LETTER TO THE EDITOR
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Response Comment on "Association between Interleukin-32 and Interleukin-17A Single Nucleotide Polymorphisms and Serum Levels with Polycystic Ovary Syndrome"

Behrouz Gharesi-Fard[1,2], and Fatemeh Hesampour[1,2]

[1] *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
[2] *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

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To The Editor

We thank the authors for arguing our article and it is so worthful for us that they found this study interesting. We have closely reconsidered the mentioned points and this letter is in response to the comments of Zakeri et al in this issue. In following our responses are given point-by-point.

Comment 1

Among polycystic ovary syndrome (PCOS) patients obesity is a common characteristic and more than 50% of PCOS patients are overweight. Also, obesity affects the secretion of the inflammatory cytokines from adipose tissue. However, in the article by Hesampour et al. the weight and body mass index (BMI) of patients (overweight) were significantly higher than controls (normal BMI) that might affect the obtained results.

Our Response

We have four reasons to argue this comment:

First, they have mentioned that obesity rate is more than 50%, but based on the given reference (no. 4) in the letter,the mentioned rate is reported regarding the criteria of the international life science association of China (BMI <25: normal and BMI ≥ 25: obesity) in the Chinese population, so it maysimply not be referred to the other populations.

Corresponding Author: Behrouz Gharesi-Fard, PhD; Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: (+98) 7132351575 , Fax: (+98), 7132351575E-mail: gharesifb@sums.ac.ir

Second, there is a difference between obesity and overweight terms. Indeed, having a BMI≥25 Kg/m^2 is considered as overweight, and having a BMI≥30 Kg/m^2 is defined as obesity (https://www.who.int/topics/obesity/en/). Moreover, concerning some studies, infertile PCOS women have increased BMI which results in anovulation.[1-4] As we had selected the patients referred to the infertility center, so it is obvious that most of them were overweight (BMI=26.38), not obese.

Third, to rule out the effect of BMI, we investigated the correlation between BMI and interleukin-32 (IL-32) serum level, as an inflammatory cytokine. Results indicated thatthere was not any correlation between BMI and IL-32 serum level in our patient group (p-value=0.48 and r = 0.107, data have not been shown in the article).

Last, some studies showed that despite the association between obesity and some diseases, BMI cannot be used for determining the central and/or abdominal fatwhich are the major causes of chronic metabolic diseases. BMI is used as an indicator of overweight and this should be considered as a limitation of measuring BMI as a dangerous factor for those diseases.[5-7]

Comment 2

Thus, not only controls and patients should be BMI-matched but also be free of any inflammatory diseases.

Our Response

As mentioned in the Materials and Methods section, the patients and controls in our study had no

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immunological disorders or any kinds of infectious diseases.

Comment 3

As the authors have mentioned in the introduction this syndrome occurs in women during gestational ages. The correct age ranges for occurring the syndrome is between 20 to45 years old.

Our Response

We were aware of the age range for occurring PCOS and we have mentioned this point in the introduction section (20-35 years old). Our aim had been reproductive age and the word gestational in our sentence was a typographical error.

Comment 4

Also, in the introduction, It is inferred that mothers and sisters of those women were affected with PCOS by 35 and 40%, respectively. However, these values show the chance for developing this syndrome.

Our Response

Our sentence in the introduction means that a woman's risk of developing PCOS is 40% if her sister is affected, and regarding the references that we used[2,3] in our article, we believe that the meaning of the sentence is correct.

Comment 5

The methods of detection and also the time of sampling (the day of the menstrual cycle) affect the level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that should be considered in discussing about the obtained levels of FSH and LH levels. The levels of FSH and LH need to be detected in days 2-5 of the menstrual cycle.

Our Response

We were aware of the effects of the mentioned factors on the levels of the FSH and LH, and our sampling was done on days 2-4 of the menstrual cycle. Moreover, serum levels of two aforementioned hormones were measured by a local laboratory in Ghadir Mother and Child Hospital and the method of detection was Chemiluminescence immunoassay (CLIA) technology. Moreover, the measurement of these hormones was not one of our aims in this study and we detected no association between levels of them and different genotypes, so we did not debate this subject in the discussion section.

Comment 6

The authors should consider that interleukin (IL)-17A rs2275913 in both patients and controls deviated from Hardy-Weinberg equilibrium [(χ^2=88.76, p<0.001 and (χ^2=30.18, p<0.001, respectively)] that could influence on the interpretation of results and the obtained results need to be commented with caution.

Our Response

Thank you for the correct point. Indeed, this is a calculating error based on the use of Arlequin software (version 3.5.2.2). We did the analysis again and as the authors have mentioned, IL-17A rs2275913 in both patients and controls deviated from Hardy-Weinberg equilibrium. This error has no effect on the calculated p-value, but the obtained results should be cautiously mentioned.

Comment 7

It is not clear that the frequency of GC haplotype in comparison with which haplotype is different (p=0.05).

Our Response

The frequency of GC haplotype has been assessed versus the frequency of the other haplotypes (GC vs. TT + GT + TC) and the reported p-value is calculated by the Chi-Square test between two studied groups.

Comment 8

Also, as the authors have mentioned in the statistical analysis the p values<0.05 are considered as statistically significant. So, this level of difference should not be considered as a significant level in discussing obtained results.

Our Response

The standard level of significance used to support a claim of statistically significant value is 0.05. Dealing with p-value=0.05, there is a lot of discrepancies[8-10] and some scientists consider it as a significant value. It would be better if we mentioned the p-value≤0.05 as statistical significant level in our study to avoid misconception.

Comment 9

The authors, in conclusion, have explained that the IL-32 rs9927163 (G>T) might be associated with

susceptibility to PCOS among Iranian women. However, according to Table 3, this polymorphism has a protective role against PCOS. Since the frequency of T allele is lower than G allele and the odds ratio (OR) for T allele compared to G allele is 0.696 (95%CI 0.501-0.967, p=0.03), this allele could be protective against PCOS.

Our Response

Despite the claim of the authors about the allelic frequency of IL-32 rs9927163, regarding our reported results in Table 3, the frequency of T allele is higher than G allele in both groups. Our object of the mentioned association of this single nucleotide polymorphism (SNP) with susceptibility to PCOS had been about G allele. It would be better if we mentioned the G allele in our sentence to avoid misconceptions.

Comment 10

The authors have not detected the IL-17A level in most patients and there are no available levels for it among patients and controls, so serum levels of this interleukin in the title could be considered as a typographic error.

Our Response

Since we had measured the serum levels of IL-17A and IL-32 in our study, we used the term “serum level” in the title of our article based on one reviewer's comment: “*The title is not sufficiently clear. Add more indications such as "polymorphisms of" or "serum level" to clarify better the argument.* ”

Comment 11

Further, due to presenting IL-32 level in a graph and not in a Table, the exact values of this interleukin in patients and controls are not clear and the obtained results should be commented with caution.

Our Response

The values of IL-32 in patients and controls can be seen in Figure 2. Presentation of obtained results using a graph or a table is usually determined by authors and is confirmed by reviewers and editors. We believe that our results in Figure 2 are enough illustrative, but we also provide a table in this letter if there is any vague issue for the authors (Table 1).

Table 1. IL-32 serum levels in control and polycystic ovary syndrome (PCOS) groups

Group		N	Mean	Std. Deviation	Std. Error Mean
Concentration	control	41	83.0710	45.04972	7.03558
	case	47	84.4513	53.20482	7.76072

p-value was calculated by Independent Sample T-test (p-value=0.9)

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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