5

SUMMARY AND CONCLUSION

Cervical cancer remains a significant public health issue in Tamil Nadu, India, driven by an interplay of socio-demographic, clinical, and genetic factors. Despite being a preventable disease, many women are unaware of HPV, its role in cervical cancer, and the importance of early detection. Socio-demographic inequalities include disparities in education, economic adversity, and cultural barriers, which strongly limit the uptake of prevention measures such as vaccination and regular screening programs.

From a clinical perspective, several challenges further compound the problem. Delays in early diagnosis, limited access to affordable and quality healthcare services, and the absence of personalized treatment protocols often result in suboptimal outcomes. Addressing these clinical gaps requires a thorough understanding of the barriers that prevent effective care delivery and management.

On the genetic front, cervical cancer exhibits unique molecular characteristics in the Tamil Nadu population that are not yet fully understood. By employing advanced techniques such as whole exome sequencing, this study explores the genetic landscape of cervical cancer, identifying specific mutations and biomarkers associated with the disease. These findings are further validated through Sanger sequencing to ensure accuracy and reliability, offering deeper insights into the genetic underpinnings of the disease. Such an approach has the potential to pave the way for personalized medicine, enabling more targeted and effective treatment strategies.

This study adopts an integrated, multi-phased approach, bringing together analysis of socio-demographic variables, the clinical perspective, and genetic studies. Living with inadequately addressing these interventional factors, this strategy aspires to fill important knowledge gaps and facilitate the rectification of the health inequality sphere while promoting wider-reaching prevention and management strategies.

The results of this research will provide useful information toward the better health outcome for cervical cancer patients.

Phase I focused on examining women's awareness and understanding of cervical cancer and HPV infections. Our study found alarming knowledge gaps among participants with only a minimum college education. Only 63.1% of rural respondents and 71.3% of urban respondents could identify cervical cancer. The percentage of people who were aware of HPV was only slightly higher in rural areas (74.5%) compared to urban areas (70.38%).

Exposure to information on preventive measures is very low, especially regarding Pap tests and HPV vaccinations. Only 18.6% of the rural women and 27.23% of the urban women knew about Pap test. Similarly, the percentage of awareness of HPV vaccines was very low, indicating that only 26.75% of the rural and 19.76% of the urban women knew about the vaccines. In addition, while a large proportion of the respondents, 61.75% of rural women and 53% of urban women did not know symptoms of cervical cancer, there was a need for increased educational access and exposure.

The study indicated that while some risk factors like HPV infection, having multiple sexual partners, and having a weakened immune system were recognized, many others—such as smoking, prolonged use of oral contraceptives, and early pregnancies—were often overlooked.

These findings emphasize the need for education and awareness campaigns, community outreach, health promotion interventions, free or subsided screening of educational initiatives, social media and digital platforms—the essential role of academic initiatives and media in disseminating critical information about cervical cancer and HPV. Probably, such interventions could promote an early identification capability and enhance general preventive measures likely to lower rates of death from cervical cancers by raising alertness about cervical disease, possible symptoms, technique of prevention of the disease itself, and the relevance of routine screen tests.

Phase II of the study would have involved gaining more information on the patient journey with cervical cancer. A hospital-based study conducted at Sri Ramakrishna Hospital, Coimbatore, Tamil Nadu, in the period of 2019-2022 was undertaken. It would focus on assessing the disease from multiple standpoints such as socio-demographic profiles, clinical characteristics, and treatment methods so that it is possible to extract valuable insights into enhancing management and prevention strategies.

The median age of patients diagnosed with cervical cancer was 54.8 years. This means that a large number of the patients affected by this disease are women in their middle ages. Also, most of these patients are from rural backgrounds. This rural demographic is particularly vulnerable, as rural working women face heightened risks due to several factors, including occupational exposure to carcinogens—substances that can lead to cancer—and barriers such as limited access to healthcare facilities and lower socioeconomic status. These factors can impede timely diagnosis and treatment, contributing to poorer health outcomes.

In terms of clinical presentation, the study identified the most prevalent symptoms among cervical cancer patients, which included abdominal pain, leucorrhoea (abnormal vaginal discharge), weight loss, and post-menopausal bleeding. These symptoms often indicate advanced stages of the disease, underscoring the need for earlier detection and intervention.

The pathological analysis indicated that squamous cell carcinoma (SCC) was the most common type of cervical cancer, accounting for 77.96% of cases, followed by adenocarcinoma, which made up 15.46%. The histological diagnosis, which involves examining tissue samples under a microscope, was pivotal in determining appropriate treatment strategies tailored to individual patient needs. Such personalized approaches are crucial for improving treatment efficacy.

