ORIGINAL ARTICLE

CD_{64} EXPRESSION IN PATIENTS WITH CHRONIC PROSTATITIS AND ITS IMPLICATION IN DIAGNOSIS

JINGHE CAO [1], BAOGANG SUN [1], YINGYING FAN [1]*, ZHUANG LIU [1], NING ZHANG [1]

[1]*Reproductive Center, Affiliated Hospital of Jining Medical University, Jining, Shandong, 272029, China*

**corresponding author: yingyfan2015@sina.com*

Manuscript received: December 2017

Abstract

The aim of this study was to investigate the expression of neutrophil granulocyte cluster of differentiation 64 (CD_{64}) in patients with chronic prostatitis and the significance for diagnosis and treatment of chronic prostatitis. Seventy-two patients with chronic prostatitis were selected and included in the experimental group. Moreover, thirty-five healthy individuals represented the control group. Pathogen detection and the level of CD_{64} were determined in the prostatic fluids of all individuals included in the study. Fifty-four pathogen stains were detected in the specimens of prostatic fluid of patients in the experimental group and none in the control group. Patients infected with *Staphylococcus simulans* had the highest expression level of CD_{64} (3912.237 ± 767.416/cell), followed by *Staphylococcus auricularis* (2764.371 ± 141.261/cell), *Staphylococcus warneri* (1916.356 ± 127.284/cell), *Enterococcus faecalis* (1893.636 ± 314.458/cell) and *Staphylococcus haemolyticus* (1874.328 ± 125.212/cell) the difference being significant compared to the control group ($p < 0.01$). There was no significant difference between the expression levels of CD_{64} in control the group and in patients from the experimental group infected with *Chlamydia trachomatis, Ureaplasma urealyticum, Staphylococcus epidermidis, Staphylococcus xylosus, Staphylococcus capitis urealyticum* and *Staphylococcus hominis.* CD_{64} can be a potent marker for the diagnosis of bacteria chronic prostatitis.

Rezumat

Scopul prezentului studiu a fost de a investiga expresia CD_{64} la pacienții cu prostatită cronică și asocierea acestuia cu prognosticul și alegerea tratamentului personalizat. Șaptezeci și doi de pacienți cu prostatită cronică au fost incluși în grupul experimental, iar treizeci și cinci de indivizi sănătoși au fost incluși în grupul martor. Au fost identificate microorganismele patogene din lichidul prostatic, precum și expresia CD_{64}. Cincizeci și patru de tulpini patogene au fost detectate la pacienții din lotul experimental și nici una la lotul martor. Pacienții infectați cu *Staphylococcus simulans* prezintă cel mai crescut nivel al CD_{64} (3912,237 ± 767,416/celulă), urmați de cei infectați cu *Staphylococcus auricularis* (2764,371 ± 141,261/celulă), *Staphylococcus warneri* (1916,356 ± 127,284/celulă), *Enterococcus faecalis* (1893,636 ± 314,458/celulă) și *Staphylococcus haemolyticus* (1874,328 ± 125,212/celulă) diferența fiind semnificativă comparativ cu lotul de control. Nu a fost observată o diferență semnificativă între valoarea CD_{64} observată la lotul de control și la cei din lotul experimental infestați cu *Chlamydia trachomatis, Ureaplasma urealyticum, Staphylococcus epidermidis, Staphylococcus xylosus, Staphylococcus capitis urealyticum* și *Staphylococcus hominis.* CD_{64} poate fi un marker potent pentru diagnosticul prostatitelor bacteriene cronice.

Keywords: neutrophil granulocyte, bacterial chronic prostatitis, CD_{64}

Introduction

Chronic prostatitis, the most common male urinary disease, is most frequent in males aged from 30 to 40 years [10]. Pathogen infection is the main cause for chronic bacterial prostatitis, and *Staphylococcus* is the main infection pathogen.

Diabetes is an important factor in favouring prostatitis, degeneration of seminiferous tubule epithelium and alteration of spermatogenesis [6].

