Chapter

Cytologic Monitoring, Management of Cervical Cancer, and Control of Human Papillomavirus

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Abstract

Cervical cancer is the second most common cause of cancer-related death among women that is caused by Human Papillomavirus, a double-stranded virus that leads to cellular alterations in the cervical squamocolumnar junction. Most HPV infections are cleared by the host immune system, while very low cases progress to invasive carcinoma due to persistent infection and other contributory risk factors. Several screening techniques have been devised over the years to detect Human Papillomavirus at an early stage, the most common being the Pap smear test, which is capable of detecting benign cellular changes and also squamous intraepithelial neoplasias. Other important techniques involve visual inspection with acetic acid (VIA), colposcopy, and HPV DNA testing. In addition, recent advances have led to the development of new techniques such as biosensor and bioreceptor technology and loop-mediated isothermal amplification (LAMP). Several methods have been in place to prevent the increased incidence of cervical cancer. Among these is the development of Prophylactic HPV vaccines, which elicit a humoral immune response against about 15 HPV genotypes but have the limitation of not curing an established cancer. Several trials are underway on developing a therapeutic vaccine that will be effective in curing cervical cancer.

Keywords: cervical cancer screening, cervical cancer management, HPV vaccine, HPV prevention, cervical cancer control

1. Introduction

Cervical cancer is the second-leading cause of global gynecological cancer-related deaths in women [1]. Human papillomavirus (HPV), a double-stranded DNA tumor virus, is the major cause of cervical cancer. Even though a strong immune system can normally fight off HPV infections, chronic infections with high-risk (HR) HPVs lead to cervical cancer [2]. However, in recent years, cancer patient assessment methods have changed from actual decision-making, based on the clinic-pathological characteristics of the patient, to biomarker-driven treatment plans, focusing on genetics and specific targeted medicines. Furthermore, the molecular alterations that

occur during the development of cervical carcinogenesis have been examined using high-throughput technologies and gene expression profiling based on microarrays. The found genes with abnormal expression may aid in the diagnosis of cancer, the subtyping of cancer, and the design of chemotherapy [3, 4]. Also worthy of mentioning is the Papanicolaou (Pap) test that has been the primary option for gynecological screening for almost 60 years due to its ease of use, low cost, and lack of major side effects, which has likely contributed to its success in recent years in lowering overall incidence and mortality in the world [5]. Despite this, recent screening studies from several nations have showed the advantages of HPV testing over the Pap test for detecting cervical cancer, as HPV-based screening was 70% more efficient in reducing the incidence of invasive cervical carcinomas than cytology-based screening [6]. This study's goal is to highlight the importance of cytologic surveillance, cervical cancer treatment, and HPV prevention.

2. Current cervical cancer screening initiatives

HPV viral gene integration in the host genome promotes the expression of E6 and E7, with subsequent deadly genetic changes leading to neoplastic transformation. HPV-infected cells can advance from normal to preinvasive to invasive cancer in about 10 years or more [7]. However, this lengthy timeframe allows for the detection of early preinvasive neoplastic lesions and the prevention of cancer development through screening. Cervical cancer screening can be done in three ways, namely, VIA, Pap smear, and HPV DNA testing.

2.1 Pap test

George Papanicolaou established the Pap smear test in the early 1940s. A qualified cytotechnologist or pathologist will collect a liquid biopsy from the squamocolumnar junction and smear it on a glass slide for microscope examination. With a sensitivity of 70–80%, it is the earliest screening technique that became widely used in the 1960s [8]. The adequacy of the specimen is absolutely essential for the accuracy of the Pap test [9]. The cellular changes are reported in accordance with the "The Bethesda System for Reporting Cervical Cytology," which establishes consistent and reproducible criteria for detecting preinvasive and invasive cancer [10]. The most significant benefit of a traditional Pap smear technique is its cheapness and affordability [11]. Although the Pap test is successful in cities, it has failed in rural areas because it is an extremely competent personnel-intensive scheme. Key drawbacks include the low sensitivity to detect early preinvasive lesions, the complex logistical and care network necessary for executing quality control, and the succeeding relevant medical management (like colposcopy, histology, and endocervical curettage) of women who screen positive [12]. As a result, there is an urgent need to develop a reliable, highly sensitive, and cost-effective cervical cancer screening technique.