Regarding treatment methods, the study found that cisplatin-based combination therapy was the most frequently utilized treatment approach. This type of chemotherapy involves using cisplatin in conjunction with other drugs to enhance therapeutic outcomes. In some cases, monotherapy, which predominantly consisted

of cisplatin and radiotherapy, was also employed. These treatment modalities are guided by the histological classification and staging of the cancer, highlighting the importance of accurate diagnosis.

The study analysed survival data for 138 patients and found that combination therapy offered the highest survival probabilities, followed by chemotherapy, while radiation-only treatment showed the poorest outcomes. Despite these insights, the analysis is limited by missing data, short follow-up duration, and the absence of adjustments for confounding factors like age, comorbidities, and cancer stage. Further research with a larger cohort and longer follow-up is needed to confirm these findings.

The results emphasized the critical need for targeted interventions, particularly for rural working women, who are disproportionately affected by cervical cancer. Improving access to healthcare services is essential to ensure timely screening and treatment. Furthermore, the study suggested that exome sequencing of Squamous Cell Cervical Carcinoma samples could yield significant insights into genetic alterations associated with the disease. This genomic approach can potentially drive personalized therapeutic strategies, ultimately leading to more effective treatments tailored to the individual genetic profile of patients.

Overall, Phase II of the study not only emphasized the clinical and socio-demographic factors associated with cervical cancer in Tamil Nadu but also underscored the need for targeted strategies to enhance awareness, access, and treatment options, particularly for at-risk populations.

Phase III of the study focused on utilizing Whole Exome Sequencing (WES) to delve into the genetic complexities associated with squamous cell carcinoma (SCC), a prevalent subtype of cervical cancer. This phase aimed to shed light on the molecular underpinnings of the disease. Still, the study faced significant challenges rooted in the overall lack of awareness about cervical cancer among the target population.

The pervasive knowledge gap about cervical cancer significantly impacted the research's feasibility. Many women in the target population were either unaware of the disease or misinformed, which led to a tendency to delay in seeking medical help. As a result, by the time many patients did seek assistance, their cancer had often progressed to more advanced stages. This delay reduced the window for effective interventions and limited the availability of biopsy samples necessary for genetic analysis.

Additionally, many individuals chose non-surgical treatment options, further restricting the collection of biopsy samples. Most patients presented to medical facilities only in the final stages of their illness, which resulted in a stark limitation in accessing early-stage samples for research purposes. Consequently, the study could only include five patients (CC1-CC5) in its analysis, significantly hindering the validity and reliability of the findings.

These experiences emphasize the critical need for comprehensive public awareness campaigns and educational initiatives that aim to enhance knowledge about cervical cancer. Such efforts could promote early detection and treatment, potentially reducing the disease's morbidity and mortality rates.

Despite the limited sample size, the study provided valuable insights into the genetic basis of cervical cancer. DNA was successfully isolated from the biopsy samples of the five patients, and sequencing revealed a complex mutational landscape that included 6,658 single-nucleotide variations (SNVs). Among these variations, 2,389 were identified as nonsynonymous mutations, meaning they alter the amino acid sequence of proteins and potentially impact their function. After applying a read-depth filter to ensure data quality, 2,356 variants were retained, of which 2,174 were cross-referenced with the dbSNP database. Pathogenicity analysis using computational tools identified 37 damaging and deleterious variants for detailed functional investigation, while predictions for other variants were documented for future reference. Unlike previous studies on cervical cancer in Tamil Nadu, India, our research focused on squamous cell carcinoma with a small cohort of five cases. This limitation stemmed from late-stage diagnoses, low

awareness, and a preference for non-surgical treatments, highlighting the need for larger-scale studies to explore these findings further.

A comprehensive analysis of the mutation spectrum in cervical cancer identified G>A and C>T transitions as the most prevalent among nonsynonymous SNPs, followed by moderate occurrences of A>G and T>C transitions and less frequent transversions like A>T and T>A. The findings provide valuable insights into the genetic alterations driving tumorigenesis, offering potential avenues for targeted therapies and improved diagnostic strategies in cervical cancer.

The study focused on nonsynonymous mutations to evaluate their functional implications in squamous cell cervical cancer and compared findings with the COSMIC database. Key genes, including MUC12, TTN, AHNAK, LRP5L, and OR2T34, exhibited high mutation rates in both datasets, with 100% mutation rates in the study cohort for all analysed genes. MUC12 and TTN showed consistently high mutation frequencies, highlighting their potential relevance in cancer pathology. These results underscore the importance of specific genes in the molecular mechanisms underlying squamous cell cervical cancer.

