Biomarkers for the Early Detection of Cervical Cancer

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10.1 Introduction

Cancer is a genetic disease caused by a multistep process involving activation of oncogenes, loss of function of tumor suppressor genes, and alteration of modifier genes, for instance, genes involved in DNA repair and genomic stability. Cervical cancer is the fourth most common cancer affecting women worldwide [1]. India alone accounts for one-quarter of the worldwide cervical cancer burden [2]. In last decade, significant advancement in understanding the causes of cervical cancer and identification of biomarkers have been achieved for its early diagnosis, prevention, and treatment. The human papillomavirus (HPV) is considered as one of the major etiological factors for cervical cancer along with other factors [3]. HPVs are epitheliotropic viruses and possess a small, circular double-stranded DNA. These viruses cause a variety of benign epithelial lesions such as warts or condylomata acuminata and neoplasia of the lower genital tract in humans [4, 5]. Presently, more than 120 HPV types have been described, of which at least 40 are associated with anogenital lesions, 15 of these have been classified as high risk (HR-HPV) (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and 12 as low risk (LR-HPV) (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89) [6, 7]. Among these, infection of HPV types 16 and 18 [8, 9] is found to be the most oncogenic type that leads to the development of cervical cancer, while the infection of low-risk HPV types 6 and 11 is mainly associated with the development of benign lesions and genital warts. This causal relationship between HR-HPV infection and cervical cancer has been proved from various epidemiological and experimental studies [10, 11]. These HR-HPVs have been detected in almost 100% of cervical squamous cell carcinomas (SCCs) [7, 12] and 94–100% of cervical adenocarcinoma and adeno-squamous carcinoma [13, 14]. In India, cancer of the uterine cervix is the major cancer harboring HPV in almost 98%, and more than 90% of them are infected with specifically HPV type 16 [15].

PCR detection of HPV DNA by L1 consensus primers and typing by HPV type-specific primers should be performed to detect the presence of high-risk HPVs. Most widely used MY9 and MY11 consensus primers are capable of detecting about 27 HPV types which include all 15 high-risk HPVs (HPV 16, 18, 31, 35, 39, 45, etc.) and 6 low-risk HPVs [16].

Cytology-based screening for cervical cancer has shown to reduce the incidence and mortality rate since the last few decades. Addition of HR-HPV DNA screening in cervical cancer

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screening has improved the sensitivity, but it is also associated with low specificity. Thus, other markers are needed to triage test for maintaining acceptable sensitivity and specificity. Various protein biomarkers for the detection of cervical cancer have been identified. Most of them are involved in cell cycle regulation, signal transduction, DNA replication, and cellular proliferation [17–19]. The altered expression of these proteins is a consequence of the binding of the high-risk HPV E6 and E7 oncogenes to host regulatory proteins, resulting in the degradation of the p53 tumor suppressor gene product, ultimately leading to dysregulation of the cell cycle. The evidence regarding the use of these biomarkers have shown their ability to triage mildly abnormal and indeterminate cytology cases, with those found to have elevated levels of biomarkers staining more likely to represent cases with true high-grade cervical cancer.

10.2 Biomarker Principles and Cervical Cancer

The biomarkers help in improving the management of cervical cancer at every point from screening and prognosis to assessment of treatment response. A significant advancement in understanding the causes of cervical cancer and identification of many different biomarkers have been achieved for its early diagnosis, prevention, and treatment.

Cytology still forms the mainstay of screening programs in most parts of the world, especially the USA. It is used either as a stand-alone test or as a co-test with HPV testing [20–23]. Presently with evidence building up, some European countries are using HPV as a primary screening modality and triaging positive results with cytology [24].

New biomarkers would be potentially useful in triaging women with primary cytology or HPV testing positive. The hallmark of cervical screening is to identify lesions which are most likely to progress to cancer. The biomarkers have a crucial role to play as they can identify significant changes that occur during any of the important steps of the functional progression model. There are two main groups of markers, viral or cellular markers (Table 10.1). Table 10.2 lists the commercially available viral and cellular biomarkers.

