ISSN: 03875547
Volume 45, Issue 08, October, 2022

Evidence fore no involvement of cytomegalovirus in prostate cancer

Kawtar ABOULALAA[1], Youssef ENNAJI[1], Berjas ABUMSIMIR[1,2], Kawtar NABIL[1], Moulay Mustapha ENNAJI[1*]

Laboratory of Virology, Oncology, Biosciences, Environment and New Energy (LVO BEEN), Faculty of Science and Technology, Mohammedia, Hassan II University of Casablanca, Casablanca, Morocco[1]
Pharmacological and Diagnostic Research Centre (PDRC), Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University(AAU), Amman 19328, Jordan[2]

Corresponding Author: 1*

Keywords:
Prostate cancer, cytomegalovirus, molecular detection, herpesviridae

ABSTRACT

Prostate cancer (PCa) is the cancer that registers the highest incidence and mortality rate in men. Epidemiological studies indicate the existence of an association between high exposure to Sexually transmitted diseases (STDs) and an increased risk of prostate cancer. Cytomegalovirus (CMV) is a member of the sexually transmitted herpesviridae family. Studies carried out on prostate biopsies revealed the presence of the virus on pre-neoplastic and neoplastic tissues. Thus, and given that the virus is transmissible through all body fluids including saliva and breast milk, our study aims to detect CMV in the blood of subjects with prostate cancer looking for a possible association between the infection and the risk of prostate cancer. Blood samples were collected from a population of 45 people with prostate cancer, viral DNA testing was performed using conventional PCR. We were unable to detect CMV DNA in the blood samples studied. Based on these results, a first suggestion to be made is the non-implication of CMV in prostate cancer. But, considering that the results of previous studies are controversial between detection and and non-detection of the virus, it would be opportune to increase the sample size and to consider other parameters related to the therapy administrated for a possible correlation between the anti-cancer protocol that can cause both immunosuppression and the reactivation of CMV of its latency.

1. INTRODUCTION

Prostate cancer remains a serious public health problem, reflected by the alarming statistics it records, in particular its prevalence on a global scale. Actually, it is the most diagnosed cancer among men in 12 regions of the world, and it is the second cause of death after lung cancer in 5 regions [1]. In 2020, the number of diagnostic cases worldwide reached 1,414,249 [2].

Indeed, despite therapeutic advances and the emergence of new targeted therapies through the development

of personalized medicine. Reaching the metastatic stage is frequently observed leading to a high rate of death cases, which reached 375,000 cases in 2020 [2].

A good knowledge of the risk factors associated with this cancer will help to better elucidate and understand the mechanisms of carcinogens and metastasis. Several studies have proposed a causal link between prostate carcinogenesis and chronic inflammation of the prostate, which could be induced by environmental factors and also by infectious agents, in particular those causing sexually transmitted infections [3], [4]. Although the mechanisms elucidating the association of infections and inflammation have not been clearly established [5] but several studies have revealed the presence of infectious agents in the prostate, in particular oncogenic viruses such as HPV, BKV polyomavirus, and herpesviruses. Several viruses of the herpesvirus family have been suspected to be involved in carcinogenesis, this is the case of Epstein Barr virus and human herpesvirus-8 [6]. Cytomegalovirus (CMV), or human herpes virus-5, belonging to the beta-herpesvirinae subfamily, is a very widespread virus whose infection can affect between 50% and 100% of the population [7], [8]. This virus, known for its latency period like other viruses of the herpesvirus family, can spread via various biological fluids (saliva, blood, urine, vaginal secretions and breast milk), also, several organs may be prone to infection but the preferred site is thought to be the salivary glands [9].

Several studies on different tumor locations have reported the detection of CMV in various human epithelial tumors such as brain, breast, colon, cutaneous tumors, prostate and ovarian [10- 15]. For PCa, the experiment performed by Roman et al on a cell culture model demonstrated that CMV acts on the invasive properties of infected PC3 cells [16]. Also, an involvement of the virus in the prostate inflammation has been reported [17]. CMV has also been detected in neoplastic and preneoplastic lesions [18], [19].