Even some foods (selected cultivated fruits and vegetables) containing aluminium, cadmium, arsenic and gamma-orizanol may have potential health risks, acting as immune disruptors and favouring the occurrence of infections [1, 11].

Chronic bacterial prostatitis manifests as continuous and recurrent lower urinary tract infection for at least three months. Currently, there is no fast and appropriate method for accurate diagnosis of chronic prostatitis [14]. Neutrophil granulocytes are part of the defence mechanism against the infection.

CD_{64}, a transmembrane glycoprotein [4], can eliminate pathogenic microorganism through antibody dependent cellular cytotoxicity, phagocytosis and immune complex elimination [9]. Usually, CD_{64} are distributed on the surface of antigen presenting cells such as macrophage, monocyte and dendritic cells [5]. CD_{64} has almost no expression on the surface of neutrophil granulocyte [15, 17], and there is no difference between genders. Once proinflammatory cytokines are released due to an infection and neutrophil granulocyte are activated, CD_{64} is highly expressed [16]. Therefore, a high expression of CD_{64} occurs in infections, suggesting

that it can be used as a marker for the diagnosis of infectious prostatitis.

This study aims to determine whether CD_{64} could be regarded as an indicator for the diagnosis of chronic prostatitis through its detection in the prostatic fluid of patients with chronic prostatitis.

Materials and Methods

Patients. The study has been approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University, Shandong, China, and all the patients have signed an informed consent. Seventy-two patients with chronic prostatitis who were admitted to the Affiliated Hospital of Jining Medical University from November 2015 to February 2017 were selected and set as an experimental group. The average age was (40.21 ± 10.64) years. All patients were confirmed to suffer from chronic prostatitis. Moreover, thirty-five healthy subjects were selected and set as a control group. The average age of this group was (38.15 ± 9.73) years.

Instruments and reagents. Instruments included FC-500 flow cytometry (Beckman Coulter Company, USA), fully-automatic polymerase chain reaction (PCR) amplifier (Thermo Fisher Scientific Inc., USA), fully-automatic chemiluminescence apparatus (MAGLUMI 4000 Plus, Shenzhen Snibe Diagnostic Inc., China) and BD Phoenix™-100 Automated Microbiology System (BD, USA). The reagents used were *Chlamydia trachomatis* (Ct) and *Ureaplasma urealyticum* (Uu) kits (Wuhan Moshake Biotechnology Company, China).

Experimental methods.

Bacterial culture. Bacterial culture followed the national inspection standardization operation regulations and requirements. Bacterial colony was observed and identified after 24 h of specimen culture. The culture procedures referred to the National Clinical Laboratory Procedures. Prostatic fluid was incubated into a goat blood plate, and the species of the growing bacteria were identified using a WalkAway40 full-automatic bacterial analyser (Siemens, Germany) according to the standard requirements. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were taken as standard bacterial strains.

Detection of CD_{64}. 50 μL of prostatic fluid was absorbed by a sterile tip and added to a TRU-COUNT tube. After the addition of 10 μL of CD_{64}-FITC (fluorescein isothiocyanate), the tube was shaken slightly. It was then placed in dark at room temperature for 20 min, followed by the addition of 500 μL of ACK Lysis Buffer. Afterwards, it was placed again in dark at room temperature for 10 min, followed by the addition of 500 μL of phosphate buffer solution (PBS). Finally, after it was placed in dark at room temperature for another 10 min, the specimens were analysed with the flow cytometer.

Chlamydia trachomatis (Ct) and *Ureaplasma urealyticum* (Uu) detection. Detection strictly followed the specifications of the reagents.