Table 10.1 Markers for cervical cancer screening

Viral markers	Cellular markers
HPV DNA detection	$p16^{ink4a}$
E6/E7 mRNA	Proliferation—Ki67, MCM2, Top2a
Viral integration	Chromosomal instability—3q, 5p
Viral and host methylation	

10.3 Viral Biomarkers

10.3.1 HPV DNA Detection and Genotyping

With time the importance of molecular basis of HPV infection and HPV genotyping has been recognized. Studies have shown that the sensitivity of high-risk HPV DNA testing is more sensitive than cytology [25, 26]. The National Comprehensive Cancer Network (NCCN) recommends co-testing with Pap and HPV every 5 years in women between 30 and 65 years as the preferred option for cervical cancer screening [27].

The ASCUS-LSIL Triage Study (ALTS) trial which was conducted by the National Cancer Institute concluded that doing reflex HPV testing in cytology reports of ASCUS identified 96% of CIN3+ cases, and only 56% of cases were referred for colposcopy [28].

Follow-up is mandatory for women after treatment for CIN, and HPV testing is a good option for doing so due to its high sensitivity in picking up recurrences [29, 30]. The prediction for recurrences can be further improved if genotyping for the type of HV infection is also done [31]. But the specificity for the test is low which can be improved by triaging positive results with cytology or methylation markers [32].

Table 10.2 Commercially available assays targeting viral as well as cellular biomarkers

Available assays approved	Manufacturer	Target	HPV genotypes	Genotyping	FDA
Viral assay HPV DNA					
COBAS 4800	Roche	L1 DNA	13 HR HPV and HPV66	16 and 18	Yes
Cervista	Hologic	L1 DNA	13 HR HPV and HPV66	16 and 18	Yes
Hybrid capture 2	QIAGEN	Full genome	13 HR HPV and HPV66	No	Yes
Amplicor	Roche	L1 DNA	13 HR HPV	No	No
careHPV	QIAGEN	L1 DNA	13 HR HPV and HPV66	No	No
Digene HPV eHC	QIAGEN	Full genome	13 HR HPV, HPV66 and 82	No	No
EIA kit HPV GP HR	Diassay	L1 DNA	13 HR HPV and HPV66	No	No
INFINITI HPV-HR QUAD	AutoGenomics	E1 DNA	13 HR HPV and HPV66	No	No
RT HPV	Abbott	L1 DNA	13 HR HPV and HPV66	16 and 18	No
Digene HPV eHC 16 18/45	QIAGEN	Full genome	13 HR HPV, HPV66 and 82	16, 18, and 45	No
CLART	Genomica	L1 DNA	13 HR HPV and 22 no HR	Yes	No
InfinitiTM	Genomica	L1 DNA	13 HR HPV and 12 no HR	Yes	No
INNO-LiPA	Innogenetics	L1 DNA	13 HR HPV and 15 no HR	Yes	No
Linear array	Roche	L1 DNA	13 HR HPV and 24 no HR	Yes	No
Multiplex HPV genotyping	Multimetrix	L1 DNA	13 HR HPV and 11 no HR	Yes	No
PapilloCheck	Greiner bio-one	E1 DNA	13 HR HPV and 11 no HR	Yes	No
HPV RNA					
Aptima	Gen-probe	E6/E7 mRNA	13 HR HPV and HPV66	No	Yes
NucliSens EasyQ	Biomerieux	E6/E7 mRNA	5 HR HPV	16, 18, 31, 33, and 45	No
OncoTect	IncellDx	E6/E7 mRNA	13 HR HPV	Yes	No
PreTect proofer	Norchip	E6/E7 mRNA	5 HR HPV	16, 18, 31, 33, and 45	No
HPV proteins					
Cytoactiv	Cytoimmun diagnostics	L1	All known HPVs	No	No
OncoE6	Arbor Vita	E6	3 HR HPV	16, 18, and 45	No
Cellular assay					
CINtec	Mtm laboratories	p16ink4a			No
CINtec plus	Mtm laboratories	p16ink4a/ K1-67			No
Ki-67 (MIB1)	DakoCytomation	Ki-67			No
ProEx C	Becton Dickinson	TOP2A/ MCM2			No

10.3.2 HPV E6/E7 mRNA

As established HPV DNA testing has an important role in cervical cancer prevention, other biomarkers with higher specificity and prognostic value need to be used to identify patients who are at higher risk of this disease. There are evidences which suggest that HPV messenger RNA transcripts' detection proves to be a more specific method for diagnosing clinically important infection than detection of viral DNA. It has been found that HPV E6/E7 mRNA testing for high-risk types correlates better with the severity of the lesions as compared to HPV DNA testing and is considered as a potential marker for the identification of women who are at high risk of contracting cervical cancer [33]. Various studies supported the above finding that the detection of E6/E7 mRNA expression is much helpful in predicting the risk of cervical cancer than HPV DNA testing [34] as mRNA expression profile shows better correlation with the severity of the lesions. The persistent and regressive infections cannot be distinguished by HPV DNA detection methods. Hence, such methods are not specific enough to identify patients at risk of cervical cancer [35].

