Review Article

Comprehensive Details on Human Papillomavirus (HPV) Infections and Associated Malignancies: Awareness about Prophylaxis & Treatment Options Available for HPV Infections

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ABSTRACT

Human Papilloma virus (HPV) role has now been widely accepted as a causative agent for cervical as well as head and neck squamous cell carcinomas. Screening and early detection of cervical cancer remains the key element to eradicate this deadly disease. Since it has a long natural history and disease progression, this gave an opportunity for early detection through screening of precancerous lesions which was adopted in high income countries (HIC) like UK which has led to the significant reduction of the cases. Unfortunately, Low- and middle-income countries are facing huge burden because of the lack of effective screening program. FDA approved Cervarix, Gardasil and Gardasil 9 are used as prophylaxis for cervical cancer & genital warts but screening is still essential after vaccination because these vaccines are not effective against all types of HPV types. HPV 16 & HPV 18 are highly oncogenic types and accounts for 70% of cervical cancers. The use of vaccine reduces the risk of getting HPV infection and decrease the rate of morbidity and mortality due to the cervical cancer. In addition to vaccines, many peptides or protein-based & cell or DNA-based vaccines are still in clinical trials; these prevention and management available in vaccines are likely to come up with significant benefits of health in future. Although it's usually cured by immune system but presence of certain cofactors such as smoking, HIV etc. reduces the probability of an individual to eradicate the infection effectively. The aim of this study is to review literature regarding HPV related infections and malignancies. Moreover, it will make individuals aware about prophylaxis & treatment options available for HPV infection. This review article evaluates the HPV related diseases, there screening, evaluation and management currently available.

Keywords

Human Papilloma virus (HPV); cervical cancer; Papanicolaou test (Pap smear test); Cervarix; Gardasil

INTRODUCTION

Cervical cancer is the fourth most common cancer in women globally with around 660,000 new cases and around 350,000 deaths in 2022. [1] Since natural history of cervical cancer is well known and hence HPV is essential for causing Cervical Cancer in 99.8% of cases. Cervical HPV infection leads to the development of a precancerous lesion called Cervical Intraepithelial Neoplasia (CIN) which in the presence of co-factors such as smoking, increases the likelihood of disease progression to the cervix.[2]

Human Papilloma virus (HPV) role has now been widely accepted as a causative agent for cervical as well as head and neck squamous cell carcinomas (Fig. 1). [3] HPV 16 and 18 are most common strains of virus responsible for cervical cancer especially among women less than 30 years. Importantly, in more than 90% of women who acquire the infection the virus clears off but in remaining 10 % where the infection persist the

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virus remains latent in the basal layer of the cervix and leads to the progression to precancerous and cancerous lesion respectively. In developed countries the incidence of cervical cancer decreases but in developing countries the incidence rate of cervical cancer is still on peak. The reason behind increase incidence of cervical cancer is lack of awareness, [4] low income, and insufficient screening & vaccination programs. [5] The development of cervical cancer from HPV infection typically takes 15 to 20 years.

Harald Zur Hausen was the one who identify, clone, and sequence high risk HPV types 16 and 18 & made great contribution for showing the strong correlation between HPV infections and cervical cancer.[6] Furthermore in another study it is also revealed that HPV also play part in causing cancer of oral cavity, esophagus, vagina, vulva and anal cavity and penis. [6, 7]

METHODOLOGY

In this study, articles from Pub Med, Science Direct, Google Scholar & Cochrane library between the months of September 2021till December 2021 were screened out by using different keywords like "human papilloma virus" or "HPV and cervical cancer", "diagnosis of HPV", "screening methods for HPV", "epidemic of HPV", "HPV in South Asia", "treatment of HPV" & "HPV vaccination". Additionally, articles from reference list of other articles were extract out. [4] Initially, 1200 articles were retrieved since the beginning of HPV infection till up to date. After first screening, we include 385 of articles and then out of which 297 articles were excluded as they didn't match the inclusion criteria & the number of articles remains were 88. Inclusion criteria included relevance to HPV infection in women, cervical cancer, HPV types, diagnosis, screening, epidemiology, treatment, vaccination. Information about head and neck cancers by HPV were excluded from the current study.

EPIDEMIOLOGY

During 1990s cervical cancer was ranked as 2nd highest kind of cancer after breast cancer and large number of cases was reported in Brazil and Colombia (70/100,000). [1]

In 2022, cervical cancer becomes the 4th leading cause of death due to cancer among women world widely. Approximately 660,000 new cases have been recorded in 2022 & more than 300,000 peoples have been died due to cervical cancer. [1] The greatest rate of mortality and incidence were recorded in Sub-Saharan Africa.

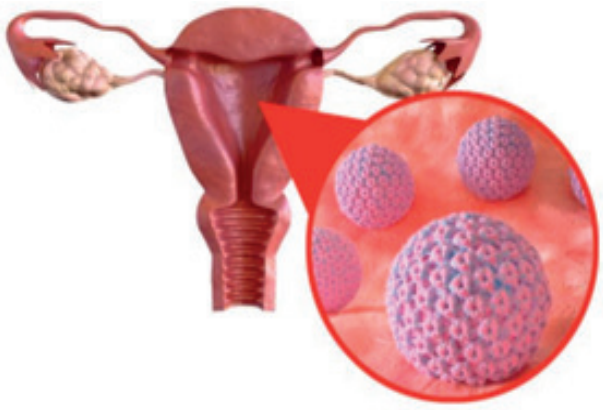

Fig. 1: Cervix & Human Papilloma Virus

Malawi (present in southeastern Africa) has the world highest recorded cases and increased mortality rate.[8] While in Northern America, Australia/New Zealand, and Western Asia (Saudi Arabia and Iraq) which are considered as developed countries, the incidence rates were 7-10 times lower. [8] In sub-Saharan Africa, Melanesia, South America, and South-Eastern Asia, cervical cancer becomes the leading cause of cancer death. Cervical cancer is epidemic in sub-Saharan Africa, Melanesia, South America, and South-Eastern Asia since 2009. [8]

HPV alone is not enough for causing cervical cancer, some other co-factors like HIV (Human Immunodeficiency Virus) [9] and other sexually transmitted diseases along with HPV play important role in causing cancer of cervix. From previous studies it has been evaluated that having sex at early stage and having multiple sex partners also increase the risk of cervical cancer. [10,11] Women of middle age (usually child bearing age) are at higher risk of having cervical cancer. Using Contraceptives for the long term also increases the incidence of cervical cancer. [12]. Smoking is another co-factor which also increases the risk of cervical cancer. [13] Low income causes the non-compliance for screening and these people also unaware of screening programs; thus, low income & illiteracy also increases is responsible for the risk of cervical cancer indirectly.[14]

