EVALUATING THE RELATIONSHIP BETWEEN HUMAN PAPILLOMA VIRUS INFECTIONS, PROSTATE CANCER AND INTERLEUKIN-12 LEVELS

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Abstract

Prostate cancer (PC); is the second leading cause of cancer mortality among men. Human papillomavirus (HPV) is the most common cause of cervical cancer, strongly associated with anal and vaginal cancers. Also, interleukin-12 (IL-12) induces antitumor immunity. This study aimed to investigate the role of HPV in PC; and determine its effects on serum IL-12.

Between 2018 and 2022 in Ahvaz, researchers obtained 55 paraffin samples of malignant prostate lesions and 55 control samples of benign hyperplasia tissues from the prostate. Blood samples were collected from 24 diagnosed cancer patients to assess IL-12 levels before treatment initiation. Additionally, 24 patients with benign prostatic hyperplasia participated as controls. We performed DNA extraction using the phenol-chloroform method and examined the presence of papillomavirus DNA in tissues through Nested-PCR. Subsequently, IL-12 levels in serum were measured using ELISA.

The findings did not show the relationship between HPV and PC; HPV infection was not correlated to the presence of IL-12 secretion. However, with the progression of cancer, the level of IL-12 decreased significantly in patients compared to the control group ($P<0.05$).

HPV infection can exist in prostate tissue, although this does not mean that it contributes to P.C. development. The most significant strains infecting prostate tissue are types 16 and 18. Compared to the control group and with different Gleason scores, prostate cancer patient's levels of interleukin-12 secretion are significantly lower. One can make effective measures to assess the prognosis, regulate the condition, or aid in treating individuals using this crucial cytokine.

Keywords: Cytokine, Tumor, Nested-PCR, ELISA, Gleason score.

1 Introduction

Papilloma is a small virus of the Papilloma Viridae family with a double-stranded, lipid-free DNA genome that can infect both the nucleus and the cytoplasm in superficial squamous cells, cause structural and morphological changes in these cells, and produce non-cancerous tumors. The virus is called "papillomavirus" (HPV) because non-cancerous tumors of the skin are known as papillomas [1].

Human papillomavirus is the most common sexually transmitted virus infection in humans. Most men and women are most likely to become infected sometime after sexual activity, and skin-to-skin contact can also transmit the virus. The virus has various types, and most of them do not cause any problems. Also, most HPV infections clear without treatment. They clear after a few months. Specific HPV types can cause a small percentage of infections to linger in the body for years and progress into cancer, but medical treatment resolves 90% of cases within two years [2] .

Human papillomavirus is the cause of almost all cervical cancers. It is also strongly associated with other types of cancers, including anal, vaginal, and ovarian cancers [3, 4].

Previous studies show that prostate tissue is also susceptible to some sexually transmitted viruses, including human papillomavirus [5] . At some point in their lives, sexually active individuals can become infected [6]. The involvement of papillomavirus in the development of cervical cancer was first proposed by Zur Hausen [7] . Cervical and prostate cancers are similar in factors such as sexual activity and the location of the infection [8]. Therefore, since reports have linked papillomavirus to cancer over the years, papillomavirus infection may be linked to prostate cancer [9, 10] .

Interleukin-12 (IL-12), on the other hand, is a bridge between nonspecific primary immunity and specific immunity. In response to pathogens, monocyte cells, macrophages , Dendritic Cells (DCs) , B lymphocytes, neutrophils, and microglia secrete IL-12, which also acts via STAT4 to stimulate the production of IFN in T and NK cells. IFN-γ mediates the activation of pro-inflammatory agents and activates T-bet [11] . After identifying and linking it to TH1 commitment, researchers placed high hopes on IL-12 to become a target for immunotherapy and cancer treatment programs. In vitro, Interleukin-12 induces tumor eradication, prevents metastasis, and enhances antitumor immunity [12, 13]. This cytokine performs the above functions by activating CTLs, TH1-dependent cellular response, and NK cells [14]. Treating the IL-12 gene in a cervical tumor model from human papillomavirus type 16 activates cellular immunity. It also boosts the expression of TH1 cytokines. This activation is linked to the inhibition of tumor growth [15]. Numerous studies have shown the benefits of IL-12 in controlling tumors in mouse models[16-19]. In the advanced stages of many cancers, including cervical cancer, TH2 levels increase [20-24].

