INTRODUCTION

Uterine cervical cancer is the second most common cancer among women worldwide, with an estimated 529,409 new cases and 274,883 deaths in 2008 **(*WHO/ICO, 2010).*** About 86% of the cases occur in the developing countries with highest incidence rates in Eastern and Western Africa (age-standardized rates [ASR] greater than 33/100, 000), Southern and Middle Africa (ASRs 23 and 26.8 / 100, 000, respectively), South-Central Asia (ASR 24.6/ 100, 000) and South America (ASR 23.9/ 100, 000)and lowest in Western Asia (4.5 /100, 000), North America (5.7 /100, 000) and Australia/New Zealand (ASR 5.0/100, 000) ***(Ferlay et al., 2010).***

Egypt has a population of 25.76 millions women ages 15 years and older who are at risk of developing cervical cancer. In Egypt, no national data is available on incidence or mortality from cervical cancer, incidence rates have been estimated as the simple mean of the incidence rates from Aswan (1999-2003) and Gharbiah cancer registry (1999-2002) and applied to the 2008 population. It was estimated as 514 annual number of new case and 299 deaths from cervical cancer as estimated by International Agency For Research on Cancer (IARC), GLOBOCAN, 2008 and published on September, 2010 **(*WHO/ICO, 2010)***, however, these data appeared to be actually underestimating incidence and mortality rates.

Risk of cervical cancer is mainly a function of Human Papillomavirus (HPV) infection and lack of effective screening ***(Schiffman et al., 2007)***. Early onset of intercourse and multiple partners play key role as these increase exposure to HPV and other sexually transmitted diseases as HIV, Chlamydia trachomatis ***(Smith et al., 2004)*** and herpes simplex ***(Munoz et al., 2006).*** Other risk factors include Tobacco smoking and high parity as they enhance viral persistence and decreasing rate of clearance ***(Inter. Coll. of Epid. Studies of Cervical Cancer, 2006; ZurHausen, 2006b)***, long-term hormonal contraceptive use ***(Smith et al., 2003)*** and low socioeconomic status ***(Khan et al., 2005)*** are estimated to be risk factors.

Human Papillomavirus is a small double-stranded DNA virus ***(de Villiers et al., 2004)*** that infects the metaplastic epithelium at the cervical transformation zone. E6 and E7 are the primary HPV oncoproteins, E6 inhibits p53 tumor suppressor gene of the host cell blocking apoptosis, whereas E7 inhibits retinoblastoma (Rb) gene abrogates cell cycle arrest ***(Doorbar, 2006)***. Papillomaviruses are classified by genotype not by serotype and at present about 130 HPV types are identified ***(Stanley, 2010). Munoz et al. (2006)*** classified the mucosal types of HPV by their carcinogenic effect into high risk, probable high risk, low-risk HPV types and types of undetermined risk. HPV type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are high risk types**,** (26, 53, 66, 68, 73, 82) are

probable high risk and (6, 11, 13, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89) are low-risk types and (30, 32, 34, 62, 67, 69, 71, 74, 83, 84, 85, 86, 87, 90, 91) are types of undetermined risk.

HPV infection can remain latent for long periods (up to 20 years) with 80%-90% clearance within 2 years due to the cellular immune response. Persistant HPV infection occur in 10-20% of cases and is the most important factor involved in the development of primary invasive cervical cancer with prevalence as high as 100% ***(Parkin and Bray, 2006)***. HPV type is the strongest factor that affects the absolute risk of viral persistence and progression to precancer ***(Schiffman et al., 2005; Kjaer et al., 2006)***. Worldwide, HPV-16 and 18 contribute to over 70% of all cervical cancer cases, 41% - 67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions. Then come the six most common HPV types namely 31, 33, 35, 45, 52 and 58 that account for an additional 20% of cervical cancers worldwide ***(Clifford et al., 2006)***. Differences in geographic distribution of HPV types have been reported to exist between countries ***(Clifford et al., 2005).*** In Egypt, data is very limited, ***Abdel Aziz et al (2006)*** estimated the prevalence of HPV in women with normal cytology to be about 10.3% and ***Abdel All et al, (2007)*** estimated the prevalence of HPV in women with epithelial abnormalities to be 65.9% with HPV-16 and 18 infecting 33.3% of cases,