(1) Ct detection. 1 mL of sterile physiological sodium chloride solution was added to the tube and mixed evenly. After centrifugation at 12000 rpm for 6 min, it was washed. After several minutes of standing, 50 μL of DNA extracting solution was added. It was processed by boiling water bath for 10 min and then placed at 4°C for 7 h. Finally it was centrifuged at 12000 rpm for 3 min, and 2 μL of supernatant was collected for PCR. Each specimen was added with 24 μL of CT PCR reaction liquid and 2 μL of Taq polymerase. The mixture was transferred to a centrifuge tube and vortexed for 10 seconds. Each reaction tube was added with 26 μL of the mixture. The supernatant which was collected from the last procedure was vortexed for several seconds, and then 4 μL of the supernatant was added to each reaction tube, followed by low-speed centrifugation. PCR amplification was performed according to the procedures described in Table I.

Table I

Procedures of PCR amplification for Ct detection

Procedure	Number of cycles	Temperature	Time
1	1	37°C	2min
2	1	93°C	5min
		93°C	30sec
3	40	55°C	30sec
		72°C	40sec

(2) Uu detection. Specimen processing was the same as the above. Each specimen was added with 35.6 μL of Uu reaction mixture and 0.4 μL of Taq polymerase. Then the mixture was transferred to a centrifuge tube and vibrated for 10 seconds. Then 36 μL of the mixture was added in each reaction tube. The supernatant which was collected from the last procedure was vortexed for several seconds, and then 4 μL of the supernatant was added to each reaction tube. The procedures of PCR amplification are described in Table II.

Table II

Procedures of PCR amplification for Uu detection

Procedure	Number of cycles	Temperature	Time
1	1	95°C	3 min
2	40	94°C	15 sec
		60°C	30 sec

Statistical method. Experimental data were analysed and processed by SPSS version 20.0. Measurement data were expressed as mean ± standard deviation. The comparison of means between groups was performed using t test. Difference was considered as statistically significant if $p < 0.05$.

Results and Discussion

Pathogens detection.

54 strains of pathogens were detected out of the specimens of the experimental group (75%), including 44 strains of bacteria (81.48%), 7 strains of Uu (12.96%) and 3 strains of Ct (5.56%). No pathogens were detected in the control group. The distribution of pathogens detected in the experimental group is shown in Table III.

Table III

The distribution and constituent ratio of pathogens detected in the experimental group

Pathogen	Number of strains	Constituent ratio (%)
Bacteria	44	81.48
Staphylococcus epidermidis	9	16.67
Staphylococcus simulans	8	14.81
Enterococcus faecalis	8	14.81
Staphylococcus haemolyticus	5	9.26
Staphylococcus warneri	5	9.26
Staphylococcus auricularis	4	7.41
Staphylococcus hominis subspecies *hominis*	3	5.56
Staphylococcus capitis urealyticum	1	1.85
Staphylococcus xylosus	1	1.85
Ureaplasma urealyticum	7	12.96
Chlamydia trachomatis	3	5.56
Total	54	100.00

Quantitative expression of neutrophile granulocytes CD_{64}

The expression level of CD_{64} in the prostatic fluid of the subjects in the experimental and control groups is shown in Table IV.

Table IV

Comparison of expression level of CD_{64} in the prostatic fluid

Group	Pathogens	Expression level of CD_{64} (N/cell, mean ± SD)
Control group		951.605 ± 112.254
Experimental group	*Staphylococcus simulans*	3912.237 ± 767.416**
	Staphylococcus auricularis	2764.371 ± 141.261**
	Staphylococcus warneri	1916.356 ± 127.284**
	Enterococcus faecalis	1893.636 ± 314.458**
	Staphylococcus haemolyticus	1874.328 ± 125.212**
	Staphylococcus hominis subspecies *hominis*	1627.835 ± 107.546*
	Staphylococcus epidermidis	1301.691 ± 143.227
	Staphylococcus xylosus	1237.246 ± 125.248
	Staphylococcus capitis subspecies *urealyticum*	1212.727 ± 107.899
	Chlamydia trachomatis	951.33 ± 131.244
	Ureaplasma urealyticum	947.17 ± 127.561

* indicated $p < 0.05$ compared to the control group; ** indicated $p < 0.01$ compared to the control group