In light of the potential for cancer in other organs, such as the prostate, and the function of the human papillomavirus in some cancers, such as cervical cancer, as well as the significance of interleukin-12 in promoting immunity and limiting the growth of malignancies, this study analyzed the correlation between prostate cancer and the human papillomavirus. Furthermore, the Gleason grade (a grading system for prostate cancer that assesses the aggressiveness of the cancer cells) and the age of prostate cancer patients were utilized to compare their interleukin-12 levels with those of individuals diagnosed with

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benign prostatic hyperplasia. Furthermore, researchers compared the levels of this interleukin in persons with prostate cancer and papilloma infection to those with cancer but no papilloma infection.

2 **Materials AND METHODS**

Subjects

We collected samples of malignant prostate lesions from 55 patients with prostate cancer and collected 55 control samples of prostate tissue from patients with benign hyperplasia. These samples were collected from laboratories in the Pathology of Ahvaz City between 2018 and 2022. Out of the 55 patients with malignant prostate lesions, 24 newly diagnosed cases were selected for the study. Blood samples were taken from these patients to measure the levels of interleukin-12 before their treatment began. In addition, we selected 24 patients with benign prostatic hyperplasia as controls and also took blood samples from them. We cut each tissue sample into four sections with a diameter of five microns. An experienced pathologist examined the first and last sections and confirmed that they were cancerous. ***Nested-PCR***

We used the second and third sections to extract DNA, using the Phenol-Chloroform method . Then, used the Nested-PCR method to determine the presence of papillomavirus DNA in tissues. The HPVL1 gene was done in two stages by an outer primer called MY09 / 11 (450bp) and a nested primer called GP5 / 6 (150bp). In this method, 12.5 µL of Master Mix (ampliqon taq2x, Denmark), 1µL of Forward and Rivers primers (10 pmol), and 9.5µL of sterile distilled water were mixed with 1 µL of extracted DNA for the first and 0.5 µL for the second steps, respectively. The first temperature cycle or outer PCR consisted of the 30s at 95 $^{\circ C}$, 1 min at 50 $^{\circ C}$, 1 min at 72 $^{\circ C}$, and 5 min at 95 $^{\circ C}$ as pre denaturation and an additional expansion step for 8 min at 72 $^{\circ}$. This stage consists of 35 cycles. The nested PCR step began with an initial denaturation at 95 ° C for 5 min. It ended with 30 cycles: 30s at 95ºc, 30s at 50ºc, 30s at 72ºc, and 8min at 72ºc. The product of the second stage was examined using gel electrophoresis . DNA extracted from HPV_18 from HeLa cell tissue was used as a positive control .

We sent product to the Genetics Research Laboratory for sequencing, then it was subjected to BLAST to determine the type (NCBI database):

My09 primer: 5’CGTCCMARRGGAWACTGATC3’ ,

My10 primer: 5’GCMCAGGGWCATAAYAATGG3’ ;

Gp 5 primer: 5TTTGTTACTGTGGTAGATACTAC3’ ,

gp6 primer: 5’GAAAAATAAACTGTAAATCATATTC3 ;

Enzyme linked immunosorbent assay (ELISA)

The amounts of IL-12 in the patients’ and controls’sera were measured at the same time by the same technician, using ELISA-kits (Thermo Fisher, USA) . Briefly, the standard stocks were serially diluted in Reagent Diluent to generate 7 points for the standard curves. Diluted capture antibody was added to a microtiter plate. Plates were sealed and incubated overnight at room temperature, then washed with Wash Buffer. Premixed standards or samples were added to each well, and incubated for overnight at 4ºC. After incubation and washing, premixed Detection Antibody was added to each well and the plate was incubated for 2 h at room temperature. After incubation and washing, Streptavidin-HRP was added to each well. The incubation was terminated after 20 min at room temperature. Then, Stop

Solution was added to each well, and the optical density of each well was immediately determined using a microplate reader set to 450 nm. The results were expressed in pg/mL.

