Serum levels of hypersensitive-C-reactive protein in moderate and severe acne

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ABSTRACT

Background: Elevation of C-reactive protein (CRP) has been reported to occur in psoriasis, urticaria, acne, rosacea and many other dermatological and nondermatological conditions. Chronic systemic inflammation has been implicated in the development of neuropsychiatric/degenerative disorders, atherosclerosis, coronary artery disease, diabetes mellitus and even carcinogenesis. The present study is designed to determine whether the level of inflammation created by acne vulgaris could be high enough to raise the serum levels of high-sensitive CRP. **Materials and Methods:** Forty-two patients with moderate and severe acne vulgaris were enrolled, along with 44 age and sex matched healthy blood donors as controls. Hypersensitive-CRP (Hs-CRP) was measured in both groups. **Results:** Hypersensitive-C-reactive protein levels in the case group varied between 0 and 28.1 μg/ml with an average of 2.24 ± 4.87 μg/ml (mean ± standard deviation) and a median of 0.6 μg/ml (interquartile range [IQR] =0.3, 1.4 μg/ml). Hs-CRP levels of the control group varied between 0 and 14 μg/ml with an average of 3.12 ± 3.67 μg/ml and a median of 1.5 μg/ml (IQR = 0.55, 5.0 μg/ml). No significant difference of Hs-CRP level between the two groups was seen (t = –0.961, 95% confidence interval: Lower = –2.6942, upper = 0.9377; P = 0.339). Additionally, no significant difference in the level of Hs-CRP was noted between the moderate and severe acne groups (95% confidence interval: Lower = –5.2495, upper = 1.6711; P = 0.165). **Conclusion:** Acne vulgaris, even in its severe grades (excluding acne fulminans and acne conglobata), does not induce significant inflammation at the systemic level.

Key words: Chronic inflammation, hypersensitive-C-reactive protein, inflammatory acne

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INTRODUCTION

Acne vulgaris is one of the most common skin disorders to affect humans, characterized by both inflammatory (papules, pustules, nodules and cysts) and non-inflammatory lesions (comedones). *Propionibacterium acnes* is known to be implicated in the pathogenesis of inflammatory acne. Infiltration of $CD4^+$ T-cells of $\alpha\beta^+$ phenotype and T-helper 1 (Th1) cytokine profile (high levels of interferon-γ and low levels of interleukin-4 [IL-4]) have been demonstrated within the acne follicle. This could be regarded as a cellular immune response against the *P. acnes* antigens within the follicular lumen. Furthermore, *P. acnes* contributes to the inflammatory nature of acne by inducing monocytes via toll like receptor 2 dependent pathways to secrete pro-inflammatory cytokines such as IL-8, and IL-12.[1] IL-8 along with other *P. acnes*-induced chemotactic factors may play an important role in attracting neutrophils to the pilosebaceous unit. Consequent release of lysosomal enzymes by the neutrophils leads to rupture of the follicular epithelium and further inflammation. IL-12 promotes the development of Th1-mediated immune responses. In addition, *P. acnes* releases lipases, proteases, and hyaluronidases that contribute to tissue injury and potentiate the inflammation further.[2] Furthermore, *P. acnes* induces the monocytes in acne lesions to produce high levels of IL-1 and tumor necrosis factor-α (TNF-α).[3] Thus, acne is local chronic inflammatory state that could potentially become systemic.

C-reactive protein (CRP) is one of the best indicators of systemic inflammation, considering that its serum levels show no circadian change across the 24 h.[4] IL-1, IL-6 and TNF-α that are found in the acne lesions are also major inducers CRP production by the liver.[5] Thus, CRP levels could be elevated in acne if the amount of local inflammation is high enough. The present study was designed to test the aforementioned theory. The advent of highly sensitive methods

to measure CRP has made it possible to detect very small increases of this inflammatory marker.

MATERIALS AND METHODS

This was a case–control study. Because CRP distribution is positively skewed and statistical analysis on the basis of available data from other studies stipulated a very large sample size that would be impractical, our statistical consultant suggested that all eligible cases and controls reporting within a predicted period of six months be selected. We arrived at a minimum sample size of 40 each for cases and controls.

Forty-two otherwise-healthy patients with moderate to severe inflammatory acne vulgaris living in Fars province, Iran were sequentially enrolled in the study. Exclusion criteria included systemic or topical anti-acne therapy in the previous month and conditions that could affect CRP levels such as smoking, hypertension, sedentary life-style, elevated body mass index, strict diet, moderate alcohol consumption, increased activity/endurance exercise, coexistent chronic inflammatory conditions other than acne such as rheumatic disease, chronic infections, cancer, sleep disorders, diabetes mellitus, polycystic ovary syndrome, metabolic syndrome, hyperlipidemia, and intake of estrogen/progesterone pills, statins, fibric acid derivatives or niacin.[6] Additionally, patients with acne fulminans and acne conglobata were excluded from the study. Participants were excluded by meticulous history taking and physical examination; no laboratory investigations were done in this regard.

