to each participant, read to their understanding, and assisted in answering it.

3.4 Reliability of the instrument

A pre-test was completed on the thermocycler, polymerase chain reaction (PCR), and Applied Biosystem Prism7500 Fast machine by calibrating the ABI FAST 7500 RT using a dedicated dye calibration plate such as FAM, JOE, ROX, SYBR provided by the manufacturer to confirm the results matched with the manufacture’s results and also the instrument's consistency. To avoid false positives and negatives, appropriate precautions and procedures were used to conduct the test on each sample.

3.5 Ethical Consideration

The ethical clearance approved for this current study was obtained from the institutional review boards of the University of Cape Coast, Cape

Coast Teaching Hospital, and Komfo Anokye Teaching Hospital with the clearance identification numbers UCCIRB/CHAS/2021/294, CCTHERC/EC/2022/023 and KATH IRB/AP/025/23 respectively.

Detailed information about the study methodology and a promise of confidentiality were given to the study participants. Prior to the collection of data and samples, each participant's informed consent was requested and acquired.

3.6 Data Collection Procedures

3.6.1 Collection of Socio-demographic data

Data on age and gender were collected through a well-structured questionnaire. The questionnaire was divided into two sections: socio-demographic characteristics, sample collection, and laboratory investigation.

3.6.2 Blood Sample Collection

Patients with cervical cancer and non-cancerous patients had their venous blood samples obtained by a registered gynecologist. Using a syringe, approximately 5mL of whole blood was taken from each participant and deposited into EDTA tubes that were kept at -80°C for additional testing. Blood samples were taken by a registered gynecologist.

3.7 Laboratory methods

3.7.1 Plasma collection and storage

Blood samples were brought to the Department of Biomedical Sciences Laboratory, UCC, and centrifuged at 1500 x g at 10°C for 15 minutes to obtain plasma. Plasma was collected with a Pasteur pipette into a 1.5ml disposable cryovial tube (Jiangsu Huida Medical Instruments Co., Ltd.), labeled, well corked, and kept at -80°C, until assayed for immunological

analysis at the Department of Virology, Noguchi Memorial Institute for Medical Research.

3.7.2 RADI RNA kit for RNA extraction

Extraction of RNA was done using a RADI RNA kit (KH MEDICAL, 201, Jinwiseo-ro, Jinwi-myeon, Pyeongtaek-si, Gyeonggi-do, 12982, Republic of Korea). Nucleic acids of bacteria and viruses found in the samples were extracted using the RADI RNA kit. The nucleic acid that was extracted can be used in any experiment that calls for nucleic acids, including real-time PCR. RADI RNA kit is a spin column method that separates nucleic acids using a silica-based membrane. The sample is inactivated by the provided lysis buffer, the exposed nucleic acid is bound to the silica-based membrane, and impurities are removed by the washing buffer. After this, the purified DNA/RNA is eluted with an Elution buffer.

3.7.3 Protocol for the Extraction of RNA

A total of 200µl of pre-treated sample was collected into a 1.5ml centrifuge tube. Lysis buffer (KSB) of 255µl containing carrier molecule and 20µl of proteinase K were added and vortex thoroughly for 10 seconds to mix. Incubation was done at 56 ° C for 10 minutes. 350µl of Absolute ethanol was added and vortexed thoroughly for another 10 seconds to mix. The mixture was spun, dispensed, and centrifuged for one minute at 10,000 rpm. The spin column was then transferred to a new collecting tube, and the tube containing the filtrate was discarded. The spin column was then transferred to a fresh collecting tube, and the tube containing the passing liquid was discarded. Next, 500 µl of wash buffer 1 (KSW1) was dispensed into the spin column, which was then centrifuged at 10,000 rpm for one minute. The spin column

was filled with 500 µl of Wash buffer 2 (KSW2), which was then centrifuged for three minutes at a full speed of 13,000 rpm. The passing liquid in the dispensing tube was discarded after centrifugation, and the spin column was transferred to a clean 1.5ml microcentrifuge tube. The spin column was filled with 70 µl of KSE buffer and left to sit at room temperature for 1 minute. The extracted RNA was then centrifuged at 10,000 rpm for 1 minute before being used right away.

3.7.4 Brief introduction of the kit used for the cDNA synthesis

The SYBR-Green microRNA reverse transcription kit from Applied Biosystems, Sangon Biotech was used for the cDNA synthesis of the extracted RNA. To convert particular microRNAs to cDNA after RNA extraction, this kit's components work with the appropriate RT primers in the SYBR-Green microRNA Assays.

Table 3A: Constituent of MiRNA cDNA synthesis kit

Constituents	Volume
2X miRNA L-RT solution mix	10 µl
MiRNA L-RT Enzyme mix	1.5 µl
RNase free water	4.5 µl
Total RNA/ microRNA	3 µl
Stem-loop primer (RT primer)	1 µl

3.7.5 Process for cDNA synthesis

A master mix was prepared using the constituents from the complementary DNA (cDNA) kit as shown in the table above. A quarter reaction was prepared from each constituent from the kit for the master mix for cDNA synthesis. Samples were run in duplicate. A quarter reaction was calculated against the initial volume of each constituent for preparing the

master mix as shown in the table below. For example, 11 samples were used against eight different primers and then aliquoted into a standard plate in the master mix room. Primers aliquoted into the standard plates were sent to the Pre-RNA room and the RNA sample extracted was also aliquoted into the same standard plate, mixed thoroughly, and then placed in a thermocycler. The thermocycler, was gently harmonized for 3-5 seconds, warmed at 16 ° C for 30 minutes, incubated at 37 ° C for 30 minutes, and heated at 85 ° C for 5 minutes to inactivate the enzyme and kept at 4 ° C.

Table 3AI: Constituents for preparing quarter reaction of master mix for cDNA synthesis

Reagents	**The volume of reaction for a sample**	**Volume for a quarter reaction for 11 samples**
2XmiRNA L-RT solution mix	10 µl	2.5x11=27.5 µl
MiRNA L-RT enzyme mix	1.5 µl	0.375x11= 4.13 µl
RNase free water	3.5 µl	0.875x11= 9.63µl
Stem-loop primer (RT primer)	1 µl	0.25x11=2.75 µl
Total	16 µl	44µl

44 µl volume of master mix for cDNA synthesis was used for a sample.

Table 3AII: Additional constituents to be added to the master mix for cDNA synthesis to run a sample

Reagents	**The volume of reaction for a sample**	**The volume of a reaction for 11 samples**
Total RNA/microRNA	4 µl	1 µl
Total	4 µl	1 µl

The total volume of the master mix and the additional constituents used for a sample should sum up to 45 µl.

3.7.6 Polymerase chain reaction (PCR)

After the complementary DNA (cDNA) was created, a fluorescent RT PCR was performed using the created cDNA as a template. When compared to non-cancerous patients, the target microRNAs for these assays were found to have distinct expression patterns, suggesting that they may promote the spread of cancer (Esquela-Kerscher et al., 2006; Volinia, et., al., 2006). A microRNA qPCR kit, a SYBR-Green MicroRNA Reverse Transcription Kit from Applied Biosystems, Sangon Biotech was used to amplify the generated DNA after the addition of PCR chemicals. On the Applied Biosystems SYBR-Green Prism7500 Fast qPCR equipment, each sample was run in duplicate.

Table 3AIII: Constituent of qualitative Polymerase Chain Reaction (qPCR) kit

Constituent	Volume
2X MiRNA of PCR master mix	10 µl
ROX Dye(L)	1 µl
RNase free water	6 µl
Forward primer	0.5 µl
Reverse primer	0.5µl

3.7.7 Process for qualitative Polymerase Chain Reaction (qPCR)

Constituents in the PCR kit were used to prepare the Master mix for the PCR run. A quarter reaction was calculated against the initial volume of each constituent for preparing the master mix as shown in the table below. For example, 11 samples were used against eight different primers and then aliquoted into the fast trip plate in the master mix room. The fast trip plate was sent to the Pre-RNA (2) room and the template (cDNA) was also

aliquoted into the same fast trip plate. It was then mixed thoroughly and placed in the Applied Biosystems Prism7500 Fast machine. All the experiments were repeated two times. The reaction mixtures were incubated at 95°C for 5secs, followed by 40 cycles of 5 secs at 60°C and 30 secs at 64°C.

The specificity of each of the seven microRNAs (miR-specific) used in the qRT-PCR experiment was examined using melt curve analysis. The Ct values for each microRNA and the qRT-PCR were linearly correlated with the amplification of a particular target microRNA. In terms of the seven microRNAs' dissociation curves, the melting curve analysis just had one peak produced by the qRT-PCR experiment with no further peaks. This shows that a single PCR-amplified product was present and that the qRT-PCR experiment was specific. In both groups of cervical cancer patients in this investigation, the melting temperatures for all seven microRNAs were between 78°C ± 1°C for miR-146a, 79°C ± 1°C for miR-155, 80°C ± 1°C for miR-29a, 80°C ± 1°C for miR-29b, 80°C ± 1°C for miR-34a, 78°C ± 1°C for miR-233 and 80°C ± 1°C for miR-27a in both groups of cervical cancer patients in this study.

Table 3AIV: Constituents for preparing quarter reaction of master mix for PCR

Reagents	**The volume of reaction for a sample**	**The volume of a reaction for 11 samples**
2X MiRNA of PCR master mix	10 µl	2.5x11= 27.5 µl
ROX Dye(L)	1 µl	0.25x11= 2.75 µl
RNase free water	6 µl	1.5x11= 16.5 µl
Forward primer	0.5 µl	0.125x11= 1.38µl
Reverse primer	0.5 µl	0.125x11= 1.38µl
Total	18 µl	49.5µl

49.5µl volume of master mix for PCR was used for a quarter reaction for 11 samples.

Table 3AV: Additional constituents to be added to the master mix for PCR to run a sample

Reagents	The volume of reaction for a sample	The volume of a reaction for 11 samples
Template DNA	2 µl	0.5µl
Total	2µl	0,5µl

The total volume of the master mix and the additional constituents used for a sample should sum up to 50 µl

3.7.8 MicroRNA primers

These are the list of the eight (8) microRNAs used. In cycle thresholds (Ct) for cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients, seven microRNAs were calculated and normalized to one microRNA, that is miR-16, which was found to be the literature's most often used endogenous control microRNA for RT-qPCR (Schrauder et al.,2012). However, only seven microRNAs were screened from the plasma of all cervical cancer patients, both on therapy and not on therapy, and non-cancerous patients. These micro RNAs were used because most of them were associated with cervical cancer cases in other studies.

Table 3AVI: MicroRNAs with their RT primer

Oligo name	Stem loop RT primer and qPT-PCR Foward-primer 5' to 3'
hsa-miR-146a-RT	Gtcgtatccagtgcagggtccgaggtattcgcactggatacgacaaccca
miR-146a-F	gccgtgagaactgaattcca
hsa-miR-155	Gtcgtatccagtgcagggtccgaggtattcgcactggatacgacacccct
miR-155-F	Cggcttaatgctaatcgtgat
hsa-miR-29a	gtcgtatccagtgcagggtccgaggtattcgcactggatacgac taaccg
miR-29a-F	cggctagcaccatctgaaat
hsa-miR-29b	gtcgtatccagtgcagggtccgaggtattcgcactggatacgacaacact
miR-29-F	cgcgtagcaccatttgaaatc
hsa-miR-34a	gtcgtatccagtgcagggtccgaggtattcgcactggatacgacacaacc
miR-34a-F	atcgtggcagtgtcttagct
hsa-miR-223	gtcgtatccagtgcagggtccgaggtattcgcactggatacgacaactca
miR-223-F	gcaacgtgtatttgacaagc
hsa-miR-27a	gtcgtatccagtgcagggtccgaggtattcgcactggatacgacgcggaa
miR-27a-F	cgcattcacagtggctaag
U6	ctcgcttcggcagcaca (forward) aacgcttcacgaatttgcgt (reverse)
Universal reverse primer	Gtgcagggtccgaggt

3.8 Data Analysis

Microsoft (MS) Excel (version 2016) was used to load all obtained data, and GraphPad Prism 9.3.1(471) (GraphPad Software Inc., CA) was used for statistical analysis. The sociodemographic features and additional relevant aspects were described using descriptive statistics.

The Kruskal-Wallis test was employed to examine the variations in microRNA expression levels among the fold change of cervical status, including non-cancerous patients, positive patients with cervical cancer who are not on therapy, and positive patients with cervical cancer who are on therapy. Each median was given together with its standard deviation (SD).

When the p-value is less than 0.05 (p-value < 0.05), statistical significance was taken into account.

3.8 Chapter Summary

Participants for this cross-sectional study were drawn from the Cape Coast Teaching Hospital (CCTH) and the Komfo Anokye Teaching Hospital (KATH) located in Ghana's Central and Ashanti regions, respectively. Using a convenient sample technique, 40 adult females between the ages of 24 and 60 who consented to participate in the study were selected.

The study was first given the go-ahead by the UCC, CCTH, and KATH IRB boards, with clearance identification numbers of UCCIRB/ CHAS/2021/294, CCTHERC/EC/2022/023 and KATH IRB/AP/ 025/23, respectively.

Both cervical cancer and non-cancerous patient samples were extracted, and all of the samples taken were run through complementary DNA (cDNA) synthesis and Polymerase Chain Reaction (PCR) using particular primers. GraphPad Prism 9.3.1(471) was used to examine the data after being entered into MS Excel 2016 for analysis (GraphPad Software Inc., CA).

CHAPTER FOUR

RESULTS AND DISCUSSION

After seven different microRNAs were analyzed on each of the 40 samples used in this study, the fold change of the data was calculated using the Shapiro-Wilk test. The study employed non-parametric tests because the data obtained did not conform to normal distributions. The normality of the data was evaluated by the Shapiro-Wilk test. Numbers were used to express categorical data. The frequency was used to categorize ages, marital status, and cervical cancer stages into percentages. Results for each parameter were displayed as a median and interquartile range (IQR). Individuals' microRNA expression levels were compared using Dunn's multiple comparisons test depending on whether they had cervical cancer, including cervical cancer patients on treatment, cervical cancer patients not on therapy, and non-cancerous patients. The differences in the microRNA expression levels among cervical cancer status groups, including cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients were performed using Kruskal-Wallis analysis. To examine the stages of cervical cancer patients' microRNA expression, the receiver operating characteristic curve (ROC) was used. MS Excel and GraphPad Prism 6.0 (GraphPad Software Inc., CA) were used for all statistical analyses. A 95% confidence interval and a p-value of less than 0.05 were required for the results to be considered significant.

4.0 Socio-demographic characteristics of participants

After receiving consent and a 100% response rate, all forty patients were included in the research. The ages of participants ranged from 24 years to 60 years and the majority of the participants, 57.5% (n=23) were within the age range of 46 years to 60years, followed by 25% (n=10), within the age range of 35years to 45years and 17.5% (n=7) within the age range of 24 years to 34years. As shown in **Table 4.1,** 70% (n=7) of patients were in stage III, 20% (n=5) were in stage IV, and 10% (n=3) were in stage II. Age showed a significant difference of p=0.41 to the cervical cancer status; cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients. For each experiment, duplicate samples were conducted. Cycle thresholds (Ct) for cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients were determined and standardized to miR-16, which was found to be the literature's most widely utilized endogenous control microRNA for RT-qPCR (Schrauder et al.,2012). The comparative Ct approach was used to calculate the levels of microRNA expression in cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients. The Ct value was computed by deducting the Ct values for each unique mature microRNA response from the average Ct values of the non-cancerous patients' miR-16 for each sample. The -ΔΔCt value was determined by deducting the -ΔCt value of a normal sample from the respective -ΔCt values of the patient samples. The relative expression levels of all the seven microRNAs used were evaluated using the $2^{-\Delta\Delta}C^{t}$ method. (Ohysahiki et al.,2011, Schrauder et al.,2012).

Table 4.1: Characteristics of the subjects participating in the study

Characteristics	Total Number	Frequency (%)	non-cancerous patients(n=21)	Cervical cancer patient not on therapy (n=10)	Cervical cancer patient on therapy (n=9)	P value
Age range/years	40		21-60	34-60	30-60	0.4057
Median ± SD			42.0±11.67	49.5±8.7	46.0±8.4	
Age range						
24-34		7(17.5)				
35-45		10(25)				
46- 60		23(57.5)				
Marital status						
Single	14	(35)				
Married	8	(20)				
Divorced	18	(45)				
FIGO stages						
II	3	(10)				
III	7	(70)				
IV	5	(20)				

Kruskai-Wallis test was used to calculate the median and standard deviation of all ages of the cervical status thus, cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients with a p-value p=0.41.

4.1 Expression patterns of microRNA to the fold change of non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy.

In Table 4.2; the study analyzed the difference in the expression patterns of microRNA to the fold change of non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy. Among the seven microRNAs used, the values of only three microRNAs, such as miR-146a, miR-155, and miR-27a, were statistically significant to the fold change of cervical cancer patients on therapy, control, and cervical cancer patients not on treatment with p values of, p=0.0443, p=0.0130 and p=0.0085 respectively.

