International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.7, pp 218-225, 2016

Cytomegalovirus correlation with bladder cancer patients by using insitu hybridization technique in Baghdad city

Noor Al-Huda Ali A.H. Saeed[1]*, Inas Waleed Shakir[1], Rana Sabah Jawad[1]

[1]Dept. of Biology,College of Science. University of AL-Mustansiriya,Iraq

Abstract: Worldwide, one of the most common cancers is bladder carcinoma which is infect mostly the older people with highest incidence especially in the industrialized countries. The most important factor associated with many human cancers including bladder carcinoma is viral agent, that's why, this study was resolved to determine the correlation of human cytomegalovirus in some Iraqi patients with bladder carcinoma by using insitu hybridization technique. A series of fourty bladder tissue block from different sites(transitional cells and squamous cells) from patients who had undergone cystactomy and already diagnosed by specialist as a bladder cancer patients. Samples were collected during the period between 2012 until 2014> The mean age of those patients was 62.6 years ranged between 45-85 years of 20 women and also 20 men . The histological grade included 21,9 and 10 of well, moderate and poor differentiated type, whereas, the tumor staging included 30 patients falling in stage I-II and the other 10 patients falling in III-IV tumor stage, most of cases 25 out of 40 were transitional cell carcinoma and 15 of them were squamous cell carcinoma. Human cytomegalovirus was detected in 17(42.5%) out of 40 patients , while, according to the positive results of CMV, it was related in highly significant association with each of sites and the grade of the tumor and with significant correlation with age, gender and the stage , but there was no significant relation found with smoking patients.
Keywords: Bladder cancer, Cytomegalovirus, Insitu hybridization.

Introduction

Human cytomegalovirus is a double stranded DNA virus, it's a member of the herpes virus family (1). Infection is worldwide usually asymptomatic (2). Many agent like radiation, chemicals and viruses that found to induce human cancer(3, 37). So, the most important infectious agent associated with human cancers are viral factors(4, 38).

Recently it suggests that chronic viral infection play a role in cancer etiology(5, 42). It was estimated that 17-20% of cancers incidence worldwide attributable to viral cause(6). Infection of cytomegalovirus was implicated in malignant disease by different cancer entities(7).

Previously , it proposed that the concept of oncomodulations in human cytomegalovirus may favor tumor progression without being an oncogenic virus to explain the presence of HCMV in tumor tissues(8). Oncomodulation of HCMV means that it may infect tumor cells and modulate their malignant properties(9), furthermore, HCMV genes and proteins have been detected in many kinds of human cancers (10). One of the most common human cancers worldwide is bladder cancer(11).

Approximately 90-95% of bladder cancers are transitional cell carcinoma(12) and about 5% of it are squamous cell carcinoma , less than 2% of all bladder cancers are adenocarcinoma or small cell carcinoma(13). The urinary bladder cancer typically seen in older patients, more than 90% of bladder cancer cases occur in patients older than 55 years of age(14). Bladder carcinogenesis probably related with viral infection also bacterial infection, commonly relation with bilharzial infestation rather than parasite(15).

Increasing exposure to the chemicals, population aging progression of tobacco epidemic in developing communities it is expected that asignificant increase of occurs of bladder cancer incidence in the future(14). The smoking habit is also a major cause of bladder cancer especially in middle and low income countries(14).

Materials and Methods

The present study involved fourty bladder carcinoma patients with mean age 62.6 years ranged between (45-85) years, male-female ratio 1:1, whom already diagnosed as a bladder cancer patients by specialist and undergone surgical operation in Baghdad Medical City between the period of 2012 until 2014.furthermore, No one of those patients had received any anticancer therapy before the surgery . fourty paraffin embedded tissue blocks were collected randomly from Teaching Laboratories/Baghdad Medical City.

Cytomegalovirus were determined in specimens using Insitu hybridization method and performed as recommended in leaflet with kits.

- DNA probe Hybridization/ Detection System : Highly sensitivity Insitu kit. A complete hybridization and immunodetection system were purchased from Maxim Biotech , USA. Cat. No. (IH-60001), (IHD-OOSO).
- Biotinylated Long DNA probe for CMV matrix Protein was purchased from Maxim Biotech, USA .Cat No. IH-60043(CMV-6001-B)

Insitu Hybridization Method

Three steps of ISH for detection of CMV in bladder cancer patients (39).

