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UROONCOLOGY

Original Article

Ductal variant of prostate adenocarcinoma harbor Xenotropic murine leukemia virus related virus (XMRV) infection: a novel finding in subtype of prostate cancer

Prostat adenokarsinomunun duktal variyantı ksenotropik fare lösemisi virüsüyle ilişkili virüs (XMRV) enfeksiyonu barındırmaktadır: Prostat kanseri alt tipinde yeni bir bulgu

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ABSTRACT

Objective: Xenotropic murine leukemia virus related virus (XMRV), is the first gammaretrovirus identified a decade ago, in human tissue bearing adenocarcinoma of prostate, followed by several researches documenting little or no prevalence of XMRV in prostate cancer samples. However, the status of XMRV within subtype of prostate adenocarcinoma has not been investigated yet. In this study, we investigated the relationship between XMRV and broad spectrum morphological entities of prostate adenocarcinoma, including acinar, ductal and other rare subtypes.

Material and methods: The prevalence of XMRV DNA in different histological subtypes of prostate adenocarcinoma was examined after characterizing the tumors into groups, using formalin-fixed, paraffin-embedded tissue samples from newly diagnosed prostate adenocarcinomas and archival prostate cancer tissue from our XMRV case control analysis. Broad-spectrum XMRV DNA amplification was performed by end-point polymerase chain reaction, using commercially available primer set.

Results: The study included 100 patients with prostate cancer. XMRV DNA was detected in 4 of 8 (50%) ductal adenocarcinomas, exhibiting papillary and cribriform histological features. XMRV DNA was not detected in any other variant of adenocarcinoma including acinar (0/91) and mucinous carcinomas (0/1). Majority of XMRV positive cases were biologically aggressive and present cancer at an early age upon diagnosis.

Conclusion: Ductal adenocarcinomas demonstrate a significant association of XMRV DNA while other histological variants of prostate adenocarcinoma seem unrelated to XMRV infection.

Keywords: Acinar; adenocarcinoma; ductal; XMRV.

ÖZ

Amaç: Ksenotropik fare lösemi virüsüyle ilişkili virüs (XMRV), insan prostat adenokarsinomunda 10 yıl önce tanımlanmış ilk gama retrovirüstür. Daha sonra yapılan araştırmalar, XMRV'nin prostat kanseri örneklerinde çok az olduğunu ve yaygın olmadığını belgelemiştir. Ancak prostat adenokarsinomlarının alt tiplerinde XMRV'nin durumu henüz araştırılmamıştır. Bu çalışmada XMRV ile prostat adenokarsinomu (asiner, duktal ve diğer nadir görülen tipler) arasındaki ilişkiyi araştırdık.

Gereç ve yöntemler: Yeni tanı konmuş prostat karsinomlarının formalinle tespit edilmiş, parafine gömülmüş doku örnekleri ve XMRV olgu kontrol analizimizde arşivlenmiş prostat kanseri dokusu örnekleri kullanılarak, prostat kanserinin farklı alt tiplerinde XMRV DNA 'nın prevalansı araştırılmıştır. Ticari primer set kullanılarak polimeraz zincir reaksiyonuyla - geniş spektrumlu XMRV DNA amplifikasyonu yapılmıştır.

Bulgular: Bu çalışmaya prostat kanserli 100 hasta dahil edilmiştir. Papiller ve kribriform histolojik özellikler gösteren sekiz duktal adenokarsinomun dördünde (%50) XMRV DNA saptanmıştır. Asiner (0/91) ve müsinöz karsinomlar (0/1) da dahil diğer adenokarsinom variyantında XMRV DNA saptanmamıştır. XMRV pozitif olguların çoğunda kanser biyolojik olarak agresif olup, erken yaşta tanı almıştır.

Sonuç: Duktal adenokarsinomlar XMRV DNA ile anlamlı olarak ilişkiliyken prostat kanserinin diğer histolojik variyantlarının XMRV enfeksiyonuyla ilişkili olmadığı görünmektedir.

