INTRODUCTION

Warts are benign proliferations of skin and mucosa caused by the human papillomavirus (HPV) ***(Cohen et al., 1993).***

Over 100 genotypes of viruses have been identified and a number of these can result in benign tumors of skin and mucosa. The clinical lesions encountered with such infections can be broadly divided into cutaneous, genital, oral and laryngeal warts ***(Peate, 2006).***

There is no cure for warts. Current therapies are divided into two groups: destructive therapies and immunomodulators. There were many techniques encompass most traditional prescription interventions, such as podophyllin, podophyllotoxin, mono-chloroacetic/ trichloroacetic/ bichloroacetic acid, 5-fluorouracil, bleomycin, retinoids, salicylic acid, glutaraldehyde, formaldehyde and cantharidin, as well as physical modalities such as surgical excision, cryotherapy, lasers or adhesive/duct tape and immunomodulators such as interferon, imiquimod, cidofovir and vaccines ***(Tyring and Rivera, 2004).***

There is a trend towards the use of immunotherapy in treatment of warts, as the immune system seems to play an important role in the control of warts infection ***(Frazer, 1998; Contant, 2000)*** and also due to the mounting evidence that cellular mediated immunity (CMI) has the ability to the

resolution of human wart disease, although the exact mechanisms by which this occurs are not yet fully understood ***(Friedmann et al., 2004).***

Intralesional injection of mumps and candida skin test antigens has been shown to be effective in the treatment of resistant warts & also warts at distant sites ***(Marchese et al., 2003).*** Recently the intralesional mumps, measles and rubella (MMR) vaccine has showed an efficacy in an Egyptian study ***(Nofal and Nofal, 2010).***

In a study done on 18 patients with different types of warts, the tuberculin purified protein derivative was injected intralesionally. Sixty one percent of patients showed a complete resolution and 16.67% had partial resolution. So tuberculin seems to be a promising therapy especially in countries where vaccination against tuberculosis is performed routinely ***(Salem et al., 2010).***

The Bacille Calmette–Guérin (BCG) vaccine is one of the most widely used of all current vaccines. BCG is given routinely in vaccination in Egypt ***(Kotb and Azza, 1993).*** BCG vaccine is of proven efficacy in the control of leprosy and probably also protects against cancer ***(Mandeville et al., 1983).*** It is also a potent inducer of T-cell responses ***(Siegel et al., 2000).***

Although tumors may be resolved due to host immune response, it is difficult to obtain direct evidence of this in man ***(Tagami et al., 1983).*** In animals, BCG can delay or prevent

the development of cancers induced with chemical carcinogens, oncogenic viruses as HPV or radiation ***(Bast et al., 1974).*** Also treatment with BCG can delay the onset of leukemias ***(Lemonde et al., 1971)*** and mammary cancers in animals genetically predisposed to the development of these neoplasms ***(Weiss et al., 1966).***

Many clinical researchs were attempted to prove the efficacy of BCG vaccine as an immunotherapy in warts whether by topical applied ***(Metawea et al., 2005)***, intradermal administration ***(Sharquie et al., 2008)*** or intralesional as in a study of ***Yuan et al. (2007).***

AIM OF THE WORK

To evaluate the efficacy & safety of intradermal injection of BCG vaccine (Bacillus Calmette-Guerin) in treatment of warts with or without the tuberculin antigen intralesional injection (purified protein derivative of Mycobacterium tuberculosis).

Chapter 1

1. HUMAN PAPILLOMA VIRUS

1.1. General properties

Human Papilloma Virus (HPV) is a member of the papoviridae family which comprises a large number of double stranded DNA viruses ***(Munger, 2002).***

The HPV is responsible for wide range of diseases from common warts to the epidermodysplasia verruciformis (EV). Dermatotropic HPVs are demarcated with hyperplasia and hyperkeratosis of skin ***(Sterling and Kutz, 1998).***

If the lesions are left untreated, they may regress spontaneously, or progress to precancerous lesions and eventually, cancer ***(Stephen et al., 2000).*** Carcinogenesis 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).***

The HPV is extremely hard 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).***

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 (cells of both upper stratum spinosum and stratum granulosum) ***(Jorge and Plasencia, 2000).***

The characteristics of the lesions depend on the type of HPV causing the infection ***(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).***

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, 15, 17, 19, 25, 36, 46 and 47 ***(Sterling and Kutz, 1998).***

Genital infections caused by HPV, which is known as condylomata acuminata (CA) are most commonly caused by types 6 and 11 ***(Bernard et al., 2006).***

1.2. Virology of HPV

HPVs are non-enveloped, double-stranded DNA viruses with 55 nm in diameter. The viruses are spherically shaped viral particles (virons) which consist of an outer

protein shell (capsid) surrounding a single closed super coiled circle of double stranded DNA of about 8000 nucleotide base pairs (8 kb) that comprises the viral genome ***(Van Ranst et al., 1996).***

