Human Pappilomavirus (HPV) induced cancers and prevention by immunization

Sheikh Abdul Khaliq[1]*, Syed Baqir Shyum Naqvi[2] and Anab Fatima[1]
[1]Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[2]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

Abstract: Incidences of different types of cancer are increasing in Pakistan, among which cancer of Cervix and Respiratory pappilomatosis are of great concern because of their association with human Pappilomavirus (HPV). Cervical cancers typically distress women of middle age or older; however it may affect women in any age after the puberty. Two serotypes of HPV (16 & 18) accounts 70% of cervical cancer cases, while HPV (6 & 11) are considered low-risk viruses associated with genital warts (*Condyloma acuminata*) and Respiratory pappilomatosis in both gender. Generally, there is transient role of HPV in human body and are removed by immune system in or around 1 year. Data from different Pakistani hospitals provides sound evidence for increasing trends of cervical cancer, which is, being developing country imperative for us. As the cost of cancer management is increasing day by day with poor survival rate and its burden is borne by patient, their family or society in-large, so if screening or prevention is possible then there would be need to identify target population for screening and vaccination. By quality adjusted life year (QALY) measurement, the data from different sources indicates that adolescent age is the appropriate target population and is cost effective for vaccination. Two vaccines manufactured by recombinant DNA technology are licensed in some parts of the world for prevention of HPV related cancers, however both have certain advantage over another, as one of the vaccines contains viral like proteins of two HPV serotypes 16 & 18 and provide additional cross protection against HPV type 13 and 45 with 100% seroprotection, while the other vaccine, being quadrivalent offers protection against four serotypes 6, 11, 16 and 18. Both vaccines tolerability and safety profiles are similar and acceptable, however bivalent vaccine appears to provide long-lasting immunity by the development of memory B-cells hypothetically due to difference of adsorbing agent used by manufacturer, on the other hand, quadrivalent vaccine offers protection against cervical cancer but also offers additional protection against *Condyloma acuminata* and respiratory Pappilomatosis. As these vaccines are new in the market and initial trials indicate availability of antibodies for up to around 5 years i.e. why it is controversial at the moment that whether booster dose is recommended or not, however it is assumed that, there is no harm to have booster dose at 5th year of vaccination.

Keywords: Cancer incidences, pappilomatosis, HPV, Serotypes, low risk HPV, cancer cost, cost burden, society, screening, rDNA technology, vaccines, quadrivalent, bivalent, QALY, memory B-cells, adsorbent, booster dose.

INTRODUCTION

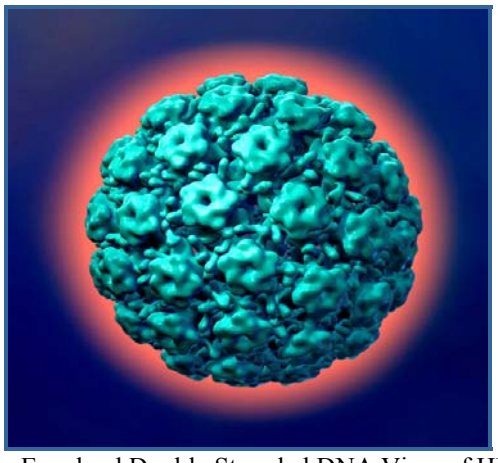
Pakistan is rising state with whole population 160.9 million. Per capita income is $2410 & life expectancy in male and female are 62 and 63 correspondingly. Possibility of dyeing involving 15 to 60 years men and women are 218 and 194 respectively/1000 inhabitants. Overall spending on health is 2% of GDP (Gross Development Product). Being developing country it is imperative for us to keep close focus on the diseases which require expensive treatment and in addition to this where survival rate is low. As we know cost of cancer treatment is growing substantially day by day i.e. why society, families and patients; all have to borne economic burden in broad sense. Cancer of Cervix exists in our society and its vaccination is available in certain parts of world, so we need to determine on the base of prevalence of Cervix cancer that better is either treatment or prevention. The data from 1998 to 2002 indicates the rising incidence of cancer of Cervix. The data of JPMC (Jinnah Post Graduate Medical Centre study) from 1985 to 1995 and of another state owned hospital of Karachi represents increasing trends of cervical cancer with high proportion in Sindhi and Immigrants females, while lowest proportion in Siraiki and Pukhtoon females. Human papillomavirus DNA has been detected in most cervical cancer biopsies, yielding the largest causative relationship of any cancer (Wallboomers *et al*., 1999). Human Pappilomavirus (HPV) is DNA virus, non-enveloped, composed of viral capsid proteins and are classified as E-proteins (E_1, E_2, E_5, E_6 & E_7) and L-proteins (L_1 and L_2). More than 120 HPV (human papilloma virus) serotypes identified, and among them 15 are linked to cancer. The virus serotypes which affect human Cervix are classified into 2 categories. The low risk serotypes, HPV 6 and 11, and high risk serotypes, mainly HPV serotypes 16 and 18. However there are some other serotypes including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 also be considered carcinogenic. Each year diagnosis of new cases of cancer of cervix

**Corresponding author:* e-mail: sheikh1974@gmail.com

globally is ≈500,000. The incidence of genital infection with HPV peaks between adolescence and age 29 years, with at least 80% of women acquiring an infection with one or more HPV subtypes by age 50 years (Schiffman *et al*., 2005, Weinstock *et al*., 2004). Generally, there is transient role of HPV in human body and are removed by immune system in or around 1 year (Ho *et al*., 1998, Sataiya *et al*., 2007).

