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# Chromogenic in Situ Hybridization for Human Cytomegalovirus-DNA Detection in Tissue Subsets with Prostatic Adenocarcinoma and Benign Hyperplasia

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**Abstract**

Human cytomegalovirus (HCMV) infects a wide range of human cells, resulting in both benign and malignant tumors. In the last few decades, proteins and/or nucleic acids of the virus were found to be often highly expressed in in patients with basal cell hyperplasia and prostatic neoplasia.

This research aimed to unravel the rate of HCMV infections among prostatic tissue subsets from Iraqi patients with adenocarcinoma and benign hyperplasia.

One hundred, formalin-fixed and paraffin embedded prostatic tissues were obtained from 40 tissue samples collected from different grades of prostate carcinoma; 40 from benign prostatic hyperplasia and 20 from apparently healthy prostatic tissues. These tissue specimens were collected from the archives of different public and private histopathological laboratories in Baghdad. Detection of HCMV-DNA was achieved by a highly sensitive version of chromogenic in situ hybridization technique.

The signals of chromogenic in situ hybridization reactions for HCMV-DNA detection in prostatic adenocarcinoma tissues were found in 65% (26 out of 40) of the tissues, whereas in BPH (Benign Prostatic Hyperplasia), HCMV-DNA was detected in 57.5% (23 out of 40) of the tissues, and in the healthy control group in 25% (5 out of 20) of the tissues. The highest percentage of positive- HCMV- DNA-CISH reactions (57.5%) was found in prostatic adenocarcinomatous tissues that showed poor differentiation.

Our results could show that HCMV might contribute to the development of the studied subsets of prostatic adenocarcinoma and benign prostatic hyperplasia.

**Keywords**: HCMV; prostatic adenocarcinoma, benign prostatic hyperplasia, chromogenic in situ hybridization.

# التهجين الموضعي لدنا فايروس المضخم للخلايا البشرية في سرطان البروستات واورام البروستات الحميدة

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**الخلاصة**

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الخلفية النظرية: يصيب الفيروس المضخم للخلايا البشرية (HCMV) مجموعة واسعة من الخلايا البشرية مما يؤدي إلى أورام حميدة وخبيثة، وفي العقود الأخيرة تم الكشف عن بروتينات HCMV و/ أو الأحماض النووية على وجه التحديد وبشكل كبير يظهر تعبيرها الجيني في تضخم الخلايا القاعدية والأورام داخل الظهارة للبروستاتا.

الأهداف: يهدف هذا البحث إلى الكشف عن معدل الإصابة بفيروس HCMV بين المجموعات الفرعية للأنسجة البروستاتية من المرضى العراقيين المصابين بسرطان البروستات والتضخم حميد.

المرضى وطرق العمل: جمعت مئة (100) عينة من نسيج البروستات المحفوظة بالفورمالين والمطمور بشمع البارافين منها 40 عينة مأخوذة من الخزع النسيجية من سرطان البروستات بدرجات تمايز نسيجي مختلفة، و40 عينة من حالات التضخم الحميد في البروستات، و20 عينة لخزع نسيجية البروستاتا السليمة جمعت هذه العينات من ارشيف ٢مختبرات الانسجة المرضية في المستشفيات العامة وبعض من المختبرات الخاصة في بغداد. تم العمل بطريقة التهجين الموضعي ذات الحساسية العالية للكشف عن الفايروس المضخم للخلايا البشرية.

النتائج: كشفت النتائج الموجبة لفايروس المضخم للخلايا البشرية (HCMV) بتقنية التهجين الموقعي اللوني ذات الحساسية العالية في انسجة غدة البروستات المسرطنة 65٪ (26 من أصل 40 نسيج)، بينما كشفت في انسجة غدة البروستات الحميدة 57.5٪ (23 من أصل 40 نسيج) وفي مجموعة السيطرة الصحية كشفت 25% (5 من أصل 20 نسيج). وجدت نسبة عالية (57.5%) من نتائج التهجين الموضعي الموجبة لفايروس المضخم للخلايا البشرية في حالات سرطان البروستات في ذات التمايز النسيجي العالي.

الاستنتاج: نتائج الدراسة الحالية تشير إلى أن الفايروس المضخم للخلايا البشرية قد يلعب دورا مهما في حدوث تطوير في حالات سرطان البروستات والتضخم الحميد.

