Iraqi Journal of Science, 2021, Vol. 62, No. 1, pp: 96-107
DOI: 10.24996/ijs.2021.62.1.9

ISSN: 0067-2904

Molecular Detection of Human Herpes Virus-8 in Prostatic Adenocarcinoma and Benign Prostatic Hyperplasia Tissues by DNA -In Situ Hybridization

Zainab Mahdi Saleh[1], Jinan Al-Saffar[1]*, Saad Hassan
[1]Department of Biotechnology, Collage of Science, University of Baghdad, Baghdad, Iraq
[2]Pathology Research Unit, College of Medicine, University of Baghdad, Baghdad, Iraq

Received: 13/3/2020 Accepted: 26/4/2020

Abstract

Human Herpes Virus-8 (HHV-8) is a sexually transmitted viral infection that can infect the prostate epithelium in immunocompromised adults. Recently, HHV-8 was related to the development and progression of several human malignancies like prostatic adenocarcinoma. This retrospective research was designed to analyze the distribution and possible impact of HHV-8 infection on prostatic adenocarcinogenesis. A total number of one hundred formalin-fixed prostatic tissues were enrolled in this research; forty Prostate Adenocarcinoma (PAC) biopsies, forty biopsies from Benign Prostatic Hyperplasia (BPH), and twenty Apparently Normal Prostatic Tissues (ANPT) as a control group. Detection of HHV -8 DNA was achieved by a highly-sensitive variant of Chromogenic In Situ Hybridization (CISH) technique. In this study, the mean age of PAC patients was 64.08±7.54years. Detection of CISH reactions for HHV 8- DNA was observed in tissues of 70% (28 out of 40) of PAC patients and 30% (12 out of 40) of BPH tissues, whereas no positive reactions were detected in the ANPT group. Detection of CISH reactions for HHV 8- DNA was observed in 55.6% of tissues of PAC patients with well grade histopathological examination, 87.5% of moderate grade, and 69.6% of poorly differentiated grade. It can be concluded that HHV-8 infection might contribute in prostate oncogenesis, together with other essential oncogenic viruses.

Keywords: HHV-8; Prostatic Adenocarcinoma; Benign Prostatic-Hyperplasia; Chromogenic In Situ Hybridization.

الكشف الجزيئي لراشح الحلأ البشري- 8 في انسجة مأخوذة من غدة البروستات واورام البروستات الحميدة بأستخدام تقنية التهجين الموضعي للحامض النووي (دنا)

زينب مهدي صالح[1]، جنان الصفار*[1]، سعد حسن[2]
[1]قسم التقنيات الاحيائيه، كلية العلوم ، جامعة بغداد، بغداد، العراق
[2]وحدة بحوث الامراض الانتقاليه ،كلية الطب ، جامعه بغداد، بغداد، العراق

الخلاصة

راشح الحلأ البشري النوع-8 يسبب عدوى تنتقل عن طريق الاتصال الجنسي . تصيب باستمرار الخلايا الطلائية المبطنة لغدة البروستات في الاشخاص البالغين الذين يعانون من نقص في المناعة. وجد في الآونة

*Email: jsjsjzhsb@gmail.com

الاخيرة ان راشح الحلأ البشري النوع −8 له ارتباط في خطوات تطور وتقدم عدد من الاورام السرطانية التي تصيب الانسان بما في ذلك سرطان غدة البروستات .صممت هذه الدراسة كبحث بأثر رجعي لتحليل التوزيع والتأثيرات المحتملة للإصابة براشح الحلأ البشري النوع −8 في سرطان غدة البروستات .وقد كان العدد الكلي في هذه الدراسة 100 عينة نسيجية أخذت من غدة البروستات،40 عينة نسيجية لمرضى شُخِّصَ لديهم سرطان غدي خبيث في انسجة غدة البروستات ،الى جانب 40 عينة نسيجية لمرضى شُخِّصَ لديهم التهابات مزمنة او تضخم في انسجة غدة البروستات و 20 عينة نسيجية استخدمت كشواهد وقد استخدمت بغرض التموضع الجزيئي للترددات المبكرة الكامنة لراشح الحلأ البشري النوع−8 في انسجة سرطان غدة البروستات اجيال فائقة التحسس من تقنية التهجين الموضعي اللوني للحامض النووي (دنا) . وقد توصلت الدراسة الى ان معدل عمر الاشخاص المصابين بسرطان غدة البروستات 64.08±7.54 سنة. .كما اظهرت الدراسة وجود لراشح الحلأ البشري النوع −8 في انسجة سرطان غدة البروستات 70%(28 من مجموع 40 قطعة نسيجية محفوظة) و في الانسجة المتضخمة لغدة البروستات 30%(12 من مجموع 40 قطعة نسيجية محفوظة) ولا يوجد هناك أي نموذج من أنسجة غدة البروستات ايجابية له .كما اظهرت الدراسة وجود لراشح الحلأ البشري النوع −8 في انسجة سرطان غدة البروستات ذات التمايز النسيجي الجيد وبنسبة , 55.6% ، وفي الانسجه ذات التمايز النسيجي المتوسط كانت % 87.5 و ذات التمايز النسيجي الفقير69.6%. يمكن الاستنباط من خلال الكشف عن راشح الحلأ البشري النوع الثامن صلته وتأثيره الملحوظ في تمايز أنسجة الاورام السرطانية لغدة البروستات و تسليط الضوء على الاصابة المبكرة لأنسجة البروستات المسرطنة براشح الحلأ البشري النوع الثامن ونسبه المتصاعده مرورا بدرجات التمايز النسيجي الاخرى ،دعما لنظرية اداء الراشح ودوره السببي ، المولد او/ و كعامل مرافق في احداثه.

