WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 2536-2551. Research Article ISSN 2277– 7105

DETECTION HIGH RISK OF HUMAN PAPILLOMA VIRUS GENOTYPE (16/18) IN IRAQI WOMEN PATIENTS WITH CERVICAL CARCINOMA BY USING CHROMOGEN - INSITU HYBRIDIZATION (CISH) TECHNIQUE

[1]*Athraa Zaidan Hassan, [2]Assist Prof. Dr. Sajaa J. AL-Khalidi, [3]Dr. Ali Hussein Mohammed Ali AL- Khafaji and [4]Dr. Basher S. Mohammed

[1]M. Tech. Medical Laboratory Science Technology/ College of Health and Medical Technology

[2]Ph. D. Medical Microbilogy /Molecular Virology/College of Health and Medical Technology.

[3]Head of Histopathology Unit/ Central public Health Laboratories/ Baghdad

[4]Head of Laboratories Department/ Ministry of Health.

Article Received on
12 Aug 2015,

Revised on 04 Sept 2015,
Accepted on 27 Sept 2015

***Correspondence for Author**
Dr. Athraa Zaidan Hassan
M. Tech. Medical Laboratory Science Technology/ College of Health and Medical Technology, Iraq.

ABSTRACT

AIM: To investigate the association between human papilloma virus (HPV) infection and cervical cancer among Iraqi women patients.

Method: To determine the relationship between HPV and cervical carcinioma, a retrospective study was done. This study was carried out on 30 patients with hisopathologically confirmed primary cervical cancer. Samples were collected from each patients, as well as ten (20) cervical tissues from control individuals with no cancer. Chromogen In situ hybridization (CISH) was used to detect HPV DNA (HPV 16 /18 DNA CISH in cervical tissues. **Results:** HPV 16/18 was detected in 26/30 (86.7%) tumor sample while negative cases in 4/30 (13.3%) of tumor sample. and HPV 16/18 was detected in 5/20(25.0%) of cervical control group while negative cases in 15/20 (75.0%) of cervical control group. **Conclusion:** Our results suggest that cervical HPV infection is common in patients with carcinoma of the cervix with HPV16 / 18 being the most prevalent type in Iraqi women patients. HPV infection may play a role in cervical carcinogenesis.

KEYWORDS: cervical cancer, HPV, chromogen in situ hybridization.

INTRODUCTION

Papillomaviruses are a group of genetically related organisms, which infects cutaneous and mucosal epithelia and causes a variety of lesions ranging from benign tumors (plantar, flat and common warts, genital condylomas and papillomas) to cervical neoplasia and cancer. (Chow et al, 2010). Currently, 120 different HPV genotypes that infect humans have been classified and are allocated a type number according to the order of discovery (Bernard et al., 2010). Genital tract HPV types are classified by their relative malignant potential into low risk include (6, 11, 42, 43, 44, 55, 81, 83) and high risk types include (16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82). (Chaturvedi et al., 2011; Doorbar, 2006). Persistent infection with high- subtype of HPV is associated with the development of cervical cancer (Castle et al., 2011). HPV16 is the predominant HPV type of squamous cell cervical carcinomas and HPV18 predominates within adenocarcinomas (Bulk et al., 2006). The HPV infected epithelial cells and after integration with host DNA, the production of oncoprotein mainly, E6 and E7 disrupts natural tumor suppressor pathway and is required for proliferation of cervical cells (Nebesio et al., 2001).

Human papilloma virus also believed to play a role in other human cancer such as head and neck tumor (Marurs et al., 2010), skin cancer (Blomberg et al., 2012). Lung cancer (Srininvasan, 2009). Anal cancer (De vuyst et al.,2009; Kreimer et al.,2005). oropharyngeal squamous cell carcinoma (Panwar et al. 2013).

Cervical cancer is an important health problem for all women. Worldwide, in 2008, it was estimated that there were 473,000 cases of cervical cancer, and 253,500 deaths per year. in the United States in 2014 it was estimated that there new 12,360 and 4,020 death. (American Cancer Society, 2014). Cervical cancer accounts for 15% of female cancers in addition are more common and approximately 85% of cervical cancers occur in developing countries than in the developed countries (Kent,2010). HPV type 16 and 18 are the cause of 70% of cervical cancer globally (Parkin, 2002; Schiffman et al., 2007; Gadducci et al., 2011). The fundamental role of human papilloma virus in the etiology of cervical cancer has been firmly established. Accordingly, infection with oncogenic HPV types is now being considered as the primary cause of almost all cervical carcinoma (99%). A recent report shows that HPV types 16, 18, 31, 33, 35, 45, 52, and 58 account for 91% of all HPV DNA positive cervical cancers in the world, of which HPV16 and 18 are the most common (71%). HPV16, 18, and 45 were

found in 94% of the adenocarcinomas. (Bosh, 2002; Munoz et al.,2003; cogliano et al.,2005; de Sanjose et al., 2010).

