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# Identification of the *Propionibacterium acnes* using Polymerase Chain Reaction (PCR) in the Acne vulgaris lesions

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## Abstract

**Background**: Acne vulgaris is an inflammatory chronic disease of pilosebaceous unit. One of the most important factors playing a role in occurrence of acne is presence of *Propionibacterium acnes*. With the aim of molecular identification of the P. acnes from the acne vulgaris lesions, current research was carried out.

**Methods**: In this study, contents within the lesions was collected from 70 patients. The presence of the *P. acnes* was examined by a specific PCR technique.

**Results**: Of 70 samples, 58 samples (82.85%) were determined to be positive in terms of presence of *P. acnes*. No significant relationship was observed between presence of *P. acnes* and each one of the studied demographic factors, including gender, age, disease period, family background and treatment background.

**Conclusions**: The adopted molecular technique has obviated the limitations associated with the culture method for identification of the bacteria. To overcome the problems with conventional culture techniques for *P. acne*, this PCR method is promising for better identification of this bacterium.

**Keywords**: Identification, Polymerase Chain Reaction (PCR), *Propionibacterium acnes*, Acne vulgaris

## Introduction

Acne vulgaris is the most prevalent skin disorder of follicle sebaceous (Bhambri et al., 2009) that observed in 80% of the juveniles (Ray et al., 2013; Jahns et al., 2012) at the age ranged from 14 to 19 years old. Due to earlier onset of the puberty age, this disease is more prevalent in the women, while it's more acute shapes are observed in the man (Adityan and Thappa, 2009). Pathogenesis of acne is multifactorial; obstruction of sebaceous pores, increase of producing the sebum, *P.acnes*, inflammation and androgens are the factors supposed to play a role in formation of the acne (Bhambri et al., 2009). For more than a century, the clinical relationship between *P. acnes* and appearance of the acne vulgaris has been identified (McDowell et al., 2013). So that increase of number of this bacterium leads to acuteness of the acne and decrease of number of that leads to improvement of the

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acne (Bhambri, et al., 2009).

*P. acnes* is a polymorphic (Cauich et al., 2001; Perry and Lambert, 2006), gram positive, facultative anaerobic, without spore (Cauich, et al., 2001; Skinner et al., 1978), non motile (Skinner et al., 1978) and slowly growing bacilli (Portillo et al., 2013; Perry and Lambert, 2006). It has the best growth in the oxygen limitation conditions (Cove et al., 1983) and rate of it's growth decreases in the high concentration of oxygen (Perry and Lambert, 2006; Cove et al., 1983). This bacterium is an opportunistic pathogen (McDowell et al., 2013) and, through extracellular products, including Hyaluronidase, Protease and Lipase and, also, Chemotaxis factors for the Neutrophils, Lymphocytes and Macrophages, plays a role in the pathogenesis of acne (Burkhart et al., 1999).

Today, PCR technique is used to diagnose an extensive spectrum of the biological factors. Compared to the traditional methods of diagnosis, PCR method is of a high speed, accuracy and specificity and sensitivity. Considering the weak points of the traditional methods in this study, PCR method was used in order to diagnose quickly and accurately *P.acnes* in the lesions isolated from the patients with acne vulgaris.

## Materials and Methods

### Sample collection

The current research has been carried out cross sectional analysis. In this study, 70 patients with acne vulgaris referred to the skin clinic in the Tonekabon township within the years ranged from 2013 to 2014 were studied. Of total 70 collected samples, 49 cases were female and 21 cases were male who placed in the age limits ranged from 14 to 32 years. The studied samples were collected from the face (forehead, cheek and chin) of the studied individuals. The collected samples were transferred to the micro tube containing TBE buffer (Tris-borate-EDTA buffer) and kept under -20˚C until enforcement of the DNA extraction process. Of a number of the samples, microbic culture was prepared randomly. After identification of bacillus of *P.acne*, the purified colonies were used to perform process of sequencing.

### DNA extraction

300 µl of the taken collected samples were solved in 200 µl of the TE buffer (Tris-EDTA buffer) and, following the addition of 2 µl of lysozyme, kept for 30 minutes in the room temperature. Then, DNA extraction was conducted by use of the instruction of maker company of the extraction kit (Qiagen, Germany). In order to study accuracy of the DNA extraction, photoabsorption of the samples in the range of 260 and 280 nm was evaluated by biophotometer apparatus (Eppendorf, Germany).