Introduction

Worldwide, prostatic adenocarcinoma and benign prostatic hyperplasia are reported as the most common pathological conditions affecting male urothelial tissues, while their incidence was increasing annually [1-3]. Prostatic adenocarcinoma is ranked as the fifth most common male cancer and the second higher cause of cancer mortality in the United States and Europe, as well worldwide [4, 5, 2, 6]. Viral factors have been recognized as the most significant class of infectious agents linked to human cancers [7]. This was determined among all worldwide incidences of cancers, where 17-20% were attributed to viral etiology [8]. Among the DNA viruses, Herpesvirus is associated with Kaposi's sarcoma (human herpesvirus 8; HHV-8), which is a gammaherpesvirus implicated in many human cancers [9, 10, 11]. The first among the human herpesvirus-8-associated malignancies is Kaposi's sarcoma, while the role of this virus in causing cancers has been well-established [12]. Although the prostatic pathogenesis is still unclear, yet it was observed that both benign prostatic hyperplasia and prostatic adenocarcinoma share similar pathogenesis and both have their relations to either hormones and/or inflammation [10-13]. In addition, chronic inflammation of prostatic tissues was revealed as one of the risk factors in both these prostatic lesions [2, 3, 4, 7, 9-13]. An evidence-based knowledge was found to support a role for inflammatory responses in tumor microenvironment control. These inflammatory cells are indeed releasing growth factors and cytokines in the tumor microenvironment that promote angiogenesis and remodeling of the extracellular matrix. Also, more inflammatory cytokines were found to be released into the reactive stroma [14]. Human Herpes Virus 8 (HHV 8) infects various cell types, including macrophages, B-cells, and endothelial and epithelial cells. HHV 8 infection establishes latency in B cells and glandular epithelial cells. The seroprevalence rate in HHV 8 infection was found to differ among different geographical regions [15]. The HHV 8 can persistently infect adult prostate epithelium especially in those infected with Human Immunodeficiency Virus-1 (HIV-1) [16]. This research aimed at unraveling the percentage of HHV-8 infections in prostatic adenocarcinoma as well as benign prostate hyperplasia as compared to apparently normal prostatic tissue by using a recent version of Chromogenic In Situ Hybridization test. This study was designed to analyze the distribution and possible impact of HHV 8 infection in prostatic adenocarcinogenesis. The present results indicated that HHV 8 might participate in the development of a subset of prostatic tumors. A significant percentage of HHV 8 in prostate adenocarcinoma could shade light on the important role of viral factors in prostatic carcinogenesis.

Materials and Methods

Study population

In this study, one hundred samples of formalin-fixed, paraffin-embedded prostatic tissues were investigated. These tissue blocks were collected from the archives of histopathological laboratories of Ghazi Al-Hariri Teaching Hospital / Baghdad, Medical City Hospital Teaching Laboratories, and Al-Kindy Teaching Hospital. The tissue blocks were related to the archives of the past 3 years (i.e. 2017, 2018, and 2019) and are including 40 biopsies from PAC, 40 from BPH, and 20 from APNT subjects. The diagnoses were based on the accompanied pathological reports of the corresponding patients.

Chromogenic In Situ Hybridization (CISH)

The ultrasensitive version of CISH technique, detecting HHV 8-DNA, was employed. The research was planned as a background analysis. The related guidelines were followed for the treatment of these tissue blocks. A part of each paraffin-embedded tissue block was placed on an ordinary glass slide and stained with hematoxylin and eosin, in order to further validate its diagnosis. For other tissue slices, charged slides were fixed to be used to identify the ISH of HHV 8-DNA. Detection of HHV8-DNA by digoxigenin-labeled oligonucleotide probe was performed on 4μm tissue sections, included in ISH Chromogenic Zyto Vision detection kit (Zyto Vision GmbH., Fischkai, Bremerhaven, Germany). The slides were deparaffinized for the Chromogenic In Situ Hybridization method by placing them overnight in a hot-air oven at 60 °C,, then incubated twice in Xylene for 15 min. Treatment with graded alcohol (through 5 min in 100% of ethanol incubation, 2 times) was then performed. The slides were fully dried and incubated for 5 minutes at 37 ° C, followed by processing by the addition of proteinase K, and incubation for 15 minutes at 37 °C. The slides were dehydrated by immersing them serially in different solutions for specified times below room temperature; distilled water for one minute, 70 % ethanol for one minute, 95 % ethanol for one minute, and 100 % ethanol for 5 minutes at 37° C. Then, 20 μl cDNA probe was added to each portion and the slides were carefully covered with coverslips so that no air bubbles are detected. The DNA was denatured after the slipping of the cover in a preheated oven at 95 °C for 8-10 minutes. The slides were taken to the pre-hot humid hybridity chamber and incubated at 37 °C overnight. During hybridization and staining, the slides were not allowed to dry at any time. Both hybridization and detection reagents were warmed up to normal temperature. On the following day, the slides were submerged in preheated protein blocks at a temperature of 37 °C, before the coverslips were cut off and no tear tissue was taken care of. The slides were placed in the buffer at 37 ° C for 3 minutes after the removal of the cover. Tissue sections were coated with streptavidin-alkaline phosphatase. Next, the slides were held in a humid chamber at 37 ° C for 20 minutes and rinsed and drained in a wash buffer for (5) minutes. Following this, 1-2 drops of 5-bromo3-chloro3-indole / phosphate / nitro-tetrazolium substrate-solution (BCIP / NBT) were added to the tissue section. The slides were incubated at 37 ° C for 30 minutes, or until the color production was finished. The advancing of color was controlled by a microscopic viewing of the slides. Deep blue precipitates were developed at the complementary site of the probe in positive cells. The slides were rinsed for 5 minutes in sterile water and exposed to Nuclear Fast Red stain for 30 seconds. After washing, the slides were submerged in the purified water for 1 minute. Then the sections were dehydrated with ethyl alcohol (95%, once a min, twice for two min, 100%) and cleansed of Xylene. The next permanent medium assembled (DPX). Dewax protocol was then used regularly, e.g. 15 min xylene (twice), 5 min 100% ethanol (twice), 5 min 96% ethanol (once), 5 min 70% ethanol(once), and lastly soaking into 5 minutes of distilled water to eliminate any residual alcohol.

