



# Is *Propionibacterium acnes* a Causative Agent in Benign Prostate Hyperplasia and Prostate Cancer?

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## Abstract

**Background:** Prostate cancer (PCa) is the most common cancer and the second cause of death among men worldwide. Recent documents have disclosed that chronic inflammation can be a major risk factor for PCa. Based on recent studies, the presence of *Propionibacterium acnes* (*P. acnes*) as an anaerobic gram-positive bacterium in prostate tissue can be a predisposing factor for PCa. The aim of this study was to evaluate the *P. acnes* presence in patients with PCa compared to patients with benign prostatic hyperplasia (BPH).

**Methods:** In a descriptive study that was conducted from January 2015 to December 2016, 95 paraffin-embedded prostate tissue samples (57 PCa and 38 BPH) were evaluated. All samples were collected from the pathology unit of a hospital. DNA was extracted with an extraction kit (GeneAll, Korea) and then PCR was carried out using specific primers for *P. acnes*. Sequencing was performed on the PCR products to confirm the presence of *P. acnes*. Demographic data were analyzed using statistical package for social sciences (SPSS) software (version 21).

**Results:** Out of 95 patients, 57 (60%) were patients with PCa and 38 (40%) were patients with BPH. 39 (41%) and 22 (23%) samples were *P. acnes* positive in cancer and BPH groups, respectively.

**Conclusions:** The results suggest that the spread of *P. acnes* in males with PCa may be common. This finding reflects the possible role of *P. acnes* in the carcinogenesis of PCa. *P. acnes* infection may play a relative role in the pathogenesis of PCa or it could facilitate the PCa progression.

**Keywords:** *Propionibacterium acnes*, Prostate Cancer, PCR, Iran

## 1. Background

Prostate cancer (PCa) is one of the most common cancers among men in the world (1, 2). In 2012, more than one million men suffered from prostate cancer worldwide (3). Among Iranian men, gastric cancer and prostate cancer are the first and second most common cancers, respectively (4). Chronic inflammation is an important risk factor, which can increase the chance of developing prostate cancer in individuals (5). It has been suggested that bacterial infections by inducing long-term inflammation can facilitate cancer development (6, 7). Some studies showed a significant correlation between the presences of *Propionibacterium acnes* (*P. acnes*) and inflammation in prostate tissue. *P. acnes* is an anaerobic Gram-positive ubiquitous bacterium and is a member of skin normal flora. This bacterium has four main types IA, IB, II, and III based on DNA

sequence comparison of the *recA* or *tly* genes (8). *P. acnes* can be unambiguously implicated in a variety of manifestations including sarcoidosis, systemic infections folliculitis, and chronic inflammatory (9, 10). Furthermore, some byproducts of *P. acnes* such as free fatty acids produced as a result of the metabolism of triglyceride can annoy the enforce inflammation through immune cells chemotaxis to the site of infection (11). Some recent research has reported the high incidence of *P. acnes* in prostate tissue samples of patients with PCa (12-15). These studies have shown that *P. acnes* has the ability to annoy the cells of the prostate tissue to secrete Interleukin 6 (IL-6) and Chemokine (C-X-C motif) (16, 17). In addition, a number of other studies assert that chronic and acute inflammation has been associated with the presence of *P. acnes* in prostate tissue (13). Some investigations show the transformation of prostate cells after being infected with *P. acnes*, supporting the hypothesis that

*P. acnes* is a predisposing factor in *P. acnes* development (14-18). Since there is no evidence of the prevalence of *P. acnes* in prostate tissue in Iran, in the present study, we sought to investigate the presence of *P. acnes* strains in prostate tissue samples from men with PCa and benign prostate hyperplasia (BPH) who referred to shohada-ye tajrish hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2016.