It has been noticed that the developing countries are at higher risk (7-10%) of having cervical cancer as compared to developed countries. In developed countries the incidence rate is 18.8 out of 100,000 while in developed countries the incidence rate is 11.3 out of 100,000 women.[1] From past few decades, the decrease

in incidence and mortality rate was observed in many countries due to the development of different screening techniques but cervical cancer still remains the major health problem. It has been observed in developed countries like in Europe, Oceania and Northern America that there is a dramatic declining in incidence of cervical cancer with increase in screening programs but in some regions like in Eastern Europe and in Central & South-Asia mortality rate of premature cervical cancer increases in younger generation due to inadequate screening programs (Fig. 2) .[1] Western Asia has the lowest morbidity rate of cervical cancer in the whole world, which is 4.1/100,000; while in Australia & New Zealand the lowest mortality rates were recorded, which is 1.6/100,000.[1] In Asia nearly 315,346 women infected with cervical cancer per year, out of which 116,369 cases were recorded from Southern-Asia and the incidence rate is 13/100,000 women. In Southern-Asia; Maldives & Nepal have increased incidence rate that is 23.2/100,000 and 21.5/100,000 respectively followed by India (14.7/100,000), Bhutan (14.4/100,000), Bangladesh (10.6/100,000), Sri-Lanka (7.8/100,000), Pakistan (7.3/100,000), Afghanistan (6.6/100,000) and lowest incidence rate of cervical cancer were recorded in Iran (2.2/100,000).[15] The annual number of recorded deaths in Asia is 168,411. [15] According to report of ICO/IARC Information Centre on HPV and Cancer (2019), the current scenario designates that every year in Pakistan, approximately 5000 women diagnosed with cervical cancer and out

of which approximately 60-70% die from cervical cancer. HPV 16 or 18 attributes about 88.1% of all invasive cervical cancers. [16]

PATHOGENESIS

Oncogenic types are mostly involved in causing cervical cancer but it's not necessary that HPV infections always convert into invasive cervical cancer. Sometimes they convert into transient infection (Fig. 3). The ratio of conversion of HPV persistent infection from oncogenic types to cancer is less than half. [17] Mostly HPV infections are converted into transient infection within 4-18 months & it clears within a year or two. From previous studies, it was concluded that clearance rate of non-oncogenic type is quite high than oncogenic types. [18]

Before 2001 the term "Cervical intraepithelial Neoplasia (CIN)" was used for 'cervical dysplasia' then this term was flipped into 'Squamous Intraepithelial Lesion' (SIL) by Bethesda Classification of cervical dysplasia (Table 1). SIL further classified as Low grade intraepithelial lesions (LSIL) and high grade intraepithelial lesions (HSIL). However, many biopsy reports are still using the term CIN. [10] The severity of HPV infections is based on the grades like there are 3 grades CIN1, CIN2 and CIN3. CIN1 is low grade & relapse without treatment or sometime it's not able to detect, [20,21]as basically it's an infection not a cancer [22]. CIN2 and CIN3 are examined as high-grade dysplasia. Progression of CIN2 is slow as compare to CIN3 (Table 2).

Table 1: Classification of Cervical Cancer

Cytology		Histology		Natural history model
Papanicolaou classification[34]	Bethesda system	Dysplasia nomenclature	CIN nomenclature	
I	NILM	Negative	Negative	
II	ASC-US	squamous atypia	Squamous atypia	
III	LSIL	Mild dysplasia Moderate dysplasia	CIN 1 CIN 2	Infection Precancerous
IV	HSIL	Severe dysplasia carcinoma in situ	CIN 3	
V	Carcinoma	Carcinoma	Carcinoma	Cancer

Table 2: Comparison of Cervical intraepithelial Neoplasia; CIN1, CIN2 &CIN3

	Regression %	Persistence%	Progression%
CIN 1	57%	32%	1%
CIN 2	43%	35%	5%
CIN 3[22]	32%	47%	12%

CIN3 have greater chances to develop into invasive cancer but it takes usually up to 10 years. There are 12-30% chances of untreated CIN3 to develop into invasive cancer but if CIN3 is treated properly so the chance of developing cancer will reduce up to 1%. [23, 24, 25]

HPV TYPES & VARIANTS

Human Papillomaviruses are small size non-enveloped, double stranded DNA viruses; Papilloma viruses have about 10 open reading frames (ORFs): 8 of them are called early ORFs and written as E1, E2, E3, E4, E5, E6, E7 and E8 & 2 of them are called late ORFs and written as L1 & L2. (An open reading frame is a part of DNA molecule that, when converted to amino