## Introduction

The prostate cancer (PC) is a very common malignancy in men and makes up a major public health problem in all countries [1]. It is the second most common cancer among men worldwide after the cancer of lung [2].

The benign prostatic hyperplasia (BPH), represents the prevalent abnormal, non-carcinomatous growth of prostatic cells in aging men [3]. It is considered as a general health problem, affecting men's life that qualified via clinical and pathological methods [4].

Almost all PC developments originate from the glandular cells that add the prostate fluid to the semen. The prostatic cancer is identified as an uncontrolled proliferation of these prostatic glandular cells [5]. Cancer in humans is mostly associated with infectious agents of virus factors [6]. A bulk of evidence accumulated over the past 50 years revealing several viral contributions to the progression of malignancies. It is estimated that viral infections contribute to about 12-20% of all human cancers [7].

Human Cytomegalovirus (HCMV), referred as human herpesvirus 5 (HHV-5), belongs to the Herpesviridae family. Its name is derived from the tendency of HCMV-infected cells for the massive enlargement (cytomegaly) and induction of distinctive inclusion bodies [8].

During the last decades, an increased evidence focused on the relationship between the presence of HCMV infection (as shown by genome and antigen analyses) with many human cancers. Human cytomegalovirus genomic products have been detected in several cancers, including malignant glioma, cervical carcinoma, breast cancer, brain cancer, colon cancer, salivary gland cancer, and Kaposi's sarcoma [9, 10]. In addition, there is a significant possibility that HCMV might contribute to the natural history of prostatic cancer, where HCMV proteins and/or nucleic acid are highly expressed in prostatic basal cell hyperplasia and intraepithelial neoplasia [11].

A chronically stressed immune system is common in HCMV - persistent and latent infections, that is reactivated sporadically or periodically. Seropositivity of HCMV is considered as a parameters for an "Immune Risk Profile" which is associated with syndromes and diseases comprising an inflammatory component, including cardiovascular disease, cognitive impairment, functional impairment, and cancers [12]. Therefore, activation or reactivation of a latent state of HCMV infection is considered as a potential cofactor for inflammatory diseases [13].

Cytomegalovirus infection may lead to an immune deterioration by inducing aggregation of late differentiated CD8 + and CD4 + T cells following the production of pro-inflammatory cytokines and the formation of an increased pro-inflammatory condition [14].

Various parameters, e.g. oxidative stress, inflammatory genes, dietary factors, hormones and inflammatory mediators might play roles in the development of prostate cancer. The growth of prostatic tissue and progression of PC an be influenced by important factors associated with prostatic tissues inflammation [15].

This research study was planned to assess the prevalence of HCMV infection in prostatic tumorous archival tissues (prostatic adenocarcinoma and benign prostate hyperplasia), as compared to normal control prostatic tissue, via the identification of HCMV-DNA through a newly developed version of chromogenic in situ hybridization test.

**Materials and Methods**

The prostatic tissues used in this study were divided into three groups: the first group included prostatic adenocarcinoma, the second group included benign prostatic hyperplasia, and the third group included normal prostate tissues (selected as an apparently healthy control group), obtained from archives of Baghdad Institute of Forensic Medicine.

This work received the approval of the ethical committees of the hospitals and the College of Science, University of Baghdad.

The data of patients to be analyzed were focused on age, histopathological type, and tumor grade, taken from histopathological reports that accompanied the blocks of tissues collected from the archives.

All the tissues used in this research were previously processed according to Bancroft and Steven technique [16]. Paraffin blocks of tissues were sectioned serially at 4 µm thickness. The first section was fixed on ordinary glass slide, stained with Eosin and Hematoxyline, and re-examined by a histopathologist for final definitive diagnosis. The other following tissue sections were fixed on positive charge slides to be used for in situ hybridization.

In the chromogenic in situ hybridization test for HCMV DNA, the Zyto fast P.L.U.S C.I.S.H Implementation kits (Zyto Vision, Germany) with AP-NBT/BCIP were used for the detection of Digoxigenin-Labeled Zyto Fast CISH Probe. The presence of certain nucleic acid sequences in cells or tissues can be detected by the hybridization that results in the duplex formation of sequences present in the test object and the specific probe.