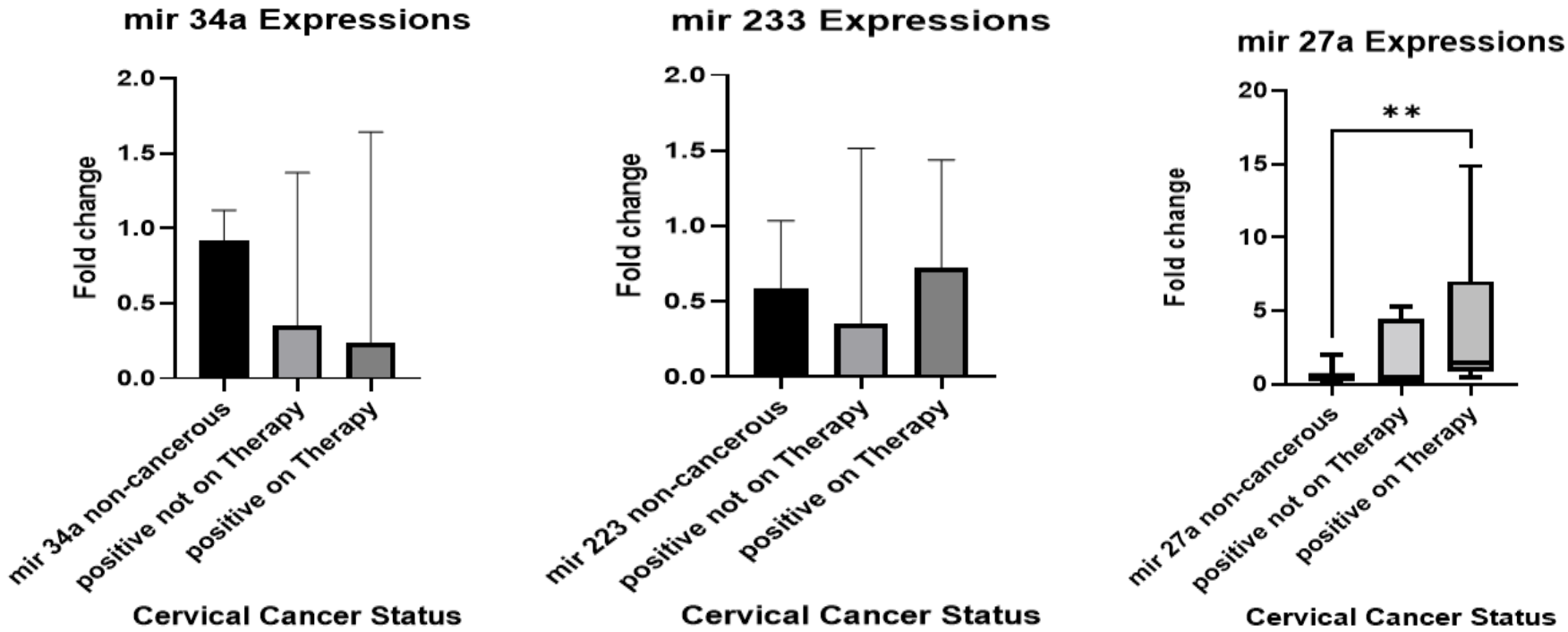
Table 4.2: Median, IQR, and p-value of the seven microRNAs used

MICRORNA	non-cancerous patients Median	POSITIVE CC PATIENT WITHOUT THERAPY Median	POSITIVE CC PATIENT WITH THERAPY Median	P value
MiR-146a	0.54(0.41-0.90)	1.42(1.08-2.80)	0.37(0.12-6.05)	**0.04**
MiR-155	0.59(0.25-1.36)	0.10(0.00-0.31)	0.66(0.34-1.47)	**0.01**
MiR-29a	0.70(0.27-1.59)	0.60(0.09-1.76)	0.31(0.10-0.81)	0.35
MiR-29b	0.72(0.32-0.96)	0.09(0.01-2.44)	0.62(0.19-2.71)	0.28
MiR-34a	0.92(0.35-1.12)	0.35(0.02-1.37)	0.24(0.14-1.64)	0.25
MiR-233	0.58(0.35-1.04)	0.35(0.14-1.04)	0.72(0.29-1.44)	0.65
MiR-27a	0.38(0.14-0.79)	0.42(0.07-4.41)	1.44(0.83-7.02)	**0.01**

The variations in microRNA expression levels between the fold change of the cervical status, including non-cancerous patients, Positive cervical cancer patients not on therapy, and Positive cervical cancer patients on therapy were analyzed using the Kruskal-Wallis test. Data from the table represent the median with IQR with p-value, <0.05, and values of only miR-146a, miR-155, and miR-27a were significantly different. IQR – Interquartile range, CC-Cervical Cancer.

4.2 Expression patterns of microRNA among cervical cancer status

MicroRNA expression levels were compared with the three categories- non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy. However, in **Fig 4.1B,** miR-155 expression level was found to be upregulated in non-cancerous patients when compared to cervical cancer patients, not on therapy, while cervical cancer patients not on therapy were found to be downregulated with a p-value of 0.02. In **Fig. 4.1G**, the expression level of miR-27a was also elevated in the non-cancerous patients compared to cervical cancer patients on therapy, with a p-value of 0.01. The remaining genes' expression levels of microRNAs, such as miR-146a, miR-29a, miR-29b, miR-34a, and miR-233, were not statistically significantly different among cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients using (Dunn's multiple comparisons test).



E **F** **G**

Figure 4.1: Expressions pattern of microRNAs among cervical cancer status thus, positive cervical cancer patients not on therapy, positive cervical cancer patients on therapy, and non-cancerous patients

A graphical representation of microRNA expression in plasma of study participants grouped into three categories thus, non-cancerous patients, cervical cancer patients on therapy (Positive on therapy), and cervical cancer patients not on therapy (Positive not on therapy). The seven different microRNAs analyzed among the three categories are miR-146a (a), miR-155 (b), miR-29a (c), miR-29b (d), miR-34a (e), miR-233 (f), miR-27a (g). The expression patterns of microRNAs, miR-155 and miR-27a showed a statistically significant difference with p values of ($p = 0.02$, and $p = 0.01$ respectively), while miR-29a, miR-29b, miR-233, miR-146a, miR-34a were not significantly different with a p-value of ($p = >0.99$, $p >0.99$, $p >0.99$, $p > 0.07$, $p = > 0.65$) respectively between the study groups according to **Fig. 4.1**

4.3 microRNA among the different statuses of cervical cancer

In **Table 4.3**; with cervical cancer classified into some FIGO stages, non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy, the expression patterns of microRNAs such as miR-146a, miR-155, and miR-29b were highly expressed (p values, $p=0.03$, $p=0.00$, and $p=0.02$, respectively). However, results in the table were presented in the median and inter-quartile range, but some were only in the median without the inter-quartile range because only one participant was present in those FIGO stages of cervical cancer patients as shown in Table 4.3.

Table 4.3: Showing the median with IQR, and p-value of microRNA among the different FIGO stages of cervical cancer patients on therapy and cervical cancer patients not on therapy

FIGO STAGES	miR-146a	miR-155	miR-29a	miR-29b	miR-34a	miR-233	miR-27a
CC not on Thpy S2b	0.32(0.19-0.46)	0.06(0.00-0.06)	0.89(0.13-1.66)	0.07(0.01-0.14)	0.29(0.13-0.57)	1.21(0.13-0.57)	0.31(0.06-0.56)
CC on Thpy S2b	1.41	0.03	0.16	0.00	0.21	0.79	0.82
CC not on Thpy S3a	0.28	0.02	0.60	0.01	0.01	0.14	0.03
CC on Thpy S3a	0.89	0.66	0.05	0.24	0.09	0.37	2.95
CC not on Thpy S3b	0.12	0.43	0.60	2.30	0.59	1.37	5.305
CC on Thpy S3b	1.34(0.35-1.34)	1.115(0.382-1.467)	0.239(0.843-0.973)	1.61(0.73-2.99)	0.45(0.29-2.23)	0.87(0.25-1.46)	2.294(0.714-11.930)
CC not on Thpy S4a	0.11	0.003	0.004	0.01	0.12	0.03	0.289
CC on Thpy S4a	2.80(2.34-3.26)	0.30(0.30-0.98)	0.44(0.44-0.44)	0.21(0.15-0.26)	0.13(0.07-0.19)	0.40(0.03-0.72)	1.058(0.830-1.286)
CC not on Thpy S4b	10.46	0.15	2.08	5.70	37.14	0.49	0.275
CC on Thpy S4b	8.89	3.92	3.52	12.36	2.97	4.38	10.890
p-value	**0.03**	**0.00**	0.41	**0.02**	0.10	0.54	0.0964

Using the Kruskal-Wallis test, the variance in microRNA expression levels between non-cancerous patients, cervical cancer patients not on therapy, and cervical cancer patients on therapy among different categories of stages. Data on the table are presented in median with IQR. Among all seven microRNAs compared to non-cancerous patients, cervical cancer on therapy stages and cervical cancer not on therapy stages, mir-146a, mir-155, and mir-29b showed a significant difference with p-value, $p < 0.05$. IQR – Interquartile range; CC- Cervical cancer; Thpy-Therapy; S- Stages; miR-MicroRNA; p-value, <0.05.

4.4 MicroRNA expression among cervical cancer patients in International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer

The graphs below represent microRNA expression according to clinical grading with cervical cancer status, thus positive cervical cancer patients not on therapy (Pos no thpy), positive cervical cancer patients on therapy (Pos with thpy), and non-cancerous patients. The International Federation of Gynaecology and Obstetrics (FIGO) stages were used to determine the clinical state. The study samples came from cervical cancer patients at various clinical stages (stage 2b, stage 3a, stage 3b, stage 4a, and stage 4b).

Using one-way ANOVA, statistical analysis revealed significant expressions between the cervical cancer status and its stages for the following targeted microRNAs: miR-155, miR-146a, miR-29b, miR-34a, miR-223, and miR-27a (**Figure 4.2**). With the help of the statistical analysis, we contrasted the various stages of each microRNA using the t-test, non-parametric test, and Wilcoxon test. Most of the microRNAs' expression patterns when compared to the stages were found that all but miR-29a had a statistically significant difference.

Figure 4.2 (A): The expression profile of miR-146a indicated a significant difference when compared among positive cervical cancer patients with therapy (Pos with thpy), positive cervical cancer patients without therapy (Pos no thpy), and non-cancerous patients to the International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer. Among all the cervical cancer patients in the FIGO stages of cervical cancer, when

comparing positive cervical cancer patients with therapy (Pos with thpy) to positive patients without therapy (Pos no thpy) and the non-cancerous patients, the expression profile of miR-146a in stage 4a of the disease was found to be highly expressed. The p-value was found to be 0.01 and a fold change of 2.26.

When compared to non-cancerous patients and positive cervical cancer patients without therapy in connection to the FIGO stages of cervical cancer, however, pos without thpy was elevated in stage 4b with a p-value of 0.00 and a fold change of 2.26, but pos with thpy was downregulated.

Figure 4.2 (B): In stage 4b, the expression profile of miR-155 showed a significant difference, when pos with thpy was found to be upregulated to pos no thpy and non-cancerous patients with a p = 0.00 and a fold change of 3.34, while pos no thpy, downregulate to pos with thpy and control.

Figure 4.2 (D): When pos no thpy was found to be elevated in stage 3b, with a p value of 0.00 and a fold change of 0.88 in comparison to pos with thpy and non-cancerous patients, there was a statistically significant difference in the expression pattern of miR-29b. Though pos with thpy was downregulated in stage 3b, it was found to be upregulated in stage 4b with p = 0.00 and fold change of 1.58 when compared to pos no thpy and non-cancerous patients, however, pos no thpy was downregulated.

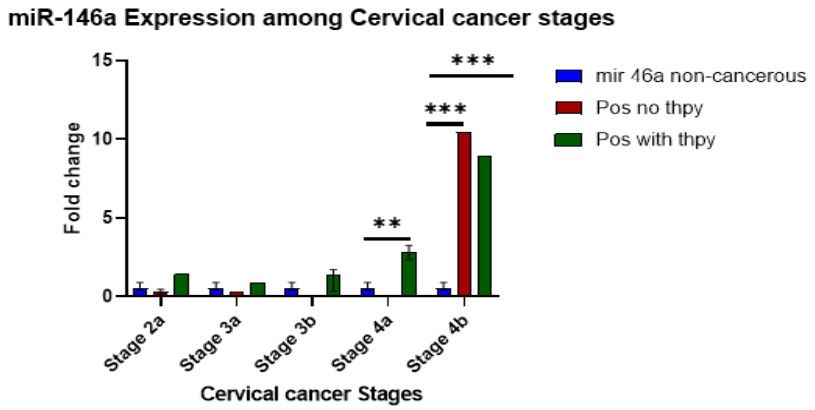
Figure 4.2 (E): MiR-34a showed a significant difference in the expression pattern in stage 4b, in comparison to non-cancerous patients, pos without thpy was upregulated (p = 0.00 and fold change of 9.54) while pos with thpy was downregulated (p = 0.00 and fold change of 8.35).

Figure 4.2 (F): The expression pattern of miR-233 also showed a significant difference in stage 4b, when pos with thpy was upregulated with a

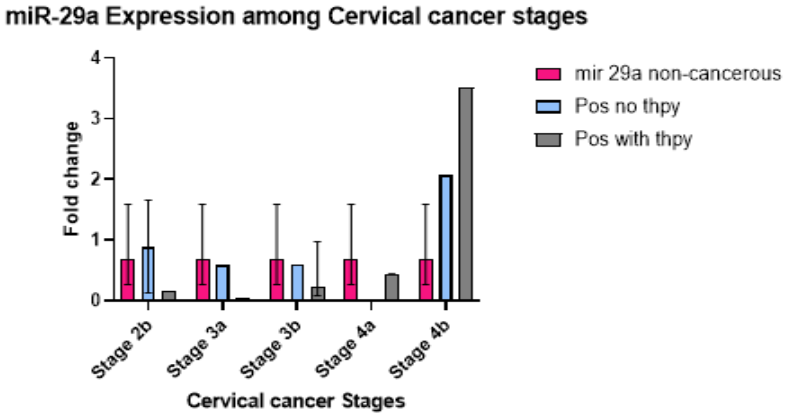
p = 0.01 and a fold change of 3.80, and pos no thpy was downregulated in comparison to non-cancerous patients.

Figure 4.2 (G): The expression profile of miR-27a was highly expressed when pos with thpy was found to be upregulated with p= 0.00 and fold change of 0.34 and pos no thpy was downregulated to non-cancerous patients in stage 3a. In contrast, it was discovered that in stage 3b, pos without thpy was downregulated while pos with thpy had an upregulated fold change of 4.93 and a p-value of p=0.00.

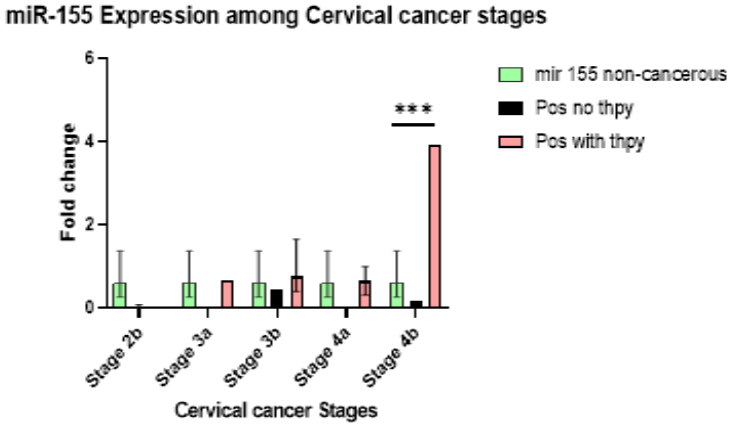
Meanwhile, in **Figure 4.2 (B):** When compared to both pos with thpy, pos without thpy, and non-cancerous patients at all stages, miR-29a's expression profile did not exhibit any significant differences.



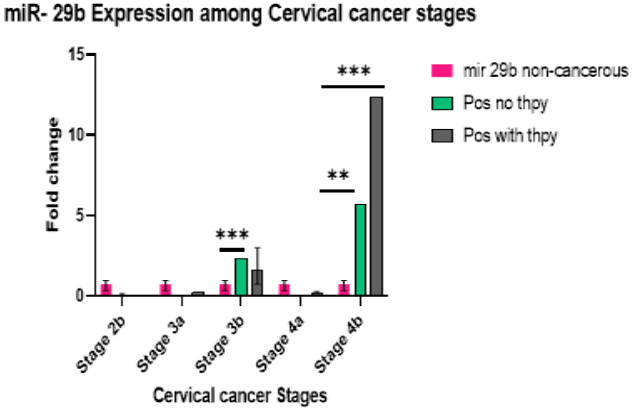
A



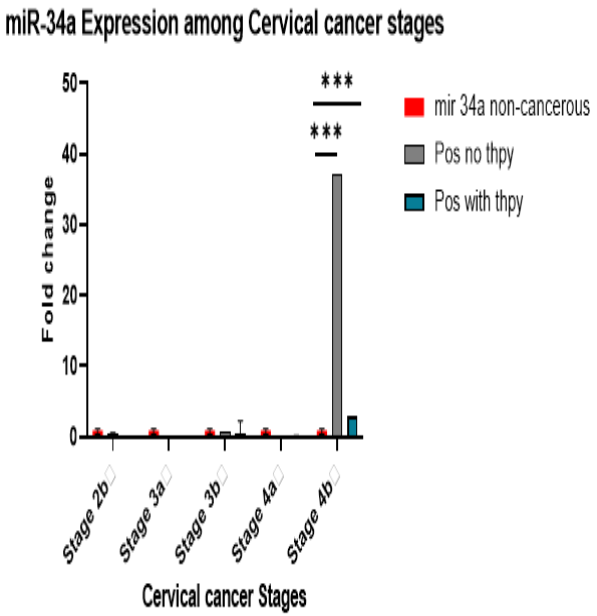
B



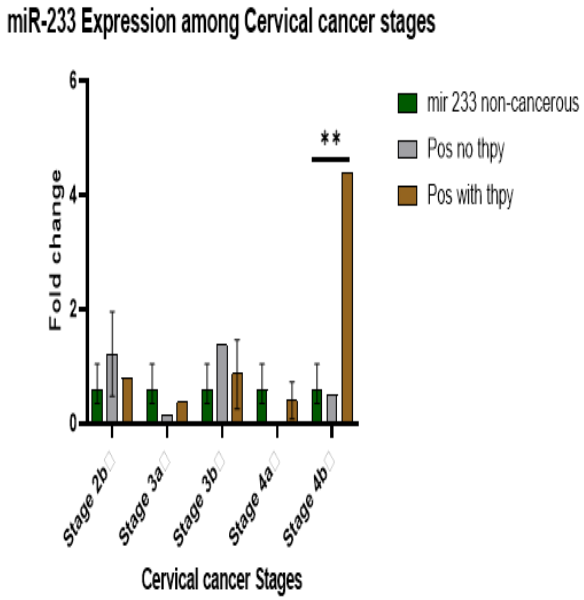
C



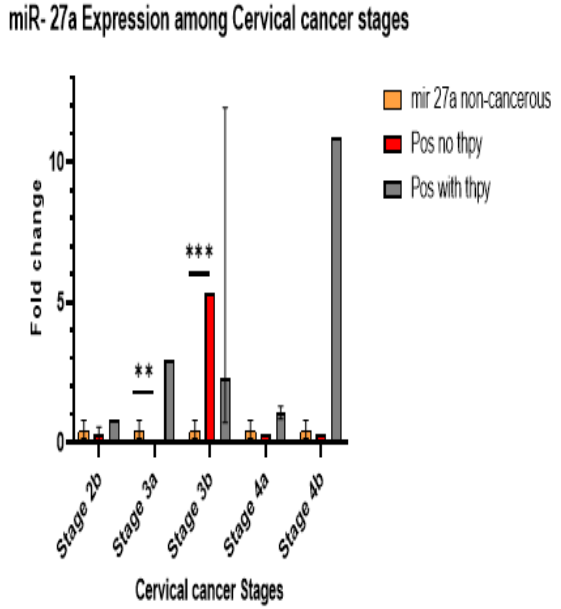
D



E



F



G

Figure 4.2: microRNAs expression among cervical cancer patients in International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer

4.5 Correlation, linear and multiple linear regression against the expression pattern of microRNAs

Among all the seven microRNAs used against the effects of treatments, only miR-29a showed a significant correlation with a p-value of p=0.01, and correlation coefficient (r) = -0.82, whilst the remaining microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a did not show any significant correlation against the effect of treatment with p-value > 0.05, using non-parametric spearman correlation. A crude analysis using simple linear regression was used for the four microRNAs miR-29a, miR-34a, miR-27a, and miR-155 against the effect of treatment and none of the microRNAs showed statistically significant difference but when age was adjusted against the effect of treatment using adjusted analysis thus multiple Linear Regression, miR-34a, miR-27a and miR-155 showed a statically significant difference with p-value 0.04, 0.01 and 0.05 respectively and parameter estimate **(β_2)** of -0.31, -1.43 and -0.31 respectively against the effect of treatment but miR-29a did not show any significant difference.

