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Case report

Proteomic analysis of elevated intraocular pressure with retinal detachment

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ABSTRACT

Purpose: To report a case of elevated intraocular pressure with retinal detachment.
Observations: Liquid chromatography and tandem mass spectrometry was performed on the patient aqueous biopsy. Protein levels were analyzed with 1-way analysis of variance (ANOVA) and unbiased clustering. High levels of rod outer segment proteins were not detected, suggesting that this was not a case of Schwartz-Matsuo syndrome. Instead, elevated levels of Hepcidin (HEPC) and Cystatin C (CYTC; candidate biomarkers for primary open angle glaucoma) were detected, suggesting a different, unknown etiology.
Conclusions and importance: Molecular diagnoses can differentiate between clinical diagnoses and point to common biomarkers or disease mechanisms.

1. Introduction

The majority of eyes with rhegmatogenous retinal detachment present with relative hypotony, while elevated intra-ocular pressure (IOP) is less frequent.[1] In a large retinal detachment case series, glaucoma was present in 9.5% of patients, ocular hypertension in 6.5%, and an estimated 2.1% of patients with an elevated IOP due to Schwartz-Matsuo syndrome.[2] The elevated IOP in Schwartz-Matsuo syndrome occurs during chronic retinal detachment when liberation of photoreceptor outer segments block the trabecular meshwork, as shown by electron microscopy.[3]

To our knowledge there are no proteomic studies on patients with elevated IOP following retinal detachment, which could easily confirm the presence of photoreceptor outer segment proteins in the anterior chamber.[4] Here we present a case of elevated IOP in a patient with chronic retinal detachment who was clinically diagnosed with Schwartz-Matsuo syndrome, but molecular analysis of the aqueous humor suggested a different etiology.

2. Case report

A patient with presumed Posner-Schlossman syndrome (glaucomatocyclitic crisis) presented with a one-year history of recurring, mid-day “haloes” in his left eye. There was no history of trauma. His visual acuities were 20/20 OD and 20/40-2 (pinhole 20/25) OS. The IOP at presentation was 19 mmHg OD and 52 mmHg OS. There appeared to be 1 + cells in the anterior chamber and the angles were normal without recession or synechiae. Fundus examination revealed a cup-to-disc ratio of 0.20 OD and 0.65 OS. Posterior B-scan ultrasonography revealed a retinal detachment (Fig. 1A). Fundoscopic exam further showed a shallow macula-on rhegmatogenous retinal detachment extending from 6:00–1:30 in the nasal hemisphere with a retinal hole at 11:00 (Fig. 1B). There

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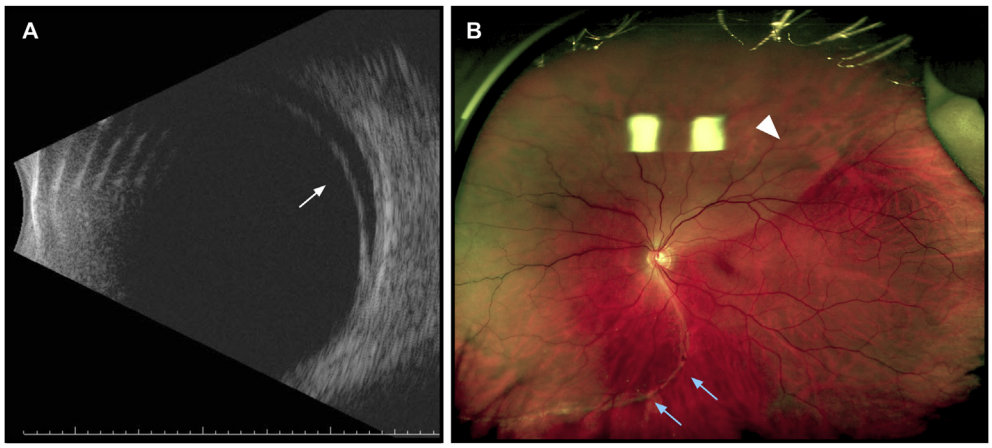

Fig. 1. Clinical Phenotype of a Patient with Elevated IOP and Retinal Detachment: (A) Posterior B-scan ultrasonography reveals retinal detachment (white arrow). (B) Fundoscopic exam of the patient's left eye revealing a shallow nasal macula-on rhegmatogenous retinal detachment (white arrow). There is a demarcation line at 7:00, which indicated chronicity (blue arrows). On examination, his acuity was 20/20 in the right eye and 20/40-2 in the left eye.

was a pigmentary line along the detachment, indicating chronicity, and a diagnosis was consistent with Schwartz-Matsuo syndrome.