Analysis

According to the kit specifications, for proper use of the detection system of CISH by light microscope, the positive tissues of the hybridization probe showed a strong blue signal at some locations. The signal was analyzed using 100 X lens to count the positive cells. CISH tests were calculated by intensity and percentage based on the positive signal strength and cell size, respectively. The positive cells for each sample were counted in 10 separate 100 cell fields and the average percentage of positive cells was measured in the 10 fields. A scale of 0-3 was used for the relative power, with 0 being equal to non-detectable CISH reactivity and I, II, and III equal to weak, moderate, and strong reactivity, respectively. Cases were assigned to each group of scores as follows: 1-25% (Score I), 26-50% (Score II) and > 50% (Score III) [17]. Statistical analysis was conducted

and Chi-squared analysis was employed in this study by the SPSS program (version–23) and differences were considered significant when $P < 0.05$.

Results and discussion

The mean age of prostatic adenocarcinoma patients was 64.08±7.54 years , while that of patients with benign prostatic hyperplasia was 67.53±9.77 years and of healthy control group was 57.05±9.65 years . The statistical analysis showed highly-significant differences ($P \leq 0.01$) among the groups, as shown in Table-1.

Table 1-The distribution of the studied groups according to age**.**

Studied groups	No.	Mean age/ years	Std. Deviation	Std. Error	Range Age/ years		*p* value
					Minimum	Maximum	
PAC	40	64.08	7.54	1.19	48	75	Highly-significant0.0001**
BPH	40	67.53	9.77	1.54	48	85	
Control	20	57.05	9.65	2.16	43	77	
Total	100						

The present research, up to our knowledge, is the first attempt to study the eighth type of herpesviruses (HHV-8) in Iraqi male patients with invasive prostatic adenocarcinoma as well as benign prostatic hyperplasia tissues. This study is aiming to assess whether human herpesvirus type 8 is associated with such male genital tract neoplasms. The present results of the enrolled 40 archival tissue blocks are related to those patients who have previously prostatectomies for an invasive prostatic adenocarcinoma, as well as to other 40 counterpart tissues of men who have been biopsied for benign prostatic hyperplasia, and compared to 20 control tissues. The results revealed that the age of the prostatic tumor patients ranged from 48 to 85 years while the mean age for the studied prostate adenocarcinoma patients was 64.08±7.54 years. These findings are consistent with the age of the vast majority of prostate adenocarcinoma males which were included in the 2018 Global Cancer Statistics [18]. These findings are also consistent with the worldwide age presentation of invasive prostatic adenocarcinoma patients with an age range of 50-80 years and an average age of 59.3 years, as it is rare for younger men to experience either in situ adenocarcinoma or invasive adenocarcinoma. [19]. Table-2 shows the distribution of the age strata by the histopathological diagnosis of the sample group. It can be observed that the age stratum of the patients primarily affected by the prostatic disease is 61-70 years (45 %, 18 cases), followed by the age strata of 51-60 years (32.5%, 13 cases), > 70 years (20%, 8 cases), and 40-50 years (2.5 %, 1 case). Regarding the patients with benign prostatic hyperplasia, the most affected age group was 61-70 years (40%, 16 cases), followed by >70 years (35%, 14 cases), 51-60 years (17.5%, 7 cases), and 40-50 years (7.5%, 3 cases). Regarding the age of the included control group of apparently healthy prostatic tissue individuals, the most affected age group was 40-70 (30%, 18 cases), followed by >70 years (10%, 2 cases). The statistical analysis showed highly-significant differences ($P<0.01$) in the distribution of age strata, while a non-significant difference was found between the groups of benign prostatic hyperplasia and prostatic cancer.

Table 2-Age strata according to histopathological diagnosis of studied groups

Age (year) / Group	40-50 No. (%)	51-60 No. (%)	61-70 No. (%)	>70 No. (%)	Chi-squared [a]	*P*-value	Sig.
PAC (No. 40)	1 (2.5%)	13 (32.5%)	18 (45%)	8 (20%)	15.8	0.001	**
BPH (No. 40)	3 (7.5%)	7 (17.5%)	16 (40%)	14 (35%)	11	0.012	*
Control (No. 20)	6 (30%)	6 (30%)	6 (30%)	2 (10%)	2.4	0.494	NS

Chi-Square [b]	**X^2**	***P*-Value**	**Sig.**
Control vs. BPH	8.95	0.03	*
Control vs. PC	10.219	0.17	*
BPH vs. PC	4.554	0.208	NS

Also, in reviewing Table-2, it was noticed that the percentages of PAC cases are increased with the increase of the age of patients. The present results could have their importance when realizing that the age of prostate cancer patients is an important factor both in the occurrence and management of this disease [20]. These findings may indicate that age is a significant risk factor in tumor changes that are affecting lesions of the prostate epithelial tissues. Overall, aging raises the incidence of malignant changes in prostatic epithelial tissues and their incidence with age has been found to rise [21]. This indicates that these two lesions are affecting the group of old-age Iraqi patients, which is consistent with earlier results [22]. Prostate cancer is the sixth most common cancer in the world and the third major cause of male cancer. Prostate cancer occurs in one in nine males over 65 years of age in the developing countries. The incidence is increased by about almost 60 percent of people over 65 years of age [23].

Nevertheless, the profile of prostate cancer among the Arab population is defined by low frequency (WHO study 1997). It accounts for only 0.99 percent of all malignant tumors in males in Iraq, according to the Iraqi Cancer Registry in 2012 [24]. The possible explanation for such differences can be multifactorial, where genetic factors have been involved. The incidence of prostate cancer also differs among populations that are ethnically similar but live in different locations. Nutritional and hormonal factors have also been related to the changes in the risk of prostate cancer. Environmental factors may also play a role in such an issue [25].