Anahtar Kelimeler: Asiner; adenokarsinom; duktal; XMRV.

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Introduction

The incidence of prostate adenocarcinoma (PCa) has been increasing, particularly in younger men during the last few decades.[1] Histologically, PCa is confirmed by the presence of neoplastic glands lined by a single layer of cuboidal cells. Several different histological types of PCa exist, based on the variation in tumor morphology.[2] The majority of tumors are acinar followed by ductal and mucinous varieties, and the latter is predominant among rare variants.[3] Previously, in a large series of PCa specimens, we successfully characterized acinar and ductal variants based on the typical adenocarcinoma morphology and papillary and cribriform histological patterns, using H&E and immunohistochemistry.[4] We have determined that, acinar component coexists with rare morphological characteristics, however 40 percent or more tumor volume exhibits the ductal pattern on biopsy.[4] Whereas, for mucinous tumor, pool of extravasated mucin in stroma with suspended nests, cords or groups of carcinoma cells is standard diagnostic criterion.[3] Additionally, the grading of acinar component varies; however, the ductal and mucinous components are usually seen in Grade 4 PCa on Gleason scoring system.[4]

Age, hormonal status, diet and genetics are established risk factors in development of PCa; whereas the association of oncogenic infection with tumor has not been confirmed yet.[5,6] In the last few years, some studies have reported a positive link of novel oncogenic virus, termed; Xenotropic murine leukemia virus related virus (XMRV) with the etiology of PCa, however its pathogenesis remains unclear.[7,8]

Xenotropic murine leukemia virus related virus is the only gamma retrovirus identified in humans till date. Initially, virus was detected in non-malignant prostatic stromal cells of cancer tissue bearing RNaseL gene polymorphism; R462Q.[9] This finding suggests that an oncogenic infection is involved in the development of tumor via paracrine mechanism.[9] However, following investigation revealed viral antigens in malignant prostatic epithelial cells especially in higher-grade tumors, independent of the RNaseL polymorphism, suggesting a direct role of XMRV in tumorigenesis.[7] Later, demonstration of specific viral integration sites near cancer breakpoints, micro-RNA genes and XMRV neutralizing antibodies in serum of PCa patients further strengthened the hypothesis of viral oncogenesis.[10]

On the contrary, recent studies have reported the same virus, as a mere contamination of a PCa cell line.[6,11,12] Although, XMRV infection has been reported in prostate biopsies bearing adenocarcinoma, low prevalence of viral DNA was documented in our previous case control study, which included archival prostate biopsies diagnosed with cancer.[13]

To date, extensive burden of PCa research is focused on broad spectrum of morphological differentiation; however investigation in etiopathogenesis of different histological subtype is largely neglected. Taking this opportunity into account, we expand our ongoing project to further investigate the relationship between XMRV and broad spectrum morphological entities of PCa, including acinar, ductal and other rare subtypes in a large number of representative tumors, using novel and sensitive polymerase chain reaction (PCR) assay. We also sought to compare the association of XMRV infection with biological behavior and age at diagnosis of different tumor varieties independently.

Material and methods

We sought the ethical approval to study 100 tissue biopsies bearing PCa from Institutional Review Board of Dow University of Health Sciences. The samples included 20 cases from "The Laboratory Saddar- Karachi" and 80 cases from "Dow Diagnostic Research and Reference Laboratory- Karachi", of which 50 cases have been investigated previously for XMRV infection.[13] The cases were recruited after informed consent.

All cases were evaluated by a panel of histopathologist and diagnosticians and graded according to standard histological criteria. The presence of an acinar component associated with other histologic differentiation was recorded. For newly diagnosed tumors, a representative tissue block of each case was selected for XMRV analysis, whereas the XMRV screening results of published cases were retrieved from our previous case-control outcome. The clinicopathological parameters of all cases were acquired from pathology reports.