The patient underwent surgical repair. First, an undiluted 200 μL anterior chamber biopsy was obtained, followed by an anterior chamber washout. During a 25 gauge pars plana vitrectomy, his posterior hyaloid was noted to be firmly adherent. A scleral buckle, air-fluid exchange, endolaser, and 24% SF6 gas tamponade was used to reattach the retina. One week post operatively his IOP improved to 19 mmHg and remained normal during a 6-month follow up. For comparison, control biopsies were taken from two other non-glaucomatous patients: one with retinal detachment and another with a posterior vitreous detachment.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) identified 239 proteins in our patient's aqueous. A total of 208 proteins were shared among the three samples (Fig. 2A). There were 9 unique proteins in our patient sample (Fig. 2A). Highly abundant levels of rod outer segment proteins, like rhodopsin, were not detected, suggesting that this was not a case of Schwartz-Matsuo syndrome. The only retinal protein present was very low levels of PDE6G. Pathway analysis of the 239 proteins in our patient's aqueous revealed that the complement cascade pathway was represented, but no other inflammatory cell pathways were detected (Fig. 2B).[5–7]

Protein levels were then analyzed using 1-way ANOVA and unbiased clustering. Eighteen proteins were upregulated (Fig. 2C) and 63 were downregulated in our high IOP case compared to normal IOP controls ($p < 0.05$; Fig. 2D). There were significantly elevated levels of Hepcidin (HEPC) and Cystatin C (CYTC), two candidate biomarkers previously identified in primary open angle glaucoma (POAG).[8]

3. Discussion

It is not clear why our patient's IOP was elevated, but based on the proposed mechanism of Schwartz-Matsuo syndrome, we expected to identify significant numbers of rod outer segment proteins in the aqueous humor of a clinically similar patient.[3] LC-MS/MS is a very sensitive, un-biased method for detecting proteins.[9,10] Rhodopsin constitutes as much as 80% of outer-segment proteins and is expressed at 10-fold higher levels than PDE6G in the human retina (unpublished observation). This makes it highly likely that LC-MS/MS would detect rhodopsin if rod-outer segments were present in the aqueous.[11] However, our proteomic analysis of the aqueous humor did not detect rhodopsin or other highly abundant levels of rod outer segment proteins, suggesting a different etiology.

Instead we identified the candidate POAG biomarkers, HEPC and CYTC, in our patient. This suggests that these proteins could be biomarkers for other etiologies of elevated IOP or markers for different forms of glaucoma. The mechanism of HEPC in POAG is unknown, but it is thought to mediate local inflammation that damages retinal ganglion cells.[8] Interestingly, elevated levels of iron-regulated genes, like HEPC, have been previously found in glaucomatous eyes as well as other ocular diseases.[12]

Our molecular diagnostic approach suggests that not all chronic retinal detachment cases with elevated IOP are Schwartz-Matsuo syndrome. Although the potential glaucoma proteins were elevated, it is possible that this might be a response to elevated IOP rather than the cause of IOP elevation itself. Regardless, it will be interesting to follow this young patient to see whether he develops risk factors for POAG in the eye without retinal detachment. If these markers (HEPC and CYTC) are a result of high IOP in susceptible individuals, a future study could determine if expression levels of these proteins return to normal following retinal detachment repair and normalization of pressures. In this case, the treatment would have remained similar regardless of the diagnosis. Nevertheless, as our technologies advance, understanding the molecular basis of disease and identifying protein biomarkers could help differentiate clinical diagnoses, point to common disease mechanisms, and determine prognoses and best approaches.

4. Patient consent

The study protocol was approved by the Institutional Review Board for Human Subjects Research (IRB) at the University of Iowa, was HIPPA compliant, adhered to the tenets of the Declaration of Helsinki. The patient underwent informed written and verbal consent for research and report publication.