2.2 Visual inspection with acetic acid

Visual examination of the cervix while using 3% acetic acid offers an alternate, economically advantageous screening method [7]. The cervical dysplastic regions are highlighted by the application of acetic acid, which causes instantaneous color changes that are evident to the unaided eye. Any alteration in hue is considered a sign

of cervical cancer that has not yet spread. Since the 1990s, visual inspection with acetic acid (VIA) has been employed, particularly in rural and remote areas. It is a straightforward, user-friendly method. The VIA technique has the distinct advantage that care providers can fulfill their duties without the need for sophisticated infrastructure or equipment [13]. According to the findings of Arbyn et al., VIA has a specificity of 81–89% as well as a sensitivity of 73–85% for identifying high-grade cervical preinvasive lesions [14]. Nevertheless, the VIA technique has limitations such as provider dependence and subjectivity, as well as lower sensitivity for women over the age of 40. This necessitates immediate action to develop an alternative approach with greater sensitivity, specificity, and noninvasiveness.

2.3 HPV DNA testing

The only circulating tumor DNA tests that have received clinical approval to date are quantitative PCR-based tests; however, other studies have shown that digital PCR and sequencing are preferred due to their enhanced capacity to identify uncommon variations. By finding variations without knowing their precise sequence, sequencing-based approaches dramatically boost flexibility. Due to the high expense and complexity of NGS, especially those approaches used for low-abundance mutations, sequencing studies must frequently concentrate on narrower regions of the genome where mutations are likely to occur [15]. Despite this, targeted assays have no sensitivity to mutations that they are not targeted to study; NGS approaches have a distinct benefit in that they discover variants with little to no prior information of the mutation's existence or position. This benefit is less pronounced when taking into account a number of prospective uses for ctDNA, such as companion diagnostics, where the pertinent mutations and related cautions are well-known and scarce [16]. In these circumstances, focused assays may outperform sequencing, especially if they can be carried out quickly, easily, and affordably. This is corroborated by the fact that digital PCR (dPCR), despite being virtually exclusively utilized for singleplex tests, is currently quite popular in preclinical research of ctDNA [17]. However, The Cobas 4800 HPV/DNA automated PCR equipment was effectively used in previous research from Sri Lanka to show that HPV-DNA testing can be used as a primary screening tool in low-resource settings [18]. Rapid molecular point-of-care assays for identifying HPV DNA have been developed recently [19]. While the HPV-DNA test is highly sensitive, it is less specific. Thus, it may be able to identify clinically inconsequential infections in women who are at risk of developing cervical cancer. Therefore, in order for these procedures to be truly effective in a diagnostic set, they ought not to be time-consuming and expensive. Also, they need to be highly specific.

2.4 Biosensor and aptamer technology

The most crucial aspect of a biosensor is likely selectivity. The capacity of a bioreceptor to identify a particular analyte in a sample that contains various admixtures and impurities is known as selectivity. The relationship between an antigen and an antibody provides the best illustration of selectivity. Traditionally, antibodies function as bioreceptors and are immobilized on the transducer's surface. The antigen-containing solution is then exposed to the transducer, where antibodies only interact with the antigens. The solution is typically a buffer including salts. Selectivity is the primary factor to be taken into account when selecting bioreceptors for a biosensor [20]. Furthermore, reproducibility, or the biosensor's capacity to

