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# Role Of Human Papilloma Virus And EBV With Prostate Cancer In Tikrit Governorate

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**Abstract**

**Background:** Prostate cancer is one of the greatest common types of cancer in men. Commonly prostate cancer develops slowly and is firstly confined to the prostate gland, where it may not cause severe harm.

**Objectives**:- The present studyfind relationship between Epstein –Bar virus (EBV) and Human Papilloma virus (HPV) with prostate tumor patientin Tikrit governorate **by** using immunohistochemistry (IHC) for detecting cancer protein of (LMP-1) for EBV and (L-1) for HPV and using Hematoxylin and Eosin stain for diagnosis of prostate tissue (normal, benign and malignant)

**Methods**:- The study included 100 paraffin impeding block tissue from archives of tikrit TeachingHospital insalah din province and some of archive of private histopathology laboratories in tikrit city, the datafrom January to December 2019whose ages were between40-89 years.

**Results:** the resultsdetection of 30 blocks(33.3%)malignant and 60(66.7%)benign prostate cancer.the malignant tissue with viruswas 7/30 ( 23.33%) in EBV , 12/30(40%) in HPV and the remind 11/30 ( 36.7%) was malignantwithout virus, while in HPV while the benign tissue with virus was 10/60 (16.7%), 5/60(8.33) for EBV and the remind 45/60 (75%) was benign without virus. The present study shows an increase in the incidence of prostate cancer at the age groups (60-69) years old patients with (33.33%) from the total percentage. Also the classification of malignant prostate according to Gleason Grade (1, 2, 3, 4 and 5), and show the high percentage was in Grade 1. also the result showed high percentage for positive LMP-115/90( 16.7%) and negative LMP-175/90, (88.33%) for EBV and also high percentage in L-1 22/90(42.44 %) in positive, and 68/90 (75.6%) in negative for HPV.Thepercentage of EBV in Grade of prostate malignant was higher in Grade 3 as 8/15 (53.33%) while in Grade (1, 4)was 2/15(13.33 %) for each, and in Grade (5) was 3/15(20%) and in Grade (2) was zero when compared with healthy control .Also the percentage of L-1 for HPV in Grade was high percentage inGrade 16/22(27.3 %)

and the Grade 2, 3,4,5 was 4/22(18.2%) when compared with control groups.More ours the EBV and HPV are higher percentage in Urban than in Rural. In Urban they are 10/65(15.4%) and 2/25(8%) in rural for EBV while the HPV in Rural they are 14/55 (25.5 %) and 8/ 35(22.9%) in in rural for HPV .

**KEYWORDS**:- Human papilloma virus ,EBV, Prostate, cancer, immunohistochemistry

**Introduction**

Prostate cancer (PCa) is the second cause common cancer in men. According to recent estimations, over a million men are diagnosed with the disease yearly, with the highest incidence in more developingcounties such as Australia, New Zealand, North America, and Western and Northern Europe. Frequency rates are also relatively high in the Caribbean and sub-Saharan Africa, which also has the peak prostate cancer mortality rates [1].

PCa pathogenesis includes both hereditary and environmental factors. The latter have been implicated from studies in migrant Asian populations to the West that shows a greater incidence of PCa than their counterparts still living in Asia. As in cancers of the stomach, intestine, and liver, chronic inflammation secondary to infection and other ecological factors such as diet, may also play a role in the increase of prostate cancer [2].

Mechanisms of prostate carcinogenesis and character of infection have been reviewed in detail previously [3]. Here we focus specially on probable viral etiology of prostate cancer and sign of viral-mediated genetic changes and related immune dysregulation.

Several studies appearance that the EBV and HPV are the chief causes of prostate cancer[4].Virus secreted ingenital secretions and viasaliva to the stayed of aninfected people'slife.This virus mainly infects B-cellsand epithelial cells. Then the genomic is transported intothe nucleus of cell and replication with a viral DNApolymerase. HPV like HBV and other viruses is sexual transferred there for it wascategorized either asnononcogenic or “low-risk,” HPVtype (e.g., HPV type11 and 6), or as oncogenic or “high-risk, ”(e.g., HPV type 18 and 16) . [5].

**Material and Method**:-

The study included 100 paraffin impeding block tissue from stores of tikrit Teaching Hospital in salah din province and some of store of private histopathology laboratories in tikrit city, the data from January to December 2019whose ages were between40-89 years.