The signals of in situ hybridization were tested by light microscopy at 100 and 40, with the application of oil immersion. At the specific sites, the test showed a strong blue–violet signal in positive test sections of the hybridization probe. The counting of positive cells was performed at oil immersion. The signal intensity and percentage of scoring were measured based on a positive signal strength and signal number, respectively. The counting of positive signal was performed in 10 different fields of one hundred cells to each tissue section. The mean of positive signals was scored according to one of the following score categories [17]: Score (1) = 1-25%, Score (2) = 26-50%, Score (3) > 50%.

A 0-3 scale was used to record the relative intensity, corresponding to not detected, low, medium, and high signal intensity values, respectively [18].

Analysis of data statistically was performed by utilizing Statistical Package for Social Sciences (SPSS) for Windows, version 25 (SPSS Inc. Chicago, Illinois, United States). Different parameters and variables (standard error, mean standard deviation, percentages, and frequencies) were analyzed in this study, while the relationships among them were tested by using $\chi^2$ (Chi-Square) test, one-way ANOVA test, Odd ratios, and 95% confidence interval (CI)). The p-value lower than 0.05 considered as reflecting significant differences, while that lower than 0.01 reflected highly significant differences [19].

**Results and Discussion**

**The studied groups**

The total number of one hundred studied prostatic tissues included in this research were distributed on three groups: the first group comprises forty samples collected from cases with different grades of prostate carcinoma, the second group comprises forty prostate tissue samples obtained from patients with BPH, and the third group comprises twenty prostatic tissues used as control group, obtained from apparently healthy prostatic tissues on histopathological examination.

**Clinico-pathological distributions of the studied groups**

The samples involved in our study were distributed according to the range and mean of age (years), as shown in Table-1. The age of prostate cancer patients ranged 48-75 years and their mean age was 64.1 years. While the age range of patients with benign prostatic hyperplasia was from 48 to 85 years and the mean age was 67.5 years. The age of healthy individuals from whom the control prostatic tissues were obtained ranged from 43 to 77 years with a mean age of 57.1 years.

**Table 1-** The studied group's characteristics

| | | **PC N= 40 (%)** | **BPH N= 40 (%)** | **Control N= 20 (%)** |
|---|---|---|---|---|
| **Age (years)** | **Mean of Age** | 64.1 | 67.5 | 57.1 |
| | **Range of Age** | 48-75 | 48-85 | 43-77 |
| | **<50 years** | 1 (2.5) | 1 (2.5) | 5 (25) |
| | **50-60 years** | 13 (32.5) | 9 (22.5) | 7 (35) |
| | **61-70 years** | 18 (45) | 16 (40) | 6 (30) |
| | **>70 years** | 8 (20) | 14 (35) | 2 (20) |
| **PC Grade** | **Well** | 9 (22.5) | | |
| | **Moderate** | 8 (20) | | |
| | **Poor** | 23 (57.5) | | |

Our results were consistent with those of Waheed, 2018 [20], who recorded the mean age of 72 years for prostatic adenocarcinoma patients, as compared to the mean age of benign prostatic hyperplasia patients of 66.5 years. However, our results are not consistent with those of another study [21], which recorded the mean age of prostatic adenocarcinoma patients of 40.9±2.02 years, as compared to the mean age of benign prostatic hyperplasia patients of 41.25±2.05 years, whereas the value for the control was 60.45±2.6 years.

The mean age of Iraqi prostate cancer patients included in this analysis were also in line with the results of other previous studies of Abbas, 2014 [22], Abid *et al*., 2017 [23], and Hobi *et al*., 2019 [24] who found values of 57.3 years, 59.3 years, and 70.6 years, respectively.

The mean age of Iraqi benign prostatic hyperplasia patients included in this analysis were also in line with other previous studies of Abdul-Sattar, 2017 [25] and Abid *et al*., 2017 [23] who found values of 68.7 and 59.3 years, respectively. Such results suggest that the prostatic lesions affected the old-aged group of Iraqi patients.

In addition, it is observed from the data listed in Table-1 that the highest percentages of affected subjects for both prostate cancer (45%, 18 out 40 cases) and benign prostatic hyperplasia (40%, 16 out 40 cases) patients were recorded in the age of 61-70 years. In the control group, the highest percentage of subjects was in the age range of 50-60 years (35%, 7 out 20 cases). The less affected age group in both prostate cancer and benign prostatic hyperplasia patients was that of lower than 50 years (1%, 1 out 40 cases), whereas the lower proportion of subjects in the control group was at the of age over 70 years (10%, 2 out 20 cases). In both PC and BPH, there was an increase in the number of cases with the advance of the age of patients, followed by a decrease in the age of less than 50 years.