- Prehybridization : Tissue blocks were cut 4 µm , all samples were deparaffinized and dewaxed in xylene, series of ethanol (100,90,70%) and D.W. respectively, immersed in pre heated (98 Cº) citrate buffer (pH:6). Tissue deprotinization performed by placing it in proteinase K solution, dehydration by immersing the slides in D.W. then ethanol (70,90,100%).
- Hybridization step done by added CMV probe into each sections , denature the DNA probe by placed it in oven 98 Cº and removed from oven then incubated over night at room temperature to allow hybridization of probe with target nucleic acid.
- Post hybridization step: fall off the coverslip by using protein block buffer , conjugate were placed onto sections ,then substrate used , counterstained by Nuclear Fast Red (NFR), dehydration by 90,100% ethanol and xylene then finally mounted with DPX.

Statistical analysis

The statistical analysis system SAS was used to effect of different factors in study parameters. Chi square test was used to significant compare between percentage in this study(16).

Results and Discussion

Bladder cancer incidence has increased with highest rate in the last decades (7), similar to other types of cancer, bladder cancer increased occur in Iraqi people after exposure to depleted uranium in highly levels on the two gulf wars(17, 41).

It is commonly prevalent in older population with age over 60 years of old(14,18,19)and(20), this finding compatible with our studied patients mean age 62.6 ranged between 45-85 also confirmed with (21) with age ranged from 36-83 years of their patients and mean age 66 years.

Previous studies reported that more than 90% of all bladder cancers are transitional cell carcinoma which is the most common kind of bladder malignancies (22,23,24),this is confirmed to our studied group of patients which was involved (62.5%) 25 out of 40 biopcies of transitional cell carcinoma and the remaining 15 samples(37.5%) were squamous cell carcinoma which was agreed with (13) who reported that 90% of bladder cancer are TCC, and 5% SCC and the rest are uncommon bladder cancer like adenocarcinoma.

According to the histological grade of the tumor in this study, the cases consist of 21 well differentiated type, 9 moderate type and 10 were poorly differentiated carcinoma .Our finding compatible with (25) who reported the highly incidence in the moderate and the lowest occurrence with poorly differentiated carcinoma samples(26).

A lot of studies refers to fact that bladder cancer occurs in male more than in female (27), in contrast with our finding of male to female ratio 1:1.

Regarding to the staging , the results showed that the majority of cases 30 out of 40 were falled in stage I-II whereas, the remaining 10 of the biopcies were falled in stage III and IV, this was confirmed with (28) who reported similar to our finding that 43% of their cases were in stage II, whereas, 39% of stage III. All of these finding suggested that because of early detection of the types of bladder cancer that's why the majority falling in the earlier stages.

One of the most important cause involving in bladder cancer was smoking , in the current study it was including 20 smoker (50%), previous reports like(29) recognized smoking as an important risk factor for bladder carcinomas, it is account for about 50% of all tumors. So, smoking can develop bladder cancer three times more in smoker than non- smoker people(30).

Previous studies suggested that cytomegalovirus acts as a cofactor in the pathogenesis of some human cancers (31)

By using insituhybridization method, fourtyparaffine embedded tissue blocks of bladder cancer patients were involved in this study to detection for cytomegalovirus infection. As seen in figure(1), the positive results of CMV in 40 cases were detected in 17 out of them (42.5%). This finding approach to (32) who detected CMV in 48% of bladder cancer patients.

By using polymerasechain reaction, some reporters detected CMV in 30% of bladder cancer(33) also documented that 11% of their bladder cancer patients examined to detect CMV by using PCR.

One reporter (34) represented a highly significant rate between CMV infection and bladder cancer tissue(43%) in group of schistosomal patients , whereas, in non schistosomal patients was detected in 12% both of them at $p<0.009$.It suggested that CMV implicated in the development of bladder carcinoma and play a major role in the etiology of bladder cancer.