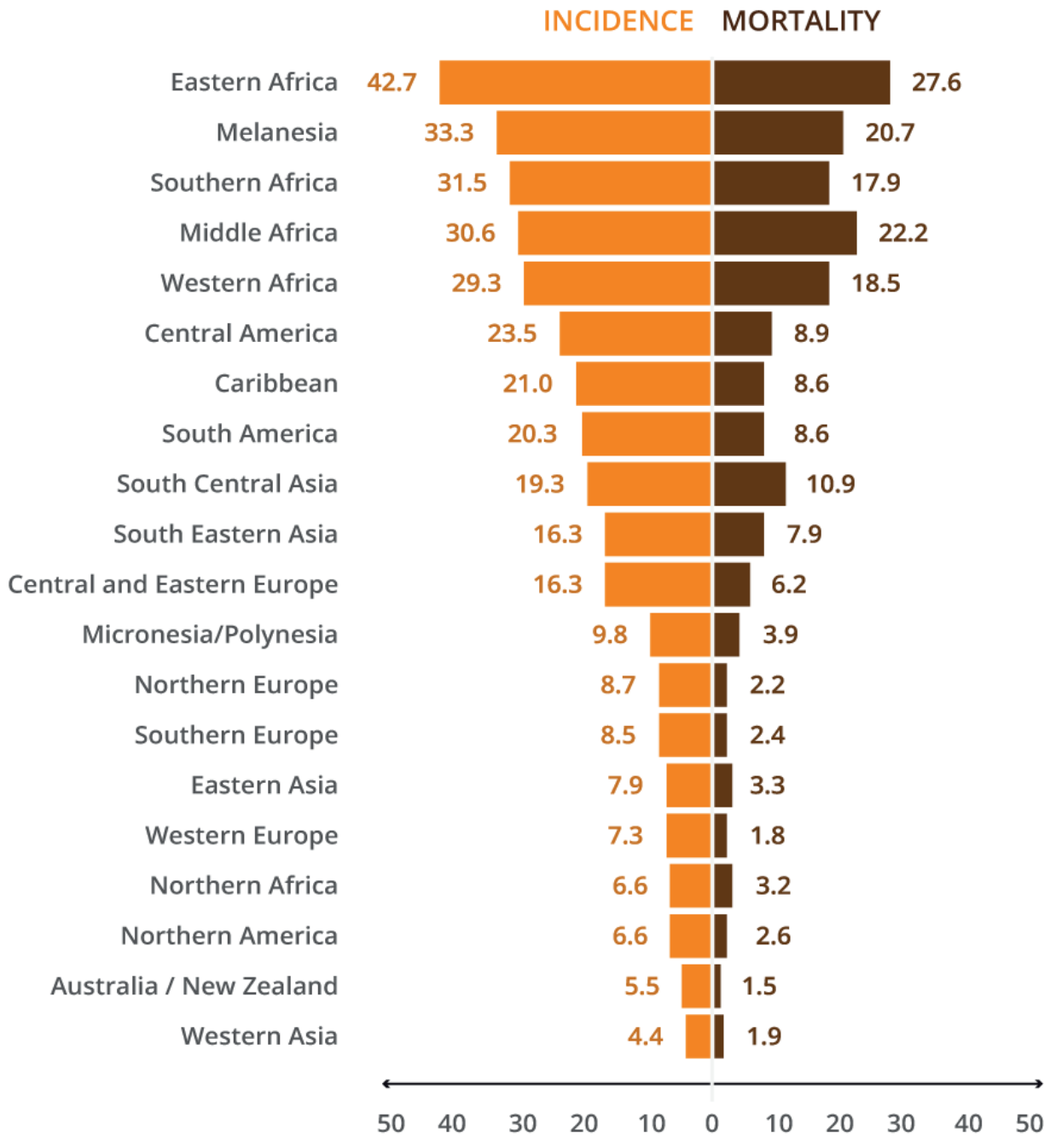

Fig. 2: Global Incidence & Mortality Rate of Cervical Cancer (Torre etal.,2016).

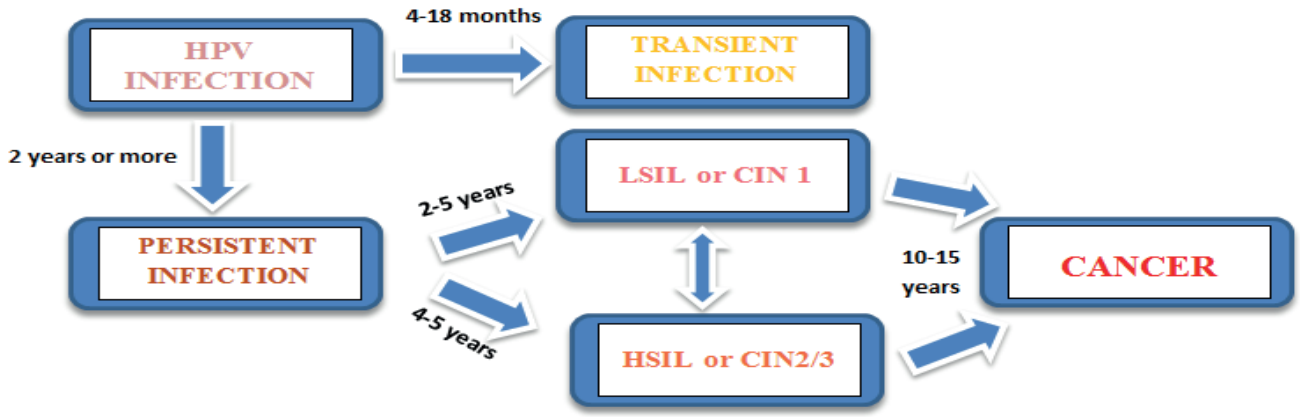

Fig. 3: Pathogenesis of HPV Infection and Cervical Cancer [19]

acids, contains no stopping codons). [26]L1 is a major component of capsid & assemble themself into virus-like-particles (VLPs) while L2 is a minor component & have no capacity to form VLPs thus involves in infectious processes. The oncogenic potential is based on the DNA of a viral gene that binds to the host genome and takes control over regulation of native genes. Once the viral DNA has been implanted in the host cell genome, viral sub-particles, particularly E6 and E7, replicate without regulation disturb normal cell growth and growth control.[27]

Control yet now approximately 200 types of genetically mutated HPV have been discovered, [23, 28] but not all of them associated with infections or malignancies. Approximately 40 types are able to cause infections & only 13-15 are known to cause cervical cancer and other malignancies. There are 12 high risk oncogenic types of HPV classified as group 1 carcinogens [29] involved in the development of cervical intraepithelial Neoplasia.

On the basis of difference in nucleotide sequence of L1 different types of HPV are classified, usually the difference is of at least 10% so they allocate separately. [10] Initially HPV 6 discovered in 1982 form locally invasive rare Buschke-Lowenstein tumor which is a huge size genital wart,[30] although HPV6 is now routinely found in benign condyloma accuminata genital warts. After the very next year HPV 11 and 16 were discovered and isolate from lesions of cervix. HPV 11 is found to be consistently isolated from laryngeal lesions and genital warts.[10]

On the basis of malignancies HPV types are categorized into low risk & high-risk types (Table 3). From previous studies, it has been concluded that HPV6 and 11 are present in 90% of benign lesions of genital wart that's why they are classified as low risk HPV, while HPV 16 and 18 are found in pre-cancerous lesions and classified as high risk HPVs, having a greater chance to cause invasive cancer. According to international agency for research on cancer hpv53 and hpv66 are the most potential carcinogens of cervical cancer. [30]

Table 3: Low & High Risk Types of HPV

Low Risk HPV Types	6, 11, 40, 42, 43, 44, 54, 61, 72, 81
High Risk HPV Types [22]	16, 18, 31, 33, 35, 39, 45, 51, 52, 53,56, 58, 59,66, 68, 82

The neoplasia of cervix, vulva, vagina, penis & anus are usually caused by HPV 6 and 11 (these are low risk or benign types of HPV) but more routinely these neoplastic conditions are caused by HPV 16, 18, 31 and 45(which are high risk oncogenic forms of HPV). [31] 90% of infections are caused by low risk HPV types become inactivated within 12-24 months after introduction into the body but the infections due to high risk pathogens are developed into the cancer. [23]