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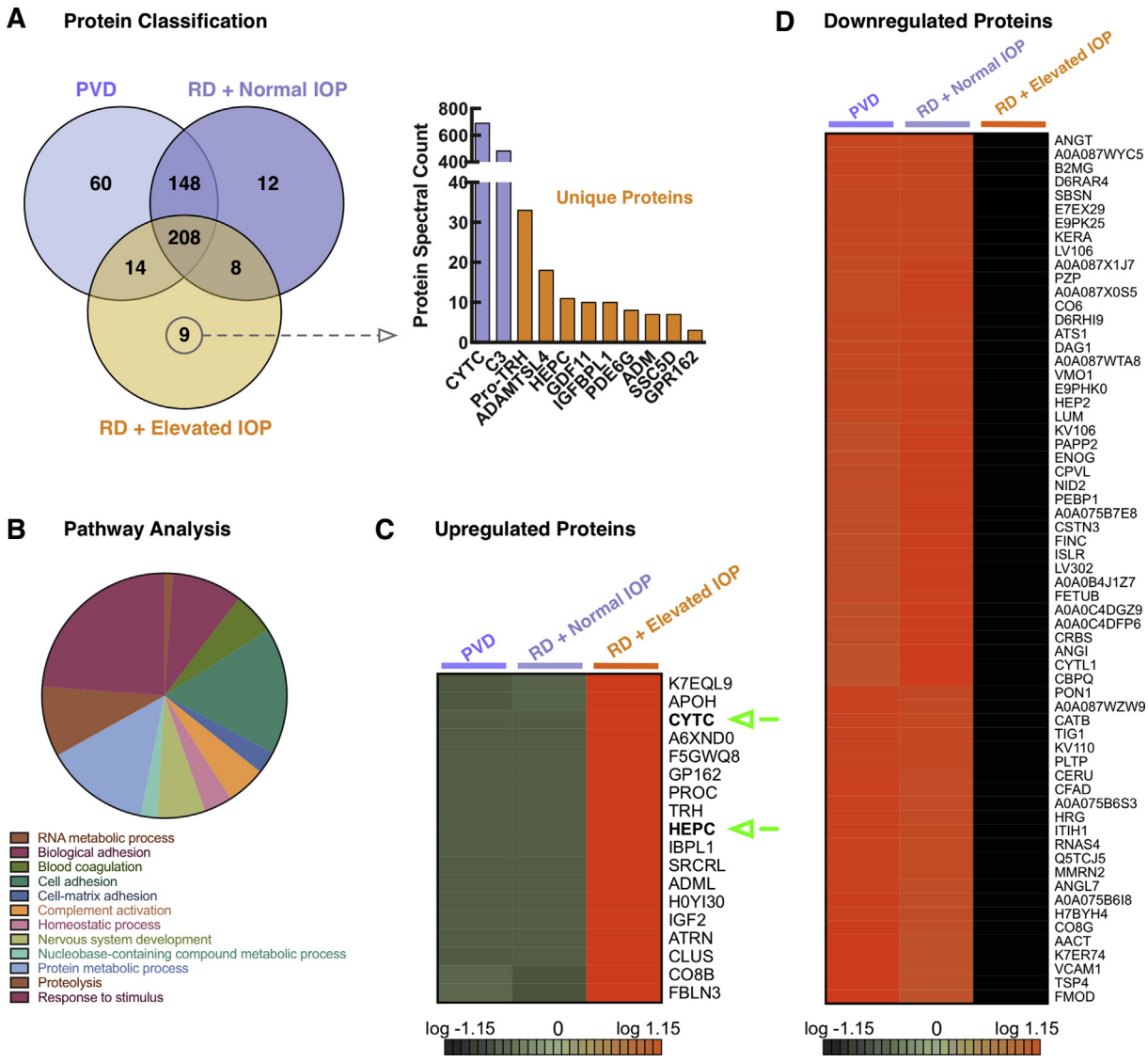

Fig. 2. Proteomic Analysis of Aqueous Humor Suggests that Elevated Intraocular Pressure (IOP) is not Secondary to Schwartz-Matsuo Syndrome: (A) Proteins from each patient sample were compared with Venn diagrams to identify common and unique proteins. PVD – posterior vitreous detachment; RD – retinal detachment. The 9 unique proteins in our patient were thyrotropin-releasing hormone (TRH), ADAMST-like protein 4 (ADAMTSL4), hepcidin (HEPC), growth/differentiation factor 11 (GDF11), insulin-like growth factor binding protein-like 1 (IBPL1), phosphodiesterase gamma subunit (PDE6G), adrenomedullin (ADML), soluble scavenger receptor cysteine-rich domain-containing protein (SSC5D), and G-protein coupled receptor 162 (GPR162). These proteins were expressed at comparatively low levels to more abundant proteins (complement C3 and cystatin-C) (B) Top twelve represented pathways in our patient's aqueous. Pathway analysis was performed using PANTHER. (C) Hierarchal clustering of proteins differentially expressed ($p < 0.05$) in our patient compared to normal intraocular pressure (IOP) controls. Results are represented as a heatmap and display protein expression levels on a logarithmic scale. Orange indicates high expression while dark green/black indicates low or no expression. A total of 18 proteins were upregulated, including HEPC and CYTC (green arrow). No rod outer segment proteins were elevated. (D) A total of 63 proteins were downregulated. The downregulated proteins represent glycolysis, coagulation, fibroblast growth factor (FGF), endothelial growth factor receptor (EGFR) signaling, integrin signaling, and G-protein signaling pathways. These proteins are likely being consumed and not replenished in our patient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Conflict of interest

The authors have no commercial or financial interests associated with this article.

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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