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Original Article

Ophthalmology Section

Electron Microscopy in Phacolytic Glaucoma: A Cross-sectional Study

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ABSTRACT

Introduction: Cataracts when allowed to progress become hypermature. In patients with hypermature cataracts the liquified lens cortex tends to leak through the micropores of lens capsule. It is this leaked material which causes blockage of trabecular meshwork and thus an acute rise in Intraocular Pressure (IOP) leading to phacolytic glaucoma. Though the incidence of phacolytic glaucoma has reduced, it is still common in developing countries, in patients with limited access to healthcare.

Aim: To analyse the aqueous humour cells and study the characteristics of the macrophages in phacolytic glaucoma using electron microscopy.

Materials and Methods: This was a cross-sectional observational study conducted in the Department of Ophthalmology at Schell eye hospital (Christian Medical College) Vellore, Tamil Nadu, India from December 2012 to November 2013. Patients presenting with gradual progressive loss of vision, acute onset of eye pain, redness, vision less than or equal to 3/60, IOP of ≥ 21 mm Hg, hypermature cataract, macroscopically intact anterior capsule and flare of 2+ were included in the study. Patients were admitted and paracentesis was done. A 0.1-0.2 mL of aqueous was aspirated using a 26 gauge customised cannula and collected in Eppendorf tubes. This was then sent for Electron Microscopic (EM) analysis where the aqueous humour was studied for the cellular content and its characteristics. Categorical variables were expressed as n (%). The statistical software Statistical Package for the Social Sciences (SPSS) version 16.0 was used for analysis wherever applicable.

Results: A total of 10 phacolytic glaucoma patients were recruited as a part of a larger study in whom the aqueous was sent for histopathological analysis. Amongst them four patients had enough sample and these were studied by electron microscopy. From the sample obtained, blocks were prepared by the pathologist. The pathologist picked 10 consecutive macrophages from each block to study. Numerous macrophages were identified in the first two cases. In addition to this the second case demonstrated four neutrophils. Majority of the macrophages showed distension of the cytoplasm with phagosomes containing lens proteins. Many membrane bound granules containing uniformly electron dense material, ranging in size from 100 nm to >1000 nm were also seen in the macrophages. These were consistent with melanin pigment granules. Only one monocyte with no engulfed material was seen. The 3rd sample showed no cells which were confirmed on light microscopy as well and the 4th sample showed cell lysis.

Conclusion: Macrophage was the predominant cell in patients with phacolytic glaucoma though occasional neutrophils and one monocyte were found. All the macrophages were overwhelmed with lens protein and melanin pigments.

Keywords: Aqueous humour, Lens proteins, Macrophages, Melanin pigment

INTRODUCTION

Opacification of lens or its capsule which can be developmental or acquired is known as cataract. This is graded into immature, mature, hypermature depending on the extent of opacification [1]. Lens Induced Glaucoma (LIG) includes a group of secondary glaucomas where the crystalline lens plays a common role in the mechanism of raised IOP [2]. Phacolytic glaucoma is one of the most common LIG, seen in patients with hypermature cataract. The raised IOP here is due to the lens protein that leaks through the macroscopically intact capsule and blocks the trabecular meshwork [3,4]. Phacolytic glaucoma is more common in developing countries due to delayed presentation or backlog of cataract cases and limited access to healthcare [5,6] especially in the rural areas [7]. Phacolytic glaucoma can sometimes present with severe anterior chamber reaction and hypopyon, closely mimicking infectious endophthalmitis, which may lead to diagnostic confusion and delayed management [8]. Cataract surgery is known to produce good visual outcome in patients with phacolytic glaucoma if performed early [9-11].

Light microscopy [6,12] and immunohistochemistry findings [12] of the phacolytic material as well as the lens capsule have been previously reported. There are very few studies which have looked at the EM picture of the cells in the anterior chamber in phacolytic glaucoma [13,14]. These studies are mostly case reports and have identified the cell type alone.

Hence, this novel study was conducted to analyse the aqueous humour in detail, focusing on its cellular content and their characteristics in particular using EM. This information may further elucidate the pathogenesis of phacolytic glaucoma and thus improve its management as well.

The present EM study of phacolytic material was done on a subset of patients recruited as a part of a larger study for histopathological and biochemical analysis of aqueous humour in phacolytic glaucoma [15].

