

A Thesis submitted to the Atmiya University

For the degree of **Doctor of Philosophy** in **Microbiology**

By **Riya Mashru** Enrolment No. [190682003]

Under the guidance of **Dr. Debashis Banerjee** Department of Biotechnology

ATMIYA UNIVERSITY Yogidham Gurukul, Kalawad road, Rajkot (360005), Gujarat, India

DECEMBER 2023

DECLARATION BY THE CANDIDATE

I declare that thesis entitled "*Study of Molecular Markers of Cervical Cancer and its Clinical Applications*" is my own work conducted under the supervision of **Dr**. **Debashis Banerjee** at Department of **Microbiology**, **Faculty of Science**, **Atmiya University**, **Rajkot**, **Gujarat**, **India** and approved by the Director of Research.

I further declare that to the best of my knowledge the thesis does not contain any part of any work which has been submitted for award of any degree either in this University or any other University without proper citation.

Date: 17/10/202 3 Place: Rajkot

Signature of Candidate [Riya Mashru]

Study of Molecular Markers in Cervical Cancer and its Clinical Applications CERTIFICATE OF SUPERVISOR

This is to certify that work entitled "*Study of Molecular Markers of Cervical Cancer and its Clinical Applications*" is a piece of research work done by **Riya Mashru** under my supervision for the degree of Doctor of Philosophy in Department of Microbiology, Faculty of Science, Atmiya University, Rajkot, Gujarat, India

To the best of my knowledge and belief the research work and thesis

- I. Embodies the work of candidate himself / herself,
- II. Has duly been completed,
- III. Fulfils the requirement of ordinance related to Ph.D. degree of the University &
- IV. is up to the standard both in respect of content and language for being referred to the examiner.

Date:

Place:

Signature of the Research Supervisor

Thesis Approval Form

The Viva-Voce of the Ph.D. thesis entitled "*Study of Molecular Markers of Cervical Cancer and its Clinical Applications*" submitted by **Riya Mashru** (190682003) was conducted on 24/12/2023 (Saturday) at Atmiya University, Rajkot, Gujarat

Based on the Performance of the Candidate, We, the panel of examiners, recommend that (Please tick any of the following)

- 1. He / She be awarded Ph.D. Degree
- 2. The following modifications are to be incorporated in the thesis within one month and viva-voce be re-conducted by the same panel of examiners

Please Suggested Modification

3. He / She should not be awarded Ph.D. Degree

Please mention Reason for Rejection

Date: Place:

Signature of Research Supervisor

Name of Research Supervisor

Signature of External Examiner

Name of External Examiner

Declaration by Research Scholar for submission of thesis

I declare that the submitted thesis entitled "*Study of Molecular Markers of Cervical Cancer and its Clinical Applications*" submitted by me incorporates,

- 1. All the suggestions /directions/modifications/additions/deletions received from the supervisors and the external examiners through their thesis evaluation reports.
- All the suggestions /directions/modifications/additions/deletions received from the panel of examiners during the public viva-voce (Open Defense) conducted on Dt. 23/12/2023.

Date:

Place: Rajkot

Signature of Research Scholar

ACKNOWLEDGEMENT

I would like to acknowledge SHOADH scholarship for their financial support and also like to thank Multi-Disciplinary Research Unit at Shri M. P. Shah Government Medical College, Jamnagar for their Valuable cooperation.

I would take this opportunity to thank my PhD supervisor, Dr. Debashish Banerjee. His support and guidance throughout the PhD work was invaluable. I would also like to thank Dr. Ashish Kothari sir, Deputy Register of Atmiya University, who helped me to get this stage. I am really thankful to Dr. Minaxi Parmar, my former supervisor who always provided guidance and inspiration whenever I needed.

My humble gratitude to Dr. Dhaval Parmar, Scientist C at MDRU, who helped me in each and every step during my research work. He also helped me in publications. His valuable guidance helped me to complete my PhD work.

I would love to thank my family, my parents; they not just supported but believed in my dream. I always and eternally thankful to them for always trusting me. I am elated to thank My brother Dhruvil, who always helped me in editing and merging my thesis. Words fall short for my husband Samip who always supported me in each and every possible way and also bearing with my stress level throughout my work period.

