Original Article

Chlamydia pneumoniae Seropositivity in the Iranian Patients with the Skin Inflammatory Disorder of Rosacea

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Abstract

Background: *Rosacea* is a skin chronic inflammation with an unknown cause and cure. Environmental and genetic factors could not entirely explain the disease pathogenesis. Recently, infections like *Chlamydia pneumoniae* are of more attention in the rosacea progression. This study investigated the relationship between the *C. pneumoniae* seropositivity and the rosacea disorder.

Materials and Methods: We aimed at a cohort of 100 patients with the rosacea disorder (60 active and 40 inactive) and from 100 sex- and age-matched healthy controls in Isfahan and determined the immunoglobulin M (IgM)/IgG antibodies titers to *C. pneumoniae* in the serum using the enzyme-linked immunosorbent assay method. The groups were compared using the analysis of variance procedure at the significant level of $P < 0.05$, statistically.

Results: The mean of IgG in the controls was significantly higher than the levels in both the active and the inactive rosacea patients ($p < 0.022$). Also, the titer of serum IgM to *C. pneumoniae* in the controls was different, compared with the active ($p < 0.019$) and the inactive ($p < 0.02$) rosacea patients. In addition, the median titer of serum IgG (not IgM) to *C. pneumoniae* in the females with the inactive rosacea disorder was lower than the active rosacea disorder ($p < 0.019$) and controls women ($p < 0.008$). Furthermore, the serum level of IgG or IgM to *C. pneumoniae* in the controls males was higher than the males with the rosacea disorder ($p < 0.05$) and ($p < 0.02$), alternatively.

Conclusion: *C. pneumoniae* seropositivity in the rosacea patients and controls was insignificant.

Keywords: *Chlamydia pneumonia*, ELISA, IgG, IgM, *rosacea*, seropositivity

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Introduction

Rosacea as a chronic cutaneous inflammation affects all races, but people with colored skin throughout Africa, Asia, and South America have significant proportions up to 10%. The prevalence of rosacea disorder in Iran seems to be on the rise but there is no scientific evidence. Rosacea is more common in women 40–50 years old, but it may be more severe in men. There are four subgroups of rosacea that include phymatous, papulopustular, erythematotelangiectatic, and ocular.[1] It involves cutaneous manifestations such as temporary or sustained skin erythema, focal cataracts due to dilatation of

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capillaries, edema, papules, and acne commonly in the central part of the skin.[2] The therapeutic options for rosacea include topical agents, oral treatments, laser therapy, and surgical procedures. Although the pathogenicity of the disease is still unknown, genetics, environment, and microorganisms such as *Helicobacter pylori*, *Demodex folliculorum*, and so on have been considered the cause of the rosacea disorder.[1]

Recently, studies indicate that chronic inflammation with host pre-inflammatory cytokines (tumor necrosis factor-α, interleukin-1β), reactive oxygen species, nitric oxide, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP-1, MMP-3, MMP-9) could be associated with the symptoms of rosacea.[3,4] In fact, the cytokines associated with rosacea are adapted to the known pathogenicity of the gram-negative bacteria.[5] Endotoxins (lipopolysaccharides), as parts of the external membrane of the gram-negative bacteria, induce various inflammatory responses (moderate to severe).[6] Although past studies have linked bacteria like *H. pylori* and *Bacillus oleronius* to rosacea, these bacteria cannot enter the bloodstream and create the same pathogenicity.[7] More recently, other known inflammatory diseases suggest the association of *Chlamydia pneumoniae* with inflammatory diseases that may be involved in the rosacea etiology.[8] *C. pneumoniae* as a gram-negative intracellular bacterium is belonging to the Chlamydiaceae family. *C. pneumoniae* as a prevalent cause of respiratory disease is transmitted by the respiratory tract with a relatively long incubation period (3–4 weeks). Studies show *Chlamydiae* have a unique biphasic developmental cycle that leads to persistent infection causing chronic inflammatory diseases,[9] as infected macrophages transmit this bacterium from the lungs to the bloodstream and *C. pneumoniae* with the presence in the epithelial cells of the bloodstream creates chronic inflammation.[10] Elementary bodies like spores of *C. pneumoniae* circulate in the whole body through the bloodstream for infecting organs such as bone marrow, kidneys, liver, spleen, and pancreas. As *C. pneumoniae* has been found in cheek skin biopsy of rosacea patients and this may explain how *C. pneumoniae* reaches the skin and creates rosacea after entering to the body through the respiratory system.[8] Another key that links rosacea and *C. pneumoniae* is the recent studies about antimicrobial peptides of cathelicidin and their activity in the rosacea that have shown *C. pneumoniae* induces an unusually high level of cathelicidins activity.[8,11] Thus, concerning the probable role of *C. pneumoniae* in the pathogenicity of the rosacea disorder, the present study evaluated the seropositivity of *C. pneumoniae* in the rosacea patients.

