Evaluation of Emerging in-Vitro Assays in Cervical Cancer

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***Abstract*— Cervical cancer is one of the most common types of gynaecological cancer in women. It's also one of the most frequent cancers in women, and it's one of the few that may be detected and treated entirely at the precancerous stage. Cervical cancer is caused mostly by sexually transmitted diseases (STDs), as well as the Human Papillomavirus (HPV), particularly HPV-16 and HPV-18. In India, it is still a big public health issue for women. Cervical cancer strikes women between the ages of 55 and 59, and a large percentage of those diagnosed are in advanced stages of the disease. Cervical cancer vaccinations include prophylactic vaccines against HPV-16 and 18 therapeutic v. Premature sexual activity, teen pregnancy, family history, and oral contraceptives are all epidermal risk factors. This page discusses the history of cervical cancer, histological types, risk factors, prevention, therapy, and drugs approved to prevent cervical cancer. Squamous cell carcinoma is the most frequent kind of cervical cancer. Only persons who have never been infected with HPV benefit from the vaccines.**

***Index Terms:* Cervical cancer, human papillomavirus, prevention.**

INTRODUCTION

It is the most frequent cancer in underdeveloped countries, accounting for around 86 percent of all deaths. It is seen in countries with a low and middle income. Surgery and chemotherapy are beneficial at the primary stage. Surgery can also help patients with cervical cancer live longer. Chemotherapy and radiation therapy are not appropriate for many patients. In the 1523 patients who received radiation or chemotherapy, 179 had a favourable response and 1344 had a negative reaction, with an overall rate of 88.6% being negative to surgery. According to one study, the removal of primary uterine cancer, metastatic cervical cancer, was almost certainly limited to the lymph nodes, thus the development of new cancer cells was in balance with the elimination of existent cancer cells, probably by the immune system. According to kamura.et al., the typical treatment plan for cervical cancer is radical hysterectomy with pelvic lymph node examination. Certain drugs, such as corticosteroids, may disrupt the fragile balance between cancer cell renewal and death by the immune system, whether innate or not. In addition, it acts as a distinguishing factor between cervical cancer that is persistent and cervical cancer that is less progressing. Physicians should make greater use of therapeutic options such as radiation and chemotherapy. According to a study in India, 122,844 women were diagnosed with cervical cancer, with 67,477 of them dying. India is one of the world's most populous Approximately 432.2 million women under the age of 15 are diagnosed with cancer. [1,2, 3].

HISTORY OF CERVICAL CANCER

Pericles Hippocrates, a Greek physician, was the first to describe cervical cancer in 400 B.C. It was thought to be incurable at the time. An Italian surgeon recognised the pathogenic process some 2000 years later, thanks to pioneering study. Dr. Rigoni Stern found that nuns had a low prevalence of cervical cancer in the mid-nineteenth century (Rigoni-stern, 1842). All of this research suggests that sexual intercourse is associated to the development of cervical cancer. As a result, cervical cancer was thought to be highly transmissible. The discovery of human papillomavirus (HPV) DNA in cervical cancer and warts by German scientist ZurHausen in 1976 was the first report of transmitting agents. ZurHausen, Gissmann, and their colleagues discovered the structure and sequence of HPV in 1985. The later discovery of the HPV vaccination resulted in a watershed moment in the disease's treatment. [1].

PATHOLOGY

The abnormal development of cells in the cervix characterises cervical cancer. The most common location is where the uterus meets the vaginal canal. Although HPV is a prevalent cancer in women, the use of a pap smear lowers the mortality rate. In poor countries, cervical cancer is very common.

HISTOPATHOLOGICAL TYPES OF CERVICAL CARCINOMA

1 Squamous cell carcinoma is detected in 66 percent of cervix squamous epithelial cells.
2 Endocervicaladenoocarcinoma mucus-producing glandular cells are detected in 28% of patients.
3 Adenosquamous carcinoma and neuroendorine carcinoma are rare cancers that account for just 6% of all cases. [1].