This study aimed to investigate the presence of HPV 16/ 18 and DNA by using chromogen situ hybridization (CISH) in Cervical cancers in order to determine if a relationship exists between HPV infection and cervical neoplasm.

MATERIALS AND METHODS

The study was designed as a retrospective one. Cervical tissues were obtained from thirty patients with cervical cancer. Specimens belong to the period from June 2010 until November 2013. From each patient two blocks was taken formalin fixed, paraffin embedded cervical carcinoma and twenty cases from individual cervical tissue were proved to be free from any significant pathological changes were considered as a negative control groups for this study. Tumor, control blocks were collected from the archives of histopathology laboratories of Teaching Laboratories of the Medical City/Baghdad and Teaching Alkarmaa hospital, Teaching AlYarmouk hospital, AlWiya hospital for delivery as well as many private laboratories.

The diagnosis of these tissue blocks were based on the obtained pathological records of these cases from hospital files as well as histopathological laboratories records. A confirmatory histopathological re-examination of each obtained tissue blocks was done. Four µm thick sections were made and sticked on positively charged slides. Chromogenic in Situ Hybridization (CISH)/Detection system (Zytovisions GmbH. Bremerhaven. Germany) used to target DNA sequences using Digoxigenin– labelled long DNA probe for HPV types 16/18. Method was conducted according to the instructions of manufacturing companies leaflet. Positive control reactions were performed by replacing the probe with Biotinylated and Digoxigenin housekeeping gene probe. For the negative control, all reagents were added except the probe. Proper use of this hybridization/detection system gave an brown blue signal at the specific site of the hybridization probe in positive test tissue. Quantification of different molecular markers in situ hybridization signal was evaluated under light microscopy and the counting of positive cells was performed at X1000. Chromogen In situ hybridization was given intensity and percentage scores, based on intensity of positive signals and number of signals, respectively.

The intensity score included low, moderate, and high intensity of reaction. Positive cells were counted in ten different fields of 100 cells for each sample and the average of positive cells of the ten fields was determined assigning cases to one of the three following percentage score categories: Score(1)=1-25%, Score(2)= 26-50%, Score(3)>50% (10). Chi-square test was used to detect the significances between variables of our study. All the statistical analysis was done by SPSS program (version-18). P-value was considered significant when < 0.05.

RESULT

The archival specimens collected in this study were related to cervical cancer patients whom ages were ranged from twenty five to seventy three years. The mean age of the patients with cervical carcinoma was (50.6± 11.98) while cervical control groups were ranged from twenty three to sixty nine years. The mean age of the cervical control groups was (47.10± 9.28). There are non statistical significant difference (pvalue>0.05) between different groups according to age.(Table 1).

Study groups typed according to the histological diagnosis of cervical cases after investigation by Hemotoxillin & Eosin (H&E) stained slides and the studied cervical carcinoma has been classified into two histopathological types:- squamous cell carcinoma and adenocarcinoma. Histological diagnosis of cervical carcinoma cases showed that high percentage of squamous cell carcinoma (25 cases; 83.3%) followed by the adenocarcinoma which made (5 cases; 16.7%). The statistical analysis shows highly significant difference (P<0.05) on comparing histopathological types of cervical carcinomas.(Table 2).

the distribution of cervical carcinoma cases according to histopathological grades also evaluated in the present study. It was found that moderate differentiated grade in cervical carcinoma cases constituted of (60%) (18 out of total 30 cases), whereas cases with well and poorly differentiated grades constituted of (30%) (11 out of 30 cases) and (10%) (1 out of 30 cases) respectively. the statistical analysis of grading distribution of cervical cases shown non significant difference (p>0.05) on comparing histopathological grades of cervical. (Table 3).

Regarding cervical cancer group, the total percentage of positive HPV16/18 – CISH detection was (86.7%) (26 out of 30 cases), whereas in the cervical control group, it was (25%) (5 out of 20 cases) Statistically, highly significant difference (p<0.05) was found on comparing the percentage of HPV16/18 among the study groups.(Table 4).

