COMPARATIVE HISTOPATHOLOGY AND BIOCHEMICAL ANALYSIS OF AQUEOUS HUMOR IN PHACOLYTIC GLAUCOMA AND IMMATURE CATARACT

SUBMITTED BY

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BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"Comparative Histopathology and Biochemical analysis of Aqueous Humor in Phacolytic Glaucoma and Immature Cataract"** done towards fulfillment of the requirements of the Tamil Nadu Dr MGR Medical University**,** Chennai for MS Branch III Ophthalmology examination to be conducted in 2014, is the bonafide original work of **Dr. Reetha B T**, Post Graduate student in Ophthalmology, Christian Medical College, Vellore.

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Opacification of lens or its capsule which can be developmental or acquired is known as cataract. This is graded into immature, mature and hypermature depending on the extent of opacification. Cataract if left untreated become hypermature and eventually leaks out liquefied material into anterior chamber. The lens protein which thus leak cause blockage of trabecular meshwork resulting in an acute raise in intraocular pressure (IOP) with pain, leading to a condition called Phacolytic Glaucoma (1) Lens induced glaucoma include a group of conditions where in the crystalline lens plays a common role in the mechanism of raised intra ocular pressure, leading to glaucoma.(2). Phacolytic glaucoma...

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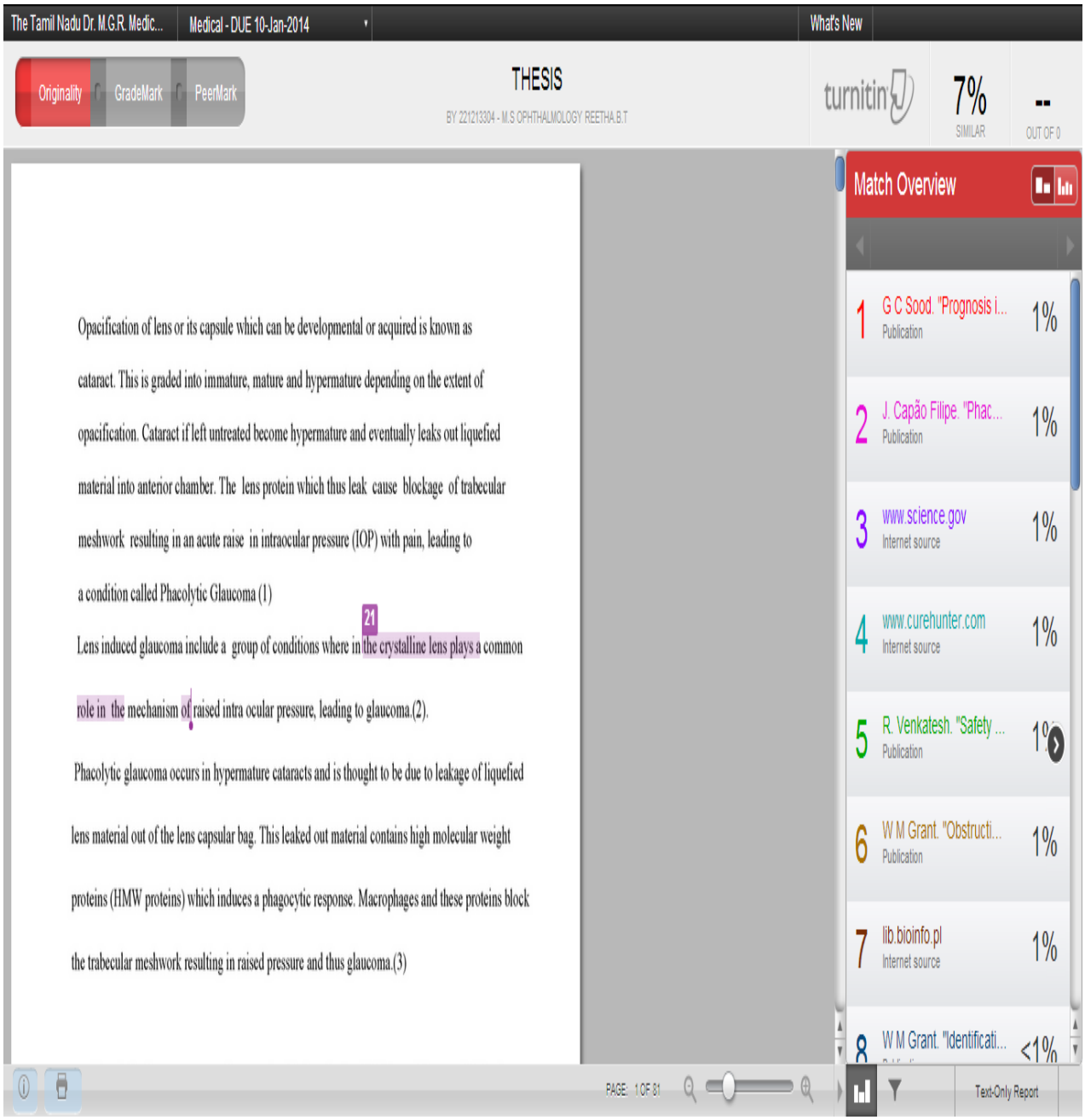

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ABSTRACT

TITLE : COMPARATIVE HISTOPATHOLOGY AND BIOCHEMICAL ANALYSIS OF AQUEOUS HUMOR IN PHACOLYTIC GLAUCOMA AND IMMATURE CATARACT

Department : Ophthalmology

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Aims &objectives : The aim of this study was to characterize Phacolytic glaucoma by doing a comparative study of the clinical features, Ultrasound biomicroscopy and aqueous humor (biochemical & histopathological) study in phacolytic glaucoma and immature cataract and also to study the characteristics of the macrophages in Phacolytic glaucoma using electron microscopy(EM)

Material and Methods: This was a case- control study was conducted from December 2012 – November 2013. Patients attending the outpatient department or casualty diagnosed to have phacolytic glaucoma or immature cataract(up to nuclear sclerosis Grade-3), fulfilling the inclusion and exclusion criteria were included in the study. Ultrasound biomicroscopy was performed on all patients at presentation followed by paracentesis to analyse aqueous humor for presence of cells and to estimate protein concentration and results were analyzed. In 3 cases aqueous humor was studied by electron microscopy.

Results : Aqueous humor study showed cells in 75% of cases. Neutrophils were seen in 58.3 % and macrophages in 50% of samples. This high incidence of Neutrophils is reported rarely in literature. The protein concentration in cases of phacolytic glaucoma was four times that of controls. Electron microscopy study revealed macrophages as the predominant cell with only 1 monocyte and few neutrophils. UBM revealed AC echoes only in the two cases which had clinically evident fluffy material in the AC.

Keywords : Phacolytic glaucoma , Aqueous humor ,Electron microscopy, Ultrasound biomicroscopy

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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. Cataract if left untreated become hypermature and eventually leaks out liquefied material into anterior chamber. The lens protein which thus leak cause blockage of trabecular meshwork resulting in an acute raise in intraocular pressure(IOP) with pain ,leading to a condition called Phacolytic glaucoma.[1]

Lens induced glaucoma include a group of conditions where in the crystalline lens plays a common role in the mechanism of raised intra ocular pressure, leading to glaucoma.[2]

Phacolytic glaucoma occurs in hypermature cataracts and is thought to be due to leakage of liquefied lens material out of the lens capsular bag. This leaked out material contains high molecular weight proteins (HMW proteins) which induces a phagocytic response. Macrophages and these proteins block the trabecular meshwork resulting in raised pressure and thus glaucoma.[3]

Phacolytic glaucoma commonly occurs in elderly patients with history of decreased vision in one eye for a long duration usually, months to years, and in patients with minimal access to facilities of health care.[4]

Clinical features of phacolytic glaucoma is attributed to raised intraocular pressure associated with inflammation and include the following:

1) Raised intraocular pressure with acute onset corneal edema
2) Hypermature cataract with presence of exudates and cellular reaction in anterior chamber

3) Pseudohypopyon and polychromatic hyperfringent crystal like particles in the anterior chamber along with a semi dilated pupil and open angles.[5]

In rare instances phacolytic glaucoma can have a sub-acute onset where in there is intermittent leakage of lens proteins resulting in repeated episodes of inflammation and glaucoma. This is mostly seen in cases where cataractous lens has dislocated into vitreous. Vision in phacolytic glaucoma is usually low, to the extent of light perception being inaccurate.[4]

Phacolytic glaucoma may also be associated with the presence of soft spots on the lens surface-which actually are macrophages sticking to anterior capsule of the lens in order to seal the micro ruptures in lens capsule after engulfing the lens proteins.[6,7]

Diagnosis in case of phacolytic glaucoma is usually clinical but diagnosis maybe confirmed by techniques such as phase contrast microscopy and cytological examination of aqueous.[4]

Definitive treatment in case of phacolytic glaucoma involves eliminating the triggering factor which in this condition is the cataractous lens. But prior to surgery control of intraocular pressure and inflammation is a must which is done by medical management using anti-glaucoma medications such as carbonic anhydrase inhibitors or hyperosmotic drugs and topical steroids.[4]

Surgical intervention involves extra-capsular or intra-capsular cataract extraction. In earlier days intra capsular technique was followed and examination of these lens on microscopy revealed the presence of calcium oxalate crystals. In case

of capsular rupture during surgery thorough irrigation of anterior chamber should be done in order to ensure that no protein material is retained.[4]

Limited studies (3,8,9,11,13) have looked into the nature of the material leaking into the anterior chamber (AC) in cases of phacolytic glaucoma. It is thought that high molecular weight proteins (HMW proteins) / macrophages block the trabecular meshwork and induce glaucoma.[3]

Ultrasound Biomicroscopy (UBM) to study the anterior segment in patients with phacolytic glaucoma has not been done so far to the best of our knowledge. This is a newer diagnostic modality and we have tried to characterize the UBM features in this study.

Electron microscopy studies of aqueous humor in phacolytic glaucoma are rare, here we have looked at the electron microscopic features of the centrifuged contents of aqueous humor in a small subset of our patients with phacolytic glaucoma.

In this study we relooked at the histopathological and biochemical composition of the aqueous in phacolytic glaucoma and compared it with the findings in immature cataract in addition to adding newer diagnostic modalities to characterize phacolytic glaucoma.

AIM OF STUDY

The aim of this study was to characterize Phacolytic glaucoma by doing a comparative study of the clinical features, Ultrasound biomicroscopy and aqueous humor (biochemical & histopathological) study in phacolytic glaucoma and immature cataract.

Objectives:

1) Compare the clinical features - IOP, AC finding in phacolytic glaucoma and immature cataracts.

2) To analyze aqueous humor cells in phacolytic glaucoma and immature cataracts.

3) To estimate protein content in aqueous humor in phacolytic glaucoma and immature cataracts.

4) To study the characteristics of the macrophages in Phacolytic glaucoma using electron microscopy(EM).

5) To study the anterior segment characteristics using the ultrasound biomicroscope (UBM) in phacolytic glaucoma and immature cataracts.

REVIEW OF LITERATURE

In human lens, protein concentration is about 33%,[14] which is twice the concentration seen in other tissues.

Lens proteins are classified into 2 groups[14]

1)Water soluble: Alpha, beta and gamma crystallins.

2)Water insoluble :Urea soluble and urea insoluble proteins.

60 % of the wet weight and most of the dry weight of lens is attributed to lens proteins .The water soluble portion includes cytoplasmic proteins and the water insoluble fraction includes cytoskeletal and plasma membrane proteins.[1]

Among the various crystallin proteins α –crystallin has largest molecular size.(with a molecular weight of 600 -900k Daltons (Da)

Beta crystallin can be divided into 2 fractions by gel chromatography:

1)Beta –light (β L -52 k Da)

2)Beta heavy(β H :150 -210 k Da)

β –L can be separated into β L -1, β L-2.

γ-crystallin are smallest among various crystallin fraction of proteins and can be subdivided into 6 fractions γ-A to γ-F , with molecular weight of 20 k Da.[1]

α –crystallin are the first of crystallins to be synthesized and they are found in all the cells of lens unlike β and γ-crystallin which are first detected in elongated cells. α –crystallin are found in epithelial cells and lens fibres but β and γ-crystallin are present in lens fibres only. α –crystallins are found in dividing and non –dividing cells, where as β and γ-crystallin are found in non dividing cells only. The transformation of a lens

epithelial cell into lens fibre acts as a triggering factor for synthesis of β and γ-crystallins. The high concentration of crystallins with their refractive gradient is responsible for the refractive property of lens. The crystallins also function as chaperone under conditions of oxidative stress.[1]

During the natural process of maturation there is conversion of water soluble proteins into water insoluble proteins ,which is exaggerated in a lens which is cataractous . In brunescent cataract there is an increase in amount of water insoluble proteins which co-relate well with degree of opacification.[15]

The components of WS-HMW(water soluble high molecular weight proteins) and WI(water insoluble) protein fractions of cataractous lenses differs from that in normal lenses. Selective insolubilization of fragments of betaA3/A1- and betaB1-crystallin occur during cataract development compared to normal lenses. Further, the crystallin species of cataractous lenses showed increased truncation, deamidation of Asparagine(Asn) to Aspartic acid (Asp) residues, and oxidation of Tryptophan(Trp) residue.[16]

Glaucoma in Greek means 'CLOUDED' and is defined as the disturbance in integrity of optic nerve both in structure as well as its function which can be arrested or reduced by decreasing the intraocular pressure to an adequate level .It includes a group of conditions which have certain common features in terms of optic nerve atrophy, optic disc cupping ,corresponding visual field loss and all these usually occur in relation to intraocular pressure level.[17]

Primary open angle glaucoma is a condition in which there is optic neuropathy which is multi-factorial and acquired optic atrophy associated with retinal ganglion cell loss which develop in presence of anterior chamber angles being open , which is manifested by characteristic abnormalities of visual field . They do not have any association with any ocular conditions or systemic diseases which can result in aqueous outflow obstruction .Though primary open angle glaucoma has been found to be associated with many risk factors, raised intraocular pressure is the most important factor among all which is common to both primary and secondary glaucoma and is also the only factor which can be modified with intervention.[17]

Secondary glaucoma's are defined by the presence of raised intraocular pressure and not by the presence of optic neuropathy that is seen in association with raised intraocular pressure. They are associated with certain ocular conditions or systemic diseases which attribute for the raised intraocular pressure .They are usually acquired conditions and are unilateral.[17]

Lens induced glaucoma include group of conditions wherein the crystalline lens plays a common role in the mechanism of raised intra ocular pressure, leading to glaucoma.[2]

Lens induced glaucoma can be divided into two broad groups:

1) Obstruction of trabecular meshwork by the Iris tissue due to pupillary block :

a)Phacomorphic glaucoma(pupil block)

b)Ectopia lentis

2) Obstruction of trabecular meshwork due to lens proteins or debris :

a)Phacolytic glaucoma[2]

Phacomorphic glaucoma is a type of lens induced glaucoma in which pupillary block results from an abnormal lens or the peripheral iris is mechanically pushed forward by the lens into the angle structures .Phacomorphic glaucoma is described in association with an intumescent lens.[18]

Lens particle glaucoma is a condition in which penetrating trauma results in lens capsule disruption leading to release of lens material which tend to obstruct the trabecular meshwork thereby resulting in glaucoma .Glaucoma thus resulting from the liberated lens material depends on the quantity of lens material released and the inflammatory response it incites in the eye and also on the trabecular meshwork's ability in removal of these lens material.[4]

Phacoanaphylaxis is a condition which is uncommon and occurs due to sensitization to one's own lens proteins which usually is seen following an extra-capsular surgery of cataract extraction or penetrating trauma .Histopathological examination revealed granulomatous inflammation with presence of lymphocytes, giant cells, polymorphonuclear leucocytes and epitheloid cells. They are managed conservatively with medications to reduce intraocular pressure and control inflammation.[4]

In 1900 it was Gifford[3] who described an entity of open angle glaucoma in association with hypermature form of cataract. Flocks et al proposed that the glaucoma in this condition is due to lens material inducing a macrophagic response. Epstein et al have shown that obstruction to aqueous flow in this condition is primarily due to heavy molecular weight proteins of the lens.

The characteristic features of phacolytic glaucoma includes acute onset of raised intraocular pressure in association with hypermature cataract. The proposed mechanism for pathogenesis of phacolytic glaucoma is leakage of heavy molecular weight proteins through the anterior capsule of the lens which in turn triggers an inflammatory response and causes blockage of trabecular meshwork, leading to raised intraocular pressure.[12]

The clinical features of Phacolytic are:

1) Acute onset corneal edema with raised intra ocular pressure

2) Exudates and cellular reaction being present in anterior chamber

3) Hypopyon and polychromatic hyperfringent crystal like particles in the anterior chamber and open angles on gonioscopy

4) Hypermature cataract[5]

The differential diagnosis of PHACOLYTIC GLAUCOMA includes:

1) Glaucoma secondary to trauma,

2) Open angle glaucoma with uveitis,

3) Neovascular glaucoma,

4) Acute angle closure glaucoma.