Statistical analysis

We used SPSS 24 software to analyze the collected data and, evaluated the normality of the research data by the Kolmogorov-Smirnov test. We also ran the Mann-Whitney-U test to compare the means in the two groups and used the Kruskal-Wallis test to compare the means in several groups. Finally, we utilized the Chi-square test to compare the ratios.

3 **Results**

Evaluation of the relationship between human papillomavirus and prostate cancer incidence

The chi-square test showed that there was no significant relationship between human papillomavirus and prostate cancer (P> 0.05). Also, among the 12 positive cases, the virus was type 16 in 10 cases (83.3%) and type 18 in 2 cases (16.7%) (Table 1) .

Comparison of serum interleukin-12 between patients and control group

Serum level of interleukin-12 in the patients with prostate cancer and controls showed that there was a significant difference between the mean of IL-12 in the cases and control groups, and the mean of IL-12 in the control group was significantly higher (p<0.05). Also, we compared the mean level of serum IL-12 in various stages (Gleason score 7 to 9) of prostate cancer. It showed a significant difference between the mean IL-12 levels in the stages. (p<0.05). In addition, the results of the serum interleukin-12 levels in positive or negative papilloma cases showed no significant difference between the mean of IL-12 in the two groups of positive and negative papilloma cases (p<0.05) (Table2, Fig.1) .

Comparison of interleukin-12 levels by age groups in control and experimental groups

The mean age of patients in the control and experimental groups showed no significant difference between the two groups. Also, no significant difference was observed between the levels of IL-12 in different age groups. (P> 0.05) (Table3) .

4 **Discussion**

We analyzed records for 110 patients referred to Ahvaz hospitals from 2018 to 2022 in this study. First, the case study results revealed that no significant relationship exists between the prevalence of prostate cancer and the human papillomavirus (P>0.05) .

The research also revealed that, out of the 12 cases where the human papillomavirus was detected, 3.83% had the virus type 16 and 7.16% had the virus type 18. Comparing the amount of interleukin-12 in the serum of patients with prostate cancer and healthy controls is another objective of this study. The mean levels of interleukin-12 in the experimental and control groups differed significantly (P<0.05), with the mean levels in the control group being substantially higher than those in the experimental group. The study investigated the quantity of serum interleukin-12 in patients with various Gleason scores and found a significant difference between the mean levels of interleukin-12 in different stages of prostate cancer. The level of serum interleukin-12 was then evaluated to determine whether the papilloma was present in individuals with prostate cancer. The findings indicated that Interleukin-12 averages in the two Papilloma positive and negative groups did not significantly differ (P>0.05). Finally, the mean age of the respondents was analyzed. It showed that there was no difference in their ages between the patients in the

two groups. Additionally, neither the control nor the experimental groups' levels of interleukin-12 varied significantly by age group (P>0.05).

According to an analysis of these findings, the human papillomavirus typically modifies tumor suppressor and proto-oncogene genes, changes cellular structure, and induces cancer. Oncogenes activate cellular proto-oncogenes. These genes produce proteins that are specifically necessary for controlling cell division and growth. Proto-oncogenes can turn into oncogenes through mutation or improper expression, and as a result of these changes, oncogenes can lead to abnormal cell division and the growth of tumors [25]. Papillomavirus infection influences cancer development in two different ways: through cell death and persistent inflammation [26]. The prostate gland, surrounding tissues, and the urinary system get contaminated as a result of this viral infection, which also causes chronic inflammation of the prostate [27, 28]. The primary role of the prostate gland is that of a reservoir for the sexual transmission of the papillomavirus through a seminal fluid. Infection of the reproductive system's mucous membrane can result in the growth of mucous cells and cancer. Consequently, HPV may cause prostate cancer [29]. However, further study is necessary before it can be said with certainty that this virus causes cancer in the prostate tissue.