The expression levels of CD_{64} in patients infected with Ct and Uu in the experimental group had no significant difference compared to the control group ($p > 0.05$); in the experimental group, patients infected with *Staphylococcus simulans* had the highest expression level of CD_{64} (3912.237 ± 767.416/cell), followed by *Staphylococcus auricularis* (2764.371 ± 141.261/cell). There was a very significant difference between the experimental group and control group in the expression level of CD_{64} in patients infected with *Staphylococcus simulans*, *Staphylococcus auricularis*, *Staphylococcus warneri*, *Enterococcus faecalis* and *Staphylococcus haemolyticus* ($p < 0.01$). The expression level of CD_{64} in patients infected with *Staphylococcus hominis* subspecies *hominis* in the experimental group was significantly different compared to the levels of control group ($p < 0.05$). The quantitative expression of CD_{64} for *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus capitis urealyticum* infected patients in the experimental group was not significantly different from that in the control group ($p > 0.05$).

Detection rates of CD_{64} and C-reactive protein (CRP)

Fifty patients were randomly selected from the 72 patients of the experimental group to assess the positivity rates of CD_{64} and CRP according to the bacterial culture results (Table V).

Table V

Results of analysis of CD_{64} and CRP positivity rates as marker for diagnosis of chronic prostatitis

Bacterial culture	Total	CD_{64} positive*	CD_{64} negative*	CRP positive*	CRP negative*
Positive	23	35	1	28	5
Negative	27	11	3	6	11
Total	50	46	4	34	16
p		< 0.01		< 0.05	

0 < CRP < 3 mg/L indicated negative and CRP > 3 mg/L indicated positive results; 0 < CD_{64} < 1000 indicated negative and CD_{64} > 1000 indicated positive results

In Table V, the rate of positive bacterial cultures was 46% (23/50), whereas the rate of CD_{64} detection was 92% (46/50), and the rate of CRP detection was 68% (34/50). The rate of CD_{64} detection was higher than that of the other two experiments, and the difference was statistically significant ($p < 0.05$). It indicated that the rate of CD_{64} detection was high and the rates of CD_{64} and CRP detection were higher than that of bacterial culture.

Sensitivity and specificity of CD_{64} and CRP.

The sensitivity and specificity of CD_{64} and CRP in the diagnosis of chronic prostatitis were calculated. Receiver operator characteristics (ROC) analysis was made on the data of the experimental and control groups (Table VI).

Table VI

The sensitivity and specificity of CD_{64} and CRP

Item	Critical value	Sensitivity (%)	Specificity (%)
CD_{64} (N/cell)	3071.32	91.6	71.4
	2691.84	97.9	43.2
CRP (mg/L)	0.73	84.8	30.7
	2.8	29.1	88.9

As shown in Table VI, the sensitivity and specificity of CD_{64} in the diagnosis of chronic prostatitis were 91.6% and 71.4% respectively when the critical value was 3071.32 (N/cell) and 97.9% and 43.2% respectively, when the critical value was 2691.84 (N/cell).

The sensitivity and specificity of CRP in the diagnosis of chronic prostatitis were 84.8% and 30.7% respectively, when the critical value was 0.73 mg/L and 29.1% and 88.9% respectively, when the critical value was 2.8 mg/L.

Both CD_{64} and CRP suggested obvious sensitivity and specificity for the diagnosis of chronic prostatitis. Moreover, the sensitivity of CD_{64} was higher than the specificity.

Therefore the combination of CD_{64} and CRP could improve the detection rate.

Chronic prostatitis is a common disease among males and has an increasingly higher incidence in recent years [7, 8], and it tends to occur in younger people. Up to date, there are no accurate determination standards for the diagnosis and classification of chronic prostatitis in clinical practice [12]. Hence a more high-efficient and accurate method is needed for the diagnosis and treatment of chronic prostatitis, which is the main goal of the present study.