The findings of the present study in relation to age are consistent with those shown in the study of the American Cancer Society (2018), which found that prostate cancer is more diagnosed in men of 65 years of age or older and is rare in men younger than 40 years [26].

The data also show that most of the Iraqi patients with prostatic tumors (benign and malignant) were in the age group of 48-85 years. The reported findings are consistent with other studies that showed that prostate cancer has a high incidence in this age group[22, 27]. This is confirmed by another research which found that prostate cancer affects some elderly men who are usually over 50. Seventy-five percent (75%) of patients with this diagnosis are between 60-80 years of age. Patients below the age of 50 represented less than 1% of the cases [28].

Prostatic cancers are the world's sixth greatest communal cancers and the third leading cause of men's cancer. Prostate cancer occurs in one in every nine men over age 65. In developed countries, prostate cancer incidence varies by area and population [29]. Prostatic adenocarcinoma incidence increases with age [30]. In the Arab population, occurrence rate of prostate cancer is characterized by low frequency [31] and accounts for only 3.1% of all malignant tumors in Iraqi males, according to the Iraqi Cancer Registry in 2016 [32].

The present results are consistent with other studies on western populations which also found prostate

cancers to be common in older men, where two-thirds of those who died of prostate cancers were older than 76 years[33, 34].

The most common condition within men over the age of 50 is benign prostatic hyperplasia. The small deviation from the current study may also be clarified by the sample size that is not representing the entire population. It may also be linked to patients' knowledge that led them to postpone their medical examination, as some of them typically seek medical advice after complications occur [35]. Probably, the explanation for those variations is multifactorial. The incidence of prostate cancer often differs among ethnically related populations but living in different locations. Links were reported between the nutritional and hormonal statuses and the changes in disease risk. Environmental factors are also likely to play a role [36]. The agreement in the results also suggests that these two factors (genetic and environmental) affect the community of elderly patients in Iraq. The present findings are consistent with the findings of an earlier work [37]. The occurrence of changes of the malignant epithelial prostatic tissues is increased by getting older[38, 39]. It was found that the highest incidence of prostate cancer occurs in the eighth decade, which is in unconformity with our study [40].

Moreover, the current study also includes the distribution of patients in the prostate cancer group according to their Gleason's grading (Table-1). The results revealed that differentiated prostatic adenocarcinoma (1-10 according to Gleason's grading system). The well differentiated prostatic cancer cases (with score of 2-4 according to Gleason's grading system) constituted 22.5% (9 out of total of 40 cases), whereas cases with moderately and poorly differentiated prostatic cancer (5-7 and 8-10 according to Gleason's grading system) constituted 20% (8 out of total of 40 cases) and 57.5% (23 out of total of 40 cases), respectively. The statistical analysis of Gleason's grading of prostatic carcinoma showed highly significant differences (< 0.01).

An earlier study [20] showed that grade III had the highest distribution among patients, accounting for 43.3%, while the lowest was grade II (10%), with the differences being significant ($p < 0.05$).

Our findings showed that 22.5% and 57.5% of patients with prostatic adenocarcinoma showed well and poorly-developed grades of disease, respectively, according to Gleason's classification. These results are in agreement with Trujillo *et al*., 2017 [41] who found that 3% of prostatic adenocarcinoma patients had well grade (lowest) and 33.3% had poor grade (majority) of disease development. In addition, our study is in agreement with Ali and Al-Alwany, 2014 [42] who reported a highest number and percentage of prostate carcinoma patients within the poor grade (40%:16), followed by moderate grade (32.5%:13) and well grade (27.5%: 11). The study reported by Al-Lebawy, 2018 [21] reported a highest number and percentage of prostate carcinoma patients in grade I (50%:15), followed by grade II (33%:10) and grade III (17%:5).

**Results of HCMV-DNA-CISH in the studied prostatic tissues**

In the present study, the positive results of HCMV–CISH detection in prostatic adenocarcinoma patients were found in 65% (26 out of 40 cases), while in the benign prostatic hyperplasia group the value was 57.5% (23 out of 40 cases) and in the group of prostatic healthy control it was 25% (5 out of 20 cases). The statistical analysis revealed highly significant differences ($p<0.01$) in HCMV expression among those studied groups (Table- 2).