Fifty newly diagnosed PCa tissues were prepared for DNA extraction in a separate laboratory free from contamination, followed by conventional end-point PCR. To authenticate a successful extraction β-Globin gene was amplified by conventional PCR. The same protocol was followed, as described in our previous case- control analysis.[13]

Briefly, for XMRV and β-Globin PCR; 20 μL PCR reaction mixture was prepared comprising of 5 μL sample, 10 μL 2x PCR master mix (Merck), 2 μL primers mix (XMRV; Norgen Biotek Corp. and β-Globin; PG04/GH20), and 3 μL nuclease free water. The XMRV PCR thermal profile was: 95°C for 3 minutes, 40 cycles of 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 45 seconds and final extension of 5 minutes at 72°C whereas, the PCR cycle used for β-Globin amplification were; 94°C for 5 minutes, and 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and 10 minutes final extension at 72°C.

The details of primers used in the study are given in Table 1. Cloned XMRV RNA fragments (Norgen Biotek Corp.) were used to generate XMRV cDNA stock for PCR controls. Agarose gel (2%) stained with ethidium bromide was used to run the amplified product. The amplified products were recognized on the basis of their representative fragment size.

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Statistical analysis

The one-sample t-test was used to determine differences between the means for XMRV infection versus continuous variables and noncontinuous clinicopathological variables. Statistical Package for the Social Sciences software package (IBM SPSS Statistics; Armonk, NY, USA) version 21.0 was used for all statistical analysis. P values of less than 0.05 were taken as cut-off for statistical significance.

Results

Based on morphological differentiation, 8 out of 100 cases (8%) were characterized as ductal adenocarcinoma and 91 cases (91%) exhibited morphological features of acinar adenocarcinoma; however one sample bearing mucinous morphological pattern was also identified in present research. Figure 1 compares histological features of XMRV positive ductal adenocarcinoma and other variants of PCa. No other variant of the disease was documented in our analysis. The clinicopathological characteristics are presented in Table 2. Majority of the patients with ductal adenocarcinoma were almost a decade younger than those with acinar adenocarcinoma (58.7 years vs. 69.3 years) and presented with a high -grade PCa upon diagnosis.

β globin DNA was amplified in all whereas, XMRV DNA in 4 of 100 cases. None of the newly diagnosed cases included in the present research were positive for XMRV DNA. XMRV DNA was detected in 4 of 8 tumors with ductal differentiation (p=0.03), whereas tumors exhibiting morphological features other than ductal characteristics were XMRV DNA-negative (Figure 2). The results of XMRV DNA detection in different histological tumor subtypes are summarized in Table 2.

The median ages of the patients with XMRV DNA-positive and XMRV DNA-negative tumors were 54.7 and 62.7 years, respectively. Thus, majority of ductal adenocarcinoma patients with XMRV infection had aggressive behavior and were younger than those without infection. However, no association between age (p=0.18) and tumor grade (p=0.05) with the presence of XMRV DNA in ductal tumors was detected.

Discussion

To the best of our knowledge, the present research is the first study comparing the association of XMRV with histopathologic characterization of PCa worldwide. We have determined that the frequency of each tumor subtype was similar to our previous

Table 1. Primers used for XMRV screening

Primer	Sequence / Description	Amplimer length
β-Globin		268bp
PG04	CAACTTCATCCACGTTCACC	
GH20	GAAGAGCCAAGGACAGGTAC	
XMRV	Catalog # 34710; ISO Certified (Norgen Biotek Corp.)	300bp

XMRV: Xenotropic murine leukemia virus related virus

Table 2. Clinicopathological data and XMRV DNA detection rates

Tumor	n	Gleason ≤7	Gleason >7	XMRV DNA positive
Acinar adenocarcinoma	91	43	48	0
Ductal adenocarcinoma	8	1	7	4†
Mucinous adenocarcinoma	1	0	1	0
Total	100	44	56	4

