International Journal of Research Studies in Biosciences (IJRSB)
Volume 9, Issue 1, 2021, PP 33-38
ISSN No. (Online) 2349-0365
DOI: https://doi.org/10.20431/2349-0365.0901004
www.arcjournals.org

Different Expression of Vitamin D and Vitamin D Receptor Stimulated by Cigarette Smoking

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Objective

Objective to investigate the level of vitamin D in serum and the expression of vitamin D receptor in lung tissue of smoking patients, so as to provide reference for clinical treatment.

Methods

Serum and lung tissues of patients with lung cancer were collected and divided into smoking group and control group. Enzyme linked immunosorbent assay (ELISA) was used to detect the level of vitamin D in serum. Real time quantitative polymerase chain reaction (PCR) and Western blot were used to detect the effect of smoking on the expression of vitamin D receptor.

Results

The level of vitamin D and the expression of vitamin D receptor mRNA and protein in smoking group were higher than those in control group.

Conclusion

The increased expression of vitamin D and its receptor may be involved in the enhancement of lung fluid clearance in patients with chronic obstructive pulmonary disease (COPD), which will provide a theoretical basis for seeking suitable drugs to reduce airway mucus hypersecretion in COPD patients in the future.

Keywords: *Chronic obstructive pulmonary disease (COPD); Smoking; Vitamin D*

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a kind of chronic bronchitis and / or emphysema with the characteristics of airflow obstruction. Due to its slow progressive development, it seriously affects the labor ability and quality of life of patients. To study the pathogenesis of COPD is of great significance to the prevention and treatment of COPD[1-3]. It is of great significance.

Smoking and smog are high risk factors for COPD. COPD is related to the abnormal inflammatory reaction of the lung to the harmful gases or harmful particles such as cigarette smoke. The morbidity and mortality are very high. The incidence rate of the disease is over 9% to 10% worldwide over the age of 40. In recent years, most areas of China have been affected by the haze weather, the air has reached a serious pollution or serious pollution, the number of patients with respiratory tract infection

has increased significantly, the number of outpatients and the hospitalization rate of COPD patients in most areas are increasing. COPD is a kind of disease with the characteristics of airflow limitation[4]. Airflow limitation is not completely reversible and progressive. In addition to stopping smoking, no other treatment can change the course of COPD [5-6].

Increased airway mucus secretion is the main manifestation of bronchitis and COPD caused by smoking. Studies have shown that the clearance rate of alveolar fluid in COPD patients is increased, and its mechanism may be related to the regulation of epithelial sodium channel protein. A large number of literatures have reported that there is a close dose-dependent relationship between vitamin D and lung function [7-8]. The purpose of this study was to investigate the levels of vitamin D in serum and the expression of vitamin D receptor in lung tissue of smoking patients, so as to clarify the possible role of vitamin D in smoking patients and provide theoretical basis for the mechanism of airway mucus hypersecretion in COPD patients.

2. DATA AND METHOD

2.1 Clinical data: with the approval of the ethics committee of Hunan Provincial People's Hospital and the informed consent of the patients, this study applied the lung segment specimens of patients with bronchial lung cancer undergoing surgical resection. Preoperative chest CT showed no fibrosis and emphysema, and all patients had normal pulmonary function. In the morning, blood samples were collected on an empty stomach for anticoagulation. The samples were centrifuged at 4 ℃ 1000 ×g for 15 minutes within 30 minutes after collection. The patients were divided into control group and smoking group according to whether they smoked or not (at least 20 cigarettes a day, more than 10 years of smoking history).

2.2 Instruments and reagents: vitamin D detection kit (Nanjing senbeijia Biotechnology Co., Ltd.); Trizol (American Life Technologies Co., Ltd.); reverse transcription kit, real-time quantitative polymerase chain reaction (PCR) kit, primer sequence synthesis (Takara company of Japan); mouse anti vitamin D receptor (VDR) monogram. The results showed that the anti-β-actin monoclonal antibody, anti-β-actin monoclonal antibody and electrochemi luminescence (ECL) luminescence solution were used.

2.3 Enzyme linked immunosorbent assay (ELISA) was used to determine the level of vitamin D in serum: 30 minutes before the detection, the ELISA kit and human blood samples were taken out from the refrigerator and balanced to room temperature, the sample diluent was used to dilute the standard and samples, and the experiment was carried out according to the operation steps of the manual. The absorbance (a) value was read at the detection wavelength of 450nm, and the actual a value of each sample was calculated. The concentration of vitamin D in serum was calculated by software.