Our investigation in the cervical carcinoma group, monitor the highest percentage of HPV 16/18 score signaling was made (46.7%: 14 out of 30 cases) in the low scores (score I), whereas (30%: 9 out of 30 cases) and (10%: 3 out of 30 cases) were found within score II and score III respectively. While in cervical control group, it was found that (20%: 4 out of 20 cases) and (5%: 2 out of 20 cases) were distributed in score I and score II , respectively . The HPV16/18 DNA was detected in a higher percentage in cervical carcinoma than their cervical control counterpart group. Statistically, highly significant difference ($p<0.05$) were found in comparing the percentage of HPV16/18 in the study groups according to the signal score numbers as shown in (Table 5).

the percentage of HPV- infected cells that were evaluated for the intensity of HPV 16/18 – CISH reactions was shown in current study .It was found that High signal intensity were in 12 cases (40%), moderate signal intensity in 8 cases (26.7%) and low signal intensity in 6 cases (20 %). In the cervical control group the low signal intensity was found in 3 cases (15 %) followed by 2 cases (10%) with high signal intensity. Statistically, highly significant difference ($p<0.05$) were found in comparing the results of these study groups according to their signal intensity.(Table 6).

In the current study, the pattern of HPV 16/18 replication in cancerous and non cancerous cervical tissues revealed that there was a highly significant difference ($p<0.05$) between the HPV16/18 positive cases and patterns of DNA replication. The HPV16/18 DNA signals were detected in cervical carcinoma cases with high percentage (46.7%) as dot (integrated DNA) and mixed patterns (episomal and integrated) in (30.0%) of the cases and (10.0%) of the cases as diffused (episomal DNA) while all the positive HPV16/18 signal in cervical control group were showed diffused pattern of replication which represented in (25%) of them (Table 7).

Histopathological features were studied between positive and negative HPV with cervical carcinomas. The results are shown in (Table 8). It was found the positive results of ISH reactions of HPV16/18 HPV according to tumor grade of cervical cancer tissues were found 66.7% that have well differentiated. While the positive results of CISH reactions of HPV16/18 according to tumor grade of cervical cancer tissues were found 94.4% that have moderate differentiated. Lastly, the positive results of ISH reactions of HPV16/18 according to tumor grade of cervical cancer tissues were found 100.0% that have poorly differentiated.

Statistically, no significant differences ($p > 0.05$) on comparing the results of HPV genotypes with tumor grade of cervical cancer. (Table 8).

According to the association between CISH results of HPV16/18 DNA signals and histological types of cervical carcinoma, show that there were high positive results of HPV16/18 DNA signals in squamous cell carcinoma and it was detected in 23 cases (92.0%). No significant association was found between cervical carcinoma types and HPV 16/18 CISH positive signals ($P<0.05$) (Table 8).

Table (1): Distribution of cervical carcinoma patients &control group according to their age

The study groups	Number	Mean age	SD	SE	Minimum	Maximum
Cervical carcinoma	30	50.60	11.98	2.19	25.0	73.0
Cervical control	20	47.10	9.28	2.08	32.00	69.0
Statistical analysis	(p_{value} = 0.26>0.05) Non Significant					

Table (2) Distribution of cervical cases according to the histopathological types

Histological types	Cervical carcinoma		p- value
	NO	%	
Squamous cell carcinoma	25	83.3%	0.001<P Highly significant
Adenocarcinoma	5	16.7%	
Total	30	100	

Table (3): Distribution of cervical carcinoma cases according to histopathological grades

Histological grades	Cervical carcinoma		p-value
	NO	%	
Well Differentiated	9	(30.0%)	0.54 non Significant
Moderate Differentiated	18	(60.0%)	
Poorly Differentiated	3	(10.0%)	
Total	30	(100%)	

Table (4): The frequency of distribution HPV16/18 signals among study groups by using CISH assay

Study groups	HPV16/18 signal – CISH				P- value
	Positive		Negative		
	NO	%	NO	%	
Cervical cancer (30 cases)	26	86.7%	4	13.3%	<0.001 Highly Significant
Cervical control (20 cases)	5	25.0%	15	75.0%	

Table (5): Frequency distribution of positive HPV16/18 DNA signal between study groups according to the score reading