MATERIALS AND METHODS

This was a cross-sectional observational study performed in the Department of Ophthalmology at Schell eye hospital, CMC Vellore from December 2012 to November 2013. The study was done in accordance with the tenets of the Declaration of Helsinki. An ethics committee approval was obtained for performing the study. (IRB NO-8164).

The EM study of phacolytic material was done on a subset of patients of a larger study recruiting patients for histopathological and biochemical analysis of aqueous humour in phacolytic glaucoma [15].

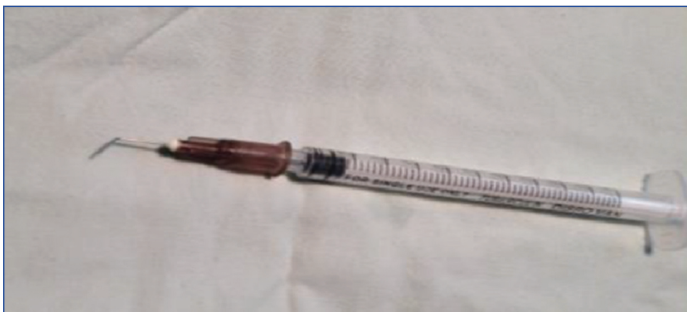
Patients in whom there was enough aqueous humour (≥0.1 mL) for EM study after collection of sample for histopathological analysis were recruited for this study. All consecutive cases fulfilling the inclusion criteria presenting to the Department of Ophthalmology over a 12-month period were included after obtaining an informed consent.

Inclusion criteria: Patients presenting with a history of gradual progressive loss of vision, acute onset of mono-ocular pain and redness, having the clinical features of visual acuity less than 3/60, IOP of ≥ 21 mm Hg, hypermature cataract, macroscopically intact anterior capsule and flare of 2+ or more in the absence of keratic precipitates were included in the study.

Exclusion criteria: Patients with a history of trauma, pre-existing glaucoma, previous use of long term (two week) topical medications-steroids; uveitis; previous ocular surgeries, usage of antiglaucoma medications prior to enrolment were excluded from the study.

Study Procedure

Patients were admitted and initiated only on tablet Acetazolamide 250 mg every six hours until the initial aqueous tap to prevent any possible cellular modification of the aqueous humour by topical medications like steroids, anti-glaucoma medication etc., Within 24 hours of starting the treatment, under aseptic precautions in the operating room and under peribulbar anaesthesia with 2% lignocaine a controlled anterior chamber paracentesis was done at the temporal/nasal limbus based on the eye affected with a Microvitreoretinal Blade (MVR) blade taking care to avoid blood vessels. About 0.1-0.2 mL of aqueous was aspirated using a unique customised 26 gauge cannula mounted on a tuberculin syringe [Table/Fig-1].



[Table/Fig-1]: Customised 26 g cannula mounted on Tuberculin syringe.

The cannula was specially designed to enable aspiration of aqueous with minimal trauma to intraocular structures. The aqueous sample was sent for EM study by collecting the aqueous in Eppendorf tubes containing 3% glutaraldehyde as the fixative agent. This was transported in ice lined packs to the lab for further study.

Electron microscopy of aqueous humour- The sample was initially fixed in 3% glutaraldehyde and washed in a buffer and then fixed with 1% osmium tetroxide and washed in buffer. This double fixation gives stability during dehydration, embedding and electron bombardment. The sample was then dehydrated by ascending series graded alcohol (50-100%) and cleared with propylene oxide which was then embedded in siliconised rubber mould with epoxy resin. This embedded mould was kept in an incubator at 60°C for 48 hours and blocks were sectioned. One micron thick sections cut through ultra-microtome using a glass knife was subsequently stained with toulidine blue. These ultra-thin sections were taken on copper grid and stained with uranyl acetate and Reynold's solution to enhance contrast. Once the sample was fixed, blocks were prepared by the pathologist for each case. These blocks were then studied in detail. In one of the cases since sufficient sample was available, two blocks were prepared. In each block the pathologist picked 10 consecutive macrophages and studied them in detail.

The cytological data analysis using electron microscopy was represented using both quantitative and qualitative parameters. Every macrophage that was studied was assessed for the following variables:

1) Cell viability- viable/non viable/not assessable ;
2) Position of the nucleus- central/peripheral/mid-peripheral/not visualised;
3) Amount of intracellular lens protein within phagosomes - expressed as percentage fill;
4) Extent of clear fluid vacuoles- expressed as percentage fill; and
5) Number of melanin pigment granules in each cell.