Table 4.4: Correlation, linear regression, and multiple linear regression among the effects of treatment against the expression pattern of microRNAs such as MiR155, MiR-29a, MiR-34a, and MiR-27a.

	CORRELATION	CRUDE ANALYSIS		ADJUSTED ANALYSIS	
		Linear Regression		Multiple Linear Regression Weeks (β_2)	
microRNAs	P value and r	P value and (95 CI)		P value and (95 CI)	Parameter Estimate (β_2)
		Age	Weeks		
MiR-155	**0.20**(-0.47)	**0.46**(35.39 to 54.15)	**0.13(**2.42 to 7.88)	**0.05**(-0.61 to -0.01)	-0.31
MiR-29a	**0.01**(-0.82)	**0.76**(37.73 to 54.75)	**0.13(**92.36 to 7.15)	**0.09**(-0.58 to 0.06)	-0.26
MiR-34a	**0.69**(-0.15)	**0.33**(36.41 to 52.94)	**0.16**(2.24 to 7.35)	**0.04**(-0.58 to -0.02)	-0.31
MiR-27a	**0.15**(-0.52)	**0.22(**35.60 to 52.17)	**0.13**(2.37 to 7.59)	**0.01**(-2.42 to -0.45)	-1.43

4.6 Effects of treatments against the seven microRNAs

Among all the seven microRNAs used against the effects of treatments, only miR-29a showed a significant correlation with a p-value of p=0.01, and correlation coefficient (r) = -0.82, whilst the remaining microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a did not show any significant correlation against the effect of treatment with p-value > 0.05, using non-parametric spearman correlation. Simple linear regression was used to identify the direction of the fold change of miR-29a against the effect of treatment, and it was discovered that miR-29a's fold change was decreasing, suggesting that miR-29a downregulates in opposition to the effects of treatment. Table 4.4 has the detailed information.

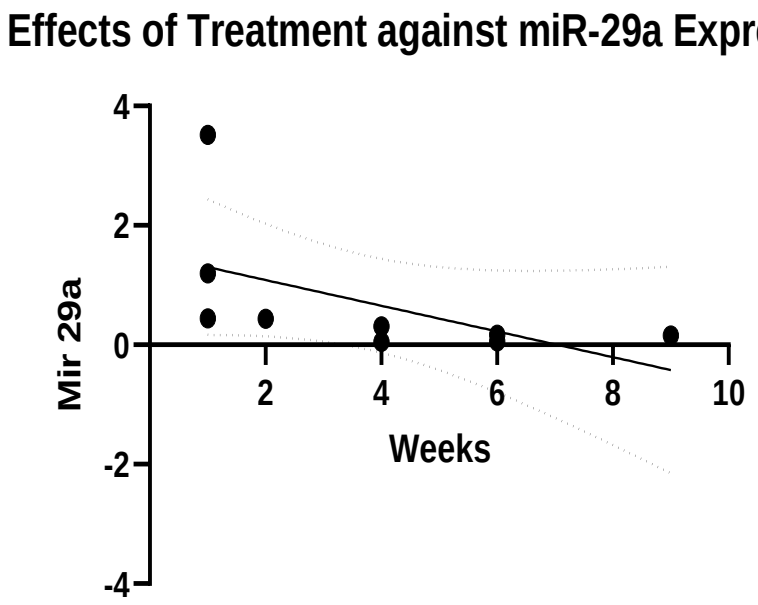


Figure 4.3: Correlation of Effects of treatment on the fold change of miR-29a.

4.7 Diagnostic Potential values of microRNAs

The diagnostic utility of each microRNA in this study was evaluated by creating ROC curves and the area under the curve (AUC). The cut-off points of each microRNA for patients with cervical cancer who were not on therapy

based on FIGO stages of cervical cancer patients were examined. According to Nahm. (2022) among the seven microRNAs examined, miR-155 showed extremely good diagnostic performance, and miR-146a, miR-34a, and other microRNAs demonstrated fair diagnostic performance to identify cervical cancer patients not on therapy at various FIGO stages of cervical cancer. The AUC was considered independently after ROC curves for the chosen microRNAs were created. According to Table 4.5, the AUC and the 95% CI of microRNAs such as miR-146a, miR-155, and miR-34a were 0.77 (0.47-1.00), 0.91 (0.78-1.00), and 0.71 (0.42-0.10), respectively. The area under the ROC curve calculates a test's usefulness; a larger area indicates a more beneficial test. The utility of the microRNAs in comparison to non-cancerous patients and cervical cancer patients not on therapy was assessed using the area under ROC curves. Four of the seven microRNAs used, including miR-29a, miR-29b, miR-27a, and miR-233, had AUC values that were less than 0.6, with an AUC value of 0.5 indicating that the ROC curve is diagonal (i.e., 45-degree line) The remaining three microRNAs, miR-146a, miR-155, and miR-34a, have shown to be effective diagnostic tools with p values, $p < 0.5$ indicating that the ROC curves were above the diagonal line, and as a result, the diagnostic test has no discriminatory ability to identify a patient that has the disease or not. However, ROC curves above the diagonal line are thought to have the ability to distinguish between patients who have the condition and those who don't. The information in Table 4.5 also showed that the examined microRNAs had the following accuracy in their sensitivity and specificity: miR-155> miR-146a> miR-34a. Table 4.5 provides a detailed breakdown of the three microRNAs' diagnostic values.

Table 4.5: Receiver operating characteristic curves for microRNAs of the fold change of cervical cancer patients not on therapy based on FIGO stages

MIRORNAs	AUC	95% CI	CUT-OFF	SENSITIVITY	SPECIFICITY	P VALUE	LIKELIHOOD RATIO
MiR-155	0.91	0.78 to 1.00	<0.22	83.33%	80.95%	**0.00**	**4.38**
MiR-146a	0.77	0.47 to 1.00	<0.48	83.33%	66.67%	**0.05**	**2.50**
MiR-34a	0.71	0.42 to 1.00	<0.60	83.33%	66.67%	**0.11**	**2.50**

microRNA of cervical cancer patients not in therapy stages; AUC, Area under the curve; CI, Confidence Interval 95%; p-value, < 0.5.

4.8 Receiver operating curves (ROC) of microRNAs among the fold change of cervical cancer patients not on therapy according to the FIGO stages of cervical cancer

The receiver operating characteristic curves for the 3 distinct microRNAs of cervical cancer patients not on therapy are shown in Figure 5. According to **Fig 4.4 (A),** sensitivity was 83.3 percent and specificity was 80.95 percent when the cut-off value was set to the optimal level of 0.22, and AUC of 0.91 which depicts the ROC curve of miR-155. **Fig 4.4 (B)** Sensitivity was 83.33 percent and specificity was 66.67 percent on the ROC curve for miR-146a, which had an AUC of 0.77 when the cut-off value was adjusted to the ideal level of 0.48 and **Fig 4.4 (C)** ROC curve of miR-34a, the AUC was 0.71 when the cut-off value was set to the optimal point of < 0.60, sensitivity was 83.33%, and specificity was 66.67%. Table 4.5 provides a detailed breakdown of the three microRNAs' diagnostic values.

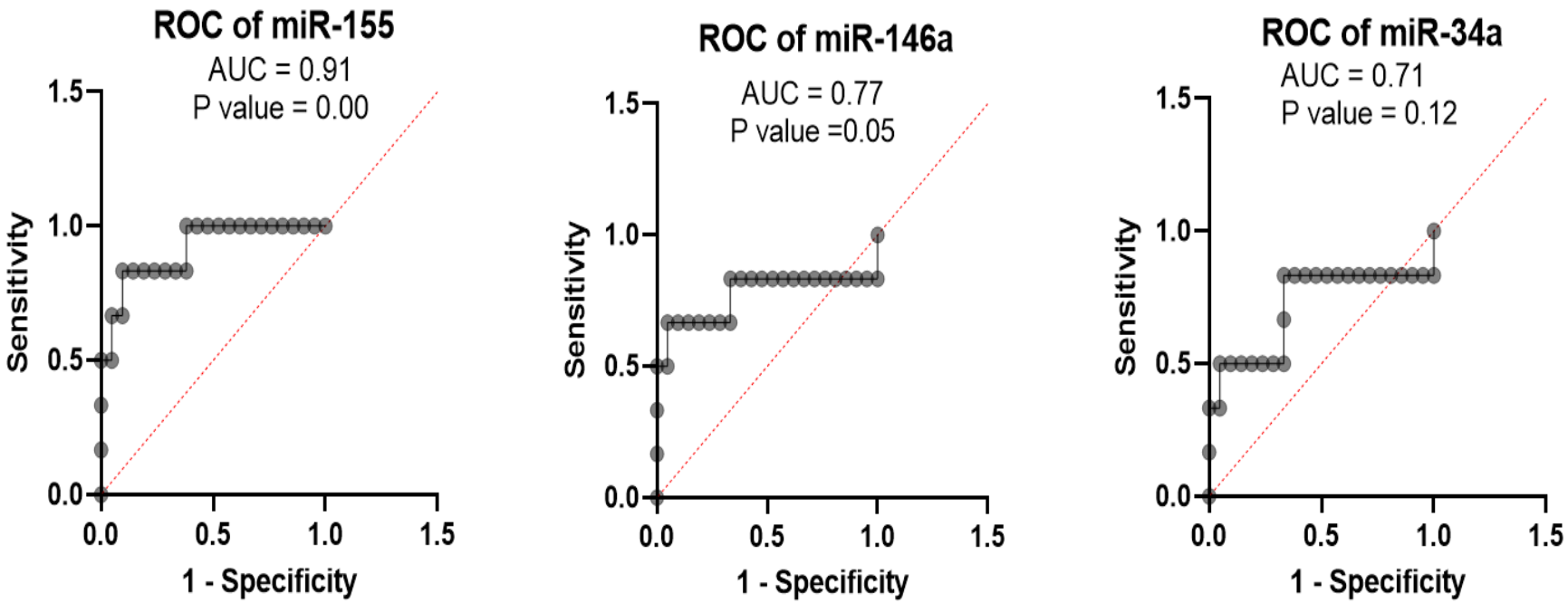


Figure 4.4: Receiver operating curves (ROC) of microRNAs among the fold change of cervical cancer patients not on therapy according to the FIGO stages of cervical cancer and non-cancerous patients showing their diagnostic potential.

4.9 Discussion

Cervical cancer is associated with significant rates of mortality and morbidity, which disproportionately impact women that are young between the ages of 30 and 50, the disease is still considered a public health concern (Shah et al.,2016). Although the most common preventive approach for identifying cervical cancer is the Pap smear test, it has certain drawbacks (Zhang et al.,2015). Therefore, novel ways are needed for early detection, prognosis, and therapy to prevent this disease.

MicroRNAs have been acknowledged as a new class of minimally invasive biomarkers because of their consistency and potential clinical utility in the early identification of the cervical intraepithelial neoplasia stage. The level of microRNA can be altered in either direction when there are genetic and epigenetic changes in malignancies (Chen et al., 2014; Dundea-Simon et al.,2022). However, this study was intended to determine the circulating microRNA in the plasma of cervical cancer patients at some selected teaching hospitals in Ghana.

In this study, the ages of participants ranged from 24 years to 60 years and the majority of the participants, 57.5% were within the age range of 46 years to 60years, followed by 25% within the age range of 35years to 45years and 17.5% within the age range of 24 years to 34years. The majority of age participants for cervical cancer were between the ages of 46 to 60 years, which relates to why the screening recommendations by WHO,2021, state that women should begin to do HPV DNA screening at the age of 30 years since HPV DNA testing has higher sensitivity for detecting precancer and cancers. A study by Forman et al., (2012) suggests that about **11-12%** of sexually

active individuals globally are infected with HPV at any given time. By age 50, up to **80%** of sexually active women may have been infected with at least one type of HPV (Smith et al.,2008). HPV infection rates in West Africa vary widely, with studies reporting prevalence rates between **20% and 50%** among sexually active women (Smith et al.,2008). High-risk HPV types, particularly HPV 16 and 18, are commonly detected and are associated with cervical cancer (Ramakrishnan et al.,2015). Specific countries such as Ghana and Nigeria report higher prevalence rates, often exceeding **30%** among women attending gynecological clinics (Konadu et al.,2019). A study by Schubert et al., (2023) suggests that cervical cancer is the fourth most common cancer among women worldwide, with an estimated **604,000 new cases** diagnosed in 2020. The global age-standardized incidence rate is approximately **9.2 per 100,000 women (**Zhang et al.,2019). Cervical cancer accounts for about **342,000 deaths** annually, making it a significant cause of cancer-related mortality among women (Hosono, S. 2024). The global mortality rate is approximately **7.5 per 100,000 women (**Sopik et al.,2015). Cervical cancer is a significant health issue in West Africa, with incidence rates ranging from **20 to 13.1 per 100,000 women** in various countries (Arbyn et al.,2020). Countries like Ghana, Nigeria, and Sierra Leone report some of the highest rates in the region. The region experiences high mortality rates associated with cervical cancer, with an estimated **341,831 deaths** annually attributed to the disease (Bogdanova et al.,2022). Cervical cancer poses a significant health threat to women worldwide, necessitating effective screening strategies. Traditional methods, such as Pap smears and HPV testing, are essential for early detection but present several inconveniences that limit their

effectiveness. Therefore, the exploration of microRNAs (miRNAs) as biomarkers offers a promising alternative that can address these issues, which will help reduce the mortality and morbidity rates in our developing countries.

Identifying the expression patterns of specific microRNAs in cervical cancer patients on or not on therapy and healthy individuals could serve as diagnostic, prognostic, and treatment markers. The expression pattern of seven different microRNAs such as miR-146a, miR-155, miR-29a, miR-29b. miR-34a, miR-233, and miR-27a among cervical cancer statuses such as non-cancerous patients, positive cervical cancer patients on therapy, and positive cervical cancer patients not on therapy were investigated. However, only two microRNAs (miR-155 and miR-27a) showed statistically significant differences in their expression profiles among cervical cancer status, thus, positive cervical cancer patients not on therapy, positive cervical cancer patients on therapy, and non-cancerous patients. According to research by Papaconstantinou et al. (2013) and Wang et al. (2009), there was a significant difference in the expression pattern of miR-155 between the non-cancerous patients and positive cervical cancer patients who were not on therapy in this study, which suggested that plasma and tumor tissues had elevated expressions of miR-155 and that tissue expressions were linked to tumor stage and a poorer prognosis for patients with cervical cancer undergoing therapy than for non-cancerous patients. Further study by Lv et al. (2018) discovered that patients who reacted to the medication had much lower levels of miR-155, and this downregulation was linked to a stronger response to the therapy.

However, in this study, when cervical cancer patients were generally analyzed in the non-cancerous patients, the expression pattern of miR-155,

was found to be upregulated in cervical cancer patients than the non-cancerous patients which was similar to a study by Sun et al., (2016), who found that the expression of the miR-155 was substantially higher in cervical cancer tissues than it was in the non-cancerous tissues. In cervical cancer tissues, Wang et al. (2008) also discovered that the expression pattern of miR-155 was up-regulated. A study by Lei et al. (2012) demonstrated that ectopically overexpressed miR-155, which has been associated with cervical cancer, acts as a tumor suppressor. It prevents the epithelial-mesenchymal transition (EMT) and makes cervical cells chemo-sensitive while impeding cell expansion, migration, and invasion. The expression pattern of miR-155 mimics has the potential to partially correct the down-regulation of E-cadherin brought on by EGF (epidermal growth factor) therapy in cervical cells. It specifically binds to two binding locations in the Smad2 gene's 3' UTR. MiR-155 was overexpressed, which causes the suppressor of mothers against decapentaplegic (SMAD2) to be downregulated. Moreover, miR-155 prevents cervical cancer's EGF-induced EMT, and by preventing EMT, miR-155 increases the cervical cells' chemo-sensitivity to cisplatin treatment. The expression pattern of miR-155 in cervical cancer patients in comparison to non-cancerous patients, was generally observed to be elevated in these patients (Lei et al.,2012). Ferrajoli et al. (2013) found that monoclonal B-cell lymphocytosis patients' B cells have greater levels of miR155 than B cells from non-cancerous patients.

This study also demonstrated that, in contrast to the non-cancerous patients, the expression pattern of miR-27a was elevated in cervical cancer patients on therapy and downregulated in cervical cancer patients not on

therapy and this was similar to recent studies showing that miR-27a levels are consistently elevated in a wide range of cancer types, supporting the malignant properties of cancer cells (Wu et al.,2015; Su et al.,2019). Moreover, the expression pattern of miR-27a promoted cell invasion, migration, and mortality in ovarian cancer by being overexpressed (Li et al., 2019). According to Wei et al. (2020), cervical cancer was found to have an overexpression of miR-27a, and its elimination significantly decreased the malignant features of cervical cancer cells.

When comparing the expression pattern of the microRNAs to the International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer patient status. Cervical cancer patients are patients with cervical cancer on therapy and patients with cervical cancer not on therapy. Six microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a showed statistically significant difference among the cervical cancer status with only miR-29a showing no significance among the cervical cancer status. To begin with, the expression pattern of miR-146a was found to be upregulated in stage IVB of cervical cancer patients not on therapy and downregulated in stage IVA whilst the expression pattern of miR-146a was upregulated in stage IVA of cervical cancer patients on therapy and downregulated in stage IVB in this study. In general, the expression pattern of miR-146a was upregulated in cervical cancer patients to non-cancerous patients in this study. This was similar to a study by Brase et al. (2011) which proposed that the expression pattern of miR-146a was noticeably upregulated in cervical cancer tissue samples than non-cancerous patients. Age-matched normal and cervical cancer tissues were compared using microRNA array

analysis by Wang et al. (2006), who found that miR-146a was overexpressed in cervical cancer tissues and increased cell viability, whiles Sathyanarayanan et al. (2016) noticed that miR-146a prevented human cervical cancer cells from migrating, invading, or surviving. However, no studies were found to group cervical cancer status into FIGO stages of cervical cancer but rather compare cervical cancer patients to non-cancerous patients. This study was generally discussed to get studies to support since our study seems to be the first study to group cervical cancer patients into those on or not on therapy based on the FIGO stages of cervical cancer to the expression pattern of miR-146a.