produce the same results under equal experimental conditions, is another quality of a successful biosensor. The transducer and electronics in a biosensor are precise and accurate, which defines reproducibility. When a sample is tested more than once, accuracy refers to the sensor's capability to offer a mean value that is close to the true value, while precision refers to the sensor's ability to produce identical findings every time. The inference made on a biosensor's response is highly reliable and robust when the signals are reproducible. A biosensor's sensitivity is one of its key characteristics. Besides this, a biosensor's limit of detection (LOD), or sensitivity, is determined by the smallest amount of analyte that it can detect. Additionally, the stability of a biosensing system determines how susceptible it is to environmental disturbances both inside and outside of it. A biosensor under measurement may experience a drift in its output signals as a result of these disruptions. This could skew the concentration being measured and compromise the biosensor's precision and accuracy. In applications where a biosensor needs lengthy incubation periods or ongoing monitoring, stability is the most important component. The reaction of electronics and transducers may be temperature-sensitive, which could affect a biosensor's stability. To achieve a steady response from the sensor, proper tuning of the electronics is necessary as shown in **Figure 1**. Likewise, linearity is the property that demonstrates the precision of the measured response to a straight line for a measurement set with various analyte concentrations. It is mathematically represented as y = mc, where c is the analyte concentration, y is the output signal, and m is the sensitivity of the biosensor. The

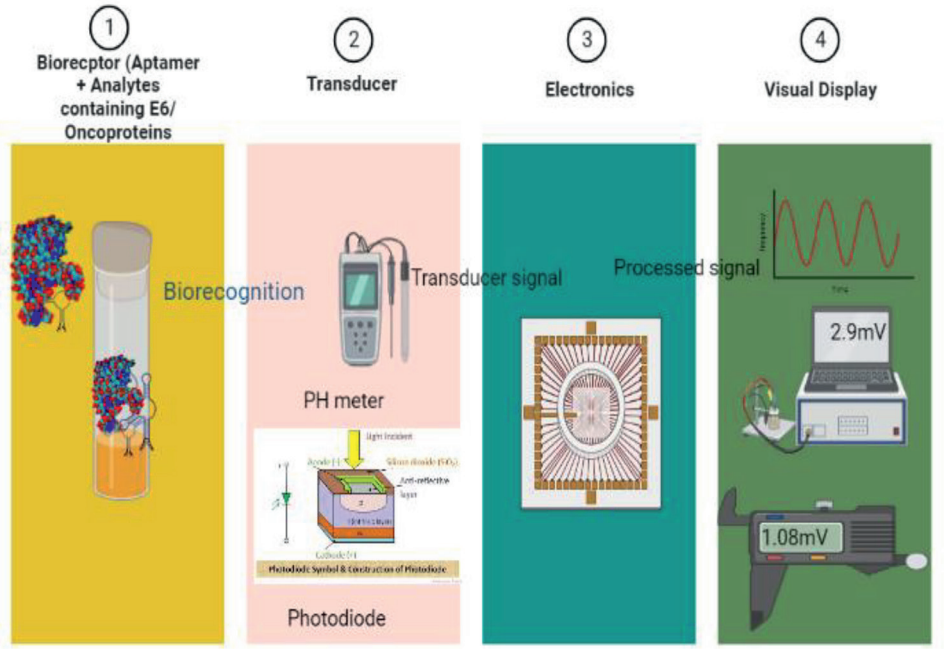

Figure 1.
An illustration of a biosensor in diagrammatic form. 1. The analyte E6/7 oncoproteins are particularly recognized by the aptamer molecule. Upon the engagement of the bioreceptor with the analyte, the process of bio-recognition occurs, during which a signal is produced (in the form of light or pH). 2. The transducer converts the bio-recognition event into a measurable signal. 3. The transduced signal is processed by the electronics and made ready for display. It is made up of intricate electrical circuitry that carries out signal conditioning tasks like signal amplification as well as conversion from analog to digital form. 4. The display produces user-friendly numerical results.

resolution of the biosensor and the range of analyte concentrations under test can both affect the biosensor's linearity. The smallest change in an analyte's concentration necessary to cause a change in the biosensor's response is known as the resolution of the biosensor. A strong resolution may be necessary depending on the application since biosensor applications call for the detection of analyte concentrations over a large operating range [20]. Generally, there are different ways that biosensors can be used to enhance the quality of life. This area includes their application for a variety of purposes, including environmental monitoring, disease detection, food safety, and drug development. The detection of biomolecules that are either disease indicators or medication targets is one of the main uses for biosensors. For instance, clinical tools for the detection of protein cancer biomarkers can be developed using electrochemical biosensing techniques [21]. Yet these methods have the ability to use aptamers to detect these biomolecules [22]. Aptamers are easily synthesized and available for purchase once they have been created. Aptamers not only have a high affinity and specificity for their target while undergoing a conformational change, but they can also maintain the majority of their functionality even after going through several regeneration steps [23]. Moreover, they are easily adaptable to other functional groups, allowing for a wider range of applications [24]. Electrochemical biosensors have always been a hot topic in research because of their ease of use, low cost, high sensitivity, quick turnaround, and resilient nature. They also only need basic apparatus. There are numerous studies in the literature on impedimetric aptasensors that take advantage of the aptamers' conformational change capabilities as well as other elements that alter the system's impedance [25].