Materials and Methods

Patients

In the present case–control study, 100 rosacea patients (60 active and 40 inactive) were identified by the skin diseases clinics of the Isfahan University of Medical Sciences in the Isfahan province, the central area of the Islamic Republic of Iran. This work was done from November 2020 to December 2021. The following formula was used to calculate the sample size: $N = (Z_{1-\alpha/2} + Z_{1-\beta})^2 [P_1(1-P_1) + P_2(1-P_2)/d^2$

As, in this formula, $\alpha = 0.05$, $\beta = 0.2$, and $d = 0.3$.

Patients were chosen randomly based on a pre-existing list prepared by a computer program. The patients with common symptoms such as red bumps, facial itching, burning, and swelling were being treated by the dermatologists of the skin center with or without considerable improvement. The course of the disease was defined as "active" if there was an alternation of worsening and improvement phases in the last 1 year, and "inactive" otherwise.

The rosacea patients in the age range of 17–60 years did not take the antibiotics (erythromycin, clarithromycin, azithromycin, levofloxacin, and moxifloxacin) for 1 month before the initiation of the study.

Patients who were reluctant to donate their blood samples/fill out a questionnaire.

There were 100 age and sex-matched healthy controls from the Isfahan province, the center of the Islamic Republic of Iran. Controls had not a family member or relative with the rosacea patients or a history of malignant, allergic, or autoimmune diseases.

Enzyme-linked immunosorbent assay (ELISA)

The samples of peripheral blood (5–10 ml) of patients and controls were collected and centrifuged at RPM for minutes and the separated sera were stored at − 20°C. Then, the serum levels of immunoglobulin M (IgM) and IgG to *C. pneumoniae* were evaluated by the ELISA assay, using the *C. pneumoniae* IgG and IgM kit (Order no: EI2192-9601 G, Euroimmun state, Germany). Tests were conducted according to the manufacturer's instructions and according to the laboratory kit, *C. pneumoniae* IgM titer >1.1 RU/ml and *C. pneumoniae* IgG titer >22 RU/ml were regarded as seropositive.

Statistical analysis

The serum levels of IgG and IgM to *C. pneumoniae* were mentioned as the mean ± standard deviation (SD). In this regards the serum levels of IgM and IgG *to C. pneumoniae* levels (including) were compared in the rosacea and control groups by Student's *t*-test. Furthermore, in each group, the serum levels of IgM and IgG were compared with other factors by non-parametric analysis of covariance and Chi-square tests. A P value < 0.05 was considered significant.

Ethical standards

The present study was approved (in accordance with the ethical guidelines of the 1975 Declaration of Helsinki) by the Ethical Committee of Isfahan University of Medical Sciences for Clinical Research with the code number IR.MUI.REC.1393.2.343, and the participants signed their informed written consent after the researchers explained the purpose and protocol of the study to them, and the baseline data was obtained by a researcher-made questionnaire.