EPIDEMIOLOGY

In the last two decades, cervical cancer has been the most common malignancy among women. In India, this occurrence is most common in those aged 55 to 59. Between 2009 and 2011, the Aizawl district in India's north-eastern region had the highest risk of cervical cancer, with an age-adjusted rate of 24.3 percent, followed by Barshi Expanded at 19.5 percent, and Bangalore at 18.9 percent. Squamous cell carcinoma, which starts in the ectocervix, and adenocarcinoma, which starts in the endocervix, are the two most prevalent histological types of cancer. Even in the absence of the control programme, the complete population-based registry has demonstrated a continuous increase in age-adjusted rates. Cervical cancer rates declined by 1.8 percent each year on average among women aged 30 to 64, but they still accounted for 16 percent of all female cancers. Cervical cancer was the second most frequent malignancy in Odisha, with a 3.1 percent increase from 2001 to 2011. The north eastern district of Tamilnadu in southern India shows a disnictive pattern with a high incidence of cervical cancer. This could be caused by a human papillomavirus infection (HPV). The high burden of cervical cancer in south and south east Asian countries is due to a high prevalence of HPV (More than 10% in women aged more than 30 years) and due to lack of screening [3,5].

RISK FACTORS

Approximately all cervical cancer cause by HPV. The climax age of cervical cancer is about 47 ages of years. HPV usually transmit through sexual contact, it can spread without sex, by skin-to-skin contact with infected area of body. HPV infection diagnosed in young women lasts from 8-13 months [1].

OTHER RISK FACTOR

- Dietary habits: A diet lacking in fruits, vegetable as well as being in excess of weight, greater than before increases risk of cervical cancer.
- Sexual activity: HPV infection is most common route passes through sexual contact. Particularly early onset sexual activity, high risk sexual partners. Multiple partner and failure to use condoms.
- Family history: It is transmitted genetically from mother to her baby or to the sister has 2-3 times development of cancer is occurring.
- Smoking: Smoking also increases risk of squamous cell cancer by sensational body to cancer causing chemical change and also by weakening immune system.
- Multiple pregnancies: Women with 3 or more pregnancies cause hormonal changes and immune system is weak during period of pregnancy.
- Diethylstilbestol: DES increases risk of adeno carcinoma in cervix, especially in women whose mother took DES when pregnant.
- Oral contraceptive: Some research studies recommend that use of oral contraceptives for birth control, may be connected with an raise the risk of cervical cancer [1].

Causes

1 The majority cervical cancer cases are cause by the sexually transmitted human papilloma virus (HPV).
2 HPV is the same virus that causes genital warts. There are about 100 different strains of HPV. Only definite type’s causes cervical cancer, these 2 types that most commonly caused cancer are HPV- 16 & HPV-18. Being infected with a cancer causing strains of HPV.

HPV can also cause other cancers in women and men this include:-

1. Vulvar cancer
2. Vaginal cancer
3. Penile cancer
4. Alal cancer
5. Rectal cancer
6. Troat cancer

HPV

is a prevalent infection among sexually active adults who may contract it at any moment during their lives [4]. HPV Human papillomavirus (HPV) is the most prevalent viral infection of the reproductive system, infecting nearly all sexually active women and men at some point in their lives. Some people may become infected multiple times. Humans and males are most susceptible to infection shortly after becoming sexually active. HPV is sexually transmitted, however penetrative intercourse does not necessitate skin-to-skin transmission. The most common form of transmission is genital touch. With certain kinds of HPV, a small part of the virus can remain and proceed to cervical cancer. The infection with certain HPV types also causes a proportion of cancer of the of the anus, vulva, vagina, penis and oropharynx, which are preventable using similar primary prevention strategy as those for cervical cancer [7].

HPV Vaccine

HPV vaccine is given start from age of 9 years to 45 years. Most people who having age above 26 will not benefit of HPV vaccination. Under the age of 15 years children, they require 2 doses of HPV vaccine [8].