The Papillomaviruses initially infect basal epithelial cells, which constitute the only cell layer in an epithelium that is actively dividing. The nature of the HPV receptor(s) remains unclear, although integrin α4β6 has been implicated ***(Evander et al., 1997).***

Similarly, the processes that mediate virus uptake, decapsidation, and nuclear import of the viral genome remain largely unknown. The viral DNA is maintained at a low copy number in the nuclei of infected host cells as they undergo differentiation and move toward the surface of the epithelium. In terminally differentiated cells, the virus replicates to a high copy number, late genes are expressed, and progeny virus is produced ***(Stubenrauch and Laimins, 1999).***

HPVs are nonlytic viruses, and progeny virus is shed into the environment as a cargo within epithelial squamae. The HPV early gene (E4) protein associates with keratin intermediate filaments, which affects the mechanical stability of the keratin network and may facilitate the release of viral particles ***(Doorbar et al., 1991).***

The design of the HPV infection cycle is tightly fitted to the differentiation program of its natural host, the keratinocyte. This has important consequences for the role of antigen-presenting cells in the priming of antiviral immunity. The confinement of HPV infection to epithelia puts the epithelial dendritic cell, the Langerhans cell (LC), in charge of the induction of T cell-dependent immunity. Because HPV-infected keratinocytes cannot reach the regional lymphoid organs, and HPV-infection of LCs does not result in viral gene expression, priming of antiviral T cells exclusively depends on cross-presentation of viral antigens by the LC ***(Offringa et al., 2003).***

Only one of the two strands of the circular papillomavirus DNA genome is actively transcribed. The genome can be divided into three major portions: a ~4-kb early (E) region that encodes nonstructural proteins, a ~3-kb late (L) region that encodes the two capsid proteins, and a ~1-kb noncoding long control region (LCR) that contains a variety of *cis* elements, which regulate viral replication and gene expression. E and L genes are numbered according to size; the higher the number, the smaller the corresponding ***(Munger et al., 2004).***

1.2.1. Early genes

Include E1, E2, E4, E5, E6, and E7, which code for proteins involved in viral DNA replication, transcriptional control, and cellular transformation ***(Doorbar, 2006).***

The E genes are transcribed at low levels in the basal cell layer and stratum malpighii. The first genes to be expressed following infection are the E1 and E2 genes, which are viral regulatory proteins that are responsible for controlling the transcription of the viral genes and replication of the viral genome. The E4 protein is expressed in the terminally differentiated keratinocytes where it causes cytokeratin collapse, thus facilitating the assembly and maturation of the virus. E4 is probably a late gene because it is expressed late in the cycle of virus replication ***(Tyring, 2000).***

One major effect of HPV proteins E5, E6 and E7 is that the epidermal cell cycle, which is normally blocked for cells that are supra-basal, continues so that HPV genome copy number can be amplified to high levels during viral replication for assembly into virions ***(Pfister, 2003).***

Some data show an association between HPV genotypes and cell cycle regulators. The cell cycle is governed by a family of cyclins, cyclin dependent kinases (CDKs) and their inhibitors (CDKIs) through activating and inactivating phosphorylation events. Key among these are p53, the cdk inhibitors (p15, p16, p18, p19, p21, p27) and retinoblastoma (Rb), all of which act to keep the cell cycle from progressing until all repairs to damaged DNA have been completed ***(Skomedal et al., 1999).***

An E6 and E7 protein of HPV induces immortalization of cells through their inhibitory effects on the tumour suppressor proteins pRb and p53 and disturbing cell cycle control ***(Howley et al., 1999).*** 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).***

1.2.2. Late genes

The 72 viral capsomeres are composed of L1 protein pentamers, and the capsomeres are associated with 12 or more copies of the L2 protein. Recombinant L1 or L1 and L2 can be generated in a variety of expression systems to produce self-assembled virus-like particles (VLPs), which approximate the structure of native virions. The HPV L1 the major capsid surrounds the viral DNA, thereby protecting it from degradation and it also enables the virus to bind efficiently to target cells ***(Kimbauer et al., 1992),*** while the minor capsid protein L2 plays important roles in the generation of infectious viral particles and in the initial steps of infection ***(Marusic et al., 2010).***

The VLP of papillomavirus display type-specific and neutralizing surface epitopes and are therefore useful as an

antigen to detect serum antibodies and as a subunit prophylactic vaccine ***(Lowy and Frazer, 2003).***

1.3. Pathogenesis

Human papillomavirus infect the cells of the epithelium whether on the genitalia or elsewhere. Although the skin is the most common site of extra genital HPV infection, infection can occur within the mouth, oesophagus, larynx, trachea and conjunctiva ***(Stephen et al., 2000).*** The HPVs are highly species-specific, and productive infection has never been observed outside their natural host tissue (no known infection of man by animal HPV) ***(Howley and Lowy, 2001).***