Therefore, this study tested and analysed the pathogens in prostatic fluid and the levels of CD_{64} [2]. The results demonstrated that 54 strains of pathogens were detected out from the 72 specimens (75%), among which, 44 strains were bacteria (81.48%), 7 strains were Uu (12.96%) and 3 strains were Ct (5.56%). Hence Gram positive bacteria are the main pathogens inducing chronic prostatitis.

Chesnokova MG *et al*. [3] made a microbiological study on the urine and prostatic secretion of 35 patients with chronic bacterial prostatitis, aged from 35 to 75 years, and performed the bacterial examination. One hundred and sixty-three strains were isolated, among which, 13 strains were *Staphylococcus*. Their study confirmed the importance of *Staphylococcus spp.* in the aetiology of chronic bacterial prostatitis.

In our study there was a significant difference between the experimental and control groups in the expression of CD_{64} in *Staphylococcus simulans*, *Staphylococcus auricularis*, *Staphylococcus warneri*, *Enterococcus faecalis* and *Staphylococcus haemolyticus* ($p < 0.01$); the expression of CD_{64} of *Staphylococcus hominis* subspecies *hominis* in the experimental group was significantly different compared to the control group ($p < 0.05$).

Zhu J *et al*. [18] detected the prostatic fluid specimens of 123 patients who were diagnosed with type III prostatitis and 84 healthy individuals, analysed the difference of neutrophil elastase, chronic prostatitis syndrome index score and count of white blood cells between the two groups, and found that the level of neutrophil elastase (NE) in expressed prostatic secretion was an important marker for the diagnosis of IIIA and IIIB prostatitis.

Qian L *et al*. [13] cultured prostatic fluid collected from 116 patients with chronic prostatitis and 27 healthy individuals, then detected the expression of CD_{64}, and found that the expression of CD_{64} in white cells in prostatic fluid was associated to bacterial infection and could be regarded as a marker for the early diagnosis of chronic prostatitis.

The above findings suggested that the apparent increase of CD_{64} expression in the prostatic fluid of patients with chronic prostatitis was associated to bacterial infection; hence it may have a biomarker potential.

Conclusions

In conclusion, Gram positive bacteria are the major pathogens inducing chronic prostatitis. The expression of CD_{64} in prostatic fluid of patients with chronic prostatitis is in a positive correlation with bacterial infection, and the detection rate of CD_{64} is high. Hence it can be regarded as a potent biomarker for the diagnosis and monitoring of chronic prostatitis.

Acknowledgement

This study was supported by the Project of Science and Technology Development of Jining [No. (2015)57 -39], the Project of Science and Technology Development of Medicine and Health of Shandong (2015WS0410) and the Fund for Natural Science of Shandong (ZR201702220088). The authors acknowledge the financial support provided by the Natural Science Foundation Projects of Shandong Province (No. ZR2013HL013).