Moreover, the FIGO stages of cervical cancer status were compared to the miR-155 expression pattern in this study, and was found that, in stage IVB, patients with cervical cancer undergoing therapy were shown to have an increased expression pattern of miR-155 whereas cervical cancer patients not on therapy had a downregulated expression pattern of miR-155 and this corresponds to the expression pattern of miR-155 which was markedly up-regulated in cervical cancer tissues (Wang et al., 2016) and high miR-155 level could be associated with FIGO stages of cervical cancer (Fang et al.,2016) which was in line to the study. Therefore, miR-155 may be used as a novel prognostic marker and/or effective therapeutic target in cervical cancer (Fang et al.,2016). On the other hand, a study by Lao et al. (2014) discovered that downregulating miR-155 results in apoptosis, cell cycle arrest in the G1 phase, and inhibits the growth of cervical cancer cells. The target gene of miR-155, a significant tumor suppressor in various malignancies, has been discovered to be liver kinase B1 (LKB1). Cervical cancer tissues have

significantly lower levels of LKB1 mRNA and protein expression, whereas LKB1's luciferase activity and protein expression are increased when miR-155 expression is downregulated. Thus, by controlling LKB1, miR-155 encourages the growth of cervical cancer cells.

According to this study, the expression pattern of miR-29b was compared among cervical cancer status thus, cervical cancer patients on therapy, cervical cancer patients not on therapy, along with their FIGO stages. The expression patterns of miR-29b in cervical cancer patients on therapy were reported to be increased in stage IVB and downregulated in stage IVB of cervical cancer patients not on therapy, while in stage IIIB the expression pattern of miR-29b of cervical cancer patients not on therapy was found to upregulated and downregulated in stage IIIB of cervical cancer patients on therapy which was similar to a study by Li et al. (2017), which proposed that the overexpression of miR-29b significantly reduced the ability of cervical cancer cells to proliferate and progress through the cell cycle. The clinical phases of cervical cancer and the regulation of tumor invasion were both correlated with the expression pattern of miR-29b. A study by Han et al. (2017) investigated the role of miR-29b in cervical cancer and revealed that miR-29b overexpression considerably reduced cervical cancer cell migration, invasion, and angiogenesis. These results suggest that in cervical cancer, miR-29b may function as a tumor suppressor. However, a study by Li et al. (2011) proposed the hypothesis that miR-29b may contribute to cervical carcinogenesis by targeting YY1 and CDK6 in the same way that E6 and E7 oncoproteins generated by the HPV genome can deregulate cellular proliferation and apoptosis through targeting p53 and pRb, respectively.

According to Goa et al. (2017), cervical cancer tissues and plasma samples have a different miR-29b expression pattern from healthy persons. The miR-29b's promoter region was hypermethylated, which was linked to its downregulation (Teng et al., 2016). These studies (Goa et al., 2017; Teng et al., 2016) do not support the findings of this study. However, studies used in backing the study were the expression pattern of miR-29b of cervical cancer to non-cancerous patients, and not categorized into FIGO stages of cervical cancer. This study was found to be the first study to categorize cervical cancer status based on FIGO stages to the expression pattern of miR-29b.

Furthermore, in this study, the expression pattern of miR-34a was compared to the FIGO stages of cervical cancer status, thus, cervical cancer patients were on therapy and cervical cancer patients were not on therapy. The purpose of these categories was to identify the patterns of expression for the microRNA that circulated in the plasma of patients with cervical cancer who were on therapy and those who were not on therapy. The expression pattern of miR-34a of cervical cancer patients on therapy was found to be upregulated in stage IVB while downregulated in stage IVB of cervical cancer patients not on therapy. The downregulation of miR-34a expression pattern in cervical cancer patients not on therapy was in line with the findings of a study by Li et al. (2010) which discovered that precancerous lesions and cervical malignancies have decreased expression of miR-34a. Thus, the suppression of miR-34a may have been caused by the HR-HPV E6 oncoprotein and the p53 pathway, and its early initiation may have contributed to the development of cervical cancer (Li et al., 2010). By blocking oncogenic pathways, several microRNAs restrict tumor growth in cervical cancer. For instance, miR-34a targets

numerous oncogenes, including Notch1, c-Met, and E2F3, and its decreased expression promotes tumor invasion and growth (Wang et al., 2013). A tumor suppressor microRNA called miR-34a controls cell cycle progression and apoptosis at a moderately high level in healthy cervical tissue. Its expression was frequently downregulated in cervical cancer, promoting the growth and spread of the tumor (Chen, 2015). Recent research has shown that several microRNAs, particularly miR-34a, can influence the response to chemotherapy in a variety of tumor types, including cervical cancer (Fujita et al., 2008; Wang, Yang, Li, & Han, 2015; Yu et al., 2015). Even though stomach, liver, prostate, and cervix malignancies typically had miR-34a down-regulated, Zhang et al. (2016) discovered that it was a well-known tumor suppressor gene. Tumour suppressive gene expression was also found to be downregulated in cervical tumors and cancer-derived cell lines carrying oncogenic HPVs, according to Wang et al. (2009)'s study on miR-34a and cervical cancer. Before this, it was believed that the viral protein E6, which also destabilizes the tumor suppressor p53, activates the miR-34a trans activator (Wang et al., 2009). However, studies used to back this study were discussed among the expression patterns of miR-34a of cervical cancer patients to non-cancerous patients, and not the expression pattern of miR-34a grouped into cervical cancer status according to FIGO stages of cervical cancer. This study seems to be the first study to research cervical cancer status based on the expression pattern of miR-34a to FIGO stages of cervical cancer.

Moreover, to understand the expression patterns of miR-233 in FIGO stages of cervical cancer, our investigations compared the FIGO stages of cervical cancer among the cervical cancer status to the miR-233 expression pattern. The expression pattern of miR-233 of cervical cancer patients on therapy was found to be upregulated in stage IVB while downregulated in stage IVB cervical cancer patients not on therapy. However, the upregulation of the expression pattern of miR-233 of cervical cancer on therapy found in this study was similar to a study by Yin et al. (2016) that proposed that Forkhead box protein O1 (FOXO1) was the target of miR-223, which was under-expressed in cervical cancer cells. Cervical cancer cell growth was markedly slowed by miR-223 overexpression. MiR-223 reduces the growth, proliferation, and colony formation of cervical cancer cells by targeting Akt/mTOR/p70S6K and hypoxia-inducible factor-1 (IGF-1R) (Jia et al.,2011). A study by Goa et al. (2017) found that miR-223 played a significant role in controlling cervical cancer, mostly through targeting the disease's related genes and interacting with various signaling pathways.

Finally, in this study, the expression pattern of miR-27a was in comparison to the FIGO stages of cervical cancer status, thus, cervical cancer patients were on therapy, and cervical cancer patients were not on therapy. The expression pattern of miR-27a of cervical cancer patients on therapy was found to be upregulated in stage IIIA and downregulated in stage IIA while that of cervical cancer patients not on therapy was downregulated in stage IIIA and upregulated in stage IIB of the FIGO stages of cervical cancer. The upregulation of the expression pattern of miR-27a of a cervical cancer patient on therapy was in line with the findings of the study by Li et al. (2023), which

found that miR-27a bound to the 3′UTR of TAB3, increases TAB3 synthesis, which consequently activates the NF-kB signaling. It was shown that miR-27a promoted cervical cancer cells' ability to become malignant, and the study revealed that the elevation of TAB3 by miR-27a overexpression was what caused the cells' increased ability to become malignant (Li et al.,2023). Elevated miR-27a inhibits tumor progression by inhibiting TGF-RI expression and TGF-signaling (Fang et al., 2018). The downregulation of the expression pattern of miR-27a in cervical cancer patients not on therapy corresponds to a study by Wang et al. (2008), who discovered that miR-27a was downregulated in cervical cancer tissues and cancer cell lines when compared to normal cervical tissue, proving that miR-27a has a tumor-suppressing role in the growth of cervical cancer. In a study by Fang et al. (2018), it was discovered that the expression of miR-27a was lower in cervical cancer cells than in normal cervix squamous epithelia or glandular epithelia, indicating that miR-27a was downregulated in this disease.

This study looked at comparing the expression pattern of the microRNAs to the International Federation of Gynaecology and Obstetrics (FIGO) stages of cervical cancer patient status. Cervical cancer patients are patients with cervical cancer on therapy and patients with cervical cancer not on therapy. Six microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a showed statistically significant differences among the cervical cancer status. This study is the first study to group its findings into cervical cancer status based on FIGO stages, since, studies used to support this study discussed their findings in general without breaking them into cervical cancer status based on FIGO stages of cervical cancer.

To identify the expression patterns of specific microRNAs in cervical cancer patients on therapy. Only miR-29a expression pattern was statistically significant among the seven microRNAs examined in this study which showed that the expression pattern of the microRNA was correlated to the effect of treatment. The results of the study indicated that the expression pattern of miR-29a was downregulated in response to the effects of the treatment and that these effects are closely correlated with it. This study's finding was in support of a study by Li et al. (2011) which proposed that numerous microRNA expression patterns are either up- or down-regulated in cervical cancer, affecting how sensitive the disease was to chemotherapy and radiation treatment, however, he demonstrated that miR-29a expression levels were lower in CIN and ICC. According to Gong et al. (2019), the expression level of miR-29a methylates the tumor suppressor SOCS1, which could stop cervical cancer from spreading. Furthermore, it was shown that miR-29a may act as a tumor suppressor in the emergence of cervical cancer (Li et al.,2011).

Moreover, a study by Dinh et al. (2016) discovered a connection between the radiation dose used to treat non-small-cell lung cancer and the amount of miR-29a present in the blood. By controlling the PAK1/LIMK signaling pathway, which directly binds to the 3′-UTR, and downregulating CDC42, Chen et al. (2019) found that miR-29a might reduce cell proliferation, migration, and invasion, and increase apoptosis of cervical cancer cells. Additionally, a study by Chen et al. (2019) revealed that excessive expression of the tumor suppressor miR-29a in cervical cancer may inhibit cell growth and migration as well as trigger cell apoptosis. Tumors and HPV-positive LSILs have been revealed to have downregulated miR-29a (Yamamoto et al.,

2013; Jia et al., 2015; Servn-González et al., 2015). A recent study has demonstrated that miR-29a inhibits DNMT3A and DNMT3B, which affects how p16 was methylated in cervical cancer (Wang et al., 2021).

Finally, it has been discovered that miR-29a controls HSP47, a protein required for the synthesis of collagen molecules. The miR-29a-HSP47 pathway appears to be involved in cervical squamous cell carcinoma (SCC) metastasis since reduced HSP47 inhibited cancer cells' invasion and migration (Yamamoto et al., 2013). When invasive squamous cell carcinoma was present, miR-21 was overexpressed and miR-29 was downregulated, which distinguishes tumors from healthy cervical tissue and creates a molecular signature that can be used to diagnose cervical cancer (Bauelos-Villegas et al., 2021). MiR-29a levels were much lower in tumor samples than in control groups, according to Sara et al. (2019). This finding raises the possibility that miR-29a functions as a tumor suppressor in the course of cervical cancer development.

The ROC calculation was done to identify the expression patterns of the microRNAs when compared to cervical cancer patients, not on therapy, and non-cancerous patients. There were only three microRNAs that were highly expressed with good AUC among cervical cancer patients, not on therapy, and non-cancerous patients. The values for these microRNAs such as miR-155, miR-146a, and miR-34a showed good AUC, sensitivity, specificity, and cut-off point in Figure 4.4. Therefore, the expression of these three microRNAs could accurately distinguish healthy individuals from patients with cervical cancer, with excellent sensitivity and specificity. miR-34a showed a good AUC in this study and this was similar to a study by Azimi et

al. (2021) which assessed the possibility of employing miR-92a-5p and miR-155-3p levels found in Pap smears as diagnostic biomarkers for separating patients with LSIL and HSIL from healthy people, using the ROC curves. The expression of these two molecules could reliably separate healthy persons from patients with LSIL since miR-155-5p and miR-92a-5p exhibited nearly identical AUC, sensitivity, specificity, and cut-off points showing great sensitivity and specificity. In this study, miR-34a showed good specificity and sensitivity and this corresponds to a study by Wang, P., Zhai, G., & Bai, Y. (2018) which proposed that the ROC curve analysis on miR-34a and miR-218 had good sensitivity and specificity, indicating that the diagnosis of cervical cancer using miR-34a and miR-218 would be substantially more accurate.

Finally, miR-146a showed good specificity and sensitivity after the ROC curve was determined in this study and this aligned with the research by Ma et al. (2019) which revealed that the AUCs of miR-146a-5p, miR-151a-3p, miR218 2110, and miR-21-5p displayed good sensitivity and specificity according to ROC curves and their ideal cut off values for the four microRNAs were established. Showing that they were consistently greater in 237 individuals with cervical cancer than in normal controls.

4.10 Chapter Summary

In all, the expression pattern of seven microRNAs assessed among non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy, was discovered that miR-155 and miR-27a showed statistically significant differences with p-value < 0.05 in this study, and could serve as a diagnostic, prognostic and treatment marker.

However, microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a showed statistically significant differences when the expression patterns of microRNAs were compared to non-cancerous patients, and the stages of cervical cancer patients on therapy and cervical cancer patients not on therapy. The expression pattern of some of the microRNAs such as miR-155, miR-29b, and miR-233 were also found to be upregulated in stage IVB of cervical cancer patients on therapy and downregulated in stage IVB of cervical cancer patients not on therapy to the non-cancerous patients. The expression of certain microRNAs, such as miR-146a and miR-34a, was observed to be upregulated in stage IVB of cervical cancer patients not on therapy and downregulated in stage IVB of cervical cancer patients on therapy. Nevertheless, it was discovered that the expression pattern of miR-27a was upregulated in stage IIIA of cervical cancer patients on therapy and downregulated in stage IIIB of cervical cancer patients on therapy, whereas it was upregulated in stage IIIB of cervical cancer patients not on therapy and downregulated in stage IIIA of cervical cancer patients not on therapy.

The effect of treatment was also assessed to know if it has any correlation to the expression patterns of the microRNAs, and only miR-29a out of the seven microRNAs used showed statistically significant correlation to the effects of the treatment. In this investigation, it was discovered that the expression pattern of miR-29a was downregulated, with a p-value of less than 0.05 against the treatment effect.

In contrast, the ROC curve generated along the expression patterns of the seven microRNAs, with non-cancerous patients, and cervical cancer patients not on therapy, miR-155, miR-146a, and miR-34a recorded a good AUC, sensitivity, specificity, and cut-off point. Therefore, the expression of these three microRNAs could accurately distinguish healthy individuals from patients with cervical cancer, with excellent sensitivity and specificity.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to evaluate the expression patterns of microRNAs among non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy. The study also evaluated the expression patterns of microRNAs among non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy based on the FIGO stages of cervical cancer, as well as assessing the expression pattern of microRNAs on the effects of treatment of cervical cancer patients on therapy and generating ROC curve along the expression patterns of the seven microRNAs for non-cancerous patients, and cervical cancer patients not on therapy.

5.0 Summary

When it comes to cancer-related deaths among women from underdeveloped nations, cervical cancer has historically been one of the causes. Persistent infection with the high-risk HPV strains 16 and 18 was one of the main risk factors for the emergence of cervical cancer. Despite this, a very small number of women who have morphologic symptoms of an HPV infection progress to develop an invasive illness indicating that additional variables may be involved in the development of cervical cancer. MicroRNAs are short non-coding RNAs that are conserved and help regulate the expression of genes, particularly those related to essential biological processes and human cancer. In all, the expression pattern of seven microRNAs was assessed among non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy, and it was discovered that miR-

155 and miR-27a had statistically significant expression patterns, which can be explored to be used as markers for diagnosis, prognosis, and treatment.

Moreover, when the expression patterns of microRNAs were compared to the non-cancerous patients, cervical cancer patients on therapy, and cervical cancer patients not on therapy based on FIGO stages of cervical cancer, microRNAs such as miR-146a, miR-155, miR-29b, miR-34a, miR-233, and miR-27a expression patterns were statistically significant. In comparison to non-cancerous patients, it was discovered that the expression pattern of several microRNAs, including miR-155, miR-29b, and miR-233, were increased in stage IVB of cervical cancer patients on therapy and downregulated in stage IVB of cervical cancer patients not on therapy. MicroRNAs like miR-146a and miR-34a, for example, were shown to have upregulated expression patterns in stage IVB of cervical cancer patients not on therapy and downregulated expression patterns in stage IVB of cervical cancer patients on therapy. Nevertheless, it was discovered that the expression pattern of miR-27a was upregulated in stage IIIA of cervical cancer patients on therapy and downregulated in stage IIIB of cervical cancer patients on therapy, whereas it was upregulated in stage IIIB of cervical cancer patients not on therapy and downregulated in stage IIIA of cervical cancer patients not on therapy.

The effect of treatment was also assessed to determine whether it correlates with the expression patterns of the microRNAs. Only the expression of miR-29a out of the seven microRNAs analyzed had a statistically significant correlation with treatment effects. MiR-29a's expression pattern was shown to be downregulated in response to the treatment, with a p-value of less than 0.05.

In contrast, the ROC curve generated along the expression patterns of the seven microRNAs for non-cancerous patients, and cervical cancer patients not on therapy, miR-155, miR-146a, and miR-34a showed a good AUC, sensitivity, specificity, and cut-off point. Therefore, with great sensitivity and specificity, the expression of these three microRNAs could successfully distinguish between healthy people and people with cervical cancer.

5.1 Conclusion

Currently, this study is the first in Ghana to assess the expression patterns of the circulatory microRNA in freshly diagnosed cervical cancer patients not on therapy and cervical cancer patients on therapy, and also discuss them according to the International Federation of Obstetrics and Gynaecology (FIGO) stages of cervical cancer. Similar to other studies, some of the microRNA expression patterns were downregulated or upregulated in cervical cancer patients in general, without grouping them into cervical cancer patients on therapy or not on therapy, and also not grouped into FIGO stages of cervical cancer. However, in this study, cervical cancer patients were grouped into those on therapy and those not on therapy and also in FIGO stages of cervical cancer. These groupings have helped us to easily identify the expression patterns of microRNAs in FIGO stages of cervical cancer, for example, the expression pattern of miR-27a was found to be upregulated in stage IIIA of cervical cancer patients on therapy and downregulated in stage IIIB of cervical cancer patients on therapy whiles it was upregulated in stage IIIB of cervical cancer patients not on therapy and downregulated in stage IIIA of cervical cancer patients not on therapy. It was discovered that the expression pattern of miR-34a in stage IVB cervical cancer patients on therapy

was increased. In contrast, it was downregulated in stage IVB of patients not on therapy. When cervical cancer patients were on therapy, the expression patterns of miR-29b were reported to be increased in stage IVB and downregulated in stage IVB of cervical cancer patients not on therapy, while in stage IIIB the expression pattern of miR-29b of cervical cancer patients not on therapy was found to be upregulated and downregulated in cervical cancer patients on therapy. This study has made us appreciate how the expression patterns of microRNAs work in the FIGO stages of cervical cancer, grouped into patients with cervical cancer on therapy and cervical cancer patients not on therapy. It has also helped us know the expression patterns of microRNAs in cervical cancer patients on therapy, cervical cancer patients not on therapy, and non-cancerous patients. Moreover, to identify the expression patterns of specific microRNAs in cervical cancer status, the expression pattern of miR-155 was compared to positive cervical cancer patients, not on therapy, and non-cancerous patients, and there was a substantial difference in the expression pattern of miR-155. It was also found that miR-29a expression pattern was statistically significant among the seven microRNAs examined in this study, which showed that the expression pattern of the microRNA was correlated to the effect of treatment. MiR-29a's expression pattern was demonstrated to be downregulated in contrast to the treatment's effects, and it was strongly linked with the treatment's effect. Lastly, the ROC analysis in this study suggested that miRNAs like miR-146a, miR-155, and miR-34a could be useful as prospective biomarkers for the accurate and timely detection of patients with cervical cancer.