In figure 2, the positive results of CMV including 7 out of 18 patients were falling in age of ≤ 60, whereas 10 out of 22 patients were falling in > 60 years of age. In figure 3 , the results revealed that 10 out of 20 patients were male that showed positive CMV correlation , while, 7 out of 20 cases were female that showed positive results .In figure 4, Out of 21 cases, 8 well differentiated were positive CMV relation,whereas,4 out of 9 of moderately differentiated and 5 out of 10 poorly differentiated showed positive CMV association. According to the correlation between CMV and the stage of bladder carcinoma, and as shown in figure 5, out of 30 cases falling in the I and II stages, 13 sample were showed positive expression of CMV, while, out of 10 cases falling in stage III and IV, 4 out of them were showed positive expression. In figure 6, and according to the site distribution, 8 cases out of 25 were expressed positive relation of transitional cell carcinoma with CMV and 9 out of 15 were showed positive correlation of squamous cell carcinoma, otherwise, and as we can see in figure 7, nine out of 20 smokers patients were expressed positive and 8 out of 20 nonsmoker patients were positive CMV expression

To best of our knowledge ,there is no Iraqi study referred for the role of CMV in bladder cancer with the use of insituhybridization . In our study and as shown in table (1). Cytomegalovirus have been associated with different parameters statistically in highly significant relation with grade and site ($p = 0.0153$, $p= 0.0144$)

at ($p \leq 0.01$), and with each of age ($p=0.0277$), gender($p=0.0466$), stage ($p=0.0272$) respectively at ($p \leq 0.05$), but with non- significant correlation with smoking .

It suggested that CMV make a great role in the etiology of immunodeficient than in immunocomproment bladder cancer patients(35). The highly significant correlation of CMV with bladder cancer in this study explain the meaning of viral agent is the most important infection factors associated with human malignancies(36, 40).

Table 1: The distribution of bladder cancer patients regarding their age, gender, histological grade, stage, smoking habit and the site of the tumor correlation with cytomegalovirus virus (CMV).

Factors	**CMV positive results = 17 (42.5%)**	**CMV negative results = 23 (57.5%)**	**p- value χ^2 test chi square**
Age ≤ 60 > 60	 7(41.1%) 10(58.8%)	 11(47.8%) 12(52.1%)	 0.0277 4.972 *
Gender Male Female	 10(58.8%) 7(41.1%)	 10(43.4%) 13(56.5%)	 0.0466 4.015 *
Grade Well Moderate Poor	 8(47%) 4(23.5%) 5(29.4%)	 13(56.5%) 5(21.7%) 5(21.7%)	 0.0153 8.824 **
Stage I – II III - IV	 13(76.4%) 4(23.5%)	 17(73.9%) 6(26%)	 0.0272 4.042 *
Site TCC SCC	 8(47%) 9(52.9%)	 17(73.9%) 6(26%)	 0.0144 7.926 **
Smoker Non smoker	9(52.9%) 8(47%)	11(47.8%) 12(52.1%)	0.255 1.038 NS.

*(significant)
**(higly significant)
NS(non significant)

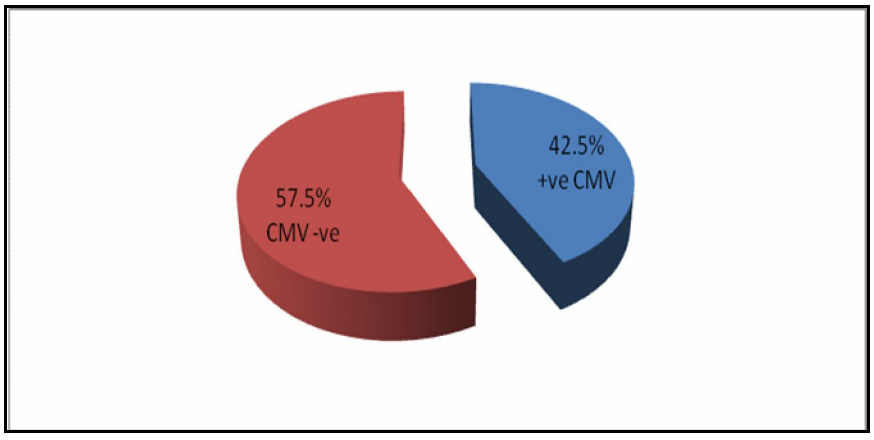

Figure (1): Distribution of bladder cancer patients in relation with cytomegalovirus