The results of a study by Javid Sadri et al. in 2020 that evaluated the potential relationship between HPV-mediated inflammation, apoptosis, and angiogenesis in prostate cancer revealed that there is no significant relationship between the human papillomavirus and the development of prostate cancer [30], which is in line with the results of the current study. However, in 2020, a study was conducted by Shariat et al. in Ahvaz hospitals to investigate the relationship between human papillomavirus (HPV) and prostate cancer. The study used immunohistochemistry and PCR methods to analyze the samples. The research showed that there is a significant correlation between HPV infection and prostate cancer, which contradicts the findings of our study [31].

In addition, in different studies, it was reported a positive significant relationship between HPV infection and prostate cancer [32-35], which is not in line with the results of the present study. Additionally, ALICE-C-HChen et al., in 2017 [36] Mahmoudi et al., in 2022 [37] and two studies with the help of the Bradford Hill criteria found no relationship between this virus and prostate cancer [38, 39], which is in line with results of current research. The use of various techniques to identify viral infection in cancer samples, as well as technical issues with these techniques, such as contamination of the PCR product and variations in HPV genome detection, as well as limitations like the small sample size, maybe the reason why this study's findings differ from those of earlier studies. Additionally, the location, patient's age, immunological health, and unique genetic variables all influence the frequency and prevalence of HPV infection in men with prostate cancer

The study also revealed that among 12 instances where the human papillomavirus was detected, viruses' types 16 and 18 affected 3.38% and 7.16%, respectively. These results are in line with those of the study by Javid Nasr et al., in 2020, which revealed that high-risk HPV 16 and 18 were present in the majority of positive papilloma samples taken from prostate cancer tissue [30]. Our study supports previous research, indicating that high-risk HPVs such as HPV16 and HPV18 were primarily responsible for prostate infection [33, 40].

Also, we compared the amount of interleukin-12 in the serum of men with prostate cancer and healthy controls and, the mean levels of interleukin-12 differed significantly ($P<0.05$). The mean levels in the control group are much higher than in the experimental group. The findings show that interleukin-12 is a natural interleukin. Dendritic cells and macrophages produce it in response to antigenic stimulation. Because of its immune-stimulating and angiogenesis-inhibiting properties, it is thought to be a potential cancer treatment [41]. As a result, its quantity will be lower in cancer patients than in healthy individuals. In a 2017 study by J Salimu et al., it showed clearly how exosomes from prostate cancer suppress the production of IL-12 [42]. However, these findings are not in line with those of M Kundu et al. in 2017, Shokrabi et al. in 2008 and, Kovach in 2001, showing that in line with the progression of the illness, IL-12 serum levels were higher in cancer patients than in healthy people [43-45].

Our investigation results showed a significant difference in the mean level of serum interleukin-12 among patients with different Gleason scores in various stages of prostate cancer. Subsequently, we examined the amount of serum interleukin-12 to determine whether a patient had a positive or negative papilloma. The results of this study revealed that there was no statistically significant difference between the mean levels of interleukin-12 in the two Papilloma positive and negative groups ($P>0.05$). Analyzing the results mentioned above and evaluating interleukin-12 serum levels may lead to the conclusion that a decrease in interleukin-12 serum levels in patients with severe illness could indicate a lack of increase in inhibitory responses. Additionally, it can indicate the lack or improper operation of TH1+ CD4 cells, other cytokine-secreting cells, or tumor microenvironment inhibitory agents such as MDSCs, which would suppress the immune response against the tumor. Additionally, the non-significance of the mean of interleukin-12 in the two Papillomavirus positive and negative groups may suggest that the human papillomavirus has little or no impact on immune system suppression and IL-12 production.

The findings mentioned above are in line with those of S Murakami et al. in 2004, who found that patients with metastasized gastric cancer had significantly lower levels of IL-12 [46], and A Jebreel et al. who analyzed head and neck cancer [47]. Finally, the study compared the mean age of the respondents between the patients of the control and experimental groups. The results showed no significant difference in the mean age of patients in the two groups. Furthermore, the levels of interleukin-12 did not vary significantly by age group in either the control or the experimental groups ($P>0.05$).

