

suggested by Călugăru M and Călugăru D would have overstressed the statistical possibilities.

Compounding numerous factors onto statistical analyses creates problems. With an increasing number of analytes, the risk of Type 1 error increases. Rare factors have a risk for Type 2 error if not analyzed in a very large cohort. Thus, a study needs to carefully evaluate to what degree it can include potentially interesting factors. It needs to consider the sample size, the prevalence of the factors, the biological plausibility for an association, the adequacy of the given material, and potential bias factors. An extremely broad approach would be possible in very large cohorts only.

Having said so, we do not mean that the additional factor analysis as suggested by our Romanian et al would not be interesting. Indeed, within the list of additional suggestions, we would specifically emphasize the flat irregular pigment epithelium detachment as promising. It is known to be a sign of occult CNV. It is currently an open debate, whether occult CNV would have a supportive role for retinal pigment epithelium survival, with the idea of being an effective replacement of degenerated choriocapillaris.^{5,6}

A recently suggested new classification system for atrophy in AMD uses predominantly OCT characteristics to identify complete and incomplete forms of atrophy, involving either the photoreceptors only, or the retinal pigment epithelium with the photoreceptors.⁶ This new classification system is a promising approach for more detailed understanding of progressive atrophy development. However, expert opinions differed significantly regarding presence or absence of complete atrophy.⁷ This challenge would arise in case of use of this system for atrophy incidence.

In conclusion, there is room for further investigations on atrophy incidence and progression in nAMD. Our study contributed evidence that higher treatment need and the choice of the anti-vascular endothelial growth factor drug (ranibizumab or aflibercept) did not impact the development of atrophy. The identified risk factors were mostly related to the underlying degenerative process of AMD.

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None of the authors has any financial/conflicting interests to disclose.

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To the Editor:

We read a systematic review and meta-analysis of Pinna et al¹ entitled “Malondialdehyde levels in patients with age-related macular degeneration” with great interest. The authors have done a comprehensive review work and tried to formulate the association between age-related macular degeneration (AMD) and malondialdehyde (MDA) levels by meta-analysis of 12 published reports. Serum/plasma levels of MDA in control groups varied in the range of 1.00 to 33.29 $\mu\text{mol/L}$ and those of AMD groups were in the range of 2.18 to 29.36 $\mu\text{mol/L}$. The ratio of AMD groups/control groups varied from 0.88 to 3.00. Extreme heterogeneity was correctly reported by Pinna et al, and a number of possible sources of heterogeneity were investigated, including type of sample matrix (serum or plasma), geographical area where research was conducted (eight reports in Turkey, and one report each in China, Italy, South Korea, and Japan), and AMD form (wet or dry) without any significant changes in heterogeneity. The large values of relative standard deviations in both control (varying between 10.6% and 40.2%) and AMD (varying between 5.1% and 46.4%) groups reveal that there are serious confounding factors affecting MDA levels in both groups.

Pinna et al¹ correctly mentioned the effects of age, type of sample, sample pretreatment, and standardization of the analytical method for quantification of MDA as other sources of heterogeneity. In addition to the above-mentioned sources of heterogeneity, one may consider the effects of sex, diet, circadian rhythms, cigarette, drugs, other diseases, cross reactions of MDA, cross reactions of thiobarbituric acid, and effects of pH as further confounding factors in using MDA as a biomarker² in any disease, including AMD. In addition, low reproducibility, low repeatability, less sensitivity, and low specificity of spectroscopic methods should be critically evaluated before conducting anymore clinical investigation. Validation of the analytical method to be used in future studies is highly recommended, and a combination of separation techniques such as chromatographic or capillary electrophoresis methods could improve a part of analytical results; however, the problems associated with high chemical reactivity of MDA still remain unresolved.³

As an example of the nonreliability of MDA determination, if one considers the reported levels for MDA in three articles^{4–6} reviewed by Pinna et al¹ which were measured using a single analytical method proposed by Jain et al,⁷ there are significant differences between MDA levels of both control (1.00 ± 0.36^4 , 4.30 ± 1.30^5 , and 2.83 ± 0.43^6) and AMD (2.76 ± 1.28^4 , 6.90 ± 1.30^5 , and 3.69 ± 0.88^6) groups, as revealed by the results of the *t*-test (Figure 1). It is the same for MDA data of two other works^{8,9} in which the analytical method was reported by Wasowics et al.¹⁰ There are other similar observations dealing with the variations in MDA levels using the same analytical method and even the same research group, which have been discussed elsewhere.¹¹ The most interesting point is that in all these works, the derivatization of MDA was performed using thiobarbituric acid, and after, spectrophotometric/spectrofluorimetric assay was used for quantification.

