Introduction

Human papilloma virus (HPV) infections are very common and cause various benign and malignant lesions, most notably condylomata acuminata, anogenital carcinoma, laryngeal papilloma and cutaneous warts ***(Gross, 1997).***

There are more than 100 types of HPVs. HPV-1,-4,-27,-57 and-63 cause common warts ***(Kilkenny and Marks, 1996).***

Common warts are characterized by the formation of thick, hyperkeratotic lesions. Virus particles reside in the basal layer of epithelia, but replicate only in the well differentiated, superficial layer i.e. cells of the upper stratum spinosum and stratum granulosum. The ensuring superficial cellular proliferation gives rise to the characteristic morphology of warts ***(Jorge and Plasencia, 2000).***

While many warts resolve spontaneously over years, most patients seek treatment for painful, tender or even psychologically affecting cosmetic appearance. Regression of warts appears to represent an effective host response to HPV infection ***(Coleman et al., 1994).***

The persistence of HPV at cutaneous sites may be due to impaired trafficking of immune effector cells to superficial epidermis ***(Gross et al., 1997).***

Treatment of common warts involve 2 basic approaches, destruction of the warts and induction of local immune reactions ***(Sterling, Handfield-Jones, and Hudson, 2001).***

Destructive methods are most commonly used as initial therapy by most practitioners ***(Sterling, Handfield-Jones and Hudson, 2001).*** Cryotherapy is a reasonable first-line therapy for most common warts. Products containing salicylic acid with or without lactic acid are effective patient-applied treatments. These have an efficacy comparable to that of cryotherapy ***(Gibbs et al., 2002).***

Surgical ablation of warts can be effective treatment, but even complete destruction of a wart and the surrounding skin does not guarantee that the wart will not recur ***(Berth-Jones and Hutchinson, 1992).***

Oral cimetidine has been anecdotally reported for resolution of common warts, perhaps because of its immuno-modulatory effects ***(Berth-Jones and Hutchinson, 1992).***

Immunotherapy with topical and intralesional agents has become a mainstay of wart therapy. The commonly used agents are topical dinitrochlorobenzene, squaric acid dibutyl ester, and diphencyprone, as well as intralesional candida or mumps antigen ***(Parton and Sommerville, 1994).*** Imiquimod has been reported to be effective for common warts in small series or single cases ***(Atzori, Pinna, and Ferreli, 2003).***

No type of therapy has provided constantly effective results in treatment of common warts. Combining surgical ablative methods with immunomodulatory agents seems to be promising in treatment of recalcitrant warts ***(Berth-Jones and Hutchinson, 1992).***

A new approach is local immunotherapy of genital warts with viable Bacillus Calmette-Guerin (BCG). In the initially reported adjuvant postablative treatment, the annual recurrence rate in patients with refractory recurrent condylomata acuminata decreased significantly after BCG therapy ***(Bohle et al., 1998).***

Topical intravesical instillation of BCG is a standard adjuvant therapy for recurrent superficial bladder cancer, and its wide use may have contributed to decreased mortality, although the incidence of superficial bladder cancer has been increasing ***(Silverberg, Boring, and Squire, 1990).***

Because of the similarity of the immune response against malignant and virally transformed cells, researchers have extended the application of BCG to untreated genital condylomata acuminata ***(Bohle, Buttner, and Jocham, 2001).***

Aim of the Work

The present work aims to assess the result of topical application of BCG in treatment of common and plantar warts, its effectiveness, and its possible complications and side effects.

Human Papilloma Virus

General properties:

Human Papilloma Virus (HPV) is a member of the papoviridae family which comprises a large number of double stranded DNA viruses of approximately 8kb that are packaged into non enveloped particles of about 55nm ***(Munger, 2002).***

The HPV is extremely hardy and is difficult to eradicate from surfaces because it resists freezing, inactivation and desiccation on account of the lack of encapsulation ***(Schiller and Lowy, 2004).***

HPV is involved in the development of wide range of diseases from common warts to the rare epidermodysplasia verruciformis (EV) ***(Zur-Hausen, 1996).***

HPV infects the basal cells of the epithelium and multiplies in its upper more differentiated layers of the epithelium ***(Resnick et al., 1990).*** Hyperplasia and hyperkeratosis are the hallmarks of skin infection by dermatotropic *HPV* ***(Sterling and Kutz, 1998).***