Study groups	Total HPV16 CISH -ve results	Positivity according to score numbers			Total HPV16 CISH +ve results	p-value
		Score I	Score II	Score III		
Cervical cancer (30 cases)	4 (13.3%)	14 (46.7%)	9 (30.0%)	3 (10.0%)	26 (86.7%)	< 0.001 Highly significant
Cervical control (20 cases)	15 (75%)	4 (20%)	1 (5.0%)	0 (0.0%)	5 (25.0%)	

Table (6): Frequency distribution of positive HPV16/18 DNA signal intensity among study groups

Study groups	Total HPV16/18 CISH -ve results	Positivity according to intensity			Total HPV16/18 CISH +ve results	p- value
		low	Moderate	High		
Cervical cancer (30 cases)	4 (13.3%)	6 (20.0%)	8 (27.6%)	12 (40.0%)	26 (86.7%)	<0.001 Highly significant
Cervical control (20 cases)	15 (75.0%)	3 (15%)	0 (0.0%)	2 (10.0%)	5 (25.0%)	

Table (7): Frequency distribution of positive HPV16/18 DNA among study groups according to patterns of replication

Study groups	Total HPV16/18 CISH –ve results	Positivity according to patterns			Total HPV16/18 CISH + ve results	p- value
		Dot	Diffused	Mixed		
Cervical cancer (30 cases)	4 (13.3%)	14 (46.7%)	3 (10.0%)	9 (30.0%)	26 (86.7%)	<0.001 Highly significant
Cervical control (20 cases)	15 (75.0%)	0 (0.0%)	5 (25.0%)	0 (0.0%)	5 (25.0%)	

Table (8): Relationships between HPV16/18 DNA CISH results in correlation with clinico-pathological findings in cervical carcinoma

characteristics		No.	Total HPV16/18 CISH RESULTS				P- value
			Positive		Negative		
			No.	%	NO.	%	
Tumor types	SCC	25	23	92.0%	2	8.0%	0.11 Non Significant
	AD	5	3	60.0%	2	40.0%	
	Total	30	26	86.7%	4	13.3%	
Tumor Grades	Poorly	3	3	100%	0	0.0%	0.10 Non Significant
	Moderate	18	17	94.4%	1	5.6%	
	Well	9	6	66.7%	3	33.3%	
	Total	30	26	86.7%	4	13.3%	

DISCUSSION

The causal role of human papillomavirus infections in cervical cancer has been documented beyond reasonable doubt. The association is present in virtually all cervical cancer cases worldwide.(Bosch *et al*.,2002). The carcinogeneity of HPV is related to the activity of two oncoproteins, E6 and E7. E6 inhibits p53 in the blocking of apoptosis, and E5 inhibits pRB (retinoblastoma suppression protein) in abrogating cell-cycle arrest. (Doorbar, 2007)Both proteins are expressed at low levels during the infectious phase, but at some point in the progression to precancer the expression of E6 and E7 is deregulated, leading to their over expression and in unregulated cellular proliferation (Schiffman et al., 2007). In western Asia, the region Iraq belongs to about 2.5% of women in the general population were estimated to harbor cervical HPV16/18 infection at given time and (72.0%) of invasive cervical cancer are attributed to HPV16/18 (WHO/2010).

In the current study, the mean age of patients with cervical carcinoma was 50.6± 11.98 . this results were comparable to reported by lena, 2011, Shirish et al 2014. In Iraq Liqaa et al (2008) had observed that in Iraq the mean age of squamous cervical carcinoma was (49.6±10) years and those results are in consistence with the current study.

Our data show the most common type of cervical carcinoma was squamous cell carcinoma. .A similar trend to that frequency was reported in another studies (Boyle &Levin, 2008; Misra et al, 2009, Kalyani et al, 2010; Dikshit et al, 2012; Lotten, 2013) In contrast Bray et al, 2005; Martins et al, 2009 who observed that there are an increase trends in cervical adenocarcinoma among young women and those results was found to be incompatible to the present results. It was proposed that the reason for increasing rates of cervical adenocarcinoma in other countries may likely due to unintended effects of cytology-based cervical cancer screening which cannot adequately detect the pre-cancerous lesions leading up to adenocarcinoma (precursor lesion). Cervical adenocarcinoma precursor lesion were typically higher in the adenocarcinoma precursor lesion were typically higher in the endocervical canal, which are making them less accessible the squamous cell carcinoma precursor lesion during pap test screening and are allowing invasive cervical cancer to be developed. (Castellsague et al, 2006; Parkin & Bray, 2006).

According to histopathological grades, Our investigation was found that the most prominent grades among cervical carcinoma cases were Moderate differentiated grade these finding

agreement with other studies.(vaida et al., 2011, Elizabeth et al 2009, IKechebelu et al., (2010, Pannayana, 2014).