5) Endophthalmitis[5]

Sowka et al[19] report a case of phacolytic process with clear time delineation. They reported a case of mature cataract which became hypermature and subsequently underwent phacolysis with an associated rise in intraocular pressure due to ongoing inflammatory process over a period of 17 days. This was thought to be a first time in which the time process involved in development of phacolysis was studied. This case was also considered as the one having the fastest onset of phacolytic process among the cases reported at that period of time . Although maturation of cataract is usually considered as a slow and insidious process, this study laid emphasis on the fact that the onset of phacolytic process may not be slow always. Once a lens becomes hypermature, phacolysis might occur rapidly over a period of several days. This case stressed on the importance of management in terms of removal of cataractous lenses in cases of advanced cataract. This case might have been be the one with shortest reported time from initial diagnosis of a mature cataract to the development of inflammatory phacolysis and secondary glaucoma, which developed over a short time span of only 17 days.[19]

Sahu S K et al [5] report an unusual case where a dislocated cataractous lens which was found to be firmly adherent to the endothelium of the cornea which resulted in a cellular reaction similar to phacolytic glaucoma but it appeared clinically as a deep corneal abscess. This case report is of a 73-year-old lady who

presented with complaints of severe photophobia, pain, and redness in left eye since two months despite the patient being on antibiotics and anti-fungal. An anterior chamber wash was done which revealed an infiltrate with the cataractous lens being buried within it, which was subsequently removed and sent for histopathological analysis. Postoperatively patient was treated with topical ofloxacin, homatropine, dorzolamide, timolol, and tapering dose of steroids. Histological examination showed the presence of inflammation, histiocytic response, and also giant cells lying around the lens material which established the ongoing phacolytic process. Following removal of the lens photophobia, pain, and redness subsided.[5]

Mavrakanas Net al reported a case of acute phacolytic glaucoma in which the anterior chamber was found to contain only protein in the absence of macrophages. They suggested that this case represented a form of phacolytic glaucoma which was characterized by a hyper acute presentation and was due to the rapid leakage into the aqueous humor of lens proteins which were degenerated as compared to another form of phacolytic glaucoma which presents with an onset which is more gradual and is associated with the presence of phacolytic macrophages in the aqueous humor which results from an immunologic reaction seen in response to the lens proteins. Thus they suggested the existence of perhaps 2 forms of phacolytic glaucoma, with distinct characteristics and pathophysiology.[20]

Barnhost D et al[21] observed and report an unusual presentation of lens-induced glaucoma which occurred in a patient who had undergone congenital cataract extraction surgery 65 years prior. This patient was successfully treated by them by pars plana vitrectomy through which the retained lens material was removed.

Examination of the intraocular specimen revealed the presence of lens material, epithelial cells as well as macrophages. It was suggested that it might have taken years for denaturation of residual lens material and for it to break down into smaller pieces, which thus led to the development of phacolytic glaucoma.[21]

Bremond D et al[22] report a case of an eighty five year old man who presented with a picture similar to acute endophthalmitis; associated with raised intraocular pressure (60 mm Hg) and visual acuity being perception of light only. Diagnosis of Phacolytic glaucoma due to leakage of lens proteins through the lens capsule perforations which were pre-existing was made . Intra-capsular lens extraction was performed to remove the lens without any placement of intraocular lens. Examination on the first post-operative day revealed normal intraocular pressure with visual acuity improving to 6/10 P2.[22]

Peddi A et al[23] report two cases who presented to them with the combination of phacolytic glaucoma and Fuch's uveitis . Both of these patients were females who were 50 and 55 years old respectively and presented with sudden onset of painful diminution of vision of few days duration only in the right eye with the left eye being completely normal in both. The right eye on examination showed morgagnian cataract associated with features typical of phacolytic glaucoma in the form of congestion of conjunctiva, corneal edema, anterior chamber flare as well as particles floating in the anterior chamber. There was also an associated loss of iris pattern along with patchy hypochromia. Though the first patient underwent an extra-capsular cataract extraction surgery with an intraocular lens implantation in the posterior chamber which was uneventful ,it did not help achieve control of the intraocular pressure although control of the inflammation was achieved and

maximal anti-glaucoma medications were used. The intraocular pressure in this patient was 35 mmHg at 6 weeks post –operative follow up. Based on this experience a suggestion of an extra-capsular cataract surgery with intraocular lens implantation in the posterior chamber along with trabeculectomy was made to the second patient but the patient failed to show up for surgery. In a case of phacolytic glaucoma, usually cataract extraction is considered as a definitive treatment and following this complete remission of glaucoma is usually seen, where as a case of phacolytic glaucoma secondary to Fuch's do not respond to medical treatment hence needs surgical intervention. Diagnosis of Fuch's was made in addition to phacolytic glaucoma based on the presence of typical iris changes and unilaterality. Glaucoma in this condition appears refractory to a cataract surgery alone hence a combined trabeculectomy may be advised.[23]

Aqueous humor in phacolytic glaucoma and other grades of cataract have been studied in order to understand the mechanism leading to phacolytic glaucoma.

In a study conducted by Epstein DL et al on six patients with phacolytic glaucoma and six controls –which included patients who had immature cataract but clear aqueous humor. About 0.1-0.2 ml of aqueous humor was obtained during cataract surgery and analyzed by phase contrast microscopy for cellular content and remainder of aqueous humor was analyzed for protein concentration by chromatography –which revealed presence of heavy molecular weight proteins(>150 x 10^6) in all 6 patients with phacolytic glaucoma with none being found in the control patients. Three of these hypermature cataract lenses among the cases of phacolytic glaucoma were further examined and found to have around 14-fold greater amount of HMW protein in their cortex in comparison to the cortex of lens with immature

cataract. These findings also correlated with the experimental studies conducted where in HMW protein perfusion was done in excised human eyes which indicated direct obstruction to aqueous outflow channels by these HMW soluble lens protein as a significant and unappreciated factor in the pathogenesis of phacolytic glaucoma in the earlier studies.[13]

According to a study conducted by Goldberg MF et al, cytological diagnosis in patients with phacolytic glaucoma was made using millipore filtration of the aqueous .This study revealed presence of macrophages (several 100 large rounded cells seen singly and few in clumps), large number of erythrocytes and one polymorphonuclear leucocyte with absence of plasma cells and fibroblasts. Lens capsule revealed presence of grayish white opacities which stained positive for calcium.[8]

In a study conducted by R.S.Bartholomew et al on three patients with characteristic signs of phacolytic glaucoma, aqueous humor was analyzed which revealed presence of viable spheroidal cells-macrophages, notched rhomboid plate like crystals in two samples-morphologically typical of cholesterol, and octahedral crystals in third sample-which were identified as calcium oxalate based on their morphology and chemical properties. Crystals of calcium oxalate have been identified in cases of hypermature cataract in the lens nucleus as well as in cases of long standing retinal detachment but were not seen free in the aqueous humor. The probable oxalate source in the eye is not clear. The possible sources from which it could be derived include carbohydrates, purine ,amino acids, creatinine. Vitamin-C which in the presence of light degrades to oxalic acid. The concentration of Vitamin-C in aqueous humor is quite high compared to that found in plasma. Obstruction to

outflow leading to aqueous humor stasis may result in high concentration of calcium or oxalate from either of the sources which can crystallize when suitable nidi are present.[11]

Anne.M.V .Brooks et al conducted a study in which comparison of specular and direct microscopy of wet aspirate of aqueous in 5 cases of phacolytic glaucoma was done. Four of these patients underwent specular microscopic examination which revealed a blurred endothelial mosaic picture suggestive of sub endothelial edema .In all cases during cataract surgery aqueous humor sample was collected and analyzed. It revealed presence of macrophages(regular round cells about 3 times size of an erythrocyte)seen in the relief mode of specular microscopy, and a single hyperfringent crystals which were morphologically identical with cholesterol crystals. These findings confirmed the presence of cholesterol crystals in the exudates of phacolytic glaucoma which are responsible for their hyperfringent nature.[9]

In a study[24] on the chemotactic activity of lens proteins and pathogenesis of phacolytic glaucoma, Rosenbaum et al (24)suggested that lens proteins might account for the monocyte response. Concentration based monocyte migration was induced by a sonicated lens induced concentration-dependent migration in Boyden chamber assay system and a Checkerboard analysis indicated that the monocyte movement was directed rather than being merely random. In relation to a control chemoattractant, N-formyl-methionyl-leucyl-phenylalanine, the lens induced monocyte migration was more potent than that of neutrophil migration. This ability to induce migration was markedly reduced when the lens was incubated with either trypsin or papain. Chemotactic activity was also readily demonstrable in lens without

cataract from young donors . Separation of lens proteins by gel filtration with high-performance liquid chromatography indicated the chemotactic activity was most consistent with the gamma crystallin fraction in comparison to other fraction of proteins. The chemotactic activity of lens proteins may contribute to the pathogenesis of phacolytic glaucoma or the uveitis resulting from retained cortical material after cataract extraction.[24]

Hypermature cataract is usually associated with leakage of lens proteins. This leak usually occurs secondary to alteration in permeability of lens capsule, but it can also be seen in conditions such as trauma to the crystalline lens resulting in formation of cataract and lens matter leakage thus acting as a precursor for occurrence of phacolytic glaucoma.[25]

Enucleated human eyes were perfused by Epstein DL et al via the anterior chamber at 25 mm Hg pressure with lens particles (which were whole lens homogenates) in one series and soluble lens proteins which were obtained from human cataractous lenses in the other series . Addition of 1% homogenate of single cataractous lens into the anterior chamber resulted in decrease of outflow by 68%. However HMW soluble lens proteins (MW more than 150 million) perfusion decreased the outflow by 60% in about 1 hr. The outflow obstruction was not relieved by anterior chamber irrigation either with balanced salt solution or alpha-chymotrypsin in neither of the series. These results suggest that it is possible for both lens particles as well as soluble lens proteins to directly obstruct the aqueous outflow pathways of human eyes which may be a significant factor in certain lens-induced glaucoma.[26] Cytological analysis of aspirate from the anterior chamber obtained following the procedure of needling was done in about 28 eyes from 23

patients(27).The time interval between the procedure of needling and aspiration was directly proportional to the cellular response elicited which comprised purely of macrophages. The cell type obtained in the aspirate after needling was similar to that seen in phacolytic glaucoma but were much lesser in terms of number of cells obtained when compared to phacolytic glaucoma .This suggested that the lens proteins which escape from hypermature cataracts are mostly altered which leads to a more toxic or an aggressive response in comparison to the lens particles which are released into the anterior chamber, post needling procedure done on soft cataractous lens. It was concluded that additional risk factors such as genetic factors might be involved in the occurrence of Phacoanaphylactic endophthalmitis rather than the mere presence in the eye of lens proteins which are found to be altered as a result of lens capsule which was disrupted.[27]

In a study conducted by Hayasaka S et al biochemical determination of degrading activity exhibited by lens protein in aqueous humor was done. The aqueous humor of patients with Morgagnian cataract, traumatic cataract, after cataract, traumatic lens subluxation and phacolytic glaucoma demonstrated proteolytic activity. This activity was seen at pH of 3.8 and it also showed inhibition partly by pepstatin and leupeptin.It was also suggested that probably cathepsin D as well as lysosomal thiol proteases which are present in the macrophages may actually play a major role in degrading the lens protein which leak under various pathological conditions.[28]

Ueno H et al[29] studied a case of phacolytic glaucoma in a 63 year old man . The cells floating in the anterior chamber as well as the specimen obtained by trabeculectomy were examined under a light microscope as well as a

transmission electron microscope. It was noted that the anterior chamber contained numerous macrophages which had engulfed phagocytized degenerated lens material (phacolytic cells) as well as free-floating degenerated lens material . In addition melanin-laden macrophages were also seen in the anterior chamber which were accompanied by few erythrocytes, ghost erythrocyte, lymphocyte, and macrophages showing erythrophagocytosis and leukophagocytosis and phagocytizing degenerating macrophages. This is the only article on EM studies in phacolytic glaucoma and there was none in the English literature.

Filipe J C et al report a case of phacolytic glaucoma in left eye of a patient who presented with bilateral dislocation of the lens into vitreous. The aqueous and vitreous were aspirated and studied which showed lenticular fragments and macrophages with lipofuscin granules and phagocytic vacuoles containing lens proteins. Further Immunocytochemistry study was done which revealed the presence of foamy macrophages which were immunoreactive for CD68 and HLA-DR. About a year later the right eye showed anterior granulomatous uveitis which was mild along with mutton-fat keratic precipitates on the cornea which remained unchanged in the follow-up period without any further treatment. These findings suggest the possibility that, besides their mechanical and inflammatory roles in the impairment of the outflow system of the (exciting) left eye, phacolytic macrophages might also have been involved in the afferent phase of the mild chronic uveitis of the (fellow) right eye.[30]

The records of 135 patients with Phacolytic glaucoma were retrospectively studied in order to determine if vitreous opacities were present by Thomas R et al. [31]

Five eyes of patients with PG demonstrated vitreous opacification, which was initially noted during surgery and later confirmed in the post operative period. In all these patients symptoms of phacolytic glaucoma were present for 7 or more days (mean +/- SD, 10.6 +/- 2.4 days) before medical attention was sought. During pre-operative examination in three of these eyes hypopyon was present and among them the anterior chamber showed refractive crystals in two eyes. In all these five eyes the vitreous opacities showed spontaneous resolution in about 12 weeks duration with visual acuity being affected in them during immediate postoperative period only. Thus it was concluded that in phacolytic glaucoma vitreous opacification being a self-limiting process may not require any surgical intervention unless it is desired to attain a rapid visual rehabilitation and the vitreous opacification seen in these cases is probably due to liquefied lens material that has leaked through the posterior capsule.[31]

In a case report by Blaise P et al[32] in which a patient of phacolytic glaucoma refused surgery but was still able to achieve a normal intraocular pressure and recovery of vision to a satisfactory level due to spontaneous absorption of the hypermature cataractous lens.

A 79 yr old lady who presented to their hospital with complaints of pain and redness in the left eye for a duration of five days and on examination was found to have phacolytic glaucoma in left eye with initial pressure recorded being 62 mm Hg and vision being light perception only with presence of iridescent particles in the anterior chamber along with intense flare. Patient was started on oral and topical anti-glaucoma medications following which her pain subsided, hence patient refused surgery. Patient was lost to follow up and when she presented after a duration of

seven months, spontaneous absorption of lens with only thin white opacity left behind in the capsular bag with normal pressure of 20 mmHg and disappearance of flare. On examination her visual acuity with +12.00 D lens was found to be 4/10.[32]

In Indian population ,lens induced glaucoma account for a majority of the secondary glaucoma. In a retrospective study which was conducted on about forty patients who presented to Nepal eye hospital for a span of two years between 2002 and 2004 ,analysis of control of intraocular pressure ,patient's age as well as sex and time interval between onset of symptoms and initiation of surgical treatment was done. Prevalence was found to be greater in females.