In order to study the involvement of CMV in PCa, we conducted this study aimed at searching for the virus in the blood of a population target, to assess the risk between infection and prostate cancer.

2. Material and method

2.1 Patient population and sampling

The sampling for this study corresponds to blood samples taken at the Urology Department, Military Hospital teaching Mohammed V, Rabat, Morocco, from a population of 44 subjects who had been diagnosed with prostate cancer. Informed consent was obtained from all participating patients and the study was carried out with the approval of the Ethical Review Committee of the Ethics Committee of Biomedical Research Committee in Morocco (No. 3/2018/April 30/2018). The clinicopathological data of the patients are available, some of which are summarized below (table 1):

Table 1: the clinicopathological data of patients

	Age		PSA			Gleason score		
	< 60	≥ 60	≤10	>10 et <20	≥20	≤6	7	>7
Case number	6	38	13	17	14	13	14	17

2.2 DNA extraction from blood sample

Peripheral blood samples were collected in EDTA containing tubes and stored at -20C. Samples were de-frosted once. Genomic DNA was extracted from a volume approximately 200 µl using the Blood Kit Roche Applied System. For a concentration assay and verification of the purity of the DNA, we measured the absorbance at 260/280 nm using the NanoDrop spectrophotometer 2000 (Thermo Scientific). DNA extracted

from whole blood is then stored at -20C until use.

2.3 Viral DNA amplification and detection

In order to assess the DNA quality and integrity of our samples, the samples were tested through β-globin gene amplification using. The sequence of the primers used 5'-CAACTTCATCCACGTTCACC-3' (PC03) and 5'-GAAGAGCCAAGGACAGGTAC-3' lGH20/PCO4 [20]. Only samples positive for amplification were retained in the experiment. The amplification protocol adopted for β-globin begins with denaturation for 10 min at 94°C, 35 cycles of denaturation at 94°C for 45 s, hybridization at 54°C for 45 s, extension at 72°C for 1 min, at the end an extension at 72°C for 10 min.

The samples selected from this step were subjected to a chain polymerization reaction, and the search for CMV was carried out by targeting a fragment of the sequence encoding the open reading frames for pp65 (UL83) using the primer sequences previously described (21). The sequence of the primers used for this gene is 5'-TCGCGCCCGAAGAGG-3' for the forward and 5'-CGGCCGGATTGTGGATT-3' for the reverse. The amplification program consisted of 10 min at 94°C, then 35 cycle of denaturation at 94°C for 1 min, hybridization at 61°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 10 min. The verification of the amplification product, which is the pp56 size 57bp gene, is ensured through electrophoresis at 70V on a 1.5% concentrated agarose gel (DNA SUB CELTM, Bio-RAD, Italy) and visualization of the after addition of the ethidium bromide.

3. Results

Our work covered a population of subjects diagnosed with prostate cancer during the period between February 2020 and June 2021. Given the clinicopathologic data, the majority of patients, 86%, are aged 60 and over. PSA values are variable but 38% of subjects have values between 10 and ng/ml 20ng/ml. As for the Gleason score, 38% of the population had a score greater than 7. In fact, for the stage of the disease, 38% of patients have prostate cancer classified as T1c and T2a corresponding to a localized low-risk cancer, 16% of subjects have a T2B tumor stage, meaning that more than half of one lobe is affected but without the other being affected (intermediate progressive risk according to Amico). 29% of patients are at the T2C stage, meaning both lobes are affected. 3 patients had stage T3 reflecting extension of the cancer beyond the capsule, while 4 had stage T4 synonymous with dissemination of the cancer to structures other than the seminal vesicles.

In our study aiming at the detection by PCR of CMV in the blood of subjects with PCa with a view to a possible reconciliation with the above data, however, all the results of the amplification were negative, and the virus wasn't detected in the studied population.