The natural development of cervical cancer involves reversible changes in the cervical tissue from a normal state to

various cellular abnormalities that ultimately lead to cervical cancer, this sequence of events underlies the cytological screening for cervical cancer.

Primary prevention aimed at eliminating the risk factors for development of the disease such as sexual education to prevent the spread of the HPV and motivation to change the lifestyle that includes risk factors for the development of cervical cancer ***(Cronjé, 2010).*** Another method to decrease the burden of the disease is the introduction of HPV vaccine ***(Stanly, 2008).*** The Quadrivalent vaccine provides protection up to 99% from the development of cervical intraepithelial neoplasia (CIN) grade II/III related to HPV-16, 18 infection after 3 years if given before acquiring the infection, and 44% if given after acquiring the infection ***(Ault, 2007)***. It is not clear how long immunity lasts but may last for 5-9.5 years ***(Rowhani et al., 2009; Stanley, 2010).*** Although Quadrivalent vaccine offers some cross protection against other HPV types ***(Browen et al., 2009; Pavoonen et al., 2009)***, vaccinated women should continue cervical cancer screening according to the guidelines ***(National Comprehensive Cancer Network, 2011).*** Recent Data suggests that the quadrivalent vaccine decreases abnormal cytology, colposcopy and biopsy ***(Munoz et al., 2010)***

In secondary prevention several effective strategies for cervical cancer screening exist ***(Gravitt, 2008)***. Established cytology-based programmes are also gradually moving towards a greater use of HPV DNA testing to improve their efficacy as

the addition of HPV high risk types screening to cervical cytology for women aged above 30 years increase the detection rate of CIN3 ***(Bulkmans et al., 2007; Kitchener et al., 2009; Naucler et al., 2009)*** and safely lengthens the screening interval to be every 3 years if both are negative ***(ACCSP, 2012).*** HPV DNA testing compared to cytology has consistently shown higher sensitivity and positive predictive value ***(Cuzick et al., 2006)*** that argues strongly for using HPV DNA testing as the primary screening test in newly implemented programmes, except where resources are extremely limited and only programmes based on visual inspection are affordable, However, in such countries the use of a simple HPV DNA test followed by immediate 'screen and treat' algorithms based on visual inspection in those who are HPV positive can minimize the number of visits and make best use of limited resources**.** Also high-risk HPV DNA testing is considered to be potentially useful as a triage test to select which women who have minor cytological lesions in their Pap smears are in need of referral for colposcopic diagnosis and treatment, in the continuing management of women referred for colposcopy for whom no lesion could be visualized; and as a follow-up test for women treated for high-grade intraepithelial lesion with local ablative or excisional therapy to more rapidly and accurately identify women who have or have not been cured by their treatment ***(Cuzick et al., 2008).***

AIM OF THE WORK

The aim of the work was to estimate the prevalence of Human papillomavirus and distribution of genotypes in a population of Egyptian females with cervical carcinoma and pre-invasive lesions.

1.1) Epidemiology of HPV Infection in anogenital Lesions in Females

A) Epidemiology of Cervical HPV infection:

Worldwide about 11.4% (11.3-11.5%, CI 95%) of women with normal cytology are estimated to harbor cervical HPV infection at a given time in their lives ***(De sanjose, 2007)***. HPV is found in nearly 100% of cases of cervical carcinoma ***(Parkin and Bray, 2006***), HPV-16 and 18 contribute to over 70% of all cervical cancer cases, 41% - 67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions. Then comes the six most common HPV types namely 31, 33, 35, 45, 52 and 58 that account for an additional 20% of cervical cancers worldwide ***(Clifford et al., 2006)***

In Africa, IARC, GLOBOCAN, 2008 indicated that about 21.3% of women in the general population were estimated to harbor cervical HPV infection at a given time, and 69.7% of invasive cervical cancers are attributed to HPV-16 or 18 **(*WHO/ICO, 2010)***.