Symptoms

1 Blood spots or light bleeding occurs during menstrual cycle.
2 Menstrual bleeding that is longer and heavier than typical.
3 Bleeding after intercourse, douching or a pelvic assessment.
4 Increased vaginal release. 5. Pain occurs during sexual intercourse.
5 Blood loss after menopause.
6 Mysterious constant pelvic and or back pain [7].

Prevention

1 Avoid smoking and avoid using oral contraceptive for long time
2 It is also can be prohibited by avoiding hazard factor and by getting regular pap test also known as Pap smear.
3 A vaccine is a most important avoidance for cervical cancer..
4 Avoid many sexual partners during sex.
5 Change in life style or eating habbits.
6 Avoiding other risk factors like early marriage/ child bearing and smoking [2].

Treatment

- Surgery is a useful for treatment to most cervical cancer.
- If the cancer has spread locally within the tissue, one of two type hysterectomy may be required. A straight forward hysterectomy that removes the Uterus and cervix will be enough in some Cases.

- Radical HysterectomyThe principal connective tissue (parametrium) and ligaments, as well as the upper portion of the vagina, must be removed. If necessary, either of these surgeries can be combined with the removal of the fallopian tubes and ovaries. The removal of the ovaries leads in infertility, while the removal of the fallopian tubes causes the female to enter menopause. During the surgery, lymph nodes may also be removed.

- Radiation Therapy It's also regularly used in conjunction with surgery in the treatment of cervical cancer. If the cancer has engulfed and spread beyond the cervix's surface.

- Brachytherapy Uses radioactive rods or pellets placed in the body to focus radiation on the cancer and decrease side effects. Premature menopause can also be a side effect of pelvic radiation therapy. Bladder inflammation or vaginal constriction caused by scar tissue buildup [6].

Chemotherapy

It is commonly used in cervical cancer of the uterus. Such chemotherapy is essential to search for and destroy as various cancerous cells as possible [6].

Drugs

Treatment with drugs that target gene changes in cells causing cancer is often called targeted therapy. Cisplatin, carboplatin, (chemotherapeutic agents and bevacizumb) targeted therapy are available treatment choice [9].

Drugs used to treat cervical cancer drug name brand name Cisplatin. Platinol, platinol-AQ. Carboplatin. Paraplatin. Topotecan. Hycamtin. Bevacizumb. Avastin, Mvasi.9 Cyclophosphamide. Ifex .

CELL CULTURE AND CHEMICALS

ATCC, Manassas, VA, USA, provided the ME-180 cervical cancer cell line. The cells were kept at 370C in a humidified 95 percent air/5 percent CO2 incubator in DMEM (50:50, v/v) media supplemented with 10% FBS, 1% antibiotic (100 U/mL penicillin, 10 mg/mL streptomycin in 0.9 percent normal saline), and 0.5 mg/mL hydrocortisone (Chatterjee and Kundu, 2020).

GIBCO, ThermoFisher Scientific, India provided all of the cell culture chemicals. Antibodies were obtained from Abcam in Cambridge, England. MTT and -Lipoic acid were acquired from Sigma Chemicals. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) were utilised in this work (Sigma, St Louis, MO, USA)

CELL PROLIFERATION AND VIABILITY ASSAY

MTT assay was used to determine the anchorage-dependent short-term cell viability of -Lipoic acid treated cells (Chatterjee et al., 2021). ME-180 cells that were exponentially proliferating (8000–10,000 cells/well) were sown in 96-well plates and allowed to develop to 70–80% confluence. For 24 hours, cells were exposed to increasing concentrations of -Lipoic acid. After rinsing the cells with 1X PBS, each well was filled with 0.05 percent MTT and incubated at 37°C for 5 hours to generate formazan crystals. . The crystals were dissolved by adding 100L of 0.2 percent NP-40 detergent after 1 hour of incubation in the dark. Using a microplate reader, the colour intensity was then measured spectrophotometrically at 570nm. The results were represented graphically as percentage viability vs plant extract concentrations. Each data point was calculated three times, and all assays were carried out at least three times.