2.5 Loop-mediated isothermal amplification

Digital PCR and NGS have often been preferred for ctDNA studies. However, LAMP could be used to replace these approaches because it has proven to be inexpensive, efficient, and highly specific, in addition to having reasonable sensitivity. Meanwhile, the method begins with cell-free DNA extraction and analysis as described in earlier studies [26]. As the HPV ctDNA can be amplified without the DNA extraction stage, therefore this step can be avoided. Even though PCR was employed to amplify the HPV ctDNA genes disclosed in the listed papers, LAMP can be used as its substitute. Milan et al. recommended the use of an electrochemical LAMP-based test to identify HPV16/18 infection in cervical samples. The LAMP reaction was performed in the aforementioned study using a premixed mixture of WarmStart LAMP 2 Master Mix, DIG-dUTP, 100 ng of DNA template, and LAMP primers. They performed the process at 66°C for 40 min, following a polymerase inactivation at 80°C for 5 min. Subsequently, Agarose gel electrophoresis was used to detect the LAMP reaction on 1.5% agarose gel stained with GelRed nucleic acid. Nevertheless, based on the identification of the amplicons, there are numerous LAMP models available. Anton et al., on the other hand, preferred to combine electrochemical measurements with a geomagnetic technique, using streptavidin magnetic beads to assess DIG-labeled LAMP amplicons in a working electrode [27]. In yet another work by Mudhigeti et al. the LAMP mixture with sample-containing tubes was placed in a water bath that had been preheated to 63°C for 60 minutes [28]. Following a brief exposure to blue light, 1 l of SYBR Green I was added to each tube, gently mixed, and checked for bright green fluorescence. Bright green fluorescence denotes the presence of the target (HPV) or successful amplification, while no fluorescence denotes the target's absence. Likewise, Yu et al. used digital LAMP assays on a self-digitization