In the present study, HPV DNA 16 /18 was detected in (86.7%) of cervical carcinoma cases. this is comparable with Clifford et al, 2003; Robert et al, 2012; Yasmine, 2012; Das et al, 2013; Zhang, 2013; Nahid et al, 2014. They found that the percentage of HPV16/18 in cervical carcinoma was made 77%, 81.5%, 88%, 88%, 89%, 100%, respectively. In Iraq. Jasim Mohammed, (2013) detected HPV16 DNA in (70%) of squamous cervical carcinoma cases by using in situ hybridization technique.

we found highly significant evidence between cervical carcinoma and HPV infection in the role of HPV infection between cervical carcinoma and cervical control group in the analyzed samples, this is keeping compatibility with the findings of Arora et al., (2005); parkin et al., (2005), Hossein et al., (2014). According to the Smith et al., 2007. Detected prevalence of HPV16/18 rate in Europe which made (57.6%) and in North America (55.1%). In current study we found that the incidence of HPV16/18 show higher rate in comparison with those results.

Difference in studies in showing a significant association between HPV infection and cervical cancer may be due to the difference in the type of pathological lesion, the viral load, duration of infection, heterogeneity of laboratory assays employed for HPV DNA detection and population studied, variation in HPV type– specific sensitivity of different PCR protocols. Iftner and villa, (2003) explained those variations, in addition factors could also influence variation in prevalence rates, include the lack of screening programs, sexual behaviors of both men and women (such as early age at first marriage to older men or to men with several concurred partners and poor hygienic conditions might be some of these factor. (Bayo et al., 2002).

In current study, high percentage of HPV 16/18 signal in the cervical were found in low score categories (Table 5) this may reflect a low reproduction (replication) rate of the virus in cervical as well as, the low intensity and high intensity of HPV16/18 DNA signals may also reflect viral latency or past infection and continuous replication (Giordano et al., 2008). Our results revealed that positive cases in cervical control groups were in a low score with low intensity categories (Table 5 & Table 6). This may reflect that HPV16/18 may have

precancerous effect and considering problem in the future which could be development of malignancy (NelbaTabora, 2008).

In direct way this could be related to the certain host genetic factors, such as polymorphism or variation in specific human leukocyte antigen (HLA) class II have been implicated in the natural history of HPV infection, some associated with positive risk of HPV persistence whereas other are associated with negative risk of persistence HPV infection (Scheurer, 2005). It has been reported that distribution of HPV variant varied in different geographical areas suggested that the virus and the host have coevolved over time, in addition to the host and pathogen genetic variation, the difference of detection methods used in the studies might also account for data discrepancy (Stewart, 1996).

It was evident from our data that dot or mixed chromogenic in situ hybridization signals were highest in the HPV positive in cervical carcinoma cases(Table 7) which revealed that the integration of the viral genome is considered a critical step in the progression to cancer (Mc Murray et al.,2001). Our observation show compatibility with Janine et al., (2006), Shirish et al., (2014). In this respect, in low grade lesion HPV was found to be in episomal form whereas in high grade lesions and cancer, the HPV DNA is more likely to have been integrated into the host– cell chromosome. The integration of HPV DNA into the host DNA increases cellular proliferation and the chance of malignancy. (Scheurer et al.,2005).

In the current study, the presence of episomal signals in all positive cases in cervical control group may reflect non progression intraepithelial lesion (Kalof et al., 2005; Jung, 2010).

Anton et al., (2005) concluded that the punctuate patterns which was assumed to be an indication of integration of the virus into the genome, correlates strongly with the transition of a premalignant lesion which maintain the HPV 16/18 as episomal form to microinvasive carcinoma.

According to Lee et al., (2004), HPV DNA presentation was not found to be significantly associated with other clinico - pathological parameters include tumor types, tumor grades. This results were likewise to the results of current study as shown in (Table 8).

Also in current study, no significant relationship was found between HPV16/18 CISH positive signals in correlation with cervical carcinoma types, this results show agreement with previous studies reported by (De sanjose et al., 2010; fernandes et al., 2010; cristina et

al., 2013). But other studies observed that HR- HPV presentation show that statistically significant difference (pvalue <0.05) according to histological grades of cervical dysplasia (Xu et al., 2009; Borna et al., 2015). Those results incompatibility with the present study. Those variation in percentage of detection HPV when compared to their grades might be related to difference in host and pathogen genetic variation, cellular immune response states. the CD4 helper cells are known to play a role in the control of HPV at the mucosa and when impaired cellular immunity in groups such as: renal transplant patients and HIV (Human immune deficiency virus) positive individual, auto immune disease give more room for the establishment and persistence of HPV infection (Clifford et al., 2006; Banura et al., 2008).