The oncogenic potential of the HPV early genes E6 and E7 is well known. It is widely accepted that HPV can cause cancer only if there is persistent infection and a cellular environment which allows high-level expression of viral E6 and E7 genes. The E6 and E7 proteins are essential for the replication of the virus and are expressed during the productive normal life cycle, where their regulation is under tight control. When this regulation is disrupted and E6 and E7 are overexpressed, they can evade normal tumor suppressive function and cell cycling [36]. This may lead to a disturbance in cell cycle control and a deficiency in DNA repair, causing genomic instability and an elevated risk of malignant transformation [37]. Thus, targeting E6/E7 mRNA may lead to more trusted outcomes than detecting the presence of viral DNA.

10.3.3 HPV Viral Load and Integration

Several studies suggest that there exists a close link between HPV viral copy number and integration of viral genome into the host cell which increases the risk for progression to invasive cancer [38]. The grade of the lesion is directly linked to the HPV viral, and a much higher number have been found in high-grade lesions. Integration of the viral DNA to host cell genome is yet another biomarker as persistent HPV infection leads to integration of viral DNA into the host cell genome, leading to tumorigenic transformation of cervical epithelium.

The tests for viral DNA detection, E6/E7 mRNA, and viral integration have been discussed in detail in Chap. 9, and out of the viral markers, only DNA methylation will be discussed here.

10.3.4 DNA Methylation as Biomarkers

Tumorigenesis involves modifications in the epigenes within the promoter genes which is crucial for progression to cancer. There are reports showing evidence of hypermethylation of DNA of tumor suppressor gene causing its activity to cease and thereby leading to progression of the lesion [39]. This methylation is nonrandom, with certain genes being methylated in some tumor types and others are not. Also, some reports show contradictory results with DNA hypomethylation of oncogenes in cancers [40–42].

Hypermethylated markers are DNA based as they are inherently more stable than RNA. As gene promoter hypermethylation is common to many cancers, so marker panels can be made which would pick up 70% of all major cancers [43].

Hypermethylated CpG islands are very sensitive tumor markers which utilize methylation-specific polymerase-chain-reaction (MS-PCR) methods to detect methylated DNA sequences [44, 45]. By utilizing these approaches, abnormally methylated gene sequences have been detected in DNA from serum [46, 47].

Host Methylation Methylation of many genes has been studied in cervical cancer, and these are listed in Table 10.3. As these genes are negative regulators of cell growth, they are most probably methylated and silenced in cervical cancer and its precursor lesions. Also, the frequency of DNA methylation increases with increasing severity of precursor lesions. These genes have been studied