†p=0.03; t-test

XMRV: Xenotropic murine leukemia virus related virus

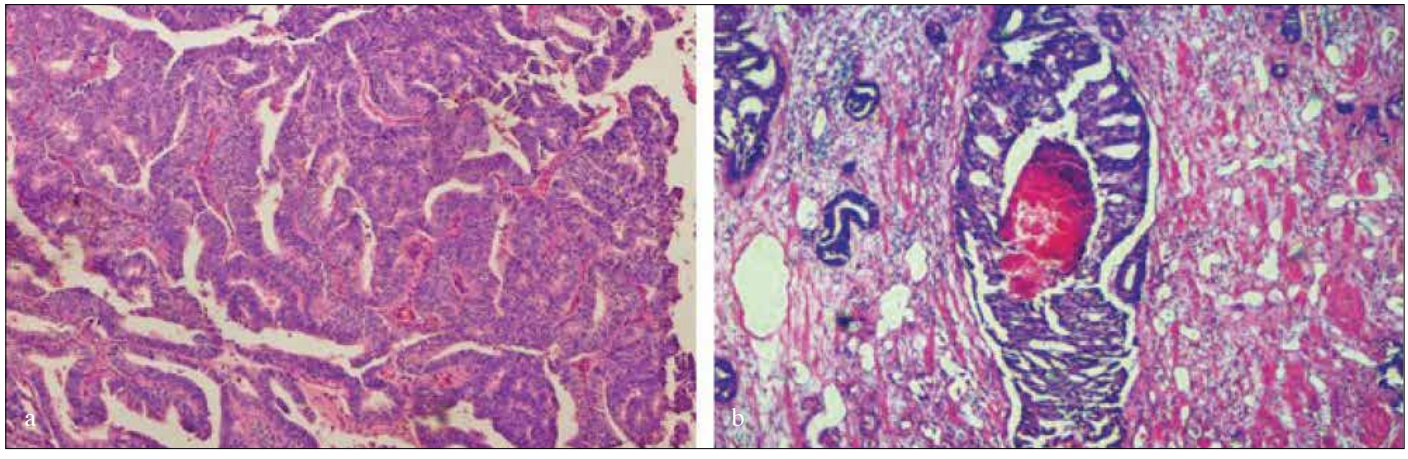


Figure 1.a, b. Xenotropic murine leukemia virus related virus (XMRV) positive tumor sections exhibiting (a) papillary architecture (b) cribriform pattern with central necrosis, 10 X magnifications

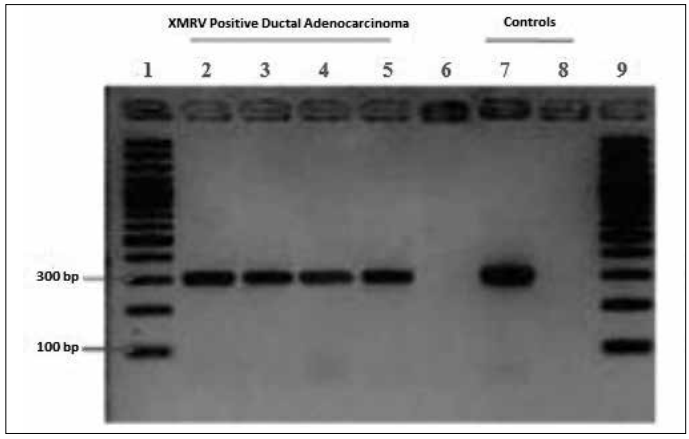


Figure 2. Representative gel of Xenotropic murine leukemia virus related virus (XMRV) PCR. Lanes 1 shows the 100 bp molecular weight ladder. Lanes 2 - 5 are results from XMRV positive ductal prostatic adenocarcinoma specimens. Lanes 7 - 8 are negative and positive controls respectively for XMRV PCR

finding and with the Western investigations, bearing predominance predominance of acinar (91%) subtype, followed by ductal (8%), and one case of mucinous (1%) tumor.[2-4] However, we have observed a high prevalence of XMRV infection in ductal tumors (50%). We also noted a marked preponderance of age <65 years in patients bearing XMRV infection (3 vs. 4) cases. To our knowledge, there is no comparable data on PCa subtype with XMRV infection.