Results

Patients and controls

A total of 100 rosacea patients (82 females and 18 males F:M = 4.5:1) and 100 controls (70 females and 30 males F:M = 2.3:1) were studied ($p < 0.04$). The mean age was 44.7 ± 12.4 for the rosacea group and 34.8 years (SD 11.3) for the controls. Among patients with rosacea, 40 patients (40%) had the inactive course and 60 patients (60%) had the active course of rosacea. The most common clinical manifestations of rosacea were red bumps and sensory symptoms like facial itching and burning. The baseline demographic in the rosacea patients is shown in Table 1.

ELISA analysis

The median titer of the serum levels of IgG and IgM to *C. pneumoniae* in the controls was higher than in the rosacea patients [Table 2 and Figure 1]. Baseline data and the serum levels of IgG and IgM to *C. pneumoniae* in 200 subjects (patients and controls groups) are compared in Table 2.

In addition, the median titer of the serum level IgG to *C. pneumoniae* was significantly different between the inactive and active rosacea patients, as the mean serum level of IgG in the inactive rosacea patients was significantly lower than the levels in the active rosacea patients. In contrast, the median titer of the serum level of IgM to *C. pneumoniae* in the inactive rosacea patients statistically was not different in comparison with active patients [Table 3 and Figure 2].

Furthermore, the analysis of data based on sex showed an insignificant difference in the median titer of the serum levels IgG and IgM to *C. pneumoniae* between the females and males in the rosacea patients and controls. However, the serum levels of IgM and IgG in the males and females of the controls were higher than in the males and females of rosacea patients. In addition, the serum level of IgG in males of each group was higher than women of the same group [Figure 3].

Furthermore, there was not any correlation between either *C. pneumoniae* IgG or IgM and disease duration, disease symptoms, taking supplements, menopause, history of autoimmune, sexual, and breathing diseases, but there was a statistical difference between both *C. pneumoniae* IgG or IgM and sex, age. Unlike IgM, there was a correlation between IgG and onset age and the progression of the disease. Regarding the progression of the disease, there was no significant difference between the progression of disease and time exposed to sunlight, skin color, and autoimmune diseases, but statistically correlation between the progression of disease and travel to the sea was significant.

Table 1: Baseline demographic of the rosacea patients

Characterization	Patients (*n*=100)
Family history of Rosacea	11%
Skin color	
White	36%
White to light brown	46%
Dark brown	18%
History of travel to the sea	48%
History of taking supplements	54%
History of autoimmune diseases	26%
History of Menopause	26%(Female)
History of Pap smear test	57%(Female)
History of respiratory diseases	19%
History of sexual diseases	24%
Smoking history	4%(Male)
Drinking history	2%(Male)

Table 2: Baseline data and the serum levels of IgG and IgM of the rosacea patients and controls

	Patients (*n*=100)	Control (*n*=100)	*P*
Age (year)	44.7 (±12.4)	34.8 (±11.3)	0.0
Sex	0.82 (±0.38)	0.7 (±0.46)	0.04
Onset age (year)	38.17 (±13.16)	-	-
Mean duration of Rosacea (year)	6.5 (±7.2)	-	-
C. pneumoniae IgG titer (RU/ML)	52.02 (±43.2)	66.76(±46.8)	0.022
C. pneumoniae IgM titer (RU/ML)	0.51(±0. 41)	0.70(±0. 67)	0.019

Discussion

The present study investigated the seropositivity of *C. pneumoniae* in rosacea patients. The results did not show any correlation between the *C. pneumoniae* infection and rosacea