This study used bioinformatics tools to determine the functional effect of the genetic variants discovered in squamous cell cervical cancer. It was only nonsynonymous mutations that the study focused on, and thus 37 deleterious variants were found within five samples and genes involved with critical biological pathways and disease mechanisms. The study also identified the high-priority genes, ABCC2, RDH12, ACVR2B, HEPHL1, and CHD4, because they had strong predictions for pathogenicity and relationships with processes including immune regulation, mitochondrial function, calcium signaling, and chromatin remodeling.

Variants mapped to multiple chromosomes had diverse functions, ranging from ion transport and DNA repair to visual processes. Conservation scores and pathogenicity predictions from SIFT, PolyPhen2, and CADD underscore the biological relevance of several mutations but leave many of the variants uncertain or clinically conflicting and, therefore, require further validation. This study has

shed light on genetic heterogeneity that, in turn, will allow the understanding of genotype-phenotype relationships and inform precision medicine approaches.

It had focused on the 31 mutated genes that showed 37 potential deleterious variants in SCC and analyzed these thoroughly by means of functional enrichment, pathway analysis, and the construction of protein-protein interaction network, providing tremendous insights into biological, cellular, and molecular mechanisms affected by mutations. Identified biological processes include transmembrane transport (involving genes like ABCC2 and SLC4A3), which is important in the movement of ions and molecules across cellular membranes, disruption of which may be a cause for cancer and metabolic disorders. Pathways such as blood-brain barrier transport, copper and iron ion transport, and visual perception were also enriched, suggesting broader implications for neurotoxicity, oxidative stress, and cancer progression. Cellular components analysis identified plasma membrane, voltage-gated calcium channel complexes, and endoplasmic reticulum lumen as key sites where the SCC's alterations were likely to impact cell signaling, adhesion, and protein folding. ATP hydrolysis and binding, related to molecular functions, also had a good number of enriched pathways. This underlines the involvement of ATP-dependent processes in cell functions such as DNA repair, ion transport, and chromatin remodeling, all crucial in the development of cancer. Metabolic pathways associated with fatty acid oxidation, which involves genes such as HADHA and EHHADH, are also shown, suggesting disturbances in energy production and metabolic reprogramming in cancer cells. These findings underscore the intricate role of altered cellular signalling, ion homeostasis, and metabolic processes in the progression of SCC, offering potential targets for future therapeutic strategies.

Constructing a protein-protein interaction (PPI) network for the 37 variants associated with 31 mutated genes identified in squamous cell cervical cancer (SCC) revealed significant insights into the functional relationships among these genes. The PPI network had 452 nodes and their interacting proteins that were connected by 509 edges. The network of protein-protein interaction was generated using 20 mutated genes out of 31 mutated genes, that were instrumental in

revealing some key proteins engaged in common biological processes and pathways important for SCC progression. Moreover, PPI network integration with Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways showed significant enrichment in several pathways, such as Hippo signalling, TGF-beta signalling, Adherens junction, Hepatitis C, and Tight junction pathways. Hippo signalling, involved in regulating cell growth and apoptosis, has the highest significance and is potentially linked to uncontrolled cell proliferation and tumorigenesis. The TGF-beta signalling pathway, which plays a role in cell differentiation and immune modulation, was also significantly enriched, suggesting its role in immune evasion and metastasis. Mutations in the Adherens junction pathway, which is important for cell-cell adhesion, indicated tissue integrity disruption and invasive behavior. The Hepatitis C pathway and Tight junction pathways indicated the role of viral infection and epithelial barrier disruption in cancer progression. These findings underscore the complex molecular mechanisms underlying SCC and thus suggest valuable targets for future therapeutic interventions.

This study focused on squamous cell carcinoma of the cervix in patients aged 50–55 years, using UALCAN database data for comparison due to the unavailability of normal sample sequencing from the study. Gene expression was compared across three conditions: Normal vs. Primary Tumor, Squamous Cell vs. Normal, and Normal vs. Age (41–60 years). Of the 31 mutated genes identified, 20 were found to be hub genes, significantly interacting with other proteins and disrupting key biological processes. The UALCAN platform validated these findings. Notably, RDH12, RFC4, and AIM1L showed highly significant changes in expression, suggesting their potential as diagnostic or prognostic biomarkers. ATP2B2 and ABCC2 demonstrated moderate significance, implicating them in tumor-related and age-related processes. Genes like KCNJ1 showed moderate importance in specific comparisons, while others like TUBA8, ACSF3, and CHD4 showed no significant changes. This study identifies RDH12, RFC4, AIM1L, ATP2B2, and ABCC2 as promising biomarkers for early detection, prognosis, and

therapeutic targeting in squamous cell cervical cancer, though further functional studies and clinical validation are needed to confirm their roles.