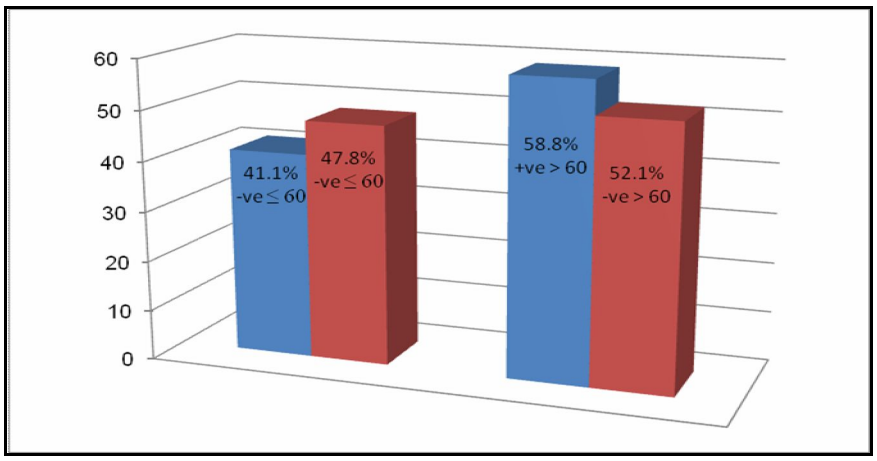

Figure (2) :Distribution of CMV according to the age of bladder cancer patients.

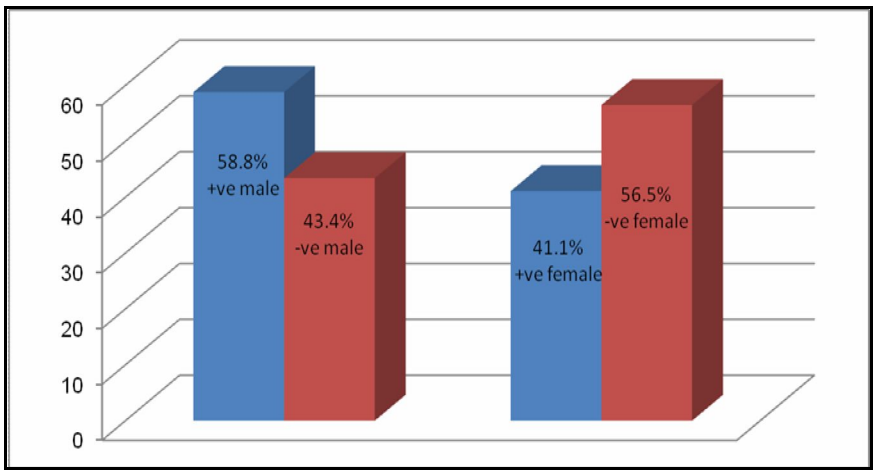

Figure (3): Distribution of CMV according to the gender of bladder patients

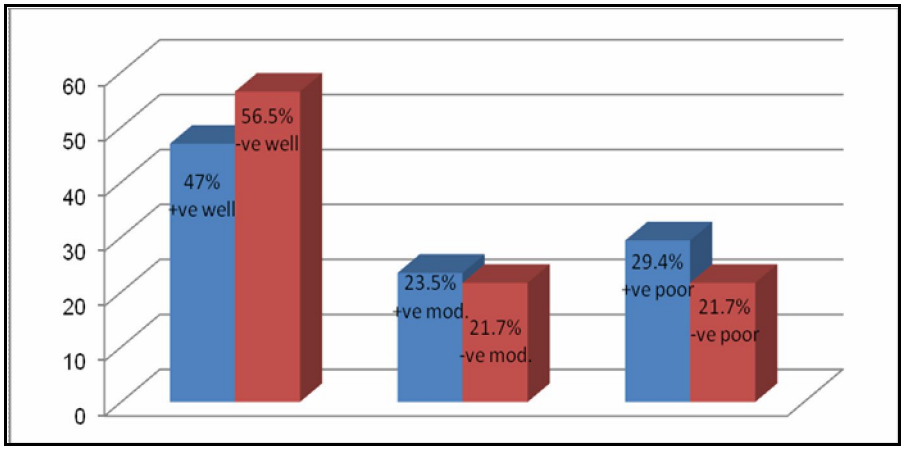

Figure (4): Distribution of CMV according to the tumor grade of bladder cancer .