These factors were collectively supported by various aging theories which claimed that the genetic material, i.e. DNA, can be easily oxidized and greatly influence the predetermined propensity towards certain types of physical functioning which control the rate at which this damage can be accumulated. The influencing factors can include the diet, lifestyle, toxins, pollution, radiation and other external influences [26]. The range and mean age findings of this analysis are consistent with the results obtained by a previous study [27]. The results of the mean age of Iraqi prostate cancer patients included in the present study are also in line with the findings of other works [28-30], which reported values of 67.4 , 63.1, and 69.87 years, respectively. In addition the actual median age outcomes of Iraqi BPH patients were also fairly consistent with other earlier findings [31, 28, 30], which found values of 70, 63.7, and 68.15years, respectively. These findings indicate that the aged group of Iraqi patients was affected by these prostatic lesions. The data also indicate that most of the herein studied Iraqi patients with prostatic tumors (benign and malignant) were in the age group of 55-65 years.

The findings presented are fairly consistent with other studies that showed that prostate cancer has a high incidence in this age group [31]. This is reinforced by reports elsewhere that have found prostate cancer as a disease affecting some elderly men who are generally over 50. Seventy-

five percent of patients with this diagnosis are between 60-80 years of age [32]. In addition, benign prostatic hyperplasia is a common condition in men over 50 years old[32,33].
The small variation from the current study may also be explained by the sample size that is not represe nting the entire population. This may also be linked to those patients who postpone their medical exam ination , as some of them typically seek medical advice after complications occur.

Grading of prostatic cancer with HHV-8 expression

Table-3 shows that the results of this study indicate that the total percent of men who have prostatic adenocarcinoma and infected with HHV-8 is 55.6% (5/9).Whereas 87.5% (7/8) of tissues had moderately differentiated grades, and 69.6% (16/23) had poorly differentiated grades .

Table 3-Association between the grade of prostatic adenocarcinoma and HHV-8 expression.

Grade of Prostatic adenocarcinoma (No. 40) / Expression of HHV-8	Well differentiation No. (%)	Moderate differentiation No. (%)	Poor differentiation No. (%)	Total	Chi-squared [a]	*P*-value	Sig.
Positive	5 (55.6%)	7 (87.5%)	16 (69.6%)	28 (70%)	2.063	0.356	NS
Negative	4 (44.4%)	1 (12.5%)	7 (30.4%)	12 (30%)			
Total	9 (100%)	8 (100%)	23 (100%)	40 (100%)			

Since the variables of the grading system (due to its subjective assessment), Nottingham The most popular and dependent grading system used is Scarf Bloom-Richardson (SBR) system modification, based on the tubular shape, pleomorphic nuclear and mitotic figures [34]. This study was also dependent on this common grading system for fixing or recording the histopathological grades of our prostatic adenocarcinoma series of tissues [35]. Prostatic HHV-8 infection may pose a risk of progression to the imitation transcriptional profile of differentiated prostate tumors. Findings derived from these efforts would be useful in achieving the important aim of isolating specific cancer biomarkers, which will then direct the creation of successful therapies or methodologies for improved detection and stratification of potentially high- cases of prostate cancer [36].

DNA –CISH signal detection of the HHV-8

Table-4 shows positive results of HHV-8 DNA-CISH signal detection, where 28/40 prostatic adenocarcinoma tissue (70%) showed positive blue nuclear signals at sequence complementary sites. Furthermore, 12 out of 40 benign prostatic hyperplasia tissues (30 %) showed positive signals, while no positive signals for the HHV-8 DNA –CISH test were present in healthy prostatic tissues. Of the positive cases, 4/28 (10%) adenocarcinoma tissues had score 1, while score II was revealed in 19/28 (47.5%)and score III in 5/28 (12.5%). Benign prostatic hyperplasia showed positive signals in 7.5% (3/12) of patients with score I, 15% (6/12) with score II, and 7.5% (3/12) with score III. The scoring and strength scoring of positive DNA– CISH blue HHV-8 signals of prostatic adenocarcinomas, in contrast to the negative CISH microscopic presence in the healthy tissue, are illustrated in prostatic adenocarcinoma tissue (Figures-(1 to 6).

Table 4-Signal scoring of HHV-8 DNA-CISH detection among prostatic adenocarcinoma tissues

Score / Studied group	Negative No. (%)	Positive No.(%)	I No. (%)	II No. (%)	III No. (%)	Chi-squared [a]	*P*-value	Sig.
PAC (No. 40)	12 (30%)	28(70%)	4 (10%)	19 (47.5%)	5 (12.5%)	14.6	0.002	**
BPH (No. 40)	28 (70%)	12(30%)	3 (7.5%)	6 (15%)	3 (7.5%)	43.8	<0.0001	**
Control (No. 20)	20 (100%)	0(0%)	0 (0%)	0 (0%)	0 (0%)	-	-	-

Chi-Square [b]	X^2	*P*-Value	Sig.
Control vs. BPH	7.5	0.58	NS
Control vs. PC	26.25	<0.0001	**
BPH vs. PC	13.803	0.003	**

In Figures- 1 to 6 and Table-5, the signal intensities of the detected HHV-8-DNA-CISH signal are demonstrated. Positive signal intensities in the prostatic adenocarcinoma group showed high signal intensities in 35% (14/28), while 22.5% (9/28) had moderate intensities and 5/28 (12.5%) had weak intensities. In benign prostatic hyperplasia, the positive high signal of intensity was revealed in 7.5% (3/12), with 20% (8/12) showing a moderate signal intensity and 2.5% (1/12) a weak intensity.