Table 10.3 Methylation markers studied in cervical specimens

Gene	Number of studies	Methylation frequency (number positive)			Full name	Biological function
		NL	HGCIN[a]	Ca		
DAPK	22	0.068 (33)	0.296 (158)	0.582 (659)	Death-associated protein kinase-1	Serine-threonine kinase; positive mediator of IFN-γ-induced apoptosis
RASSF1	17	0.031 (10)	0.102 (31)	0.141 (175)	Ras association (RalGDS/AF-6) domain family member-1	Ras effector protein; microtubule regulation, cell migration, proliferation, and apoptosis
CDH1	15	0.159 (37)	0.129 (36)	0.521 (456)	Cadherin 1, E-cadherin	Calcium-dependent cell adhesion glycoprotein
CDKN2A/p16	15	0.049 (17)	0.131 (26)	0.220 (187)	Cyclin-dependent kinase inhibitor 2A	Inhibits CDK4 kinase; regulation of cell cycle control in G1
MGMT	12	0.091 (33)	0.124 (37)	0.183 (124)	0-6-Methylguanine-DNA methyltransferase	DNA repair
RARB	12	0.045 (15)	0.130 (40)	0.343 (169)	Retinoic acid receptor-β	Regulates gene expression in response to thyroid-steroid hormones
CADM1	10	0.256 (43)	0.385 (106)	0.657 (236)	Cell adhesion molecule 1	Intracellular adhesion
FHIT	10	0.072 (21)	0.020 (2)	0.398 (268)	Fragile histidine triad gene	Diadenosine 5′,5‴-P1,P3-triphosphate hydrolase; purine metabolism
TIMP3	9	0 (0)	0.107 (6)	0.189 (82)	TIMP metallopeptidase inhibitor 3	Matrix metalloproteinase; degradation of the extracellular matrix
TERT	7	0.156 (12)	0.388 (73)	0.628 (120)	Telomerase reverse transcriptase	Enzymatic component of telomerase; responsible for the addition of short repeats to the ends of chromosomes or telomeres
CDH13	5	0.177 (25)	0.047 (7)	0.391 (79)	Cadherin 13, H-cadherin	Calcium-dependent cell adhesion glycoprotein
PAX1	4	0 (0)	0.356 (36)	0.917 (33)	Paired box 1	Pattern formation during embryogenesis
TFPI2	4	0.200 (20)	0.342 (13)	0.721 (88)	Tissue factor pathway inhibitor 2	Regulation of plasmin-mediated matrix remodeling
CCNA	3	0.108 (8)	0.387 (24)	0.696 (94)	Cyclin A2	Activates CDK2 kinases; promotes G1/S and G2/M transitions
MAL	3	0.098 (4)	0.577 (71)	0.942 (227)	T-lymphocyte maturation-associated protein	Candidate linker protein in T-cell signaling; implicated in myelin biogenesis and function in the nervous system; formation, stabilization, and maintenance of glycosphingolipid-enriched membrane microdomains
TWIST	3	0.0928 (4)	0.403 (27)	0.362 (68)	Twist homolog 1	Transcription factor; differentiation and cell lineage determination

Inclusion criteria: Genes that have been studied in normal, high-grade, and cancer samples; genes that showed a low level of methylation (<20%) in normal samples that increased in precancerous lesions and/or cancer samples; genes that have been reported in at least three studies; or genes that have been utilized in a marker panel.
Ca cervical cancer, *HGCIN* high-grade cervical intraepithelial neoplasia, *NL* no lesion
[a]Includes CIN2, CIN3, and HSIL in calculations.

as single markers as well as marker panels, but further studies are needed to confirm their role as markers in cervical cancer prevention.

Viral Methylation Detecting methylation of the HPV genome can add to the list of biomarkers for detection of CIN and its progression. E6 and E7 promoter regions get methylated late in the tumor cycle. Also, methylation of CpGs within L1 has been shown to be increased in high-grade lesions. The clinical relevance of these findings is still under research [48, 49].

10.4 Cellular Biomarkers

10.4.1 p16

p16 (also known as $p16^{INK4a}$), a cyclin-dependent kinase inhibitor, is a cell cycle regulatory protein. This tumor suppressor protein, p16INK4a, plays a critical role in regulation of the cell cycle. It is a cellular correlate of the increased expression of HPV E7 oncoprotein and causes disturbance in the cell cycle regulator pRb. This further leads to compensatory overexpression of $p16^{INK4a}$ through negative feedback. It is clearly identified from the result of several studies that $p16^{INK4a}$ is a useful diagnostic marker for squamous and glandular epithelial dysplasia in the uterine cervix [50, 51] (Fig. 10.1). A recent study showed that a $p16^{INK4a}$ immunocytochemical assay has much better specificity as compared to HPV DNA testing to predict underlying high-grade dysplastic lesions [52]. The sensitivity ranges between 59 and 96% and the specificity between 41 and 96% for the detection of CIN2+ lesions. It has been evaluated as a stand-alone and as an adjunct to cytology and HPV testing. p16 overexpression has been found in majority of the cases of cervical precancers and cancers, while it is rarely expressed in normal tissue [50]. It is commercially available as CINtec (mtm lab) and has been widely validated. It is also available as a dual immunostain with Ki67 as CINtecPlus.

10.4.2 Markers of Abnormal Cell Proliferation

10.4.2.1 Ki-67

Ki-67 is a nuclear protein which is expressed during all active phases of the cell cycle, and its expression is directly linked with cellular proliferation. Increased expression of Ki-67 can be found in superficial layers of the cervical epithelium in CIN [53]. Several studies have concluded that Ki-67 can be used as an independent prognostic marker to identify women who are at high risk for progression and/or recurrence of CIN [54].