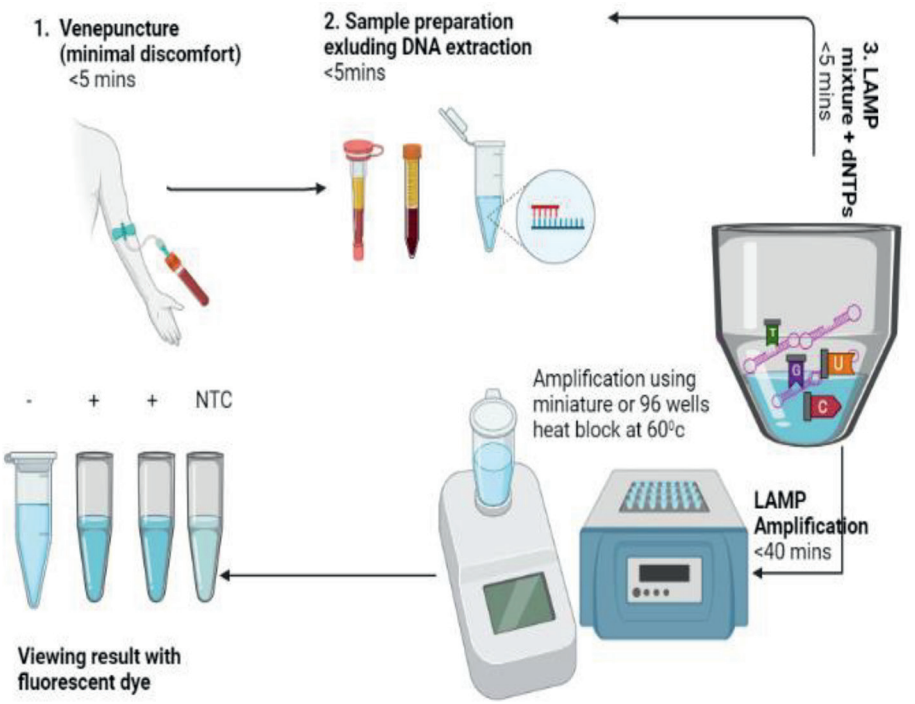

Figure 2.
Employing LAMP in the diagnosis of HPV ctDNA Summary of the procedure. About 5mls of blood will be collected. 2. Sample will be separated, it can either be extracted or amplified directly. 3. LAMP mixture will be added to an Eppendorf tube containing the sample. 4. At a single temperature of about 600c the HPV ctDNA will be amplified 5. The positive sample can be viewed with the naked eye either through turbidity of color intensity using fluorescent dye.

chip to detect 14 high-risk human papillomaviruses [29]. All of these studies used LAMP to detect HPV DNA in the cervical sample, but so far, no report has yet been published on the detection of HPV ctDNA using LAMP. The summary of this procedure is depicted in **Figure 2**.

3. Cervical cancer control

The global strategy developed by WHO for cervical cancer elimination has proposed three essential ways to fight HPV; 90% of girls fully vaccinated with the HPV vaccine by the age of 15 years, high-performance cervical screening of 70% of women at 35 and 45 years of age, and 90% treatment of infected women with confirmed cervical cancer. These targets must be met by 2030 for countries to be on the path toward cervical cancer elimination [30]. To achieve this goal, WHO with its partners is developing a global strategy toward the elimination of cervical cancer. Given the substantial global burden of cervical cancer, the increasing inequalities, and opportunities for effective and cost-effective primary and secondary prevention, the WHO Director-General made a global call in May 2018 for action toward eliminating cervical cancer as a public health problem [31]. A comprehensive national or sub-national surveillance system for elimination would encompass long-term surveillance of both processes and outcomes across the three core activities of vaccination, screening, and

treatment as outlined in the WHO Global Strategy toward eliminating cervical cancer as a public health problem [32].

3.1 HPV vaccine

Large international randomized control clinical trials have proved that HPV vaccines are safe and highly effective against vaccine-type infection and cervical precancerous lesions in women (with vaccine efficacy ≥93%). These vaccines target high-risk HPV types that cause about 70% (bivalent and quadrivalent vaccines: HPV types 16 and 18) and 90% (9-valent vaccine: HPV types 16, 18, 31, 33, 45, 52, and 58) of cervical cancers [31]. Large-scale international randomized control clinical trials have demonstrated the safety and great efficacy of HPV vaccinations in preventing vaccine-type infections and cervical precancerous lesions in females (vaccine efficacy of 93%). These vaccines target high-risk HPV types that account for 70% (HPV types 16 and 18 in the bivalent and quadrivalent vaccines) and 90% (HPV types 16, 18, 31, 33, 45, 52, and 58 in the 9-valent vaccine) of cervical malignancies, respectively [31]. The currently available HPV vaccines were created using the virus-like particles (VLPs) of the primary papillomavirus capsid protein L1, which are essentially empty viral shells made up of one or more different polymeric shells or capsid proteins [33]. All three HPV vaccines were developed based on L1 VLP [33]. VLPs are not infectious or carcinogenic because they lack a viral genome that is capable of eliciting a humoral immune response with significant and persistent neutralizing antibodies [33].

Early genes are thus targeted during the viral life cycle and aid in regulating the emergence of HPV-related premalignant and malignant abrasions. Because E6 and E7 proteins are continually produced and connected to the malignant development of HPV-linked malignancies, they provide two promising targets for therapeutic HPV vaccines. Other proteins E1 (viral helicase) and E2 are useful for focusing on early viral abrasions, and these proteins are expressed at a faster rate than E6 and E7 before viral genome incorporation at early stages [34].

