

Traditional approach, an alternative to human papillomavirus therapy

Abstract

HPV been a major cause of cervical cancer, with cervical cancer been one of the leading cause of annual death in adult women, there is need for prevention of cervical cancer by finding a cost effective therapy for the major causative agent (human papillomavirus). The major antiviral therapy against HPV is the cidofovir (CDV) which aside been expensive as side effects ranging from renal condition to ocular disturbances. Though alternative medicines approaches ranging from cervical cancer symptoms treatments using globally accepted Homeopathic approaches, to the allopathic approach by Indian, Chinese and Japanese were adopted ways of treating HPV associated diseases but there mechanism of action is not known. Therefore there is need for more researches to be done on antiviral agent against HPV, Echinacea therapy has been reported to not only reduce viral replication but also boost the immune system, it also has promise of been easily accessible and less expensive in Nigeria. Though there is no basic scientific evidence of Echinacea therapy against HPV been reported as at time of this review, research in this field therefore is recommended.

Keywords: human papillomavirus, therapy, allopathic, immune system

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Introduction

Human papillomavirus (HPV) is one of the leading cause of diseases transmitted through sexual activities but not gender specific, HPV occurs globally but more common in developing countries, asymptomatic infection account for almost 44% of the population.¹ HPV is a small, double-stranded DNA virus; the viral tropism is the epithelium. Over 200 HPV type species have been discovered, their outer capsid protein L1 are differentiated by its genetic sequence which serve as the basis of classifying the various HPV types. Studies showed that the sexually active individuals are more at risk to be exposed to Human Papillomavirus as the virus is mostly found in the genitalia.² Majority of Human Papillomavirus types invades the cutaneous epithelium (stratified epithelium) causing skin warts. While some types invade mucosal epithelium; they are classified in to two, base on severity of disease they cause, infection with low-risk (non-oncogenic) types, such as types 6, 11, 40, 42, 43, 44, 54, 61, 72 and 81. Can cause benign reactive alterations or Atypical Squamous cell of undermined significance or low-grade cervical cell abnormalities, genital warts and laryngeal papillomas. High-risk (oncogenic), HPV types, such as type 16, 18 31, 33, 35 39, 45, 51, 52, 56 and 59 are the major cause of cervical cancer, it also causes anogenital cancers. The oncogenic HPVs can cause low-grade cervical intraepithelial lesion, high-grade cervical intraepithelial lesion that are precursors to cancer of the cervix, and anogenital cancers. Oncogenic HPVs are seen in about 99% of cancer of the cervix, it is also reported to be involved in the pathogenesis of penile, vulvar, anal, and head and neck carcinomas, and oral malignant disease. Despite the strong proofs that relate infection with a high-risk HPV type to pathogenesis of cancer of the cervix, most immuno-competent women have the ability to clear this virus. Also detection of this virus at an early stage will aid in the prevention of the malignancy.³ Papillomaviruses possess oncogenes (E5, E6, and E7 genes) that bind the products of tumor suppressor genes.⁴

Properties and biology of HPV

Human papillomavirus is a DNA virus belonging to family *Papillomaviridae*, with a diameter of about 55nm and genomic length of 8 kbp, it is a circular double stranded DNA virus, with an envelope. There are two distinct regions within the circular genome: one that codes for the regulator proteins produced early in the replication cycle and another that codes for the structural proteins synthesized later. The icosahedral capsid of HPV is formed by two proteins which are L1 and L2 proteins; each of the capsid is made up of its building block of 72 capsomeres. Each capsomere has 5 monomeric units forming a pentamer. L1 pentamers are arranged to form a network of pentameric disulphide bonds aiding the capsid stability. In other for the viral capsid to assemble, the pentamers join to copies of L2 that occludes the center of each pentavalent capsomere.⁵ Thus, each virion contains 72 copies of the L1, which serve as the major component of the capsid, while the number of L2 varies.⁶