10.4.2.2 TOP 2A and MCM2

A combination of antibodies against minichromosome maintenance protein 2 (MCM2) and topoisomerase II alpha (TOP2A) has been studied as a biomarker for detection of CIN [55–58]. Both MCM2 and TOP2A regulate different steps of DNA replication, and their expression is increased in situations of aberrant cell cycle and cellular proliferation including cervical neoplasia related to high-risk HPV. Rather than testing for either one of them, which will leave out some dysplastic lesions, it is generally approved to apply the combination of MCM2 and TOP2A immunostaining (ProExC) for diagnosis of CIN. The sensitivity of the test ranges from 67 to 99% and specificity between 61 and 85% according to the few studies done which had a limited sample size [57, 58].

10.4.3 Chromosomal Aberrations

Cervical cancers and precancers are associated with a high degree of genomic instability in the form of recurrent chromosomal amplifications and deletions. The regions typically lost are 2q, 3p, 4p, 5q, and 18q, while regions amplified are 1q, 3q, 5p, and 8q [59–61].

Gain of 3q is the most consistent abnormality seen in cervical cancer; one gene TERC within this region is of particular importance. According

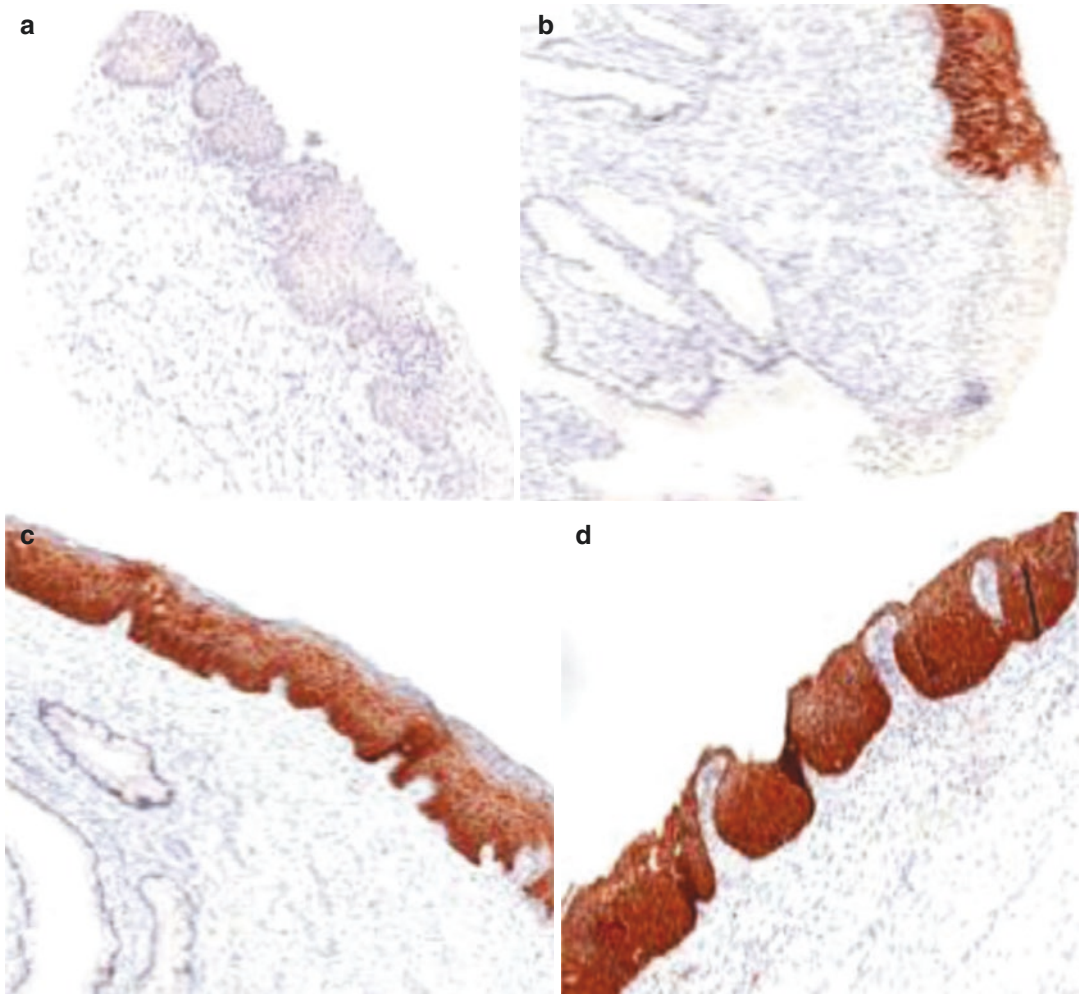

Fig. 10.1 p16 staining: (**a**) normal, (**b**) CIN 1, (**c**) CIN 2, and (**d**) CIN 3. Reprinted with permission from Kaur S. (2017) Pathology of Preinvasive Lesions of the Cervix. In: Mehta S., Sachdeva P. (eds) Colposcopy of Female Genital Tract. Springer, Singapore