In Summary high risk HPV16/18 infection seems to be common in Cervical cancer tissues suggesting that HPV might play a role in the pathogenesis of cervical cancer and can be studied effectively by chromogen in situ hybridization (CISH) technique.

REFERENCES

1. Chow LT, Broker TR, Steinberg BM. The natural history of human papilloma virus infections of mucosal epithelia. APMIS, 2010; 118: 422-449.
2. Doorbar J, Molecular biology of human papillomavirus infection and cervical cancer. Clinical Sciences, 2006; 110: 525-541.
3. Chaturvedi AK, katki HA, Hildesheim A, et al. Human papillomavirus infection with multiple types: pattern of coinfection and risk of cervical disease. J Infect Dis, 2011; 203(7): 910-20.
4. Castle PE, Rodrigue AC, Burk RD, et al. Long term persistence of prevalently detected human papillomavirus infections in the absence of detectable cervical precancer and cancer. J Infect Dis, 2011; 203(6): 814-22.
5. Bulk, S., Berkhof, J., Bulkmans, N. W., et al. "Preferential risk of HPV16 for squamous cell carcinoma and of HPV18 for adenocarcinoma of the cervix compared to women with normal cytology in The Netherlands", Br. J. Cancer, 2006; 94(1): 171-175.
6. Nebesio CL, Mirowski G., and Chuang TY. Human papillomavirus: clinical significance and malignant potential. Int J Dermatol, 2001; 40(6): 373-9.
7. Marurs D, Souza G, Westra WH, et al. Human papillomavirus associated head and neck cancer:a virus –related cancer epidemic .Lancet oncol, 2010; 11(8): 781-789.
8. Blomberg M, Friis S, Munk c,et al. Genital warts and risk of cancer :a Danish study of nearly 50000 patients with genital warts. J Infect Dis, 2012; 205(10): 1544-53.

9. Srininvasan M, Taioli E., and Ragin CC. Human papillomavirus type 16 and 18 in primary lung cancers-amta- analysis .carcinogenesis, 2009; 30(10): 1722-1728.
10. De vuyst H, Clifford D, Nascimento MC, et al. Prevalence and type distribution of human papillomavirus carcinoma and intra epithelial neoplasia of the vulva, vagina and anus : ametaa- analysis .Int J cancer, 2009; 124: 1626-36.
11. Kreimer AR, Clifford GM, Boyle p, et al. Human papillomavirus types in head and neck squamous cellcarcinomas worldwide: asytematic review. Cancer Epidemiol Biomarkers Prev, 2005; 14: 467-75.
12. Panwar A, Batra R, Lydia AA, et al. (2013). Human papillomavirus positive oropharngeal squamous cell carcinoma: Agrowing epidemic. Cancer Treat Rev.
13. American Cancer Society. Cancer Facts and Figures 2014. Atlanta, Ga: American Cancer Society; 2014.
14. Kent, A. "HPV Vaccination and Testing". Reviews in obstetrics and gynecology, 2010; 3 (1): 33–34. PMC 2876324.
15. Parkin D.M. et al. Global cancer statistics, 2002. CA Cancer J Clin., 2005; 55(2): 74-108.
16. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet, 2007; 370(9590): 890-907.
17. Gadducci A, Barsotti C, Cosio S, et al. (2011). "Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature". Gynecological Endocrinology, 2011; 27(8): 597–604.
18. Bosch X, Lorincz A, Munoz N, et al. The causal relation between human papillomavirus and cervical cancer. J clin pathol, 2002; 55(4): 244-65.
19. Munoz N, Bosch F X, de Sanjose S, et al. Epidemiological classification of human papillomavirus types associated with cervical cancer. N Engl J Med, 2003; 348: 518–27.
20. Coglian V, Baan R, Straif K, et al. Carcinogenecity of human papillomaviruses. Lancet oncol, 2005; 6(4): 204.
21. De Sanjose S, Quint W, Al emany L, et al. Human papillomavirus genotype altribution in invasive cervical cancer: aretrospective cross- sectional worldwide study . Lancet oncol., 2010; 11: 1048-1056.
22. Doorbar J and Griffin H .Intrabody strategies for the treatment of human papillomavirus-associated disease. Expert Opin Biol Ther, 2007; 7: 677-89.
23. Lena S, (2011). cervical cancer prevention studies on outcome of cervical screening and on management of abnormal cytology findings. PHD thesis submitted to university medical dissertation, New series No1402, Umea university.