Non-enveloped double-stranded DNA virus of HPV[17]

Persistent infection with HPV subtypes can cause variety of diseases ranging from invasive cancer to genital warts and respiratory papallomatosis. Cervical cancers usually affect women of middle age or older, but it may be diagnosed in any reproductive-age. The analysis of 932 specimens from women in 22 countries indicated prevalence of HPV DNA in cervical cancers worldwide 99.7% (Wallboomers *et al*., 1999). It is hypothesized that most infected individuals are unaware that they are infected and may unknowingly spread the virus (Anhang *et al*., 2004). Humoral immune response against HPV Infection include; Two types of antigen responses (Howley *et al*., 2001, Moscicki *et al*., 1999). Late capsid proteins (L1/L2) induce and humoral response to early (E) viral proteins. Antibodies against HPV is mainly type-specific (Carter *et al*., 2003). Subsequent to natural infection, only ≈50% infected women develop neutralizing antibodies and even their levels were low and found to be ineffective. That is why, an efficient vaccination followed by the development of long lasting neutralizing antibodies is needed. Two vaccines Licensed in some parts of the world: Bivalent vaccines and Quadrivalent vaccine. Bivalent Vaccine is characterized by inducing long lasting titers of neutralizing antibodies and a higher level of memory B cells and Quadrivalent Vaccine vaccine reveals a decrease in the titer of neutralizing antibodies, namely those against HPV 18. In addition, to the high titer of antibodies against HPV serotypes 16, 18, Bivalent Vaccine offers 100% protection against additional HPV serotypes mainly 13 and 45. Both vaccines present a strong safety profile.



Non-Envelped Double Stranded DNA Virus of HPV

MATERIAL AND METHODS

The data is collected from several sources to analyze association and incidences of HPV with Cervical cancer in Pakistan and rest of the world. The data also collected from two retrospective cancer registries of JPMC (1985-1995) and another state owned hospital (2003-2010) for true number of cases. Different clinical trials obtained from reputed medical journals to establish the efficacy and safety of two different vaccines available for prevention of cancer of cervix. Although, there are no head to head clinical trial available to compare efficacy and safety of both vaccines, however indirect comparison has been made by evaluating composition, seroconversion, seroprotection, antibody titres produced, their levels, cross protection from other HPV serotypes, effect on previously exposed individuals, persistence of antibodies in serum, and development of immune memory, economic analysis.

Observations

More than 120 HPV (human papilloma virus) serotypes have been known, and among them, only 15 are linked to cancer of cervix. The proven serotypes of human papilloma virus family (HPV) which cause cervical intraepithelial neoplasia and cancer are mainly serotype 16, 18 and less frequently serotype 31, 45. The virus serotypes which affect human Cervix are classified into 2 categories:

- The serotypes which linked to low grade SILs (Squamous Intraepithelial Lesions) are considered low risk serotypes, HPV 6 and 11, and never found in invasive cancer.
- The serotypes which are found in up to 90% of invasive cancers and 50-80% of SILs are considered high risk serotypes, mainly HPV 16 and 18.

However there are some other carcinogenic serotypes including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. Following natural infection, ≈50% women develop neutralizing antibodies and even these were of very low titer and found ineffective (Kidon MI. *et al*., 2011). Cervical cancers distress women of middle age or older, however it may be diagnosed in any age after the puberty. Abnormal vaginal bleeding, postcoital vaginal discomfort, malodorous discharge, and dysuria are main clinical symptoms. Endometrial cavity, downward vagina, and pelvic wall may be affected by extension of tumor. Bladder and rectum can also be affected by direct invasion. Constipation, hematuria, fistula, and urethral obstruction are the main symptoms with or without hydroureter or hydronephrosis. The pelvic wall association can be linked to triad of leg edema, pain, and hydronephrosis.. Extrapelvic lymph nodes, liver, lung, and bone are the sites of distant metastasis. Sexual contact or sexual intercourse is considered the main mechanism

of transmission of HPV (Kiaer *et al*., 2001), in addition to this genital-genital, manual-genital, oral-genital should also be considered (Winer *et al*., 2003, Fairly *et al*., 1995, Herrero *et al*., 2003, Manhart *et al*., 2002). Non-penetrative sexual contact may also be considered, however it is rare in virgins (Winer *et al*., 2003), the use of condom may cut down the risk, but not fully protective. Nonsexual routes include (Manhart *et al*., 2002). Mother to newborn (vertical transmission) (Smith *et al*., 2004), Fomites (e.g, undergarments, surgical gloves, biopsy forceps) (Ferenczy *et al*., 1989, Roden *et al*., 1997), it is hypothesized that many carriers spread the virus unknowingly as they are not aware that they are infected (Anhang *et al*., 2004).

In broader picture of HPV associated cancers, 80-85% anal cancer, which may leads to colorectal cancer. Anal cancer is mainly caused by HPV 16 while HPV 16 and 18 also cause penile, vulvar and vaginal cancers (Watson *et al*., 2008). Among 20% oropharyngeal cancer, which is a class of Head and Neck cancer, 50% are associated with HPV 16 in United States (Jayaprakash *et al*., 2011) and around 10% larynx and aerodigestive tract cancer probable (Wallboomer *et al*., 1999, Herrero *et al*., 2003). Constant infection with various HPV subtypes causes variety of diseases ranging from invasive cancers (by high risk strains 16 and 18) to genital warts and respiratory papallomatosis (by serotypes 6 and 11). The incidences of recurrent respiratory pappilomatosis are 4.3 cases per 100,000 children and 1.8 cases per 100,000 adults (Jansen *et al*., 2004). Many HPV serotypes can readily infect the respiratory tract, resulting in respiratory papillomatosis, oral mucosa, leading to cancerous and precancerous lesions and the anal and perianal areas, resulting in Condylomata acuminata (genital warts) as well as cancer formation (Smith *et al*., 2009).

Immune Response (Humoral) Against HPV natural Infection include: Two types of antigen responses (Howley *et al*., 2001, Moscicki, 1999):

1 Consistent and strongest neutralizing antibody response induced by Late capsid proteins (L1/L2) (Howley *et al*., 2001, Bonnez *et al*., 2002, Jansen *et al*., 2004).
2 Humoral response to early viral proteins is typically modest or absent in most women without cervical cancer (Bonnez *et al*., 2002, Tindle, 2002).

Antibodies are shown to be mainly type-specific (Wang *et al*., 2003). Antibody response to infection is slow and weak (Carter *et al*., 2000, Castle *et al*., 2004). Some evidence for presence of immunologic memory to specific HPV types (Nakagawa *et al*., 2002, Welters *et al*., 2003). Following natural infection, ≈50% of the infected women develop neutralizing antibodies and even these were of a very low antibody titer and found ineffective. Hence, an efficient vaccination followed by the development of long lasting neutralizing antibodies is needed.