In Egypt, There are four published studies estimated the prevalence of HPV.***Abd El Salam et al.,(1998)*** estimated the prevalence of HPV in cervical preinvasive lesions to be 72.2% and in cervical cancer to be 58.8%. ***Abdel Aziz, (2006)*** estimated the prevalence of HPV in women with normal cytology to be 10.3%***. Abd El All et al., (2007)*** estimated the prevalence HPV

in cervical preinvasive lesions to be 65.9% with HPV-16/18 being the most prevalent genotypes (33.3***%). Abd El-Azim et al. (2011)*** estimated the prevalence of HPV-DNA to be 85.7% in CIN2/III and to be 93.3% in invasive cancers with HPV-16, HPV-18, HPV-31 and 33 being the most prevalent genotypes.

B) Epidemiology of HPV in Other HPV related lesions:

HPV DNA is detected among 91% of invasive vaginal carcinomas and 82% of high-grade vaginal neoplasia (VAIN3). HPV-16 is the most common type in at least 70% of HPV-positive carcinomas ***(De Vuyst et al., 2009)***

The majority of vulvar carcinomas are of the basaloid warty type (>55%), which occur mainly in younger women and are associated with HPV DNA in 27.3-100% of cases and 72-100% among cases of high-grade vulvar neoplasia (VIN3). Similarly, HPV-16 is the most common detected followed by HPV-18 ***(IARC, 2007).***

Overall HPV DNA positivity is 85% among cases of anal cancer. HPV-16 is the most common detected type, representing 87% of all HPV-positive tumors and HPV-18 is the second most common type detected. The prevalence of HPV in Anal intraepithelial neoplasia (AIN) increases with the severity of the lesion being 75% in AIN1, 86% in AIN2, and 94% in AIN3 ***(Parkin and Barly, 2006; IARC, 2007)***

1.2) Basic characteristics of papillomaviruses

Papillomaviruses are a family of DNA viruses that have a double-stranded, closed, circular genome of 7000–8000 base pairs and a non-enveloped, icosahedral capsid ***(IARC, 2007)***, only one DNA strand is transcriptionally active ***(Chow and Broker, 1994; De villiers, 2001)***

Nine open reading frames (ORFs) were identified in the genome of papillomaviruses, which code for two groups of proteins ***(Kisseljov et al., 2008):***

- Late genes encoding two structural proteins, major capsid protein (L1) and minor capsid protein(L2),
- Early genes (E1–E7) that play key role in DNA replication control and in initiation and maintenance of the transformed phenotype in the cells.

Both groups of genes are controlled by the upstream regulatory region (URR), also called the long control region (LCR), with binding sites for different transcription factors of viral or cellular origin. In accordance with this, two ribonucleic acid (RNA) poly-A addition sites, one for the early protein transcripts and one for the late protein transcripts ***(Kisseljov et al., 2008).***