Table 5-Grade of HHV-8 DNA –CISH signal intensities in prostatic adenocarcinoma tissues

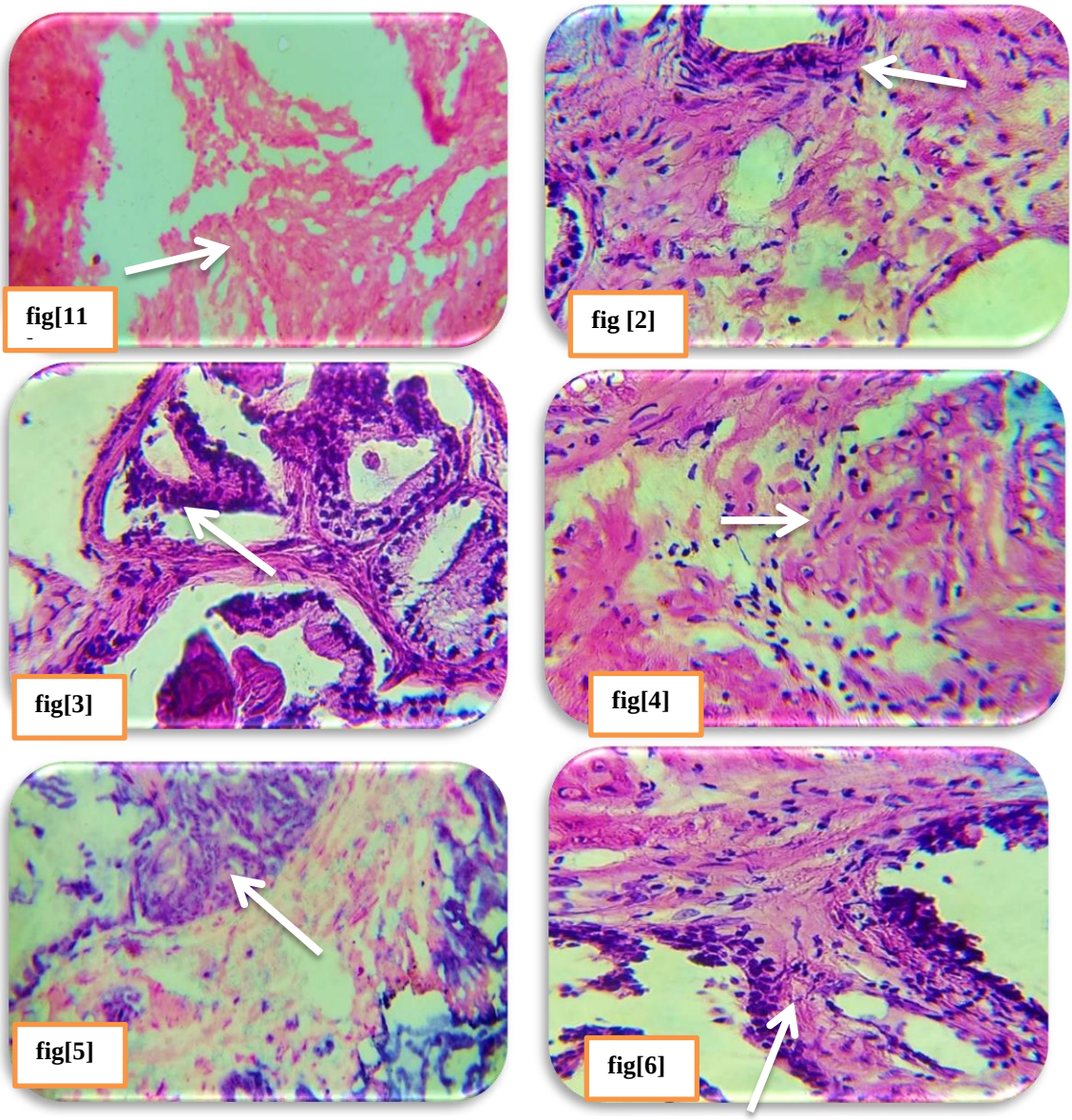
Intensity / Group	Negative No. (%)	Weak No. (%)	Moderate No. (%)	High No. (%)	Chi-squared [a]	*P*-value	Sig.
PC (No. 40)	12 (30%)	5 (12.5%)	9 (22.5%)	14 (35%)	4.6	0.204	NS
BPH (No. 40)	28 (70%)	1 (2.5%)	8 (20%)	3 (7.5%)	45.8	<0.0001	**
Control (No. 20)	20 (100%)	0 (0%)	0 (0%)	0 (0%)	-	-	-

Chi-Square [b]	X^2	*P*-Value	Sig.
Control vs. Benign	7.5	0.58	NS
Control vs. Malignant	26.25	<0.0001	**
Benign vs. Malignant	16.243	0.001	**

Table-6 shows the results of the positive –HHV-8 DNA –CISH screens for the histopathologic stage of prostatic adenocarcinomas tested. In the case of adenocarcinoma is distinct, 3/9 (33.4%) of the score I and 2/9 (22.2%) of score II patients revealed positive blue nuclear signals for HHV-8. Also, 12.5% (1/8), 50% (4/8) and 25% (2/8), respectively, of score I, II and III patients among moderately differentiated groups revealed positive-HHV-8 results. As for patients with low grade of differentiation, 56.5% (16/23), 13% (13/23), and 3/23 (of the patients belonged to scores I, II and III, respectively .

Table 6-Association of the HHV-8 signal scoring distinction of prostatic adenocarcinoma with DNA CISH**.**

Prostate cancer		HHV-8 Signal Scoring				Total
		Negative	I	II	III	
Grade	Well	4 (44.4%)	3 (33.4%)	2 (22.2%)	0 (0%)	9 (100%)
	Moderate	1 (12.5%)	1 (12.5%)	4 (50%)	2 (25%)	8 (100%)
	Poor	7 (30.4%)	0 (0%)	13 (56.5%)	3 (13%)	23 (100%)
Total		12 (30%)	4 (10%)	19 (47.5%)	5 (12.5%)	40 (100%)
Chi-Square		X^2		*P*-Value		Sig.
		12.44		0.053		NS



Microscopic involvement in prostatic adenocarcinoma epithelial tissues for positive reactions of HHV-8-DNA-CISH; NBT / BCIP stained (blue signals) and by Nuclear Fast Red (red signals) as counter-stain.
Figure-[1]: Negative Signal Score(400X) .
Figure[2]: high signal intensities with signal score III (400X).
Figure [3]: moderate signal intensities and signal score III (400X).
Figure[4]: moderate signal intensities and score II (400).
Figure [5]: high signal intensities and signal score I (400X).
Figure [6]: high signal intensities and signal score II (200X).