Vaccines

For the prevention of infection associated with HPV, two vaccines approved by United States Food and Drug Administration (FD&A), which are manufactured by recombinant DNA technology. Neither of the current approved HPV vaccines provides therapeutic effect against preexisting infections with HPV serotype-16 and 18 (Hildesheim *et al*., 2007). The quadrivalent HPV vaccine contains recombinant L1 proteins from serotype 16, 18, 6 and 11 (Merck & Co., Inc. 2009). A bivalent is approved for prevention of cervical cancer in females 10-25 years caused by serotypes 16 and 18. This bivalent vaccine contains recombinant L1 protein from HPV serotype 16 and 18 (US Food & Drug Administration. 2009, Glaxo Smith Kline, 2009). Both vaccines contain recombinant forms of type-specific L1 major capsid proteins of Human Pappilomavirus, capable of in vitro self-assembly to form an HPV virus-like particle (VLP), identical to the capsid structure of the native virion (Schmiedeskamp *et al*., 2006). Interaction between the recombinant L1 proteins and host immune system occurs in type-specific manner, providing protection against the specific HPV type (Stanley, 2006). The L1 proteins in the bivalent vaccine are produced with use of a baculovirus-insect cell expression system. In the quadrivalent vaccine, the four types of VLPs present are assembled from recombinant HPV serotypes 6, 11, 16, and 18 L1 proteins, which are expressed in the yeast cells called Saccharomyces cerevisiae (Stanley, 2006, Ault, 2007). Other than the strains covered by each vaccine and the method of production, the key difference between the two product is the use of adjuvant to boost the immune response. The quadrivalent vaccine uses a traditional amorphous aluminum hydroxyphosphate sulfate adjuvant (Merck & Co., Inc. 2009). The bivalent vaccine uses a proprietary adjuvant, aluminum hydroxide combined with 3-deacylated monophosphoryl lipid A (GlaxoSmithKline, 2009). A more robust antibody response is attributed in bivalent vaccine because of new adjuvant, which perhaps have potential for a more durable immunity (Ault, 2007).

Efficacy of vaccine

Both vaccines clinical efficacy and safety were evaluated in phase II and phase III randomized, double-blind, multicenter clinical trials (Villa *et al*., 2005, Girland *et al*., 2007, The Future II study group, 2007, Paavonen *et al*., 2009). The study end points evaluated were seroconversion, prevention of persistent infection, prevention of different grades (1-3) of CIN (Cervical Intraepithelial Neoplasia), prevention of vulvar and vaginal lesions, and for the quadrivalent vaccine, prevention of Condyloma acuminata (genital warts) (Girland *et al*., 2007, The Future II study group, 2007, Paavonen *et al*., 2009, Mao *et al*., 2006, Harper *et al*.,

2006). In the evaluation of safety, injection site pain and erythema were the most common adverse events reported in both vaccines and more frequent in vaccine groups compare to placebo (Merck & Co., Inc., 2009, GlaxoSmithKline, 2009). Other adverse events common in both vaccine and placebo groups were fever and malaise (The Future II study group, 2007, Paavonen *et al*., 2009, Mao *et al*., 2006, Harper *et al*., 2006, Harper *et al*., 2008). In >20,000 vaccinees received the quadrivalent vaccine and the >18,000 women who received the bivalent vaccine, the development of antibodies was 100% after three doses (The Future II study group, 2007, Mao *et al*., 2006). The analysis of per-protocol population reported quadrivalent vaccine was >97% effective in the prevention of lesions of cervix, vulva, and vagina caused by HPV serotypes 16 and 18, as well as Condyloma acuminata (genital warts) caused by HPV serotypes 6 and 11 (Gerland et. al., 2007), while bivalent vaccine was >90% effective in prevention of HPV serotypes 16 and 18 associated cervical cancers (Paavonen *et al*., 2009).

Cervical cell abnormalities

Invasive cancer of cervix require long period of time to form i.e. why development of cytologic abnormalities is the best marker for effectiveness of vaccines. FD&A designated CIN (Cervical intraepithelial Neoplasia) as effective marker for clinical trials and primary outcome in all clinical efficacy studies. However, assessment of CIN1 is not a marker of cancer prevention, as CIN1 resolves over time. The CIN2+ and most especially CIN3+ lesions serve as the markers for cancer prevention, as their development to cancer is more likely. In the per-protocol populations for both vaccines, efficacy against CIN2+ was high for HPV serotypes 16 and 18, and their lesions (Garland *et al*., 2007, The Future II study group, 2007, Paavonen *et al*., 2009).

Durability of immune response

Because of the short follow up periods, it is difficult to say vividly that how long patients will be protected. Both vaccines show high immunogenicity. Nearly 100% seroconversion seen in patients received the three-dose regimen, and peak geometric mean antibody titers (GMT) were 10-100 times higher when compared with patients with natural HPV infection (Garland *et al*., 2007, Paavonen *et al*., 2009, Villa *et al*., 2006). Peak GMT response was cut down with increasing age after vaccination, but remained above that of natural exposure. 1 month after the all three doses, peak GMT for both vaccines were observed (Smith *et al*., 2007). Another study on quadrivalent vaccine reported that reduction in antibodies was seen in a 5-year follow-up of 241 vaccinees (Olsson *et al*., 2007). A GMT reduction was seen over the first 24 months and then plateau phase of much slower decline. Throughout the plateau phase, up to the end of follow up, GMT remained above that of patients with natural HPV infection for HPV serotypes 6 and 16. The HPV serotypes 11 and 18 GMT were not significantly different compared with placebo at 60 months. Referred to the same study of Olsson *et al*. (2007) about bivalent vaccine mention sustained seropositivity in >98% of patients receiving all 3 shots of bivalent vaccine, contrast to reported five year follow-up with the quadrivalent vaccine discussed above (Olsson SE. *et al*., 2007). Differences in immune responses and durability could be attributed to the number of strains covered in quadrivalent vaccines and differing adjuvant used in their formulation. There is no evidence exist uptil now which confirms the availability of memory cells, however it is hypothesized that like Hepatitis-B vaccines, there would be availability of memory B-cells.

