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Research article

Anti-citrullinated peptide antibodies in rheumatoid arthritis patients exposed to wood smoke

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Abstract: *Background and Aims:* Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune disease with an unknown etiology, which attacks the synovial tissue more than any other organ. Citrulline has been observed in the joints of RA patients. Anti-citrullinated peptide antibodies (ACPAs), which are recognized as the most specific serologic markers of RA, are synthesized against unusual citrullinated peptides during the disease. There is little information about the increased production of antibodies against citrullinated peptides in RA patients exposed to wood smoke. Therefore, in this study, we aimed to compare the serum level of ACPA in two groups of exposed and non-exposed RA patients to wood smoke. *Materials and Methods:* A total of 110 RA patients, including 55 exposed patients to wood smoke and 55 non-exposed patients, were enrolled in this study. The serum level of ACPA, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and disease activity (based on the 28-joint Disease Activity Score) were determined in patients and compared between the two groups. *Results:* In this study, there was no significant difference in the serum level of ACPA between the exposed and non-exposed groups (P = 0.73). On the other hand, RF (P = 0.03), ESR (P = 0.007), and disease activity index (P = 0.01) were significantly higher in the exposed group, compared to the non-exposed group. *Conclusion:* According to the results of the current study, pollutants from wood smoke significantly increased the RF, ESR, and disease activity

index in RA patients. Nonetheless, there was no significant difference in the serum level of ACPA between the two groups.

Keywords: rheumatoid arthritis; wood smoke; anti-citrullinated peptide antibodies

1. Introduction

Rheumatoid arthritis (RA), affecting approximately 1% of the world's population, is a chronic, systemic, autoimmune disease with an unknown etiology, which attacks the synovial tissue more than any other organ. Progression of this disease shows a slow and very destructive course in patients. Therefore, timely diagnosis and treatment can prevent joint damage and disability in RA patients [1]. Moreover, it has been demonstrated that environmental and genetic factors play important roles in predisposing a person to the disease [2].

Despite the unclear source of inflammation in the immunopathogenesis of RA, evidence suggests the possibility of inflammation in areas far from the synovial joint [3]. Considering the pathogenesis of RA, recent studies have paid more attention to the involvement of immune disorders in the lungs [4], and there is evidence regarding the role of inhaling cigarette smoke and possibly other atmospheric pollutants in the progression of RA [5]. Cigarette smoking has been identified as an important environmental risk factor for RA. It results in the increased expression of HLA-DRB1, which leads to immune responses and increased production and release of inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) [6–8]. Moreover, some evidence suggests that cigarette smoking may induce citrullination in the lung cells [9,10].

Approximately three billion people use fossil fuels (biomass fuels) worldwide, including wood for home cooking and heating [11]. This type of contamination is a major risk factor for chronic obstructive pulmonary disease (COPD) in developing countries [12]. A recent study indicated that the serum levels of anti-citrullinated peptide antibodies (ACPA) significantly increased in patients with COPD induced by wood smoke, compared to patients with cigarette smoking-induced COPD and healthy individuals [12]. Consequently, wood smoke may be a risk factor for the production of ACPA.

Citrulline is a post-translational modification of amino acid arginine by peptidylarginine deiminase, which increases in inflammatory processes [13]. Citrullination is recognized as a key process in the pathogenesis of RA, especially in the joints of RA patients [14]. During the disease, ACPAs are synthesized against these unusual citrullinated peptides and represent the most specific serological marker of RA. In the early stages of the disease and before the onset of clinical symptoms, the ACPA titer begins to rise with a high predictive value. Measurement of this antibody is beneficial for the prognosis of RA, as it is closely related to the destructive forms of arthritis [15–17].

A large number of studies on pollutants (e.g., smoking cigarettes) have addressed their role in the pathogenesis of RA, their involvement in disease activity and severity, their effects on response to treatment, and possibility of mortality. However, there is a dearth of research on wood smoke, its related harms, and chronic inflammatory reactions, which may lead to the production of ACPA. Considering the impact of exposure to wood smoke, in this study, we aimed to investigate the serum level of ACPA in RA patients.

2. Materials and methods

2.1. Study design

This cross-sectional, case-control study was performed on patients above 16 years, who were referred to the Rheumatology Clinic of Tohid Hospital in Sanandaj, Iran, from October 2013 to March 2014. Diagnosis of RA was based on the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) criteria (ACR-EULAR 2010). The implementation protocol was approved by the Ethics Committee of Kurdistan University of Medical Sciences, Sanandaj, Iran (No.: IR.MUK.REC.1392.81). In addition, a written informed consent was obtained from each participant prior to the research.