In addition to this, based on the qualitative analysis the intracellular contents of macrophages mainly melanin pigment, lens proteins, and clear fluid vacuoles were divided into three groups- numerous/moderate/few.

Only four cases were recruited for the study, as ultrastructural analysis by EM was done only in patients from whom sufficient sample was first obtained for histopathological analysis (as part of larger study) followed by EM when adequate material remained humour [15].

STATISTICAL ANALYSIS

Categorical variables were expressed as number of patients and percentage of patients. The statistical software SPSS version 16 was used for analysis wherever applicable.

RESULTS

Aqueous humour was sent for ultrastructural analysis by EM in four cases where enough sample could be obtained during the paracentesis. From the sample obtained a single block was prepared by the pathologist in each case where in 10 consecutive cells were studied in detail. However, in one of the cases there was enough sample to prepare two blocks hence 20 macrophages were studied [Table/Fig-2]. In the 3rd case no cells were seen on electron microscopy which was confirmed on light microscopy as well. The sample in the 4th case revealed cell lysis which might have been due to delay in transferring the sample to the lab. Among the 26 cells analysed, the majority demonstrated melanin pigment counts ranging from 1 to 37. In contrast, only two cells showed markedly higher pigment accumulation, with one cell containing 50 pigments and another containing more than 50 pigments [Table/Fig-3-5].

Cell no.	Viability	Nucleus position	Lens protein	Vacuoles	Melanin pigment
M1	Viable	Periphery	>75% fill	<25% fill	6
M2	Viable	Not seen	>75% fill	<25% fill	37
M3	Viable	Periphery	>75% fill	<25% fill	14
M4	Viable	Periphery	>75% fill	<25% fill	28
M5	Viable	Periphery	>75% fill	<25% fill	15
M6	Non viable	-	-	-	-
M7	Non viable	-	-	-	-
M8	Viable	Not seen	>75% fill	<25% fill	15
M9	Viable	Central	>75% fill	<25% fill	22
M10	Viable	Periphery	>75% fill	<25% fill	12

[Table/Fig-2]: Cellwise quantification of intracellular contents on EM, Case-1.
*M: Macrophage

Cell no.	Viability	Nucleus position	Lens protein	Vacuoles	Melanin pigment
M1	Viable	Not seen	>75% fill	<25% fill	26
M2	Viable	Not seen	50-75% fill	25-50% fill	3
M3	Viable	Not seen	50% fill	50% fill	3
M4	Viable	Periphery	25-50% fill	>50% fill	5

M5	Viable	Mid-periphery	>75% fill	<25% fill	11
M6	Viable	Not seen	>50% fill	25-50% fill	1
M7	Cell in cell phagocytosis	--cannot comment	-	-	-
M8	Viable	Periphery	<50% fill	<50% fill	50
M9	Non viable	-cannot comment	--cannot comment	--cannot comment	--cannot comment
M10	Viable	Mid-periphery	>75% fill	<25% fill	3

[Table/Fig-3]: Cellwise quantification of intracellular contents on EM, Case-2: Block-I.

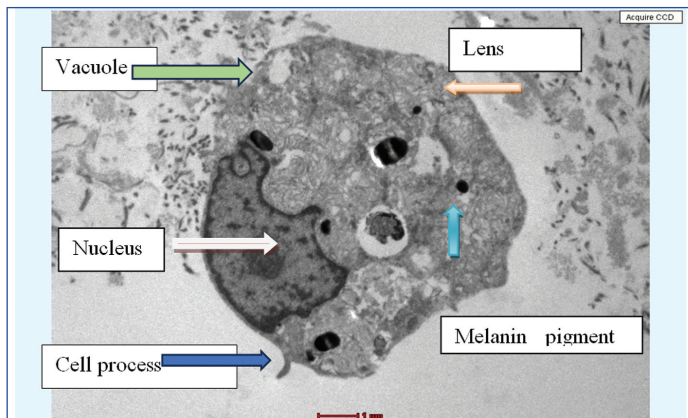
Cell no.	Viability	Nucleus position	Lens protein	Vacuoles	Melanin pigment
M1	Viable	Not seen	>75% fill	<25% fill	4
M2	Viable	Not seen	>75% fill	<25% fill	3
M3	Viable	Mid-periphery	50% fill	50% fill	5
M4	Viable	Periphery	50-75% fill	25-50% fill	5
M5	Viable	Central	>75% fill	<25% fill	11
M6	Viable	Not seen	50% fill	50% fill	5
M7	Viable	Not seen	>50% fill	<25% fill	5
M8	Viable	Not seen	<50% fill	50% fill	11
M9	Viable	Not seen	50% fill	50% fill	6
M10	Viable	Not seen	<25% fill	>50% fill	>50

[Table/Fig-4]: Cellwise quantification of intracellular contents on EM, Case No-2: Block-II.