5.2 Recommendations

The following are some recommendations made in light of the study's findings.

1. The Ministry of Health should promote the use of microRNAs as a biomarker for detecting cervical cancer and increase screening participation rates among women since conventional methods require clinical visits, can cause discomfort, and may lead to false results, which deter women from participating in regular screening.
2. miRNAs are involved in regulating gene expression and can provide insights into the molecular pathways involved in cervical cancer, helping to identify potential therapeutic targets. However, WHO and the Ministry of Health should encourage the efficient use of the microRNA biomarker to reduce mortality and morbidity rates in developing countries.
3. For research purposes: More research should be carried out on microRNAs as cervical cancer biomarkers to further validate it for clinical implementation.
4. More research should be carried out to elaborate more on the microRNAs that could serve as biomarkers for cervical cancer patients based on the International Federation of Obstetrics and Gynaecology stages.

REFERENCES

Abotchie, P. N., & Shokar, N. K. (2009). Cervical cancer screening among college students in Ghana: knowledge and health beliefs. *International Journal of Gynecologic Cancer*, *19*(3).

Adams, A. R., Nortey, P. A., Dortey, B. A., Asmah, R. H., & Wiredu, E. K. (2019). Cervical human papillomavirus prevalence, genotypes, and associated risk factors among female sex workers in Greater Accra, Ghana. *Journal of Oncology*, *2019*(1), 8062176.

Afroj, S., Banu, M. A., Sultana, S., Jahan, R., Rahman, S., & Begum, N. (2017). Clinicopathological profile of cervical cancer patients attending in a specialized hospital. *Journal of Dhaka Medical College*, *26*(2).

Akpabla, G. S., & Sun, K. (2021). The State of Cervical Cancer Screening and HPV Vaccination in Africa: In the Advent of Advanced Health Care. *Journal ISSN*, *2766*, 2276.

Almeida, A. M., Queiroz, J. A., Sousa, F., & Sousa, Â. (2019). Cervical cancer and HPV infection: ongoing therapeutic research to counteract the action of E6 and E7 oncoproteins. *Drug discovery today*, *24*(10), 2044-2057.

Ali Syeda, Z., Langden, S. S. S., Munkhzul, C., Lee, M., and Song, S. J. (2020). Regulatory Mechanism of MicroRNA Expression in Cancer. Ijms 21, 1723

Allouch, S., Malki, A., Allouch, A., Gupta, I., Vranic, S., & Al Moustafa, A. E. (2020). High-risk HPV oncoproteins and PD-1/PD-L1 interplay in human cervical cancer: recent evidence and future directions. *Frontiers in Oncology*, *10*, 914.

Ambros V. (2008). The evolution of our thinking about microRNAs. Nat Med. 2008 14:1036–40.

Amoako, Y. A., Awuah, B., Larsen-Reindorf, R., Awittor, F. K., Kyem, G., Ofori-Boadu, K., ... & Laryea, D. O. (2019). Malignant tumours in urban Ghana: evidence from the city of Kumasi. *BMC cancer*, *19*, 1-12.

Appiah, M. T. (2023). *Knowledge and Uptake of Cervical Cancer Vaccine among Community Health Nurses in Takoradi, in the Western Region of Ghana* (Doctoral dissertation, Ensign Global College).

Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., & Bray, F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *The Lancet Global Health*, *8*(2), e191-e203.

Arga, K. Y., & Kori, M. (2023). Current status of viral biomarkers for oncogenic viruses. In *Oncogenic Viruses* (pp. 221-252). Academic Press.

Azimi, T., Paryan, M., Mondanizadeh, M., Sarmadian, H., & Zamani, A. (2021). Pap Smear miR-92a-5p and miR-155-5p as potential diagnostic biomarkers of squamous intraepithelial cervical cancer. *Asian Pacific Journal of Cancer Prevention: APJCP*, *22*(4), 1271.

Baciu, A., Negussie, Y., Geller, A., Weinstein, J. N., & National Academies of Sciences, Engineering, and Medicine. (2017). The state of health disparities in the United States. In *Communities in action: Pathways to health equity*. National Academies Press (US).

Bak, R. O., & Mikkelsen, J. G. (2014). miRNA sponges: soaking up miRNAs for regulation of gene expression. *Wiley interdisciplinary reviews: RNA*, *5*(3), 317-333.

Balasubramaniam, S. D., Balakrishnan, V., Oon, C. E., & Kaur, G. (2019). Key molecular events in cervical cancer development. *Medicina*, *55*(7), 384.

Bañuelos-Villegas, E. G., Pérez-yPérez, M. F., & Alvarez-Salas, L. M. (2021). Cervical cancer, papillomavirus, and miRNA dysfunction. *Frontiers in Molecular Biosciences*, *8*, 758337.

Barrett, R. M., & Wood, M. A. (2008). Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. *Learning & Memory*, *15*(7), 460-467.

Barroso, L. F., Stier, E. A., Hillman, R., & Palefsky, J. (2022). Anal cancer screening and prevention: summary of evidence reviewed for the 2021 centers for disease control and prevention sexually transmitted infection guidelines. *Clinical Infectious Diseases*, *74*(Supplement_2), S179- S192.

Bartel, D. P. (2004) MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. Cell 116, 281–297, https://doi.org/10.1016/S0092- 8674(04) 00045-5 (2004).

Bartel, D. P. (2009). MicroRNAs: target recognition and regulatory functions. *cell*, *136*(2), 215-233.

Berindan-Neagoe I, Monroig Pdel C, Pasculli B, Calin GA. (2014) MicroRNAome genome: a treasure for cancer diagnosis and therapy. CA Cancer J Clin. 2014 64:311–36.

Bhattacharjee, R., Dey, T., Kumar, L., Kar, S., Sarkar, R., Ghorai, M., ... & Dey, A. (2022). Cellular landscaping of cisplatin resistance in cervical cancer. *Biomedicine & Pharmacotherapy*, *153*, 113345.

Binka, C., Nyarko, S. H., Awusabo-Asare, K., & Doku, D. T. (2019). Barriers to the uptake of cervical cancer screening and treatment among rural women in Ghana. *BioMed research international*, *2019*(1), 6320938.

Bogdanova, A., Andrawos, C., & Constantinou, C. (2022). Cervical cancer, geographical inequalities, prevention and barriers in resource depleted countries. *Oncology letters*, *23*(4), 1-11

Bouyssou, J. M., Manier, S., Huynh, D., Issa, S., Roccaro, A. M., & Ghobrial, I. M. (2014). Regulation of microRNAs in cancer metastasis. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1845*(2), 255-265.

Brase, J. C., Johannes, M., Schlomm, T., Fälth, M., Haese, A., Steuber, T., ... & Sültmann, H. (2011). Circulating miRNAs are correlated with tumor progression in prostate cancer. *International journal of cancer*, *128*(3), 608-616.

Brawley, O. W. (2014). Lung cancer screening: Summary of the evidence and the 2013 US Preventative Services Task Force recommendations. In *Cancer Forum* (Vol. 38, No. 3, pp. 224-226).

Bray, F. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 68, 394–424, ://doi.org/10.3322/ caac.21492 (2018).

Brinton, L. A., Hamman, R. F., Huggins, G. R., Lehman, H. F., Levine, R. S., Mailin, K., & Fraumeni Jr, J. F. (1987). Sexual and reproductive risk factors for invasive squamous cell cervical cancer. *Journal of the National Cancer Institute*, *79*(1), 23-30.

Bruni, L., Saura-Lázaro, A., Montoliu, A., Brotons, M., Alemany, L., Diallo, M. S., ... & Bloem, P. (2021). HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010–2019. *Preventive medicine*, *144*, 106399.

Btoush, R., Kohler, R. K., Carmody, D. P., Hudson, S. V., & Tsui, J. (2022). Factors that influence healthcare provider recommendation of HPV vaccination. *American Journal of Health Promotion*, *36*(7), 1152-1161.

Cancer Genome Atlas Research Network Albert Einstein College of Medicine Burk Robert D. 1 Chen Zigui 1, Analytical Biological Services Saller Charles 2 Tarvin Katherine 2, Barretos Cancer Hospital Carvalho Andre L. 3 Scapulatempo-Neto Cristovam 3 Silveira Henrique C. 3 Fregnani José H. 3, Baylor College of Medicine Creighton Chad J. 4 Anderson Matthew L. 4 Castro Patricia 4, Beckman Research Institute of City of Hope Wang Sophia S. 5, Buck Institute for Research on Aging Yau Christina 6 Benz Christopher 6, ... & Washington

University in St Louis Schwarz Julie 57 Grigsby Perry 57 Mutch David 57. (2017). Integrated genomic and molecular characterization of cervical cancer. *Nature*, *543*(7645), 378-384.

Cao, H., Pan, G., Tang, S., Zhong, N., Liu, H., Zhou, H., ... & Zou, Y. (2020). miR-145-5p regulates the proliferation, migration and invasion in cervical carcinoma by targeting KLF5. *OncoTargets and therapy*, 2369-2376.

Cai, L., Gong, X., Sun, X., Li, S., & Yu, X. (2018). Comparison of chemical and microbiological changes during the aerobic composting and vermicomposting of green waste. *PloS one*, *13*(11), e0207494.

Castellsague, X. (2008). Natural history and epidemiology of HPV infection and cervical cancer, *Gynaecologic Oncology*,110(3), S4-S7.

Centers for Disease Control and Prevention. (2017). Genital HPV infection-fact sheet. *See https://www. cdc. gov/std/hpv/stdfact-hpv. htm*.

Chan, C. K., Aimagambetova, G., Ukybassova, T., Kongrtay, K., & Azizan, A. (2019). Human papillomavirus infection and cervical cancer: epidemiology, screening, and vaccination—review of current perspectives. *Journal of oncology*, *2019*(1), 3257939.

Chandrasekaran KS, Sathyanarayanan A, Karunagaran D. (2016) Downregulation of HMGB1 by miR-34a is sufficient to suppress proliferation, migration and invasion of human cervical and colorectal cancer cells. Tumour Biol. 2016 37:13155–66.

Chen, J. (2015). Signaling pathways in HPV-associated cancers and therapeutic implications. *Reviews in medical virology*, *25*, 24-53.

Chen, L., Zhou, Y., Sun, Q., Zhou, J., Pan, H., & Sui, X. (2017). Regulation of autophagy by MiRNAs and their emerging roles in tumorigenesis and cancer treatment. *International Review of Cell and Molecular Biology*, *334*, 1-26.

Chen, Q., Zhu, X. Y., Li, Y. Y., & Meng, Z. Q. (2014). Epigenetic regulation and cancer. *Oncology reports*, *31*(2), 523-532.

Chen, R., & Zhang, L. (2019). MiR-29a inhibits cell proliferation and migration by targeting the CDC42/PAK1 signaling pathway in cervical cancer. *Anti-cancer drugs*, *30*(6), 579-587

Chen, T. A. O. (2010). The role of MicroRNA in chemical carcinogenesis. *Journal of environmental science and health, part C*, *28*(2), 89-124.

Cheng, T., & Huang, S. (2021). Roles of non-coding RNAs in cervical cancer metastasis. *Frontiers in Oncology*, *11*, 646192.

Cheng, Y., Li, S., Gao, L., Zhi, K., & Ren, W. (2021). The molecular basis and therapeutic aspects of cisplatin resistance in oral squamous cell carcinoma. *Frontiers in oncology*, *11*, 761379.

Chen, X., Wang, L., Li, Y., Chen, H., Wei, G., & Ni, W. (2016). MiR-205 as a promising biomarker for cervical cancer patients. Cancer Cell International, 16(1), 73.

Cho, W. C. (2012). MicroRNAs as therapeutic targets and their potential applications in cancer therapy. *Expert opinion on therapeutic targets*, *16*(8), 747-759.

Cohen, L., Manion, K., & Morrison, L. (2007). Research methods in education (6th edition). New York, NY: Routledge Taylor & Francis group.

Cohen, J. (2013). *Statistical power analysis for the behavioral sciences*. routledge.

Copen, C. E., Daniels, K., Vespa, J., & Mosher, W. D. (2012). First Marriages in the United States: Data from the 2006-2010 National Survey of Family Growth. National Health Statistics Reports. Number 49. *National Center for Health Statistics*.

Cottrell, K. A., Szczesny, P., & Djuranovic, S. (2017). Translation efficiency is a determinant of the magnitude of miRNA-mediated repression. *Scientific reports*, *7*(1), 14884.

Croce CM. (2009). Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009 10:704–14.

Deligeoroglou, E., Giannouli, A., Athanasopoulos, N., Karountzos, V., Vatopoulou, A., Dimopoulos, K., & Creatsas, G. (2013). HPV infection: immunological aspects and their utility in future therapy. *Infectious diseases in obstetrics and gynecology*, *2013*.

De Martel, C., Plummer, M., Vignat, J., & Franceschi, S. (2017). Worldwide burden of cancer attributable to HPV by site, country and HPV type. *International journal of cancer*, *141*(4), 664-670.

Deng, Y. W., Hao, W. J., Li, Y. W., Li, Y. X., Zhao, B. C., & Lu, D. (2018). Hsa-miRNA-143-3p Reverses Multidrug Resistance of Triple-Negative Breast Cancer by Inhibiting the Expression of Its Target Protein Cytokine-Induced Apoptosis Inhibitor 1 In Vivo. *Journal of breast cancer*, *21*(3), 251-258.

Denny, L., Adewole, I., Anorlu, R., Dreyer, G., Moodley, M., Smith, T., ... & Schmidt, J. (2014). Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. *International journal of cancer*, *134*(6), 1389-1398.

De Vincenzo, R., Conte, C., Ricci, C., Scambia, G., & Capelli, G. (2014). Long-term efficacy and safety of human papillomavirus vaccination. *International journal of women's health*, 999-1010.

De Vuyst, H., Alemany, L., Lacey, C., Chibwesha, C. J., Sahasrabuddhe, V., Banura, C., ... & Parham, G. P. (2013). The burden of human papillomavirus infections and related diseases in sub-saharan Africa. *Vaccine*, *31*, F32-F46.

Dinh, T. K. T., Fendler, W., Chałubińska-Fendler, J., Acharya, S. S., O'Leary, C., Deraska, P. V., ... & Kozono, D. (2016). Circulating miR-29a and miR-150 correlate with delivered dose during thoracic radiation therapy for non-small cell lung cancer. *Radiation oncology*, *11*(1), 1-11.

Dixon, K., & Kopras, E. (2004, December). Genetic alterations and DNA repair in human carcinogenesis. In *Seminars in cancer biology* (Vol. 14, No. 6, pp. 441-448). Academic Press.

Doghish, A. S., Ali, M. A., Elyan, S. S., Elrebehy, M. A., Mohamed, H. H., Mansour, R. M., ... & Moustafa, H. A. M. (2023). miRNAs role in cervical cancer pathogenesis and targeted therapy: Signaling pathways interplay. *Pathology-Research and Practice*, 154386.

Dudea-Simon, M., Mihu, D., Pop, L. A., Ciortea, R., Malutan, A. M., Diculescu, D., ... & Berindan-Neagoe, I. (2022). Alteration of gene and miRNA expression in cervical intraepithelial neoplasia and cervical cancer. *International Journal of Molecular Sciences*, *23*(11), 6054.

Dunne, E. F., Unger, E. R., Sternberg, M., McQuillan, G., Swan, D. C., Patel, S. S., & Markowitz, L. E. (2007). Prevalence of HPV infection among females in the United States. *Jama*, *297*(8), 813-819.

Du, S., Zho, Y., Lv, C., Wei, M., Gao, Z., & Meng, X. (2020). Applying Serum Proteins and MicroRNA as Novel Biomarkers for Early -Stage Cervical Cancer Detection. *Scientific Reports, 001,20-22*

Elliott, D. B., & Simmons, T. (2011). *Marital events of Americans: 2009*. Washington, DC: US Department of Commerce, Economics and Statistics Administration, US Census Bureau.

Esquela-Kerscher A, Slack FJ (2006). Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 6(4):259−269.

Etzioni, R., Urban, N., Ramsey, S., McIntosh, M., Schwartz, S., Reid, B., ... & Hartwell, L. (2003). The case for early detection. *Nature reviews cancer*, *3*(4), 243-252.

Fabian, M. R., Sonenberg, N., & Filipowicz, W. (2010). Regulation of mRNA translation and stability by microRNAs. *Annual review of biochemistry*, *79*, 351-379.

Fang, F., Huang, B., Sun, S., Xiao, M., Guo, J., Yi, X., ... & Wang, Z. (2018). miR-27a inhibits cervical adenocarcinoma progression by downregulating the TGF-βRI signaling pathway. *Cell Death & Disease*, *9*(3), 395.

Fang, H., Shuang, D., Yi, Z., Sheng, H., & Liu, Y. (2016). Up-regulated microRNA-155 expression is associated with poor prognosis in cervical cancer patients. *Biomedicine & Pharmacotherapy*, *83*, 64-69.

Faraldi, M., Gomarasca, M., Banfi, G., & Lombardi, G. (2018). Free circulating miRNAs measurement in clinical settings: the still unsolved issue of the normalization. *Advances in clinical chemistry*, *87*, 113-139.

Farazi, T. A., Spitzer, J. I., Morozov, P., & Tuschl, T. (2011). miRNAs in human cancer. *The Journal of pathology*, *223*(2), 102-115.

Faridi, R., Zahra, A., Khan, K., & Idrees, M. (2011). Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. *Virology Journal*, *8*, 1-8.

Ferrajoli, A., Shanafelt, T. D., Ivan, C., Shimizu, M., Rabe, K. G., Nouraee, N., ... & Calin, G. A. (2013). Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. *Blood, The Journal of the American Society of Hematology*, *122*(11), 1891-1899.

Finnegan, E. F., & Pasquinelli, A. E. (2013). MicroRNA biogenesis: regulating the regulators. *Critical reviews in biochemistry and molecular biology*, *48*(1), 51-68.

