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LETTER TO THE EDITOR

Letter to the Editor: Metabolomics of Aqueous Humor in Diabetes Mellitus

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Dear Editor:

We have read with great interest the article by Wang et al. entitled ''Metabolomic profile of diabetic retinopathy: a GC-TOFMS-based approach using vitreous and aqueous humor.''[1] The authors identified novel metabolites and disturbed metabolic pathways. This is not the first article presenting the metabolic profiles of aqueous humor (AH), which are characteristic for diabetes mellitus.[1–4] However, each of those studies identified different metabolic pathways as the most disturbed (Table 1), which might cause some confusion among the readers. We would like to explain these discrepancies and to demonstrate that they represent a characteristic feature of metabolomic studies, rather than a methodological error.

The significance of prevention, treatment, and slowing down the progression of diabetic complications raises no doubts in the context of reducing health and economic burden. From an ophthalmological perspective, diabetic retinopathy should be considered a potentially blinding disease. In our article,[2] we emphasized that diabetes might accelerate cataract development and increase complication rates after cataract surgeries.

Metabolomic studies aim to identify the underlying mechanisms of disease. The composition of small molecules can be considered a chemical marker of a current phenotype.

Table 1. A Summary of the Methodology and Results of Previous Studies of Aqueous Humor Metabolic Composition in Diabetes Mellitus Patients

Reference	*Analytical method*	*Study participants*	*Number of detected and distinctive to DM metabolic features or metabolites*	*Disturbed metabolic pathways distinctive to DM*	
				Count	*The names of the most relevant pathways*
[1]	GC-TOFMS	23 PDR, 25 controls	200—detected metabolic features 137—detected metabolites 8—metabolites distinctive to DM	3	Glycolysis or gluconeogenesis Galactose metabolism Ascorbate-aldarate metabolism
[2]	LC-MS	16 DM, 19 controls	1222—detected metabolic features 125—metabolic features distinctive to DM 29—metabolites distinctive to DM	13	D-Arginine and D-ornithine metabolism One carbon pool by folate Arginine and proline metabolism Purine metabolism Taurine and hypotaurine metabolism
[3]	^{1}H-NMR	13 DR, 14 DM, 7 controls	26—detected metabolites 10—metabolites distinctive to DM	11	Alanine, aspartate, and glutamate metabolism
[4]	GC-TOFMS	15 DM, 15 controls	263—detected metabolites 20—metabolites distinctive to DM	10	Fatty acid biosynthesis Fatty acid metabolism Linoleic acid metabolism

DM, diabetes mellitus; DR, diabetic retinopathy; GC-TOFMS, gas chromatography coupled with time-of-flight mass spectrometry; ^{1}H-NMR, proton nuclear magnetic resonance spectroscopy; LC-MS, liquid chromatography-mass spectrometry; PDR, proliferative diabetic retinopathy.

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In diabetes, metabolomic studies involved a plethora of body fluids, among them serum, vitreous humor, and AH. Similar to Wang et al.,[1] this is AH, which represents our primary interest.

As mentioned earlier, a total of four untargeted metabolomic studies of AH in diabetic patients have been indexed in PubMed on July 10, 2020.[1–4] We were the first to report the differences in AH composition in diabetic and nondiabetic patients.[2] There are various analytical platforms that could be used in metabolomic studies. The most commonly used methods include nuclear magnetic resonance or mass spectrometry (MS), coupled with various separation techniques: liquid chromatography (LC)-MS, gas chromatography (GC)-MS, and capillary electrophoresis (CE)-MS. Importantly, each of those methods is suitable for the detection of different metabolite classes. For example, GC-MS is the best method to detect volatile metabolites or the metabolites, which can be transformed into volatile derivatives. Meanwhile, LC-MS is more suitable for nonpolar or medium polar metabolites, and CE-MS for highly polar metabolites. Furthermore, even using the same analytical method, different metabolite classes could be detected depending on the sample preparation procedure. This is demonstrated in Table 1, comparing the results of two studies that used the same GC-MS approach, but with different extraction protocols.[1–4] In conclusion, specific methods are more suitable for the detection of particular metabolites. It also needs to be stressed that none of these methods could provide information about the entire metabolome. This seems to be a primary reason behind the discrepancies in the results of published metabolomic studies of AH in diabetes.

In summary, metabolomics has the potential to detect altered metabolic pathways and to identify novel biomarkers. However, various techniques provide complementary results rather than equivalent ones, and hence, the metabolomic studies of AH and other biofluids should involve a multiplatform approach. This will allow us to detect a broader spectrum of metabolites, providing a better insight into the problem in question.

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