The three HPV prophylactic vaccines currently available are, respectively, Gardasil®4, a quadrivalent vaccine available in 2006 [35]; Cervarix™, a bivalent vaccine available in 2007 [36]; and Gardasil®9, a nonavalent vaccine available in 2014 [37]. Both the quadrivalent and bivalent vaccines show varying degrees of protection against oncogenic HPV types not included in the vaccines [38]. Data from clinical trials showed that these three vaccines all achieved good preventive effects on people infected by HPV from different regions, of different races, and in different age groups. Moreover, the majority of trial data for several vaccines provided vaccine titers data against advanced cervical cancer precursors (CIN 2, CIN 3, and adenocarcinoma *in situ*) [33].

To eliminate tumors or lesions, the vaccine unleashes T-cell immunity by directly targeting HPV antigens exposed around infected and malignant cells [34]. These proteins can be good targets for therapeutic vaccines since vaccines can target cytotoxic T lymphocytes (CTLs) and cancer-specific T cell type 1 that can destroy cancer and infected cells [34].

3.2 Health education

The World Health Organization (WHO), as well as numerous studies and clinical registries on cancer survival, claims that developed nations with well-managed cervical cancer programs have seen a significant decline in cervical cancer incidence and

mortality, but developing nations with low vaccination rates and weak cervical cancer screening programs have not [30]. For the past few decades, mortality and incidence rates have decreased in the majority of the world's regions. The decreases are attributed to elements that are associated with either rising socioeconomic averages or a declining risk of persistent infection with high-risk HPV as a result of advances in genital cleanliness, decreased parity, and a declining prevalence of sexually transmitted diseases [39]. Even though there are currently efforts to expand HPV vaccination as a means to prevent cervical cancer, in most African countries, it is still limited to research settings or poorly organized and with a slow rollout. In some countries, it is unavailable due to inadequate infrastructure, finances, and availability of healthcare workers. There are few countries that offer HPV vaccination through subsidized national immunization programs [40].

Many studies have suggested that low levels of public health education and knowledge of the disease, inadequate and inaccessibility of cervical cancer screening services, cultural beliefs, and perceived susceptibility contribute to the low cervical cancer screening rates [41, 42]. Lack of knowledge and awareness has been identified as one of the main reasons associated with low cervical cancer screening. Nonadherence to cervical cancer screening has been associated with a knowledge deficit [40]. Women living in rural settlements are mostly of low socioeconomic status, and this has been shown to be associated with a higher risk of cervical cancer, poor health knowledge, and poor access to health services [43].

Interventions utilizing peer health educators and culturally tailored methods were the most effective in improving screening uptake. Innovative approaches such as self-collected HPV testing can also be employed as they demonstrate the potential to influence changes in the uptake of screening [40]. Olubodun and coworkers reported that there is a need for increased cervical cancer awareness and promotion campaigns. Women's partners should also be targeted for health education. Improving access to cervical cancer prevention services is also crucial among this underserved population. Health education interventions increased knowledge and awareness of cervical cancer and boosted cancer screening. Therefore, a comprehensive approach to cervical cancer prevention and control should therefore include health education interventions [43].

4. Conclusion

Several efforts have been made to curtail the surge of cervical cancer incidence worldwide including early screening, vaccination, health education, HPV DNA testing, phylogenetic studies, and new molecular diagnostic techniques such as loop-mediated isothermal amplification and DNA-Aptamer-based biosensors. However, most of the new techniques are impracticable in resource-limited settings. Due to this, several efforts have been placed including health education and development of therapeutic HPV vaccines with ongoing clinical trials that lasted almost 20 years to date. It is expected that these vaccines will be able to cure or cause the regression of established cervical cancer, in addition to preventing recurrence. Therefore, hands on deck are needed to prevent the increased incidence of cervical cancer.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

VIA	visual inspection with acetic acid
LAMP	loop-mediated isothermal amplification
HPV	human papillomavirus
Pap	papanicolaou test
PCR	polymerase chain reaction
NGS	next-generation sequencing
LOD	limit of detection
VLPs	virus-like particles
CIN	cervical cancer intraepithelial neoplasia

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