Forman, D., de Martel, C., Lacey, C. J., Soerjomataram, I., Lortet-Tieulent, J., Bruni, L., ... & Franceschi, S. (2012). Global burden of human papillomavirus and related diseases. *Vaccine*, *30*, F12-F23.

Fraenkel, J. R., Wallen, N. E., &Hyun, H. H (2012). How to design and evaluate research in education (4th edition). New York, NY: Mc Graw-Hill, Inc.

Fujita, Y., Kojima, K., Hamada, N., Ohhashi, R., Akao, Y., Nozawa, Y., ... & Ito, M. (2008). Effects of miR-34a on cell growth and chemoresistance in prostate cancer PC3 cells. *Biochemical and biophysical research communications*, *377*(1), 114-119.

Gao, L., Cai, X., Zhou, Y., & Xiao, L. (2018). Downregulation of miR-375 in cervical cancer and its biological significance. Gynecologic Oncology, 150(3), 593-600.

Gao, Y., Lin, L., Li, T., Yang, J., & Wei, Y. (2017). The role of miRNA-223 in cancer: Function, diagnosis and therapy. *Gene*, *616*, 1-7.

Garland, S. M. (2002). Human papillomavirus update with a particular focus on cervical disease. *Pathology*, *34*(3), 213-224.

Garnett, G. P. (2005). Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. *The Journal of infectious diseases*, *191*(Supplement_1), S97-S106.

Ginindza, T. G., Dlamini, X., Almonte, M., Herrero, R., Jolly, P. E., Tsoka-Gwegweni, J. M., ... & Sartorius, B. (2017). Prevalence of and associated risk factors for high risk human papillomavirus among sexually active women, Swaziland. *PloS one*, *12*(1), e0170189.

Goodwin, J. S., Hunt, W. C., Key, C. R., & Samet, J. M. (1987). The effect of marital status on stage, treatment, and survival of cancer patients. *Jama*, *258*(21), 3125-3130.

Gong, Y., Wan, J. H., Zou, W., Lian, G. Y., Qin, J. L., & Wang, Q. M. (2019). MiR-29a inhibits invasion and metastasis of cervical cancer via modulating methylation of tumor suppressor SOCS1. *Future oncology*, *15*(15), 1729-1744.

Gray, P. G. (2022). Human Papillomavirus Type Replacement Following the Implementation of HPV Vaccination.

Griffiths-Jones S. (2004). The microRNA Registry. Nucleic Acids Res. 2004 32: D109–11.

Gu, X., Sun, G., Zheng, R., Zhang, S., Zeng, H., Sun, K., ... & He, J. (2022). Incidence and mortality of cervical cancer in China in 2015. *Journal of the National Cancer Center*, *2*(2), 70-77

Guil, S., & Esteller, M. (2015). RNA–RNA interactions in gene regulation: the coding and noncoding players. *Trends in biochemical sciences*, *40*(5), 248-256.

Han, B. R., & Park, W. H. (2017). MAPK inhibitors enhance cell death in pyrogallol-treated human pulmonary fibroblast cells via increasing O2•-levels. *Oncology letters*, *14*(1), 1179-1185.

He, B., Zhao, Z., Cai, Q., Zhang, Y., Zhang, P., Shi, S., ... & Wang, X. (2020). miRNA-based biomarkers, therapies, and resistance in Cancer. *International journal of biological sciences*, *16*(14), 2628.

Honarvar, Z., Kermani, S. V. N., & Robati, F. K. (2021). Comparison of Sensitivity and Specificity of Papanicolaou Test, Visual Inspection of Cervix with Acetic Acid (VIA) and Colposcopy in Cervical Cancer Screening in Patients with Secondary Immunodeficiency. *Indian Journal of Gynecologic Oncology*, *19*(2), 1-6.

Hosono, S. (2024). Recent Epidemiologic Trends in Cervical Cancer. In *Recent Topics on Prevention, Diagnosis, and Clinical Management of Cervical Cancer* (pp. 23-40). Singapore: Springer Nature Singapore.

Hrdlickova B, de Almeida RC, Borek Z, Withoff S. (2014) Genetic variation in the non-coding genome: Involvement of micro-RNAs and long non-coding RNAs in disease. Biochim Biophys Acta. 2014 1842:1910–22.

Hu, H. Y., Yan, Z., Xu, Y., Hu, H., Menzel, C., Zhou, Y. H., ... & Khaitovich, P. (2009). Sequence features associated with microRNA strand selection in humans and flies. *BMC genomics*, *10*(1), 1-11.

Hu, X. (2020) A microRNA expression signature for cervical cancer prognosis. Cancer research 70, 1441–1448, https://doi. org/10.1158/ 000 8-5472.CAN-09-3289 (2010).

Hu, Z., & Ma, D. (2018). The precision prevention and therapy of HPV-related cervical cancer: new concepts and clinical implications. *Cancer medicine*, *7*(10), 5217-5236.

Hummel, R., Hussey, D. J., & Haier, J. (2010). MicroRNAs: predictors and modifiers of chemo-and radiotherapy in different tumour types. *European journal of cancer*, *46*(2), 298-311.

Jedy-Agba, E., Joko, W. Y., Liu, B., Buziba, N. G., Borok, M., Korir, A., ... & Parkin, D. M. (2020). Trends in cervical cancer incidence in sub-Saharan Africa. *British journal of cancer*, *123*(1), 148-154.

Jia, C. Y., Li, H. H., Zhu, X. C., Dong, Y. W., Fu, D., Zhao, Q. L., ... & Wu, X. Z. (2011). MiR-223 suppresses cell proliferation by targeting IGF-1R. *PloS one*, *6*(11), e27008.

Jia, W., Wu, Y., Zhang, Q., Gao, G. E., Zhang, C., & Xiang, Y. (2015). Expression profile of circulating microRNAs as a promising fingerprint for cervical cancer diagnosis and monitoring. *Molecular and clinical oncology*, *3*(4), 851-858.

Jiao, P., Wang, X. P., Luoreng, Z. M., Yang, J., Jia, L., Ma, Y., & Wei, D. W. (2021). miR-223: an effective regulator of immune cell differentiation and inflammation. *International Journal of Biological Sciences*, *17*(9), 2308.

Jiang, H., Liu, Y., Li, W., Zhang, X., Chen, Q., & Liang, Y. (2021). Downregulation of plasma miR-222-3p predicts poor response and survival in cervical cancer patients treated with oral chemotherapy. Cancer Biomarkers, 30(4), 485-493.

Jiménez-Wences, H., Peralta-Zaragoza, O., & Fernández-Tilapa, G. (2014). Human papillomavirus, DNA methylation and microRNA expression in cervical cancer. *Oncology reports*, *31*(6), 2467-2476.

Jin, X. H., Lu, S., & Wang, A. F. (2020). Expression and clinical significance of miR-4516 and miR-21-5p in serum of patients with colorectal cancer. *BMC cancer*, *20*(1), 1-7.

Johnson, A., & Jackson, J. B. (2021). Sexually transmitted infections among college students. *Microbiology and Infectious Diseases*, *5*(1), 1-4.

Kannappan, S., Lee, J. H., Lakshmanakumar, M., Rayappan, J. B. B., & Nesakumar, N. (2021). Potential biomarkers for early diagnosis of cervical cancer. *Biomarkers and biosensors for cervical cancer diagnosis*, 23-46.

Katsenos, S., & Becker, H. D. (2011). Recurrent respiratory papillomatosis: a rare chronic disease, difficult to treat, with potential to lung cancer transformation: apropos of two cases and a brief literature review. *Case reports in oncology*, *4*(1), 162-171

Kim, D. H., Chang, M. S., Yoon, C. J., Middeldorp, J. M., Martinez, O. M., Byeon, S. J., ... & Woo, J. H. (2016). Epstein-Barr virus BARF1-induced NFκB/miR-146a/SMAD4 alterations in stomach cancer cells. *Oncotarget*, *7*(50), 82213. sectional study. BMC Public Health, 20(1), 1416.

Kim, H., Yang, J. M., Ahn, S. H., Jeong, W. J., Chung, J. H., & Paik, J. H. (2018). Potential oncogenic role and prognostic implication of microRNA-155-5p in oral squamous cell carcinoma. *Anticancer Research*, *38*(9), 5193-5200.

Kim, V. N. (2005). MicroRNA biogenesis: coordinated cropping and dicing. *Nature reviews Molecular cell biology*, *6*(5), 376-385.

Konadu, D. G., Owusu-Ofori, A., Yidana, Z., Boadu, F., Iddrisu, L. F., Adu-Gyasi, D., ... & Asante, K. P. (2019). Prevalence of vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis in pregnant women attending antenatal clinic in the middle belt of Ghana. *BMC pregnancy and childbirth*, *19*, 1-10.

Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ (2005). Combinatorial microRNA target predictions. Nat Genet. 2005 37:495–500.

Krings, A., Dunyo, P., Pesic, A., Tetteh, S., Hansen, B., Gedzah, I., ... & Kaufmann, A. M. (2019). Characterization of Human Papillomavirus prevalence and risk factors to guide cervical cancer screening in the North Tongu District, Ghana. *PLoS One*, *14*(6), e0218762.

Kulkarni, S. S., Kulkarni, S. S., Vastrad, P. P., Kulkarni, B. B., Markande, A. R., Kadakol, G. S., ... & Gai, P. B. (2011). Prevalence and distribution of high-risk human papillomavirus (HPV) Types 16 and 18 in Carcinoma of the cervix, the saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. *Asian Pac J Cancer Prev*, *12*(3), 645-648.

Kyrgiou, M. & Shaf, M. I. (2010) Invasive cancer of the cervix. Obstetrics, Gynecology & Reproductive Medicine 20, 147–154, https://doi. org/10.1016/j.ogrm.2010.02.003 (2010).

Lao, G., Liu, P., Wu, Q., Zhang, W., Liu, Y., Yang, L., & Ma, C. (2014). Mir-155 promotes cervical cancer cell proliferation through suppression of its target gene LKB1. *Tumor Biology*, *35*(12), 11933-11938.

Larbie, J. M. (2023). *Association between Knowledge of Human Papillomavirus, Related Cancers, and Barriers to Information*.

Lea, J. S., & Lin, K. Y. (2012). Cervical cancer. *Obstetrics and Gynecology Clinics*, *39*(2), 233-253.

Lee, S. I., & Atri, M. (2019). 2018 FIGO staging system for uterine cervical cancer: enter cross-sectional imaging. *Radiology*, *292*(1), 15-24.

Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH (2004). MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004 23:4051–60.

Lee, J.-W. (2008). Altered MicroRNA Expression in Cervical Carcinomas. Clinical Cancer Research 14, 2535–2542, https://doi. org/10.1158/ 1078-0432.ccr-07-1231 (2008).

Leedy, P. D.& Ormrod, J. E. (2010). Practical research: Planning and design (9th edition). Upper Saddle River NJ: Pearson.

Lei, C., Wang, Y., Huang, Y., Yu, H., Huang, Y., Wu, L., & Huang, L. (2012). Up-regulated miR155 reverses the epithelial-mesenchymal transition induced by EGF and increases chemo-sensitivity to cisplatin in human Caski cervical cancer cells. *PLoS One*, *7*(12), e52310.

Lewis BP, Burge CB, Bartel DP. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005 120:15–20.

Li, B., Hu, Y., Ye, F., Li, Y., Lv, W., & Xie, X. (2010). Reduced miR-34a expression in normal cervical tissues and cervical lesions with high-risk human papillomavirus infection. *International Journal of Gynecologic Cancer*, *20*(4).

Li BH, Zhou JS, Ye F, Cheng XD, Zhou CY, Lu WG, and Xie X (2011). Reduced miR-100 expression in cervical cancer and precursors and its carcinogenic effect through targeting PLK1 protein. European Journal of Cancer 47: 2166-2174.

Li J, Li Y, Li X (2021). Identification of a miRNA signature associated with the therapeutic efficacy of neoadjuvant chemotherapy in cervical cancer. Front Oncol. 2021; 11 :606264

Li X, Zhang Y, Zhang Y, Ding J, Wu K, Fan D. (2010). Survival prediction of gastric cancer by a seven-microRNA signature. Gut. 2010;59(5):579-585. doi:10.1136/gut.2009.181149

Li Y, Li L, Guan Y (2020). The oncogenic roles of microRNA-21 in cervical cancer development and progression. Cancer Biother Radiopharm. 2020;35(4):271-277. Oncotarget. 2016 7:1439–50.

Li Y, Wang F, Xu J, Ye F, Shen Y, Zhou J, Lu W, Wan X, Ma D, Xie X. (2011) Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29. *J Pathol.* 2011; 224:484–495

Li, B., Hu, Y., Ye, F., Li, Y., Lv, W., and Xie, X. (2010). Reduced miR-34a Expression in Normal Cervical Tissues and Cervical Lesions with High-Risk Human Papillomavirus Infection. Int. J. Gynecol. Cancer 20, 597–604. doi:10.1111/ igc.0b013e3181d63170

Li, E., Han, K., & Zhou, X. (2019). microRNA-27a-3p down-regulation inhibits malignant biological behaviors of ovarian cancer by targeting BTG1. *Open Medicine*, *14*(1), 577-585.

Li, M., Gao, Z., Wang, S., Zhao, Y., & Xie, H. (2023). miR-27a-3p upregulation by p65 facilitates cervical tumorigenesis by increasing TAB3 expression and is involved in the positive feedback loop of NF-κB signaling. *Oncology Reports*, *50*(1), 1-15.

Li, X., Chen, Y., Wang, F., & Xu, J. (2017). Expression of miR-106b in cervical cancer and its correlation with prognosis of patients treated with Intex. Journal of Southern Medical University, 37(3), 324-329.

Li, Y., Zhang, Z., Xiao, Z., Lin, Y., Luo, T., Zhou, Q., & Zhang, X. (2017). Chemotherapy-mediated miR-29b expression inhibits the invasion and angiogenesis of cervical cancer. *Oncotarget*, *8*(9), 14655.

Liao, J., Liu, R. A. N., Shi, Y. J., Yin, L. H., & Pu, Y. P. (2016). Exosome-shuttling microRNA- 21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. *International journal of oncology*, *48*(6), 2567-2579.

Lei, C., Wang, Y., Huang, Y., Yu, H., Huang, Y., Wu, L., & Huang, L. (2012). Up-regulated miR155 reverses the epithelial-mesenchymal transition induced by EGF and increases chemo-sensitivity to cisplatin in human Caski cervical cancer cells. *PLoS One*, *7*(12), e52310.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* **433**: 769– 773.

Liu L, Wu J, Ying Z (2018). Downregulated miR-214 increases the sensitivity of cervical cancer cells to radiation treatment. Cancer Biother Radiopharm. 2018;33(6):223-230.

Liu, Y., Yin, B., Zhang, C., Zhou, L., Fan, J., & Hui, Z. (2017). Low expression of miR-let-7a predicts poor prognosis in patients with cervical cancer. European Journal of Gynaecological Oncology, 38(1), 66-70.

Lowy, D. R. (2016). HPV vaccination to prevent cervical cancer and other HPV-associated disease: from basic science to effective interventions. *The Journal of clinical investigation*, *126*(1), 5-11.

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., ... & Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. *nature*, *435*(7043), 834-838.

Lu X, Wang L, Li H, (2016). MiR-143 and miR-145 regulate cervical cancer prognosis and radiotherapy sensitivity. Med Sci Monit. 2016; 22:640-647.

Lu, Y., Wang, L., He, S., Huang, X., Chen, S., & Chen, X. (2021). Elevated serum miR-21-5p is associated with better response to oral chemotherapy and improved prognosis in cervical cancer patients. Cancer Biomarkers, 30(3), 327-336

Lv, Y., Yuan, J., Xiong, X., Chen, Y., Li, H., Xia, X., ... & Li, S. (2018). Circulating miR-155 expression in cervical cancer patients undergoing chemotherapy with Intex and Outex. Cancer management and research, 10, 2187-2194.

Ma, G., Song, G., Zou, X., Shan, X., Liu, Q., Xia, T., ... & Zhu, W. (2019). Circulating plasma microRNA signature for the diagnosis of cervical cancer. *Cancer Biomarkers*, *26*(4), 491-500.

Marek, E., Dergez, T., Rebek-Nagy, G., Kricskovics, A., Kovacs, K., Bozsa, S., ... & Gocze, P. (2011). Adolescents' awareness of HPV infections and attitudes towards HPV vaccination 3 years following the introduction of the HPV vaccine in Hungary. *Vaccine*, *29*(47), 8591-8598.

Markowitz, L. E., Hariri, S., Unger, E. R., Saraiya, M., Datta, S. D., & Dunne, E. F. (2010). Post-licensure monitoring of HPV vaccine in the United States. *Vaccine*, *28*(30), 4731-4737

Martín, A., Epifano, C., Vilaplana-Marti, B., Hernández, I., Macías, R. I., Martínez-Ramírez, Á., ... & Pérez de Castro, I. (2023). Mitochondrial RNA methyltransferase TRMT61B is a new, potential biomarker and therapeutic target for highly aneuploid cancers. *Cell Death & Differentiation*, *30*(1), 37-53.

Mayya, V. K. (2021). *Understanding molecular mechanisms of microRNA-mediated gene silencing*.

Miao, J., Regenstein, J. M., Xu, D., Zhou, D., Li, C., Qiu, J., &Chen, X. (2020). The roles of microRNA in human cervical cancer. *Archives of Biochemistry and Biophysics*, *690* (March), 108480.

Morgan, E. L., Patterson, M. R., Ryder, E. L., Lee, S. Y., Wasson, C. W., Harper, K. L., ... & Macdonald, A. (2020). MicroRNA-18a targeting of the STK4/MST1 tumour suppressor is necessary for transformation in HPV positive cervical cancer. *PLoS pathogens*, *16*(6), e1008624.

Müller, S. F. (2013). *Adenocarcinoma of the cervix uteri: methods to improve diagnostics: biological markers and HPV testing*. Inst för kvinnors och barns hälsa/Dept of Women's and Children's Health.

Murewanhema, G., Moyo, E., Dzobo, M., Mandishora-Dube, R. S., & Dzinamarira, T. (2024). Human papilloma virus vaccination in the resource-limited settings of sub-Saharan Africa: Challenges and recommendations. *Vaccine: X*, 100549.

Nagamitsu, Y., Hirotaka Nishi, Sasaki, T., Takaesu, Y., Terauchi, F., & Isaka, k. (2016). *Profiling analysis of circulating microRNA expression in cervical cancer.*

Nahm FS. (2022). Receiver operating characteristic curve: overview and practical use for clinicians. Korean J Anesthesiol. 2022 Feb;75(1):25-36. doi: 10.4097/kja.21209. Epub 2022 Jan 18.

Obiri-Yeboah, D., Akakpo, P. K., Mutocheluh, M., Adjei-Danso, E., Allornuvor, G., Amoako-Sakyi, D., ... & Mayaud, P. (2017). Epidemiology of cervical human papillomavirus (HPV) infection and squamous intraepithelial lesions (SIL) among a cohort of HIV-infected and uninfected Ghanaian women. *BMC cancer*, *17*, 1-10.