1- *Structure of the viral genome:*

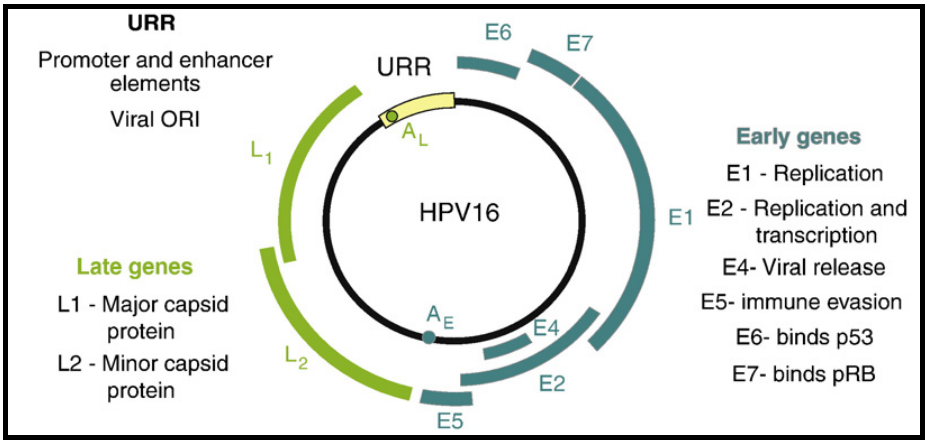

Fig. (1): Structure of the viral genome ***(Stanely, 2010).***

A) Regulatory region of the genome

The nonstructural region of the viral genome is represented by the URR, or the LCR, comprising about 12% of the genome (1Kb). The main functions of this region are to control transcription and expression of epithelial-specific genes as well as viral genes during cell differentiation, in addition to utilization of different promoters, transcription termination, and stability of viral RNAs. cis-active elements within the URR control the transcription of the major viral oncogenes E6 and E7, replication of the genome, and its packaging into virus particles. Most transcription factor-binding sites identified in the URR are common for many HPV types; however, type-specific ones have also been found ***(Kisseljov et al., 2008).***

B) Structural viral protiens:

E1 gene:

The encoded protein plays the key role in viral DNA replication and exerts a helicase activity that is required for efficient DNA replication and interaction with the cellular DNA polymerase. This protein binds to the proximal URR, where the viral origin of replication is located. The E1 primary structure is the most conserved among papillomaviruses***. (Ustav and Stenlund, 1991; Holt et al., 1994; Li et al., 1993; Deng et al., 2004)***

E2 gene:

This gene codes for at least two transcriptional factors proteins which play the main transcriptional activity of viral genome ***(Hegde, 2002).*** The URR in high-risk HPV contain four E2-binding sites, three of which are essential for the viral life cycle, that is, for the regulation of DNA replication and promoter repression ***(Stubenrauch et al., 1998).*** E2 overexpression suppresses proliferation, arrests the cell cycle, and triggers apoptosis ***(Blachone and Demeret, 2003).***

The E2 ORF often contains deletions in cervical cancer since the circular viral DNA is commonly linearized in this gene region during the viral DNA integration into the genome ***(Schwarz et al., 1985).*** Usually this leads to uncontrolled expression of the E6 and E7 genes and eventually to cancer progression ***(Romanczuk and Howley, 1992).*** Mutations in the

E2 ORF, particularly, in the URR-binding sites, increase the immortalizing potential of HPV-16 DNA. In cervical carcinogenesis, a break in the E2 ORF and the subsequent integration of viral DNA into the cell genome are relatively late events that are not observed before the CIN3 stage ***(Matsukura et al., 1989; Durst et al., 1992; Klaes et al., 1999).*** E2 protein can interact with E1 to stimulate viral DNA replication and promote E1 binding to the origin of replication ***(Seo et al., 1993).***

E4 gene:

This is the major viral transcript in HPV-infected cells. The gene product is localized in differentiating epithelial cells. Apparently, the main E4 function is related to the productive infection since this protein affects normal differentiation, and thus favors the viral particle maturation. E4 can interact and induce the collapse of the cytokeratin network in the cell and arrests the cell cycle in G2 ***(Chow et al., 1987; Doorbar et al., 1991; Roberts et al., 1993).*** This arrest allows the viral DNA amplification ***(Nakahara et al., 2002; Rai et al., 2004).***

E5 gene:

E5 protein reduces expression of major histocomptability complex (MHC) Class I ***(Ashrafi et al., 2006),*** which might impair antigen presentation. In addition, E5 induces the perturbation of MHC class II maturation ***(Cartin and Alonso, 2003).*** E5 is an important factor during early infection. However, Regular losses of this gene after viral DNA

integration confirm that E5 is hardly essential during late viral oncogenesis.