I am grateful to everyone I've collaborated externally, G. T. Sheth Cancer Hospital, Dr. Radhika Javiya, Head of Kundariya Foundation, Dr Kirit Patel sir, Sanjeevani (Metropolis), Dr. Vijay Popat sir, Dean of Faculty Saurashtra University (HoD of Pathology Department of shree M. P. Shah Government Medical College, Jamnagar)

At last, I would like to thank all my friends from Atmiya University, Deep, Binal, Shivani, Jay, and friends from MDRU Dr. Pruthvi Gohel, Kishan, Ekta, & Meera.

I am thankful to almighty God for giving me this opportunity and surrounded me with great people.

Riya Mashru

Figure No.	Figure caption	Page no.
Figure 1.1	The most common types of cancers in	2
	various organs	
Figure 1.2	Female reproductive system	3
Figure 2.1	Estimated age-standardized rates per	10
	100,000	
Figure 2.2	Cervical cancer in comparison to	11
	other types of cancer in women	
Figure 2.3	Screening and management	14
	Algorithm for Cervical cancer	
Figure 2.4	p53 and cancer progression	29
Figure 2.5	Overview of Ki-67 localisation and	31
	functions	
Figure 2.6	Phylogenetic tree of Papillomaviruses	33
Figure 2.7	Electron micrograph of Human	35
	Papillomavirus	
Figure 2.8	Genomic Structure of Human	35
	Papillomavirus	
Figure 2.9	Organization of the HPV genome and the	38
	virus life cycle	
Figure 2.10	Cervical Cancer Genesis	44
Figure 2.11	The organization of circular HPV DNA and	45
	its integration into host cell	
Figure 2.12	Effect of oncogenic HPV leading to	45
	cellular transformation	
Figure 3.1	Workflow of the research	49
Figure 3.2	AgNOR structures and configurations	52
Figure 3.3	Work flow of HPV detection and HPV	54
	typing	

Atmiya University, Rajkot, Gujarat, India

Study of Molecul	lar Markers in Cervical Cancer and its Clinical App	lication
Figure 4.1	Overview of results	59
Figure 4.2	Types of samples collected	60
Figure 4.3	Age at the time of screening years of age	61
Figure 4.4	Age of Marriage	62
Figure 4.5	Geographic area of females at the time of	62
	screening	
Figure 4.6	Community distribution	63
Figure 4.7	Occupation of the females at the time of	63
	screening	
Figure 4.8	Active sexual life	64
Figure 4.9	Menarche of females at the time of	65
	screening	
Figure 4.10	Menopause	66
Figure 4.11	Place of Delivery	67
Figure 4.12	Mode of Delivery	67
Figure 4.13	Gravidity showing from obstetrics history	69
	Maximum	
Figure 4.14	Parity from obstetrics history	69
Figure 4.15	Living from obstetrics history	70
Figure 4.16	Abortion from obstetrics history	70
Figure 4.17	Tubal ligation from obstetrics history	71
Figure 4.18	Contraception history of female	71
Figure 4.19	Common symptoms observed in cervical	72
	cancer screening	
Figure 4.20	Symptoms observed in menopausal women	75
Figure 4.21	Symptoms v/s PAP results	76
Figure 4.22	Most common symptoms in menopause	77
Figure 4.23	Pelvic Per Speculum Examination in	77
	menopausal women	

Atmiya University, Rajkot, Gujarat, India

Study of Molecul	iai Markers in Cervical Cancer and its Chincar App	incatio
Figure 4.24	Pelvic Per Vaginal Examination in	78
	menopausal women	
Figure 4.25	Pelvic per speculum examination	78
Figure 4.26	Pelvic per vaginal examination	79
Figure 4.27	Normal cervix stained with silver stain	80
	showing predominantly 1 Dot (NOR)/cell	
	(X1000)	
Figure 4.28	Low Squamous Intraepithelial Lesion	80
	(LSIL) Stained with Silver Stain Showing 2-	
	3 Dots (NOR)/ Cell (X1000)	
Figure 4.29	High Squamous Intraepithelial Lesion	81
	(HSIL) Stained with Silver Stain Showing 3-	
	4 Dots (NOR)/Cell (X1000)	
Figure 4.30	p53 showing positivity in HSIL smear	83
	(X400)	
Figure 4.31	p53 showing positivity in malignant cervical	83
	neoplasm (X400)	
Figure 4.32	Ki-67 showing positivity in HSIL smear	84
	(X400)	
Figure 4.33	Ki-67 showing positivity in malignant	84
	cervical neoplasm (X400)	
Figure 4.34	Gel picture of amplification of beta-globin	86
	gene	
Figure 4.35	Cancer patients samples positive for HPV 16	86
	by Typing	
Figure 4.36	cancer patients samples negative for HPV 18	87
	by Typing	