The results demonstrated significantly high percentage of identification of human herpes virus 8 DNA in the PAC patients group (70 percent) relative to that in BPH patients and control groups. Since the elevated levels of PSA have been well correlated to prostatic adenocarcinoma and since such prostatic carcinomas in the present study have shown significant association with HHV-8, therefore such findings are in turn consistent with Henning's study [37] who found a significant association between HHV-8 and increased serum PSA levels. In a patient-centered age, HHV-8 serology tests will take into account men with high PSA serum to determine whether HHV-8 is responsible for high PSA. A cause of HHV-8 is unknown to the practice of oncogenic effects in a single patient but may be associated with co-factors [38]. It is believed that the prostate is a host for many viral and other infectious agents that have some oncogenic potential. Besides, the present study revealed that HHV-8

might be linked to initial or further growth of prostatic adenocarcinoma. In combination with other co-factors, HHV-8 could exert its oncogenic effects in the occurrence of prostatic cancer. HHV-8 has been listed by the International Agency for Research on Cancer (IARC) as a group I carcinogen because of its oncogenic potential to public health [39, 40]. Research related to the spread of HHV-8 infection in prostatic adenocarcinoma is not only scarce but also contentious [41, 42]. The definition of the relationship between the HHV-
8 genome sequences and the tissues of prostate adenocarcinoma and the immortalization by HHV-
8 of primary epithelial cells [42]. The discrepancies between the present and previous findings could be linked to the PCR criteria as the most efficient DNA amplification technique for viral DNA detection in situ, such that PCR can potentially detect only one viral DNA particle in a tissue segment. Some tissues with negative outcomes from the latest in situ hybridization study do not have a sufficient number of copies of this virus to be identified and may have more positive outcomes by using PCR. In this current study, the direct in situ hybridization kit used to detect HHV-8 DNA showed the presence of viral DNA within the nucleus of these prostatic malignant cells in most of the cases tested. Also, since this virus appears to have an oncogenic role in the development of other malignancies, it is strongly suggested that the tissues studied were mostly incorporated into the virus. This HHV-8 is likely to be sexually transmitted, although it is not known if HHV-8 can replicate in the male genital tract [42]. Although HHV-8 is well recognized as a lymph-tropical and lymph-cryptic virus, it is increasingly apparent that HHV-8 may have an epithet-tropical tendency, as supported by the proven HHV-8 associations with gastric cancers [18, 35]. A suggestion along with that connection is that this virus may have the unique oncogenic potential within prostatic epithelial cells to play a role in the etiology or pathogenesis of prostatic carcinoma. The risks associated with the age of HHV-8 infection in the general populations revealed a pattern in the increased prevalence of HHV-8 antibodies to Orf 65 antigen and HHV-8 seropositivity to Latency-Associated nuclear antigen (LANA) with increased age in HIV negative people in Switzerland [43, 44]. Herein, the prevalence of Orf 65 antigen antibodies increased from 15 to 23 percent in those under 30 years of age and then increased to 50 percent over the next three decades of age. In Hungary, similar effects were observed [45]. As age went up every decade later, the distribution of seropositivity to LANA has also increased markedly and moderately. Also, equally close association with Orf-65 peptide reactivity was observed by another report [44]. Increased antibody response against HHV-8 lytic antigens has progressed in Taiwan, beginning with 3 percent in children under five years of age and peaking between 31 and 40 years of age (19.2 percent) [46,47]. Far more similar outcomes are seen in Africa [48], Sardinia, and Italy [49]. However, other studies [50, 51] suggested this virus to be most likely a non-sexual transmission pathway of HHV-8, as it has also been shown that children worldwide can be infected with HHV-8 . Such findings are consistent with the effect of HHV-8 infection on the age of the studied prostate adenocarcinoma as well as benign prostatic hyperplasia patients. In addition to the risk factors associated with the transmission of HHV-8, a limited and incomplete understanding of how HHV-8 is transmitted among these populations has placed a burden on the correlation of diagnostic test results with true HHV-8 infection. The existing findings are consistent with the results of Henning [35]. These results showed that prostate cancer triggers a T-helper 2 antitumor response with a suppressed T-Helper 1 response. Human herpesvirus 8 infection results in a similar immune response that supports the hypothesis that human herpesvirus 8 develops a chronic infection, in a study sample from Tobago, that can lead to an immune response that promotes the development and survival of prostate cancer. On the other hand, it may also be inferred that such HHV-8 infections could be associated with both the initiation and development of prostatic cancers, but in the latter stages of this cycle, they could exert an increased risk.

However, the lack in the present study of detailed knowledge of clinical as well as histopathological records associated with these prostatic tissue specimens leaves several questions to be answered in further studies; How does this HHV-8 initially infect these cervical tissues? Is this virus incorporated in those malignant cells or in an active or latent phase and what exactly is its role in prostatic adenocarcinogenesis? In order to incriminate HHV-8 in prostatic adenocarcinoma pathogenesis and clarify issues related to many biological aspects of HHV-8 in prostate cancer, future research should be conducted to determine if such viruses can directly transform prostatic epithelial cells and if their mechanisms for infection is sexually hematogenic. Thus, broader studies are necessary to identify the risk of HHV-8 infections and their exact initiating or assisting roles in

prostatic adenocarcinogenesis. Furthermore, researchers need to investigate the involvement of other pathogenic viral infections in prostatic carcinogenesis.