2.4 Western blot was used to detect the changes of VDR expression in the control and smoking groups: protein lysis was used to extract the protein, and after heating denaturation, the protein was separated by 10% twelve alkyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) and the protein was transferred to PVDF membrane. 5% skim milk was blocked for 1 hour, and the first antibody was 1:1000 diluted mouse anti human VDR or β-actin. After incubation at 4 ℃ overnight, the membrane was washed with tbst three times for 10 minutes each time. Then anti mouse IgG labeled with anti horseradish peroxidase was added and diluted with 1:2000. After incubation at room temperature for 1 h, tbst washed the membrane and reacted with ECL reagent. X-ray film exposure, development, analysis after fixing.

2.5 Real time quantitative PCR: Total RNA was extracted by Trizol kit. The expression of VDR was detected by real-time PCR. Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as

internal reference, and the specific oligonucleotide primer of hvdr was forward primer The reverse primer was 5′- CATGCCGATGTCCACA-3′. The specific oligonucleotide primers of hgapdh were: forward primer: 5′-ACCACAGTCCATGCATCAC-3′; reverse primer: 5′-TCCACCCTGTGCCTGTA-3′. The reaction conditions were pre denaturation at 95 ℃ for 5 min, pre denaturation at 95 ℃ for 10s, pre denaturation at 60 ℃ for 10s and pre denaturation at 72 ℃ for 10s, 40 cycles in total. Results the comparative threshold method was used for quantitative analysis. The copy number of target gene was calculated and compared for each sample.

2.6 Statistical treatment: SPSS18.0 software was used for statistical analysis, the measurement data was expressed by x ± s, and the t-test was used for the comparison between the groups. $P < 0.05$ was statistically significant.

3. Result

3.1 Effect of smoking on serum vitamin D level of patients: in this experiment, ELISA method was used to detect the serum vitamin D level of patients. The serum vitamin D levels in smoking group and control group were (1183 ± 116) nmol / L and (815 ± 36) nmol / L, respectively. Compared with the control group, the serum vitamin D level in the smoking group was significantly higher, the difference was statistically significant ($P < 0.05$).

3.2 Effect of smoking on the expression of VDR mRNA: the results of real-time quantitative PCR showed that the expression level of VDR in lung tissue of smoking group (4.61 ± 1.05) was significantly higher than that of control group (1.00 ± 0.00), and the difference was statistically significant ($P < 0.05$).

3.3 The effect of smoking on the expression of VDR protein: as shown in Figure 1, the results of Western blot showed that the expression level of VDR protein in the lung tissue of the smoking group (1.04 ± 0.09) was significantly higher than that of the control group (0.65 ± 0.07), and the difference was statistically significant ($P < 0.05$).