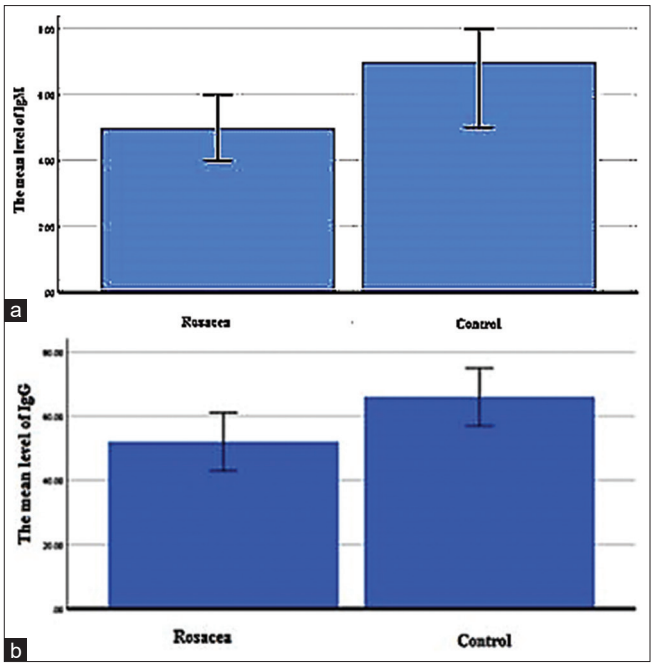

Figure 1: The mean serum levels of IgM (a) and IgG (b) to *C. pneumoniae* in the rosacea patients and controls. The serum levels of IgM and IgG to *C. pneumoniae* in the controls were higher than in the rosacea patients

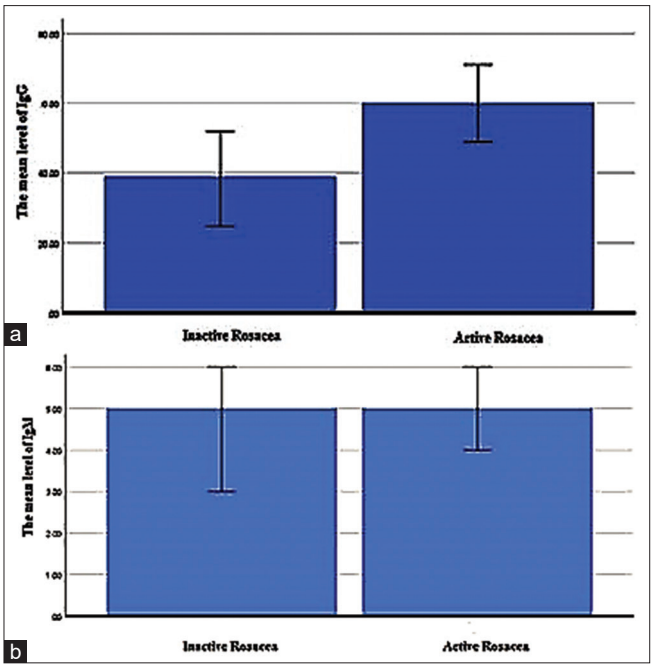

Figure 2: Comparison of the serum levels of IgG (a) and IgM (b) to *C. pneumoniae* with the rosacea disease progression. The mean serum level of IgG in the active rosacea patients was higher than the inactive rosacea patients, but the mean serum level of IgM was insignificant between the active rosacea patients and the inactive rosacea patients

Table 3: The mean level of *C. pneumoniae* IgG and IgM of the rosacea and control groups

IgG (RU/ML)			IgM (RU/ML)		
Form	**Mean**	***P***	**Form**	**Mean**	***P***
Active (*n*=60)			Active (*n*=60)		
Inactive	39.12±44.8	0.014	Inactive	0.51±0.53	0.96
Control	66.76±46.8	0.39	Control	0.70±0.67	0.02
Inactive (*n*=40)			Inactive (*n*=40)		
Active	60.6±40.2	0.014	Active	0.51±0.32	0.96
Control	66.76±46.8	0.002	Control	0.70±0.67	0.08

symptoms. The mean serum level of IgG to *C. pneumoniae* in the inactive rosacea patients was significantly lower than the serum level in the active rosacea patients and the controls; however, the difference in the mean serum levels of IgG between the active rosacea patients and the controls was insignificant.

Although other studies suggest that *C. pneumoniae* infection leads to pustular rashes and increased VEGF production that is likely to cause chronic inflammation associated with this pathogen.[12] For example, Obregon[8] suggested *C. pneumoniae* as a causative factor of rosacea. As he detected *C. pneumoniae* antigen in 4 out of 10 and also serum antibodies against *C. pneumoniae* in 8 out of 10 rosacea patients. He also revealed all patients treated with azithromycin (250 mg three times a week) without any adverse effects reduced the progression of rosacea.