Severity classification of patients was done using a modification in Combined Acne Severity Classification system:[7] As comedones are not inflammatory lesions, comedonal criteria were omitted and only inflammatory lesions were taken into account. Lesions of chest and back were also included. Acne was regarded as severe if there were more than 50 inflammatory or 5 cystic lesions. Patients with inflammatory lesion count ranging between 20 and 50 were labeled as moderate acne vulgaris. Informed consent was taken of both cases and controls. The study was approved by local University Ethics Committee.

Forty-four age and sex matched voluntary blood donors from Fars province, Iran who reported to the regional Blood Transfusion Organization were included as a control group. Blood donor controls were screened according to the available data in their pre-phlebotomy assessment charts.

The selected participants were referred to the laboratory for hypersensitive-CRP (Hs-CRP) measurement. The blood samples (5 cc clotted blood) were centrifuged, and the resultant sera were frozen at –70°C and kept refrigerated till the time of measurement. The serum from blood donors are also transferred to the selected laboratory to be frozen and kept till the time of measurement.

Hypersensitive-C-reactive protein levels of the sera of case and control groups were measured using ELISA method (Sandwich ELISA) in one session using standard calibration solutions provided by the manufacturer (Product Code: 3125–300, Monobind Inc., Lake Forest, CA 92630, USA). CRP levels were quantified by a microplate reader that measured the amount of light being absorbed at 450 nm. CRP levels are calculated as μg/ml of serum. ELISA kit sensitivity for CRP measurement was 0.2 μg/ml.

Statistical analysis of the results was performed using SPSS software (version 15 for IBM; SPSS Inc, Chicago, Illinois). Because the CRP distribution is positively skewed, median, as well as mean, standard deviation (SD) and interquartile range (IQR) of the data were calculated. Unpaired *t*-test was used to compare the means of the two groups. Because the number of cases and controls was above 30, skewness of CRP distribution had negligible effect on the results of the *t*-test. Comparison of CRP levels between moderate and severe acne cases was achieved using nonparametric methods (comparison of medians by Mann–Whitney U-test) because the number of samples for each group was below 30. $P < 0.05$ were considered significant.

RESULTS

There were 42 cases and 44 controls. No significant statistical differences with respect to sex and age were noted between the two groups ($P = 0.598$ for a sex difference in the Chi-square test and $P = 0.501$ for mean age difference in *t*-test).

Hypersensitive-C-reactive protein levels in the case group varied between 0 and 28.1 μg/ml with an average of 2.24 ± 4.87 μg/ml (mean ± SD) and a median of 0.6 μg/ml (IQR = 0.3, 1.4 μg/ml). CRP levels of the control group varied between 0 and 14 μg/ml with an average of 3.12 ± 3.67 μg/ml and a median of 1.5 μg/ml (IQR = 0.55, 5.0 μg/ml). Table 1 summarizes all the available data for the two groups. Histograms of CRP distribution in the case and control groups are presented in Figure 1.

Statistical analysis using two independent sample *t*-test showed no significant difference in the mean level of Hs-CRP between case and control groups ($t = –0.961$, 95% confidence interval: Lower = –2.6942, upper = 0.9377; 2-tailed, $P = 0.339$).

There were 22 cases with moderate acne and 20 cases with severe acne. Chi-square test for sex difference between the two groups was statistically significant ($P = 0.002$), indicating that the two groups were not sex matched. No significant statistical difference was noted in terms of age between the two

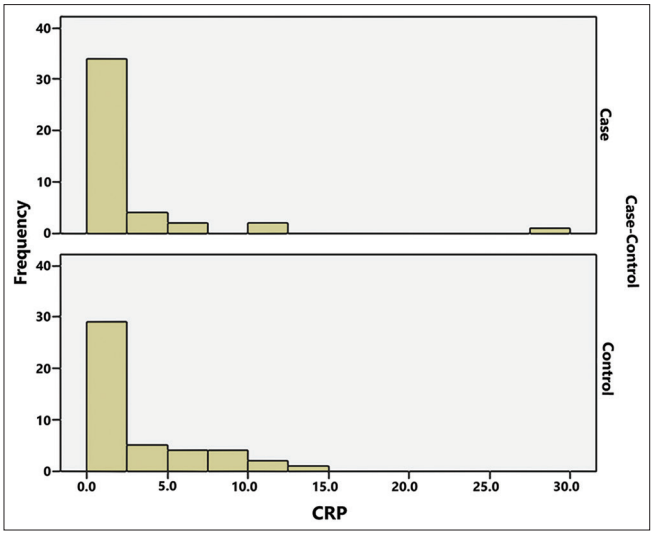

Figure 1: Histogram of C-reactive protein (CRP) level distribution in the case and control groups (CRP was measured in μg/ml units)

groups (P = 0.250). The medians of CRP levels in the moderate and severe acne groups were 0.45 μg/ml (IQR = 0.275, 0.90 μg/ml) and 0.7 μg/ml (IQR = 0.40, 3.25 μg/ml) respectively. No significant difference was detected between the two groups upon nonparametric analysis by 2-tailed Mann–Whitney U-test (95% confidence interval: Lower = –5.2495, upper = 1.6711; P = 0.165). Table 2 summarizes the data.