Hundred paraffinimbedding tissueblocks werecut bymicrotome apparatus tomake slide, and thendying byhaematoxylin and eosin stain (H&E).For staining withimmunohistochemistry, the blocks were cut to 5m andput in positive charge slides. and the slides were putting inhot air oven at 60 c for one hour for rehydrationof tissue as the following. Placed twice with xylene each time, leaving 5 minutes and left to drying then put in aserial dilution of alcohols (99%, 95%, 85%, 70%) for threeminutes after then put in D.W. then staining with (IHC)kit (LSBio’s, Denmark), (LMP-1)primaryantibody forEBVand (L-1)primary antibody forHPV(Dako Denmark), then putthe slide in retrieval in waterbath at 95 C for20 minutes then rinse slide in after

then added the peroxidesblock for5 minutesand washby bufferTrissaline 20x),primary Ab wereadded to slid for 20minutes and wash with D.W, Horseradish peroxidase (HRP)were added to slide for 20minutes, then wash them with D.W, diamino benzidine (DAB) wereadded to slide for8-10 minutes and Wash them, then put the slide in D.W.and stained with hematoxylin for few minutes, and put itin alcohol for 2minutes, left to drying, put the slid in xylene and cover the slid withDibutylphthalate Polystyrene Xylene (DPX),leftthesslidetodry andthen examining under light microscope.

**RESULTS**:

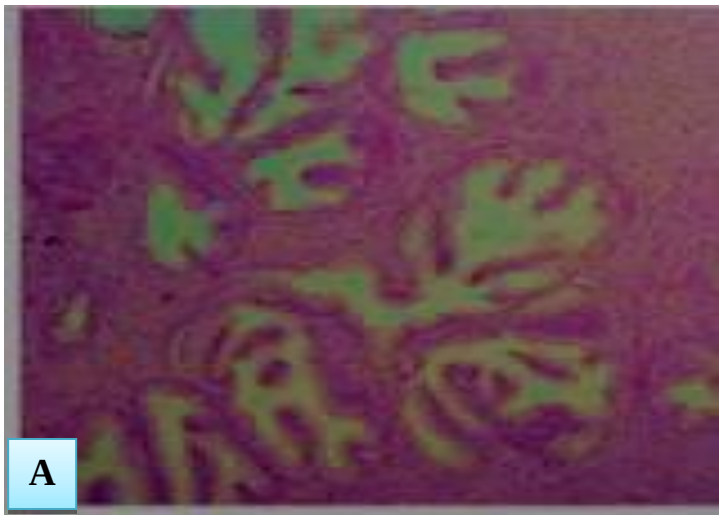
This study includes 100 block tissue samples (60benign, 30 malignant and 10 control) for patients with prostate in Cancer of tikrit Teaching Hospital in salah din province and some of archive of private histopathology laboratories in tikrit city, the data from January to December 2019whose ages were between 30-89 years.During this study we found 30 blocks (33.3%) malignant, and 60 blocks (66.7%) Benign Prostatic by using Haematoxylin and Eosin staining (H&E) (as primary stain) , Immunohistochemistry and P.C.R. technique for detecting EBV andHPV as shown in table table( 1).

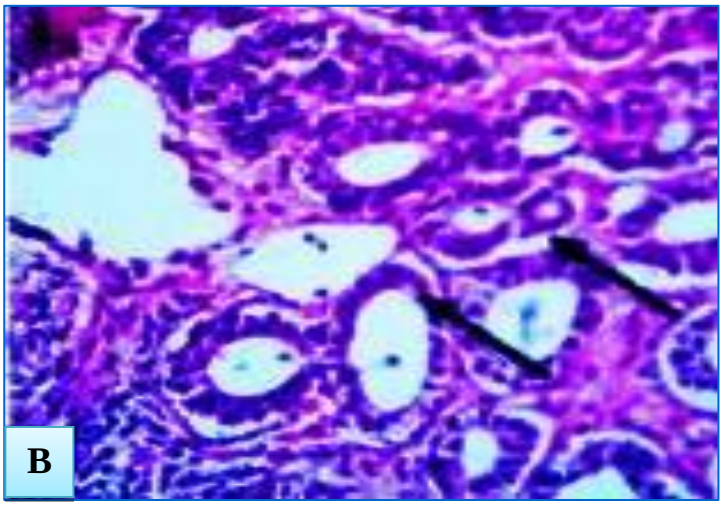
**Table (1) Distribution of prostate cancer patients according to malignant and benigntumor**

| No. of patients with PCa | Malignant | Benign S |
|---|---|---|
| **100** | **30(33.3%)** | **60(66.7%)** |