4. Discussion

Oncogenic viruses whose involvement in the cancerization process have been well established are mainly: human papillomavirus (HPV), hepatitis B and C viruses (HBV, HCV), human T-lymphotropic virus-1 (HTLV- 1), Epstein–Barr virus (EBV), Kaposi sarcoma human virus (KSHV), and Merkel cell polyomavirus (MCPyV) [22]. Although CMV is not yet classified as an oncogenic virus, but its involvement has been suspected, given its detection in several cancers [10- 15]. Also, the analysis of the functioning of the expression products of the viral genes of CMV, showed that the products of the genes, particularly those expressed early-early during the viral cycle, are involved in carcinogenesis [23]. In PCa, studies have detected the HCMV in preneoplastic and neoplastic lesions of PCa [18], [19]. Based on these findings, our study aimed to detect the virus in the blood in order to assess the risk in prostate cancer. Otherwise, the PCR carried out on 44 samples according to the protocol described above, did not detect the virus in the DNA extracted from the blood and thus found no support for the involvement of CMV in prostate cancer.

In fact, it should be recalled that there is controversy over the relationship between CMV and PCa. Considering in particular the discripancy observed between the studies, both molecular and serological, carried out in subjects with prostate cancer. In fact, although studies that have investigated the virus using different techniques (immunohistochemistry, in situ hybridization, polymerase chain reaction and DNA Sequencing) successfully detected the CMV in prostate tissue [18], [19], but other studies such as that carried out by LESKINEN et al have not found the viruses in subjects with localized PCa [24]. Another study by Bergh J et al, also detected none of the eight targeted viruses including CMV in prostate tissue and only two viruses (EBV and JCV) were found [25]. In another study the virus was detected but at a very low rate [26]. Also, several studies that aimed to assess the CMV serostatus, observed the absence of the association between CMV serostatus and the risk of PCa [27- 29].

In relation to oncogenic power, and as we have already mentioned, CMV is not classified as an oncogenic virus [22], the oncomudulation associated with this virus rather relates to the ability of HCMV to interfere with cellular properties [30]. Another finding against a possible oncogenic role that could be played by CMV is that the virus are unable to transform normal human cells in vitro, but on the other hand it has been found that the virus changes its behave under in vitro conditions compared to in vivo conditions [31].

Also, the result obtained could be explained by viral quiescence, which is mainly established is in myeloid lineage progenitor cells (CD34+ hematopoietic progenitor cell population) [32]. The ignorance of the conditions of viral reactivation often asymptomatic [33] could thus hinder the detection of the virus in the blood. In this regard, it would be very relevant to consider the data relating to the therapies administered to patients, in particular chemotherapy. n a study by Capria et al looking for CMV during first-line chemotherapy in subjects with acute myeloid leukemia, it was found that 35% of patients with positive IgG of CMV and negative IgM before treatment developed CMV reactivation [34].

5. Conclusion

In summary, we were unable to detect the CMV in blood sample of patients with PCa. A first hypothesis could be proposed on the lack of correlation of CMV with PCa. Moreover, we recommend to establish this hypothesis to widen the sample by including other parameter in relation in particular to the therapeutic protocol to evaluate the conditions of a possible recativation of the virus. A study also on tissue biopsies will provide further arguments on the possible association of CMV with the risk of prostate cancer.

6. Acknowledgments

The authors would like to thank the Minister of National Education, Vocational Training, Higher Education and Scientific Research and the members of the team of Virology, Oncology, Biosciences, Environment and New Energies of the FSTM-University Hassan II of Casablanca, for all their efforts to support us during all the stages of this research and the biopsy donors and their families.

Conflict of interest statement

The authors declare no conflicts of interest.

Contribution of the authors

K.A: conceived and designed the experiments, optimized the experimental approach, realized the experiments, wrote the manuscript, all authors approved the final version.

Y.E: manuscript Reviewing. Analysed the patient data

B.A: article reviewing

K.N: managed the collection of samples and the processing and storage of all samples, proofreading and

technical support.
M.M. E: conceived and designed the experiments, optimized the experimental approach, realized the experiments, wrote the manuscript, all authors approved the final version. Coordinate al the project,

Ethic Approval consent to participate
Agreement of the Ethics Committee of Biomedical Research in Morocco code n°3/2018/April 30/2018-Maroc.