O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in endocrinology*, *9*, 402.

Ochodo, E. A. (2010). Cervical Cancer prevention in Kenya: special considerations for HIV-infected women. *Royal tropical institute (KIT)*.

Ohyashiki, K., Umezu, T., Yoshizawa, S. I., Ito, Y., Ohyashiki, M., Kawashima, H., ... & Ohyashiki, J. H. (2011). Clinical impact of down-regulated plasma miR-92a levels in non-Hodgkin's lymphoma. *PloS one*, *6*(2), e16408.

Ojha, R., Nandani, R., Pandey, R. K., Mishra, A., & Prajapati, V. K. (2019). Emerging role of circulating microRNA in the diagnosis of human infectious diseases. *Journal of cellular physiology*, *234*(2), 1030-1043

Oliveira, C. R., & Niccolai, L. M. (2021). Monitoring HPV vaccine impact on cervical disease: Status and future directions for the era of cervical cancer elimination. *Preventive medicine*, *144*, 106363.

Orumaa, M., Kjaer, S. K., Dehlendorff, C., Munk, C., Olsen, A. O., Hansen, B. T., ... & Nygård, M. (2020). The impact of HPV multi-cohort vaccination: real-world evidence of faster control of HPV-related morbidity. *Vaccine*, *38*(6), 1345-1351.

Paavonen, J., Naud, P., Salmerón, J., Wheeler, C. M., Chow, S. N., Apter, D., ... & Dubin, G. H. P. V. (2009). Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *The Lancet*, *374*(9686), 301-314

Palanisamy, V., Jakymiw, A., Van Tubergen, E. A., D'silva, N. J., & Kirkwood, K. L. (2012). Control of cytokine mRNA expression by RNA-binding proteins and microRNAs. *Journal of dental research*, *91*(7), 651-658.

Pang, J., Pan, H., Yang, C., Meng, P., Xie, W., Li, J., ... & Xiao, S. Y. (2021). Prognostic value of immune-related multi-IncRNA signatures associated with tumor microenvironment in esophageal cancer. *Frontiers in Genetics*, *12*, 722601.

Papaconstantinou IG, Manta A, Gazouli M, Lyberopoulou A, Lykoudis PM, Polymeneas G, Voros D (2013). Expression of microRNAs in patients with pancreatic cancer and its prognostic significance. Pancreas. 2013; 42:67–71

Parkin, D. M., Almonte, M., Bruni, L., Clifford, G., Curado, M. P., & Pineros, M. (2008). Burden and trends of type-specific human papillomavirus infections and related diseases in the Latin America and Caribbean region. *Vaccine, 26*, L1-L15.

Pedroza-Torres, A., Fernández-Retana, J., Peralta-Zaragoza, O., Jacobo-Herrera, N., de Leon, D. C., Cerna-Cortés, J. F., ... & Pérez-Plasencia, C. (2016). A microRNA expression signature for clinical response in locally advanced cervical cancer. *Gynecologic oncology*, *142*(3), 557-565.

Peng, Y., & Croce, C. M. (2016). The role of MicroRNAs in human cancer. *Signal transduction and targeted therapy, 1*(1), 1-9.008.05.043 (2008).

Peralta-Zaragoza O, Deas J, Meneses-Acosta A, De la OGF, Fernandez-Tilapa G, Gomez-Ceron C, (2016) Relevance of miR-21 in regulation of tumor suppressor gene PTEN in human cervical cancer cells. BMC Cancer. 2016 16:215.

Peralta-Zaragoza, O., Deas, J., Meneses-Acosta, A., De la O-Gómez, F., Fernández-Tilapa, G., Gómez-Cerón, C., ... & Torres-Poveda, K. (2017). Relevance of miR-21 and miR-143 expression in tissue samples of cervical cancer patients. BioMed Research International, 2017.

Petersen, Z., Jaca, A., Ginindza, T. G., Maseko, G., Takatshana, S., Ndlovu, P., ... & Moyo, S. (2022). Barriers to uptake of cervical cancer screening services in low-and-middle-income countries: a systematic review. *BMC women's health, 22*(1), 486.

Pett, M., & Coleman, N. (2007). Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *212*(4), 356-367.

Pierce Campbell, C. M., Viscidi, R. P., Torres, B. N., Lin, H.-Y., Fulp, W., Abrahamsen, M., . . . Giuliano, A. R. (2016). Human Papillomavirus (HPV) L1 Serum Antibodies and the Risk of Subsequent Oral HPV Acquisition in Men: The HIM Study. *The Journal of Infection Diseases,* 214(1), 45-48. doi:10.1093/infdis/jiw083.

Ramakrishnan, S., Partricia, S., & Mathan, G. (2015). Overview of high-risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention. *Biomedicine & pharmacotherapy*, *70*, 103-110.

Re, A., Caselle, M., & Bussolino, F. (2017). MicroRNA-mediated regulatory circuits: outlook and perspectives. *Physical biology*, *14*(4), 045001.

Rosen, B. L., Bishop, J. M., McDonald, S., Wilson, K. L., & Smith, M. L. (2018). Factors associated with college women's personal and parental decisions to be vaccinated against HPV. *Journal of Community Health*, *43*, 1228-1234.

Saintibert, J. (2024). *Association Between Human Papillomavirus (HPV) Risk Factors, Protective Factors, and HPV–Associated Cancers Among Adults in the United States, NHANES 2011–2020* (Doctoral dissertation, Florida Agricultural and Mechanical University).

Sammarco, M. L., Tamburro, M., Pulliero, A., Izzotti, A., & Ripabelli, G. (2020). Human papillomavirus infections, cervical cancer and MicroRNAs: an overview and implications for public health. *MicroRNA*, *9*(3), 174-186.

Sankaranarayanan, R., Budukh, A. M., & Rajkumar, R. (2001). Effective screening programmes for cervical cancer in low-and middle-income developing countries. *Bulletin of the World Health Organization*, *79*(10), 954-962.

Sara, Z., Amir, S., Seyed Masoud, H., Marjan, R.-F., and Abolfazl, A. (2019). Deregulation of miR-21 and miR-29a in Cervical Cancer Related to HPV Infection. MicroRNA 8, 110–115

Satterwhite, C. L., Torrone, E., Meites, E., Dunne, E. F., Mahajan, R., Ocfemia, M. C. B., ... & Weinstock, H. (2013). Sexually transmitted infections among US women and men. *Sexually transmitted diseases*, *40*(3), 187-193.

Sathyanarayanan, A., Chandrasekaran, K. S., & Karunagaran, D. (2016). microRNA-146a inhibits proliferation, migration and invasion of human cervical and colorectal cancer cells. *Biochemical and biophysical research communications*, *480*(4), 528-533.

Schrauder, M. G., Strick, R., Schulz-Wendtland, R., Strissel, P. L., Kahmann, L., Loehberg, C. R., ... & Fasching, P. A. (2012). Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection. *PloS one*, *7*(1), e29770.

Schubert, M., Bauerschlag, D. O., Muallem, M. Z., Maass, N., & Alkatout, I. (2023). Challenges in the diagnosis and individualized treatment of cervical cancer. *Medicina*, *59*(5), 925.

Servín-González, L., Granados-López, A., and López, J. (2015). Families of microRNAs Expressed in Clusters Regulate Cell Signaling in Cervical Cancer. Ijms 16, 12773–1279 doi:10.3390/ijms160612773

Shah, S. S., Senapati, S., Klacsmann, F., Miller, D. L., Johnson, J. J., Chang, H. C., & Stack, M. S. (2016). Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. *Cancers*, *8*(9), 85.

Sharma, P. C., & Gupta, A. (2020). MicroRNAs: potential biomarkers for diagnosis and prognosis of different cancers. *Translational cancer research*, *9*(9), 5798.

Shenouda, S. K., & Alahari, S. K. (2009). MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer and metastasis reviews*, *28*, 369-378.

Shen, S., Zhang, S., Liu, P., Wang, J., & Du, H. (2020). Potential role of microRNAs in the treatment and diagnosis of cervical cancer. *Cancer genetics*, *248*, 25-30.

Singh, D., Vignat, J., Lorenzoni, V., Eslahi, M., Ginsburg, O., Lauby Secretan, B., ... & Vaccarella, S. (2023). Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *The Lancet Global Health*, *11*(2), e197-e206.

Smith, J. S., Melendy, A., Rana, R. K., & Pimenta, J. M. (2008). Age-specific prevalence of infection with human papillomavirus in females: a global review. *Journal of Adolescent Health*, *43*(4), S5-e1.

Smith, R. A., Brooks, D., Cokkinides, V., Saslow, D., & Brawley, O. W. (2013). Cancer screening in the United States, 2013: a review of current American Cancer Society guidelines, current issues in cancer screening, and new guidance on cervical cancer screening and lung cancer screening. *CA: a cancer journal for clinicians*, *63*(2), 87-105.

Sohel, M. H. (2016). Extracellular/circulating microRNAs: release mechanisms, functions and challenges. *Achievements in the Life Sciences*, *10*(2), 175-186.

Song L, Liu S, Zeng S, Zhang L, Li X. (2015) miR-375 Modulates Radio sensitivity of HR-HPVPositive Cervical Cancer Cells by Targeting UBE3A through the p53 Pathway. Med Sci Monit. 2015 21:2210–7.

Song T, Zhang X, Wang C, Wu Y, Dong J, Gao J,. (2011) Expression of miR-143 reduces growth and migration of human bladder carcinoma cells by targeting cyclooxygenase-2. Asian Pac J Cancer Prev. 2011 12:929–33.

Song, D., Li, H., & Dai, J. (2015). Effect of human papillomavirus infection on the immune system and its role in the course of cervical cancer. *Oncology letters*, 10(2), 600-606. doi: 10.3892/o1.2015.3295.

Song, J., Zhang, W., Wang, J., et al. (2019). Dysregulated miRNAs in plasma of patients without cervical cancer. Journal of Cancer Research and Clinical Oncology, 145(5), 1245-1252. doi: 10.1007/00432-019-02863-x.

Song, L., Huang, Q., Chen, K., Liu, L., Lin, C., Dai, T., & Yu, C. (2018). MiR-203 suppresses cell proliferation and migration by targeting BIRC5 and LASP1 in human cervical cancer cells. Cellular Physiology and Biochemistry, 45(2), 856-866.

Sonkoly, E., & Pivarcsi, A. (2009). Advances in microRNAs: implications for immunity and inflammatory diseases. *Journal of cellular and molecular medicine*, *13*(1), 24-38.

Sørensen, K. D., Ostenfeld, M. S., Jeppesen, D. K., Kristensen, H., Haldrup, C., & Ørntoft, T. F. (2013). The application of microRNAs in cancer diagnostics. *RNA Interference from Biology to Therapeutics*, 259-298.

Sopik, V., Iqbal, J., Rosen, B., & Narod, S. A. (2015). Why have ovarian cancer mortality rates declined? Part I. Incidence. *Gynecologic oncology*, *138*(3), 741-749.

Su, C., Huang, D. P., Liu, J. W., Liu, W. Y., & Cao, Y. O. (2019). miR-27a-3p regulates proliferation and apoptosis of colon cancer cells by potentially targeting BTG1. *Oncology letters*, *18*(3), 2825- 2834.

Su, K., Wang, N., Shao, Q., Liu, H., Zhao, B., & Ma, S. (2021). The role of a ceRNA regulatory network based on lncRNA MALAT1 site in cancer progression. *Biomedicine & Pharmacotherapy*, *137*, 111389.

Sun, H. L., Cui, R., Zhou, J., Teng, K. Y., Hsiao, Y. H., Nakanishi, K., ... & Croce, C. M. (2016). ERK activation globally downregulates miRNAs through phosphorylating exportin-5. *Cancer cell*, *30*(5), 723-736.

Svoronos, A. A., Engelman, D. M., and Slack, F. J. (2016). OncomiR or Tumor Suppressor? The Duplicity of MicroRNAsin Cancer. CancerRes.76,3666–3670.

Takeuchi, T., Kawasaki, H., Luce, A., Cossu, A. M., Misso, G., Scrima, M., ... & Zappavigna, S. (2020). Insight toward the MicroRNA profiling of laryngeal cancers: Biological role and clinical impact. *International Journal of Molecular Sciences*, *21*(10), 3693.

Tan, L., Yu, J. T., & Tan, L. (2015). Causes and consequences of microRNA dysregulation in neurodegenerative diseases. *Molecular neurobiology*, *51*, 1249-1262.

Tang, C., Qi, J., Wu, Y., Luo, L., Wang, Y., Wu, Y., & Shi, X. (2022). Improving the prediction for the response to radiotherapy of clinical tumor samples by using combinatorial model of MicroRNA expression. *Frontiers in Genetics*, *13*, 1069112.

Teng, Y., Zuo, X., Hou, M., Zhang, Y., Li, C., Luo, W., & Li, X. (2016). A double-negative feedback interaction between microRNA-29b and DNMT3A/3B contributes to ovarian cancer progression. *Cellular Physiology and Biochemistry*, *39*(6), 2341-2352.

Tricoli, J. V., & Jacobson, J. W. (2007). MicroRNA: potential for cancer detection, diagnosis, and prognosis.

Trottier, H., Mahmud, S., Costa, M. C., Sobrinho, J. P., Duarte-Franco, E., Rohan, T. E., ... & Franco, E. L. (2006). Human papillomavirus infections with multiple types and risk of cervical neoplasia. *Cancer Epidemiology Biomarkers & Prevention*, *15*(7), 1274-1280.

Tsikouras, P., Zervoudis, S., Manav, B., Tomara, E., Iatrakis, G., Romanidis, C., ... & Galazios, G. (2016). Cervical cancer: screening, diagnosis and staging. *J buon*, *21*(2), 320-325.

Tüfekci, K. U., Meuwissen, R. L. J., & Genç, Ş. (2014). The role of microRNAs in biological processes. *miRNomics: microRNA biology and computational analysis*, 15-31.

Ugrinska A, Bombardieri E, Stokkel MP, Crippa F, Pauwels EK. (2002) Circulating tumor markers and nuclear medicine imaging modalities: breast, prostate and ovarian cancer. Q J Nucl Med. 2002; 46:88–104.

Vaccarella, S., & Bray, F. (2015). Epidemiology and burden of disease associated with HPV infection. *Current Obstetrics and Gynecology Reports*, *4*, 181-188.

Vaidya, F. U., Sufiyan Chhipa, A., Mishra, V., Gupta, V. K., Rawat, S. G., Kumar, A., & Pathak, C. (2022). Molecular and cellular paradigms of multidrug resistance in cancer. *Cancer reports*, *5*(12), e1291.

Valinezhad Orang, A., Safaralizadeh, R., & Kazemzadeh-Bavili, M. (2014). Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. *International journal of genomics*, *2014*.

van den Berg, A., Mols, J., & Han, J. (2008). RISC-target interaction: cleavage and translational suppression. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1779*(11), 668-677.

van de Wijgert, J. H., Gill, A. C., Chikandiwa, A., Verwijs, M. C., Kelly, H. A., Omar, T., ... & HARP Study Group. (2020). Human papillomavirus infection and cervical dysplasia in HIV-positive women: potential role of the vaginal microbiota. *AIDS*, *34*(1), 115-125.

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M et al (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103(7):2257−2261.

Waite, L. J. (1995). Does marriage matter? *Demography*, *32*(4), 483-507.

Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., ... & Muñoz, N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*, *189*(1), 12-19.

Wang, A., Xu, Q., Sha, R., Bao, T., XI, X., & Guo, G. (2021a). MicroRNA-29a Inhibits Cell Proliferation and Arrests C-ell C-ycle by Modulating P-16 Methylation in Cervical Cancer. Oncol. Lett. 21, 272. doi:10.3892/ol.2021.12533

Wang, F., Liu, M., Li, X., Tang, H., & Mi, R. (2015). The expression and clinical significance of microRNA-21 in cervical cancer: a systematic review and meta-analysis. PloS One, 10(11)

Wang, F., Tan, W. H., Liu, W., Jin, Y. X., Dong, D. D., Zhao, X. J., & Liu, Q. (2018). Effects of miR-214 on cervical cancer cell proliferation, apoptosis and invasion via modulating PI3K/AKT/mTOR signal pathway. *European Review for Medical & Pharmacological Sciences*, *22*(7).

Wang J, Song Y, Zhang Y, (2019). Low plasma miR-203 predicts a poor prognosis for cervical cancer patients. Cancer Biomark. 2019;25(3):273-27e0145831.

Wang, J., Chen, J., Chang, P., LeBlanc, A., Li, D., Abbruzzesse, J. L., ... & Sen, S. (2009). MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer prevention research*, *2*(9), 807-813

Wang, J., Yang, M., Li, Y., & Han, B. (2015). The role of microRNAs in the chemoresistance of breast cancer. *Drug Development Research*, **76**(7), 368– 374.

Wang, P., Zhai, G., & Bai, Y. (2018). Values of miR-34a and miR-218 expression in the diagnosis of cervical cancer and the prediction of prognosis. *Oncology Letters*, *15*(3), 3580-3585

Wang, R., Ma, J., Wu, Q., Xia, J., Miele, L., H Sarkar, F., & Wang, Z. (2013). Functional role of miR-34 family in human cancer. *Current drug targets*, *14*(10), 1185-1191.

Wang, S., Cao, X., Ding, B., Chen, J., Cui, M., Xu, Y., ... & Jin, H. (2016). The rs767649 polymorphism in the promoter of miR-155 contributes to the decreased risk for cervical cancer in a Chinese population. *Gene*, *595*(1), 109-114

Wang, V., & Wu, W. (2009). MicroRNA-based therapeutics for cancer. *BioDrugs*, *23*(1), 15-23.

Wang X, Chen H, Liu J, (2018), Prognostic significance of plasma miR-192 expression in patients with cervical cancer. J Clin Lab Anal. 2018; 32(1): e22168.

Wang, X., Tang, S., Le, S. Y., Lu, R., Rader, J. S., Meyers, C., & Zheng, Z. M. (2008). Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PloS one*, *3*(7), e2557.

Wang, X., Tang, S., Le, S. Y., Lu, R., Rader, J. S., Meyers, C., & Zheng, Z. M. (2008). Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PloS one*, *3*(7), e2557.

Wang, X., Tang, S., Le, S. Y., Lu, R., Rader, J. S., Meyers, C., & Zheng, Z. M. (2008). Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PloS one*, *3*(7), e2557.

Wang, X., Wang, H. K., McCoy, J. P., Banerjee, N. S., Rader, J. S., Broker, T. R., ... & Zheng, Z. M. (2009). Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. *Rna*, *15*(4), 637-647.

Wang, Y., Lu, T., Wang, Q., Liu, J., & Jiao, W. (2018). Circular RNAs: crucial regulators in the human body. *Oncology reports*, *40*(6), 3119-3135.