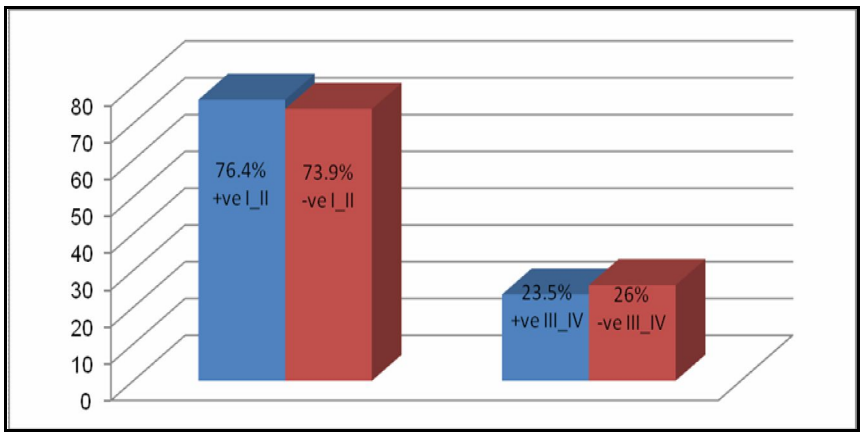

Figure 5:Distribution of CMV according to the stage of the tumor of bladder cancer patients.

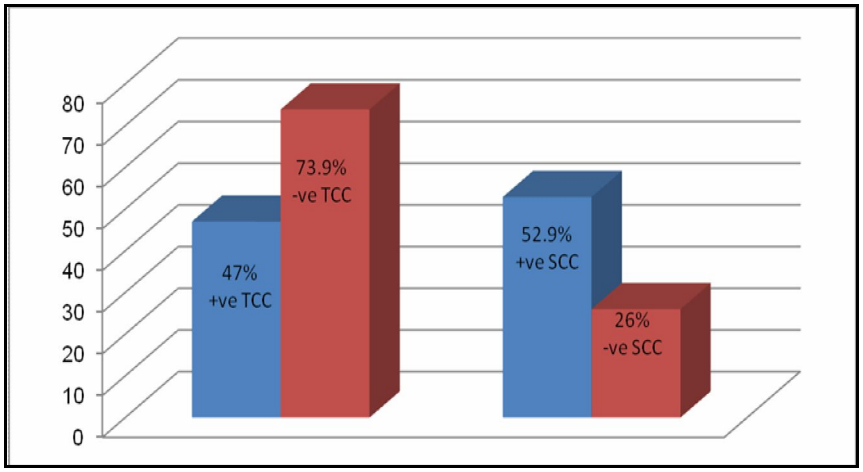

Figure (6): Distribution of CMV according to the site of the tumor of bladder cancer patients.

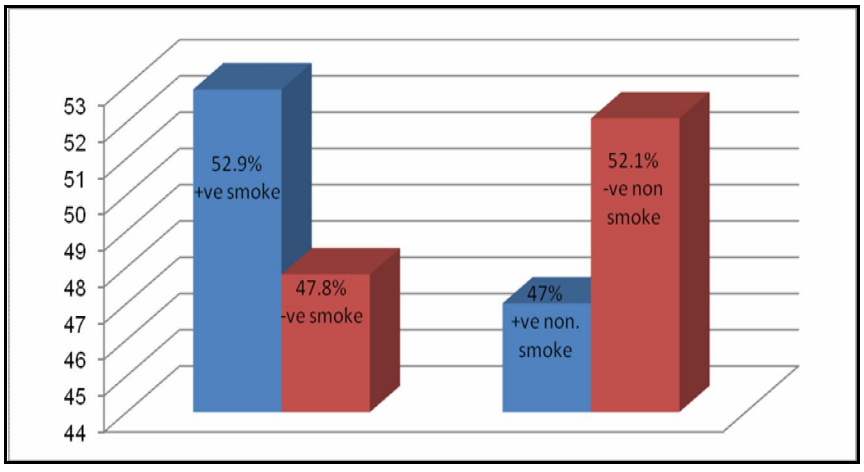

Figure (7): Distribution of CMV according to the smokers of bladder cancer patients