7. References

[1] Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021 Apr 5. doi: 10.1002/ijc.33588. Epub ahead of print. PMID: 33818764.

[2] Leslie SW, Soon-Sutton TL, Sajjad H, Siref LE. Prostate Cancer. 2022 Jul 3. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 29261872.

[3] Cheng, I. et al. Prostatitis, sexually transmitted diseases, and prostate cancer: the California Men's Health Study. Plos One 5, e8736 (2010).

[4] Caini S, Gandini S, Dudas M, et al. Sexually transmitted infections and prostate cancer risk:A systematic review and meta-analysis.Cancer Epidemiol.2014;38(4):329-338.

[5] A.M. De Marzo, et al., Inflammation in prostate carcinogenesis, Nat. Rev. Canc. 7 (4) (2007) 256–269.

[6] Howley, P. M., Ganem, D. and Kieff, E.: DNA viruses. In: Cancer: Principles and Practice of Oncology, 6th ed. Edited by V. T. De Vita, S. Hellman and S. A. Rosenberg. Philadelphia: Lippincot Williams and Wilkins Co., pp. 168–173, 2001

[7] Michaelis M, Doerr HW, Cinatl J. The story of human cytomegalovirus and cancer : increasing evidence and open questions. Neoplasia 2009; 11(1) : 1-9 2.

[8] Georges H, Amit K. The oncogenic potential of human cytomegalovirus and breast cancer. Front. Oncol. 2014; 4: 230. doi:10.3389/fonc.2014.00230

[9] Koichi Y, Arvin Am, Gabriella CF, Whitley R. Human herpes viruses : biology, therapy and immunoprophylaxis. Cambridge, UK; Cambridge University Press. 2077; ISBN O-521-82714-O.

[10] Baryawno N, Rahbar A, Wolmer-Solberg N, Taher C, Odeberg J, Darabi A, Khan Z, Sveinbjörnsson B, FuskevÅg OM, Segerström L, Nordenskjöld M, Siesjö P, Kogner P, et al. Detection of human cytomegalovirus in medulloblastomas reveals a potential therapeutic target. J Clin Invest. 2011; 121:4043–55.

[11] Taher C, de Boniface J, Mohammad AA, Religa P, Hartman J, Yaiw KC, Frisell J, Rahbar A, Söderberg-Naucler C. High prevalence of human cytomegalovirus proteins and nucleic acids in primary breast cancer and metastatic sentinel lymph nodes. PLoS One. 2013; 8:e56795.

[12] Cui J, Wang Q, Wang HB, Wang B, Li L. Protein and DNA evidences of HCMV infection in primary breast cancer tissues and metastatic sentinel lymph nodes. Cancer Biomark. 2018; 21:769–80.

[13] Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, Cobbs CS. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002; 360:1557–63.

[14] Rådestad AF, Estekizadeh A, Cui HL, Kostopoulou ON, Davoudi B, Hirschberg AL, Carlson J, Rahbar A, Söderberg-Naucler C. Impact of Human Cytomegalovirus Infection and its Immune Response on Survival of Patients with Ovarian Cancer. Transl Oncol. 2018; 11:1292–300. Melnick M, sedghizadeh PP, Allen CM, Jaskoll T. Human Cytomegalovirus and mucoepidermoid carcinoma of salivary glands : cell specific localization of active viral and oncogenic signalling proteins is confirmatory of a causal relationship. Experimental and Molecular Pathology. 2011; 92(1):118-25.

[15] Blaheta RA, Weich E, Marian D, Bereiter-Hahn J, Jones J, Jonas D, Michaelis M, Doerr HW, Cinatl J Jr. Human cytomegalovirus infection alters PC3 prostate carcinoma cell adhesion to endothelial cells and extracellular matrix. Neoplasia. 2006 Oct;8(10):807-16. doi: 10.1593/neo.06379. PMID: 17032497; PMCID: PMC1715925.