2.2. Participants

The patients were divided into the case (exposed to wood smoke) and control (non-exposed to wood smoke) groups. The inclusion criteria for the case group were diagnosis of RA, exposure to wood smoke from cooking or domestic heating appliances, and lack of occupational risk factors. On the other hand, RA patients with no history of wood smoke exposure were allocated to the control group. The exclusion criteria were pulmonary disorders, psoriasis, Sjogren's syndrome, lupus erythematosus, history of hepatitis C virus infection or other autoimmune disorders, and history of cigarette smoking or secondhand smoke.

The sample size was estimated at 55 per group based on previous studies [18] ($P_1 = 44.9\%$, $P_2 = 20\%$, 95% confidence level, and 80% power). The subjects were selected via convenience sampling among RA patients, who were referred to the Rheumatology Clinic of Tohid Hospital. Data collection continued until reaching data saturation.

2.3. Demographic and clinical data collection

Demographic data, history of chronic diseases, duration and onset of symptoms, morning stiffness, number of involved joints, RA-based radiological changes, rheumatoid nodules, severity of disease, and duration of exposure to wood smoke were collected by a physician using a questionnaire. In addition, disease activity was assessed based on the 28-Joint Disease Activity Score (DAS28), which is based on the number of joints with tenderness (0–28), number of joints with inflammation (0–28), erythrocyte sedimentation rate (ESR), and pain score according to the visual analog scale (VAS). Scores within the range of 2.6–3.2 were indicative of mild activity, whereas scores of 3.2–5.1 and > 5.1 represented moderate and high activities, respectively.

To determine the pain level, VAS was used to convert the subjective characteristics of pain to objective ones; the scores of this scale were in the range of 0–100. In this scale, score of 100 was indicative of the highest pain intensity, while score of 0 was indicative of the lowest pain severity (no pain).

2.4. Experimental evaluations

Following 8–12 hours of fasting, venous blood samples were drawn from the participants. The blood samples were divided into two citrated tubes (5 mL) for ESR measurements. They were also added separately to jelly-coated flat tubes (10 mL) for ACPA, RF, and CRP measurements. Serum samples for ACPA measurements were studied using the Euroimmun Kit (Lübeck, Germany). The levels of C-reactive protein (CRP) and rheumatoid factor (RF) were measured using the immunoturbidimetric assay on an Olympus AU2700 autoanalyzer, while ESR was measured using the Westergreen method. The main objective of the present study was to compare the serum level of ACPA between the two groups, and the secondary objective was to conduct a comparative analysis of disease severity (DAS28), serum RF level, and ESR between the groups.

2.5. Statistical analysis

Statistical analysis was done using SPSS software version 20. Kolmogrov-Smirnove test was used to evaluate the assumptions for the normalization of quantitative data. Quantitative variables were assessed for normal and abnormal distribution of data by measuring the mean (SE) and median (IQR) respectively, Qualitative variables were presented as frequency (percentage). Mann-Whitney U test, t-test, and Chi-square test were also used to evaluate data with an abnormal distribution, data with a normal distribution, and comparison of qualitative variables, respectively. P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical data

The demographic and clinical data of 110 RA patients are shown in Table 1. According to the results, a significant direct correlation was observed between living in a village and exposure to wood smoke ($P = 0.006$). The median age of the subjects in the case and control groups was 54 years (range: 30–72 years) and 52 years (range: 27–75 years), respectively, without any significant difference between the groups ($P = 0.07$). Also, no significant difference was observed between the two groups in terms of gender, duration of disease, or body mass index (BMI) ($P > 0.05$). The mean disease activity index (DAS28) was significantly higher in the case group, compared to the control group (4.12 ± 0.84 vs. 3.61 ± 1.16; $P = 0.01$). Nonetheless, evaluation of VAS showed no significant difference between the two groups ($P = 0.13$).

3.2. Blood markers

As shown in Table 2, evaluation of blood factors in the groups indicated that RF ($P = 0.03$) and ESR ($P = 0.007$) were significantly higher in the case group, compared to the control group. However, no significant difference was found between the two groups in terms of other blood markers ($P > 0.05$).

Table 1. Demographic and clinical data of RA patients in the two groups.