**Histopathological changes in prostate tissue patients:**

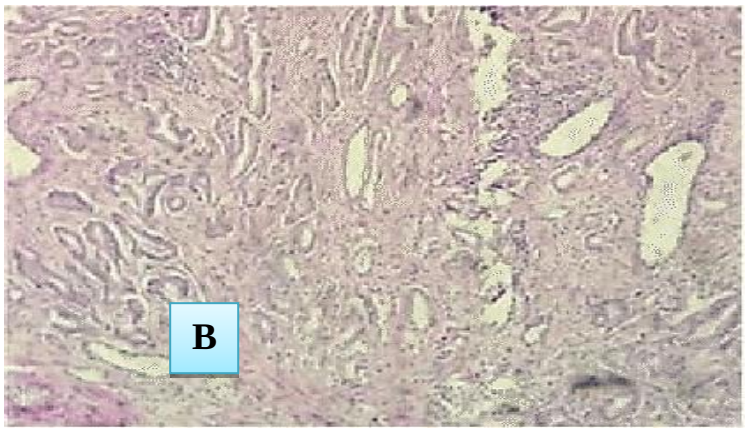
The result of histopathological test of prostate biopsy show clearly variation between benign and malignant prostate, in Benign Prostaticshow nodule prostatic and stromal cell become hyperplastic with present myoepithelial cell layer whereas in malignant tissue appearance adenocell carcinoma irregular and pleomorphic shape with present cribriform cellshows in figures (1, 2).

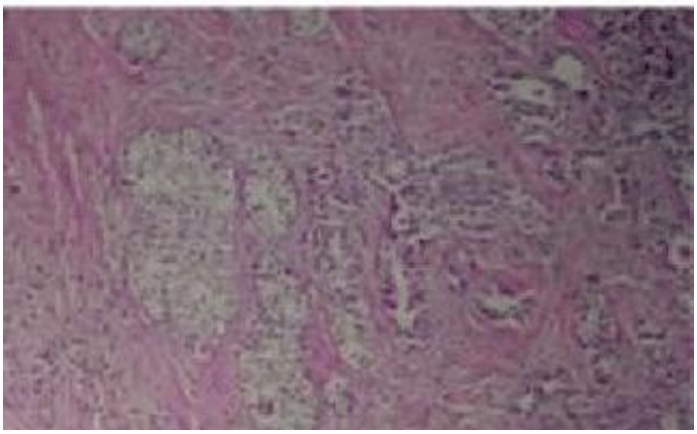


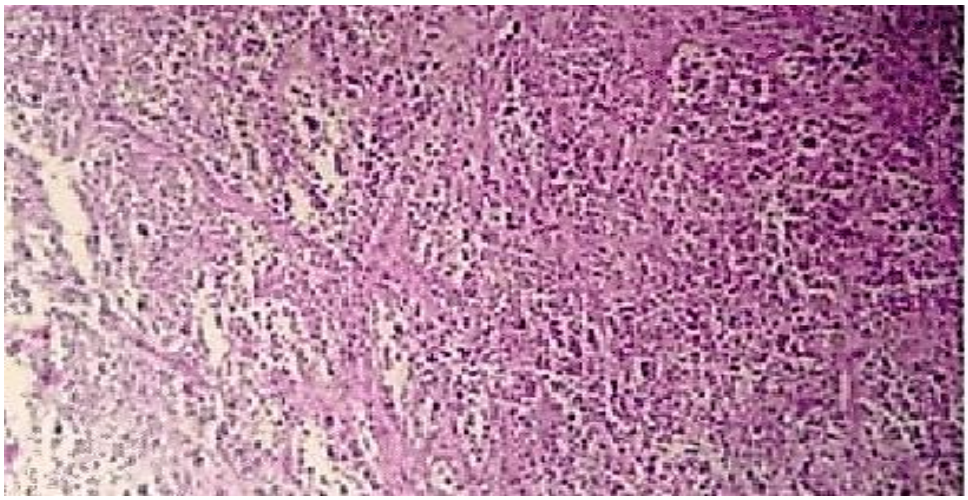



Fig(1-A)Benign ProstaticDilated gland with in folding lined with gland layer epithelial and basal cell surround by f connective tissuestoma. Two cell

Fig(1 - B) Grad (2): Slight Pleomorphic malignant prostate grad (3): adenocell carcinoma irregular and separated Fig(2-B) malignant prostate grad (4): cribriform pattern. Fig(2-A)





C

Fig(2-C) malignant prostate grad(5):diffuse pleomorphic and arrange tumors cell

**Immunohistochemical technique (LMP-1 and L-1):-**

The result of IHC appearance clear reaction withmonoclonal antibody that detects Latent membrane protein 1 in EBV and L-1(a major capsid protein)for HPV and take brow color round nuclear for LMP-1 whereas in L-1 is staining nuclear With brown color, as shown in figure (3 A,B).