Life cycle of human papillomavirus

HPV has an affinity for stratified Squamous epithelium, which enable its life cycle to be in cohort with cellular differentiation in this tissue. The basal layer of a stratified epithelium contains poorly differentiated keratinocytes which the HPV infects. But the virus has to go through the superficial cells, to the intermediate cells, then Parabasal cells, and then before it finally infects the basal cells, the HPV takes about average of 10 years or more post infection before getting to the basal layer.⁷ The HPV viral genome entry and maintenance requires active cellular division, while the basal layer cells are the only actively dividing cells in the stratified epithelium, hence the reason why HPV specifically target the basal keratinocytes.⁸ The viral genome are maintained in the basal layer at a low copy number, as terminal differentiation of infected cells occur, viral genes will reprogram the parabasal and intermediate cells to join the cell cycle. Viral replication is solely dependent on the host DNA synthesis and

repair machinery, which is followed by late gene expression and viral progeny production in and release from the superficial layers of the terminally differentiated stratified epithelium.⁹

Viral proteins and their functions

The replication and transcription of HPV has some unique features the following viral proteins are responsible for these features:

E1 protein: this protein is required by papillomavirus for their replication, as they (E1 proteins) binds to the origin of replication specifically to the E1 binding site located in the origin, E1 has the helicase ability there by aiding the DNA unwinding, it also has the ATPase function, the E1 protein can also be referred to as replication initiator protein. It also assists the E2 protein in mediating repression there by blocking transcription in the promoter region.⁹

E2 protein: they are located at the long control region (LCR). They are the transcription regulatory protein and also exact there effect by facilitating the binding of E1 to the site of origin. E2 protein has also been reported to aid in the viral packaging.

E3 protein: they are not present in most papillomavirus and are reported to have no function in the viral life cycle of the virus that expresses them.⁸

E4 protein: they are protein that in the expression of late genes, aids virus maturation and virion release.⁸

E5 protein: They are minute hydrophobic peptides, primarily located in the endoplasmic reticulum with a size of about 83 amino acids joined by peptide bonds, which function by enhancing the activity of E6 and E7 transformation, it was reported also to alter the action of Epidermal growth Factor Receptor.⁸

E6 protein: this protein binds to tumor suppressor protein P53 there by degrading its activity of preventing carcinogenesis. This depicts loss of activity of individual surveillance leading to development of tumor. E6 protein is approximately 150 amino acids in size, it is regarded as an oncoprotein in HPV due to its activity in oncogenesis, E6 protein exact it effect by forming trimetric formation with E6-Ap and P53 which lead to defective cell cycle control thus uncontrollable cell growth.⁸

E7 proteins: they are also oncoprotein as E6, they exact there oncogenic effect by binding to the tumor suppressor protein the Rb protein, Rb protein binds E2F factors to suppress tumor, the E7 protein disrupt this Rb protein-E2F factors interaction.

L1 protein: five monomeric units of 55 kDa self assembles to form a pentameric capsomers. It has immunogenic ability, as it neutralizes antibody specifically produced against the HPV type, L1 protein is the major target in prophylactic vaccination. It is believed to contain the determinant for viral attachment to surface receptor of the cell

L2 protein: it helps in packaging the viral DNA into its virion; it is also reported to aid viral binding to cell receptor, and delivering to nucleus for DNA replication.⁸

Prevalence of HPV in Nigeria

Clarke MA et al.,¹⁰ conducted a research in rural Nigeria, their research was targeted to detect HPV in relation to abnormalities that occur in the cervix, it was a population based study and Stratified random sampling was used. The age ranges 15 years and above, the research targeted females that have had sexual relationship in their

life in which their cervixes were examined. PCR technique was used to detect HPV's DNA and liquid based cytology for cervical abnormalities.¹⁰ reported a 14.7% presence of detectable high risk HPV which accounts for two third of the participants. Another research carried out by in north-eastern Nigeria reported that 48.7% of the participant are positive for HPV type 18, while HPV type 16 accounted for 13.2%, with HPV type 31, 33 and 35 together accounted for 18.5%. Making high risk HPV type 18 as the predominant HPV in that region of the country as reported by Mohammed. In a research carried out by Thomas JO et al.,¹¹ in Ibadan South-West Nigeria it was reported that, of the 932 sexually active women with age 15 years and above who participated in the research for Human papillomavirus prevalence study, shows the presence of high risk HPV (type 16, 31, 35, and 58) predominantly, accounting for 19.7% of the participant, while HPV type 16 and 35 both accounted for 3.2% each, of the sample size.¹² Conducted a study in Kano North West Nigeria, they targeted two hospitals in the capital city of Kano which are referral hospital in the state, and the study was aimed at establishing the prevalence of HPV and its risk factors in the state.¹² Conducted a study recruited 50 women with age 18+ from the department of gynaecology in the two centres (Aminu Kano teaching Hospital and Murtala Muhammad Specialist Hospital), the method for the HPV DNA detection was PCR, they reported that 76% of the participant were positive for either HPV type 16, 18 or both while 60.5% of the participant were co-infected with both HPV type 16 and 18.