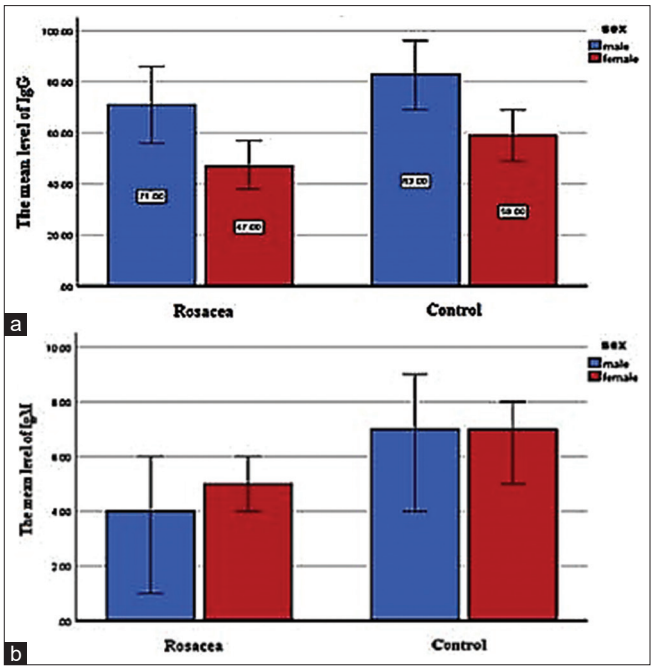

Figure 3: The mean serum levels of IgG (a) and IgM (b) to *C. pneumoniae* in the males and females of the rosacea patients and controls. The serum levels of IgM and IgG in the males and females of the controls were higher than in the males and females of the rosacea patients

It is well known that rosacea is more common in females than in males.[13] In this study, the females to males ratio was 4.5:1, a stronger female predominance in comparison with other studies.

Rosacea disorder occurs most often in adults (at the age of 30–50 years) and studies have shown that the patient's age is positively correlated with the risk of infection. For instance, Sędzikowska[14] showed that the infection risk of the rosacea in adult patients is three times higher than that of the general population. Similarly, this study showed that the serum levels of IgG and IgM to *C. pneumoniae* increased with rosacea patients' age. Also, we found a positive correlation between IgG (not IgM) and the onset age of rosacea.

Studies have shown that the rosacea progression varies from one person to another one, depending on the factors such as genetics, skin sensitivity, length of time spent in the sunlight without sunscreen, menopause, alcohol consumption, smoking, and some other factors.[15] For example, the rosacea patients showed higher genetic risks for many autoimmune diseases, such as celiac disease and type 1 diabetes.[16] Our study also included rosacea patients with a history of autoimmune diseases such as vitiligo, lupus, alopecia, type 1 diabetes, and hypothyroidism but there was no significant association between the progression of the disease and autoimmune diseases.

However, rosacea is considered a skin-limited disease, and there are associations between rosacea and systemic comorbidities.[15] As a recent study showed rosacea patients had significantly higher three odds of having urogenital

diseases, respiratory diseases, and female hormone imbalance compared with control subjects.[17] In contrast, our study did not show any association between the levels of IgG/IgM and urogenital diseases, respiratory diseases, and female hormone imbalance.

The rosacea disorder most often affects women between the ages of 30 and 60 (the average age most females begin menopause) and the menopause triggers or worsens the rosacea disorder in some sufferers.[18] Despite this matter, this study did not show any evidence of the disease progression in the 26 patients with menopause.

In addition, the rosacea disorder is often in the sun-exposed areas that probably solar radiation destructs blood vessels and connective tissue of the skin,[19] but we did not find an association between the progression of rosacea disorder and time exposed to sunlight.

Also, the epidemiologic correlation between alcohol and rosacea is unclear and inconsistent based on previous studies.[20-23] For example, Spoendlin *et al.* in the large case–control study[22] and SuYun *et al.*[24] reported an increased risk of rosacea with increased alcohol consumption based on a large cohort of women. In contrast, several case–control or case series studies,[25,26] similar to our results, did not show a significant association between alcohol intake and rosacea exacerbation.