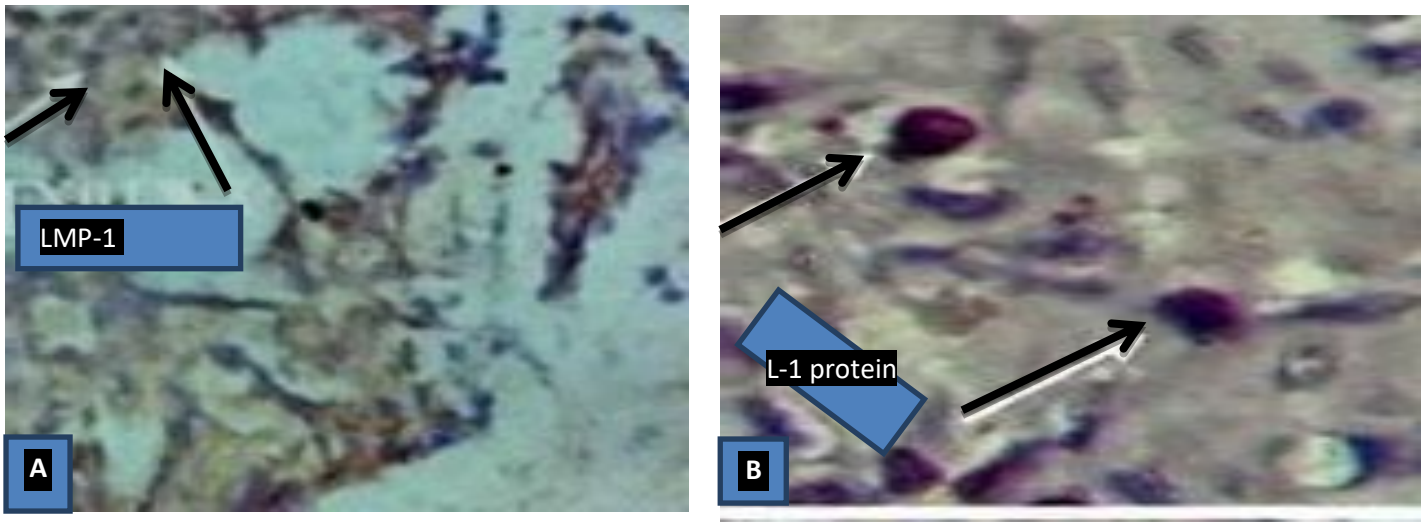



**Figure (3-A):** Positive slid for EBV cytoplasm contain LMP-1 protein. **Figure (3-B):** Positive slid forHPV nuclear contain L-1 protein

**Distribution tumor prostate in men according to viruses (EBV and HPV)** :-

The result shown in malignant prostate is 12/30(40%) in HPV, and 7/30 (23.33%) in EBV and the other percentage of 11/30(36.7%) is malignant without virus. In Benign prostate EBV is 5/60(88.33%) and 10/60 (16.7%) in HPV and other 45/60 (75%) is benign without virus as shows in table (2)

**Table (2) Frequency of HPV and EBV in men infected with PCa**

| Malignant with viruses | | Malignant without viruses | Total | Benign with viruses | | Benign without viruses | Total |
|---|---|---|---|---|---|---|---|
| HPV | EBV | | | HPV | EBV | | |
| **12(40%)** | **7(23.33%)** | **11(36.7%)** | **30** | **10(16.7%)** | **5(8.33%)** | **45(75%)** | **60** |

**Distribution ofpatient`s infected with prostatic tumor according to Age groups:**

This study includes the distribution of ages of patients with infected tumor, Table(3) shows the distribution of patients with tumor to five ages group. The group (60-69) years old is considered the highest one in viral tumor prostate with (33.33%), groups (70-79)and(80-89) years old is less than group (60-69) years old with (22.22%), then groups (50-59) -less two age groups above(16.7 %) finally group (40-49) years old with (5.6) as lowest groups.