5 Conclusion

In conclusion, we showed that HPV infection can exist in prostate tissue, although this does not mean that it contributes to PC development. The most significant strains infecting prostate tissue are types 16 and 18. In addition, compared to the control group and across different Gleason scores, levels of interleukin-12 secretion are significantly lower in prostate cancer patients. Furthermore, by measuring this cytokine, it is possible to obtain a favorable prognosis for prostate cancer and develop effective treatment plans in this area because interleukin-12 levels significantly decline as prostate cancer progresses. There is also a need for further investigations into the impact of HPV on prostate tumor tissue samples and the evaluation of additional cytokines in prostate cancer cases.

5 Declaration

Consent to participate: Ethics approval The ethics committee of Ahvaz Jundishapur University of Medical Sciences approved this study with the identification code IR.AJUMS.MEDICINE.REC.1399.021. The participants signed the consent form in this study. Also, all information about individuals is confidential. In addition, in this study, no costs were incurred by patients and controls.

Conflict of Interest*:* The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they are not employed by the government agency that has a primary function other than research and, or education.

Author contributions: Prof Ali Khodadadi and Sadegh Moradi conceived and designed the study. Sadegh moradi, D.r Mohsen Sarkarian, D.r Ladan Fatahi, D.r Maryam Seyedtabib and Moosa shariffat conducted the experiments and analyzed the data. Sadegh moradi, Mohammad Rashno and D.r Gholam Abbas Kaydani prepared the first draft of the manuscript. All authors critically revised the first draft and approved the final version.

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Availability of data and material*:* The data associated with the current study are available from the corresponding author on a reasonable request. Consent to participate: We obtained informed consent from all participants included in the study.

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Consent to publish: The authors affirm that human research participants provided informed consent for publication of the images and tables.

ТАБЛИЦЫ

Table 1. The relationship between human papillomavirus and prostate cancer and determine types of papilloma in positive cases.

Descriptors / Group	Number of cases reviewed	Condition (VirusType)	Human papillomavirus positive number	positive percent	negative number	negative percent	P-Value
Case	55	-	59	90	9.0	91.90	0.761
Control	55	-	73	27	12.73	87.27	
Total	110	-	132	98	10.91	89.09	
Positive cases	12	Tye 16 Papilloma	10	-	83.3	-	-
		Type 18 Papilloma	2	-	16.7	-	

Table 2. Comparison of interleukin-12 (Con -pg / ml) levels based on Gleason score(A) presence and absence of papilloma virus(B) in experimental and control groups.

Goup	Number (percent)	Mean±SD	Indicator	Group	Number (percent)	Mean±SD

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				(A) Gleason Score	7	8 (33.33)	3.5	140.33±1
					8	8 (33.33)	37.90	115.32±
					9	8 (33.33)	8.25	29.86±
Case	24 (50)		95.17±53.4	Kruskal Wallis Test				**P<0.001**
				(B) Papillomavirus	Papilloma poitive	4 (16.7)	11	88.83±36.
					Papilloma Negative	20 (83.3)	99	96.44±56.
				Mann-Whitney Test				0.535
Control	24 (50)	3.56	284.97±13	-				-
Mann-Whitney Test			**P<0.001**	-				-

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Table 3. Comparison of interleukin-12 levels by age groups in control and experimental groups.

group	Age	Age category	Number (percent)	Interleukin-12 level (Con - pg/ml) Mean±SD	Kruskal Wallis Test	Meaningful Level
Case	71.16±6.40	Less than 70 years	13 (54.2)	102.62±51.52	1.302	0.522
		70-75 years	5 (20.8)	105.36±63.49		
		More than 75 years	6 (25)	70.52±50.88		
Control	73.16±6.04	Less than 70 years	8 (33.3)	335.36±154.89	3.171	0.205
		70-75 years	9 (37.5)	256.75±119.82		
		More than 75 years	7 (29.2)	263.64±127.04		
P=0.227						