Age of vaccination

All published clinical data suggest that vaccination of adolescent girls will be effective strategy (Ault *et al*., 2007, Villa *et al*., 2005, Garland *et al*., 2007, The Future II study group, 2007, Paavonen *et al*., 2009, Harper *et al*., 2006). Efficacy was highest in women before initiation of sexual intercourse and decreased significantly when viewed in population already exposed (Harper *et al*., 2006, Villa *et al*., 2006, Olsson *et al*., 2007). Two studies of quadrivalent vaccine, proven to be immunogenic and safe in adolescent girls and boys (Reisinger *et al*., 2007, Block *et al*., 2006). The antibody response of boys and girls aged 9-14 years on average two times that of 15-26 years young girls and women. The titers were similar between boys and girls. There is comparison of >600 15-25 years female with 10-14 years females serum antibody levels after all three shots of vaccine of the bivalent vaccine (Pedersen *et al*., 2007). Seroconversion was complete for both groups. The GMT 1 month after the final shot which is month 7, were nearly twice high in younger cohort. There is evidence that efficacy of diphtheria, tetanus, acellular pertussis, or inactivated poliovirus vaccines is not interfered with co-administration of the HPV bivalent vaccine (Adolescents Study Investigators Network, 2010). Seroconversion rates and GMTs of the patients receiving a combination of vaccines were non-inferior to either agent given alone (HPV Vaccine Study Investigators for Adult Women, 2007).

A bridging study evaluated vaccine induced immune responses in two age cohorts outside those used in clinical efficacy trials (Schwarz, 2006). The comparison of women 26-45 years and 46-55 years with girls and women 15-25 years were done for seroconversion rates and antibody levels after bivalent vaccine. Women achieved seroconversion in all age groups for both HPV serotypes 16 and 18 at month 7 which is 1 month after the third shot. A decrease in Geometric Mean Titres was observed with increasing age, and forcing the issue of need for frequent boosters.

The quadrivalent vaccine also approved for the prevention of Condyloma acuminata (genital warts) by FDA in men and boys; however, more significant is reduction of HPV related cancers even in males. Although cervical cancer is main clinical target of vaccines, the number of men who are susceptible to HPV-related cancers and death is nearly equal to that of women. In addition to cancer prevention in men, to achieve a level of herd immunity against oncogenic HPV and further reduction of cervical cancer incidences, male sex should also be targeted for vaccination, since up to half of sexually active men are carriers of HPV.

RESULTS

Five years data from 1998 to 2002 indicates the rising incidence of cancer of Cervix 8.9% (Yasmin *et al*., 2006). Ten years data of JPMC (Jinnah Post Graduate Medical Centre) from 1985 to 1995 reported 336 cases of cervical cancer (Sanaullah *et al*., 2010). Similarly 8 years data analysis of another state owned hospital of Karachi represents around 100 cases of cervical cancer with 40% in Sindhi females, 22% in Immigrant females, while lowest in Siraiki and Pukhtoon females. 99.7% Human Pappilomavirus was found in biopsies for cervical cancer diagnosis, yielding the largest causative relationship of any cancer (Walboomers *et al*., 1999). The data of 22 countries analyze 932 specimens and indicates 99.7% HPV DNA detection in cervical cancer (Wallboomers *et al*., 1999). Polymerase chain reaction (PCR) based assays were employed to identify HPV DNA and the existence of malignant cells was established in adjacent tissue sections (Wallboomers *et al*., 1999). The available vaccines against cervical cancer and other HPV infections includes Bivalent Vaccine is characterized by inducing long lasting titers of antibodies and a higher level of memory B cells and there is decrease in the titer of antibodies against HPV 18 observed in Quadrivalent vaccine. In addition, to the high titer of against HPV 16, 18, Bivalent Vaccine offers 100% protection against additional HPV serotypes 13 and 45. This cross protection is considered to be one of the competitive edges over Quadrivalent Vaccine. Both vaccines present a strong safety profile. Both vaccines were effective for HPV serotypes covered in vaccines by preventing associated lesions >90%. The bivalent vaccine also showed significant reductions in CIN2+ caused by HPV serotype 31, 58 and composite of HPV serotypes 31, 33, 45, 52, and 58. This yielded 61.9% efficacy (96.1% confidence interval [CI] 46.7–73.2%) against CIN2+ caused by any HPV serotype in per protocol analysis (Paavonen *et al*., 2009). The quadrivalent vaccine's impact on non vaccine covered HPV serotypes was similar. A composite of efficacy against the 10 leading cancer causing serotypes excluded from the vaccine was 38% (95% CI 6-60%) (Paavonen J. *et al*., 2009, Mao *et al*., 2006). In the intention-to-treat analysis, efficacy of vaccine decreased to 30.4% (96.1% CI 16.4-42.1%) against any cause CIN2+ for the bivalent vaccine (Paavonen *et al*., 2009). The quadrivalent vaccine had efficacy of 22% (95% CI 3-38%) for CIN2 caused by any HPV type. The quadrivalent vaccine's efficacy against CIN3 caused by any HPV serotype failed to reach statistical significant at 21% (95% CI < 0-38%). Prevention of adenocarcinoma in situ did not reach a statistically significant benefit with vaccination (Mao *et al*., 2006).