Furthermore, there are different results of correlation between smoking and rosacea disorder. For example, the study of Kucukunal *et al.*[27] revealed that the prevalence of smoking among rosacea patients was significantly higher (66%) than in controls.

In contrast, the study of Drago *et al.*[28] showed that smoke plays a protective and beneficial role in rosacea and the results of the study by Li *et al.*[29] also confirmed that the rosacea risk is significantly decreased in smokers, likely in conjunction with the decreased risk for small intestinal bacterial overgrowth. Also, Li *et al.* found that past smoking in US women is associated with an increased risk of rosacea, while current smoking is associated with a reduced risk of rosacea.

Similarly, the research of Dai *et al.*[30] showed that current smoking was significantly associated with a decreased risk of rosacea. In contrast, we did not obtain any association between smoking and the progression of rosacea.

Generally, no association was found between rosacea and *C. pneumoniae* in this report.

Study limitations and strengths

However, this study was conducted in a large cohort population, and the possible involvement of *C. pneumonia* in rosacea needs further investigation in different populations with more precise diagnostic tests and methods to find more accurate evidence about the relation between *C. pneumoniae* and rosacea.

Conclusions

The preliminary studies showed an insignificant difference between the chronic cutaneous disorder of rosacea and the causal agent of *C. pneumoniae* among Iranian patients. Since *C. pneumoniae* causes co-infections with other rosacea-related pathogens (such as *Demodex* mites, *H. pylori*, *Candida albicans*, and *Staphylococcus aureus*) and induces a chronic inflammatory response, further studies can help to identify the possible cause of the rosacea.

Ethical approval

Experimental protocols of this study were approved by the Institutional Research and Ethics Committee of Medical Sciences from the Isfahan University of Medical Science with the code number IR.MUI.REC.1393.2.343.

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Conflicts of interest

There are no conflicts of interest.

References

1. Holmes AD, Spoendlin J, Chien AL, Baldwin H, Chang ALS. Evidence-based update on rosacea comorbidities and their common physiologic pathways. J Am Acad Dermatol 2018;78:156-66.
2. Lavers I. Rosacea: Clinical features and treatment. Nurs Stand 2016;30:52-9.
3. Wang L, Wang Y-J, Hao D, Wen X, Du D, He G, *et al*. The theranostics role of mast cells in the pathophysiology of rosacea. Front Med 2020;6:324.
4. Peng Q, Sha K, Liu Y, Chen M, Xu S, Xie H, *et al*. mTORC1-mediated angiogenesis is required for the development of rosacea. Front Cell Dev Biol 2021;9:751785.
5. Kim HS. Microbiota in rosacea. Am J Clin Dermatol 2020;21:25-35.
6. Daou H, Paradiso M, Hennessy K, Seminario-Vidal L. Rosacea and the microbiome: A systematic review. Dermatol Ther 2021;11:1-12.
7. Del Rosso JQ, Tanghetti E, Webster G, Gold LS, Thiboutot D, Gallo RL. Update on the management of rosacea from the American Acne & Rosacea Society (AARS). J Clin Aesth Dermatol 2020;13:S17-24.
8. Fernandez-Obregon AC. Commentary on" The role of chlamydia pneumoniae in the etiology of acne rosacea: Response to the use of oral azithromycin". J Infectiol. 2019 Mar 26; 2(2).
9. Di Pietro M, Filardo S, Romano S, Sessa R. Chlamydia trachomatis and chlamydia pneumoniae interaction with the host: Latest advances and future prospective. Microorganisms 2019;7:140.
10. Kortesoja M, Trofin RE, Hanski L. A platform for studying the transfer of Chlamydia pneumoniae infection between respiratory epithelium and phagocytes. J Microbiol Methods 2020;171:105857.
11. Yamasaki K, Gallo RL. Rosacea as a disease of cathelicidins and skin innate immunity. J Investig Dermatol Symp Proc 2011;15:12-5.
12. Manzano S, Guggisberg D, Hammann C, Laubscher B. Acute generalized exanthematous pustulosis: First case associated with a Chlamydia pneumoniae infection. Arch Pediatr 2006;13:1230-2.
13. Redd TK, Seitzman GD. Ocular rosacea. Curr Opin Ophthalmol 2020;31:503-7.