References

1. Jemal, A. et al. Cancer statistics, **2009**. *CA Cancer J Clin*. **59**: 225–249 (2009).
2. Heidenreich, A. et al. **2014**. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate. *cancer. Eur Urol*. **65**: 467–479 (2014).
3. Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D. M., Piñeros, M. and Bray, F. **2019**. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International journal of cancer*, **144**(8): 1941-1953.
4. Ferlay J EM., Lam F., Colombet M., Mery L., Pineros M., Znaor A., Soerjomataram I, et al. **2019**. Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, Accessed 02 February 2019.
5. Dillner, J. et al. **1998**. Sero-epidemiological association between human-papillomavirus infection and risk of prostate cancer. *Int J Cancer*, **75**: 564–567 (1998).
6. Cornford, P., Bellmunt, J., Bolla, M., Briers, E., De Santis, M., Gross, T. and van der Poel, H. G. **2017**. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *European urology*, **71**(4): 630-642.
7. Dennis, L. K., Lynch, C. F. and Torner, J. C. **2002**. Epidemiologic association between prostatitis and prostate cancer. Urology 60, 78–83(2002).
8. Jiang, J. et al. **2013**.The role of prostatitis in prostate cancer: meta-analysis. *Plos One*, **8**: e85179.
9. Sutcliffe, S. et al. **2006**. Gonorrhea, syphilis, clinical prostatitis, and the risk of prostate cancer. *Cancer Epidemiol Biomark Prev*. **15**: 2160–2166 (2006).
10. Orem, J. **2019**. Cancer prevention and control: Kaposi's sarcoma. *Ecancermedicalscience*, **13**.
11. Nelson, W. G., De Marzo, A. M. and Isaacs, W. B. **2003**. Prostate cancer. *N Engl J Med*. **349**: 366–381 (2003).
12. Auten, M., Kim, A. S., Bradley, K. T. and Rosado, F. G. **2017**. Human herpesvirus 8-related diseases: Histopathologic diagnosis and disease mechanisms. *In Seminars in diagnostic pathology*, **34**(4): 371-376). WB Saunders.
13. Taylor, M. L., Mainous, A. G. and Wells, B. J. **2005**. Prostate cancer and sexually transmitted diseases: a meta-analysis. *Fam Med*. **37**: 506–512.
14. Sutcliffe, S. et al. **2006**. Gonorrhea, syphilis, clinical prostatitis, and the risk of prostate cancer. *Cancer Epidemiol Biomark Prev*. **15**: 2160–2166 (2006).
15. Olsson, J., Kok, E., Adolfsson, R., Lövheim, H. and Elgh, F. **2017**. Herpes virus seroepidemiology in the adult Swedish population. *Immunity & Ageing*, **14**(1): 10.
16. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. **2018**. Global cancer statistics 2018:.GLOBOCAN estimates of incidence and mortality worldwide for 36 can-cers in 185 countries. CA Cancer J Clin., **8**(6): 394-424.
17. Blancato J., Singh B., Liu A., Liao DJ. and Dickson RB. **2004**. Correla¬tion of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridization and im¬munohistochemical analyses. *British Journal of Cancer* (2004), **90**: 1612 – 1619.
18. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A. and Jemal, A. **2018**. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, **68**(6): 394-424.
19. Fajgenbaum, D. C., van Rhee, F. and Nabel, C. S. **2014**. HHV-8-negative, idiopathic multicentric Castleman disease: novel insights into biology, pathogenesis, and therapy. Blood, *The Journal of the American Society of Hematology*, **123**(19): 2924-2933.
20. Nzivo, M. M., Lwembe, R. M., Odari, E. O., Kang'ethe, J. M. and Budambula, N. L. M. **2019**. Prevalence and Risk Factors of Human Herpes Virus Type 8 (HHV-8), Human Immunodeficiency Virus-1 (HIV-1), and Syphilis among Female Sex Workers in Malindi, Kenya. Interdisciplinary Perspectives on Infectious Diseases, 2019.
21. Gaglia, M. M. and Munger, K. **2018**. More than just oncogenes: mechanisms of tumorigenesis by human viruses. *Current opinion in virology*, **32**: 48-59.
22. Wagenlehner, F. M. E. et al. **2007**. The role of inflammation and infection in the pathogenesis of prostate carcinoma. *BJU Int*. **100**: 733–737(2007).