Current or prior infection

The definition of persistent infection is the detection of HPV DNA of the HPV serotype at two separate screening visits of the same patient. The initial assessment conducted at 36 months revealed that in quadrivalent vaccine, efficacy was >85% in persistent infection associated with HPV serotypes 16 and 18 (Harper *et al*., 2008). On further follow-up of 5 years, vaccine is 96.6% effective and 90.6% effective when cervicovaginal samples tested 4 months apart against persistent infections caused by HPV serotypes 16 and 18, respectively, in per-protocol analysis. 89% efficacy was reported in the intention-to-treat analysis for persistent infection (95% CI 73-96%; type-specific intention-to-treat efficacy was not reported). In one of the publication for bivalent vaccine, phase III study against persistent infection was evaluated 6 or 12 months time periods. 84.1% and 74% protection was reported for 6-month persistent infections caused by HPV serotype 16 and 18 respectively (Paavonen *et al*., 2009). When assessed at longer interval, in this case 12 months, 79.9% and 66.2% efficacy was reported against infection caused by HPV serotypes 16 and 18 rspectively (Paavonen *et al*., 2009). This illustrates the difference in outcomes depending on the interval chosen for sampling. In one of the report quadrivalent vaccine used a shorter interval time of only 4 months to assess persistent infection and showed a higher efficacy (Villa *et al*., 2005). In the bivalent study (samples taken 12 months apart), one third of patients were unprotected against HPV-18 and one fifth were still susceptible to persistent infection with HPV-16 (Paavonen J. *et al*., 2009).

Pre-exposed individuals

In quadrivalent vaccine's phase III study post hoc analysis assess the efficacy against CIN1+ in patients with previous exposure to HPV serotypes 6, 11, 16, and 18 (The Future II study group., 2007). Patients selected based on positive serum antibodies, but cervical swabs negative by polymerase chain reaction. This provided patient population exposed to the targeted HPV serotypes was not currently infected. 100% efficacy showed by study results in the prevention of type-specific CIN1+ caused by the HPV type of previous exposure.

Cross protection against other serotypes

70% cases of cancer of cervix accounts serotypes 16 and 18 (Moscicki, 2008). To achieve protection against 85%

of cervical cancers, HPV types 33, 45, 31, and 58 would be needed to add to the vaccine (Moscicki, 2008). To prevent 95% cases of cancer of cervix, additional 10 viral proteins need to be added (Moscicki, 2008). However, many oncogenic serotypes belong to same family and are similar in anti-genicity. This is illustrated by cross protection seen in the clinical trials of vaccines (The Future II study group, 2007, Paavonen *et al*., 2009). In the 4.5-year extended follow up period of bivalent phase II trial, 94% (95% CI 63-100%) efficacy for HPV-45 and 55% (95% CI 12–78%) efficacy for HPV 31 was reported in modified intention-to-treat analysis. Statistical difference was insignificant for other HPV serotypes (Paavonen *et al*., 2009). In quadrivalent vaccine, durin 3 year follow up, evidence of cross protection was assessed against CIN2+ (The Future II study group, 2007). To reach statistical significance, results were reported as composite of HPV serotypes 31 and 45, a composite of five HPV serotypes (31, 33, 45, 52 and 58) and a composite of 10 HPV serotypes (31, 33, 35, 39, 45, 51, 52, 56, 58 and 59). Efficacy was highest for HPV types 31 and 45-related disease at 62% (95% CI 10–85%). Efficacy against the larger composite end point including all cervical, vulvar, and vaginal diseases was 43% (95% CI 7-66%) and 38% (95% CI 6%–60%) for the 5 HPV types and 10 HPV types, respectively.

DISCUSSION

Comparison of vaccines

With the availability of two distinct vaccines, practitioners, decision makers and regulatory authorities must decide which vaccine is most appropriate for their population protection. Although both vaccines are similar in design, there are marked differences in several aspects of their clinical utility. Both vaccines provide antibodies against two oncogenic HPV serotypes 16 and 18. Protection against *Condyloma acuminata* caused by HPV serotypes 6 and 11 is the additional benefit with quadrivalent vaccine. Potentially due to multiple strains covered, the antibody response may be less durable than bivalent vaccine. Regardless of the higher antibody levels present at 5 years with the bivalent vaccine, proof of long term protection against covered HPV serotypes was not provided by both vaccines.

The safety profiles appear to be similar between both vaccines. The cost of both vaccines is also similar, with the quadrivalent vaccine approximately $360 for the three-dose series and the bivalent vaccine expected to be approxi-mately $350. Cross-protection against non vaccine covered strains appeared more robust with the bivalent vaccine; however, some cross-protection was also demonstrated by quadrivalent vaccine.

With the current clinical data, it is difficult to make decision that which vaccine is most appropriate. Some studies suggest that the protection against multiple HPV serotypes was reported with bivalent vaccine for longer period of time, but this time frame is need to be established. Bivalent vaccine's efficacy was slightly greater than quadrivalent vaccine in intention-to-treat analysis. The quadrivalent vaccine offers protection against two nononcogenic serotypes not available in bivalent vaccine. Patient preferences, as well as third-party payer coverage will also play a crucial part in the decision making. Ultimately, the decision to use either vaccine must be made by medical practitioner or pharmacist based upon efficacy and safety. For appropriate vaccine selection and decision making, long term studies are essential. The another important point is that as vaccines contains HPV type 16, so there is possibility of reduction of HPV 16 related cancers which may occurs as anal, penile, vulval, vaginal, head and neck oropharyngeal and aeridigestive tract cancers, however, the vaccines are not evaluated in clinical trials with these end points.

Screening Program

Protection against the strains of HPV causing other 30% of HPV-related cancers is not present in the vaccines i.e. why screening will remain important. If there is a misconception that vaccine will protect against all HPV-related diseases, compliance with screening could decrease, causing an increased trend of cancer related to HPV strains not covered. Currently, it has been observed that poor access to health care facilities is the major reason greater incidences of cervical cancer. Most patients diagnosed with cervical cancer were not having Pap test in the past 5 years (Franco *et al*., 2006).

Economic Benefits

As there is substantial advancement in medicine day by day, it is acceptable that resources scarcity is the major barrier to get every new medication available. One has to make choices as huge cost burdens are involved. The effective use of health care resources is possible by focusing wisely on the treatment which improve quality of life or/and extend the life. To make sure that we are right about our conclusions, it is better to adopt internationally recognized method to evaluate vaccine clinical effectiveness provided quality adjusted life year measurement (QALY). This way of measurement will provide the idea that because of vaccine, how many extra months or years of life a person will have.