14. Sędzikowska A, Osęka M, Skopiński P. The impact of age, sex, blepharitis, rosacea and rheumatoid arthritis on Demodex mite infection. Arch Med Sci 2018;14:353-6.
15. Rainer BM, Kang S, Chien AL. Rosacea: Epidemiology, pathogenesis, and treatment. Dermatoendocrinol 2017;9:e1361574.
16. Egeberg A, Hansen PR, Gislason GH, Thyssen JP. Clustering of autoimmune diseases in patients with rosacea. J Am Acad Dermatol 2016;74:667-72.
17. Rainer BM, Fischer AH, Da Silva DLF, Kang S, Chien AL. Rosacea is associated with chronic systemic diseases in a skin severity–dependent manner: Results of a case-control study. J Am Acad Dermatol 2015;73:604-8.
18. Reus TL, Brohem CA, Schuck DC, Lorencini M. Revisiting the effects of menopause on the skin: Functional changes, clinical studies, *in vitro* models and therapeutic alternatives. Mech Ageing Dev 2020;185:111193.
19. Feaster B, Cline A, Feldman SR, Taylor S. Clinical effectiveness of novel rosacea therapies. Curr Opin Pharmacol 2019;46:14-8.
20. Szentkereszty-Kovács Z, Gáspár K, Szegedi A, Kemény L, Kovács D, Törőcsik D. Alcohol in Psoriasis—from bench to bedside. Int J Mol Sci 2021;22:4987.
21. Zhang H, Tang K, Wang Y, Fang R, Sun Q. Rosacea and its comorbidities: Should be emphasized but should not be overemphasized. J Cosmet Dermatol 2020;19:3414-5.
22. Spoendlin J, Voegel J, Jick S, Meier C. A study on the epidemiology of rosacea in the UK. Br J Dermatol 2012;167:598-605.
23. Liu L, Xue Y, Chen Y, Pu Y, Zhang Y, Zhang L, *et al*. Alcohol consumption and the risk of rosacea: A systematic review and meta-analysis. J Cosmetic Dermatol 2021. doi: 10.1111/jocd. 14483.
24. SuYun L, Drucker A, Qureshi A, WenQing L. Alcohol intake and risk of rosacea in US women. J Am Acad Dermatol 2017;76:1061-7.e2.
25. Gupta M, Gupta A, Chen S, Johnson A. Comorbidity of rosacea and depression: An analysis of the National Ambulatory Medical Care Survey and National Hospital Ambulatory Care Survey—outpatient department data collected by the US National Center for Health Statistics from 1995 to 2002. Br J Dermatol 2005;153:1176-81.
26. Curnier A, Choudhary S. Rhinophyma: Dispelling the myths. Plast Reconstr Surg 2004;114:351-4.
27. Kucukunal A, Altunay I, Arici JE, Cerman AA. Is the effect of smoking on rosacea still somewhat of a mystery? Cutan Ocular Toxicol 2016;35:110-4.
28. Drago F, Ciccarese G, Herzum A, Drago F, Rebora A, Parodi A. The association between cigarettes smoke, small intestine bacterial overgrowth and rosacea. G Ital Dermatol Venereol 2019;154:727-8.
29. Li S, Cho E, Drucker AM, Qureshi AA, Li W-Q. Cigarette smoking and risk of incident rosacea in women. Am J Epidemiol 2017;186:38-45.
30. Dai YX, Yeh FY, Chou YJ, Chang YT, Chen TJ, Li CP, *et al*. Cigarette smoking and risk of rosacea: A nationwide population-based cohort study. J Eur Acad Dermatol Venereol 2020;34:2593-9.