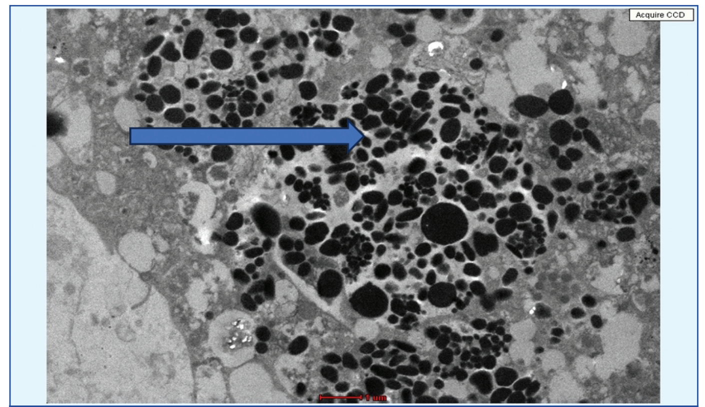
Cellular contents	Numerous	Moderate	Few
Melanin pigment	5 (19.23%)	8 (30.76%)	13 (50%)
Lens proteins	22 (84.61%)	4 (15.38%)	0
"Clear fluid" vacuoles	11 (42.30%)	4 (15.38%)	11 (42.30%)

[Table/Fig-5]: Qualitative analysis of cellular contents of macrophages (n=26).
*3 cells were non viable. 1 showed -cell in cell phagocytosis

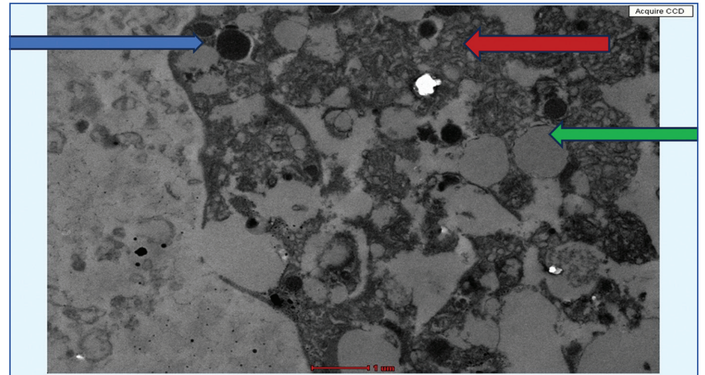
Majority of the macrophages (86.6%) which were viable, showed distension of the cytoplasm with numerous phagosomes containing predominantly a vacuolated material, possibly lens protein. Few phagosomes contained predominantly clear fluid with fine granular material. Many membrane bound granules containing uniformly electron dense material, ranging in size from approximately 100 nm to >1000 nm were also seen in the macrophages. These granules ranged in shape from round to oval, elongated and were consistent with melanin pigment granules [Table/Fig-6-8]. Majority of the macrophages showed very few cell processes and only one macrophage with a pseudopodium was seen. A few of the macrophages showed prominent cell processes actively engulfing the surrounding fluid and lens protein. Nuclei of the macrophages were often pushed to the periphery because of the abundance



[Table/Fig-6]: Electron microscopy (4.5x) showing macrophage flooded with lens proteins, few vacuoles and melanin pigments.
Arrows indicate: Vacuole[1], Lens protein[2], Nucleus[3], Cell process[4] and melanin pigment[5]
[1]Green arrow, [2]Red arrow, [3]White arrow, [4]blue arrow, [5]light blue arrow



[Table/Fig-7]: Electron microscopy (10x) showing macrophage containing large amount of melanin pigments.
Arrows indicate: Melanin pigment[1]



[Table/Fig-8]: Electron microscopy (20x) of macrophage showing plenty of lens protein, vacuoles, melanin pigment.
Arrows indicate: Melanin pigment[1], lens protein[2], vacuoles[3];
[1]Blue arrow, [2]red arrow, [3]green arrow

of engulfed material. Only minimal amounts of the macrophage cytoplasm were evident. Occasional macrophages contained lipid bodies. One monocyte with no engulfed material was seen. In the first case, only macrophages were seen though the second case showed four neutrophils on EM.