Similarly, the actual costs of vaccines vary among studies, vaccination of adolescent girls up to age of 14 is cost effective with mutual consensus where ratios of incremental cost effectiveness are under $50,000/quality adjusted life year, whereas vaccination of women aged >26 years is not considered as cost-effective, with values exceeding $100,000/QALY, since most of them are already exposed to HPV and minimal benefit to vaccinate

them (Goldie *et al*., 2004, Elbasha *et al*., 2007, Chesson *et al*., 2008, Kim *et al*., 2008, Kim *et al*., 2009). One study contested the cost-effectiveness of vaccination between ages 14 and 26 years, with the most recent studies indicating incremental cost-effectiveness ratios in this population nearing $100,000/QALY (Chesson *et al*., 2008).

One caution is that the economic analyses have overestimated the vaccine's efficacy, with efficacy rates of 75-100% (Elbasha *et al*., 2007, Chesson *et al*., 2008, Kim *et al*., 2008, Kim *et al*., 2009). With only 70% of oncogenic HPV types covered by the vaccines, and with only little cross protection seen, the best efficacy rate would still only 70%. In addition, studies assumed a lifelong immunity against covered HPV types (Goldie *et al*., 2004, Elbasha *et al*., 2007, Chasson *et al*., 2008, Kim *et al*., 2008). Additional cost of booster dose and potential loss of immunity in those do not receive the boosters would alter the cost-effectiveness to more than $200,000/QALY (Kim *et al*., 2009).

The vaccines availability against oncogenic HPV represent a significant advancement in the prevention of cancer, but many issues are still need to be addressed before adopting vaccination universally. During the current scanarios where there is economic recession and with limited health care budgets, the cost-effectiveness and access to the vaccine for individuals likely to develop cervical cancer are most important issues. It has been observed that in adequate access to health care facilities is the major reason for cervical cancer. In United States where routine Pap test is common it is unlikely to develop cervical cancer. If vaccination reduces Pap screening, it could increases the incidence of cervical cancer or untreated sexually transmitted infections.

Though, currently marketed vaccines offer seroconversion and seroprotection against vaccine covered HPV serotypes, however they have limitations. Efficacy within intention-to-treat populations was poor in clinical trials of both vaccines. Duration of antibody response needs more long-term evaluation than 5 years follow up. If the durability of the immune response is inadequate for lifelong immunity, a schedule for vaccine booster administration is needed to be developed, and the cost should be assessed again. The issues of cross-protection against non–vaccine-covered strains remain controversial.

CONCLUSION

As the incidences of cervical cancer and other head and neck cancers associated with different serotypes of Human pappilomavirus are increasing in our society, that is why immunization and its clinical significance require to optimized the health resources for prevention and screening. The available vaccines are similar in design, there are marked differences in several aspects of their clinical utility. Both vaccines provide antibodies against two oncogenic HPV serotypes 16 and 18. Protection against Condyloma acuminata caused by HPV serotypes 6 and 11 is the additional benefit with quadrivalent vaccine. Potentially due to multiple strains covered, the antibody response may be less durable than bivalent vaccine. The currently available vaccines provide protection against that HPVs which causes 70% of cancer of cervix as well as cancers of head and neck, penis, anus, vagina and vulva. There is possibility that eradication of HPV serotypes 16 and 18 could transform in the viral shift, which may increases prevalence of infections associated with other cancer causing HPV serotypes. Despite availability of HPV vaccine which is great advancement.

REFERENCES

Adolescents Study Investigators Network (2010). Immunogenicity and safety of human papillomavirus-16/18 AS04-adjuvanted cervical cancer vaccine co-administered with combined diphtheria-tetanus-acellular pertussis-inactivated poliovirus vaccine to girls and young women. *J. Adolesc. Health*, **46**: 142-151.

Anhang R, Goodman A and Goldie SJ (2004). HPV communication: Review of existing research and recommendations for patient education. *CA Cancer J. Clin.,* **54**: 248-259.

Ault KA (2007). Future II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3 and adenocarcinoma in situ: A combined analysis of four randomized clinical trials. *Lancet,* **369**:1861-1868.

Block SL, Nolan T, Sattler C, Barr E, Giacoletti K, Marchant C, Castellsague X, Rusche S, Lukac S, Bryan J, Cavanaugh P, Reisinger K. (2006). Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics,* **118**: 2135-2145.

Bonnez W (2002). *In*: Richman DD, Whitley RJ, Hayden FJ. Cervical cancer and huma pappiloma virus related disease: Epidemiology and clinical burden. American Society for Microbiology Press; Washington, DC, pp.557-596.

Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, Galloway DA. (2000). Comparison of human pappiloma virus types 16, 18 and 6 capsid antibody responses following incident infection. *J. Infect. Dis.,* **181**: 1911-1919.

Castle PE (2004). Beyond human pappiloma virus: The cervix, exogenous secondary factors, and the development of cervical precancer and cancer. *J. Low Genital. Tract. Dis.,* **8**: 224-230.

Chesson HW, Ekwueme DU, Saraiya M and Markowitz LE (2008). Cost-effectiveness of human papillomavirus vaccination in the United States. *Emerg. Infect. Dis.,* **14**: 244-251.

Elbasha EH, Dasbach EJ and Insinga RP (2007). Model for assessing human papillomavirus vaccination strategies. *Emerg. Infect. Dis.,* **13**: 28-41.

Fairley CK, Gay NJ, Forbes A, Abramson M and Garland SM (1995). Hand genital transmission of genital warts? An analysis of prevalence data. *Epidemiol. Infect.,* **115**: 169-176.

Ferenczy A, Bergeron C and Richart RM (1989). Human pappilomavirus DNA in fomites on objects use for the management of patient with genital human pappiloma virus infection. *Obstet. Gynecol.,* **74**: 950-954.

Franco EL, Cuzick J, Hildesheim A and Sanjose S (2006). Chapter 20: Issues in planning cervical cancer screening in the era of HPV vaccination. *Vaccine,* **24**(Suppl. 3): S171-S177.

Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, Tang GW, Ferris DG, Steben M, Bryan J, Taddeo FJ, Railkar R, Esser MT, Singhs HL, Nelson M, Boslego J, Sattler C, Barr E, Koutsky LA. (2007). Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N. Engl. J. Med.,* **356**: 1928-1943.

GlaxoSmithKline (2009). Bivalent Vaccine (human papillomavirus bivalent vaccine, recombinant intramuscular injection) product information. Research Triangle Park, NC.

Goldie SJ, Kohli M, Grima D, Weinstein MC, Wright TC, Bosch FX, Franco E. (2004). Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. *J. Natl. Cancer Inst.,* **96**: 604-615.

Harper D, Gall S, Naud P, Quint W, Dubin G and Jenkins D (2008). Sustained immunogenicity and high efficacy against HPV-16/18 related cervical neoplasia: Long-term follow up through 6.4 years in women vaccinated with Bivalent Vaccine (GSK's HPV 16/18 AS04 candidate vaccine). Presented at the 39th annual meeting on women's cancer of the Society of Gynecologic Oncologists, Tampa, FL.

Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA and Dubin G (2006). Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomized control trial. *Lancet,* **367**: 1247-1255.

Herrero R, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernandez L, Idris A, Sanchez MJ, Nieto A, Talamini R, Tavani A, Bosch FX, Reidel U, Snijders PJ, Meijer CJ, Viscidi R, Munoz N and Franceschi S (2003). Human pappilomavirus and oral cancer: The international agency for research on cancer multi center study. *J. Natl. Cancer Inst.,* **95**: 1772-1783.

Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, Schiller JT, Gonzalez P, Dubin G, Porras C, Jimenez SE and Lowy DR. (2007). Effect of human papillomavirus 16/18 L1 virus like particle vaccine among young women with preexisting infection: a randomized trial. *JAMA,* **298**: 743-753.

Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N. Engl. J. Med.,* **338**: 423-428.

Howley PM and Lowy DR (2001). *In*: Knipe DM; Pappiloma virus and their replication. Philadelphia, Pa: Lippincott-Raven, pp.2197-2229.

HPV Vaccine Study Investigators for Adult Women (2007). Human papillomavirus (HPV) 16/18 l1 AS04 virus-like particle (VLP) cervical cancer vaccine is immunogenic and well-tolerated 18 months after vaccination in women up to age 55 years. *J. Clin. Oncol.,* **25**(20 Suppl): 3007.

Jansen KU and Shaw AR (2004). Human Pappilomavirus vaccines and prevention of cervical cancer. *Annu. Rev. Med.,* **55**: 319-331.

Jayaprakash V, Reid M, Hatton E, Merzianu M, Rigual N, Marshall J, Gill S, Frustino J, Wilding G, Loree T, Popat S, Sullivan M (2011). Human pappilomavirus type 16 and 18 in epithelial dysplasia of oral cavity and oropharynx, a meta analysis; 1985-2010. *Oral Oncology,* **47**(11): 1048-1054.

Kidon MI, Shechter E and Toubi E (2011). Vaccination against human papilloma virus and cervical cancer, Harefuah, **150**(1): 33-6,68. Affiliation; Allergy and Clinical Immunoelogy, Sheba Medical Center, Tel Hashomer.

Kim JJ and Goldie SJ (2008). Health and economic implications of HPV vaccination in the United States. *N. Engl. J. Med.,* **359**: 821-832.

Kim JJ and Goldie SJ (2009). Cost-effectiveness analysis of including boys in a human papillomavirum vaccination programme in the United States. BMJ; 339:b3884. Available from http://www.bmj.com/content/339/bmj.b3884.long.

Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walbomers JM, Schiller JT, Bock JE, Sherman ME, Lowy DR and Meijer CL (2001). High risk human pappiloma virus is sexually transmitted: Evidence from a follow up study of virgins starting sexual activity. *Cancer Epidemiol Biomarkers Prev.,* **10**: 101-106.

Manhart LE and Koutsky LA (2002). Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta analysis. *Sex Transm. Dis.,* **29**: 725-735.

Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, Alvarez FB, Bautista OM, Jansen KU and Barr E (2006). Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: A

randomized controlled trial. *Obstet. Gynecol.,* **107**: 18-27.

Merck & Co. Inc. (2009). Quadrivalent Vaccine (human papillomavirus quadrivalent [types 6, 11, 16, and 18] vaccine, recombinant suspension for intramuscular injection) product information. Whitehouse Station, NJ.

Moscicki AB (1999). Human pappiloma virus infections in adolescence. *Pediatr. Clin. North. Am.,* **46**:783-807.

Moscicki AB. (2008) HPV vaccines: Today and in the future. *J. Adol. Health,* **43**: S26-S40.

Nakagawa M, Viscidi R, Deshmukh I, Costa MD, Palefsky JM, Farhat S and Moscicki AB (2002). Time course of humoral, and cell mediated immune responses to human pappiloma virus type 16 in infected women. *Clin. Diagn. Lab. Immunol.,* **9**: 877-882.

Olsson SE, Villa LL, Costa RL, Petta CA, Andrade RP, Malm C, Iversen OE, Hove J, Steinwall M, Riis-Johannessen G, Andersson-Ellstrom A, Elfgren K, Von Krogh G, Lehtinen M, Paavonen J, Tamms GM, Giacoletti K, Lupinacci L, Esser MT, Vuocolo SC, Saah AJ and Barr E (2007). Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine*, **25**: 4931-4939.

Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamram U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G. (2009). Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): Final analysis of a double-blind, randomised study in young women. *Lancet,* **374**: 301-314.

Pedersen C, Petaja T, Strauss G, Rumke HC, Poder A, Richardus JH, Spiessens B, Descamps D, Hardt K, Lehtinen M, Dubin G. (2007). Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. *J. Adolesc. Health,* **40**: 564-571.

Reisinger KS, Block SL, Lazcano-Ponce E, Samakoses R, Esser MT, Erick J, Puchalski D, Giacoletti KE, Sings HL, Lukac S, Alvarez FB, Barr E. (2007). Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. *Pediatr. Infect. Dis. J.,* **26**: 201-209.