It is believed that the HPV virus enters the body after slight trauma to the epithelium and needs terminally differentiated epithelial cells for replication. Late-region genes are expressed in the differentiated cells near the epithelium's surface providing an easy mode of viral transmission. Early region genes are expressed in the basal cells of the epithelium but are unable to produce virus since these cells do not make the capsid proteins encoded by the late genes. All types of HPV replicate only within the host cell's nucleus, but the mechanism by which HPV types transform cells is unclear. The HPV genome replicates as an extrachromosomal episome or plasmid in benign HPV-associated lesions. However, the viral DNA is often

integrated into the host's chromosome in malignant HPV associated lesions ***(Beutner and Ferenczy, 1997).***

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).***

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. 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 layer 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).***

Most HPV infections are asymptomatic. However lesions can develop anywhere in a time frame between three weeks and eight months after infection, with most developing 3 months after infection. Of the HPVs characterized with an oncogenic effect, it is shown that carcinoma rarely develops immediately after infection ***(Beutner and Ferenczy, 1997).***

1.4. Immune system and HPV

The epithelial cells secrete variety of cytokines including proinflammatory cytokines, growth factors and chemokines that have direct antiviral and antiproliferative effects and can be induced by various stimuli, these cytokines include the transforming growth factor β (TGF-β), tumour necrosis factor (TNF), and the type I interferon (INF-α and -β), these cellular immunity called the innate immunity. Innate immunity is a highly effective set of conserved mechanisms used by multicellular organisms to recognize and counter the constant threat of microbial infections. There is evidence to indicate that innate responses are keys to controlling most infections, as well as contributing to inflammatory responses that are central components of disease. The cytokines have the ability to inhibit the proliferation in vitro of both normal and HPV-transformed genes including the early genes E6 and E7 ***(Stadnyk, 1994).***

The various cellular components involved in the recognition and effector phases of adaptive epithelial immune responses have been demonstrated in both cutaneous and mucosal HPV infected tissues. These include Langerhans cells, which capture antigens for transport to local draining lymph nodes and presentation to naive T cells; clonally expanded T cells that have homed back to infected epithelial tissues via mechanisms involving

chemokines and adhesion molecules; and accessory cells such as macrophages. A reduced number of epidermal Langerhans cells have been documented in lesions due to HPV. It is possible that a decrease in Langerhans cell density in the epidermis of HPV-infected tissues simply represents normal egress of antigen-carrying Langerhans cells from the epidermis to draining lymph nodes for antigen presentation to T cells or due to other as yet unidentified mechanisms, may contribute to impaired immune surveillance. Some suggest that the depletion of intraepithelial Langerhans cells associated with HPV infection may, along with other local immune deficiencies, contribute to prolonged infection or possibly malignancy ***(Memar et al., 1995).***

HPV infects stratified epithelia of the skin and mucosa, and targets the different epithelial denderitic cell subsets to escape immune surveillance, HPV infection take three ways decreases both dendritic and Langerhans cells numbers in lesions and blood ***(Lee et al., 2006),*** inhibits activation and migration of Langerhans cells ***(Guess and Mc Cance, 2005)*** and induces the influx of plasmacytoid dendritic cells into the lesions. It is becoming evident that the initial interaction of pathogens with dendritic cells through specific receptors can result in either immune activation or suppression ***(Bontkes et al., 2005).***

There is an increased prevalence of warts in patients with cell-mediated immune suppression. In contrast,

patients with underlying defects in humoral immunity seem not be predisposed to HPV infections, suggesting that cell-mediated responses are primarily responsible for controlling HPV infection ***(Frazer, 1998).***

The bad response of antibody may due to the localization of virus in the outer epidermis where it is sequesterated from the immune system ***(Tindle and Frazer, 1994).*** The cell mediated immunity to HPV and warts associated antigens was found to be short-lived and therefore insufficient to prevent reinfection, but it may be significant in preventing the persistence of viral infection ***(Lee and Eisinger, 1976).***

1.5. Diagnosis of HPV

The morphology of the lesion and the site in which the lesion is found are the initial clues in classifying papillomavirus-induced neoplasia. HPV types have limited site-specificity and differ in their association with benign or malignant neoplastic development. Cytopathology, electron microscopy, antigen detection and molecular hybridization all play a role in the diagnostic methods. Although nitrocellulose blotting procedures provide the most accurate and sensitive method for detecting and characterizing viral nucleic acid sequences, also improvements in cytological hybridization methods allow for rapid detection of virus and analysis of HPV type directly in biopsied tissue and in cervical smears ***(McDougall et al., 1986).***