**Table 2-** The HCMV-DNA distribution in the tissues of prostate carcinoma and benign prostatic hyperplasia groups.

| **HCMV-DNA** | | **Studied groups** | | | **Pearson Chi Square (*p*-value)** |
|---|---|---|---|---|---|
| | | **PC** | **BPH** | **Control** | |
| **Positive** | **N** | 26 | 23 | 5 | 0.012* |
| | **%** | 65% | 57.5% | 25% | |
| **Negative** | **N** | 14 | 17 | 15 | |
| | **%** | 35% | 42.5% | 75% | |
| **Total** | **N** | 40 | 40 | 20 | |
| | **%** | 100% | 100% | 100% | |

| **Groups** | Odds Ratio | 95% C.I. | *P*-value |
|---|---|---|---|
| **PC vs BPH** | 0.729 | 0.295-1.797 | $0.491^{NS}$ |
| **PC vs Control** | 0.179 | 0.054-0.598 | 0.003** |
| **BPH vs Control** | 0.246 | 0.075-0.81 | 0.017* |

Data presented at Pearson Chi-Square. NS: Non-significant. * The correlation is significant at the $P < 0.05$ level. ** The correlation is highly significant at the $P < 0.01$ level.

**Association of HCMV-DNA expression with disease grade**

The results also demonstrated that 44.4% and 50% , respectively, of the patients who expressed positive HCMV-DNA had well and moderately differentiated Gleason's grades , while the poorly differentiated grade was recorded in 78.3% (22 out of 23 cases), as shown in Table-3. The results, however, showed non-significant differences ($p > 0.05$).

**Table 3-** Association of histological grading of prostate cancer patients to HCMV expression.

| HCMV Expression | | PC Grades | | | Total |
|---|---|---|---|---|---|
| | | Well | Moderate | Poor | |
| Positive | N | 4 | 4 | 18 | 26 |
| | % | 44.4% | 50% | 78.3% | 65% |
| Negative | N | 5 | 4 | 5 | 14 |
| | % | 55.6% | 50% | 21.7% | 35% |
| Total | N | 9 | 8 | 23 | 40 |
| | % | 100% | 100% | 100% | 100% |
| Chi-Square | | $X^2$ | *P*-Value | | Sig. |
| | | 4.241 | 0.12 | | NS |

Data presented at Chi-square independence test. NS: nonsignificant.

**Association of signal scoring of HCMV-DNA-CISH with the studied prostatic tissues**

As shown in Table-4, prostatic adenocarcinoma with high (3+) and low (1+) signal score for HCMV -CISH was noticed in 27.5% (11 out 40 cases) and 22.5% (9 out 40 cases) respectively, while prostatic adenocarcinoma with moderate (2+) scoring was found in 15% (6 out 40 cases. Negative HCMV- CISH reactions constituted 35% (14 out of 40 cases) of prostatic adenocarcinoma. In benign prostatic hyperplasia group, the percentage of tissues with low (1+) and high (3+) signal score for HCMV-CISH test were 22.5% (9 out of 40 cases) and 20% (8 out of 40 cases), respectively, while moderate (+2) scoring was detected in 15% (6 out 40 cases). The negative HCMV-CISH reactions constituted 42.5% (17 out of 40 cases) of benign prostatic hyperplasia group. The proportion of healthy control subjects with high (3+) and low (1+) scoring of HCMV-CISH reactions was 5% (1 out 20 cases) for each, while the moderate (2+) scoring was detected in 15% (3 out of 20 cases). Negative HCMV-CISH test of the healthy control group constituted 75% (15 out of 20 cases). Figure-1 illustrates the scoring of positive HCMV-DNA-CISH (stained in blue), in contrast to the negative CISH microscopic pattern in the healthy tissue of prostatic adenocarcinoma.

Statistically, there were non- significant differences ($p > 0.05$) among the patient groups. A highly significant difference ($p < 0.01$) was found between healthy control and prostate cancer groups, along with a significant difference ($p < 0.05$) between the control and benign prostatic hyperplasia groups.