Roden RBS, Lowy DR and Schiller JT (1997). Pappiloma virus is resistant to dessication. *J. Infect. Dis.,* **176**: 1076-1079.

Sanaullah G, Ishrat Bukhari S, Haroon A, Javeid I (2010). Carcinoma Uterine Cervix. *Pak. Jour. of Pharmacol.*, **27**(2): 37-42.

Sataiya M, Ahmed F, Krishnan S, Richard TB, Unger ER, Lawson HW. (2007). Cervical cancer incidence in a prevaccine era in the United States, 1998-2002. *Obstet. Gynecol.,* **109**: 360-370.

Schiffman M and Castle PE (2005). The promise of global cervical-cancer prevention. *N. Engl. J. Med*., **353**: 2101-2104.

Schmiedeskamp MR and Kockler DR (2006). Human papillomavirus vaccines. *Ann. Pharmacother.*, **40**: 1344-1352.

Schwarz TF (2006). An AS04-containing human papillomavirus (HPV) 16/18 vaccine for prevention of cervical cancer is immunogenic and well-tolerated in women 15–55 years old. *J. Clin. Oncol.,* **24**(20 Suppl): 1008.

Smith EM, Ritchie JM, Yankowitz J, Swarnavel S, Wang D, Haugen TH and Turek LP (2004). Human pappilomavirus prevalence and types in newborns and parents: concordance and mode of transmission. *Sex Transm. Dis.,* **31**: 57-62.

Smith JF, Brownlow M, Brown M, Kowalski R, Esser MT, Ruiz W, Barr E, Brown DR and Bryan JT (2007). Antibodies from women immunized with Quadrivalent Vaccine cross-neutralize HPV 45 pseudovirions. *Hum. Vaccin.,* **3**: 109-115.

Smith JS, Backes DM, Hoots BE, Kurman RJ, Pimenta JM. (2009). Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. *Obstet. Gynecol.,* **13**: 917-924.

Stanley M (2006). Immune responses to human papillomavirus. *Vaccine,* **24**: S16-S22.

The FUTURE II Study Group (2007). Prophylactic efficacy of a quadrivalent human papillomavirus (HPV) vaccine in women with virological evidence of HPV infection. *J. Infect. Dis.,* **196**(10): 1438-1446.

The FUTURE II Study Group (2007). Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N. Engl. J. Med.,* **356**: 1915-1927.

Tindle RW (2002). Immune evasion in human papilloma virus associated cervical cancer. *Nat. Rev. Cancer,* **2**: 1-7.

US Food and Drug Administration (2009). News release: FDA approves new indication for Quadrivalent Vaccine to prevent genital warts in men and boys, Available from http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm187003.htm. Accessed February 10, 2010.

Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, Brown DR, Ferenczy A, Harper DM, Koutsky LA, Kurman RJ, Lehtinen M, Malm C, Olsson SE, Ronnett BM, Skjeldestad FE, Steinwall M, Stoler MH, Wheeler CM, Taddeo FJ, Yu J, Lupinacci L, Railkar R, Marchese R, Esser MT, Bryan J, Jansen

KU, Sings HL, Tamms GM, Saah AJ and Barr E (2006). Immunologic responses following administration of a vaccine targeting human papillomavirus types 6, 11, 16, and 18. *Vaccine,* **24**: 5571-5583.

Villa LL, Costa RLR, Petta CA, Andrade RP, Ault KA, Giuliano AR, Wheeler CM, Koutsky LA, Malm C, Lehtinen M, Skjeldestad FE, Olsson SE, Steinwall M, Brown DR, Kurman RJ, Ronnett BM, Stoler MH, Ferenczy A, Harper DM, Tamms GM, Yu J, Lupinacci L, Railkar R, Taddeo FJ, Jansen KU, Esser MT, Sings HL, Saah AJ and Barr E (2005). Prophylactic quadrivalent human papillomavirus (types 6, 11, 16 and 18) L1 virus-like particle vaccine in young women: A randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol.,* **6**: 271-278.

Walboomers JM, Jacobs MV, Manos MM, Xavier F, Bosch J, Alain Kummer, Keerti V Shah, Peter JF Snijders, Julian Peto, Chris JLM Meijer and Nubia Munoz (1999). Human pappiloma virus is necessary cause of invasive cervical cancer worldwide. *J. Pathol.,* **189**: 12-19.

Wang SS and Hildesheim A (2003). Viral and host factor in human pappiloma virus persistence and progression. *J. Natl. Cancer Inst. Mongr.,* **31**: 35-40.

Watson M, Saraiya M, Ahmed F, Cardinez CJ, Reichman ME, Weir HK, Richards TB, (2008). Using population based cancer registry data to assess the burden of human pappiloma virus associated cancers in the United States, overview of methods. *Cancer*, **113**(10 Suppl.): 2841-2854.

Weinstock H, Berman S and Cates W Jr. (2004). Sexually transmitted diseases among American youth: Incidence and prevalence estimates, 2000. *Perspect Sex Reprod. Health,* **36**: 6-10.

Welters MJ, de Jong A, van den Eeden SJ, Van der Hulst JM, Kwappenberg KM, Hassane S, Franken KL, Drijfhout JW, Fleuren GJ, Kenter G, Melief CJ, Offringa R and Van der Burg SH (2003). Frequently display of human pappiloma virus type 16 E-6 specific memory t-helper cells in the healthy population as witness of previous viral encounter. *Cancer Res.,* **63**: 636-641.

Winer RL, Lee S-K, Hughes JP, Adam DE, Kiviat NB and Koutsky LA (2003). Genital human pappiloma virus infection: incidence and risk factors in a cohort of female university student. *Am. J. Epidemiol.,* **157**: 218-226.

Yasmin B, Asif B, Sania N, Ashfaq A, Ahmed U, Shahid P, Rashida A, Naila K, Ahmed R, Hadi B, Imtiaz B and Sheema H (2006) Pakistan - Country Profile of Cancer and Cancer Control. *J. Pak. Med. Assoc*., **56**(3): 2006.