Phacomorphic glaucoma was more common. In about 45% of lens induced glaucoma-visual outcome was good and ranged from 6/12 – 6/60 and in 30% of them it was moderately fair with visual acuity <6/60, time interval between onset of symptoms and the surgical treatment was as less as 1 week to a maximum of four months. Intraocular pressure prior to surgery was around 24-59 mm of mercury and post surgery around 14-22 mm of mercury.[33]

According to a study conducted by G C Sood et al[34] on Prognosis in spontaneous Phacolytic Glaucoma – in which they reported three cases of spontaneous phacolytic glaucoma which were treated successfully. In two of their cases intracapsular extraction was performed without any difficulty by upper pole delivery using forceps and in one case it was done using a erisophake once superior sector iridectomy was completed. Leakage of lens matter which decreases the tension of the anterior capsule was assumed to make it easy to catch with the help of a capsule forceps. Weakening of the zonules as a result of the phacolytic process in

these cases was suggested to be the reason of being able to perform intracapsular cataract extraction easily in them. Lowering of intraocular pressure was achieved by Diamox and by paracentesis in one case. Paracentesis is a useful procedure, in cases which are unresponsive to Diamox or other osmotic agents. The aqueous which is drained can be used for diagnostic purposes by methods as suggested by Goldberg. Though these patients presented 4 to 8 days after the phacolytic attack and the procedure of lens extraction was performed about 5 to 24 days later, good results were obtained in terms of vision. In two cases though projection of light was inaccurate ,it is not considered as a contraindication to surgery. The visual prognosis in cases of phacolytic glaucoma, even which are long standing and have inaccurate projection of light, has been found to be good.[34]

Intracapsular cataract extraction had been the initial choice of treatment in phacolytic glaucoma. According to a study conducted by Lane SS et al to establish the efficacy of extra-capsular cataract extraction (ECCE) in treatment of phacolytic glaucoma where in Five phacolytic glaucoma cases were studied after a retrospective review and ECCE (with intraocular lens placement in posterior chamber) was done with no complication reported and was found to be effective in all five cases. In all patients (100%) intraocular pressures (IOPs) was maintained at less than 20 mmHg, in the absence of medical therapy. Best-corrected visual acuity in all cases was found to be 20/50 or better (80%, greater than or equal to 20/40) with a follow-up period of 5 months to 3 years. These results have shown that ECCE can be an effective mode of treatment in a case of phacolytic glaucoma and gives the surgeons a chance to choose a procedure which they are comfortable with and also suggest that in addition

implantation of a PC IOL is a safe and effective technique in restoring visual function in these patients.[35]

In a study conducted by Rengaraj Venkatesh et al[36] to evaluate the safety, visual outcome and complications of manual small incision cataract surgery (MSICS) in the treatment of patients with phacolytic glaucoma 33 patients who were diagnosed to have phacolytic glaucoma underwent manual small incision cataract surgery using trypan blue to stain the anterior capsule. Phacolytic glaucoma is due to trabecular meshwork obstruction from lens proteins and protein-laden macrophages. The MSICS using trypan blue to stain the anterior capsule was found to have advantages in comparison to both extra-capsular cataract extraction (ECCE) as well as phacoemulsification. In ECCE a large incision is required and in an eye with raised IOP, it increases the risk of visual threatening complications like expulsive hemorrhage. Surgical steps are more challenging and it may be also be complicated by prolapsed of iris tissue through the limbal wound which is larger compared to MSICS. It has also been shown that uncorrected vision is better in MSICS when compared to ECCE because postoperative astigmatism is higher in ECCE. In phacolytic glaucoma Phacoemulsification can be difficult due to the nature of nuclei which are dense and hard ,compromise of the capsule and zonules thus giving little support. A higher risk in terms of endothelial damage, zonular dialysis, and rupture of posterior capsule is seen. In comparison MSICS causes less stress on the zonules, the need for expensive equipment as in the case of phacoemulsification is not present and also has better stability of anterior chamber because of the shelving scleral wound. In MSICS, staining of the anterior capsule using trypan blue enhances the safety as well as the ease with which nucleus prolapse can be done because trypan

blue helps in better visualization of the rim of the capsule, thus allows to detect any compromise of the capsular bag and if present, relaxing incisions will help to avoid intracapsular nucleus removal.

Braganza A et al[37] conducted a study retrospectively in which 135 eyes of patients with phacolytic glaucoma were analyzed. If symptoms were present for more than seven days and if despite maximal medical treatment an adequate control of intraocular pressure was not achieved then in addition to the standard cataract procedure trabeculectomy was performed. It was found that intra ocular pressure was comparatively lower in the group which had combined surgery(89 eyes) as against the group which had cataract surgery alone (46 eyes) ($p < 0.001$) but no difference either in terms of intra ocular pressure or visual acuity was found between these two groups at 6 months. No serious complications related to trabeculectomy were reported. Thus based on this study it was concluded that in cases of phacolytic glaucoma of long duration, cataract surgery along with trabeculectomy is safe and also prevents a rise in intraocular pressure in the post-operative period thereby reducing the need for systemic medications to decrease intraocular pressure.[37]

Mandal A K et al[38] conducted a study which included 45 eyes of patients with phacolytic glaucoma who were operated during time period of January 1990-1995 December and completed follow up period of one year. Among these 45 eyes 17 of them underwent cataract extraction by the extra-capsular technique with primary intraocular lens implantation who were included in group 1.The remaining 28 eyes underwent cataract extraction by the extra-capsular technique without any intraocular lens implantation as in these patients the contralateral eye was aphakic.

The pre-operative data such as vision ,intraocular pressure and anterior segment findings were analyzed in comparison to the post-operative data. In all these patients control of intraocular pressure was achieved without any anti-glaucoma medications during a follow up period of about 12-60 months in group-1 and a period of 12- 78 months in group- 2.In patients who presented late(2-3 weeks) though intra-ocular pressure remained controlled , desired visual recovery was not achieved despite surgical intervention due to glaucomatous disc damage. 76.5% of patients in group - 1 achieved a vision of 20/40 while 60.7% of patients in group-2 achieved a vision of 20/40. An initial visual acuity of light perception only was recorded in 18 of the total 45 patients. A visual acuity of 20/40 or even better was achieved in 8 among these 18 patients (44%). It was thus concluded that phacolytic glaucoma can be treated safely by extra-capsular cataract extraction with or without intraocular lens implantation and visual acuity of light perception without projection is not considered as a contraindication for cataract extraction in cases of phacolytic glaucoma.[38]

Ultrasound biomicroscopy studies have not been described in a case of phacolytic glaucoma, but several studies on anterior segment imaging using ultrasound biomicroscopy have reported few limitations. Most common disadvantage with this investigation reported is measurement of angle opening distance which involves placing calipers to measure values in areas of interest .This results in low reproducibility and increases inter-observer and intra-observer variability of the values thus reported.[48,49]

MATERIALS AND METHODS

This case- control study was conducted at the Department of Ophthalmology, Christian Medical College, Schell Campus, Vellore from December 2012 – November 2013. This study was approved by the Institutional review board IRB min no :8164 dated 09/01/2013.

Participants:

Patients attending the outpatient department or casualty diagnosed to have phacolytic glaucoma or immature cataract(up to nuclear sclerosis Grade-3), fulfilling the inclusion and exclusion criteria as below were invited for the study. Patients willing to participate were recruited into the study after obtaining the informed consent. (Appendix- C).

Inclusion criteria

1)Patients with phacolytic glaucoma (Cases)

Diagnosis based on history and slit lamp examination findings as described below and fulfilling all features:

a) History**:** Diminution of vision –gradual onset, long duration (over five months) , acute onset of mono-ocular pain and redness.

b) On examination: Visual acuity less than 3/60

Intraocular pressure of ≥ 21 mm Hg

Hypermature cataract and macroscopically intact anterior capsule

Flare of 2+ or more

Absence of Keratic precipitates

2)Patients with immature cataract (controls)

Diagnosis of cataract was based on history of gradual progressive painless loss of vision and slit lamp examination findings. Immature cataracts were graded based on lens opacity classification system.

EXCLUSION CRITERIA:

In the affected eye

1)History of (H/O)Trauma

2)Pre-existing glaucoma

3)History of previous use of long term (two week) topical medications-steroids

4)History of Uveitis

5)History of Previous ocular surgeries

6)Presence of corneal opacity

7)Age< 50 yrs

8)Isolated Posterior Sub capsular cataract or nuclear cataracts>grade 3 based on LOCS classification

9)Anterior chamber depth :Van hericks < 1/4 corneal thickness

Patients satisfying the above inclusion and exclusion criteria and willing to participate in the study were enrolled into the study.

The socioeconomic status of the patient was determined based on Kuppuswamy's Classification[39] and were classified into upper, middle or lower socioeconomic status.

The reason for the delay in seeking treatment by the patient was also sought and was divided into the following categories – Economic, Social, Fear for surgery , Ignorance. History of surgery and the status of vision in the fellow eye was also recorded during enrolment of the patient.

Source of data collection and Evaluation of the patient:

All the cases underwent a detailed clinical examination prior to collection of aqueous humor sample to establish the diagnosis of phacolytic glaucoma and immature grades of cataract respectively.

1)Visual acuity

On presentation initially vision was recorded in the affected eye and then the fellow eye with the help of Snellen's chart and patient at a distance of six metres from the Snellen's chart. In case of patients with vision less than counting fingers close to face perception and projection of light were examined using a bright LED torch light while occluding the other eye in a dark room.

2)Pupillary reaction:

Reaction of the pupil was assessed by using the swinging flash light test. This test was done on all patients in a dark room with illumination time which was kept at a standard of three seconds before the fellow eye was illuminated again for a period of

3 seconds. In any case of non reacting pupil, objective response to light was assessed by the indirect or the consensual reflex in the fellow eye.

Relative afferent pupillary defect is graded as follows:

Grade 1 : weak initial constriction followed by greater redilatation

Grade 2: Initial stall followed by greater redilatation

Grade 3: Immediate pupillary dilatation

Grade 4: Immediate pupillary dilatation following prolonged illumination of the good eye for 6 seconds

Grade 5:Immediate pupillary dilatation with no secondary constriction[40,41]

3) Slit lamp examination: Detailed examination of the anterior segment was done with the help of Haag Streit slit lamp to look for the following features :

a)Circumcorneal congestion(CCC)

b)Corneal edema

c)Anterior chamber – was examined by creating a 3x1 mm slit in a dark room under high magnification for the presence of flare of 2+ or more as graded by Sun's Working Group of grading for cells and Flare.[42]

If present-hypopyon and refractive particles were also noted.

c) Grading of cataract- done by slit lamp examination based on the colour of the nucleus by Lens opacity classification system -3. It consists of six slit lamp images for grading nuclear color (NC) and nuclear opalescence (NO) [43]

For this study patients with immature cataract upto Grade-3 were included.

d) <u>Intraocular pressure</u>- was recorded by Goldmann's applanation tonometry :In this technique a local anaesthetic drop(proparacaine -1%) is first instilled into the eye, followed by staining of the lower forniceal conjunctiva by a fluorescein strip and the patient is asked to blink a couple of times. Cobalt blue filter is used to direct the light on the prism head. The prism is then slowly moved forwards such that the apex of the prism just touches the centre of the cornea, when two semicircles are seen. The calibrated dial which is present in the tonometer is then adjusted such that the inner mires of the two semicircles just touch each other along the horizontal meridian ,at this point the intraocular pressure is read out.[44]

e) Examination of Fundus:

Fundus examination was done by slit lamp biomicroscopic examination using a 78 dioptre lens to look for presence of any disc changes in terms of disc pallor, size of the disc, cup : disc ratio, presence of other glaucomatous changes in form of defects of nerve fibre layer of retina. In cases of phacolytic glaucoma there was no view to the fundus due to the presence of hypermature cataract and fundus examination of the fellow eye was done to look for any glaucomatous changes, as cases with pre-existing glaucoma were not included in the study.

Detailed clinical examination was followed by:

<u>1)UBM examination using the immersion technique</u>: The machine used was Appaswamy MARVEL machine, UBM MODE was selected before starting the scan. The scan was performed using the UBM probe and 50 MHz transducer with the following settings:

Gain :100

Power:45

Zoom :4

Delay:290

Sweep:25

The patient was made to lie supine and after instilling topical anaesthetic into patient's eye, a saline holding cup of the appropriate size was placed within the palpebral fissure of the eye with normal saline used as a coupling fluid. The transducer probe oscillates in the cup and anterior segment was studied (Fig -1). The anterior segment was examined using 50 MHz transducer - to look for anterior chamber depth, angle opening distance, anterior chamber echoes and retro lental vitreous echoes.

The machine has tools(gates) to measure the various parameters like the anterior chamber depth, angle of anterior chamber, lens thickness(fig - 2,3,4). These measurements were taken after freezing the image when the pupillary diameter was maximum so as to ensure that the measurements were taken at the centre of the lens and pupil. The patient was also asked to look at the ceiling while doing the scan to relax accommodation as much as possible. All the scans were done without any cycloplegics. Presence of vitreous echoes was judged by the presence of echo signals over and above the normal noise levels by keeping the probe over the ciliary body to bypass the lens and increase the depth of scanning in the vitreous.

Patient management:

Patients were admitted in the ward and Tablet Acetazolamide 250 mg was started once every 6 hours. Within 24 hours of starting the treatment, under aseptic

precautions in the operation theatre, peribulbar anaesthesia with lignocaine (4 ml) was administered and after painting the operating eye with betadine and draping it with sterile opsite paracentesis was done at the temporal/nasal limbus with a MVR blade taking care to avoid blood vessels(Fig-6,7,). About 0.1-0.2 ml of aqueous was aspirated using a 26 g cannula mounted on a tuberculin syringe (Fig- 5,6,7).The cannula(Fig-5) was made by cutting the tip of 26G needle and the initial 3 mm of this needle was then bent to enable aspiration of aqueous with minimal trauma to intra ocular structures.

Pad was applied post-procedure. Pad was removed 2 hr later and topical drops – steroids(Predforte –Prednisolone acetate -1 %): 1 drop once every hour, Chloramphenicol 0.5% eyedrops (Dexoren)-1 drop once every 6 hours and Cyclopentolate(Cyclopent -1%) eyedrops -1 drop twice a day was started and tablet Acetazolamide(T.Diamox -250 mg) was continued if intraocular pressure remained high post paracentesis .The next day, a second sample was collected in a similar manner under aseptic precautions and cataract surgery was performed at the same time if inflammation was controlled or else the cataract surgery was deferred till inflammation settled(which was about 2-3 days)

The anterior chamber was tapped twice in case of phacolytic glaucoma. The first tap was used to study cells by histopathological examination and the second to study the proteins in the aqueous humor by biochemical analysis.

In case of immature cataract 0.1-0.2 ml of Aqueous humor was collected only once during paracentesis as a part of routine cataract surgery. In half the controls the aqueous was sent for histopathological studies and the other half it was sent for biochemical analysis. Sample of the aqueous humor (0.1-0.2 ml) was collected in

Eppendorf tube(Fig -9) and the sample was stored in the refrigerator at 4 degree centigrade and transported in an ice box to the pathology department –CMC for histopathological analysis on the same day within 2 hours of collecting the sample - to analyse the type of cells present in aqueous by Cytospin technique.

0.1-0.2 ml of aqueous aspirated for biochemical analysis was sent in a similar way to estimate the total protein content in aqueous .

Due to funding constraints only three cases could be studied with electron microscopy. In three cases of phacolytic glaucoma where there was adequate aqueous to take a second sample during the first paracentesis the aqueous was sent for EM study by collecting the aqueous sample in Eppendorf tubes(Fig-8) containing 3% glutaraldehyde as the fixative agent.

ANAYSIS OF AQUEOUS HUMOR:

1) Histopathological analysis of aqueous humor :

The cytological study of the aspirated aqueous was done using the Cytospin technique

Principle: Cytocentrifugation can be regarded as membrane filtration performed horizontally. Here cells are transferred to the slide using centrifugation in a specially designed machine. The cells directly settles on to the slide which is in the centre of a blotter filter paper through which the liquid component of the specimen passes out. Cells are flattened by cytocentrifugation allowing better enhancement of cytomorphology.

Equipments used:

1) Aqueous humor sample

2) Cell spin cytocentrifuge

3) Cell clip rotor

4) Filter cards.

5) Microscopic slide

6) W G stain

7) Diamond marking pencil

Procedure: The glass slide was labelled with diamond marking pencil. Label also included the patient's hospital number. The cell clip was opened and the microslide, filter card, cell funnel was inserted and the hook was latched .The preparation system was inserted into the cell clip rotor. About 0.1-0.2ml of aqueous was pipetted out into the funnel and sealing cap was put on and centrifuge cover closed. The sample was then subjected to spin rate of 5000 rounds per minute for 5 minutes. Then the preparation system was held in the hand, and with utmost care the filter paper was removed without ruining the smear. The slide was stained with Wright Giemsa stain and examined under oil immersion (1000X magnification) for presence/absence of cells.

2)Biochemical analysis of aqueous humor: Estimation of total protein content in aqueous was done by pyrogallol technique. The Protein measurement was performed in Roche Modular P 800 auto analyzer. The sample is centrifuged at 4500 RPM and

supernatant was used for the assay. The active ingredient in the Pyrogallol red reagent is pyrogallol red-molybdate complex. The complex binds with basic amino groups in protein causing shift in absorbance which is measured at 600 nm. The absorbance of this complex is directly proportional to the protein concentration in the sample.

3)Electron microscopy of aqueous humor –

The sample was initially fixed in 3 % glutaraldehyde and washed in buffer and then fixed with 1%osmiumtetroxide 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. This was then embedded in siliconised rubber mould with eposy resin .This embedded mould was kept in incubator at 60 degree centigrade for 48 hours and blocks were sectioned. One micron thick sections cut through ultra-microtome using a glass knife and 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.

Quantification of phagocytized material: 10 consecutive macrophages were studied in each block to semi-quantitate the phagocytized material. To give objectivity in quantification we looked at both absolute numbers and also the proportion of the cytoplasm fill. For Melanin pigment quantification only absolute numbers were used. The Pathologist also took a qualitative call viz numerous, moderate or a few based on her diagnostic impression and this was compared with the quantification data to fix numbers for each qualitative diagnostic impression. Due to paucity in the number of neutrophils in the sections these were not studied in a similar manner.