Tables (3) Distribution of PCapatients according to age .

| Age groups | No. OF patients with PCa % | EBV | HPV |
|---|---|---|---|
| **40-49** | 5(5.6%) | 1 | 5 |
| **50-59** | 15(16.7%) | 2 | 3 |
| **60-69** | 30(33.33%) | 5 | 10 |
| **70-79** | 20(22.22%) | 2 | 2 |
| **80-89** | 20(22.22%) | 2 | 2 |
| **Total** | 90 | 12 | 22 |

**Distribution of cell cancer of the tumor prostatic patient according to score grade differentiation:**

There are five types of prostatic patients according to score Grade for differentiating the prostatic cell cancer. The total number of malignant prostate is 30and the distributed as the following: Grade 1 is 12/30 (40%), Grade 2, 3 is 6/30 (20%), while Grade 42/30 (6.7%) and Grade 5 4(13.3). as showed in table (4).

| **Score of tumor** | Number | % |
|---|---|---|
| 1 | 12 | 40 |
| 2 | 6 | 20 |
| 3 | 6 | 20 |
| 4 | 2 | 6.7 |
| 5 | 4 | 13.3 |
| Total | 30 | 100% |

**Percentage of LMP-1 for EBV and (L1) for HPV in patients prostatic cancer and control groups by using IHC Techniques:-**

The total number of prostate cancer was (90) compared with (10) control group table (5) shows the negative patients for LMP-1 was 75/90( 88.33%) while the positive for LMP-1 was 15/90(16.7%) the distribution to five groups as the following in grade (1,4) there is 2/15(13.33%) for each Grades, in Grades (2) there isno infection in grade (5) is 3/15(20%) while the large percentage is found in Grade (3) there is 8/15(53.33%).

**Table (5) The percentage of LMP-1 in IHC for EBV prostatic cancer group and control group**

| **Grade** | **Positive LMP-1( IHC) EBV** | **Control group** | **Statistic (T test)** | | |
|---|---|---|---|---|---|
| 1 | 2(13.33%) | 0 | t value | D.F | Sig |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 0(0%) | 0 | 2.236 | 4 | .089 |
| 3 | 8(53.33%) | 0 | | | |
| 4 | 2(13.33%) | 0 | | | |
| 5 | 3(20%) | 0 | | | |
| Total | 15(16.7%) | 10 | | | |
| Nagative | 75(88.33%) | 10(100%) | | | |

Table (6) shows clearly the differences between prostatic tumor and healthy controlin IHC for HPV, the total number of prostate tumor is (90) case. The positive for L-1 is 22/90, (42.44%) the remainder of negative L-1 is 68/90, (75.6 %), the positive was distribution as five Grade, the less percentage found in Grade 2,3,4,5 4/22( 18.2%). For each grade and Finally the highest percentage is in Grades1 6/22 (27.3%)**as** compared with (10) control group**.**

**Table (6) percentage of L-1 in IHC for HPV prostatic cancer and control group**

| Grade | Positive L-1( IHC) HPV | Control group | Statistic (T test) | | |
|---|---|---|---|---|---|
| 1 | 6 (27.3%) | 0 | t value | D.F | Sig |
| 2 | 4(18.2%) | 0 | 11.000 | 4 | .000 |
| 3 | 4(18.2%) | 0 | | | |
| 4 | 4(18.2%) | 0 | | | |
| 5 | 4(18.2%) | 0 | | | |
| Total | 22 (42.44 %) | 10 | | | |
| Negative | 68 (75.6 %) | 10(100%) | | | |

**Distribution of patients infected withprostatecanceraccording to residence:-**

The current study showed that the highest rate of infected men with Pca were urban comparative rural areas (8.88% and 15.6%), as shown in Table**(7).**

**Table(7) Distribution of men withprostatecanceraccording to residence.**

| No. of Men pca Infected with viruses | Rural | | Urban | |
|---|---|---|---|---|
| | NO | % | NO | % |
| **EBV** | **2(2.22%)** | | **8(8.88%)** | |
| **HPV** | **10(11.11%)** | | **14(15.6%)** | |

The highest rate of men with prostate tumor who were infected with EBV and were from urban areas (HPV15.4%,25.5%), as shown in Table (8).