Variants of HPV are usually differing in sequence of L1 usually less than 3% and most often the variation occurs only in single nucleotide. [10] There are four variants of HPV16 & three variants of HPV18 are discovered. HPV16 variants are A, B, C and D; European (A1, A2, A3), Asian (A4); African-1 is B; African 2 is C; North-American, Asian-American (D1–3). [32] HPV18 variants

are A, B, and C; Asian American (A1 and A2) European (A3-A5); and African (B and C). [33]

On the basis of their similarities HPV types are either grouped in different genera or in different species. Papillomavirus types included in general are highly phylogenetic while the types included in species are the ones having lower phylogenecity. Papillomavirus is found both in human and animal and in total they are forming 16 genera out of which 5 are solely of human papillomavirus.[34] They are mainly categorized as α-papillomavirus or β-papilloma virus, and some other categories are γ, μ or papillomavirus. The α-papillomaviruses are those that infect the mucosa of genital tract while β-papillomaviruses infect the skin. [28] Cervical cancer constitute about 90-95% of squamous cell cancers, while <5% are of adenocarcinomas. [10]

Detection of HPV Infection & Cervical Cancer

Detection of precancerous lesions and HPV infections through different test or methods can be perform, such as cervicography, macroscopic examination, serologic assays, and DNA based methods. But most effective and low-cost detection method is papanicolaou (pap) smear detection of precancerous cervical lesions, since the late 1940s. [34] There are different methods having some advantages and disadvantages whice are described below.

The Pap Smear /Papanicolaou

The pap smear, is the standard method for detection of cervical cancer, which is described by Dr. George papanicolaou in 1928 for early detection of cervical carcinoma, the pap smear test was instituted in 1943.[35] In United States, before the use of pap smear, the rate of cervical cancer was 44/1000000 women but after the set down of pap smear institute, since1947, decreasing the rate of cervical cancer to 5 to 8 per 1000000 because it detected the precancerous lesions in millions of women and the mortality rate of cervical cancer reduce more than 70%.[35]

The Pap smear test is screening test of cells of uterine cervix. The smear is obtained by inserting speculum into vagina to open it and then brush/wooden scraper is used to take the sample of cells. The sample spread over a glass slide and rinsed in 95 %ethyl alcohol and sent to laboratory. [35]

In United States, annually about 50 million Pap smear test done and 3-4% reported LSIL, 0.65 as HSIL and 4-6% as ASCUS. The Pap smear test significantly identifies the dysplasia, pre-cancer or cancer with high specificity and high sensitivity. [35]

Pap smear is the easiest and most powerful method of detection of precancerous cervical disease. In Pakistan, 50.5% women have no idea about Pap smear test and 37.5%women have some knowledge about it but they thought that they have no need of Pap smear test. [36] According to another study, 54% of nurses and interns knew about the Pap smear test. Several studies show that the Pap smear testing has not been implemented in Pakistan in an organized way. [36]

COLPOSCOPY

Colposcopy was developed by Hans Hinslmann in 1925. [37]It is a procedure in which lighted, magnified instrument called a colposcope used to examine the cervix, vagina and vulva. The colposcopist should examine the vulva, vagina, and cervix grossly in the natural state and also after the application of 5% acetic acid. The entire cervix and SCJ (squamocolumnar junction) must be visualized for adequacy. Both white light and a red-free (blue or green) filter should be applied to the visual field in order to identify any lesions. [38] Colposcopy has an important role in determining the treatment modality. The International Federation of Cervical Pathology and Colposcopy (IFCPC) published the fourth edition of colposcopic terminology and guidance to increase the diagnostic accuracy of colposcopy (Table 4). [37]

Table 4: International Federation of Cervical Pathology and Colposcopy (IFCPC) colposcopic terminologies and guidance of the cervix

SECTION	PATTERN
Normal colposcopy finding	Squamous epithelium; mature, atrope Columnar epithelium; ectopy Metaplastic squamous epithelium; crypy (gland) opening
Abnormal colposcopy finding	Location of lesion; inside and outside the transformation zone Size of the lesion: number of cervical quadrants the lesion covers. Size of the lesion as percentage of cervix.

SECTION	PATTERN
Grade 1 (minor)	Fine mosaic; fine punctation; thin acetowhite epithelium; irregular, geographic border.
Grade 2 (major)	Sharp border; inner border sign; ridge sign; dense acetowhite epithelium; coarse mosaic; coarse punctuation; rapid appearance of aceto whitening; cuffed crypt (gland) openings.
Nonspecific [38]	Leukoplakia (keratosis, hyperkeratosis), erosion Lugol's staining (Schiller's test): stained or non-stained.

The accuracy of colposcopy is still controversial and its accuracy are questioned due to issues associated with biopsy number and random biopsies.

There are only four countries including China, Japan, Korea and Thailand in Asia, where the screening of cervical cancer by colposcopy is available (Table 5). [39] Till now it is the big challenge to introduce the cervical cancer screening in almost all Asian countries.

Table 5: National cancer screening programs for cervical cancer in Asian countries

Country [39]	Nationwide screening	Targeted age	Screening Interval	Confirmatory tests
China	Available	18–65	3 years	Colposcopy
India	Partially available	30–59	2 years	Observe & Manage
Indonesia	Available	30–50	3–5 years	Observe & Manage
Japan	Available	>20	2 years	Colposcopy
Korea	Available	>20	2 years	Colposcopy
Pakistan	Available	>20	3-5years	Colposcopy
Thailand	Available	35–65	2 years	Colposcopy

SEROLOGICAL TEST

HPV cannot be grown in conventional cell culture and serologic examination has limited accuracy, it is the simple and sensitive type of assay. There are many antibodies formed against HPV show variability according to type of HPV. Anti-HPV antibody mediated immunity reactions are largely estimated by enzyme-linked immune absorbent assay (ELISA) with HPV type specific virus like particles (VLPs). Recombined HPV protein and peptides are also utilized in ELISA test.[40] This assay detects antibodies with the high specificity at early protein E6 and E7. Therefore, there is great need to develop alternative antigen for HPV serology and verify positive and negative serum control. Serological diagnosis of HPV infection using genetically engineered HPV capsid virus like particles/ VLPs correlate with HPV DNA presence in the cervical smear. HPV VLP ELISAs show very high specificity (>90%), sensitivities between 50% and 60% and good inter-laboratory agreement. [40] VLP serology is used as a marker of cumulative exposure to HPV and sexual behavior.