There are over 150 distinct HPV subtypes, some tend to infect specific body sites and produce characteristic proliferative lesions at those sites ***(Bonnez and Richman, 2000).***The characteristics of the lesions depend on the type of HPV causing the infection. Type 1,2 and 4 are associated with common warts and plantar warts. Types 3,10,28,and 41 are associated with flat warts. Other HPV types are found in

patients with EV which are types 5,8,9,12,14,15,17,19-25,36,46,and 47 ***(Sterling and Kutz, 1998).***

Genital infections caused by HPV, which is also known as venereal warts or condylomata acuminata (CA) are most commonly caused by types 6 and 11 ***(Bernard, Calleja, and Dunn, 2006).*** Progression to carcinoma is rare, and carcinoma of this region is associated mainly with types 16 and 18 and are detected in almost all cases of cervical cancer ***(Weaver, 2006).***Carcinogenisis is associated with cutaneous HPV infections in certain patients such as organ transplant recipients, and patients with EV who have an underlying immune system abnormality ***(Leigh and Glover, 1995).***

Prevalence of HPV:

Regarding the prevalence of HPV in warts, the incidence of HPV in verrucae vulgaris and condylomata is estimated to be 7-10% in the European population and 1% in the American population ***(Hengge, 2004)*** , while the prevalence rates of HPV DNA in Egypt were 20%, and 60% in patients with common and genital warts respectively. This variation in prevalence rate may be either due to ethnic difference or high exposure to the virus ***(Zekri et al., 2005).***

Virology:

HPV genome has two coding regions and a non- coding regulatory region. The two coding regions are histologically divided into two groups, early (E) and late (L) genes that are

clustered in separate regions. The early genes include E1, E2, E4, E5, E6, and E7, which code for proteins involved in viral DNA replication, transcriptional control, and cellular transformation. The late genes encode (L1) and (L2) are the structural proteins of the virus ***(Doorbar, 2006).***

The function of E6 and E7 is to establish and maintain a cellular milieu that allows for viral replication. The E6 and E7 proteins of the high -risk HPV types, such as HPV 16 and 18, act as viral oncoproteins, but no such functions are associated with the corresponding proteins from the low- risk types such as HPV 6 and 11 ***(Stewart et al., 2004).***

During a common infection and in most premalignant lesions, HPV is in an episomal state ***(Daniel et al., 1997).*** However, most cervical carcinomas and the cell lines derived from them maintain the HPV genome in an integrated form or in both integrated and episomal forms ***(Cullen et al., 1991).***

The E6 and E7 oncoproteins are involved in cell transformation and their presence is necessary for carcinogenesis ***(Kado, 2001).***

Integration of the oncogenic E6 or E7 with human chromosomes has been regarded as a potentially important mechanism for tumor progression in the cervix (transformation of dysplasia into invasive carcinoma) ***(Pirami, Giache, and Becculini, 1997).***

The presence of integrated, oncogenic (E6 or E7) sequences may give the infected epithelium growth advantages

and may contribute to increased cell proliferation and genomic instability, leading to further genetic alterations ***(Kessis et al., 1996).***

The genome integration of HPV usually disrupts E2 gene open reading frames ***(Jeon and Lambert, 1995).*** It results in the lack of E2 gene suppression of the E6 and E7 products synthesis which, in turn, leads to overexpression of the E6 and E7 genes ***(Thierry and Howley, 1991).***

The E2 gene product plays various roles in HPV replication, being involved in E6 expression regulation and by indirectly controlling E1. The major role of the E2 protein in replication is to target E1, which is a weak DNA binding protein, to the ori (the origin of DNA replication) ***(Van Horn, Sheikh, and Khan, 2001),*** leading to the initial separation of the two DNA strands at the ori and local melting to generate a single stranded region of DNA ***(Patel and Donmez, 2006).***

The replication of papillomaviruses requires both viral E1 and E2 proteins ***(Van Horn, Sheikh, and Khan, 2001).*** The E2 protein positively regulates a transient replication of HPV and also represses the viral promoters ***(Fuchs and Pfister, 1994).*** The replication rate of HPV changes reciprocally, depending on the level of E2. Thus, the E1/E2 ratio is probably significant in this replication regulation ***(Van Horn, Sheikh, and Khan, 2001).***