DISCUSSION

C-reactive protein is an acute-phase protein that appears after injury, infection, or inflammation and disappears when the injury heals, or when the infection or inflammation subsides. CRP is synthesized exclusively by the liver in response to inflammatory cytokines, especially IL-1, IL-6 and TNF-α. Although IL-6 levels display circadian variability, CRP levels have been shown to be quite stable across 24 h.[4,5] When measured with high-sensitivity assays, the population distribution of CRP has generally been consistent across sex and ethnic groups: Values of 0.3, 0.6, 1.5, 3.5, and 6.6 μg/l have been reported as estimates of the 10th, 25th, 50th, 75th, and 90th percentiles respectively for middle-aged people. Furthermore, CRP levels are not altered by food intake.[8] Thus, CRP can be considered a perfect marker for assessment of systemic levels of inflammation.

Recently CRP is being shown to be more than a mere indicator of inflammation. Prospective epidemiological studies have demonstrated that CRP level is a strong predictor of future cardiovascular events[6,9] that most cases has been proved to occur independently of age, smoking, cholesterol levels, blood pressure, and diabetes. Possible mechanisms of cardiovascular dysfunction with increased levels of CRP have been described: CRP is found within atheromatous plaque, correlates with vascular dysfunction, promotes secretion of inflammatory mediators by vascular endothelium, has a direct role in cell adhesion molecular expression, and opsonizes low density lipoprotein for uptake by macrophages in the atherosclerotic plaque.[10] In addition, CRP is a powerful, independent risk determinant of insulin resistance, a known risk factor for cardiovascular disease.[11] Thus, CRP may be one of the mediators of chronic systemic inflammation.

Table 1: Demographic data and HsCRP values of case and control groups

Variable	Case	Control	*P*
Number	42	44	-
Sex			0.598
Male	20	22	-
Female	22	22	-
Age			
Range	14–34	14–30	-
Mean	20.2	20.7	0.501
SD	3.785	3.624	-
Median	20.0	21.0	-
IQR	17–22	18–23	-
HsCRP			
Range	0–28.1 μg/ml	0–14 μg/ml	-
Mean	2.24 μg/ml	3.12 μg/ml	0.339
SD	4.87 μg/ml	3.67 μg/ml	-
Median	0.6 μg/ml	1.5 μg/ml	-
IQR	0.3–1.4 μg/ml	0.55–5.0 μg/ml	-

SD: Standard deviation, HsCRP: High-sensitivity C-reactive protein, IQR: Interquartile range

Table 2: Demographic data and Hs-CRP values of moderate and severe acne groups

Variable	Moderate acne	Severe acne	*P*
Number	22	20	-
Sex			0.002
Male	6	12	-
Female	16	8	-
Age			
Range	14-34	15-27	-
Mean	21.11	19.53	0.250
SD	4.086	3.642	-
Median	21.0	19.0	-
IQR	20-22	17-23	-
Hs-CRP			
Range	0-11.7 μg/ml	0.1-28.1 μg/ml	-
Mean	1.217 μg/ml	3.006 μg/ml	-
SD	2.6838 μg/ml	6.6714 μg/ml	-
Median	0.45 μg/ml	0.700 μg/ml	0.165
IQR	0.275-0.90 μg/ml	0.40-3.25 μg/ml	-

SD: Standard deviation, hs-CRP: Hypersensitive C-reactive protein, IQR: Interquartile range

The skin is the largest organ of the body, and the inflammatory skin conditions have the potential to induce systemic inflammation. However, little work has been done in this important domain.