to a multicentric study in China, TERC amplification was seen only in women who progressed to high-grade lesions. Another study showed that amplification of 3q had a high negative predictive value for progression of LSIL to CIN2+ [61]. So, TERC amplification could be used to triage HPV-positive women with ASCUS/LSIL cytology. Other chromosomal aberrations still require further research.

10.4.4 Protein Biomarkers

Protein biomarkers help in improving the cervical screening results. Many different protein biomarkers have been identified that are involved in cell cycle regulation, signal transduction, DNA replication, and cellular proliferation. The clinically significant protein markers for the detection of cervical cancer have been summarized in Table 10.4.

Table 10.4 Protein biomarkers

S. No.	Biomarkers	Significance
1.	p53	It is a tumor suppressor protein that prevents the outgrowth of aberrant cells, by inducing cell cycle arrest, DNA repair, or programmed death. The E6 protein of oncogenic HPV types has been shown to complex with p53 and target it for rapid degradation [62]. As a consequence, p53's growth-arrest and apoptosis-inducing activities are abrogated. This suggests the potential importance of E6-p53 interaction for therapeutic intervention
2.	p16	It is considered as a surrogate marker for high-risk HPV infection according to several reports which have mainly examined HPV 16 and 18 subtypes. Its overexpression is well established in CIN and invasive cancer by many studies [50, 63–65]
3.	c-fos	It specifically shows exclusive high expression with the increasing severity of lesion. As a member of transcription factor AP-1, c-Fos has been implicated mainly in signal transduction, cell differentiation, and proliferation [66]. Many studies focused on its oncogenic functions and found that c-Fos-regulated genes are important for tumorigenesis, causing downregulation of tumor suppressor genes [67] and leading to invasive growth of cancer cells [68]
4.	Fra-1	It is normally expressed in cervical tissue, but its expression gets diminished as the lesion progresses from precancer to cancer [69]. It has been found that there is a distinct pattern of gradual increase of c-fos and a concomitant decrease of fra-1 expression that perfectly match the progression of cervical lesions
5.	NF-κB	The NF-κB family consists of transcription factors that play a complex and essential role in innate immunity, inflammation, viral replication, and the initiation and progression of cancer. The classic form of NF-κB is a heterodimer between p65 (RelA) and p50 subunits. p50 subunit of NF-κB shows enhanced expression in high-grade cervical lesions and changes in relation to disease progression [69]
6.	pRB	It is a tumor suppressor protein which plays a pivotal role in the negative control of the cell cycle and in tumor progression. It has been found that pRB is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth. The pRb protein represses gene transcription, required for transition from G1 to S phase, by associating with the E2F family of transcription factors. E7 binding to pRB releases E2F that leads to the expression of proteins necessary for DNA replication [70]
7.	Ki67	It is a marker of cell proliferation. Various studies have shown that an increased expression of Ki67 is correlated with higher cervical CIN grade and is a highly sensitive biomarker for differentiating between CIN1 and CIN2/3 [71, 72]. It can be used as an independent prognostic marker to identify women with high risk for progression and/or recurrence of cervical squamous precancerous lesions
8.	E-cadherin	It is mainly involved in the cell adhesion and is considered as an important biomarker for tumor development [73, 74]. The decrease or loss of expression of these molecules can be correlated with aggressive behavior and progression of cervical cancer. Several recent studies have already focused on changes in intercellular adhesion in different tumors, revealing the pivotal role of E-cadherin during tumor progression and invasion [75, 76]

Few important biomarkers have been discussed in detail.