Direct interaction between E2 and E1 can lower the concentration of E2 protein in the cell. It could, in addition to

the disrupting the E2 region of an integrated HPV genome, lower its regulatory influence on E6 and E7 expression ***(Titolo et al., 1999).***

In high-risk HPV types, E6 binds to p53 in a trimeric complex with an ubiquitin ligase called E6AP ***(Stewart et al., 2004)*** .Formation of this complex result in the ubiquitination of p53 and subsequent degradation by the 26S proteasome, leading to reduction in the half-life of p53 from several hours to less than 20 minutes in keratinocytes ***(James, Lee, and Klingelhutz, 2006).*** E7 binds to retinoblastoma (Rb) family of tumor supressors, as well as other proteins involved in cell cycle regulation ***(Dyson et al., 1989).***

E6 can also indirectly down-regulate p53 activity through its association with P300/CBP, which is a coactivator of p53 ***(Frame, 2002).*** Since p53 regulates both the G1/S and G2/M checkpoints of the cell cycle, its rapid turnover results in abrogation of these controls, leading to chromosomal duplications and centrosomal abnormalities ***(Thierry et al., 2004).***

Raskin (1997) found that there is no significant relation-ship between the frequency of p53 expression and either HPV type or lesion grade. HPVs express factor E6 that provokes p53 degradation via an ubiquitin-dependent pathway, hindering the apoptosis in the host cell.

Pathogenesis:

HPVs characteristically infect the cells of the epithelium whether on the genitalia or elsewhere. Although the skin is the most common site of extragenital HPV infection, infection can occur within the mouth, oesophagus, larynx, trachea, and conjunctiva ***(Stephen, Tyring, and Texas ,2000).***

Infection with HPV probably occurs as a result of exposure of the basal cells to virus particles following minor traumas to the epithelium eg, during sexual intercourse and minor skin abrasions ***(Shah and Howley,1996).***

Infection begins with viral entry followed by one of three paths: latent infection, in which there is no gross or microscopic evidence of disease, subclinical infection, in which microscopy reveals evidence of infection in the absence of clinical disease, and clinical disease ***(Brentigen et al., 2002).***

Only the more differentiated layers of the epithelium are permissive for capsid protein synthesis, vegetative viral DNA replication and assembly of viron, therefore virus particles and viral capsid antigen are found in the most superficial layers of the epithelium. Only a low number of viral genome is present in the basal and supra basal layers of the epithelium ***(Sanclemente and Gill, 2002).***

HPV multiplies exclusively in the nucleus. Viral particles and viron antigens are not found in the cytoplasm, except after injury of the mucous membrane. Koilocytosis is a characteristic

feature of many warts accompanied by nuclear enlargement, dyskeratosis and multinucleation being the major change ***(Herrington, 1994).***

Clinical and histopathologic evidence of HPV infection usually develops 1 to 8 months following initial exposure. Physical manifestations of infection include epidermal thickening, hyperplasia of the striatum spinosum, and some degree of hyperkeratosis ***(Brentjen et al., 2002).***

If the lesions are left untreated, they may regress spontaneously, or progress to precancerous lesions and eventually, cancer ***(Stephen et al., 2002).***

Various mechanisms have been described to explain the HPV immune evasion, including low antigenicity and active modulation of the immune system by the virus as recently reviewed by ***Kanodia, Fahey, and Kast (2007).*** Since HPV infects stratified epithelia of the skin and mucosa, it is thought that HPV targets the different epithelial Dendritic Cell (DC) subsets (DCs and Langerhans Cells (LCs)) to escape immune surveillance. HPV infection decreases both DC and LC numbers in HPV lesions and blood ***(Lee et al., 2006),*** inhibits LC activation and migration ***(Guess and Mc Cance, 2005)*** and induces the influx of plasmacytoid DCs into the cervical lesions ***(Bontkes et al., 2005).***

Thus, although HPV can induce an efficient immune response, it can also circumvent immune surveillance. It is becoming evident that the initial interaction of pathogens with

DCs through specific receptors can result in either immune activation or suppression ***(Bontkes et al., 2005).***

Diagnosis of human papilloma virus:

Diagnosis of HPV infection begins with a thorough clinical examination, which may need to be supplemented by histologic examination of suspicious lesions (e.g., pigmented anogenital warts) ***(Workowski and Berman, 2006).***

- Clinical evaluation:

Inspection of the external genitalia should be performed in patients of both sexes, using a bright light source and lens to aid in the evaluation of small lesions. Because cervical or vaginal lesions often accompany external lesions, all women with anogenital warts should have a speculum examination performed. Some authors also suggest anoscopy when perianal or perineal warts exist or the patient has a history of receptive anal intercourse ***(Palefsky, 2000).***

-Acetic acid:

The use of dilute solutions of acetic acid (3-5%) is valuable adjunct for delineation of disease before biopsy. HPV-associated lesions, after the application of acetic acid, develop a characteristic acetowhite appearance, which is thought to be the result of coagulation of epithelial cytokeratins ***(Sonnex, 1998).*** Clinicians often use the acetowhitening technique in conjunction with colposcopy (because of the lack of specificity

of acetowhitening alone) to identify suspicious lesions for biopsy in the cervix, vagina, and vulva ***(Von Krogh, 2000).*** The technique can also be used to aid in the diagnosis of oral and cutaneous HPV infections and to detect male genital tract disease ***(Bonnez and Wilczynski, 2000).***

-Cytology:

The papanicolaou (pap) smear is the cytologic technique routinely employed to detect HPV disease of the cervix and vagina. The sensitivity and specificity of pap smear for the detection of cervical atypia and cancer are difficult to determine, and interobserver variability has been documented as a problem in a number of studies ***(Stoler and Schiffman, 2001).***

The presence of koilocytes in conjunction with nuclear atypia and delayed maturation on the cytologic smear are hallmarks of HPV infection and correlate well with the presence of HPV DNA by polymerase chain reaction (PCR) ***(Sedlacek, 1999).***

Cytologic screening for anal HPV infection has also been recommended for homosexual men (HIV positive and negative) and women with known HPV genitourinary tract lesions ***(Palefsky, 2000).***

-Histology:

Biopsy of newly occurring, multiple, typical acuminate

lesions is unnecessary ***(Von Krogh, 2000).*** Biopsy is recommended in atypical cases or when the benign nature of the lesion is unclear (as with Bowenoid papulosis or giant condylomas). Punch biopsy, an excision technique, or biopsy forceps should be used. Histologic examination of warts demonstrates papillomatosis accompanied by the characteristic features of acanthosis, parakeratosis, hyperkeratosis and koilocytosis ***(Bonnez and Reichman, 2000).*** Koilocytosis is less often seen at sites other than the cervix ***(Sonnex, 1998).***

-Molecular-based methods:

Specific virologic methods to detect and/or confirm HPV infection are not in routine clinical use but are becoming more widely available ***(Wick, 2000).***

Molecular-based tests for HPV can be divided into three categories ***(Unger, 2000):***

-Nonamplification tests.

-Single amplification methods.

-Tests in which the target is amplified.

Examples of nonamplification methods for HPV detection include Southern blot and in-situ hybridization (with or without concomitant PCR amplification). An example of signal amplification method would be the Hybrid Capture Assay by Digene Diagnostic, Inc ***(Spitzer, 1998).*** PCR is a technique based upon amplification of target sequences with the use of general/consensus primers designed to detect a broad

array of HPVs. In situ hybridization can be performed on cell smears or histologic sections. Both PCR and the Hybrid Capture Assay can be applied directly to clinical specimens ***(Josefsson et al., 2000).***

Correlation between the viral load of HPV 16 and the development of carcinoma in situ of the cervix was described in two reports from a longitudinal study in Sweden ***(Ylitalo, 2000).*** In one study 20 percent of women with the highest HPV 16 quantities had a 60 fold increased risk of carcinoma in situ of the cervix compared to HPV negative controls ***(Josefsson et al., 2000).*** In the second study, women with an increased HPV viral load were detected as much as 13 years before the development of abnormalities on cervical cytology ***(Ylitalo et al., 2000).*** Persistence of high-risk HPV types has also been correlated with cervical abnormalities ***(Kjaer et al., 2002).***

Based on these types of studies, many experts now advise using HPV testing as an adjunct to routine screening programs for cervical cancer prevention. For example, the centers for disease control (CDC) in its most recent STD treatment guidelines, suggests that HPV testing may be useful for determining optimal follow up of women with atypical squamous cells of undetermined significance (ASCUS) and that HPV testing may have a role in screening programs for women aged more than 30 years ***(Workowski and Berman, 2006).***

HPV DNA testing is not recommended for persons with anogenital warts. There is also no indication for HPV testing