It's been demonstrated that VLPs of all HPV types induce serum antibody response which is genotype – specific with exception of HPV6 and 11, which are cross reactive. Most of the serum antibodies forms against a single type of HPV i.e. HPV 16 Virus like particles. Testing healthy and cancer patient for anti-VLP antibodies for HPV type16, 18, 31, and 58, cross reactivity was observed between HPV responses against type16 and type 31.[40]

HYBRID CAPTURE 2 ASSAY

HPV- hybrid capture assay is one of the new techniques to examine the HPV. Hybrid Capture 2(HC2) system has been used to test for high risk HPV and low risk HPV DNA. [41] Hybrid Capture 2 (HC2) uses RNA probes that are hybridized with target DNA from an individual. The resulting RNA DNA combination is captured on micro plate and coated with antibodies that are specific for DNA RNA hybrids. [41] Then the immobilized hybrids are reacted with alkaline phosphatase. Conjugated antibodies specific for the DNA RNA hybrid and detect by the chemical luminescent substrate, several antibodies capture the hybrid that cause signal amplification, and the light emit when substrate cleaved by bound alkaline phosphatase. This is measured as relative light units (RLU).

This technique analyzes the presence of high risk HPV types includes 16,18,31,35,39,45,51,52,52,58, 59 & 68.HPV type 16 DNA used as positive control

(1pg/ml) while presence and absence of hybridization reaction (HR) HPV DNA defined according the relative light unit (RLU). Hybridization reaction HPV DNA is present or positive when the RLU is equal or greater than the mean value for the PC.

POLYMERASE CHAIN REACTION (PCR):

Since the past 10 years, Polymerase chain reaction (PCR) has been considered as the 'gold standard' technique for HPV diagnosis. Polymerase chain reaction is the test to detect the genetic material from the individual. This technique uses to increase the sensitivity of HPV detection, it performed with three mostly used consensus primer sets (MY09/MY11, GP5PLUS, GP 6PLUS, SPF1/2) for different L1 regions of the viral genome. (Fig. 4)[42] Amplification with each of these primers will result in different size amplification product.[43]

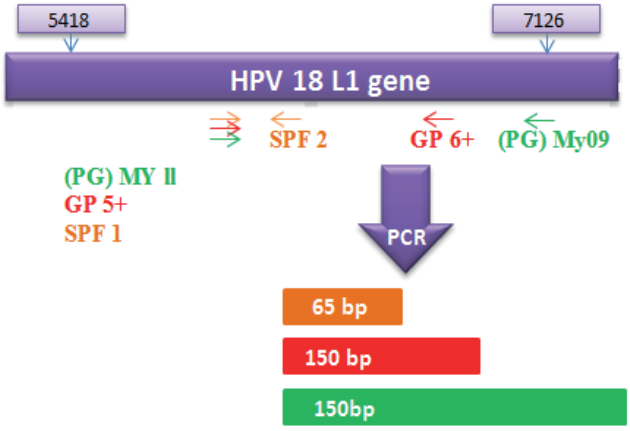

Fig. 4: L1 Consensus or General Primer PCR [42]

The primer GP 5 plus /GP 6plus method can detect the 19 different genotypes. It can detect the types 43 and 44 more efficiently than MY09/11. [44] The degenerate pool of primers MY09/MY11 amplifies a wide spectrum of HPV genotypes with various ranges of sensitivity. It detects 10 copies of viral DNA from frequently encountered genital types. The new PGMY09/PGMY11 set of consensus primer amplify a 450 nucleotide fragment and improve the sensitivity of amplification of genital HPV types over the MY primer pair. The SPF system amplifies a 65 base pair fragment with a pool of 10 diverse primer containing inosine at some positions to the optimize amplification of a few types (Table 6).

Table 6: Polymerase Chain Reaction Primer Sets for Detecting the Presence of Common L1 Region of Human Papilloma virus

Primer	Sequence	Predicted product size
GP5+/6+	F:5′TTTGTTACTGTGGTAGATACTAC-3′	150
	R:5′GAAAATAAACTGTAAATCATATTC-3′	
MY09/11	R: 5′-GCMCAGGGWCATAAYAATGG-3′	450
	F: 5′-CGTCCMARRGGAWACTGATC-3′	
SPF1A	F: 5′-GCiCAGGGiCACAATAATGG-3′	65
SPF1B	R: 5′-GCiCAGGGiCATAACAATGG-3	
SPF1C	F: 5′-GCiCAGGGiCATAATAATGG-3	
SPF1D	R: 5′-GCiCAAGGiCATAATAATGG-3′	
SPF2B-bio	F: 5′-GTiGTATCiACAACAGTAACAAA-3′	
SPF2D-bio [43]	F: 5′-GTiGTATCiACTACAGTAACAAA-3′	

STAGES OF CERVICAL CANCER

The staging of cervical cancer is based on international and federation of gynecology and obstetrics system (FIGO 1995 Montreal). [60] Cancer can spread through tissue, blood, and lymph vessels. Abnormal or carcinoma cell spread into nearby normal tissue. Clinical stage based on physical examination, biopsies, imaging test, or cystoscopy and proctoscopy. [45]

S.NO	STAGES	CONDITION
1.	STAGE I	In stage I, cancer found in cervix only and extension to the corpus should be disregarded, lesion is macroscopically visible. The depth of invasion limits 5mm and horizontal spread maximum 7mm. Stage I further divide into IA and IB based on the size and depth of tumor invasion.
2.	STAGE IA1[46]	Minor cancer can be diagnosed by microscopy found in tissue of the cervix. The depth of invasion is 3 millimeter or less and less than 7 mm in horizontal spread.
3.	STAGE IA2	Minor cancer diagnosed by microscopy found in tissue of cervix and the stromal invasion is more than 3 millimeters but less than 5 millimeters and less than 7 millimeters in horizontal spread.
4.	STAGE IB1	In stage in IB1, cancer found in cervix and diagnosed by microscopy, the stromal invasion is more than 5 millimeters and tumor is about 2 centimeters or less.