Wang Y, Yan J, Wang L. (2014). The diagnostic value of serum carcino-embryonic antigen, alpha fetoprotein and carbohydrate antigen 19-9 for colorectal cancer. J Cancer Res Ther. 2014;10(Suppl):307–9.

Wang, Y., Wang, J., Sun, Y., Zhu, X., & Chen, W. (2016). MiR-424 and miR-27a increase TRAIL sensitivity of acute myeloid leukemia by targeting PLAG1. Oncotarget, 7(31), 48230- 48244.doi:10.18632/oncotarget.10327

Wei Ben, Guangmei Zhang, Yangang Huang & Yuhui Sun (2020) MiR-27a-3p Regulated the Aggressive Phenotypes of Cervical Cancer by Targeting FBXW7, Cancer Management and Research, 12: 2925-2935,

Wei, J., Lu, Y., Wang, R., Xu, X., Liu, Q., He, S., ... & Zhang, J. (2021). MicroRNA-375: potential cancer suppressor and therapeutic drug. *Bioscience Reports*, *41*(9), BSR20211494.

Wirtz, C., Mohamed, Y., Engel, D., Sidibe, A., Holloway, M., Bloem, P., ... & Morgan, C. (2022). Integrating HPV vaccination programs with enhanced cervical cancer screening and treatment, a systematic review. *Vaccine*, *40*, A116-A123.

World Health Organization. (2021). WHO releases new recommendations to prevent cervical cancer through increased access to screenings and treatment?

World Health Organization. (2020). WHO framework for strengthening and scaling-up of services for the management of invasive cervical cancer.

World Health Organization. (2014). Human papillomavirus vaccines: WHO position paper, October 2014. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, *89*(43), 465-491.

Wright Jr, T. C., Schiffman, M., Solomon, D., Cox, J. T., Garcia, F., Goldie, S., ... & Saslow, D. (2004). Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstetrics & Gynecology*, *103*(2), 304-309.

Wu, J., Zeng, D., Zhi, S., Ye, Z., Qiu, W., Huang, N., ... & Liao, W. (2021). Single-cell analysis of a tumor-derived exosome signature correlates with prognosis and immunotherapy response. *Journal of Translational Medicine*, *19*, 1-18.

Wu W, Lin Z, Zhuang Z, Liang X, Chen J. (2014). Circulating microRNA-21 is a potential diagnostic biomarker in cervical cancer. Sci Rep. 2014; 4:5911. doi:10.1038/srep05911

Wu, X. Z., Wang, K. P., Song, H. J., Xia, J. H., Jiang, Y., & Wang, Y. L. (2015). MiR-27a-3p promotes esophageal cancer cell proliferation via F-box and WD repeat domain-containing 7 (FBXW7) suppression. *International journal of clinical and experimental medicine*, *8*(9), 15556.

Xing, D., & Fadare, O. (2021, January). Molecular events in the pathogenesis of vulvar squamous cell carcinoma. In *Seminars in diagnostic pathology* (Vol. 38, No. 1, pp. 50-61). WB Saunders.

Xu, W., San Lucas, A., Wang, Z., & Liu, Y. (2014). Identifying microRNA targets in different gene regions. *BMC bioinformatics*, *15*, 1-11.

Yamamoto N, Kinoshita T, Nohata N, Itesako T, Yoshino H, Enokida H, Nakagawa M, Shozu M & Seki, N. (2013). Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion by targeting focal adhesion pathways in cervical squamous cell carcinoma. *International Journal of Oncology* (42) 1523-1532.

Yang C, Wang C, Chen X, Chen S, Zhang Y, Zhi F, Wang J, Li L, Zhou X, Li N, Pan H, Zhang J, Zen K, Zhang CY & Zhang C. (2013). Identification of seven serum microRNAs from a genome-wide serum microRNA expression profile as potential noninvasive biomarkers for malignant astrocytomas. International Journal of Cancer 132: 116-12.

Yang, L., Liu, M., Deng, Y., Su, D., & Xiao, X. (2019). A panel of serum miRNAs as a potential biomarker for diagnosis and prognosis of cervical cancer. International Journal of Gynecological Cancer, 29(5), 930-938.

Yang, Y., Cai, J., Yin, Y., Shi, Z., Wang, J., & Wei, L. (2019). Circulating miRNA-21 as a diagnostic biomarker in cervical cancer with HIV co-infection. Frontiers in Oncology, 9,1373. doi:10.3389/fonc.2019.01373

Yao, T., & Lin, Z. (2012). MiR-21 is involved in cervical squamous cell tumorigenesis and regulates CCL20. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1822*(2), 248-260.

Yi, R., Qin, Y., Macara, I. G., & Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes & development*, *17*(24), 3011-3016.

Yin, X. Z., Zhao, D. M., Zhang, G. X., & Liu, L. (2016). Effect of miRNA-203 on cervical cancer cells and its underlying mechanism. *Genet Mol Res*, *15*(3), 1-7.

Yu, L., Xiong, J., Guo, L., Miao, L., Liu, S., & Guo, F. (2015). The effects of lanthanum chloride on proliferation and apoptosis of cervical cancer cells: Involvement of let-7a and miR-34a microRNAs. *BioMetals*, **28**(5), 879– 890.

Zen, K., & Zhang, C. Y. (2012). Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. *Medicinal research reviews*, *32*(2), 326-348.

Zhang, R., Su, J., Xue, S. L., Yang, H., Ju, L. L., Ji, Y., ... & Yu, M. M. (2016). HPV E6/p53 mediated down-regulation of miR-34a inhibits Warburg effect through targeting LDHA in cervical cancer. *American journal of cancer research*, *6*(2), 312.

Zhang, Y., Zhang, D., Wang, F., Xu, D., Guo, Y., & Cui, W. (2015). Serum miRNAs panel (miR- 16-2*, miR-195, miR-2861, miR-497) as novel non-invasive biomarkers for detection of cervical cancer. *Scientific reports*, *5*(1), 17942.

Zhang, Y., Luo, G., Li, M., Guo, P., Xiao, Y., Ji, H., & Hao, Y. (2019). Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. *BMC cancer*, *19*, 1-14.

Zhao, S., Yao, D., Chen, J., & Ding, N. (2013). Circulating miRNA-20a and miRNA-203 for screening lymph node metastasis in early stage cervical cancer. *Genetic testing and molecular biomarkers*, *17*(8), 631-636.

Zheng, F., Zhang, J., Luo, S., Yi, J., Wang, P., Zheng, Q., & Wen, Y. (2016). miR-143 is associated with proliferation and apoptosis involving ERK5 in HeLa cells. *Oncology Letters*, *12*(4), 3021-3027.

Zhou, J., Chen, Q., Yang, J., Sun, Y., Zhang, A., & Wang, Y. (2019). MiR-146a overexpression in cervical cancer patients with Outex treatment and its prognostic significance. Cancer biomarkers: section A of Disease markers, 24(3), 289-296.

Zhou, J., Gong, G., Tan, H., Dai, F., Zhu, X., Chen, Y., ... & Li, W. (2020). Diagnostic potential of a plasma microRNA signature in cervical cancer: a microRNA expression analysis Scientific Reports, 10(1), 1-10.

Zhu, L., Xu, X., Zhang, X., Ji, W., Chen, Y., Chen, J., & Wang, L. (2021). Serum miR-205-5p as a potential biomarker for predicting response to chemotherapy and prognosis in cervical cancer. Cancer Biomarkers, 30(2), 175-183.

APPENDICES

Appendix A: QUESTIONNAIRE

Declaration of Confidentiality

Hello, my name is Helena Quayson an MPhil student of University of Cape Coast offering Infection and immunity. This research is focused on identifying microRNA that can be used as biomarkers and also as diagnostic tools for cervical cancer patients and for that reason, information and samples will be needed from patients with cervical cancer in order to carry out such research. All your responses will be kept confidential and combined with other responses when reporting the results of the study.

Title of research

Circulating microRNA in plasma of cervical cancer patients at some selected teaching hospitals in Ghana.

The questionnaire has three sections with section A having part 1 and 2. Please provide the needed answers for the questions below;

Section A: Social Demographic

Part 1: Personal Details

Name……Occupation……

Age…….....Time of diagnosis…….

Telephone number……Email……

Any relative number…… Last time of menstrual flow…….

Part 2: This part is aimed at obtaining information on factors that predispose one or make one liable to cervical cancer.

1. Do your medical reports show you have cervical cancer? Yes / No.....
2. Do your medical reports show you are HIV positive? Yes / No.....
3. Has any member of your family been diagnosed of cervical cancer?.....
4. Do you smoke? Yes / No.....

Section B: Therapy

This section is focused on identifying whether you are on treatment or not. Please fill the blank spaces with the right answer.

Are you currently on medication? Yes / No.....

If Yes, what kind of medication / treatment……

Section C: Immunological profile for cervical cancer patients only

This section is to assess your current health status. You are to leave these spaces blank if you are unsure of the answer.

1. What is the stage of your cancer?.....
2. Are you anemic?.....
3. What is your current Hb level? ……

Appendix C: University of Cape Coast Ethical Clearance form

UNIVERSITY OF CAPE COAST

INSTITUTIONAL REVIEW BOARD SECRETARIAT

TEL: 0558093143 / 0508878309
E-MAIL: irb@ucc.edu.gh
OUR REF: UCC/IRB/A/2016/1519
YOUR REF:
OMB NO: 0990-0279
IORG #: IORG0009096

24TH AUGUST, 2022

Ms. Helena Quayson
School of Medical Sciences
University of Cape Coast

Dear Ms. Quayson,

ETHICAL CLEARANCE – ID (UCCIRB/CHAS/2021/294)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted Provisional Approval for the implementation of your research **Circulating Micro RNA in Cervical Cancer Patient in Ghana.** This approval is valid from 24th August, 2022 to 23rd August, 2023. You may apply for a renewal subject to submission of all the required documents that will be prescribed by the UCCIRB.

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

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
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF CAPE COAST

Appendix D: Cape Coast Teaching Hospital Ethical Clearance Form

In case of reply the reference number
and the date of this
Letter should be quoted

P. O. Box CT.1363
Cape Coast
CC-071-9967
Tel: 03321-34010-14
Fax: 03321-34016
Website:
www.ccthghana.org
email:
info@ccthghana.com

Our Ref.: CCTH

Your Ref.:

4th March, 2022

Helena Quayson
Department of Microbiology
School of Medical Sciences
College of Allied Health
University of Cape Coast
Cape Coast

Dear Madam,

ETHICAL CLEARANCE – REF: CCTHERC/EC/2022/023

The Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC) has reviewed your research protocol titled, "**Circulating Micro RNA in Cervical Cancer Patients in Central Region of Ghana**" which was submitted for ethical clearance. The ERC is glad to inform you that you have been granted provisional approval for implementation of your research protocol.

The CCTHERC requires that you submit periodic review of the protocol and a final full review to the ERC on completion of the research. The CCTHERC 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 CCTHERC for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the CCTHERC within ten (10) days in writing. Also note that you are to submit a copy of your final report to the CCTHERC office.

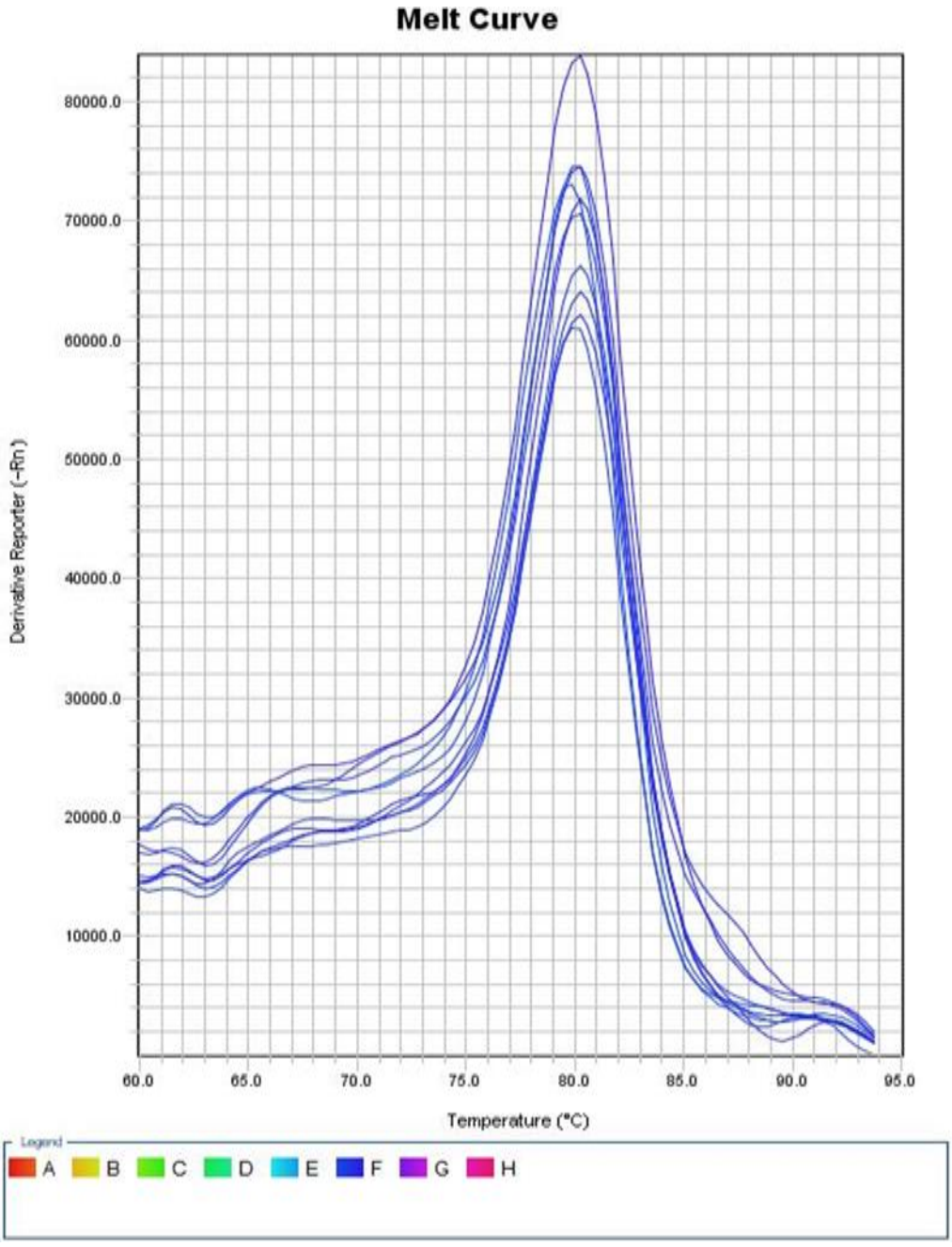
Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours sincerely,

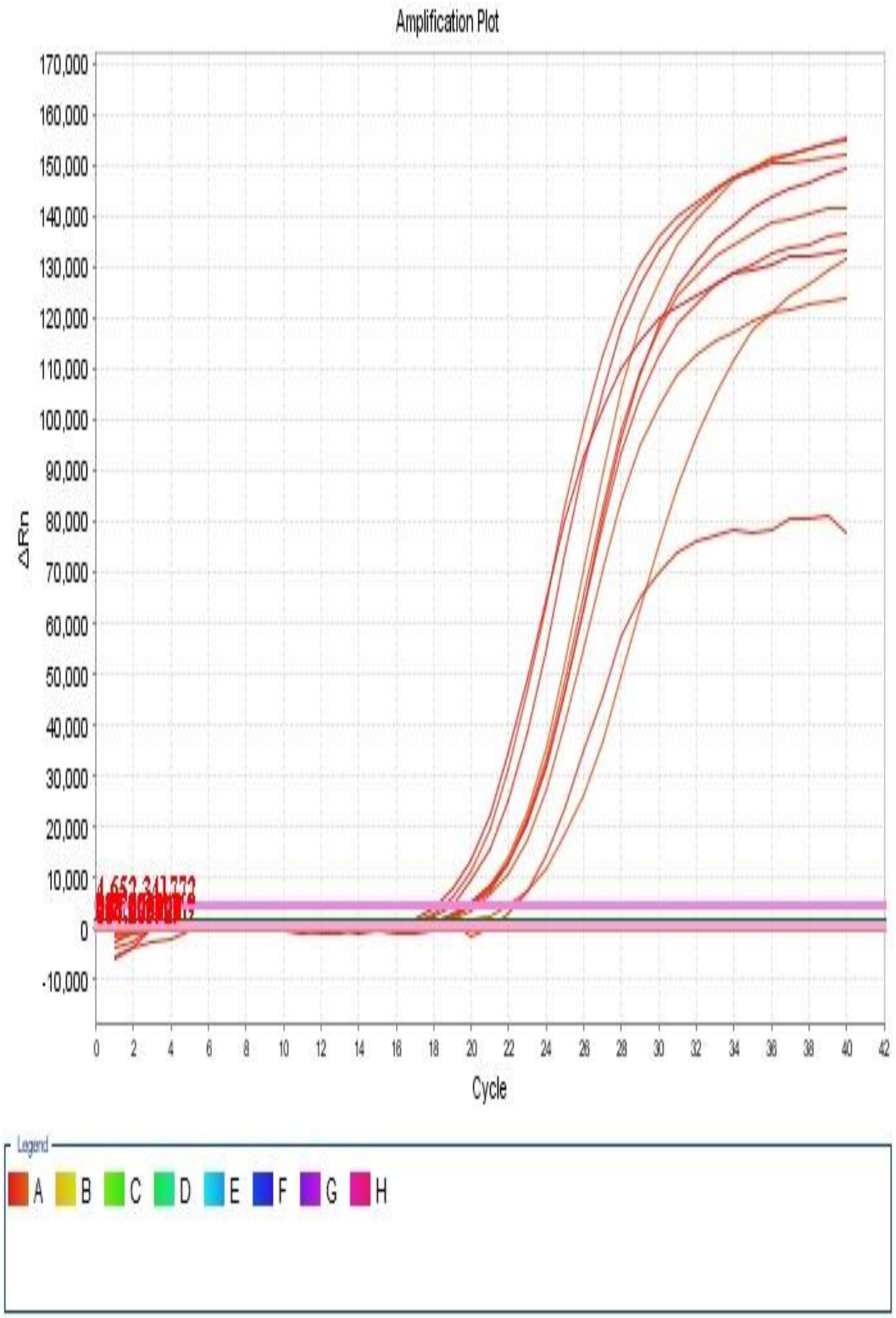
Dr. Stephen Laryea
Medical Director
For: Chairman, ERC

ETHICAL REVIEW COMMITTEE
CAPE COAST TEACHING HOSPITAL
P.O. BOX CT 1363
CAPE COAST

Appendix E: Melting curve of some of the microRNAs after the PCR run



Appendix F: Amplification plot of miR-16 used as endogenous after the PCR run



Appendix G: Amplification plot of some of the microRNAs after the PCR run