Comparison between patients with Phacolytic glaucoma (cases) and patients with immature cataract (controls) was done for the following :

1) Clinical features - IOP, AC finding in phacolytic glaucoma and immature cataracts

2) Type of cells found in the aqueous

3) Protein content in aqueous among the two groups

4)Anterior segment UBM findings of anterior chamber depth, angle opening distance, lens thickness, anterior chamber echoes and vitreous echoes.

SAMPLE SIZE CALCULATION

Sample size was calculated based on the assumption that 80% of cases and 5% of controls will have cells in the aqueous humor and the study have a 90% power at 5% level of significance.

Proportion in group I	=	0.8(CASES)
Proportion in group II	=	0.05(CONTROLS)
Risk difference	=	0.75
Power(%)	=	90
Alpha Error(%)	=	5
Side	=	2

Required sample size for each arm = 7

Alpha Error(%)	Power(%)	Sample Size(n)
1	70	7
	80	9
	90	10
5	70	5
	80	5
	90	7
10	70	3
	80	4
	90	5

Proportion in group I	=	0.8
Proportion in group II	=	0.15
Risk difference	=	0.65
Power(%)	=	90

Alpha Error(%) = 5

Side = 2

Required sample size for each arm = 10

Alpha Error(%)	Power(%)	Sample Size(n)
	70	10
1	80	12
	90	15
	70	7
5	80	8
	90	10
	70	5
10	80	6
	90	8

Sample size: Based on the above, the required sample size to show a difference was found to be 8 – 10 patients in each arm.

BIAS ELIMINATION/Randomisation: The clinical pathologists and biochemists were blinded about the diagnosis of the patients as the sample of aqueous humor were given with only patient identifiers and no clinical diagnosis.

However blinding for UBM was not possible as the diagnosis was obvious with the naked eye to the ophthalmologist who performed the UBM.

STATISTICAL ANALYSIS

Categorical variables were expressed as Number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes.

Continuous variables were expressed as Mean ± Standard Deviation and compared across the groups using one Way ANOVA test.

The statistical software SPSS version 16 was used for the analysis.

An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

RESULTS

A total of twelve cases of phacolytic glaucoma were finally recruited for the study though our sample size required only 10 cases. 20 cases of immature cataract were recruited as controls; off this 10 cases formed the histopathology control arm while the remaining 10 formed the biochemistry control arm.

A total of twenty two cases of phacolytic glaucoma presented to the department during the study period. 10 cases were excluded from the study. 4 violated thesis protocol and in 1 case there was confusion regarding the diagnosis ,as there was prior history of trauma and the contralateral eye had only an early immature cataract. In five cases there was a delay in the transport and processing of histopathology(HPE) samples which lysed the cells and thus had to be excluded from the study. For subsequent samples the transport protocol was changed as described and there were no further problems. All the 20 controls invited for the study consented for the study as patients were eager to have an additional scan(UBM) prior to cataract surgery.

CLINICAL DATA

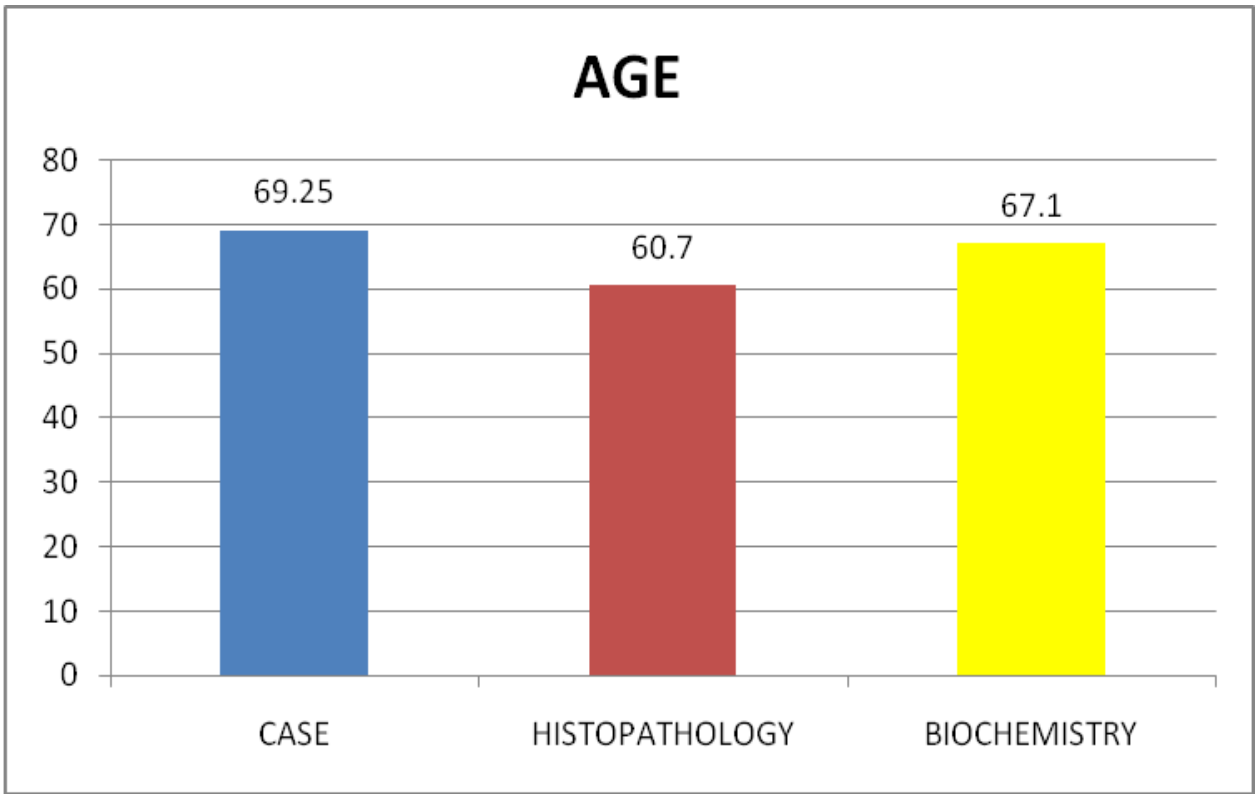
The age distribution of the cases and the controls is given in Table-1. There was no statistical difference between the cases and controls.

TABLE NO -1 : Age Distribution of Cases and Controls

	GROUP			p Value	
AGE	**Case**	**Control-Histopathology HPE**	**Control-Biochemistry BIOCHEM**	**Case Vs HPE**	**Case Vs Bio Chem**
50-60	2(16.7%)	5(50%)	2(20%)		
61-70	5(41.7%)	4(40%)	5(50%)		
>70	5(41.7%)	1(10%)	3(30%)		
Total	12(100)	10(100)	10(100)	0.141	0.852

Graph 1 shows the mean age of the cases and controls. The age of all patients in the three study group ranged from 50-80 years with mean age of 69.25years in the phacolytic glaucoma patients, the mean age of histopathology controls was 60.7 years,and biochemistry control 67.1 years.

Graph No- 1 Mean age distribution of the case and controls



The sex distribution of cases and controls is given in Table-2 and Graph-2. 13 out of 32 patients were females(40.6 %) and 19 were males (59.4%). There was no statistically significant difference between the sex distribution of cases and controls.

TABLE NO -2 : Sex Distribution of Cases and Controls in the study

	GROUP				p Value	
SEX	**Case**	**HISTOPATHOLOGY**	**BIOCHEMISTRY**	**Total**	**Case Vs HPE**	**Case Vs Bio Chem**
FEMALE	4(33.3%)	6(60%)	3(30%)	13(40.6%)	0.211	0.867
MALE	8(66.7%)	4(40%)	7(70%)	19(59.4%)		
Total	12(100)	10(100)	10(100)	32(100)		

GRAPH No -2 : Sex Distribution in study group

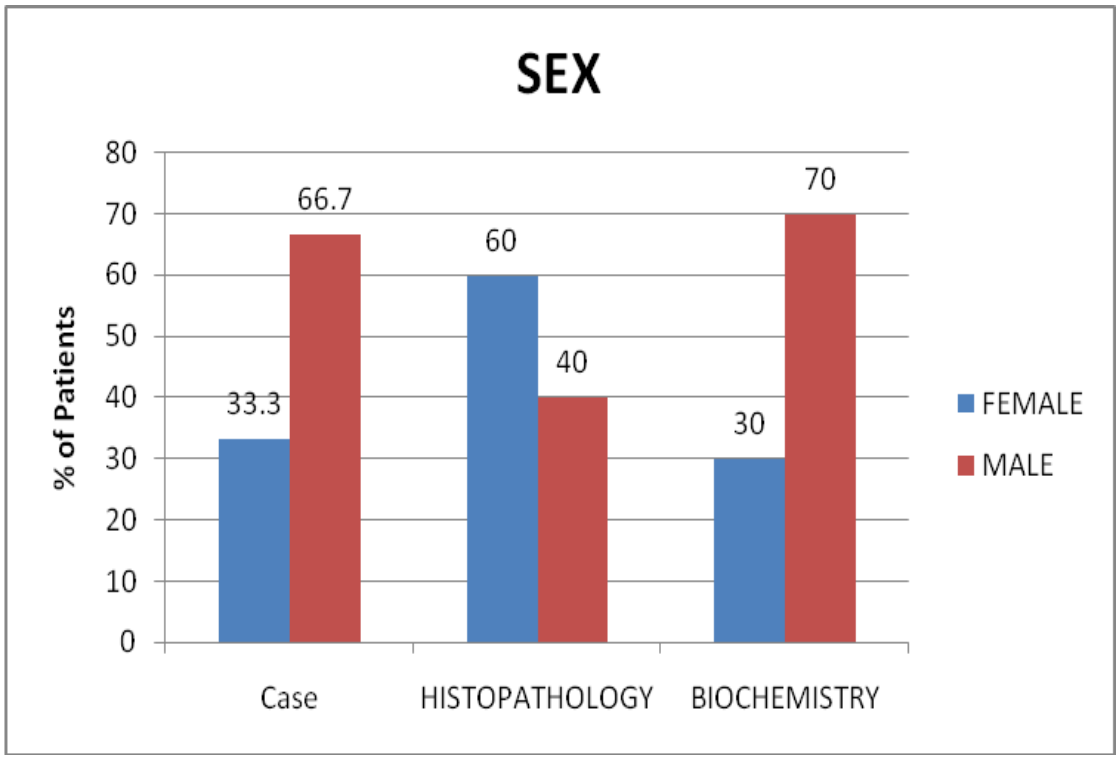


Table -3 and graph -3 shows socioeconomic status of patients in study groups .There was no statistically significant difference between the case and histopathology control group and the biochemistry control group .

TABLE – 3 : SOCIOECONOMIC STATUS OF PATIENTS IN STUDY GROUP

	GROUP			p Value	
SOCIOECONOMIC STATUS	**CASE**	**HISTOPATHOLOGY (HPE)**	**BIOCHEMISTRY (BIO)**	**Case vs HPE**	**Case vs BIO**
Lower	4(33.3%)	3(30%)	4(40%)	0.867	0.077
Middle	8(66.7%)	7(70%)	3(30%)		
Upper	0(0)	0(0)	3(30%)		
Total	12(100)	10(100)	10(100)		

GRAPH No -3 : SOCIOECONOMIC STATUS DISTRIBUTION AMONG STUDY GROUP

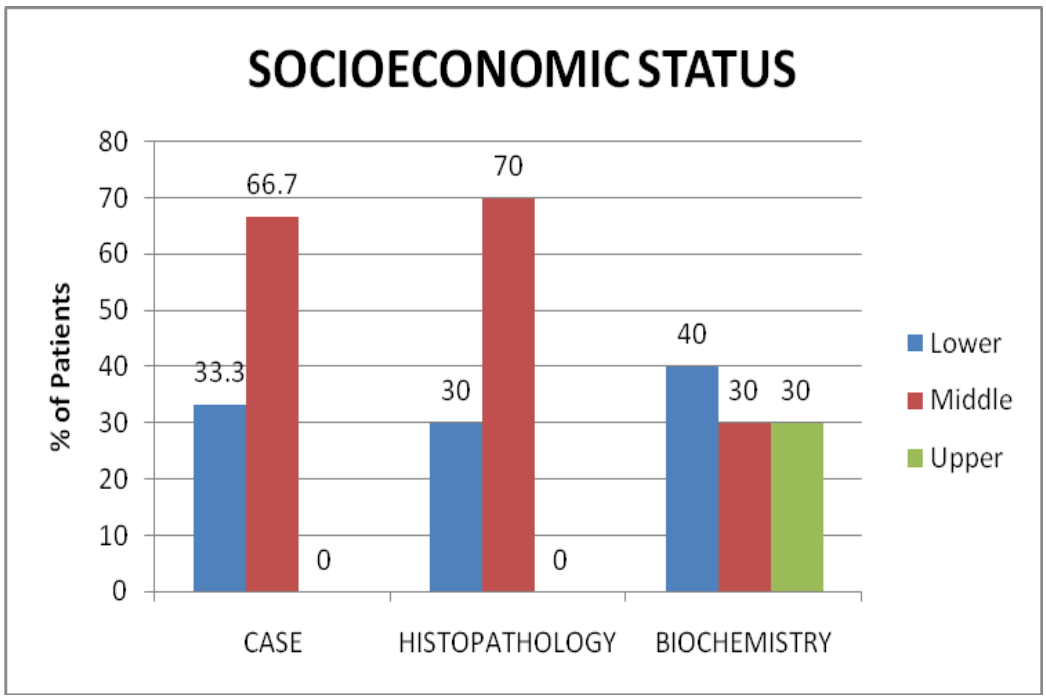


Table 4 shows the reasons for delay in undergoing cataract surgery in Phacolytic patients.

Poverty continues to be the most important cause for delay in accessing service.

TABLE – 4 :REASONS FOR DELAY IN SURGERY IN PHACOLYTIC GLAUCOMA PATIENTS

REASONS FOR DELAY IN SURGERY	Case
Socioeconomic Cause	7(58.3%)
Ignorance	4(33.3%)
Fear of Surgery	1(8.3%)
Total	12(100)

Table 5 shows the details of endothelial deposits (shiny particles; not KPs) . 1 (8.3%) of 12 phacolytic glaucoma cases showed deposits on back of the cornea ,while none of the control group had any deposits on the back of the cornea.

TABLE NO -5 : ENDOTHELIAL DEPOSITS (SHINY PARTICLES) AMONG STUDY PATIENTS

	GROUP			p Value	
ENDOTHELIAL DEPOSITS	**Case**	**HISTOPATHOLOGY**	**BIOCHEMISTRY**	**Case Vs HPE**	**Case Vs Bio Chem**
YES	1(8.3%)	0(0)	0(0)	0.350	0.350
NO	11(91.7%)	10(100%)	10(100%)		
Total	12(100)	10(100)	10(100)		

In all 12(100%) cases of phacolytic glaucoma anterior chamber flare of 2+ or more was seen on slit lamp examination while the 20 control patients had no flare in anterior chamber.

Table 6 shows the distribution of iridescent particles in AC in cases and controls. In 9 of 12 phacolytic glaucoma cases ,iridescent particles were present in anterior chamber.

None of the 20 control patients had any particulate material.

TABLE NO - 6 : PRESENCE OF IRIDESCENT PARTICLES IN AC AMONG STUDY GROUPS

	GROUP			p Value	
IRIDESCENT PARTICLES	**Case**	**HISTOPATHOLOGY**	**BIOCHEMISTRY**	**Case Vs HPE**	**Case Vs Bio Chem**
YES	9(75%)	0(0)	0(0)	<0.001	0.001
NO	3(25%)	10(100%)	10(100%)		
Total	12(100)	10(100)	10(100)		

Table 7 shows the details of fluffy material present in anterior chamber in study group. 2(16.6%) with phacolytic glaucoma was found to have fluffy material in the anterior chamber, while it was not found in the remaining 10(83.4%) cases nor in any of the control group.

TABLE NO 7- : PRESENCE OF FLUFFY MATERIAL AMONG STUDY GROUP

	GROUP			p Value	
Fluffy material in AC	**Case**	**HISTOPATHOLOGY**	**BIOCHEMISTRY**	**Case Vs HPE**	**Case Vs Bio Chem**
YES	2(16.6%)	0(0)	0(0)	0.350	0.350
NO	10(83.4%)	10(100%)	10(100%)		
Total	12(100)	10(100)	10(100)		

Relative afferent papillary defect was present in all 12 (100%) cases of phacolytic glaucoma and absent in the control groups.

Table 8 shows the details of phacodonesis in the study group . Phacodonesis was noted in 50 % of phacolytic glaucoma cases and absent in all control patients.