E6 gene (Transforming gene):

- Biological properties

The product of this major HPV oncogene is a 151-amino acid protein that can interact with many cellular proteins. The E6 protein is detected both in the cytoplasm and in the nucleus of infected cells in high risk HPV-types and confined to the cytoplasm in low risk HPV-types. Three nuclear localization signals were identified in E6 gene, and the mutations in these regions prevent E6 relocation from the cytoplasm to the nucleus, p53 degradation, and cell immortalization ***(Cooper et al., 2003).***

- Functions:

- Interaction with tumor-suppressor p53
- E6 activities not associated with p53
- Deregulation of DNA transcription and replication. E6 of both high- and low-risk viruses can modulate transcription from many viral and cellular promoters.
- Mitogenic activity of E6 as it induces cell hyperpro-liferation and epidermal hyperplasia ***(Song et al., 1999)***
- Inhibition of apoptosis.
- Interference with epithelial organization and differentiation ***(Sherman et al., 1997).***

- Interactions with PDZ proteins, conserved C-terminal domain, is an important feature of E6 from high-risk HPV.

E7 gene:

- Biological properties

E7 is detected in 98% of cervical cancers with no correlation between the expression level and tumor progression ***(Song et al., 2000; Garner-Hamrick et al., 2004).***

- Function /Role in carcinogenesis:

- Interaction with tumor growth suppressors
- Interaction with cyclin-dependent kinases and their inhibitors, E7 can also interact with some proteins crucial for the promotion of cell proliferative activity
- Interaction with histone deacetylases which can inhibit E2F-inducible promoters by binding to Retinoblastoma (pRb) gene.
- Induction of chromosomal instability. Both E6 and E7 can independently induce chromosomal instability; however, their combined action generates mitotic abnormalities and aneuploidy by inducing centrosome abnormalities as duplication of centrosomes and centrioles, whose number increases as viral infection progresses and viral DNA accumulates in the genome ***(Duensing et al., 2004; Munger et al., 2004).***

Table (1): Structural genes of the virus ***(IARC, 2007)***

E1 Adenosine triphosphatase (ATPase) and DNA helicase; recognizes and binds to the viral origin of DNA replication as a hexameric complex; necessary for viral DNA replication.
E2 Main regulator of viral gene transcription; binds the viral transcriptional promoter as a dimer; involved in viral DNA replication; interacts with and recruits E1 to the origin.
E4 Acts late in the viral life cycle; interacts with the keratin cytoskeleton and intermediate filaments; localizes to nuclear domain 10; induces G2 arrest; believed to facilitate virus assembly and release.
E5 Induces unscheduled cell proliferation; interacts with ATPase; may activate growth factor receptors and other protein kinases; inhibits apoptosis; inhibits traffic of major histocompatibility complexes to the cell surface.
E6 Induces DNA synthesis; induces telomerase; prevents cell differentiation; interacts with four classes of cellular proteins: transcriptional co-activators, proteins involved in cell polarity and motility, tumour suppressors and inducers of apoptosis, primarily p53, and DNA replication and repair factors.
E7 Induces unscheduled cell proliferation; interacts with histone acetyl transferases; interacts with negative regulators of the cell cycle and tumour suppressors, primarily p105Rb.
L1 Major viral structural protein; assembles in capsomeres and capsids; interacts with L2; interacts with cell receptor(s); encodes neutralizing epitopes.
L2 Minor viral structural protein; interacts with DNA; interacts with nuclear domain 10s; believed to facilitate virion assembly; may interact with cell receptor(s); encodes linear virus neutralizing epitopes