**Table 4-** The HCMV-CISH signal scoring distribution of patients with prostate carcinoma and benign prostatic hyperplasia and healthy control groups.

| HCMV Score groups | | Studied groups | | | Pearson Chi-Square (*P*-value) |
|---|---|---|---|---|---|
| | | PC | BPH | Control | |
| Negative | N | 14 | 17 | 15 | $0.096^{NS}$ |
| | % | 35% | 42.5% | 75% | |
| + | N | 9 | 9 | 1 | |
| | % | 22.5% | 22.5% | 5% | |
| ++ | N | 6 | 6 | 3 | |
| | % | 15% | 15% | 15% | |
| +++ | N | 11 | 8 | 1 | |
| | % | 27.5% | 20% | 5% | |
| Total | N | 40 | 40 | 20 | |
| | % | 100% | 100% | 100% | |
| Groups | | Odds Ratio | | 95% C.I. | *P*-value |

| | | | |
|---|---|---|---|
| **PC vs BPH** | 0.729 | 0.295-1.797 | 0.491 NS |
| **PC vs Control** | 0.179 | 0.054-0.598 | 0.003** |
| **BPH vs Control** | 0.246 | 0.075-0.81 | 0.017* |

Data presented as Pearson Chi-Square. NS: Non-significant. * The correlation is significant at the $P < 0.05$ level. ** The correlation is highly significant at the $P < 0.01$ level.

**Association of the signal intensity for HCMV–DNA-CISH with the studied prostatic tissues**

The percentages of HCMV infected cells were evaluated in relation to HCMV-CISH signal intensity reaction within the tissue (Table-5 and Figure-1). In prostatic adenocarcinoma group, stronger and moderate intensities of HCMV-CISH reactions were observed in 22.5% (9 out of 40 cases) for each, and weak signal intensity was found in 20% (8 out of 40 cases). Negative HCMV-CISH reactions constituted 35% (14 out 40 cases) of prostatic adenocarcinoma. In the benign prostatic hyperplasia group, strong intensity of HCMV-CISH reactions was observed in 25% (10 out of 40 cases), whereas moderate and weak signal intensities were recorded in 17.5% (7 out of 40 cases) and 15% (6 out of 40 cases), respectively. Negative HCMV-CISH reactions constituted 42.5% (17 out of 40 cases) of benign prostatic hyperplasia. In the control group, moderate signal intensity of HCMV-CISH was observed in 15% (3 out of 20 cases), whereas strong and weak signal intensities were recorded in 5% (1 out 20 cases) for each. Negative HCMV-CISH reactions constituted 75% (15 out of 20 cases) of the control group.

Statistically, there were non-significant differences ($p > 0.05$) among the studied patient groups. A highly significant difference ($p<0.01$) was found between the control and prostate cancer groups, long with a significant difference ($p<0.05$) between the control and benign prostatic hyperplasia groups.

**Table 5-** The HCMV signal intensity distribution of patients with prostate carcinoma and benign prostatic hyperplasia and healthy control groups.

| **HCMV Intensity groups** | | **Studied groups** | | | **Pearson Chi-Square (*P*-value)** |
|---|---|---|---|---|---|
| | | **PC** | **BPH** | **Control** | |
| **Negative** | **N** | 14 | 17 | 15 | 0.118 NS |
| | **%** | 35% | 42.5% | 75% | |
| **Weak** | **N** | 8 | 6 | 1 | |
| | **%** | 20% | 15% | 5% | |
| **Moderate** | **N** | 9 | 7 | 3 | |
| | **%** | 22.5% | 17.5% | 15% | |
| **Strong** | **N** | 9 | 10 | 1 | |
| | **%** | 22.5% | 25% | 5% | |
| **Total** | **N** | 40 | 40 | 20 | |
| | **%** | 100% | 100% | 100% | |

| **Groups** | **Odds Ratio** | **95% C.I.** | ***P*-value** |
|---|---|---|---|
| **PC vs BPH** | 0.729 | 0.295-1.797 | 0.491 NS |
| **PC vs Control** | 0.179 | 0.054-0.598 | 0.003** |
| **BPH vs Control** | 0.246 | 0.075-0.81 | 0.017* |

Data presented as Pearson Chi-Square. NS: Non-significant. * The correlation is significant at the $P < 0.05$ level. ** The correlation is highly significant at the $P < 0.01$ level.