TABLE NO - 8 :PRESENCE OF PHACODONESIS AMONG STUDY GROUP

	GROUP			p Value	
PHACODONESIS	**Case**	**HISTOPATHOLOGY CONTROL(HPE)**	**BIOCHEMISTRY CONTROL(BIO)**	**Case Vs HPE**	**Case Vs BIO Chem**
YES	6(50%)	0(0)	0(0)	0.009	0.009
NO	6(50%)	10(100%)	10(100%)		
Total	12(100)	10(100)	10(100)		

ULTRASOUND BIOMICROSCOPY(UBM) DATA

With UBM we tried to look at the AC depth, Angle opening distance and the central lens thickness in both the cases and controls. Table – 9 : Shows that the angle opening distance and AC depth is more in the cases compared to the controls. However only the angle opening distance was statistically significantly different from the controls. The central lens thickness in cases of phacolytic glaucoma was more than the controls and this was statistically significant .

TABLE - 9 : COMPARISON OF ULTRASOUND BIOMICROSCOPY FEATURES AMONG CASES & CONTROL

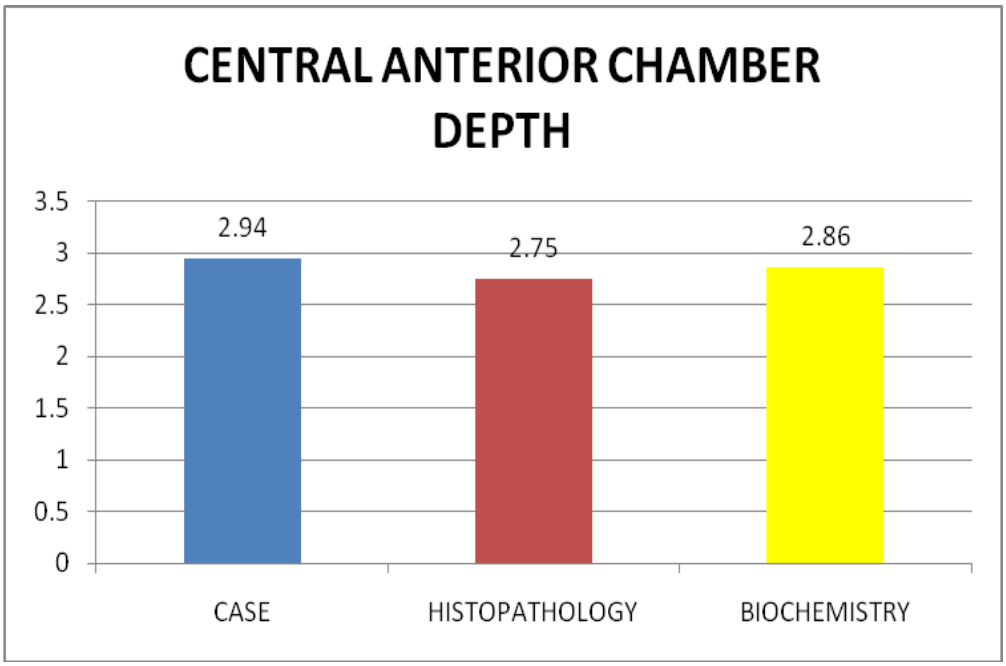
STUDY GROUP	CASE	CONTROL	p-value
CENTRAL ANTERIOR CHAMBER DEPTH	2.94 ± 0.32	2.84 ± 0.34	0.280
ANGLE OPENING DISTANCE	0.67 ± 0.08	0.57 ± 0.06	< 0.001
CENTRAL LENS THICKNESS	4.05 ± 0.4	3.71 ± 0.29	0.011

*CASE-Phacolytic glaucoma

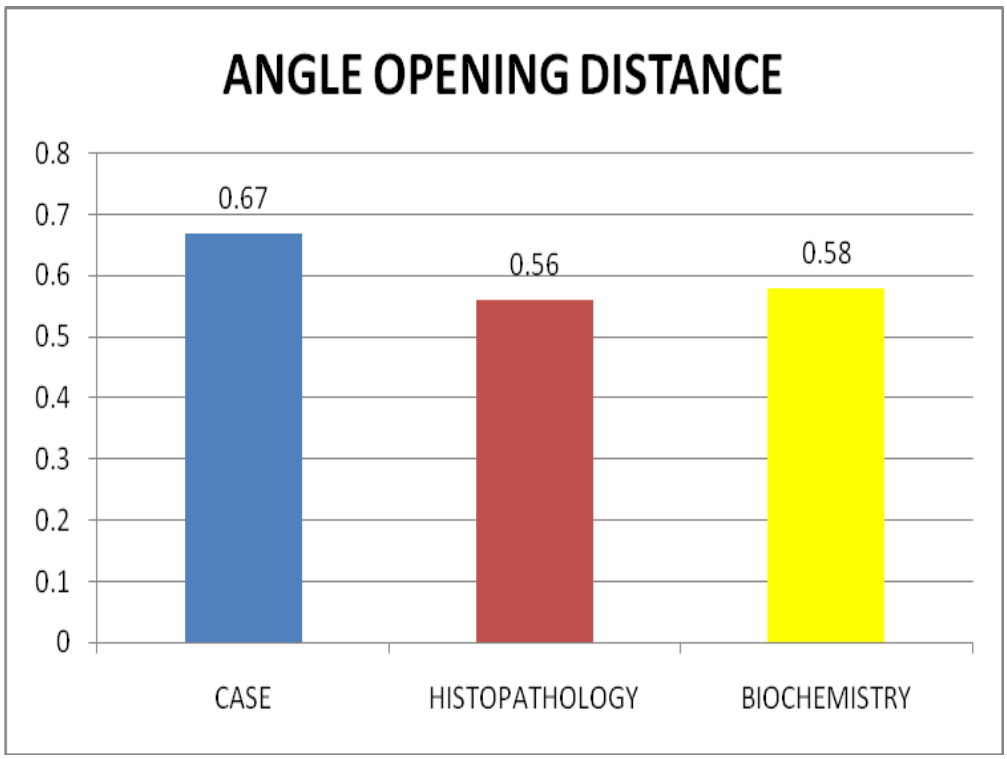
*CONTROL-Immature cataract

GRAPH -4 : MEAN DISTRIBUTION OF CENTRAL ANTERIOR

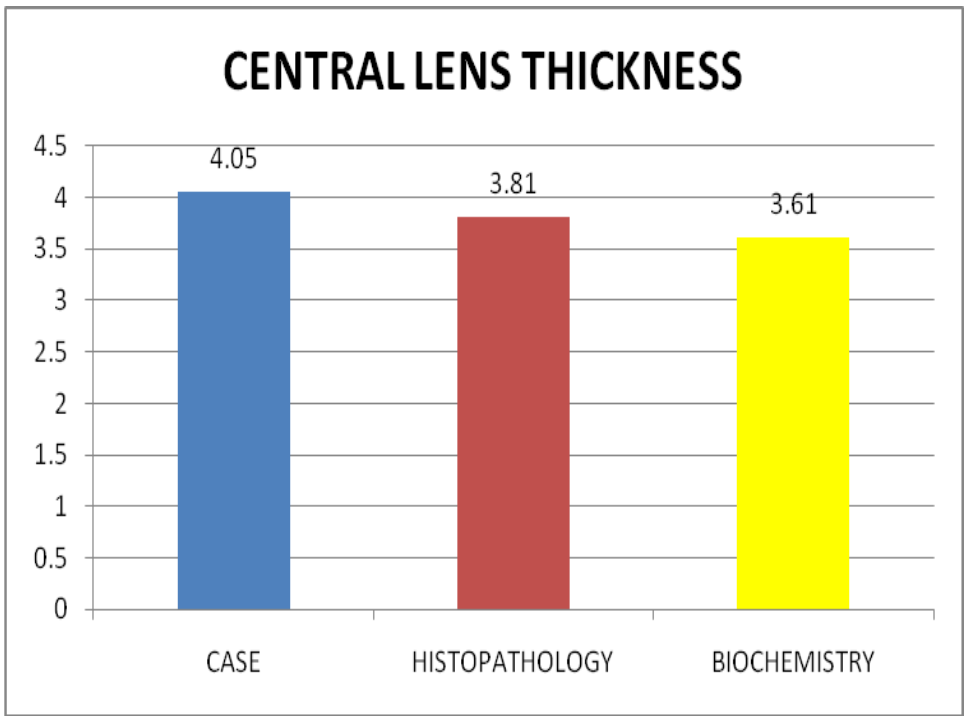
CHAMBER DEPTH IN mm AMONG THE 3 STUDY GROUP



GRAPH – 5 : MEAN DISTRIBUTION OF ANGLE OPENING DISTANCE AMONG THE 3 STUDY GROUP PATIENTS



GRAPH - 6 : MEAN DISTRIBUTION OF CENTRAL LENS THICKNESS IN mm AMONG THE 3 STUDY GROUP PATIENTS



With the possibility of picking up echoes from the leaked lens material we looked for both AC and Vitreous Echoes using UBM. Only two cases had AC echoes and none(neither cases nor controls) had vitreous echoes. The two cases which had AC echoes showed fluffy material in the AC.

HISTOPATHOLOGY DATA

Table 10 and graph 7 gives details about various types of cells seen in phacolytic glaucoma patients. In 3 (25%) of 12 phacolytic glaucoma, no cells were seen in aqueous humor on histopathology, in 2 cases(16.7%) only macrophages were seen,while 3 (25%) cases showed only neutrophils ,and in 4 phacolytic cases both neutrophils and macrophages were seen. Nocells were seen in the 10 control patients. 58% of phacolytic glaucoma cases had Neutrophils and 50% had macrophages.

TABLE - 10 : COMPARITIVE ANALYSIS OF HISOPATHOLOGICAL DATA AMONG STUDY PATIENTS

	GROUP		p Value
HISTOPATHOLOGY REPORT CELLS	**Case**	**HISTOPATHOLOGY CONTROL(HPE)**	**Case Vs HPE**
NO CELLS	3(25%)	10(100)	0.005
MACROPHAGES	2(16.7%)	0(0)	
NEUTROPHILS	3(25%)	0(0)	
MACROPHAGES & NEUTROPHIL	4(33.3%)	0(0)	
Total	12(100)	10(100)	

GRAPH : 7 - CELLS SEEN ON HISTOPATHOLOGICAL EXAMINATION IN STUDY GROUP

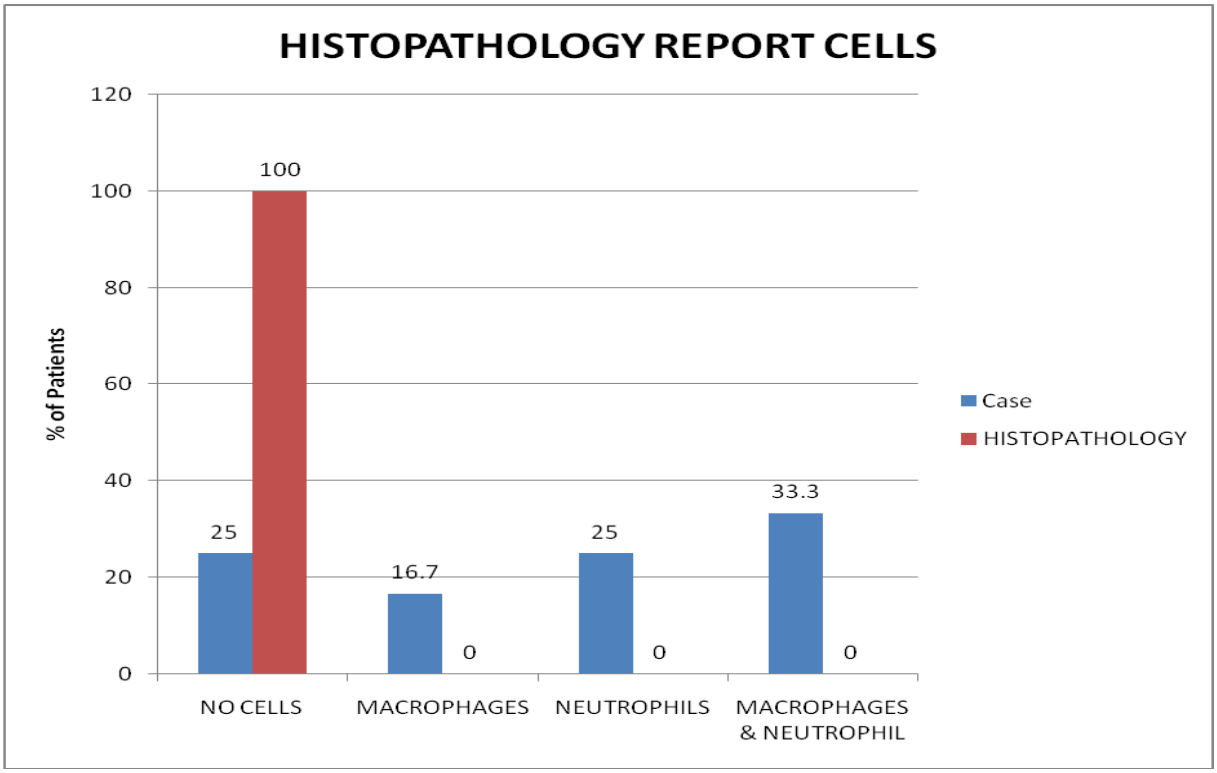


Table – 11 shows ,cell type seen in aqueous humor in each phacolytic glaucoma patient.

TABLE -11 : CELL DISTRIBUTION IN INDIVIDUAL PHACOLYTIC GLAUCOMA PATIENTS

PHACOLYTIC GLAUCOMA	ABSENT CELLS	NEUT	LYMPHO	MACRO
Case 1	-	-	-	+
Case 2	+	-	-	-
Case 3	-	+	-	-
Case 4	-	+	-	+
Case 5	+	-	-	-
Case 6	-	+	-	-
Case 7	+	-	-	-
Case 8	-	-	-	+
Case 9	-	+	-	-
Case 10	-	+	-	+
Case 11	-	+	-	+
Case 12	-	+	-	+

BIOCHEMISTRY DATA

Table -12 Shows the protein concentration in the aqeous in cases and controls. In the controls there was an outlier with unusually high protien. The mean shown in Table 12 is without the outlier value. There was a significant diffence between the two groups with and without the outlier.

Table No- 12 Showing the protein concentration in individual cases and controls

CASES	*PROTEIN CONC*	*BIOCHEM CONTROLS*	*PROTEIN CONC*
Case 1	95	**Case 1**	16
Case 2	315	**Case 2**	12
Case 3	698	**Case 3**	9
Case 4	158	**Case 4**	10
Case 5	288	**Case 5**	111.16*
Case 6	481	**Case 6**	10
Case 7	673	**Case 7**	16
Case 8	341	**Case 8**	24
Case 9	332	**Case 9**	12
Case 10	224	**Case 10**	19
Case 11	147		
Case 12	570		

*Outlier

TABLE-13 :COMPARISON OF MEAN PROTEIN CONCENTRATION IN CASES AND CONTROLS

	CASE	BIOCHEM CONTROLS	p- value
	Mean ± SD	Mean ± SD	Case vs Bio Chem
PROTEIN CONC	360.17 ± 203.2	14.22 ± 4.97*	<0.001

*The outlier in the biochemical control group was not included during calculation of mean protein concentration among the control groups.

A scatter plot was done, to see if there is any correlation between IOP and the protein concentration in the AC in phacolytic glaucoma patients. As evident from the Graph 8 the slope of the line is almost horizontal and there was no correlation between the two .