Cervical lesion preventive measures

One of the most efficient measure in preventing cervical cancer is the prevention of the high risk HPV infection, this is achieved by the administration of vaccines such as Gardasil (which target HPV16, 18, 6 and 11),¹³ Cervarix (HPV16 and 18),¹⁴ and Gardasil 9 which targets HPV 31, 33, 45, 52, 58, this vaccine when administered to females before exposure to sexual activities reduces HPV targeted by vaccine infection risk, though still faces limitation in its inability to prevent other serotypes not covered by the vaccines, it is still the favourite prevention measure. Prevention of cervical lesions can also be achieve without the use of vaccines through standard Pap smear test, visual inspection with Lugol's iodine, visual inspection with acetic acid and molecular detection of HPV DNA and RNA.¹⁵

HPV therapeutic vaccines

HPV therapeutic vaccines are administered to treat HPV associated pre malignant and malignant lesions as oppose to Gardasil, Cervarix and Gardasil 9 which are used to induce the production of neutralizing antibodies to combat L1 capsid protein (which is inactivated during viral integration), HPV therapeutic vaccines are administered in other to target onco-proteins such as E6 and E7 proteins, these are proteins HPV expresses throughout their life cycle, though this therapeutic vaccines are yet to pass clinical trials, most are in phase II and III of clinical evaluation, an example of HPV therapeutic vaccine that has passes the clinical phase III evaluation is the MVA E2 which targets HPV16, and contains the bovine papillomavirus E2 protein, since the E2 protein regulates the expression of E6 and E7 onco-proteins, truncated E2 (which the MVA E2 when introduce to the HPV infected person act as) serves has negative regulatory gene to E6 and E7 promoter sequence, therefore the E6 and E7 onco-proteins are not transcribe in HPV infected cells, thereby reducing progression to malignant. Other HPV therapeutic vaccines are reported to have local mild to moderate side effects.¹⁶

Laboratory testing of new anti-HPV agents

In vitro activity of novel 1,3-oxazole derivatives against human papillomavirus

Kachaeva MV et al.,¹⁷ Synthesized a novel 1,3-oxazole derivatives and defined their antiviral activity against human papillomavirus in vitro, after the primary, secondary and cytotoxic assay, bioassays showed that some of the synthesized compounds of 1, 3- oxazole derivatives exhibited potent antiviral activity against low-risk HPV-11 (IC₅₀ =1.7–9.6 μM) in a transient DNA replication assay and has a low cytotoxicity in HEK293 cells when compared to cidofovir (CDV), which is an antiviral agent in clinical use.

Primary assay: Transient replication of human papillomavirus-11 replication in a plasmid that contains origin, transfected in Human Embryonic Kidney293 cells

Primary assay is process of testing drugs against single drug target or small groups of drug target. Transient replication of human papillomavirus 11 replication in a plasmid that contains origins transfected in HEK293 cells is an example of primary assay. This assay uses a vector to show the expression of some Human Papillomavirus replicating protein such as E1 and E2 proteins, together with origin of replication contained in a suitable plasmid which is transfected in human embryonic kidney293 cells.¹⁷ Cells will be cultured against the test compound of choice at the different exposure time, this is done both before and after the transfecting the HEK293 cells, followed by harvesting a low molecular weight DNA after 2 days post transfection, a restriction endonuclease (Dpn1) will be used to digest it, and transfected plasmid DNA that has not replicated are removed using an exonuclease III.¹⁷ Endonuclease resistant DNA will be multiplied invitro using a real time qPCR. Two controls will be run. The first is targeted at omitting the E1 expression vector to produce undigested and un-replicated DNA. The other, positive control was treated with the known inhibitor cidofovir.¹⁷ As embryonic kidney cells may have a different susceptibility to an agent compared to human foreskin fibroblast (HFF), a toxicity assay based on cell viability at the time of harvest on the second day is performed together with each transient replication assay in human embryonic kidney293 cells. More than 1·10⁵ cells were scored in a Bio-Rad Automatic Cell Counter, with a determination of the total numbers, number of trypan blue stained (dead) cells, and % of dead cells. In determining whether each compound possess antiviral activity which exceeds its level of toxicity in HEK293 cells, the concentration of each compound at which virus replication was inhibited by 50 per cent (effective concentration, EC₅₀), and the concentration of drug cytotoxic for 50% of the cells (CC₅₀) is calculated by regression analysis. A selectivity index is calculated according to CC₅₀/EC₅₀. CDV is tested for relative comparison.¹⁷