S.NO	STAGES	CONDITION
5.	STAGE IB2[47]	The tumor is larger than 2 centimeters, and smaller than 4 centimeters, it is the clinical visible lesion.
6.	STAGE IB3	The tumor size is more than 4 centimeters.
7.	STAGE II	In stage II, the cancer extends beyond the cervix but it is not extending into the pelvic wall. The cancer spread to the upper 2/3 of vagina of tissue near the uterus. The stage II divide into stage IIA and IIB depend on the spread of cancer.
8.	STAGE IIA1	In stage IIA1, cancer extends beyond the cervix and involves to upper 2/3 vagina without parametrial invasion, it is the clinical visible lesion less than 4 centimeters in dimensions.
9.	STAGE IIA2	In stage IIA2, cancer extend beyond the cervix and involve to upper 2/3 vagina without parametrial invasion, it is the clinical visible lesion more than 4 centimeters in dimensions.
10.	STAGE IIB	In stage IIB, cancer extend beyond the cervix and involve parametrical invasion but not pelvic wall and lower 3rd vagina
11	STAGE III	The cancer spread to the lower 3rd of vagina, also involves the pelvic side wall and lymph nodes and causing hydro nephrosis or non-functioning kidney. The stage III further divides into stage IIIA, IIIB and IIIC.
12.	STAGE IIIA	The cancer extends to the lower 3rd of vagina but not to the pelvic wall.
13.	STAGE IIIB	The spread of cancer to the pelvic wall, the size of tumor become larger and block the uterus and cause kidney dysfunction.
14.	STAGE IIIC	The cancer spread to the distant lymph nodes and divide into IIIC1 and IIIC2.
15.	STAGE IIIC1	Involvement of pelvic lymph nodes.
16.	STAGE IV A	Involvement of Para aortic lymph nodes.
17.	STAGE IV	In stage IV, the cancer extends beyond the pelvis and spread to the mucosa of bladder or rectum and it can spread to the other part of body this stage further divides into 4A and 4B.
18.	STAGE IV A	The spread of tumor extends to the nearby organ such as bladder and rectum.
19.	STAGE IV B [48]	The cancer spread to the distant part of the organ such as liver, lungs, bones and distant lymph nodes.

TREATMENT OF CERVICAL CANCER

Cervical cancer is the most common cancer in women worldwide; mostly it occurs in developing countries. The rate of cervical cancer rapidly increases which correspondingly increase threat of women's health. That's why we need effective and safe biological agent to treat cervical cancer. Primarily HPV infection or cervical cancer prevents by vaccination and some prevention programs, such as cervical screening. But if the both approaches are not available, for example, in China, approx. 135000 women suffer from cervical cancer each year and around 50000 die from it. Although China is well developed country but there is less availability of vaccination. [44] The choice of treatment depends on the HPV type and the stage of cervical cancer. [49] Some additional factors that influence the choice of treatment are physical and physiological aspects such as quality of life, mental health or emotional well-being. [50]

TOPICAL MEDICATION

Warts by human papilloma virus (HPV) is considered as the most common skin disease in women. Trichloro-acetic acid is mainly used to treat the genital warts. It is used to apply topically to the warts. 80% tri-chloro-acetic acid is most effective against the human papilloma virus warts [51] and found safe to use during pregnancy. [52]

NON-SURGICAL APPROACH

Cryosurgery

The patients with the abnormal cell growth in cervix which turns into cancer/cervical intraepithelial cancer (CIN) or cervical carcinoma and unusual bleeding from the Cervix need to have cryosurgery ablation of cervix. In cryosurgery of cervix, the extremely cold nitrogen used to destroy the suspicious cells. [53] Speculum is inserted in vagina to open it, cryoprobe (hollow metal tool) also inserted in the vagina that supplies the cold nitrogen to defected cell or tissues and freeze it up to -20 °C. The process takes only few minutes and then the frozen cell either get absorbed or flushed out through vagina. The process doesn't harm the nearby cells or tissue and not affect the ability to get pregnant after.

LOOP ELECTROSURGICAL EXCISION

It is the treatment preferred for non-surgical squamous

lesions. In these procedures, the wire is use which is heated by electric current to remove the cancerous cell and tissues from the cervix. This technique is less expensive than cryosurgery. There is risk of leaving abnormal cells behind and recurrent rate is about 31% in this type of procedure.

SURGICAL APPROACH

Simple Hysterectomy

In simple hysterectomy, uterus is removed without the parametria or vagina at the stage 1A1 [54] because the risk of metastasis is less than 1%.

RADICAL HYSTERECTOMY

Radical hysterectomy involves resection of cervix, uterus, parametria and cuff of upper vagina at stage 1A2.[54] At this stage, the cancer spread 3 to 5 mm into the cervical tissue and low risk of parametrical involvement. Radical hysterectomy can be done via laparotomy or by minimally invasive surgery.

RADICAL TRACHELECTOMY:

It involves resection of entire cervix and 2 to 3 cm upper vagina and parametrium via laparotomic, vaginal laparoscopic or robotic assisted route. [54] It can be done with stage 1A1 patient with lymph vascular space invasion, stage1A2, stage 1B1 tumor without lymph node metastases.

Punch biopsy

It is the removal of a round- shaped tissue sample through a surgical procedure for pathological analysis.

Endocervical curettage

This technique is useful when a biopsy in colposcopy fails to find glandular lesion or an endo cervical squamous lesion

Loop electrosurgical excision procedure (LEEP)

It is a useful diagnostic and therapeutic technique involving removal of atypical cells from the cervix for subsequent histological examination.

Conization

It is also previously known as cone biopsy. This procedure is useful for removal of precancerous lesion or early-stage cancer. In this therapeutic technique a cone-shaped piece of tissue from the cervix and cervical canal is removed [54].

Combination Therapy

Radical hysterectomy with pelvic and para-aortic lymphadenectomy is used to treat for FIGO stage 1B1 cervical tumors. [54] These tumors are generally less than 4 cm in diameter. Alternatively, it can be treated by radio therapy.

Stage IIB to IVA is treated non-surgically. The standard care is radical radiotherapy, because surgery is unlike to be cures and the combination of radical surgery, radiotherapy and chemotherapy has a high risk of adverse event and chronic morbidity. [55]

Trachelectomy and SLN Mapping is another combination therapy in young patients (40% are under 45 years old). This combination therapy is based on preserving fertility and reducing the risk of cervical cancer. The selection criteria for this type of surgery are fertile patients of young age less than 40 years with a reproductive desire, stage IA1 with LVSI or IA2, IB1 stages (tumor size < 2 cm) and MRI not showing parametrial invasion or metastases to lymph nodes or other sites. This treatment consists of the removal of the entire cervix, parametric tissue, and vaginal cuff. This allows preserving the uterus, ovaries and tubes with SLN mapping with or without bilateral pelvic lymphadenectomy [55].