References

1. Zhang LJ.; Hanff P.; Rutherford C.; Churchill WH.; Crumpacker CS.(1995).Detection of Human Cytomegalovirus DNA, RNA, and antibody in normal donor blood. J Infect Dis. 171(4):1002-6.
2. Boeckh M.(2011).Complications, diagnosis, management, and prevention of CMV infection. Current and future. Hematology Am SocHematolEdu program.2011:305-9.
3. De Villiers EM.(2003). Relationship between steroid hormone contraceptives and HPV, cervical interapithelialneoplasia and cervical carcinoma. Int J Cancer .103:705.

4. Mao C.; Hughes JP. And Kiviat N.(2003).Clinical findings among young women with genital human papillomavirus infection. Am J Obstet Gynecol.188:677.
5. Amarante MK.; Watanabe MA.(2009). The possible involvement of virus in breast cancer. J cancer Res Clin Oncol.135:329-337.
6. Clifford GM.; Smith S.; Aguado T. etal.(2003).Comparison of HPV type distribution in high grade cervical lesions and cervical cancer a meta analysis Br J Cancer . 89:10.
7. Martin M.; Hans W. D. and Jindrich C.(2009). The story of human cytomegalovirus and cancer.Increasing Evidence and open Questions. Neoplasia. 11(1):1-9.
8. Cinatl J.; Cinatl J.; Vogel JU.; Rabenau H.; KornhuberB.andDoerr HW.(1996). Modulatory effect of human cytomegalovirus infection on malignant properties of cancer cells .Intervirology. 39:259-269.
9. Cintal J. Jr.; Vogel Ju.; Kotchetkov R.; Doerr HW.(2004).oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. FEMS Microbiol Rev.28:59-77.
10. Soroceanu L. and Cobbs CS.(2011). Is HCMV a tumor promoter. Virus Res 157:193-203.
11. Tracey EA.; Baker D.; Chen W. etal.(2007).Bishop J . Cancer in new south wales: Incidence and Mortality.2005.sydney: Cancer Institute NSW.
12. Rous SN.(1978).Squamous cell carcinoma of the bladder . J Urol .120(5):561-2.
13. Devita VT.; Hellman S. and Rosenberg SA.(2005).Cancer, Principles and Practice of Oncology. Philadelphia Lippincott Williams and Wilkins:1063-103.
14. World population prospects (2007).The 2006 Revision. Excvutivesummary .United Nations, Department of Economic and social Affairs.
15. Shokeir A.A.(2004).Squamous cell carcinoma of the bladder : Pathology, diagnosis and treatment. BJU Int.93,216-20.
16. SAS.(2012). Statistical Analysis System , User's Guide. Statistical Version 9th ed. SAS. Inst. Inc. Cary. N. C. USA.
17. Al-Azzawi SN.(2006).Depleted uranium radioactive contamination in Iraq : an overview. Global Research .vol 1:4.20-25.
18. Smith JA.; Grawford ED.; Blumenstein B., etal.(1988).A randomized prospective trial of pre- operative irradiation plus radical cystectomy versus surgery alone for transitional cell carcinoma of the bladder. A southwest Oncology Group Study. J Urol 139(4, part 2): 266 A.
19. American cancer Society (ACS)13 August 2014.
20. Mayo Clinic Cause, accessed 13 August 2014.
21. Wang X.; Jones T.D.; Maclennan G. T., etal.(2005).P53 expression in small cell carcinoma of the urinary bladder: Biological and prognostic implications. Anticancer Research 25:2001-2004.
22. Barghi MR.; Hajimohammadmehdiarbab A.; Moghaddam SM., and Kazemr B. (2005). Correlation between human papilloma virus infection and bladder transitional cell carcinoma. BMC infect Dis; 5:102.
23. National cancer institute.(2007). Bladder cancer treatment (PDQ) patient version. Bethesda, MD: National cancer institute.
24. Messing EM. (2002). urothelial tumors of the urinary tract; campbells urology; 8: 2732-2784.
25. Al Bazzaz P. H.(2009).Stage of urinary bladder cancer at first presentation.Sand of kidney dis transplant.20(4):628-631.
26. Greenlee RT.; Murray T.; Bolden S.; Wings PA.(2000).Cancer statistics,2000.CA Cancer J Clin.50:7-33.
27. Ferlay J.; Shin HR.; Bray F.; Forman D. etal (2008). Cancer incidence and mortality worldwide: IARC Cancer Base No.10 International Agency for Research on Cancer 2010.
28. Ali S.A. and Sadeq B.(2012). A study of p53 expression in transitional cell carcinoma of urinary bladder in Erbil governorate. Zanco J Med. Sd.16(3).
29. Freedman N. D.; Silverman D. T.; Hollenbeck A., etal .(2011).Association between smoking and risk of bladder cancer among men and women (published correction appears in JAMA 2011; 306:2220). JAMA. 306:737-745.
30. ACS what are the risk factors for bladder cancer (2014).
31. Meij P.; Vervoort M.; Bloemena E.,etal.(2002). Antibody response to Epstein Barr virus encoded latent membrane protein -1 (LMP1)and expression of LMP1 in Juvenile Hodgkin's disease. J Med Virol.68:370-377.