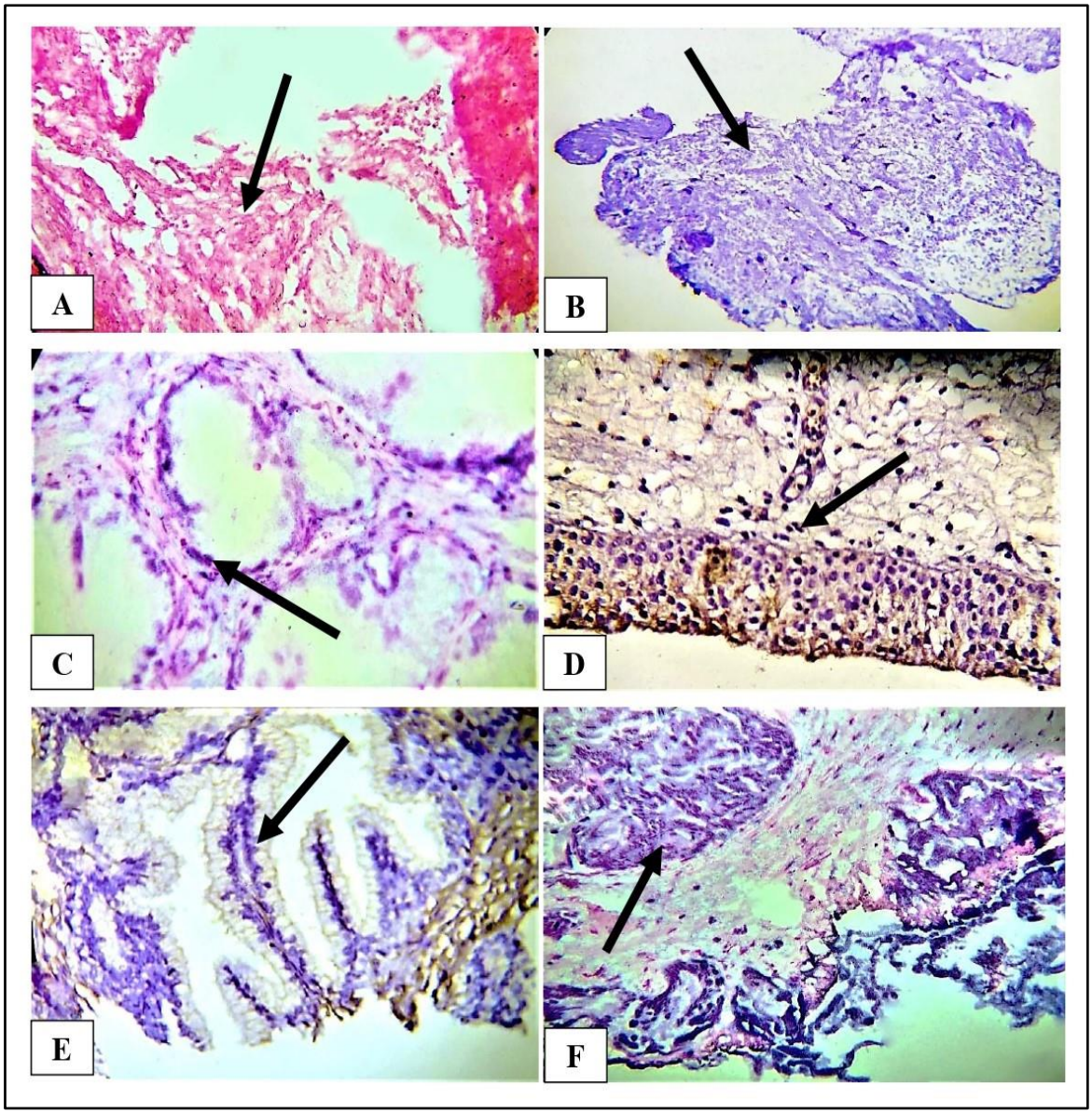


**Figure 1-** Microscopic HCMV-DNA-CISH signal appearance in prostate tumors, using Digoxigenin-labeled HCMV probe, stained with NBT / BCIP (Blue) and Nuclear Red Solution (Red) counter staining. Blue signal (arrows) is detected at sites of complementarity.
A: Prostate adenocarcinoma tissues with negative HCMV-DNA-CISH reaction (40X).
B: Prostate adenocarcinoma tissues with negative immune reactions.
C: Prostate adenocarcinoma tissues with positive HCMV-DNA-CISH reaction, weak signal intensities with signal score + (40X).
D: Prostate adenocarcinoma tissues with positive HCMV-DNA-CISH reaction, moderate signal intensity and score + (40X).
E: Prostate adenocarcinoma tissues with positive HCMV-DNA-CISH reaction, moderate signal intensity and signal score ++ (40X).
F: Prostate adenocarcinoma tissues with positive HCMV-DNA-CISH reaction, high signal intensity and signal score +++ (40X).