Characteristics	Wood smoke N = 55	Not exposed N = 55	P-value
Age (years), Med (max–min)	54 (30–72)	52 (27–75)	0.07††
Sex (M/F)	(1/54)	(7/48)	0.06 ¥
Residence (urban/rural)	(32/23)	(46/9)	0.006 ¥
BMI (kg/m^2), Mean ± SD	28.97 ± 4.61	28.14 ± 4.42	0.35†
Disease duration (years), Mean ± SD	10.2 ± 4.4	9.5 ± 3.8	0.24†
Wood smoke exposure (years), %			
< 10	9 (16%)	-	
> 10	46 (84%)	-	
DAS-28, Mean ± SD	4.12 ± 0.84	3.61 ± 1.16	**0.01†**
VAS, Med (IQR)	80 (50–100)	80 (50–100)	**0.13††**

†: Independent T test was used for analysis; ††: Mann-Whitney U test was used for analysis; ¥: Fisher's exact test was used for analysis. **Abbreviations:** BMI: Body Mass Index; DAS-28: Disease Activity Score-28; VAS: Visual Analogue Scale.

Table 2. Comparison of blood markers between wood smoke-exposed and non-exposed RA patients.

Variables	Wood smoke N = 55	Not exposed N = 55	P-value
RF (IU/ml)	10 (5–15)	5 (4–13)	**0.03††**
ESR (mm/h), Med (IQR)	28 (17–50)	17 (10–32)	**0.007††**
WBC (10^9/L), Med (IQR)	6.7 (5.5–8.5)	7.5 (6.5–8.5)	0.13††
Cr (mg/dL), Med (IQR)	0.8 (0.8–0.8)	0.8 (0.8–0.9)	0.80††
Hemoglobin (g/dL), Mean ± SD	12.92 ± 1.36	13.03 ± 1.50	0.69†

†: Independent T test was used for analysis; ††: Mann-Whitney U test was used for analysis. **Abbreviations:** RF: Rheumatoid factor; ESR: Erythrocyte Sedimentation Rate; WBC: White blood cell; Cr: Creatinine.

3.3. ACPA level and its relationship with patient characteristics

The median serum level of ACPA was 42 in the case group (IQR: 11–261) and 34 in the control group (IQR: 14–180); the difference was not statistically significant (P = 0.73) (Figure 1). Considering the positive (> 20 IU) and negative (≤ 20 IU) levels of the antibody, 34 (62%) subjects in the case group and 37 (67%) subjects in the control group had positive, no significant differences were found between them (P = 0.69).

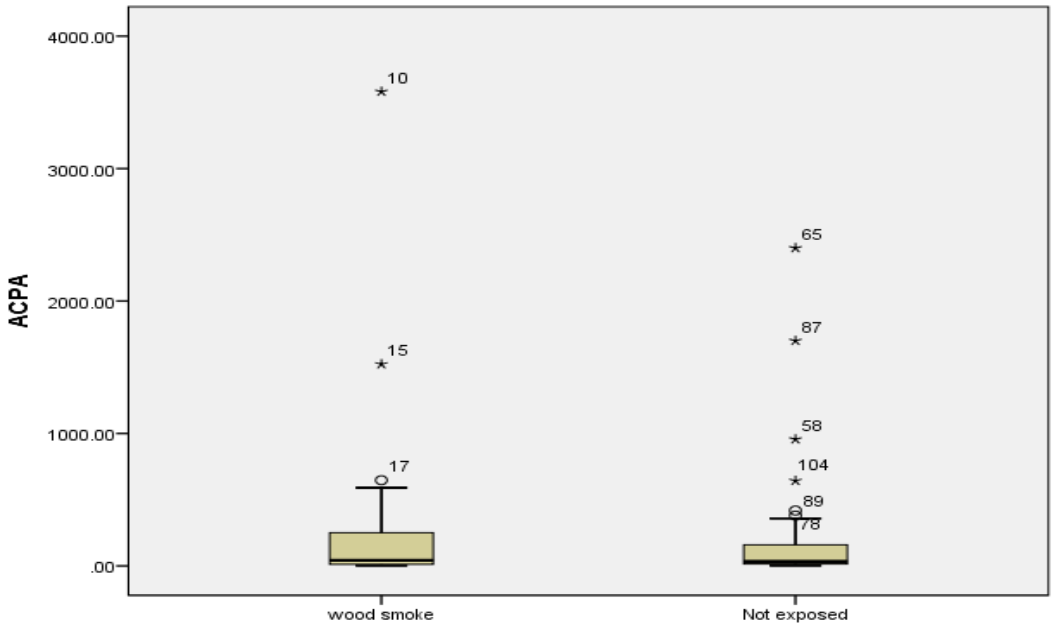

Figure 1. Median levels of anti-citrullinated peptide antibodies (ACPA) in patients with rheumatoid arthritis (RA) with or without wood smoke exposure (55 with and 55 without wood smoke exposure, P = 0.73).