References

1. Antoine JMR, Fung LAH, Grant CN, Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicology Reports*, 2017; 4:181-187.
2. Biggs CN, Siddiqui KM, Alzahrani AA, Pardhan S, Brett SI, Guo QQ, Yang J, Wolf P, Power NE, Durfee PN, MacMillan CD, Townson JL, Brinker JC, Fleshner NE, Izawa JI, Chambers AF, Chin JL, Leong HS, Prostate extracellular vesicles in patient plasma as a liquid biopsy platform for prostate cancer using nanoscale flow cytometry. *Oncotarget*, 2016; 7(8): 8839-8849.
3. Chesnokova MG, Novikov AI, Iua N, Kabanov VN, Solov'Eva TD, Characteristics of microbiological diagnosis of chronic bacterial prostatitis. *Urologiia*, 2010; (6): 47-50.
4. Corrales-Aguilar E, Trilling M, Hunold K, Fiedler M, Le VT, Reinhard H, Ehrhardt K, Mercé-Maldonado E, Aliyev E, Zimmermann A, Johnson DC, Hengel H, Human cytomegalovirus Fcγ binding proteins gp34 and gp68 antagonize Fcγ receptors I, II and III. *PLoS Pathog.*, 2014; 10(5): 1-17.
5. Cybulsky MI, Cheong C, Robbins CS. Macrophages and Dendritic Cells: Partners in Atherogenesis. *Circul Res*., 2016; 118(4): 637-652.
6. Gumustekin M, Arici A, Cilaker Micili S, Karaman M, Guneli ME, Tekmen I, HGF/c-Met pathway has a role in testicular damage in diabetes induced by streptozotocin. *Acta Endo (Buc)*, 2017; 13(1): 17-22.
7. Hedelin H, Johannisson H, Welin L, Prevalence of the chronic prostatitis/chronic pelvic pain syndrome among 40–69-year-old men residing in a temperate climate. *Scand J Urol*., 2013; 47(5): 390-392.
8. Khaikova A, Sohar S, Kazlouskaya V. P3-S1.05 incidence of STI in patients with chronic prostatitis. *Sex Transm Infect*., 2011; 87(Suppl1): A268-A268.
9. Li S, Huang X, Chen Z, Zhong H, Peng Q, Deng Y, Qin X, Zhao J, Neutrophil CD_{64} expression as a biomarker in the early diagnosis of bacterial infection: a meta-analysis. *Int J Infect Dis: Offic Publ Int Soc Infect Dis*., 2013; 17(1): 12-23.
10. Magistro G, Wagenlehner FM, Grabe M, Weidner W, Stief CG, Nickel JC. Contemporary management of chronic prostatitis/chronic pelvic pain syndrome. *Eur Urol*., 2016; 69(2): 286-297.
11. Moon SH, Kim D, Shimizu N, Okada T, Hitoe S, Shimoda H, Ninety-day oral toxicity study of rice-derived γ-oryzanol in Sprague-Dawley rats. *Toxicology Reports*, 2017; 4: 9-18.
12. Pontari M, Giusto L, New developments in the diagnosis and treatment of chronic prostatitis/ chronic pelvic pain syndrome. *Curr Opin Urol*., 2013; 23(6): 565-569.
13. Qian L, Li S, Zhou Y, Teng SJ, Guo JJ, Determination of CD_{64} for the diagnosis of bacterial chronic prostatitis. *Am J Reprod Immunol*., 2015; 74(4): 309-312.
14. Vari CE, Ősz BE, Perian M, Mărușteri MS, Miklos A, Bosa P, Tero-Vescan A, Do Aromatase Inhibitors Reduce Fertility And Impair Sexual Behaviour In An Androgen Doping Model In Rats?. *Farmacia*, 2017; 65(3): 336-342.
15. Vermassen T, Praet C V, Poelaert F, Nicolaas L, Decaestecker K, Hoebeke P, Belle SV, Rottery S, Delanghe JR, Diagnostic accuracy of urinary prostate protein glycosylation profiling in prostatitis diagnosis. *Bioch Med*., 2015; 25(3): 439-449.
16. Velescu BȘ, Anuța V, Nițulescu GM, Olaru OT, Orțan A, Ionescu D, Ghica MV, Drăgoi CM, Dinu Pîrvu CE, Pharmaceutical assesment of Romanian crops of *Anthriscus Sylvestris* (*Apiaceae*), *Farmacia*, 2017; 65(6): 824-831.
17. Xu N, Chen J, Chang X, Zhang J, Liu Q, Li A, Lin D, nCD_{64} index as a prognostic biomarker for mortality in acute exacerbation of chronic obstructive pulmonary disease. *Ann Saudi Med*., 2016; 36(1): 37-41.
18. Yang AP, Liu J, Yue LH, Wang HQ, Yang WJ, Yang GH, Neutrophil CD_{64} combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. *Clin Chem Lab Med*., 2016; 54(2): 345-351.
19. Zhu J, Yang C, Dong Z, Li L, The value of neutrophil elastase in diagnosis of type III prostatitis. *Urol J*., 2014; 11(3): 1666-1672