## 2. Methods

### 2.1. Samples collection

This descriptive study was conducted from January 2015 to April 2016. In this survey, 57 prostate cancer (PCa) and 38 BPH (as a control group) paraffin-embedded tissue blocks were evaluated. Microscopic evaluation to determine the cancerous and non-cancerous tissues and differentiation between PC and BPH was done by a pathologist. The best paraffin-embedded block containing suitable tissues of the patients were selected for examination. In order to further analysis, the samples were transported to the department of microbiology, Shahid Beheshti University of Medical Sciences.

### 2.2. DNA extraction

DNA was extracted from paraffin-embedded tissue blocks by G-spin TM total DNA extraction Kit (GeneAll, Korea). First, the paraffin blocks were sliced into thin pieces using a sterile razor blade and placed in a 1.5-mL tube (not more than 25 mg). According to the manufacturer's instruction, xylene was used to remove paraffin and then the bacterial DNA was extracted from the tissue. After measuring their concentration, the extracted DNA was stored at -20 °C.

### 2.3. Standard PCR

PCR was used to evaluate the successfulness of DNA extraction using the human beta-actin gene as the target. In the next step, the second PCR assay was done for the detection of *P. acnes* using the *recA* specific primers. The PCR reaction mixture contained 12.5 µL of PCR mix, 1 µL of each primer, and 5 µL of DNA template. PCR grade water was added to bring the final volume to 25 µL. For negative and positive controls, 5 µL of PCR grade water and 5 µL positive controls (*P. acnes* ATCC 11828) were added, respectively. The specific primers for *recA* detection were *recA* F-5'AGCTCGGTGGGGTCTCTCATC3' and *recA* R-5'GCTTCCTCATACCACTGGTCATC 3'. PCR conditions are summarized in Table 1. The PCR products were analyzed on 1.5% agarose gel. The gel was stained with ethidium bromide (0.5 µg/mL) and viewed by UV transilluminator. The

presence of 1201 bp fragments indicated positivity for *P. acnes*.

Table 1. The Amplification Protocol for the Detection of *P. acnes*

Cycle	Time	Temperature
1	5 Min	94
	30 Sec	94
30	40 Sec	55
	30 Sec	72
1	5 Min	72

### 2.4. Statistical Analysis

Demographic data were analyzed using Statistical Package for Social Sciences (SPSS) software (version 21).

## 3. Results

Most of the patients with PCa belonged to the 70 - 79 age group (29.8%) whereas 50% of the patients with BPH were 50 - 59 years-old. The characteristics of the study patients and Gleason score of tumors are summarized in Tables 2 and 3. In 3.2% of the patients, the Gleason score was 10 (the highest score). Most patients had a Gleason score of seven (33.3%). Chart 1 and 2 represent the frequency of *P. acnes* in the case and control groups based on age.

Table 2. Distribution of Gleason Score in the Study Patients

Gleason Score	No. (%)
4	1 (1.8)
5	2 (3.5)
6	13 (22.8)
7	19 (33.3)
8	7 (12.3)
9	13 (22.8)
10	2 (3.5)

Table 3. Characteristics of the Study Patients

Groups Values	Number of patients	Age, (mean ± SD) years
BPH	38	68.0 ± 8.9
PCa	57	67.1 ± 10.0

### 3.1. PCR for Beta-Actin and recA Genes

Out of 95 study patients, there were 57 and 38 patients with PCa and BPH, respectively. All tested specimens were positive for the beta-actin gene and showed PCR product bands on 1.5% agarose gel. Therefore, all specimens had suitable DNA for the next step PCR. Among the 95 samples, *P. acnes* was detected in 61 (64.2%) patients. Of 57 PCa specimens, 39 (68.4%) *P. acnes* positive specimens were detected. 22 (57.9%) specimens in the BPH group were *P. acnes* positive, as well. No significant relationship was observed between the presence of *P. acnes* and the age of patients with PCa and BPH ( $P = 0.25$  and  $0.84$ , respectively). The results of this study showed that there was no significant difference between the case group (prostate cancer) and the control group (non-cancerous samples) regarding the frequency of *P. acnes* ( $P$ -value  $> 0.05$ ). In addition, there was no significant relationship between patients with PCa and control groups in terms of clinical symptoms, stage of tumor progression, tumor type, tumor region in PCa pathological degree, and age ( $P > 0.05$ ).