Quantification of phagocytosed material: Ten consecutive macrophages were studied in each block to semiquantitate the phagocytosed material. Out of the total 30 cells studied, 22 macrophages showed abundant lens protein material within the phagosomes. Four macrophages showed small to moderate amounts of lens protein material. Three cells were non-viable, and one cell demonstrated cell-in-cell phagocytosis; hence, these could not be commented upon.

DISCUSSION

The EM has provided important insights into the cellular mechanisms of phacolytic glaucoma. This study aimed to characterise macrophages in phacolytic glaucoma using EM, an area with very limited prior literature. We anticipated that identifying ultrastructural features could improve understanding of disease pathogenesis and potentially aid in diagnosis and management.

Macrophages were the predominant cell observed in the present study though occasional neutrophils and a monocyte was noted as well. The presence of RBCs was considered insignificant and attributed to surgical trauma. The macrophages appeared markedly distended with abundant phagocytosed material that was predominantly vacuolated, most likely lens proteins with associated fluid and fine granular material. In majority of the cases the nucleus was displaced peripherally by the large amounts of lens proteins and clear vacuoles in the cytoplasm. These features suggest that the macrophages were overwhelmed with the ingested material. Such overloading of the macrophages may affect their role as effective antigen-presenting cells and limit the recruitment of other inflammatory cells like lymphocytes which are typically seen

in phacotoxic uveitis [16]. It is also possible that the phacolytic protein differs from the usual lens protein in its antigenicity which influences the inflammatory response that it elicits. These two reasons could explain the absence of lymphocytes in the present study.

In order to enable quantitative and qualitative assessment of ingested lens proteins and vacuoles in the cytoplasm definitive criteria was applied. When cytoplasmic involvement by the lens protein was 50% or more it was categorised "numerous" while an involvement of 10-50% was considered "moderate" and less than 10% as few. None of the cells demonstrated less than 10% of cytoplasmic involvement. For vacuoles it was categorised as numerous when cytoplasmic involvement was 50% or more, moderate when it was 25-50% and anything less than 25% was considered as few. With respect to melanin pigments, any number less than 10 was categorised as "few", 10-25 as "moderate" and more than 26 as "numerous". From the above the subjectivity in classification is obvious however some objectivity would allow better comparison across studies.

The presence of four non viable cells and a single monocyte amongst the 30 cells studied suggests a high cell turnover due to the excessive burden of phacolytic lens matter that these cells have to deal with. The presence of melanin pigments in the macrophages has been reported by Ueno H et al., as well [14]. It is possible that the high IOP causes iris pigment epithelial cell death leading to pigment release from these cells on the posterior surface of the iris which are then engulfed by the macrophages. Whether it is this melanin pigment which causes the cells to become more rigid thus blocking the trabecular meshwork leading to IOP spike is something worth looking into.

In the third case, no cells were identified, possibly due to prior use of over-the-counter topical steroids leading to suppression of inflammatory cellular response. In another case, cell lysis was observed which might have been secondary to a delay in sample processing.

Limitation(s)

This study had several limitations. Obtaining adequate aqueous humour for EM was challenging. Anterior chamber paracentesis in inflamed eyes with hypermature cataract carries a risk of chamber shallowing and inadvertent injury to the lens capsule or cornea, especially in eyes with already poor vision. Additionally, the high cost and technical demands of EM limited its use to cases where sufficient sample was available after routine analysis, contributing to the small sample size and potential sample bias which also limits the ability to generalise the findings of the study.

Sample degradation, including cell lysis and absence of cells in some cases, could have affected ultrastructural interpretation. Also, since the quantitative assessment of intracellular contents is subjective it affects reproducibility. Finally, the absence of correlation with clinical parameters limits the functional interpretation of the findings.

CONCLUSION(S)

The EM study revealed macrophages as the predominant cell type in patients with phacolytic glaucoma although occasional neutrophils and a single monocyte were also observed. All these macrophages were overwhelmed with lens protein, melanin pigments and vacuoles which filled more than 75% of the cell in majority of the cases. However, objectivity in classification of macrophage fill will help in comparison across studies. Nevertheless, these findings may serve as a reference framework and pave the way for future studies ideally conducted at multicentric level with standardised protocol and guidelines to further elucidate phacolytic glaucoma.

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