[16] Rouphael NG, Laskar SR, Smith A, Lyon GM. Cytomegalovirus prostatitis in a heart transplant recipient. Am J Transplant. 2011; 11:1330–3. [PubMed: 21486388]

[17] Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol. 2003 Sep;170(3):998-1002. doi: 10.1097/01.ju.0000080263.46164.97. PMID: 12913758)

[18] Boldogh I, Baskar JF, Mar EC, Huang ES. Human cytomegalovirus and herpes simplex type 2 virus in normal and adenocarcinomatous prostate glands. J Natl Cancer Inst. 1983;70:819–26).

[19] Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1986). Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele-specific oligonucleotide probes. Nature, 324(6093), 163-166.

[20] Schmolke S, Kern HF, Drescher P, Jahn G, Plachter B. The dominant phosphoprotein pp65 (UL83) of human cytomegalovirus is dispensable for growth in cell culture. J Virol. 1995 Oct;69(10):5959-68. doi: 10.1128/JVI.69.10.5959-5968.1995. PMID: 7666500; PMCID: PMC189491.

[21] Herbein G. Tumors and Cytomegalovirus: An Intimate Interplay. Viruses. 2022 Apr 14;14(4):812. doi: 10.3390/v14040812. PMID: 35458542; PMCID: PMC9028007.

[22] Herbein, G. The Human Cytomegalovirus, from Oncomodulation to Oncogenesis. Viruses 2018, 10:E408.

[23] Leskinen MJ, Vainionp R, Syrjnen S, Leppilahti M, Marttila T, Kylml T, Tammela TL. Herpes simplex virus, cytomegalovirus, and papillomavirus DNA are not found in patients with chronic pelvic pain syndrome undergoing radical prostatectomy for localized prostate cancer. Urology. 2003 Feb;61(2):397-401. doi: 10.1016/s0090-4295(02)02166-0. PMID: 12597955.)

[24] Bergh J, Marklund I, Gustavsson C, et al. No link between viral findings in the prostate andsubsequent cancer development. Br J Cancer. 2007; 96:137–9. [PubMed: 17117176]

[25] Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J, Morales-Rodriguez IB, Hernandez-Ordoñez MA, Calderon-Cardenas G, Ortiz-Lopez R, Rivas-Estilla AM, Ancer-Rodriguez J, Rojas-Martinez A. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC Cancer. 2010 Jun 24;10:326. doi: 10.1186/1471-2407-10-326. PMID: 20576103; PMCID: PMC2912861

[26] Berrington de Gonzalez A, Urban MI, Sitas F, et al. Antibodies against six human herpesviruses in relation to seven cancers in black South Africans: a case control study. Infect Agent Cancer. 2006; 1:2. [PubMed: 17150131]

[27] Huang WY, Hayes R, Pfeiffer R, et al. Sexually transmissible infections and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2008; 17:2374–81. [PubMed: 18768506]

[28] Mandel JS, Schuman LM. Sexual factors and prostatic cancer: results from a case-control study. J Gerontol. 1987; 42:259–64. [PubMed: 3553301]

[29] Cinatl, J., Jr., et al., Modulatory effects of human cytomegalovirus infection on malignant properties of cancer cells. Intervirology, 1996. 39(4): p. 259-269

[30] Wang D, Shenk T. Human cytomegalovirus virion protein complex required for epithelial and endothelial cell tropism. Proc Natl Acad Sci U S A. 2005; 102:18153–58.

[31] Söderberg-Nauclér C, Fish KN, Nelson JA. Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell. 1997; 91:119–26.

[32] Bate S., Dollard S., Cannon M. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin. Infect. Dis. 2010;50:1448–1449

[33] Capria S., Gentile G., Capobianchi A., Cardarelli L., Gianfelici V., Trisolini S., Foa R., Martino P., Meloni G. Prospective cytomegalovirus monitoring during first-line chemotherapy in patients with acute myeloid leukemia. J. Med. Virol. 2010;82:1201–1207.