The study of Poiret, 2018 [43], showed that 46.2% (128 out of 277) of pre-transplant patients were positive for HCMV. The data by Samanta *et al*., 2003 [44], demonstrated that HCMV genes were expressed specifically within a highest percentage of prostate cancer patients. Another study [45] that used in situ hybridization for human HCMV RNA e implied some degree of human HCMV association with prostate cancer. The results also suggested that the human prostate gland may harbor latent human HCMV.

The study of Hrbacek *et al*., 2011 [46], showed that HCMV positivity was higher in the BPH group than among PC cases. in the study of Sutcliffe *et al*., 2012 [47], showed that HCMV positivity was similar for PC cases and controls (67.9 versus 65.2%, respectively). In addition, no association was observed between positive HCMV and PC risk.

The results reported by Sutcliffe *et al*., 2012 [47], showed no association between HCMV and the risk for overall, high, and low -grade PC. Andabekov *et al*., 2009 [48], showed a significantly strong relationship between cytomegalovirus infection and poor prognosis of prostate cancer.

HCMV infection is related to many types of prostate, breast, and colon cancers. HCMV was previously demonstrated to infect tumor cells and modulate their malignant properties, playing an onco-modulatory function in cancers. It was presumed that tumor cells support a genetic environment that allows HCMV to exercise its onco-modulative impact. However, epidemiological and physiological findings are indicative [49].

In the concept of HCMV virus, onco-modulation is a fairly recent word, referring to the capacity of this virus to change the antitumor protection mechanisms of the host organism and to promote and/or accelerate the production of an already present neoplastic cycle. Until now, researchers could not show a clear oncogenic function of this virus. Determining whether it may play a role in the transformation from hyperplasia to BPH is both important and interesting. On the other hand, there are studies that raise the suspicion of a sexually transmitted pathogen which could play a role in prostatic neoplasia progression [11].

HCMV infections may have direct effects on tumor cells via the expression of viral proteins that influence pathways related to tumor biology and inflammation caused by viruses. Nonetheless, HCMV leads to all proven cancer hallmarks [50]. HCMV proteins and/or nucleic acids are specifically detected and often highly expressed in basal cell hyperplasia and prostatic intraepithelial neoplasia [11].

It is a reality that HCMV is found in prostate tissues. There are also several concerns regarding what function these HCMV play in either benign prostate hyperplasia or prostate adenocarcinoma in the neoplastic phase. Newly developed immunological methods are approaching the oncogenic properties of this virus. The role of this virus in mutations seems to be related its nature and the modifications about it makes to the cell cycle [11].

Sexually transmitted infections, such as those caused by herpesviruses, have long been reported to contribute to the risk of PC [51]. Sexual interaction is a significant route of transmission of HCMV in adults. Higher HCMV levels are related to increased history of sexually transmitted diseases [52]. New findings show that HCMV has several oncogenic properties, such as invasion of cells, angiogenesis, and induction of mutagenesis. The sero-prevalence of HCMV rises with age, affecting around 91% of people over 80 years of age [53]. The association between HCMV and likelihood of PC is little known. The only related research we found did not provide evidence of a relation between HCMV seropositivity and PC occurrence. Our findings suggest that cases of PC might have lower rates of antibodies to HCMV than those of BPH [46].

*In vitro* studies suggest that the production of HCMV protein could be limited to cells at a specific stage of the cell cycle and could promote anti-apoptotic pathways in prostate cancer cells. These results together indicate that persistent HCMV infection of prostate epithelial cells may be normal, and that pre-neoplastic and neoplastic prostatic epithelium may serve as a specific reservoir for persistent viral infection and gene expression[45, 54].

**Conclusions**

The high percentage of HCMV associated with PC and BPH in our findings could suggest HCMV's oncogenic potential in these cases as well as its critical function in the progression, transformation and/or development of a subset of PC and BPH cases.

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