Chemotherapy

Before the beginning of 21st century, the US National Centre Institute stated that chemotherapy should be considered together with radiotherapy for better effective treatment of cervical cancer. In 2001-2002, Radiotherapist of Royal College of UK treated few patients with radiotherapy along with surgery, few without surgery and few with radiotherapy plus chemotherapy for determining the overall survival of patients. It is found that the survival rate for only radical therapy was about 44%, with chemotherapy the survival rate was 55% but there with chemo-radiations, there was increase in number of complications occur in patients after 7 years of treatment. [56] Cisplatin is most commonly used agent in chemotherapy.

The use of Chemotherapy along with radiotherapy reduces both the local and distant sites recurrence of cancer and also improves the overall survival of patients without progression of cervical cancer.

Retreatment

After treatment, if patient recurrent the cervical cancer needs retreatment of cervical cancer. Recurrent cervical

cancer also associated with the morbidities. Recurrent cervical cancer can be treated with platinum-based combination therapy. [49] It helps to improve the survival conditions of patient when compared with no therapy. The efficacy of retreatment is less as compare the primary treatment and has high risk of adverse effects. [49]

HPV VACCINES

The aim for developing HPV vaccine is to lessen the risk of HPV infections and cervical cancer. [57] HPV vaccines are executed by 74 countries of world in their national immunization schedule and listed it as a crucial medication by WHO.[58] The coverage of HPV vaccine is quite lower in under developed or low income countries i.e. only 1.4% of women are fully vaccinated and 1.7 % of women received a single dose. The vaccination coverage in moderately developed countries is 2.7% while in highly developed countries HPV vaccine coverage is 33.6%. [59, 60]

In 2006, first vaccine of HPV becomes marketed as brand name Gardasil approved by FDA, USA. Gardasil is quadrivalent means it shows efficacy against four types of HPV that is HPV16, HPV18, HPV6 and HPV11. [61] The Cervarix is another HPV vaccine that was approved by both European Medicines Agency and Food & drug administration, in 2007 and 2009 respectively. [62] The Cervarix is bivalent and effective against HPV 16 and 18 which are highly oncogenic types of HPV and causing almost 70% of cervical cancer.[63, 64, 65] In 2014, FDA approved another nona valent (9-valent) HPV vaccine which is Gardasil 9 it shows high spectrum of activity and covers HPV6, 11, 16, 18, 31, 33, 45, 53, and 58, & Gardasil 9 have possibility to protect against 90% of cervical cancers (Table 7).[66, 67] The rate of protection from all these three vaccines vary (Fig. 5).

These vaccines are prepared from recombination of L1 subunit by yeast with protein and yeast separated from yeast cultures (not HPV cultures). These are protein components will automatically combine viral-like particles that do not have viral DNA. However, the immune system will produce a large number of antibodies to these virus-like particles than will detect the real virus when it is exposed, thus preventing infection. Thus, these vaccines don't have viral DNA, and the vaccine is not an inactive or attenuated virus in it, so there is no chance of an infection due to vaccine. Each type of HPV has different L1 subunit so the vaccine is specific to different types of HPV. [52]

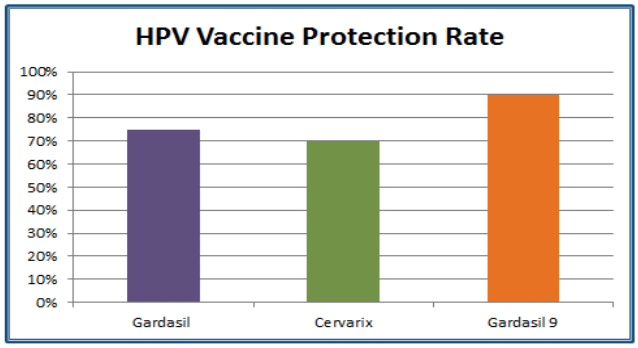

Fig. 5: Protection Rate of HPV Vaccines

All these three vaccines are proven to be safe & effective and no serious adverse events are reported [68,69] but still many countries are not using HPV vaccines due

Table 7: Comparison between Vaccinations of HPV Infections

Hpv Vaccines	Manufacturer	Approval Year	Effective Against Hpv	Dose	Injection Schedule	Protection Rate
GARDASIL	Merck & Co.	2006 by FDA	6, 11, 16, 18	0.5 ml/dose	0, 2, 6 months	70-75%
CERVARIX	GSK	2007 by EMA & 2009 by FDA	16, 18	0.5 ml/dose	0, 1 months	70%
GARDASIL 9[67]	Merck & Co.	2014 by FDA	6, 11, 16, 18, 31, 33, 45, 52, 58	0.5 ml/dose	0, 2, 6 months	90%

to isolated safety issues. [70] WHO stated that poor evidence on HPV vaccination adverse events limit the use of HPV vaccines which ultimately leads to increase incidence of HPV infection or cancers. [71]

It was concluded in one of the studies that after 12 years of vaccination the antibodies were still present in individual received either Cervarix or Gardasil, but the individual who received Cervarix have 5.1 times higher number of anti HPV-16 antibodies and 18.4 times higher HPV-18 antibodies as compared to Gardasil. [72] In about 35% of patients who received Gardasil no quantifiable antibodies were detected in their body after 5 years of follow up.[73] Studies suggest that for at least 15 years the vaccine should have to maintain 100% efficacy. [74]

Another study indicates that after 5-8 years of vaccination, the prevalence of HPV 16 and 18 decreased significantly by 83% in girls aged 13-19, and significantly decreased by 66% in women aged 20 to 24 years and also show effective results for genital warts. [75]

For greater effectiveness of HPV vaccine other strategies are also need to be taken into consideration, like educate the person about risk factors which exaggerate or help out human papillomavirus to invade HPV infection or malignancies, also making them concern towards screening programs and treatment services. [62] WHO recommended individual both girls & boys to be HPV vaccinated before their first sexual intercourse. [76] In South-Asian countries there is inadequate vaccination & screening programs due to low income which increases both the mortality & morbidity related to cervical cancer. It is proven from various studies that the most common barrier for HPV vaccination is the high cost of HPV vaccines. Furthermore, established evidence suggests that HPV vaccine have a greater impact on prevention of cervical cancer. [77]

Cecolin® is a Recombinant Human Papillomavirus Bivalent (Types 16,18) Vaccine (Escherichia coli). This vaccine is indicated for women 9 through 45 years of age for prevention of which HPV types 16 and 18. This vaccine has high efficacy against high-grade genital lesions [77]

UK National Vaccination Program

UK introduced national HPV program back on 1st September 2008 with bivalent Cervarix vaccine covering HPV 16 and 18. Initially only girls aged 12 to 13 were invited but then a catch up program for girls between 14 to 18 was offered. [78] The HPV program was further expanded and currently the HPV vaccine is being offered to all the girls and boys in a year 8 at school (ages 12 to 13 years). Those who missed the vaccination can request it till there 25th birthday.