23. Cheng, I. et al. **2010**. Prostatitis, sexually transmitted diseases, and prostate cancer: the California Men's Health Study. Plos One 5, e8736(2010).
24. Sfanos, K. S., Yegnasubramanian, S., Nelson, W. G. and De Marzo, A. M. **2018**. The inflammatory microenvironment and microbiome in prostate cancer development. *Nature Reviews Urology*, **15**(1): 11-24.
25. Afdal, A., Darwin, E., Yanwirasti, Y. and Hamid, R. **2019**. The Expression of Transforming Growth Factor Beta-1 and Interleukin-6 on Human Prostate: Prostate Hyperplasia and Prostate Cancer. *Open Access Macedonian Journal of Medical Sciences*, **7**(12): 1905.
26. Galdiero MR, Bonavita E, Barajon I, Garlanda C, Mantovani A. and Jaillon S. **2013**. Tumor associated macrophages and neutrophils in cancer. *Immunobiology*. **218**(11): 1402-1410
27. Khalaf E J. **2009**. Detection of human cytomegalorims and P53 tumor suppressor gene in prostatic carcinomas using in situ techniques M.Sc. Thesis College of Medicine of Baghdad University.
28. Achouri, Y., Noël, G., Vertommen, D., Rider, M. H., Veiga-Da-Cunha, M. and Van Schaftingen, E. **2004**. Identification of a dehydrogenase acting on D-2-hydroxyglutarate. *Biochemical Journal*, **381**(Pt 1): 35– 42. https://doi.org/10.1042/BJ20031933.
29. Karaipekli, A., Sarı, A. and Kaygusuz, K. **2007**. Thermal conductivity improvement of stearic acid using expanded graphite and carbon fiber for energy storage applications. *Renewable Energy*, **32**(13): 2201-2210.
30. Al-Badri, Z. M., Som, A., Lyon, S., Nelson, C. F., Nüsslein, K. and Tew, G. N. **2008**. Investigating the effect of increasing charge density on the hemolytic activity of synthetic antimicrobial polymers. *Biomacromolecules*, **9**(10): 2805-2810.
31. Guo, J., Kitamura, T., Ebihara, H., Sugimoto, C., Kunitake, T., Takehisa, J. and Taguchi, F. **1996**. Geographical distribution of the human polyomavirus JC virus types A and B and isolation of a new type from Ghana. *Journal of General Virology*, **77**(5): 919-927.
32. Syed A. Hoda Rana S. Hoda. **2007**. Rubin's pathology :Clinicopathologic Foundations of Medicine, 5th edition. *AMA The Journal of the American Medical Association*, **298**(17): 2073-2074
33. Umtergasser G. **2005**. Benign prostatic hyperplasia , age related tissue –remodeling. *Exp Gerontol*, **40**: 121.
34. Inamura, K. **2018**. Prostatic cancers: Understanding their molecular pathology and the 2016 WHO classification. Oncotarget**, 9(**18), 14723..
35. Marshall, C. H., Fu, W., Wang, H., Baras, A. S., Lotan, T. L. and Antonarakis, E. S. **2019**. Prevalence of DNA repair gene mutations in localized prostate cancer according to clinical and pathologic features: association of Gleason score and tumor stage. *Prostate cancer and prostatic diseases*, **22**(1): 59-65.
36. Jha, H. C., Banerjee, S. and Robertson, E. S. **2016**. The role of gammaherpesviruses in cancer pathogenesis. *Pathogens*, **5**(1): 18.
37. Henning, J. D., Karamchandani, J. M., Bonachea, L. A., Bunker, C. H., Patrick, A. L. and Jenkins, F. J. **2017**. Elevated Serum PSA is Associated With Human Herpesvirus 8 Infection and Increased Circulating Cytokine Levels in Men From Tobago. *The Prostate*, **77**(6): 617–624. https://doi.org /10.1002/pros.23308.
38. Mygatt, J. G., Singhal, A., Sukumar, G., Dalgard, C. L., & Kaleeba, J. A. **2013**. Oncogenic herpesvirus HHV-8 promotes androgen-independent prostate cancer growth. *Cancer research*, **73**(18): 5695-5708.
39. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, D. J. **2012**. Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology (pp. 133-164). Springer, Basel.
40. Sripa, B., Brindley, P. J., Mulvenna, J., Laha, T., Smout, M. J., Mairiang, E. and Loukas, A. **2012**. The tumorigenic liver fluke Opisthorchis viverrini–multiple pathways to cancer. *Trends in parasitology*, **28**(10): 395-407.
41. Tsai, S. Y. **2005**. U.S. Patent Application No. 29/211,440.
42. Naranatt, P. P., Krishnan, H. H., Svojanovsky, S. R., Bloomer, C., Mathur, S. and Chandran, B. **2004**. Host gene induction and transcriptional reprogramming in Kaposi's sarcoma-associated

herpesvirus (KSHV/HHV-8)-infected endothelial, fibroblast, and B cells: insights into modulation events early during infection. *Cancer research*, **64**(1): 72-84.

43. Abid, A. H., Mohammad, E. J. and Abdulrazaq, A. A. **2017**. Histopathological findings of prostatic autopsy in a sample of over 50 years old men in Baghdad, Iraq. *International Surgery Journal*, **4**(11): 3606-3610.

44. Regamey, N., Tamm, M., Wernli, M., Witschi, A., Thiel, G., Cathomas, G. and Erb, P. **1998**. Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. *New England Journal of Medicine*, **339**(19): 1358-1363.

45. Feiterna-Sperling, C., Königs, C., Notheis, G., Buchholz, B., Krüger, R., Weizsäcker, K. and Krüger, D. H. **2016**. High seroprevalence of antibodies against Kaposi's sarcoma-associated herpesvirus (KSHV) among HIV-1-infected children and adolescents in a non-endemic population. *Medical microbiology and immunology*, **205**(5): 425-434.

46. Beggs, P. J. and Bambrick, H. J. **2005**. Is the global rise of asthma an early impact of anthropogenic climate change?. *Environmental health perspectives*, **113**(8): 915-919.

47. Huang LM, Huang SY, Chen M, Chao M, Lu CY, Tien HF, Lee CY. and Jeang KT. **2000**. Geographical differences in human herpes virus 8 seroepidemiology: a survey of 1,201 individuals in Asia. J Med Virol. 2000;60:290–293. doi: 10.1002/(SICI)1096-9071(200003)60:3<290::AID-JMV7>3.0.CO;2-G. [PubMed][CrossRef] [Google Scholar].

48. Fowowe, B. **2017**. Access to finance and firm performance: Evidence from African countries. *Review of development finance*, **7**(1): 6-17.

49. Edelman D. C. **2005**. Human herpesvirus 8--a novel human pathogen. *Virology journal*, **2**: 78. https://doi.org/10.1186/1743-422X-2-78.

50. Dilnur P, Katano H, Wang ZH, Osakabe Y, Kudo M, Sata T, Ebihara Y. **2001**. Classic type of Kaposi's sarcoma and human herpesvirus 8 infection in Xinjiang, China. Pathol Int. **51**: 845–852. doi: 10.1046/j.1440-1827.2001.01293.x. [PubMed] [CrossRef] [Google Scholar]

51. Tangen, C. M., Goodman, P. J., Till, C., Schenk, J. M., Lucia, M. S. and Thompson Jr, I. M. **2016**. Biases in recommendations for and acceptance of prostate biopsy significantly affect assessment of prostate cancer risk factors: results from two large randomized clinical trials. *Journal of Clinical Oncology*, **34**(36): 4338.