An overall low prevalence of virus in the present research may be contributed by relative difficulty in detecting viral DNA in adenocarcinomas due to lower viral load in glandular lesions, compared to other tumors like squamous cell carcinomas. Premalignant and malignant lesions associated with viruses, in particular those other than adenocarcinoma contain integrated viral genome sequences, in addition to numerous episomal viral particles.[14] On the contrary, glandular epithelium in adenocarcinoma does not favor productive viral infection and viral DNA is usually present in only integrated form.[14] This might be the case in the present research which is attributed to integrated viral sequences with minimal replication. Therefore, the task of detecting XMRV DNA in PCa is challenging and requires the presence of intact DNA target sequences for successful amplification. Although, XMRV infections are coupled by integrating viral genome in host DNA via reverse transcription, the efficiency of XMRV detection may be reduced by two additional factors: 1) DNA fragmentation caused by formalin fixation and paraffin embedding; and 2) segmental breakup of the viral genome during integration containing PCR targeted sequence. In such cases, the intact episomal viral copies decide the outcome of PCR reaction. Thus, underestimation of XMRV prevalence in adenocarcinomas may be contributed by absence of episomal XMRV genome in glandular tumors.

On the other hand, predilection of XMRV for ductal adenocarcinoma observed in current study may be explained by several arguments. Firstly, Sharma et al.[15] reported transurethral infection of XMRV following sexual transmission in monkeys. As ductal tumors grow close to urethra; a favorable site of transurethral infections and inflammation,[16] the link between XMRV and ductal adenocarcinoma can be hypothesized. Secondly, in a previous study, XMRV infection was overwhelmingly reported in high grade malignant tumors, however the research was not targeted to access the viral status in tumor subtypes.[7] The finding may complement the aggressive morphological pattern observed in vast number of ductal adenocarcinomas. Thirdly, the contribution of XMRV in the development of ductal subtype of PCa may be related to its pathogenesis, yet to be established.

The role of XMRV in the development of PCa has remained controversial.[6] Lee et al.[11] concluded that XMRV is not a naturally acquired human infection but rather a contaminant by an XMRV-infected laboratory cell line. Schlaberg et al.[17] also shared the findings reported by Lee et al.[11] In contrast, our present and previous *in vitro* analysis did not include any cell line; instead tumor samples from patients were used for analysis. Additionally, all necessary measures to avoid contamination, discussed in our previous published case- control study, has been taken into account.[13] Interestingly, the sole prevalence of virus in a variant of tumor raises the possibility that virus might have been linked to a particular histological nature of tumor, which has not been investigated previously.

Because majority of tumors included in the current study is XMRV-negative, we suggest, two hypotheses which reconcile and explain the association of XMRV infection with adenocarcinoma of prostate. One is the hit-and-run theory, that is, the absence of XMRV infection in the negative cases may be attributed by defective viral genome or lack of integration of target viral sequence. The other possibility is the two-disease hypothesis, which proposes that adenocarcinoma seen in the ductal group have infectious etiology, whereas tumors other than ductal variety share other causes of the disease. Thus, we hypothesize two pathways for prostate tumorigenesis: one is XMRV associated and the other is XMRV-independent.

Present study has certain limitations. The unusual subtypes of PCa rarely exist and therefore we were unable to include more samples of ductal, mucinous and other rare varieties in current investigation. Furthermore, as the current study is based on the analysis of biopsy specimens, we were unable to correlate other variables such as clinical staging, PSA and patient's demographic profile. Moreover, due to lack of commercially available antibody, we were also unable to screen XMRV using immunohistochemistry, which would have provided us useful data for comparative analysis.

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In conclusion, we noted in this study that the distribution of each PCa subtype in our region is similar to that seen in Western countries. However, XMRV was present and equally distributed exclusively in ductal subtype of the disease. These findings support the hypothesis that XMRV may participate in the pathogenesis of one type of PCa, and the primary XMRV infection is a potential determinant of risk for ductal entity of PCa.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dow University of Health Sciences

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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