24. Lena S, (2011). cervical cancer prevention studies on outcome of cervical screening and on management of abnormal cytology findings. PHD thesis submitted to university medical dissertation, New series No1402, Umea university.
25. Liqaa R, Ismail I, Arwa M, et al., Astudy on human papillomavirus using in situ hybridization technique and its role in cervical Neoplasia. Iraqi J Med SCI, 2008; 6(1): 28-37.
26. Boyle P and Levin B. World cancer Report 2008. Lyon, france: IARC press; 2008.
27. Mirsa JS, Srivastava S, Singh H, et al. Risk–factor and strategies for control of carcinoma cervix in india: Hospital based cytological screening experience of 35 years Indian J cancer, 2009; 46: 155-9.
28. Kalyani R , Das D, Bindra S, et al. cancer profile in the department of pathology of Sri Devaraj urs medical college : a ten years study . Indian J cancer, 2010; 47: 160-5.
29. Dikshit R, Cupta PC, Ramasundara C, et al. Cancer mortality in India: anationally representative survey. The lancet, 2012; 379:1807-16.
30. Lotten D. Cervical cancers studies on prevention and treatment . Phd thesis submitted to university lund, Sweden, 201; P 41: 70.
31. Castellsaguev X, Diaz M, De sanjose, et al. World wide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: Implication for screening and prevention .J NAH cancer Inst, 2006 Mar 1; 9815: 303-15.
32. Parkin DM and Bray F. The burden of HPV – related cancers. Chapter 2. Vaccine. Aug 31; 24suppl 3:S3111-25.
33. Vaida A, Algidas B, Adrijus K, et al. Diffusion –weight magnetic resonance imaging of cervical cancer .Acta Medica lituanica, 2011; 18(4): p139-146.
34. Elisabeth W. Endometrial carcinoma astep closer ti individualized therapy? Exploring transcriptional alterations in relation to prognositic biomarkers. Phd thesis submitted to university of Bergen, Norway, 2013; P96.174.
35. Ikechebelu JI, Onyiaorah TV, Ugboaja JO, et al. Pthological analysis of cervical cancer seen in tertiary health facility in Nnew: South East Nigeria. Journal of obstetrics and Gynaecology, 2010, 30(3): 229-301.
36. Pannayana P, Shuresh S, Raju K, et al. Clinico pathological correlation of cervical carcinoma : Atertiary Hospital Based study. Asian pac J cancer prev, 2014; 15(4): 1671-1674.
37. Clifford GM, Smith JS, plummer M, et al. Human papillomavirus in invasive cervical cancer world wide: ameta – analysis, Brit J cancer, 2003; 88(1): 63-73.

38. Robert S. (2012) .The impact of the determination of human papillomavirus infection in cervical cancer screening strategy and follow-up strategy of precancer lesion procedures. Phd thesis submitted to university of Pecs, faculty of medicine.
39. Yasmin Leela. (2012). Risk factor for early onset cervical carcinoma and high grade cervical dysplasia are there high risk groups that required earlier papanicolau cytology screening and human papillomavirus vaccination. phd thesis submitted to faculty of medicine.
40. Das D, Rai AK, Kataki AC. et al. Nested multiplex PCR based detection of human papillomavirus in cervical carcinoma pateints of North-East India. Asian pac J cancer prev, 2013; 14(2): 785-90.
41. Zhang S. Detection of high risk papilloma virus subtypes in cervical glandular neoplasia by in situ hybridization. Int. J clin Exp pathol, 2013; 6(10): 2168-2177.
42. Nahid Yusuf, et al. (2014). Evalution of HPV-DNA test in detection of precancerous and cancerous lesions of cervix.RR J MHS volum 3 issue1.
43. Jasim Mohammed M, (2013). In situ hybridization Apporach for detection Epstein – bar virus and human papillomavirus type 16 in Agroup of Iraqi women with cervical carcinoma .Master thesis submitted to the college council of Health and Medical Technology.
44. Arora R, Kumar A, Prusty BK, et al. Prevalence of high- risk human papillomavirus (HR-HPV) types 16 and 18 in healthy women with cytologically negative pap smear . Eur J obstet Gynecol Reprod Biol, 2005; 121(1): 104-9.
45. Parkin DM, Whelan SL, Ferlay J, et al. Cancer in five continents. IARC Scientific publication 2005,. VIII.
46. Hossein A, Fatemeh H, Mohammad M, et al. Human papillomavirus 16/18 genotypes in pateints with squamous cell carcinoma of cervix in North East Iran . Niger Med J. Nov-Des, 2014; 55(6): 495-498.
47. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high grade cervical lesions:A meta analysis update .Int J cancer, 2007; (12): 621-632.
48. Iftner Tand Villa LL.(2003). Chapter 12: Human papillomavirus technologies. J NaH cancer Inst Monogr , 80-88.
49. Boyo P, Bosch FX, De sanjose S, et al. Risk factor of invasive cervical cancer in Mali. Int J Epidemiol, 2002; 31: 202-9.