10.4.4.1 p53

p53 is a tumor suppressor protein which plays an important role in the cells' response to genotoxic stresses like DNA damage, cellular senescence, and apoptosis and helps to maintain genomic stability of the cell. It has been found that disruption of p53 function by the viral E6 protein is one of the major events in cervical carcinogenesis [77]. The E6 protein of oncogenic HPV types makes complex with p53 and targets its rapid degradation [78]. As a consequence, growth-arresting and apoptosis-inducing activities of p53 are abrogated. This makes p53 as a robust prognostic biomarker in cervical cancer.

10.4.4.2 pRB

pRB is a negative regulator of the cell cycle that normally prevents S-phase entry by associating with the E2F family of transcription factors [70].

In case of cervical cancer, HPV infection affects the complex of pRB with E2F and causes its disruption upon binding of oncoprotein E7 that leads to the expression of E2F-responsive genes and degradation of pRB [77, 79]. Various studies have shown the inverse relationship between pRB levels and grade of the lesion with decreasing levels of pRB associated with higher grades of CIN [80, 81].

10.5 Newer Biomarkers

10.5.1 miRNAs

miRNAs are short noncoding RNAs and prevent translation of mRNA by negatively regulating the expression of genes. Abnormalities in their expression patterns are responsible for tumorigenesis as well as prognosis in cervical and other cancers [82]. Expression of some miRNAs (miR-21, miR-127, and miR-199a) is increased in CIN, while miR143, miR214, miR-218, and miR-34a expressions are decreased in cervical cancer compared with normal tissue [83–85]. The changes in miRNA expression is seen in the preinvasive stage of the disease, and so they can contribute as biomarkers for cervical cancer screening [86, 87].

10.5.2 Proteomics

Proteomics is a new emerging field which includes identification of differentially expressed proteins in biospecimens. Studies have shown sensitivity of 87.5% and specificity of 90% for these markers. A serum-based study which included 165 patients was able to mark out three peaks by MALDI-TOF that were different between cancer patients and healthy volunteers [88].

Alternative specimens have also been tried for the study of proteomics including cervicovaginal fluid or cervical mucus [89, 90]. However, the role of proteomics as marker in CIN or cervical cancer still needs to be validated in larger studies.

10.6 Conclusion

In cervical cancer which is associated high morbidity and mortality rates, a better understanding of the molecular mechanisms underlying tumor progression in the disease could reveal the novel pathway of high clinical relevance. Since development of cervical cancer progresses through various stages, it offers a unique opportunity to study the changes occurring at cellular and molecular levels that lead to the development of invasive cancer.

The main causative factor for cervical cancer is persistent HPV infection, but the incidence varies with the genotype of HPV. Tests for HPV genotyping thus help in the development of better screening protocols for prevention of cervical cancer. Currently, a number of molecular markers for cervical cancer screening are commercially available.

The significantly higher HPV load is a possible prognostic marker of high-grade squamous intraepithelial lesions. Integration of the viral DNA to host cell genome is yet another biomarker as persistent HPV infection leads to integration of viral DNA into the host cell genome. It has been found that HPV E6/E7 mRNA testing for high-risk types correlates better with the severity of lesions as compared to HPV DNA testing and is considered as a potential marker for the identification of women who are at high risk of contracting cervical cancer.

Various studies suggest the importance of protein biomarkers like Ki-67, p16, p53, and pRB, for use in cervical cancer screening. They help as predictive markers to identify high-grade lesions which are most likely to progress to cervical cancer.

Cancer of the cervix is the most preventable major form of cancer. The novel biomarkers not only help in screening, detection, and diagnosis of cervical cancer at an appropriate time, but they also help in prognostic evaluation, monitor treatment and predict recurrence, and also play major role in clinical decision-making.

Key Points

- New biomarkers have a potential role to play in primary screening for cervical cancer as well as for triaging primary cytology or HPV screening.
- Viral and cellular biomarkers indicating key steps of the functional progression model (HPV infection, precancer and invasive cancer) are being studied. Of these two main types of biomarkers are viral and cellular.
- The viral biomarkers include HPV DNA testing, HPV E6/E7 mRNA, HPV integration, and methylation; the cellular markers include $p16^{INK4a}$, chromosomal aberrations, and protein markers.
- Detection of HPV E6/E7 mRNA as well as cellular markers $p16^{INK4a}$/Ki-67 immunostaining is commercially available mainly as triage markers.
- Cervical cancers and precancers are associated with a high degree of genomic instability with numerous recurrent chromosomal amplifications and deletions; gain of 3q is the most consistent abnormality seen in cervical cancer.
- Role of miRNAs and proteomics is still under research and needs validation.

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