32. Badawi H.; Ahmed H.; Ismail A., etal.(2007).Bladder carcinogenesis via viral infection. European society of clinical microbiology and infectious disease. Germany.1734-205.
33. Gazzaniga P.; Vercillo R.; Gradilone A., etal.(1998). Prevalence of papilloma virus, Epstein Barr virus, cytomegalovirus, and Herpes simplex virus type 2 in urinary bladder cancer. J Med Virol., 55:262-267.
34. Badawi H.; Ahmed H.; Fadl L., etal.(2008). Performance characteristics of different assays for detection of CMV infections in patients with cancer bladder. Egyptian Journal of Medical microbiology.17(2).
35. Giffiths TRL. And Mello JK.(2000).CMV and urological tumours. Rple in bladder prostate, renal and testicular cancer. BJU Intera, 85:211-217.
36. Mao C.; Hughes JP. And Kiviat N.(2003). Clinical findings among young women with genital human papillomavirus infection. Am J Obstet Gynecol.188:677.
37. Bharti A. and Singh K. (2009) Recent Developments in the Field of Anticancermetallo pharmaceuticals.; International Journal of PharmTech Research. Vol.1, No.4, pp 1406-1420
38. Swain R. SubudhiB. MahapatraA. BolapareddI B. (2015) Bridging Between Disease, Prevalence and Treatment of Diabetes Mellitus: A Review, International Journal of PharmTech Research. Vol.7, No.2pp212- 228.
39. Alsayigh H. Ali S. M. Al mahbob T. Al salihy SH. (2016) Molecular detection of Human Papillomavirus genotype-31 intissues from patients with prostate cancer and benignprostatic hyperplasia; International Journal of PharmTech ResearchVol.9, No.5, pp 277-283.
40. Al-Terehi M. Al-kilabi L. AL –Mamoori A.. Al-Jboori M. Al-Saadi A. Zaidan H. (2015) Some Heavy Metals Concentrations in Tumor TissueInternational Journal of ChemTech ResearchVol.9, No.03 pp 407-411.
41. Poonam S. Chandana M. (2015) A Review on Anticancer Natural Drugs; International Journal ofPharmTechResearchVol.8, No.7, pp 131-141.
42. Gangwar P. SantoshKumar, Ankur Mohan (2012) In SilicoMicroarray Data Analysis of over expressed LYNGene responsible for AdultTcell leukemia/lymphoma(ATL); International Journal of PharmTech Research,Vol.4, No.4, pp1432- 1438.

*****