GRAPH -8: CORRELATION BETWEEN IOP AND PROTEIN CONCENTRATION IN AQUEOUS HUMOR

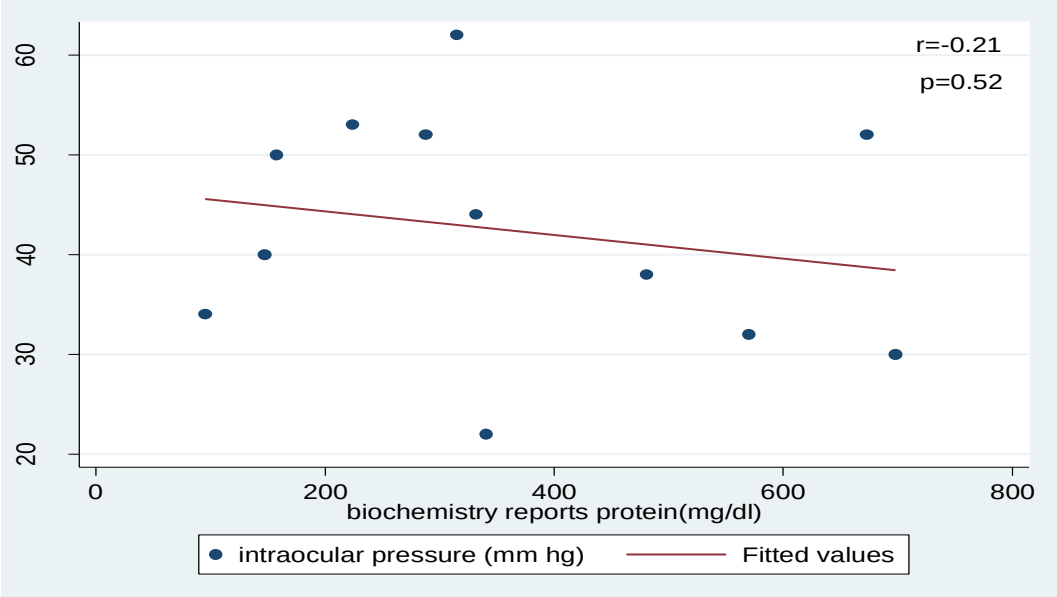


Table- 14 : shows the mean IOP in cases and controls. The mean IOP in the cases was three times higher than the controls. The range of IOP in our cases was from 30 to 62 mm of Hg and 10- 16 mm Hg and 10 – 18 mm Hg in histopathology and biochemistry controls respectively

TABLE NO - 14 :COMPARISON OF MEAN VALUES OF IOP IN CASES AND CONTROLS

	GROUP				
	CASE	**HISTOPATHOLOGY**	**BIOCHEMISTRY**	**p Value**	
	Mean ± SD	Mean ± SD	Mean ± SD	Case Vs HPE	Case Vs Bio Chem
INTRAOCULAR PRESSURE	42.42 ± 11.74	13.3 ± 3.53	13.7 ± 2.67	<0.001	<0.001

ELECTRON MICROSOPY(EM) STUDY :

In three cases where we could get enough aqueous during the first paracentesis the aqueous was sent for Ultra structural analysis by EM studies. In one case no cells were detected and in this cases the histopathology also did not show any cells. This case showed only irregular aggregates of pale grey material.

In one of the cases where the cells present were studied with EM the histopathology study did not show cells due to cell lysis as the sample was part of the original five cases mentioned earlier were the transportation protocol was faulty and there was a delay in processing for histopathology.

In one of the two cases with cells there was enough sample only for one block and 10 macrophages were studied. In the second case with cells there was enough sample for two blocks and 20 macrophages were studied here. A total of only four Neutrophils were seen in the second case in both the blocks. The first case did not show any Neutrophils. Neutrophils too contained engulfed lens proteins in large amounts with large vacuoles and few melanin pigments.(Fig :17)

Of the two cases which showed many macrophages , majority were viable and showed distension of the cytoplasm with numerous phagosomes containing predominantly a vacuolated material, possibly lens protein. Other phagosomes contained predominantly a clear fluid with fine granular material. Many membrane bound granules containing uniformly electron dense material ,ranging in size from approximately 100 nm to >1 000nm were seen in the macrophages. These granules varied in shape from round to oval to elongated and were consistent with melanin pigment granules. 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 of engulfed material and only minimal amounts of the macrophage cytoplasm was evident.

Only 1 monocyte with no engulfed material was seen.

Quantification of phagocytized material: 10 consecutive macrophages were studied in each block to semi-quantitate the phagocytized material. The pathologist categorized the ingested material in these cells as having Numerous, Moderate and a Few amount of material. To give objectivity in quantification we looked at both absolute numbers and also the proportion of the cytoplasm fill for lens proteins and vacuoles. For Melanin pigment quantification only absolute numbers were used. Of the Thirty cells studied 2 cells were obviously non viable, one showed early signs of death in that its cell membrane was not clear and one cells showed a cell in cell phagocytosis and was not included in the qualitative and quantitative analysis.

The number of Melanin pigments in the cells studied ranged from 1 to 50 and one case had more than 50.

14 out of the 26 cells (53.84%) studied for lens protein had more than 75% fill of the cell with lens protein and the nucleus when seen pushed to the periphery, 11(42.3%) cases had 25 to 75% fill and only one case had less than 25% fill.

15 out of 26 cells (57.7%) studied for vacuoles had less than 25% fill of the cell with vacuoles, 11 (42.3%) had 25- 75% fill and none showed more than 75% fill.

Table –15 , shows the cell wise quantification data

TABLE NO – 15 CELL WISE QUANTIFICATION OF INTRACELLULAR CONTENTS ON EM : CASE-1

CELL NO	VIABILITY	NUCLEUS POSITION	LENS PROTEIN	VACOULES	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

* MACROPHAGE -M

TABLE NO – 16 CELL WISE QUANTIFICATION OF INTRACELLULAR CONTENTS ON EM : CASE-2

BLOCK - I

CELL NO	VIABILITY	NUCLEUS POSITION	LENS PROTEIN	VACOULES	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

CASE No -2 :BLOCK - II

CELL NO	VIABILITY	NUCLEUS POSITION	LENS PROTEIN	VACOULES	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 -17 :QUALITATIVE ANALYSIS OF CELLULAR CONTENTS OF MACROPHAGES BY PATHOLOGIST

CELLULAR CONTENTS	NUMEROUS	MODERATE	FEW	TOTAL CELLS
MELANIN PIGMENT	5(19.23%)	8(30.76%)	13(50%)	26(100%)
LENS PROTEINS	24(92.30%)	2(7.69%)	0	26(100%)
"CLEAR FLUID " VACOULES	11(42.30%)	5(19.23%)	10(38.46%)	26(100%)

*4 cells were non viable.

DISCUSSION

Phacolytic glaucoma a type of lens induced glaucoma is seen in patients with hypermature cataract. It is postulated that it is the leaking lens matter from these fluid cortex that causes the rise in IOP.

With earlier intervention for cataracts and increasing affluence Phacolytic glaucoma will continue to get rarer and rarer though it may never be completely eradicated. Since Phacolytic glaucoma is still not so rare in our part of the world we thought we will look at aspects of Phacolytic glaucoma that has not been looked at often enough. Along with looking at the usual parameters of aqueous protein and cells in this study we looked at the ultrasound biomicroscopy in patients with phacolytic glaucoma. Electron microscopy of cells; another aspect rarely studied was also looked at in this study. In the initial stages of planning for this study we were also looking to see if characterization of the proteins could be done but this could not be taken forward due to the complexity involved and the funds needed for such an undertaking. Thus we started this study with the intention of looking at aspects of phacolytic glaucoma to enable better understanding of the clinical condition.

We excluded patients younger than 50 years of age to reduce the chances on including cataracts due to causes other than age. Since black cataracts do not develop phacolytic glaucoma we excluded controls who had nuclear sclerosis greater than grade 3 to make the groups more comparable. Based on sample size calculation with respect to macrophage presence in the aqueous, we needed 10 patients in the study group and 10 in the control group. Since we did only one tap in control patients and that did not yield enough aqueous for both protein and cytology study a second control group was needed. It was the practice in our hospital to do paracentesis in cases of phacolytic glaucoma so as to reduce IOP quickly and reduce the time needed

quieten the eye before surgery. We thus used the aqueous for cytology during the first tap and for proteins at the time of surgery. Thus 3 groups of patients ,each arm containing 10 patients were planned. The first arm included phacolytic glaucoma, the second and third arm included patients with immature cataract who formed the control group for proteins and cells. For UBM study the entire control group of 20 were used. Finally 12 patients of phacolytic patients were eligible to be part of the study and were included. In 10 control patients aqueous was analyzed for presence or absence of cells. In another 10 estimation of protein concentration only was done. The results obtained were compared with that obtained in phacolytic glaucoma in whom aqueous was analyzed for both presence of cells as well as for estimation of protein concentration ,by collecting aqueous twice, 24 hours apart . In earlier studies about 0.1-0.2 ml collected on one occasion alone was analyzed for both presence of cells and proteins ,resulting in a smaller sample volume available for analysis . On the contrary in our study we obtained about 0.1-0.2 ml of aqueous during each paracentesis, which probably enabled better analysis. Since patients were started on topical medications-antibiotics ,steroids , cycloplegics following first paracentesis, this could alter or affect the cellular contents , thus the first sample was always sent for histopathological analysis . We assumed the lens protein will continue to leak after the first paracentesis and we could find no biological reason for proteins to reduce due to anti-inflammatory medications, the second sample was sent for biochemical analysis to estimate total protein concentration. If at all there is a change in protein concentration after the first paracentesis it should only be less than the first sample. One could argue however that the hypotony created by the first paracentesis could worsen the leak.

In all the patients of phacolytic glaucoma there was an afferent pupillary defect. Such an high incidence of RAPD has not been reported before. The acute rise in pressure and the associated nerve damage could be one explanation. Reduced light entering the eye due to cataract could be another explanation.

The iridescent particles seen could have been the cholesterol or oxalic acid crystals described before.

50% of our cases had phacodonesis and this was a high proportion compared to other studies. The reason for the phacodonesis could be due to the reduced lens support as a result of leaked out lens matter. Since all of these patients had PC IOL zonular dialysis is an unlikely cause.

Studies have described white material in AC as hypopyon.[3,5] Hypopyon by definition is pus in the anterior chamber and invariably has a well defined upper border. This was not the case in our study. In one case the material was stuck to the anterior capsule and in another it appears like a fluffy material resembling organized fibrin. Cells were seen in the AC in both our cases with fluffy material. A combination of cells and lens matter could be the cause of this appearance.

In a study on 6 patients with phacolytic glaucoma by Epstein D L et al, the protein concentration in aqueous humor was estimated by chromatography and measuring its absorbance at 280 nm . According to their study around 0.8 mg/ml of high molecular weight proteins was present. This accounts for 35% of total protein (2.25 mg/ml) present in the aqueous humor of phacolytic glaucoma patients. It is postulated that it is the high molecular weight proteins which causes a decrease in aqueous outflow facility.[13] In our study we estimated the total protein concentration

in aqueous humor of patients with phacolytic glaucoma and immature cataract. The protein concentration in all phacolytic cases were high ,with minimum being 0 .95 mg/ml and a maximum of 6.98 mg/ml. In the control arm, protein concentration was within the range of 0.09-0.24 mg/ml in all except for one control in which the protein concentration was 1.11 mg/ml. This high concentration could have probably been due to presence of blood related to paracentesis. Unfortunately this could not be correlated with histology findings as the control samples were used only for either protein or histopathology study.

Normally aqueous humor does not contain any cells or high molecular weight proteins .When there is a break in blood-aqueous barrier as seen in inflammatory conditions like uveitis, phacolytic glaucoma, aqueous is flooded with cells and proteins.[27,28] Unlike in uveitis where IOP is lower than normal; in cases where there is lens matter in AC like in phacolytic glaucoma the IOP is very high. Since protein clogging the trabecular meshwork is thought to be the cause of raised IOP in phacolytic glaucoma we looked for a correlation between the protein concentration and IOP but could not find any. If such a correlation does exist one of the reasons for not finding a correlation may be the fact that the protein estimation was done at the second paracentesis and correlation with the IOP was done with the presenting pressure before even the first paracentesis. The second sample may also be the reason why our mean protein concentration was less than in the study by Epstein. Whatever be the case the amount of protein in our study was four times more than the controls and could be one of the reasons for the raised IOPs.

In previous studies looking at cells in the aqueous of patients with phacolytic glaucoma Macrophages were the predominant cells. Neutrophil was the other less

commonly reported cells.[8,9,13] Lymphocytes and other WBCs were conspicuous by its rarity. In our study among the 12 patients who were diagnosed to have phacolytic glaucoma, 75% of cases had cells in the aqueous. 58.3% of samples had Neutrophils and 50% had Macrophages (Fig-13,14,15). High incidence of Neutrophils in our study was not expected. However this is probably the largest series of Phacolytic glaucoma reported in the literature where aqueous humor analysis was done. The presence of neutrophil could have been a response to the acute inflammation and the cells may have come out with the macrophages and may not be a specific response to the lens protein unlike the macrophages. No cells were seen in the control arm consisting of patients with immature cataract suggesting that it is the leaking proteins that has caused the macrophages and Neutrophils to come into the AC.

In a study by Bartholomew et al aqueous humor analysis in 3 patients with phacolytic glaucoma showed macrophages and rhomboid plate like crystals which were morphologically typical of cholesterol in two cases. In the third case octahedral crystals were seen which were identified as calcium oxalate crystals.[11] In our study, only one case with phacolytic glaucoma showed the presence of clear ,rectangular/envelope shaped crystals which were identified as calcium oxalate crystal based on their morphology(Fig-12). There were no cholesterol crystals seen.

ELECTRON MICROSOPY STUDY

There was only one another Electron Microscopy study on the cells in phacolytic glaucoma to the best of our knowledge. The study was in the Japanese literature and the abstract mentions that the authors saw besides macrophages, lymphocyte, Erythrocytes and erythrophagocytois in the one case they studied.

Detection of blood raises the question of contamination if the case was indeed one of phacolytic glaucoma.

We studied 30 consecutive cells from the two cases which had cells and though we found macrophages (Fig:9) predominantly in our study, a few Neutrophils were also present. (Fig-17). Though both histopathology and EM studies did show occasional RBCs this was not considered significant and we attributed their presence to surgical trauma. In our study the macrophages found in phacolytic glaucoma appeared to be distended with phagocytosed material that was predominantly vacuolated; probably lens protein and fluid with fine granular material(Fig-11). Majority of the macrophages had their entire cytoplasm filled with various proportion of lens proteins and clear fluid vacuoles. This caused the nucleus to go to the periphery. The leaked lens protein was also seen extra-celluarly on EM. It appeared that these cells were overwhelmed with the ingested material. It is possible that this overwhelming of macrophages prevents it from being effective antigen presenting cells which induces recruitment of other inflammatory cells like lymphocytes which is seen in other forms of uveitis including phacotoxic uveitis (50). It is also possible that the phacolytic protein is different from the usual lens protein in its antigencity. These two reasons could be an explanation for the absences of lymphocytes even after 10 days of leakage of protein. The presence one fresh monocyte and four non viable cells in the 30 cells studied suggest a rapid turnover due to the large amounts of phacolytic lens matter these cells have to deal with.

The presence of melanin pigments in the macrophages in phacolytic glaucoma has been reported in the Japanese article too. It is possible that the high intro-ocular pressure causes iris pigment epithelial cell death and release of pigments(fig-10) from

the iris pigment cell on the posterior surface of the iris which in turn are engulfed by the macrophages. Whether this pigment causes the cell to be more rigid thus blocking the trabecular meshwork is something worth looking into.

In trying to look for a correlation between the quantitative and qualitative assessment of the ingested lens protein and vacuoles; for lens protein it appears that the pathologist have called any fill of cytoplasm over 50% as numerous and 25 to 50% as moderate. There was no cell with less than 10% lens protein fill. For vacuoles any fill more than 25% was called numerous and those less than 25% fill was variably classified as mild to moderate.

With respect to melanin pigments it looked like any number less than 10 was called few; 10 to 25 as moderate and over 26 as numerous. From the above the subjectivity in the classification is obvious and some objectivity would allow better comparisons across studies.

ULTRASOUND BIOMICROSCOPY

Ultrasound biomicroscopy (UBM) is a non-invasive procedure which ensures high resolution imaging of the anterior segment in vivo. This enables imaging of structures like the zonules and ciliary body which were previously hidden from clinical examination for better assessment of their morphology in various ocular conditions. Anterior segment architectural changes in various pathological conditions can be evaluated qualitatively as well as quantitatively.[48]

Since earlier studies have not described ultrasound biomicroscopy in phacolytic glaucoma patients, we studied anterior segment in all our patients(cases and controls) using ultrasound biomicroscopy. The procedure was performed on all

patients on the day of admission and pictures of anterior segment obtained in each patient were saved for further analysis so that medications or surgical intervention does not change the picture.

Since there was leakage of lens material in to the AC one would expect the lens thickness to reduces and AC depth to increase. Likewise one would expect the angle opening distance also to increase here. To our surprise the lens thickness increased rather than decreased in our cases compared to the controls. The possible explanation for this is that the liquefied lens material made the lens bag more turgid and spherical. This turgidity is what gives the Argentinean flag sign during capsulotomy in these cases. Alternatively the liquefaction causes the lens to lose its biconvex oval cross-section and the lens becomes more spherical. Such a spherical body, following principles of geometry will become thicker in the antero-posterior direction. This change in geometry (fig: 16 a, b) besides increasing the antero-posterior distance also reduces the equatorial diameter. This sphericity of the lens and the fact that it reduces the area of contact between the lens and the iris could explain the significant increase in the angle opening distance between case and the controls. The sphericity of the lens could also explain the higher incidence of phacodonesis in these patients (6 out of 12).