50. Giordano G, Adda TD, Gnetti L, et al. Endometrial mucinous micrglandular adenocarcinoma: morphological, immunohistochemical features and emphasis in the HPV status. Int .J.Gynecol.pathol, 2008; 25: 77-82.
51. Nelba Tabora, Annabelle F, Judith M, et al. High HPV16 viral load is associated with increased cervical dysplasia in Honduran women. AMJ Trop Med Hug, May 2008; 78: 5843-846.
52. Scheurer ME, Tortolero-Luna G and Adler- Storthz K : Human papillomavirus infection : biology, epidemiology and prevention . Int J Gynecol cancer, 2005; 15(5): 727-746.
53. Stewart ACM, Eriksson AM, Manos MM, et al; Wheeler CM. Intratype variation in 12 human papillomavirus types : a worldwide perspective . J. Virol, 1996; 70: 3127-3135.
54. Janine T B, Frank T, DeeMarie S, et al. Detection of specific Human papillomavirus Types in Paraffin- Embedded Section of Cervical Carcinomas. Journal of Medical Virology, 2006; 78: 117-124.
55. Shirish S, Sutapa M, Gauri S, et al. physical state and copy number of high of risk human papillomavirus type 16 DNA in progression of cervical cancer .Indian J M ed Res, April 2014; 139: 531-543.
56. Kalof A.N, Evans M. F, Simmons- Aroldl L, et al .p16 INK4A immunoexpression and HPV in situ hybridization signal patterns: Potenial markers of high grade cervical inteaepithelial neoplasia ".A M .J. Surg . pathol, 2005; 29: 674.
57. Jung Uee Lee, Jung Ha Shin, Jong Ok Kim, et al. Evaluation of the HPV ISH Assay in Cervical Cancer. The Korean Journal of Pathology, 2010; 44: 513-20.
58. Anton HN, Miriam AK, Frank S, et al. HPV in situ hybridization: impact of different protocols on the detection of integrated HPV. Int .J. Cancer, 2005; 115: 419-428.
59. Lee- Wen H, Shiouh- Livng C, Jiann-Loung, et al. Human papillomavirus -31 related types predict better survival in cervical carcinoma. Cancer, 2004; 100: 327-34.
60. Fernandes JV, Meissner RV, Carvalho M, et al. Prevalence of human papillomavirus in archoval samples obtained from pateints with cervical pre- Malignant and Malignant lesions from North east Brazil BMC Res Notes, 2010; 3: 96.
61. Cristina Mendes, Jose H, Jesus P, et al. Human papillomavirus genotypes distribution in 175 invasive cervical cancer cases from Brazil. BMC cancer, 2013; 13: 357.
62. Xu Y, Dotto J, Hui Y, et al. High grade cervical intraepithelial and viral load of high – risk human papillomavirus: significant correlation in pateints of 22 years old or younger. International Journal of clinical and Experimental pathology, 2009; 2: 129-175.

63. Borna NN, Tabassum S, Jahan M, et al. Genotyping of high risk human papillomavirus (HPV) among cervical precancer and cancer pateints. Acta Media International Jan -2015 volum 2 issue.
64. Clifford GM, Goncalves MA, Franceschi S; HPV and HIV Study Group. Human papillomavirus types among women infected with HIV: ameta- analysis .AIDS, 2006; 20(18): 2337-44.
65. Banura C, Franceshi S, Van D, et al. Prevalence, incidence and clearance of human papillomavirus infection among younger primiparous pregnant women in Kampala, Uganda. Int J Cancer, 2008; 123: 2180-7.