## 4. Discussion

Prostate cancer is one of the most relevant cancers and a leading cause of morbidity and mortality among men worldwide (19, 20). In Iran, there are no accurate data about the incidence of prostate cancer, but it is estimated that around 90,000 new cases of cancer are reported annually, of which 12 per 100,000 cases are prostate cancer (21). The presence of *P. acnes* was strongly correlated with chronic inflammation, suggesting that this bacterium may have a potential role in cancer development (22). Until now, there are scarce studies conducted to evaluate the prevalence of *P. acnes* in PCa or BPH. As far as we know, there is no exact data about this issue reported from Iran. Therefore, we investigated the possible role of *P. acnes* in PCa and BPH using PCR standard methods. Based on the results of the present study, 68.4% of the PCa tissue samples contained *P. acnes* DNA. About 58% of the BPH tissue samples as the control group were positive for this bacterium, too. Although the positive rate was higher in PCa than in BPH specimens, there was no statistical significance between these differences. This may be due to that we could not use healthy tissue samples as the control group. Investigators showed the role of chronic or recurrent inflammatory processes in the progression of BPH and prostate cancer (23, 24). The high rate of positive results for *P. acnes* in BPH tissues may consider this bacterium as a predisposing factor for BPH, as well as PCa. Cohen et al. in 2005 showed the presence of *P. acnes* in one-third of PCa

tissue samples as the most common detected microorganism (25). By using fluorescence in situ hybridization, another study in 2007 reported the presence of *P. acnes* in 50% of the radical prostatectomy specimens (26). *P. acnes* was the most commonly cultured microorganism (17%) from prostate samples in the study performed by Sfanos et al. (24). Unequal tissue sample size and difference in bacterial detection methods may explain the discrepancy between the results of different studies. Similar to the current study, Davidsson et al. evaluated the relationship between *P. acnes* and PCa on 100 cancerous and 50 non-cancerous samples by standard PCR. Based on the results of this study, the prevalence of *P. acnes* in patients with PCa and control group was 60% and 26%, respectively, which indicates a high prevalence of *P. acnes* in the cancer group compared to the control group (27). The results of Davidsson et al. in the cancer group confirm the findings of our study on the high prevalence of *P. acnes* in cancerous specimens. In other words, in both studies, the prevalence of *P. acnes* is lower in the control group than in the PCa group. The only difference between the results of these two studies is the difference in the prevalence of *P. acnes* in the control group, which is 58% in the present study in comparison with 26% in the Davidsson and colleagues study. The reason for this difference may be the use of benign samples instead of healthy samples, as the control group, in the present study. Although these specimens are not cancerous, they may have some degrees of malignancy that may affect the outcome of this study. That is why the prevalence of bacterial acne protein in the control samples of this study was higher than that of Davidsson et al. study. In addition, Davidsson et al. studied patients and healthy people in the age range of 42 to 81 years-old while in our study, the age range in both groups was 50 to 89 years. Regarding the fact that the study patients and control group were older in this study, it can justify the high prevalence of *P. acnes* in the control group of this study compared to the study by Davidsson et al.

### 4.1. Conclusion

Due to the global prevalence of genitourinary infections and anatomic location of the prostate, this matter that infectious agents may play a role as a risk factor in PCa it is not surprising. The results of this study support the hypothesis of a relationship between *P. acnes* infections and PCa and it can be concluded that the inflammatory effects caused by this organism, together with other risk factors, can be effective in PCa. Therefore, the early diagnosis and early treatment of *P. acnes* infections can be used as a common method of prevention and treatment of PCa.

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