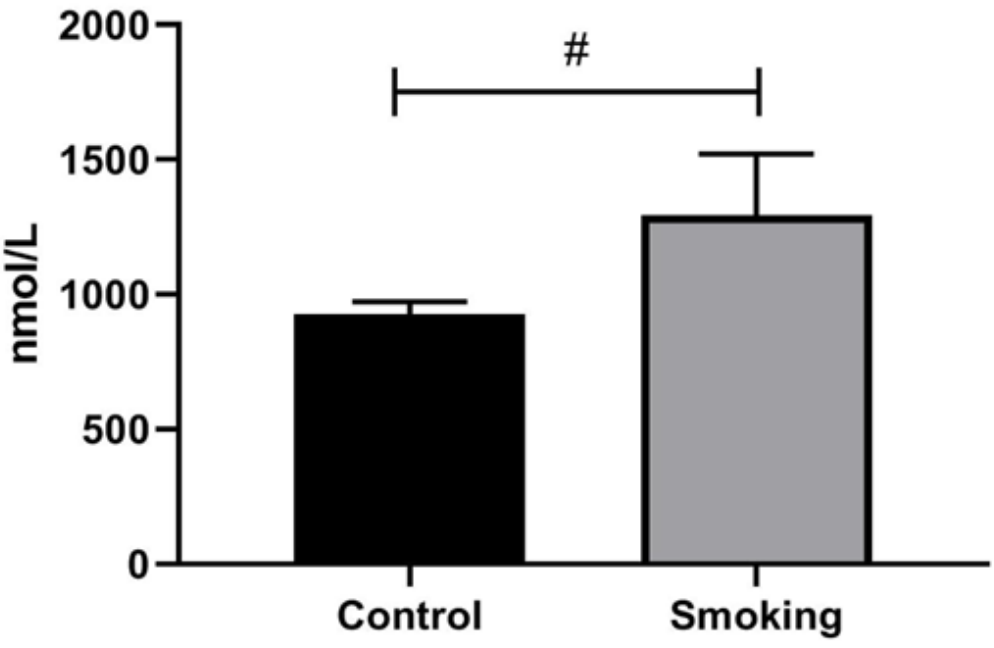

Figure 1. *Effect of smoking on serum vitamin D level of patients. The difference between the smoking group and the control group was statistically significant ($^{\#}P < 0.05$).*

4. DISCUSSION

The protective effect of vitamin D on lung function has been fully confirmed. The related research theories supporting this view are: low vitamin D content is easy to cause osteoporotic vertebral fracture, cause limitation of rib movement and functional defect of inspiratory muscle, thus affecting lung function[9-10]. Infection is the main reason of acute exacerbation of COPD, and vitamin D deficiency can reduce the effect of the body to kill some antibiotic resistant strains, viruses and atypical pathogens, which leads to respiratory tract infection and bacterial colonization[11]. In addition, if T cells want to detect and kill foreign bacteria, viruses and other pathogens, they must first be activated and transformed from harmless and inactivated immune cells into active killer cells, which need vitamin D to activate[12]. Vitamin D regulates the dynamic balance of extracellular matrix in lung tissue and contributes to emphysema caused by aging or smoking[13]. Vitamin D may be combined with stress smoking and air pollution to imbalance oxidative stress - antioxidant stress, cause lung inflammation, and thus affect lung function[14].

Figure2. *Effect of smoking on VDR mRNA expression. The difference between the smoking group and the control group was statistically significant ($^{\#}P < 0.05$).*

Vitamin D works through its receptor VDR. The results of this study showed that the levels of vitamin D in serum and VDR mRNA and protein expression in lung tissue of smoking group were significantly higher than those of control group; at the same time, previous studies showed that vitamin D could up regulate the expression of epithelial sodium channel and enhance its function. One of the main characteristics of COPD is increased mucus secretion, and smoking has been proved to be the main cause of this disease. Based on the results of this study and previous studies of our group, we believe that the increased expression of VDR may be involved in the enhancement of lung fluid clearance in COPD patients, which will provide a theoretical basis for seeking suitable drugs to reduce airway mucus hypersecretion in COPD patients in the future.

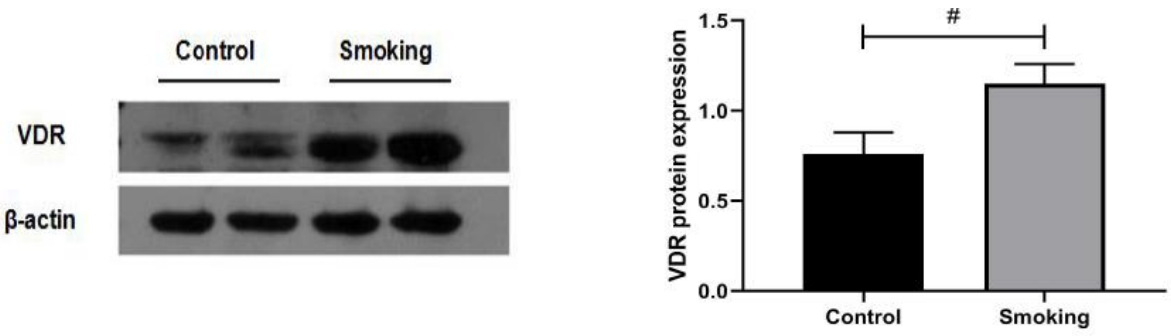

Figure3. *Effect of smoking on the expression of VDR protein. The difference between the smoking group and the control group was statistically significant ($^{\#}P < 0.05$).*

ACKNOWLEDGMENT

The present study was partially supported by the RenShu fund research project of Hunan Provincial People's Hospital (No. RS201701).

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Citation: *Shu-Ying You, et.al., Different Expression of Vitamin D and Vitamin D Receptor Stimulated by Cigarette Smoking. International Journal of Research Studies in Biosciences. 2021; 9(1): 33-38. DOI: https://doi.org/10.20431/2349-0365.0901004.*