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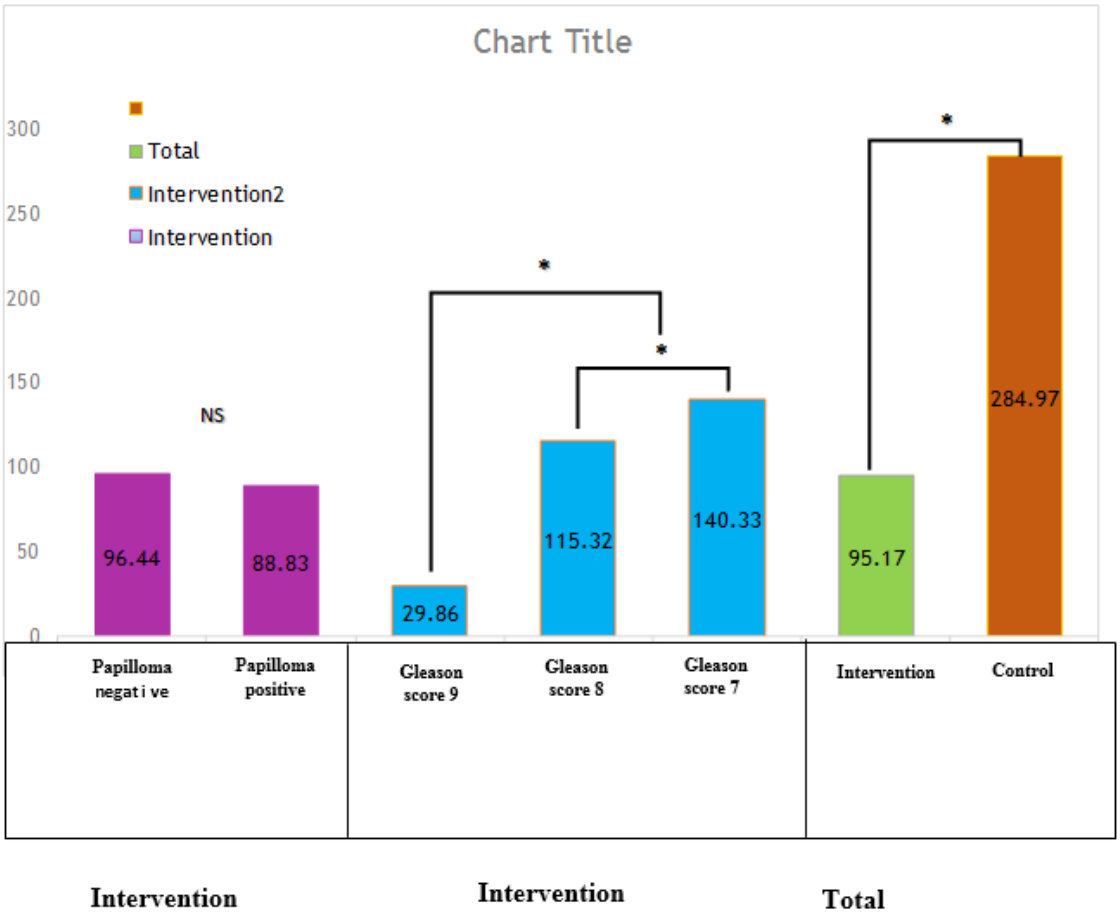
РИСУНКИ

Figure 1. Comparison of interleukin 12 levels (Con -pg / ml) in experimental and control groups, based on condition (positive or negative Papillomavirus) and Gleason score. There was no significant difference between the levels of interleukin-12 in different age groups **(P> 0.05).** The difference between the levels of IL-12 in Gleason score 7,8 and 9 was significant **(P <0.05)** and The difference between the levels of IL-12 in the experimental and control was significant too **(p<0.05). NS:** no significant.*****: significant symbol.



ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

Блок 1. Информация об авторе ответственном за переписку

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Блок 3. Метаданные статьи

EVALUATING THE RELATIONSHIP BETWEEN HUMAN PAPILLOMA VIRUS INFECTIONS, PROSTATE CANCER AND INTERLEUKIN-12 LEVELS

Сокращенное название статьи для верхнего колонтитула:
RELATIONSHIP BETWEEN HPV, PROSTATE CANCER AND IL-12

Keywords: Cytokine, Tumor, Nested-PCR, ELISA, Gleason score.

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