Another interesting finding has been reported by Matsuura et al¹² in which the MDA levels for the control group were reported to be 9.04 ± 0.96 nmol/L (which we believe it should be in $\mu\text{mol/L}$), and those for wet and dry AMD groups were 9.94 ± 1.53 and 9.30 ± 0.92 , respectively. After 3 months, supplementation with a combination of antioxidants in the wet AMD group was randomly divided into two subgroups, receiving supplementation (S+) and no supplementation (S–). The MDA levels in (S+) and (S–) subgroups were 10.34 ± 2.03 and 9.54 ± 0.70 $\mu\text{mol/L}$ (with a significant difference), which were changed to 8.88 ± 1.18 and 10.41 ± 1.36 $\mu\text{mol/L}$ (with a significant difference), respectively,

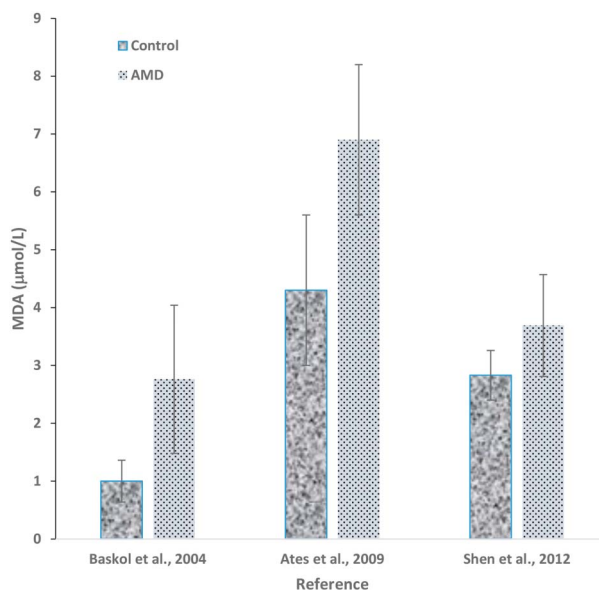


Fig. 1. Comparison of mean MDA values of three studies^{4–6} in control and AMD groups measured using a single analytical method.⁷

after 3 months. There were also significant differences before and after 3 months in both (S+) and (S–) subgroups, revealing that the supplementation decreased the MDA levels and also MDA levels were increased in the (S–) group after 3 months.

In three studies,^{6,9,13} the AMD group was investigated in early and late subgroups, and in two of those studies,^{6,13} the MDA levels were increased in comparison with the control group and also among early and late AMD subgroups. However, in one study,⁹ no significant difference was observed between early AMD and control groups.

In conclusion, the association between pathologic condition and oxidative stress is a well-accepted fact in biology and medicine, but using MDA as a reliable marker of oxidative stress is still questionable from an analytical viewpoint, as discussed in an earlier report² and also as discussed by Pinna et al.¹ There are a number of characteristics for an acceptable biomarker, and MDA does not fulfill most of the required criteria for an ideal biomarker, as shown in an earlier work.³ As stated by Wade and van Rij in 1989, problems associated with MDA specificity and reactivity have been largely ignored by many researchers¹⁴ because of the simplicity of its spectroscopic determination; however, this ignorance results in findings that are confusing and needs more attention in method validation for MDA analysis before performing a clinical study. Further discussions on this topic are open for more comments/criticisms, and we believe that clinical investigators will pay more attention to the accuracy, precision, reproducibility, repeatability,

and other validation criteria of the analytical method, as has been discussed in detail in previous works.^{2,3}

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None of the authors has any financial/conflicting interests to disclose.

Acknowledgments

The research reported in this publication was supported by a grant (64109) of Research Affairs, Tabriz University of Medical Sciences, Tabriz, Iran.

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Reply

To the Editor:

We wish to thank Khoubnasabjafari et al for their interest in our recent systematic review and meta-analysis on malondialdehyde (MDA) levels in patients with age-related macular degeneration.^{1,2} We would like to elaborate further on their valuable comments.

We fully agree on the fact that several pitfalls may affect the final data in MDA quantification and that most results reported in the literature suffer from lack of method standardization.² Overall, the unreliability of MDA determination seems quite obvious, if one takes into account that data obtained using the same analytical method, even by the same research group, often show low reproducibility, as discussed by Khoubnasabjafari et al¹ in the Comments to our article. Interestingly, their findings are consistent with previously published meta-analyses on MDA levels in several clinical conditions,^{3,4} which reported marked heterogeneity without a clear indication about the source of heterogeneity, a result probably depending on the causes so clearly described by Khoubnasabjafari et al.¹ Furthermore, we agree with Khoubnasabjafari et al¹ that most researchers ignore the analytical issues associated with the determination of MDA, which continues to be widely used as an oxidative stress biomarker, probably because of the easiness of the methodological procedure. Finally,