The anterior chamber depth though more than the controls was not statistically significant. It is possible that the increase in the AP diameter of the lens compensated for the leakage of lens matter. There was also a selection bias of the controls in the sense that patients with immature cataract and AC depth more than ¼ corneal thickness were only selected in order to avoid a misdiagnosis of angle closure glaucoma.

CONCLUSION

1) IOP in phacolytic glaucoma case was three times higher than immature cataract resulting in relative afferent papillary defect in all cases.

2) Clinical features noted in this study has all been described well in the past too and no new clinical features were noted.

3) Aqueous humor study showed cells in 75% of cases. Neutrophils were seen in 58.3 % of samples and macrophages in 50% of samples. This high incidence of Neutrophils is reported rarely in literature.

4) The protein concentration in cases of phacolytic glaucoma in our study was four times that of controls. We could not find any correlation between protein concentration and IOP values possibly due to the second sample used.

5) Electron microscopy study revealed macrophages as the predominant cell with only 1 monocyte and few neutrophils. All these macrophages were overwhelmed with lens protein and vacuoles which filled more than 75% of the cell in majority of the cases.

6) Objectivity in classification of macrophage fill will help comparisons across studies.

7) UBM revealed AC echoes only in the two cases which had clinically evident fluffy material in the AC thus adding no additional information.

8)UBM showed unexpected increase in the antero-posterior lens thickness in phacolytic glaucoma compared to controls. The angle opening distance was more in cases of Phacolytic glaucoma compared to controls.

STRENGTH AND LIMITATION

STRENGTH OF THE STUDY

1)This is one of the largest series of aqueous humor analysis in cases of phacolytic glaucoma reported in literature.

2)This is the first study using UBM to study features of phacolytic glaucoma cases in literature to the best of our knowledge.

3)There is only one another study on Electron microscopy in phacolytic glaucoma, that too in non English literature.

LIMITATIONS OF THE STUDY

1)The Sample size was small thus reducing the power of the study for some of the comparisons made between cases and controls.

2)Angle opening distance measurements is subjective and has a high intra-observer variability. This could have affected the readings especially since the examiner could not be blinded to the diagnosis

3)The lens protein determination was only basic and in this age of biochemical analysis, more could have been done to analyse this.

4)Protein measurements done during the second paracentesis does not give the correct picture with respect to the initial presentation.

5) There was some demographic difference, though not statistically significant between the cases and controls which could affect comparison between the groups.

6) High number of cases were lost due to protocol violation and mishandling of specimens collected.