Secondary assay: Human papillomavirus-18 DNA amplification in an organotypic squamous epithelial raft culture of primary human keratinocytes freshly prepared from neonatal foreskins

Primary Human Keratinocytes (PHK) harbouring whole genomic HPV-18 plasmid replicas is developed into organotypic cultures, with or without the presence compounds to be tested (selected from the primary assay) at several concentrations for different durations. In

such raft cultures, viral DNA amplification usually takes place between day 10 and day 14 after lifting the dermal equivalent (collagen with embedded fibroblasts) and the keratinocytes monolayer seeded on it to the liquid medium/air interface. Test compounds are added to the tissue culture media at three concentrations from day 6 through day 13. The media and test compounds are refreshed every other day. To label host cell DNA replication, bromodeoxyuridine is added to the culture medium at 100 μg/ml for the final 12 h before harvesting on day 13. Total viral DNA copy numbers/per cell are analyzed by real-time qPCR with HPV-specific primer pairs. Inhibition is expressed as % of viral DNA copies relative to the untreated cultures.¹⁷

Assays for cytotoxicity

For one to successfully test for an antiviral a cytotoxic assay will have to be run parallel so as to determine the lethal dose. In doing this the same drug exposure is requires, such as same drug concentration, same dosage time, same cell tropism,¹⁸ so as to ensure all compounds cell toxicity can be compared directly. Standard neutral red uptake cytotoxicity assay can be performed, if compounds involved are in confluent human foreskin fibroblasts cell line, this can be by incubation for 7 days. Human foreskin fibroblasts cells will be cultured into a ninety six (96) well tissue culture plates, with ratio of 2.5×10⁴ cells per well in a standard growth medium. This is incubated for 24 hours, medium will be substituted with a Modified Eagle Medium with the constituent 2% FBS, compounds for cytotoxic assay will be added unto the tissue culture plates first row. This is followed by serial dilutions of 5 folds, tissue culture plate will be incubated for seven (7) days, hundred (100)μl of a 0.7 mg/ml neutral red solution in phosphate-buffered saline, is added to each well and incubated for 1 hour. Excess dye is reduced by rinsing with PBS. Cell monolayers are completely suspended by shaking. The OD is measure using a multichannel spectrophotometer with a wavelength of 550 nm. Viable cells are directly proportional to the OD. The 50% cytotoxic concentration (CC₅₀) of the test compound is seen as the concentration that reduced the absorbance of cells with test compounds to 50% that of controls.¹⁷

Conclusion

HPV been a major cause of cervical cancer, with cervical cancer been one of the leading cause of annual death in adult women, there is need for prevention of cervical cancer by finding a cost effective therapy for the major causative agent (human papillomavirus). The major antiviral therapy against HPV is the cidofovir (CDV) which aside been expensive as side effects ranging from renal condition to ocular disturbances. Though alternative medicines approaches ranging from cervical cancer symptoms treatments using globally accepted Homeopathic approaches, to the allopathic approach by Indian, Chinese and Japanese were adopted ways of treating HPV associated diseases but there mechanism of action is not known. Therefore there is need for more researches to be done on antiviral agent against HPV, Echinacea therapy has been reported to not only reduce viral replication but also boost the immune system, it also has promise of been easily accessible and less expensive in Nigeria. Though there is no basic scientific evidence of Echinacea therapy against HPV been reported as at time of this review, research in this field therefore is recommended.

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None.

Conflicts of interest

The author declares there is no conflicts of interest.

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