In this study, we utilized exome sequencing to identify novel missense variants in squamous cell cervical cancer, focusing on the genome's protein-coding regions to uncover genetic variations relevant to cancer development. Among the 2,389 variants detected, 182 were nonsynonymous and unreported in the dbSNP database, suggesting the presence of novel mutations that could contribute to squamous cell cervical cancer pathogenesis. After prioritizing variants based on their potential impact, eight nonsynonymous mutations across seven genes—POM121C, PRICKLE1, SRPX2, KIF1B, BRAT1, ALOX12B, and GLIS3—were selected for validation using Sanger sequencing. These mutations, on different chromosomes, affect amino acid substitutions potentially leading to structural and functional changes in proteins. In fact, a recurrent mutation in GLIS3 (p.R747C) was noted in two samples and therefore may play a potential role in the disease. All the mutations were novel without any dbSNP IDs and therefore are unique.

Various bioinformatics tools were utilized to carry out mutational analysis of novel nonsynonymous variants in squamous cell cervical cancer to evaluate the influence of amino acid substitutions on the function of the protein. In this regard, most mutations were determined to be tolerated by the SIFT and other tools, implying little disruption of the protein's functions, including those of POM121C, PRICKLE1, and GLIS3. However, ALOX12B mutation was consistently predicted to be deleterious. In fact, all three predictions made by SIFT, PolyPhen-2, and MutationTaster suggest a high potential impact on the protein structure and function. The highest CADD score for ALOX12B was 23.9, which reinforced its likely pathogenicity, and the conservation scores also suggested its critical biological role. Mutations in PRICKLE1 and GLIS3 showed moderate to high potential pathogenicity. The mutations within BRAT1 and SRPX2 were not considered to affect the protein-coding sequence very much.

Novel genetic variants associated with squamous cell cervical cancer were validated using Sanger sequencing. Primer3Plus was used to design specific

primers so that they could be designed to be used precisely in amplifying the target regions of the gene. The designed primers targeting genes like POM121C, PRICKLE1, SRPX2, KIF1B, BRAT1, ALOX12B, and GLIS3 produced amplicon sizes ranging from 170 to 247 base pairs, thus efficiently amplifying by PCR. In-silico PCR validation through the UCSC Genome Browser tool proved specificity for primers such that these primers will hybridize to target sites exclusively and exclude any amplification that occurs away from the target.

Although the amount of DNA was limited, the PCR amplification, including touchdown PCR, was successful for three of the six variants. Partial success in amplification proved the specificity of the primers and the reliability of the PCR protocol for detecting these variants. The validation process, therefore, allows the confirmation of the presence and accuracy of these variants, thus potentially shedding more light on the genetic basis for squamous cell cervical cancer as well as guiding potential strategies for diagnosis and therapy.

Sanger sequencing confirmed novel variants in squamous cell cervical cancer, including several nonsynonymous mutations that could have functional significance. In the POM121C gene, a c.A949C mutation was identified that would change threonine to proline at position 317 and disrupt nuclear pore complex function. The mutation of c.G1118A changes arginine into glutamine at position 373 of the PRICKLE1, where it is reported to impact on planar cell polarity signalling responsible for cell migration and tissue formation. The replacement of arginine by cysteine at position 747 through GLIS3 c.C2239T has potential effects on the transcriptional regulation and on the insulin signal; both are implied for metabolic disorder diseases. Other mutations included SRPX2 (p.A124V), BRAT1 (p.V171I), KIF1B (p.L1163V), and ALOX12B (p.Y687C), which may be implicated in cell migration, DNA repair, and inflammation, which might drive SCC progression. DNA-quantity limitations impede full validation, but they point to these mutations as revealing critical molecular mechanisms in the etiology of pathogenesis of SCC and being potential biomarkers or therapeutic targets. The validation of the identification by Sanger sequencing provides for insights into personally tailored

treatment protocols designed by the research results for SCC, with early detection and subsequently improved patient results.

The study establishes the need to take public health initiatives to increase awareness about the cervical cancer prevention gap, specifically in rural setups. Improved understanding of preventive methods such as vaccinations against HPV, as well as regular screening could be facilitated early detection, consequently reducing mortality caused by cervical cancers. Clinically, the present study established better access to care and more individually tailored treatment options that could improve patients' outcomes. Although sample sizes are small, genomic analyses open the door to targeted therapies and precision medicine in SCC, based on the genetic mutations driving this disease. The results also underscore the need for further validation of these mutations and their functional significance and exploration of novel therapeutic avenues.

Recommendations for future studies

The findings of this study have paved the way for numerous opportunities for future research. A few of these recommendations are outlined below.

- Validate findings using additional patient cohorts and experimental models.
- Develop biomarkers for early detection and monitoring of cervical cancer.
- Explore potential therapeutic applications of identified genetic variants.
- To develop personalized treatment modalities based on genetic profiling
- To construct a risk prediction model using machine learning approaches