## PCR

PAR-1 and PAR-2 specific primer made by TAG Copenhagen company Denmark and PCR kit (Qiagen-Germany) were used to perform PCR. PCR mix with final volume of 20 µl including, 10 µl of Master Mix along with 0.5 µl of Forward primer, 0.5 µl of Reverse primer (10 pmol), 5 µl of extracted DNA and 4 µl of sterile distilled water was prepared in the 0.2 micro tube and placed inside the thermocycler apparatus (Eppendorf-Germany). PCR reaction was carried out in 35 cycles, including denaturation in 95˚C for 60 seconds, primer's annealing in 60˚C for 30 seconds and extension in 72˚C for 90 seconds. Also, initial denaturation with 95˚C for 5 minutes and final extension with 72˚C for 10 minutes were taken into consideration (Niazi et al., 2010).

**Table 1** Sequence of primer 5'→ 3'

| Primer | Sequence of primer 5'→ 3' | PCR Product | References |
|---|---|---|---|
| **PAR-1** | 5'-AGC TCG GTG GGG TTC TCT CAT C-3' | 1202 bp | Niazi et al., 2010 |
| **PAR-2** | 5'-GCT TCC TCA TAC CAC TGG TCA TC-3' | | |

## Electrophoresis

Electrophoresis was performed using 5 µl of each samples. A DNA molecular ladder (1Kb) was used as control (Qiagen-Germany). The electrophoresis was done on 1.5% agarose gel adjusting voltage of 75 V for 40 min. Then, the gel was transferred to Transilluminator apparatus (Gel Doc-England) to visualize the DNA bands.

## Sequencing

In order to confirm PCR data, four samples were selected for sequencing. 50 µl of the PCR product obtained by specific primer of *P. acnes* were transferred to Korea (Macrogen Company, Korea) for sequencing and identification of the isolated strains. Then, the obtained gene sequence was analyzed using BLAST software available in NCBI site.

## Results

Of 70 samples studied by use of PCR technique, 58 positive samples (82.85%) were identified in terms of *P. acnes*.

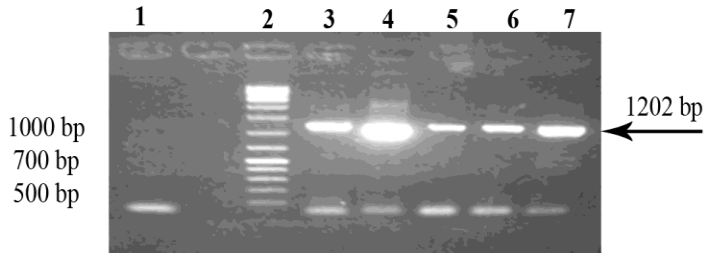


**Figure 1** Amplification products separated on agarose gel.

Electrophoresis was performed on 1.5% agarose gel. Lane 1: negative control, Lane 2: DNA molecular ladder (1kb), Lane 3: positive control and Lanes 4 to 7: P. *acne-positive samples*.

The purified colonies of the *P. acne* were used in order to sequencing. Figure 2 is the microscopic shape of the studied bacterium. The results achieved from the sequencing, also, confirmed result of PCR. In table 2, the results achieved from sequencing of the samples evaluated using BLAST software available in NCBI site are observed.

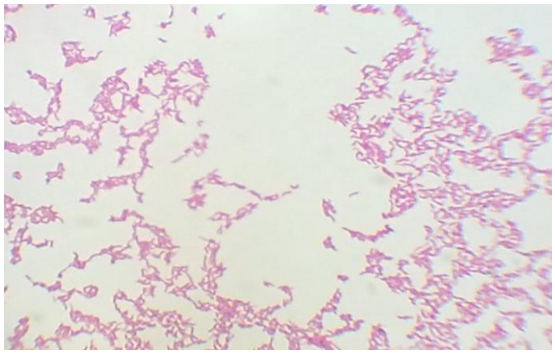

**Figure 2** Gram's staining showing Gram positive bacillus of *P. acne*

**Table 2** Sequencing data of the samples observed in NCBI site

| Sample | *P. acnes* Strain | Sequence ID |
|---|---|---|
| **1** | hdn-1 | CP006032.1 |
| **2** | PA44 | KJ572677.1 |
| **3** | hdn-1 | CP006032.1 |
| **4** | PA62 | KJ572678.1 |

Findings from research on the basis of demographic information, including gender, age, and disease period, background of acne in family and background of treatment were analyzed. In order to interpret the results, SPSS statistical software (SPSS version 18) and Chi-square independence test were used. The studies showed that there is not any relationship between rate of frequency of the *P.acnes* and each one of the studied factors.