Table No.	Table caption	Page no.
Table 1.1	Main approaches to diagnose Cervical cancer	5
Table 2.1	TNM and FIGO Classification for Cervical	15
	Cancer	
Table 2.2	Comparison of commonly used and ICTV	33
	Papillomavirus nomenclature	
Table 2.3	Members of the Alphapapilloma viruses genus	36
Table 3.1	Description of the study variables	50
Table 4.1	Personal data of the screening program	61
Table 4.2	Menstrual history of screening program	64
Table 4.3	Menopause	65
Table 4.4	Labor history	66
Table 4.5	showing Obstetric History	68
Table 4.6	Personal data of patient having Menopause	73
Table 4.7	Menstrual history of Menopausal women	73
Table 4.8	Labour history of menopausal women	74
Table 4.9	Obstetric history of menopausal women	74
Table 4.10	Clinical History of menopausal woman	75
Table 4.11	PAP staining results	79
Table 4.12	Grade wise comparison between AgNOR and	81
	PAP	
Table 4.13	Comparison of PAP Staining and AgNOR	82
	staining	
Table 4.14	Comparison between AgNOR and p53/ki67	85
Table 4.15	Sensitivity and Specificity of AgNOR	85
	considering ki67/p53 as gold standard	
Table 4.16	Cumulative results of different diagnostic	87
	methods	
Table 4.17	ANOVA test results	88

Atmiya University, Rajkot, Gujarat, India

XIV

ABBREVIATIONS

Abbreviations	Full Forms
ASC	Atypical Squamous Cells
ASC-H	Atypical Squamous Cells: Cannot Exclude a High-Grade Squamous (Intra)Epithelial Lesion
ASCUS	Atypical Squamous Cells of Undetermined Significance
CIN	Cervical Intraepithelial Neoplasia
HPV	Human Papillomavirus
HSIL	High-Grade Squamous Intraepithelial Lesion
LSIL	Low-Grade Squamous Intraepithelial Lesion
IEC	Information, Education and Communication
PID	Pelvic Inflammatory Disease
PPE	Personal Protective Equipment
STI	Sexually Transmitted Infection
UICC	Union For International Cancer Control
UNICEF	United Nations Children's Fund
VIA	Visual Inspection with Acetic Acid
VLP	Virus-Like Particles
VVM	Vaccine Vial Monitor
WHA	World Health Assembly
WHO	World Health Organization
G	Gravida
Р	Parity
L	Living Child
А	Abortion
TL	Tubal Ligation
FTLSCS	Lower Segment Cesarian Section
FTCS	First Trimester Combined Screening
FTND/FTCS	Full Term Normal Delivery/ First Trimester Combined Screening
FTVD	Full Term Vaginal Deliveries
FTVD/FTCS	First Term Vaginal Delivery/ First Trimester Combined Screening
IUCD	Intrauterine Contraceptive Device
OCP	Oral Contraceptive Pill

Atmiya University, Rajkot, Gujarat, India

AIM AND OBJECTIVES

AIM

• Molecular markers and their comparison for early detection of cervical cancer.

OBJECTIVES

- To study the socio-demography for better understanding of prevalence and progression of cervical cancer among mass.
- To assess the role of AgNORs as a cell proliferation marker.
- To study combinations of different markers relevant to cervical cancer.
- To evaluate significance of p53/Ki67 from cervical smear sample.
- To propose economical and practical approach for cervical cancer screening test from smear sample using combination of different techniques i.e., AgNOR, p53/Ki67, HPV by PCR.