Table (8). **Distribution of viral infection with prostate cancer according to residence**

| residence | No. of patient | EBVinfection | No. of patient | HPV infection |
|---|---|---|---|---|
| **Rural** | 25 | 2(8%) | 35 | 8(22.9%) |
| **Urban** | 65 | 10(15.4 %) | 55 | 14 (25.5 %) |
| **Total** | 90 | 12(13.33%) | 90 | 22(24.44%) |

**DISCUSSION:**

Prostate cancer is the third most common malignancy (after lung cancer) in men worldwide, counting 1,276,106 new cases and causing 358,989 deaths (3.8% of all births caused by cancer in men) in 2018 [6]. The incidence and mortality of prostate cancer worldwide associate with increasing age with the average age at the time of diagnosis being 66 years. Of note, for African-American men, the incidence rates are greater when compared to the White men, with 158.3 new cases diagnosed per 100,000 men and their mortality is nearly twice as White men [7]. Causes for this difference have been hypothesized to differences in social, environmental and hereditary factors. Although 2,293,818 new cases are estimated until 2040, a small difference in mortality will be observed (an increase of 1.05%) [8]. In this study, we have 100 block tissue sample after collections and stain with haematoxylin and Eosin . which are examined under light microscope for diagnosis to malignant or benign, we have found 30(33.3%) malignant ,60(66.7%) benign and 10 as control groups.

The percentage of prostatic malignant agrees with studies in Iraq with a percentage of20(33.33%) , in Kingdom Saudi Arabia KSA are (10%)(9,10). In Sweden **are**8.8% of benignand malignant prostate cancer (11), In this study, numerous specific indicators are used for detect the relation of EBV and HPV in epidemiology and the danger factor for developed of prostate cancer, by dependent on discovery of LMP-1 protein of EBV and L-1 protein of HPV by using immunohistochemistry.

This study shows the positive case for LMP-1 for EBV is cytoplasmic stain, while in protein L-1 for HPV is nuclear staining , taking brown color ofDAB Chromogenwhile the negative does not staining withDAB Chromogen, (reaction between antibody of protein with epitopes of cell). Thus agrees with a study shows nuclear stain for protein E7 which as marker for HPV infection**(12).**

Immunohistochemical staining is broadly used in the diagnosis of abnormal cells such as those found in cancerous tumors**( 13 ).** The current study showed that the highest rate of EBV and HPV infections 30(33.33%) were found in those within theage group 60-69 years.The reason for EBV to exert itsoncogenic effects in a particular patients is unknownbut is perhapsrelated with co-factors. The findingsin the research by have supported hypothesis that theprostate is a habitat for multiple viral and other infectiveagents ,some of which have oncogenic potential(14).Numerous studies were decide with this study. For example Kerman's

Southeast study of Iran show the life stage (65-74) years is highest stage of prostatic carcinoma.(15).Also (Albasri A.etal2014) indicated that most patients with prostatic cancer are between (60-69) years old(16**).**

The present study shows the high ratio of carcinoma in Gleason Grade 1, as 12/30 (40%), while in poorly ratio in low score. This agrees with a study of Mezher etal.,2017 inIraq which appearance high ratio of carcinoma in Grade 1 (30%) from total percentage for low Grade (2-4) while low percentage (10%) in high score .(9). Another study shows the high percentage of prostatic cancer in Grade (5-7) as 37/59 (62%) while in low ratio in Grade (2-4) as 14/59 (23.72%)(17). this alteration in percentage is due to hereditary, racial environmental factor and nutritional factors. (18).

The present study shows the high risk of prostatic patient in Urban in both positive and negative of IHC comparing Rural (8.88%) in EBV and (15.6%) in HPV, This agrees with a study of Mezher etal., 2017 in Iraq which showed the high risk of prostatic patient in Urban for both viruses (EBV and HPV) in both positive and negative of IHC with ($p< 0.001$) as (41%) in Urban compared with (28.6%) in Rural(9 )**.** This variance may be due to the change between Urban and Rural, the absence of information of patient in Rural areas can get or lost the panel diagnosis for prostatic diagnosis.(19). Another study shows that Jamaican men with prostate cancer is with high percentage in Rural (75%) compared with Urban (25%).(20). Another study shows the percentage of prostatic carcinoma is higher in Rural in (86%) than in Urban (21).

**conclusion** :- This study concludes :- that the EBV and HPV are of high risk for prostate men and also the age and residence are effecting in prostate men

**Conflict of Interest:** Hala .M. majeed declares that he has no conflict of interest.

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