MEASUREMENT OF LONG-TERM CYTOTOXICITY BY CLONOGENIC CELL SURVIVAL ASSAY

According to the technique, a clonogenic cell survival assay was used to investigate the effects of -Lipoic acid on the ability of cervical cancer cells to form colonies (Nayak et al., 2020). In a nutshell, 500 ME-180 cells were planted in a 12 well cell culture plate and then treated for 72 hours with the IC50 concentration of -Lipoic acid, as well as several concentrations below and above it. The drug media was then changed with new media following the time periods specified above, and the cells were allowed to double in size for an additional eight times. The media was then discarded, and the colonies were manually counted after being stained with 0.9 percent crystal violet.

MEASUREMENT OF DNA DAMAGE BY ΓH2AX IMMUNOFLUORESCENCE ASSAY

H2AX level was determined using an immunofluorescence technique to determine the DNA damaging potentiality of -Lipoic acid in cervical cancer cells (Chatterjee and Kundu, 2020). ME-180 cells were cultivated on a 6-well tissue culture plate and treated with the -Lipoic acid for the time intervals specified. The cells were then rinsed in 1X PBS and fixed for 20 minutes at -20°C in an acetone: methanol (1:1) solution. The cells were rinsed twice in 1X PBS before being treated for 2 hours at 37°C with the primary antibody anti–H2AX (1:500 dilutions in 1X PBS). Unbound antibodies were then removed by washing twice with 1X PBS before incubating for 1 hour at 37°C with a secondary antibody conjugated with TRITC (1:750 dilutions in 1X PBS).Cells were washed thrice with 1X PBS and nuclei were counter-stained with DAPI. Finally, the images were captured using an inverted fluorescence microscope (Nikon, Japan) at 20X magnification.

WESTERN BLOT ANALYSIS

The expressions of numerous apoptotic, anti-apoptotic, and angiogenic markers were checked by

Western blot analysis (Chatterjee et al., 2021). ME-180 cells were trypsinized and grown at a density of 1x105 cells per well in 6-well plates. The cells were then subjected to the treatment conditions listed above. RIPA lysis buffer (1 percent Triton X-100, 20mM Tris–HCl pH 7.5, 150mM NaCl, 1mM EDTA, 2.5mM sodium pyrophosphate, 1M-glycerophosphate, 1 mM sodium orthovanadate, 1 mg/ml PMSF) was used to isolate whole-cell extracts. SDS-PAGE was used to fractionate the extracts, which were subsequently deposited onto nitrocellulose membranes. The membranes were incubated successively with the primary (1:1000 dilution) for 4h at room temperature and then secondary (1:2000 dilution) antibodies for 3h at room temperature and exposed to the chemiluminescence reagent for signal detection. Densiometric analysis was done by using ImageJ software.

ELISA

Indirect ELISA was used to evaluate the expression of representative soluble angiogenic indicators (VEGF-A, ANG-1, and ANG-2) according to the methodology (Chatterjee and Kundu, 2020). Briefly, the protein antigen (30 g) mixed in coupling buffer was coated onto a 96 well microplate (3679, Corning, NY, USA) and stored overnight at 4°C, followed by washing with 1X PBST and blocking with super cocktail buffer. Then, at room temperature, 1° antibodies were added and incubated for 2 hours. After washing twice with 1X PBST, the wells were incubated for 45 minutes at room temperature with a 2° HRP coupled antibody, then washed twice with 1X PBST. Then, 2, 2′-azinobis (3-ethylbenzthiazoline-6- sulfonic acid) substrate solution was added, incubated for 10 min in dark and absorbance was read at 405 nm using microplate reader (Berthold, Germany).

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