No significant correlation was observed between age and antibody level in patients exposed to wood smoke (P = 0.37). However, in the control group, there was a moderate correlation between the level of antibody and age, based on Pearson's correlation test (P = 0.004, r = 0.38); in other words, the level of antibody significantly increased with aging.

In addition, there was no significant correlation between the ACPA level and gender, BMI, duration of disease, disease activity index, VAS, or blood markers in the two groups (P > 0.05). Furthermore, no significant correlation was found between the antibody level and duration of exposure to wood smoke (r = 0.25, P = 0.08). Classification of patients based on the disease activity index (DAS28) into three groups (inactive, moderate, and active) revealed no significant difference regarding the ACPA titer between the case and control groups (Table 3).

Table 3. Classification of patients in the groups based on DAS-28 score and comparison of ACPA level between wood smoke-exposed and non-exposed RA patients.

DAS-28 score	Wood smoke		Not exposed		P-value
	N	ACPA, Med (IQR)	N	ACPA, Med (IQR)	
Inactive (≤ 3.2)	11	41 (11–248)	20	30 (6–75)	0.35
Moderate (> 3.2 but ≤ 5.1)	40	40 (10–290)	30	49 (19–277)	0.26
Active (> 5.1)	4	127 (19–495)	5	14 (5–97)	0.28

Abbreviations: DAS-28: Disease Activity Score-28; ACPA: Anti-citrullinated peptide antibodies.

4. Discussion

Although several studies have indicated that pollutants, such as cigarette, play a significant role in the pathogenesis of RA [19–21], little research has been conducted on wood smoke. The present case-control study aimed to investigate the effect of wood smoke pollution on ACPA production, RF, and RA activity. The results suggested that the level of ACPA was not significantly different between RA patients exposed to wood smoke and the control group. In addition, the serum levels of ESR and RF were significantly higher in the case group, compared to the control group. Also, exposure to wood smoke was accompanied by a significant increase in the activity of the disease.

Antibody production against citrullinated peptides is significant in the synovial tissue of RA patients, leading to the spread of inflammatory diseases [22]. Although there is a scarcity of research on the effect of wood smoke on the mechanism of ACPA production, there is evidence that wood smoke can increase the production of ACPA. Recently, a significant increase was observed in the serum level of this antibody in 56 patients with COPD induced by wood smoke, compared to 56 healthy individuals and 56 patients with COPD induced by cigarette smoking [12]. Since this antibody is present for 5–10 years before the onset of RA symptoms in humans, and the cause of autoimmunity may appear before the onset of symptoms or clinical signs of RA [23–25], wood smoke may be considered a risk factor for RA [26].

In another study conducted by Vera-Pineda on 102 RA patients, including 46 patients exposed to biomass smoke (due to in-house cooking), there was no significant difference in ACPA and RF titers between the exposed and non-exposed groups. In addition, the average hours of exposure to biomass smoke per day, multiplied by the number of years, had no significant correlation with the ACPA or RF titers [27]. In the present study, despite the association of higher RF, ESR, and disease activity with wood smoke, there was no significant difference in the level of ACPA between the two groups.

There was no significant difference in the antibody titers between the two groups after their classification according to DAS-28 (Table 3). Moreover, there was no significant relationship between the level of disease activity and ACPA level in any of the groups. The results indicated that antibody production against citrullinated peptides did not depend on the level of disease activity in RA patients. To further examine the role of wood smoke in the increased production of antibodies, the level of antibodies must be compared between healthy individuals and RA patients exposed to wood smoke in future studies. In addition, the association of higher RF with wood smoke in this study may indicate that the immune system stimulates the production of RF regardless of the production of ACPA in the body.

One of the major limitations of this study is the absence of healthy people exposed to wood smoke, which could help with the interpretation of ACPA results and justification of the mechanisms. Also, there was a significant difference between the demographic characteristics of patients; in other words, there was a significant difference between the two groups in terms of place of residence. Also, pharmacological therapy and organ involvement with possible effects on the ACPA level were not investigated in patients; therefore, further analysis is suggested in future studies.

5. Conclusion

According to the results of the present study, exposure to pollutants from wood smoke could increase the RF, ESR, and disease activity index in RA patients. However, no significant difference was observed between the group exposed to wood smoke and the non-exposed group with regard to the serum level of ACPA.

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Conflicts of interest

The authors declare no conflicts of interest.

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