In the UK in 2012, the bivalent Cervarix vaccine was replaced by the quadrivalent Gardasil vaccine and was given in 2 doses. Currently, a single dose of Gardasil 9 vaccine is offered unless the child has a weak immune system. However, even after vaccine administration the girls continue to attend the routine screening of cervical cancer by liquid based cytology.

Therapeutic Vaccines

Therapeutic vaccines are in clinical trials and some of them are proving to be effective. [79] Therapeutic vaccines are of great concern for the infections that are already established because there is a need of cell mediated immunity rather than humoral immunity for the clearance of viral particles. E1, E2, E6 and E7 oncoproteins should be targeted by therapeutic vaccines because E1 and E2 oncoproteins are present highly in early infections while E6 and E7 oncoproteins are present in cancerous lesions associated with HPV.[80] The drawback of therapeutic vaccines is also the high cost due to conventional expression systems in lower-middle income countries having increases number of HPV cases; this can be overcome by using plant expressions systems in place on conventional expression systems which can reduce the cost more than 50%.[81]

Several types of therapeutic vaccines are in clinical trials to make them available for their use for treatment purposes & few of them are discuss below:

DNA Vaccines

DNA vaccines provide an alternative form of treatment for HPV. DNA vaccines produce Cytotoxic T-cells & antibodies by delivering foreign antigens that in turn stimulate CD4+ and CD8+ T-cells. As compared to live & vector vaccines, DNA based vaccines are quite safe & effective. [82] Also they are easy to prepared on large scale with high purity & stability & can be synthesized to produce antigen tumor peptides or proteins. These vaccines also help in improving immunologic memory.

They can easily be given to a single patient for multiple times; without any safety hazards. DNA sequences are used to express proteins beyond MHC inhibition and thus can work in different patients having varied MHC-l molecules. However, their immunogenicity is quite low because DNA does not have a specific cell type. Their effectiveness can be improved by identifying antigens that are DNA encoded to specialist antigen producing cells and thereby modifying their properties to enhance the body's immune response. [82, 83]

The major role in the formation of antigen specific anti-tumor and anti-viral T-cell is produced by Dendritic cells, & by increasing the number of dendritic cells, enhancing the expression of antigens & improving the interaction between Dendritic cells & T-cells, the efficacy of DNA based vaccines can be enhanced. Methods for delivery of novel vaccines increases the potency include microencapsulation, Gene gum and electroporation. Detox (pnGVL4a-CRT/E7) is in phase II clinical trials for the treatment of CIN2/3 lesions.[84]

Live Vector Vaccines

Live vector vaccines are those that include harmless bacteria or virus & can replicate within the host cells. These virus &bacteria aid in the proliferation of antigens.[85] They provide high level of immunogenicity thus unsafe in patients who are immunocompromised. Live vector vaccines deliver E6 and E7 antigens to CD8 + cytotoxic cells and CD4+ T-cells that help to attack a specific HPV antigen. In different clinical trials Listeria monocytogenes, Lactobacillus lactis and lactobacillus casei are used as bacterial vectors. ADXS-ll-001 contains Listeria monocytogenes is in phase l and or ll clinical trials. [84] Vaccinia vector, adenovirus, flow pox & alpha virus are used as viral vectors. Vaccinia vector based vaccines that produce HPV 16, 18 E6 and E7 antigens is in Phase II clinical trials.[84]

Protein & Peptide Vaccines

Peptide vaccines are specific to major histocompatibility complex (MHC) and need to match with person's body leukocyte antigens. Protein based vaccines are non-specific to MHC because the vaccine contains all antigenic human leukocytes antigenic epitopes. Due to presentation of MHC II complex, they promote antibody response over T cell responses. [85] They both are safe & can be produced easily but they show low immunogenicity that's why they required co-administration of adjuvant for better effectiveness. HspE7 vaccine is another therapeutic vaccine is in phase II clinical trials for the treatment of CIN2/3 or HSIL lesions. [86]

Adjuvant Human Papillomavirus Vaccination for Patients Undergoing Treatment for Cervical Intraepithelial Neoplasia 2+

Current observational studies and meta-analyses data show that adjuvant in the setting of surgically-managed CIN 2+, HPV vaccination reduces the recurrence of cervical dysplasia [89,90,91,92]. Another meta-analysis report showed that adjuvant HPV vaccination with surgery reduced risk of new or persistent CIN 2+ 3 . Improved quality of life and cost savings due to fewer diagnostic tests are manifestations of adjuvant HPV vaccination for CIN 2+ [93].

CONCLUSION & CONCERNS:

All countries are encouraged to incorporate standard HPV vaccines into their health care systems. For those women who are already infected with HPV various screening and treatment procedures are available to prevent them from cervical cancer. It is proven from several clinical trials that for women who are over the age of 30, HPV-based screening is the most effective method for preventing them from cervical cancer & also many prophylactic vaccines are available for the prevention of cervical cancer but the big problem is that licensed HPV vaccines are too expensive to be used worldwide and are not intended for all types of HPV. Therefore, the next generation of preventive vaccines must address two key issues such as reducing the cost of vaccines in developing countries, and increasing the number of HPV strains covered in order to increase protection against HPV-associated malignancies.

In addition, there are several factors that indicate the need for a therapeutic vaccine rather than a preventive vaccine. Most important of these factors is the widespread increase in HPV infection worldwide & for treating the HPV infection there is need for therapeutic vaccines. [87, 94] Therapeutic vaccines are of great concern for all those women who are currently infected with HPV infection & need treatment, so they can be prevented from invasive cervical cancer. Immunization Advisory Committee recommended that

only targeting the women who are sexually inactive is critical for preventing cervical cancer, evidence suggest that targeting sexually active women is of great benefit. Although in some countries where screening programs are not adequate enough so in these countries prophylactic vaccines greatly help out in decreasing the HPV burden. The high cost of current vaccines has prompted researchers to continue searching for new ways to produce and deliver vaccines for HPV. [88] The most effective way is using plant sources rather than by fermentation & cell expression systems of mammals or insects. HPV E6 and E7 are of main concern for several treatment strategies because they are fundamentally express in HPV infected tissues & plays important role in cell cycle disruption. [85]

Recommendation: Proper vaccination, screening & awareness programs in developing countries could reduce the burden of cervical cancer.

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