Psoriasis is one of the major dermatological disorders with potential to involve a large surface area of the body. It also may involve the articular system. Interestingly, patients with psoriasis are at higher risk of cardiovascular disorders which could be due to the effect of chronic systemic inflammation. Even greater risk can be seen with involvement of joints. Studies have shown that serum CRP levels in patients are increased up to 20-fold during acute exacerbations of psoriasis and decline after remission; however, levels remain continuously elevated in comparison to the normal population. Furthermore, CRP levels have a correlation with the extent of the disease and could be used as a blood marker to evaluate the severity of the disease.[12-14]

Another dermatologic disease with an increased level of CRP is chronic ordinary urticaria. Studies have shown that circulating levels of CRP and IL-6 are significantly elevated in chronic urticaria patients, and these increases corresponded to the severity and activity of the disease. Data suggests that there is a systemic inflammatory reaction in chronic urticaria.[15,16]

Studies have shown elevation of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α and IFN-γ in the blister fluid of bullous pemphigoid patients as compared to their serum levels.[17,18] D'Auria *et al*., showed an increase serum levels of IL-6 and TNF-α in BP patients. There was significant correlation between serum levels of the aforementioned cytokines and disease activity in terms of lesion count. Expectedly, the significant correlation of serum IL-6 and CRP concentrations in this study can clearly explain the observed increased level of CRP in BP patients.[19]

Inflammation is thought to contribute to the development and progression of various cancers.[20] Study of Trichopoulos *et al*., indicated that elevated plasma CRP level is a marker of cancer risk in healthy individuals. They assessed cancer risk of many organs and for the first time reported an association of non-melanoma skin cancer with elevated plasma CRP.[21]

In this study, we chose acne vulgaris because it is a very common dermatologic disease affecting many young individuals and in some cases, the involvement of skin can be very severe with intense inflammation and disfiguring scars. In addition, acne is a chronic disease which may last for a decade or more after puberty.[7,22]

A similar dermatologic condition to acne vulgaris for which CRP data is available is rosacea. One study showed that CRP level in rosacea patients was clearly above that of the control group (0.429 vs. 0.243 μg/l, P = 0.007). Only in 10% of patients was it above 0.8 μg/l. Interestingly there is evidence that patients with rosacea may be at increased risk of cardiovascular problems despite the fact that CRP is not elevated above 1 μg/l.[23]

Complicated variants of acne that may report increased levels of CRP such as acne fulminans, acne conglobata and hidradenitis suppurativa (acne inversa) were excluded from the present study.

Vergou *et al*., conducted a study to evaluate the correlation of thyroid disorder with the presence of acne. They did not find any significant statistical difference in the CRP levels between case and control groups, although they found higher median levels of CRP in the control group than in the acne group (0.8 μg/ml vs. 0.57 μg/ml), similar to our findings (1.5 μg/ml vs. 0.6 μg/ml).[24] While their study included adult women with post-adolescent acne, our study included both male and female participants, of both adolescent and adult age groups. In addition, our study applied strict exclusion criteria in order to eliminate the confounding effects of other possible elevators of CRP.

Other studies wherein CRP levels of acne patients were assessed involved those with polycystic ovary syndrome (PCOS). Increased levels of CRP were reported in such studies: Alemzadeh *et al*., reported a mean CRP level of 3.0 μg/ml and Keskin Kurt *et al*., reported mean CRP level of 5.5 μg/ml among participating PCOS patients.[25,26] In the present study, we excluded cases of PCOS because our goal was to assess the exclusive impact of acne-induced inflammation upon serum levels of CRP, while in the PCOS there is a mixture of hormonal imbalances and metabolic derangements that could exert unknown, confounding effects upon the serum level of CRP.

Our data showed that the mean CRP level in the acne group is 2.24 mg/l that is far higher than the result reported in the rosacea study. Thus, one can infer that there is more severe inflammation in the skin of acne patients than in those with rosacea. Another finding in our study was that the mean CRP level in severe acne group was higher than that of the moderate acne group (3.006 vs 1.217 mg/l). The above data potentiates the notion that Hs-CRP measurement is a practical tool to assess the severity of cutaneous inflammation in acne. In addition, 13.6% (3/22) of cases with moderate acne and 35% (7/20) of cases with severe acne had CRP levels of more than 1 μg/ml indicating possibly increased risk of cardiovascular disease.

Given that the effect of local inflammation induced by acne may induce very subtle changes in the CRP level as also the presence of many factors that may influence the CRP level, the importance of choosing an exactly matched control group appears to be very critical and must be addressed with scrutiny.

Therefore, choosing a control group comprising blood donors could be one of the limitations of our study that could have contributed to non-significant differences between the case and control groups. Another limitation could be the lack of a group of participants with mild acne that could have been used as a matched control group for comparison with the moderate to severe acne group.

Although the median CRP level in the severe inflammatory acne group was clearly above the moderate inflammatory group in our study, the lack of statistically difference between the severe and moderate acne groups could be simply due to the small number of cases in each group or the inappropriateness of the acne severity classification. Accordingly, statistical calculations based on the results of the present study propose a very large number of cases and controls (around 750).

A large study involving adequate numbers of participants with an exactly matched control group would be appropriate to elucidate the effect of inflammatory acne on CRP levels. Measuring other cytokines including IL-1, IL-6 and TNF-α would also be more informative. Delineating a serum marker for the severity of acne can help dermatologists to assess the validity of current acne severity scoring systems (more than 20) and choose the most accurate one for everyday practice.

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