**Table 3** Demographic information of the studied patients and relative and absolute frequency of the *P.acnes* in terms of each one of the mentioned factors

| Index | Status | Frequency | Percentage frequency (%) | Positive PCR | Percentage Positive PCR (%) | P-value |
|---|---|---|---|---|---|---|
| **gender** | **Female** | 49 | 70 | 42 | 85.7 | 0.261 |
| | **Male** | 21 | 30 | 16 | 76.2 | |
| **Age (years)** | **10-15** | 6 | 8.57 | 6 | 100 | 0.330 |
| | **15-20** | 32 | 45.71 | 27 | 84.4 | |
| | **20-25** | 18 | 25.71 | 13 | 72.2 | |
| | **25-30** | 12 | 17.14 | 10 | 83.3 | |
| | **30-35** | 2 | 2.86 | 2 | 100 | |
| **Time Disease (years)** | **Random** | 37 | 52.86 | 33 | 89.2 | 0.398 |
| | **1-5** | 20 | 28.57 | 15 | 75 | |
| | **5-10** | 11 | 15.71 | 8 | 72.7 | |
| | **10-15** | 2 | 2.86 | 2 | 100 | |
| **Background of acne in family** | **Yes** | 37 | 52.86 | 31 | 83.8 | 0.828 |
| | **No** | 33 | 47.14 | 27 | 81.8 | |
| **Background of treatment** | **Yes** | 29 | 41.43 | 22 | 37.9 | 0.215 |
| | **No** | 41 | 58.57 | 36 | 58.3 | |

## Discussion

In the recent years, our knowledge regarding microorganisms has been depended on the culture technique, while culture method is able to identify little minority (less than 1%) of the bacteria (Grice et al., 2008). For the purpose of identification of the viable but non culturable (VBNC), microorganisms which are not capable of growing in the usual culture media (Kong and Segre, 2012; Rhoads et al., 2012), microorganisms growing slowly (Millar et al., 2007) and, also, obligate anaerobes micro-organisms (Rhoads et al., 2012) the molecular techniques have a lot of applications.

Usage of the techniques relied on nucleic acid has obviated the limitations available on the way of identification of microorganisms through culture technique (Taravati et al., 2013). In the molecular methods, little quantity of the microorganism's DNA is identifiable and living of the studied microorganism is not to be required (Rhoads et al., 2012; Kong and Segre, 2012). Also, in order to carry out PCR, samples such as blood and liquids of the body can be used, and preparation of the fresh sample is not required. Before sampling, antibiotic consumption doesn't influence on the result achieved from PCR, while it leads to establishment of the false negative response in the results obtained from culture.

*P. acnes* is a fastidious bacterium which requires enrich medium, special nutrient factors and anaerobic conditions in order to grow. Due to it's slow growth, identification of the *P. acnes* through culture is not so much possible. With aim of molecular identification of the *P. acnes* assisted with PCR method in the acne vulgaris lesions isolated from the patients with acne, the current study was accomplished. Of 70 studied samples, 58 positive samples (82.85%) were identified in terms of presence of the *P.acnes* with assistance of PCR technique, suggesting high outbreak of the bacterium in the acne lesions. In 2003, a research was conducted by Le Page et al aiming at diagnosis of the vascular prosthesis infection through method amplification and sequencing of *16S rDNA*. In this study, of 20 studied samples, 5 positive samples (25%) of the *P.acnes* were identified with assistance of the PCR method (Le Page et al., 2003). In 2006, a research was conducted on the individuals with endophthalmitis by Bagyalakshmi et al. 30 samples were collected from the studied patients and using mPCR method, 4 positive samples (13.3%) of the *P. acnes* were diagnosed (Bagyalakshmi et al., 2006). In 2013, a study was carried out by Rollason et al, on the patients with herniated waist disk surgery. Of total 64 studied samples, presence of the *P. acnes* in 24 samples (38%) was confirmed with aid of analysis of *recA* gene sequence (Rollason, et al., 2013). In 2014, a study was carried out by Bunker et al

on the individuals with shoulder infection following the orthopedic surgery. Of 10 studied patients, 6 positive samples (60%) of *P. acnes* were diagnosed by use of PCR method (Bunker et al., 2014).

Some discrepancies are observed among the results achieved from the researches, this discrepancy may be related to sampling place, type of the studied sample (acne or other infections resulted from *P. acnes*) and conditions of PCR performance. With a general glance at the results achieved from the current study and other conducted studies, it can be concluded that, using PCR method, higher percentage of the microorganisms is to be identified. Also, usage of the molecular methods for the purpose of identification of the biologic factors compared to the traditional methods of diagnosis leads to time and cost economizing. Despite the day increasing improvements of molecular techniques for the purpose of diagnosis of a variety of infections, culture method as a standard method remains to be a special value and position (Taravati et al., 2013; Kong and Segre, 2012).

## Conclusion

With regard to the existed limitations for identification of microorganisms through culture technique as well as high speed and accuracy of the PCR technique in the diagnosis of pathogenic factors, this method, can be considered an appropriate tool for identification of the studied bacterium.

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