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PLATES

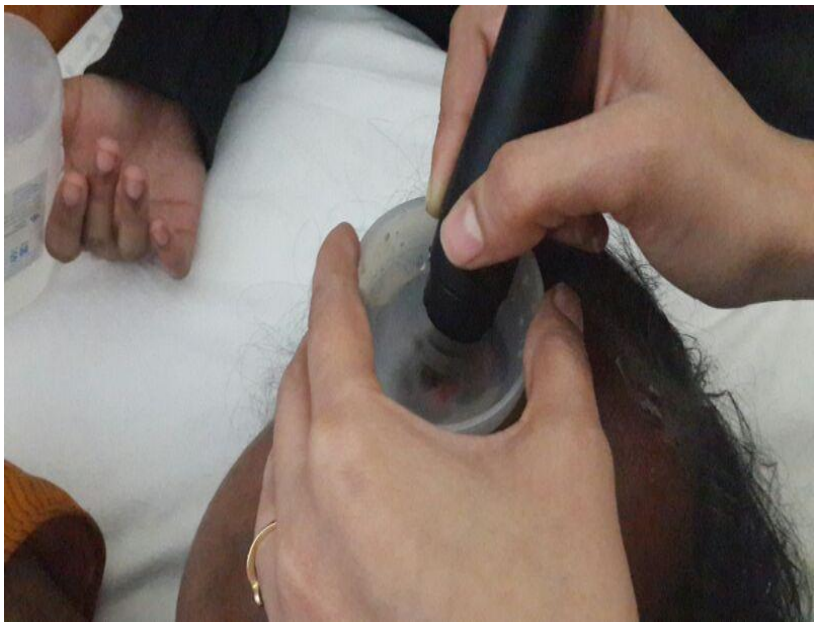


Fig :1 – ULTRASOUND BIOMICROSCOPY(UBM) PROCEDURE

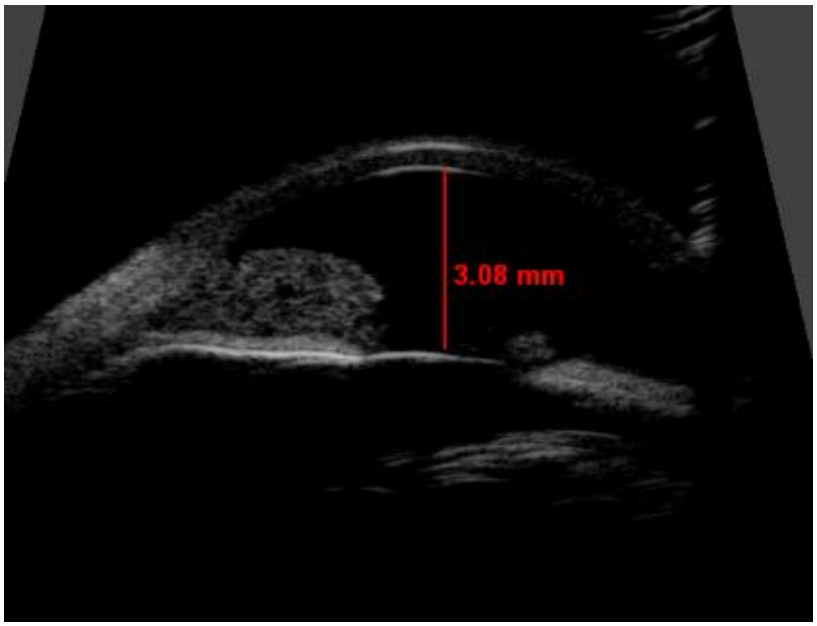


Fig : 2 – UBM – CENTRAL ANTERIOR CHAMBER DEPTH

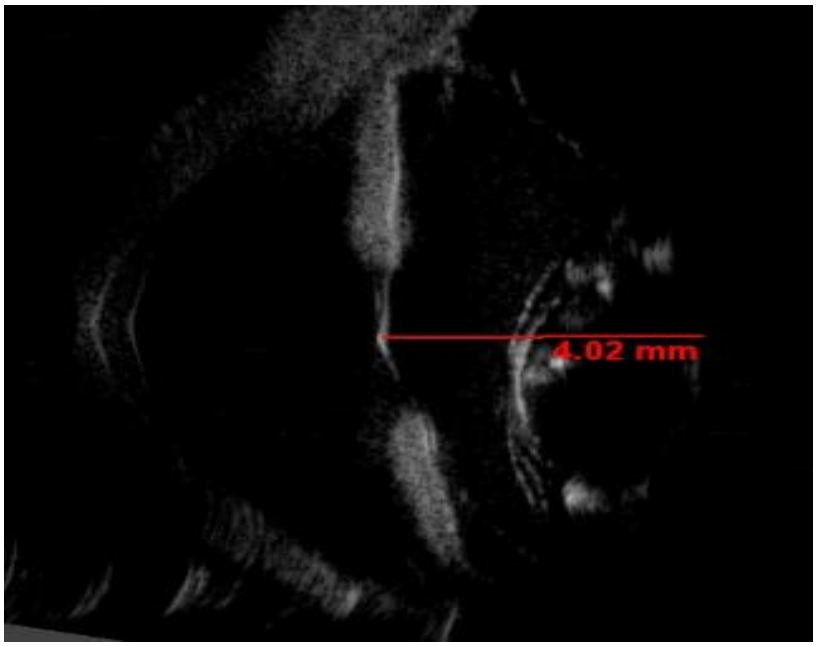


Fig : 3 – UBM - CENTRAL LENS THICKNESS MEASUREMENT

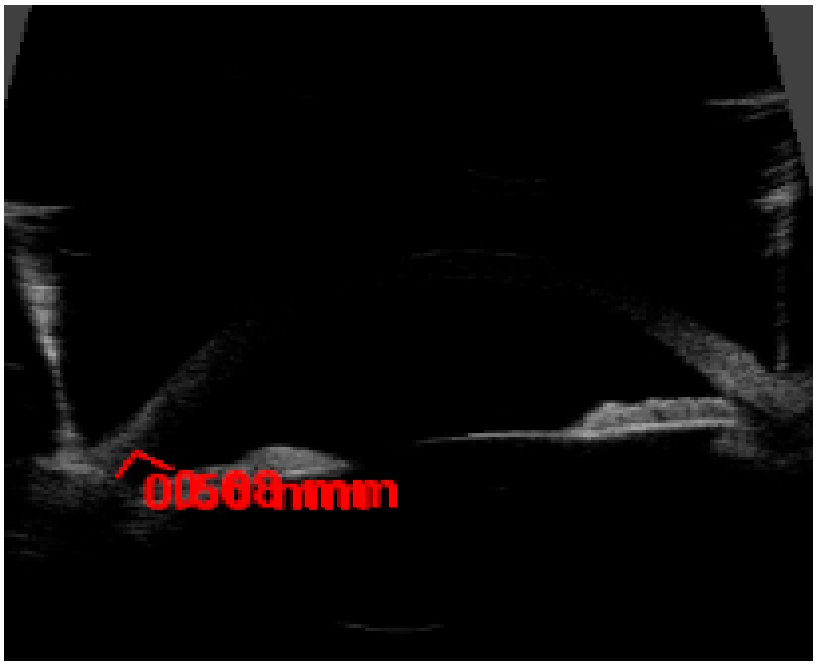


Fig : 4 – UBM –ANGLE OPENING DISTANCE MEASUREMENT

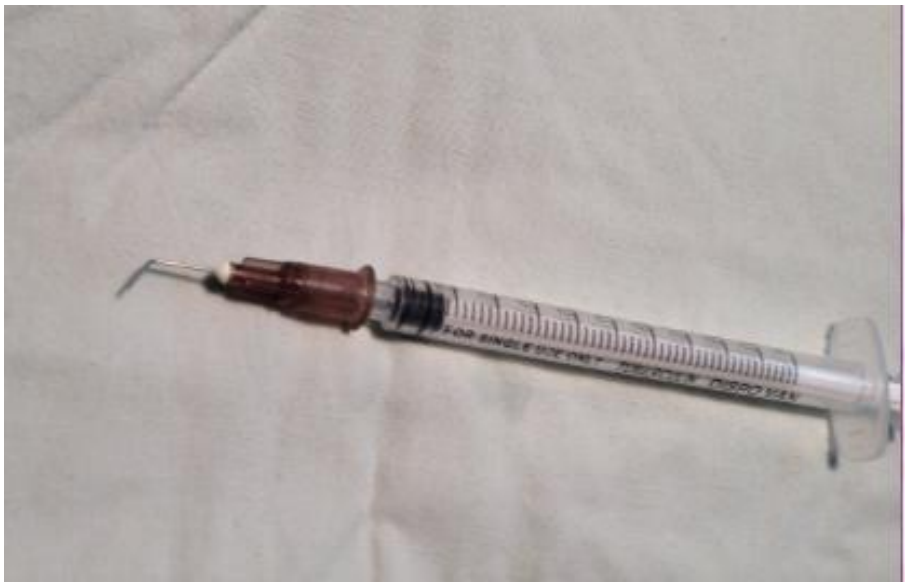


Fig 5 : CUSTOM MADE 26 g CANNULA MOUNTED ON TUBERCULIN SYRINGE

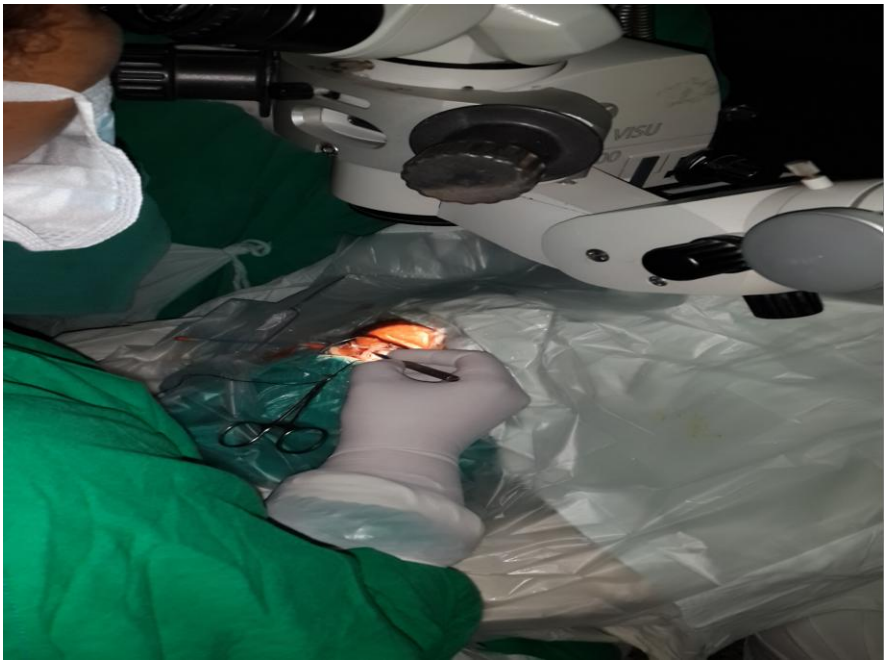


Fig : 6 – PROCEDURE OF PARACENTESIS

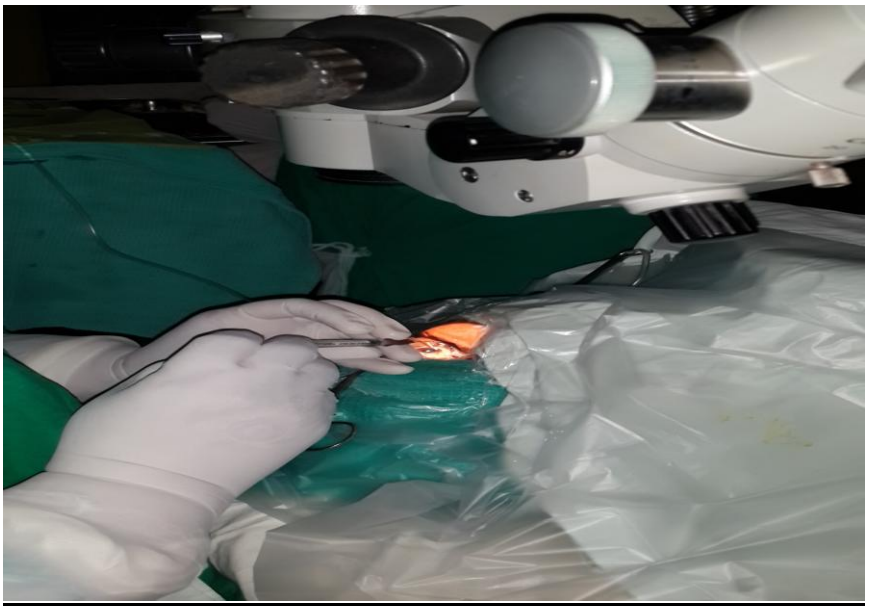


Fig : 7 –PROCEDURE OF PARACENTESIS AND COLLECTION OF AQUEOUS HUMOR

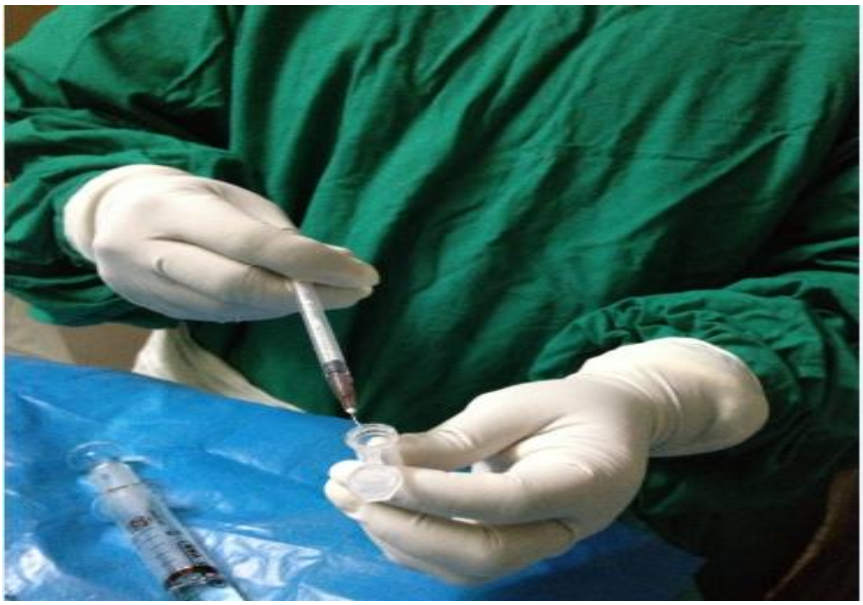


Fig 8 : AQUEOUS HUMOR COLLECTED IN EPPENDORF TUBE

ELECTRON MICROSCOPY

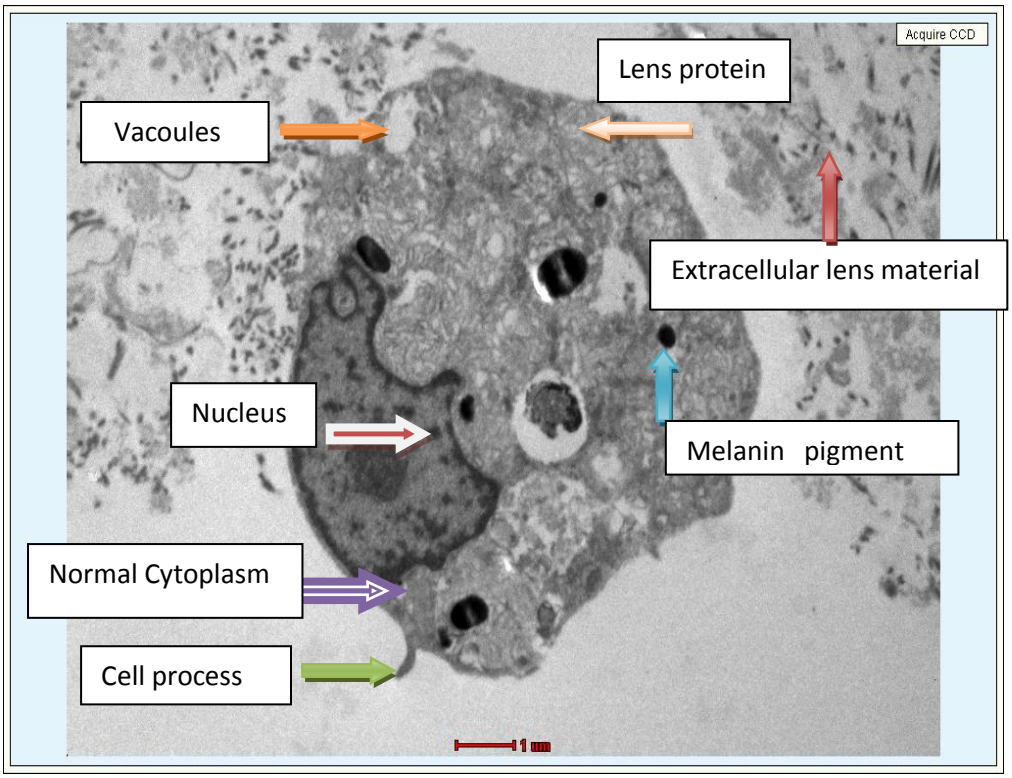


Fig 9 : ELECTRON MICROSCOPY SHOWING MACROPHAGE FLOODED WITH LENS PROTEINS, FEW VACOULES AND MELANIN PIGMENTS

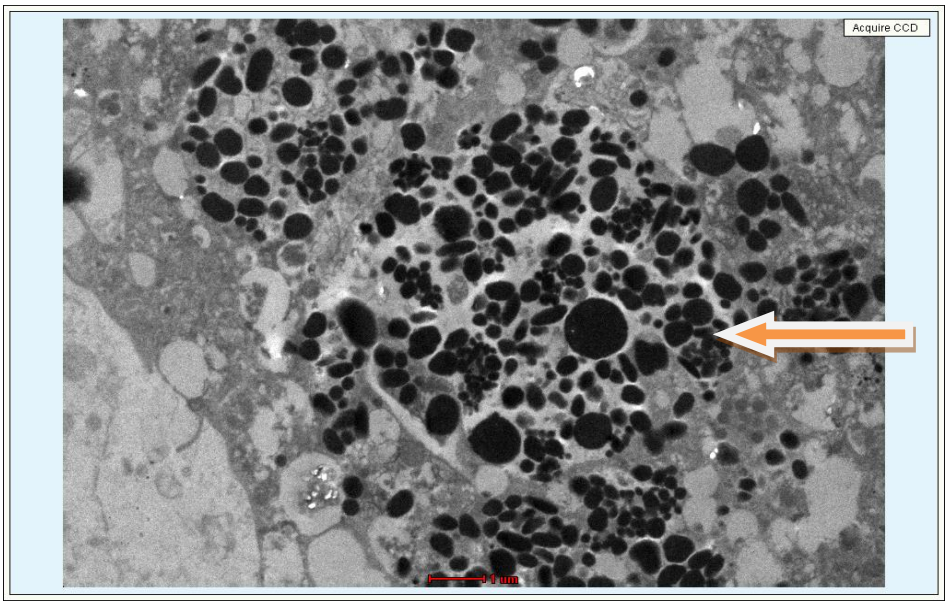


Fig 10 : ELECTRON MICROSCOPY SHOWING MACROPHAGE CONTAINING LARGE AMOUNT OF MELANIN PIGMENTS.

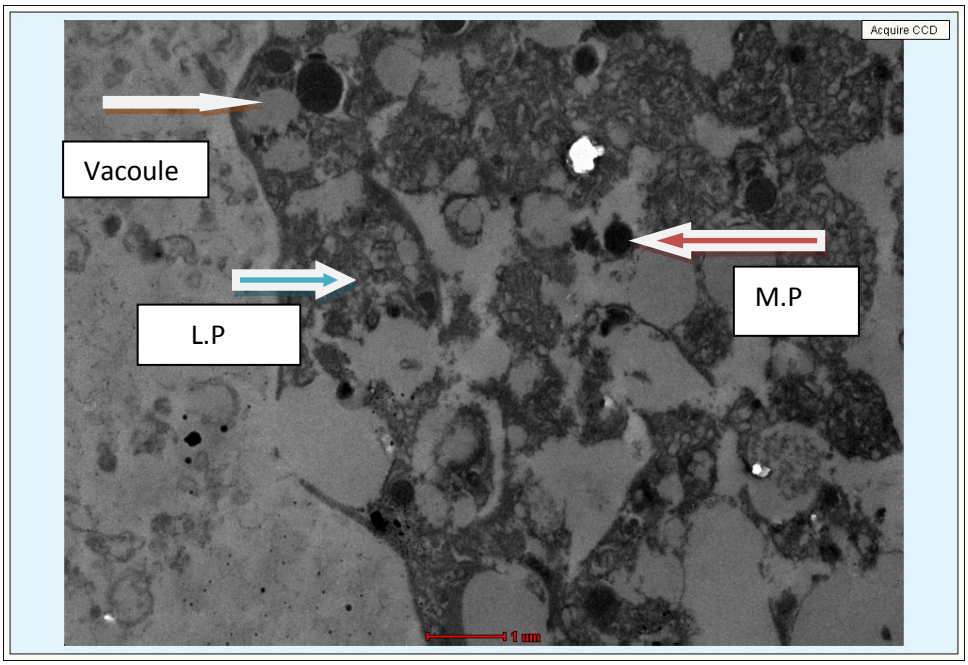


Fig 11 : ELECTRON MICROSCOPY OF MACROPHAGE SHOWING PLENTY OF LENS PROTEIN(LP), VACOULES (V), MELANIN PIGMENT (M.P)

HISTOPATHOLOGY PICTURES OF AQUEOUS HUMOR IN PHACOLYTIC GLAUCOMA

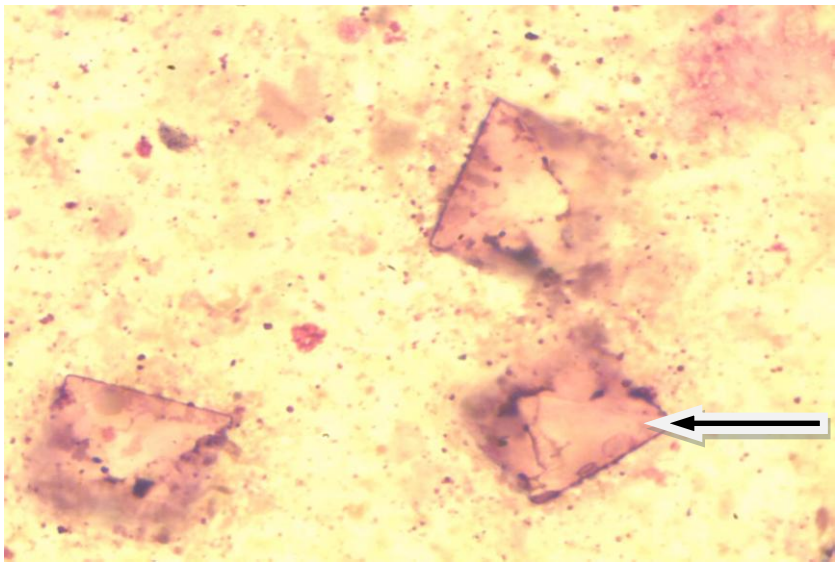


Fig 12 : CALCIUM OXALATE CRYSTALS SEEN ON HIGH POWER MAGNIFICATION

IN AQUEOUS HUMOR OF PATIENT WITH PHACOLYTIC GLAUCOMA

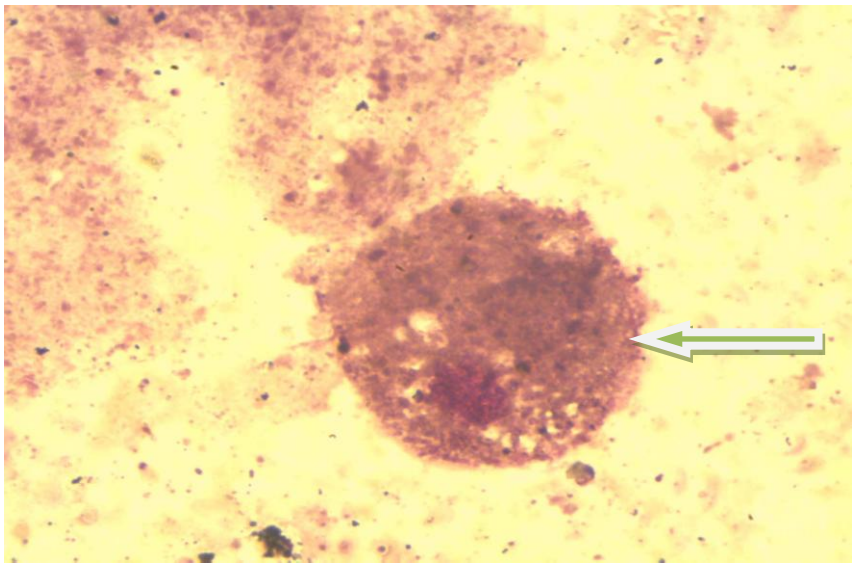


Fig 13 :HISTOPATHOLOGY PICTURE SHOWING MACROPHAGE IN A PATIENT WITH PHACOLYTIC GLAUCOMA

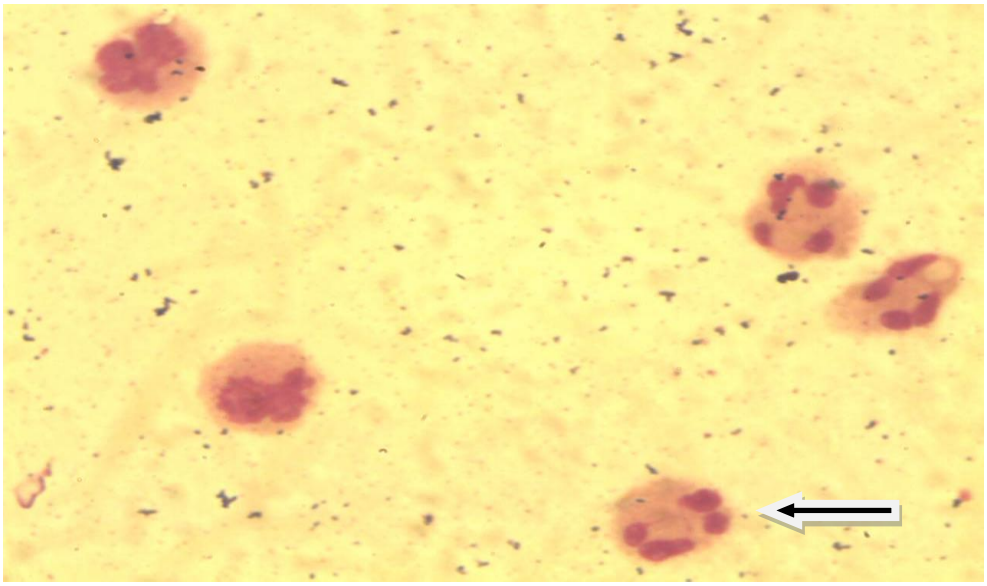


Fig 14 –HISTOPATHOLOGY PICTURE SHOWING MANY NEUTROPHILS IN AQUEOUS HUMOR OF A PATIENT WITH PHACOLYTIC GLAUCOMA

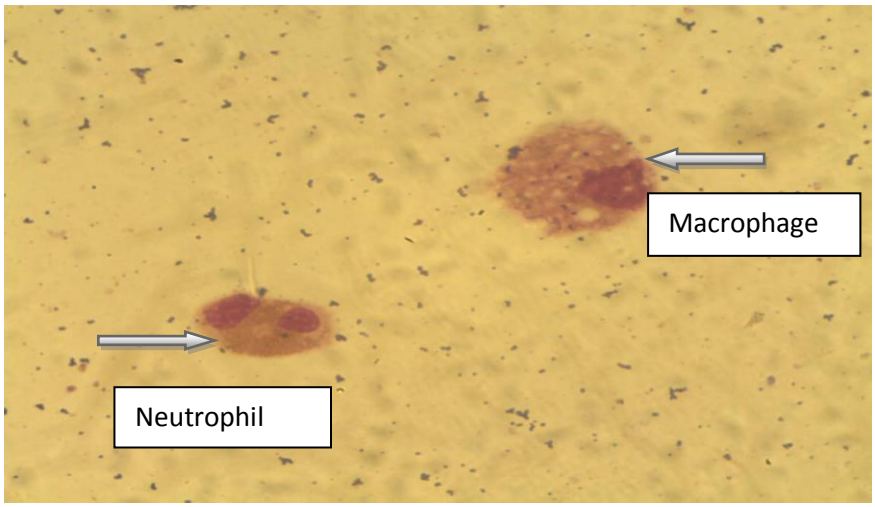
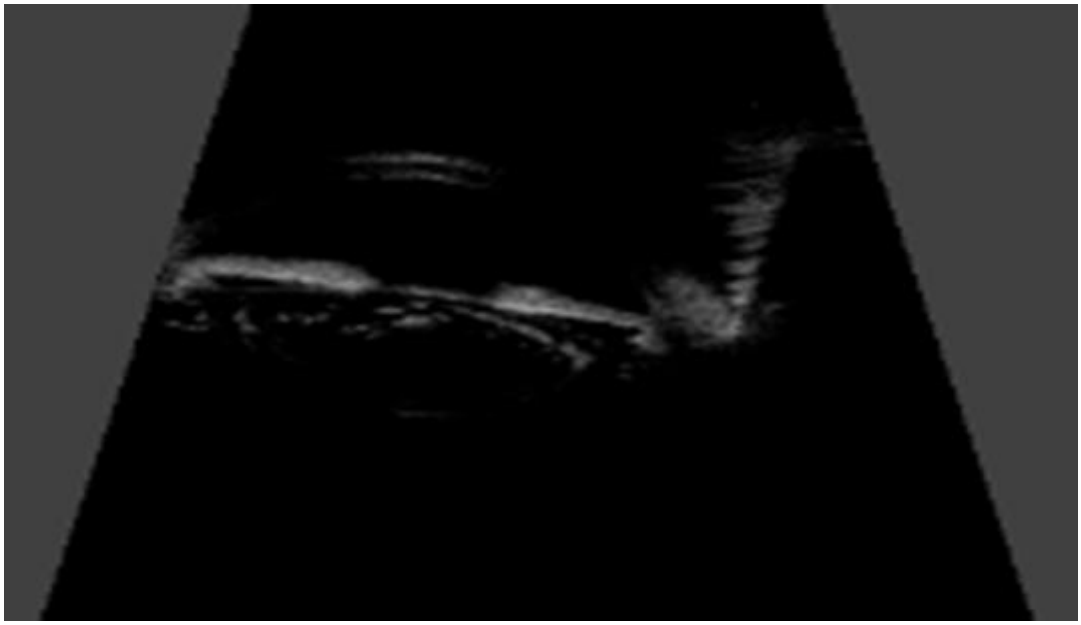
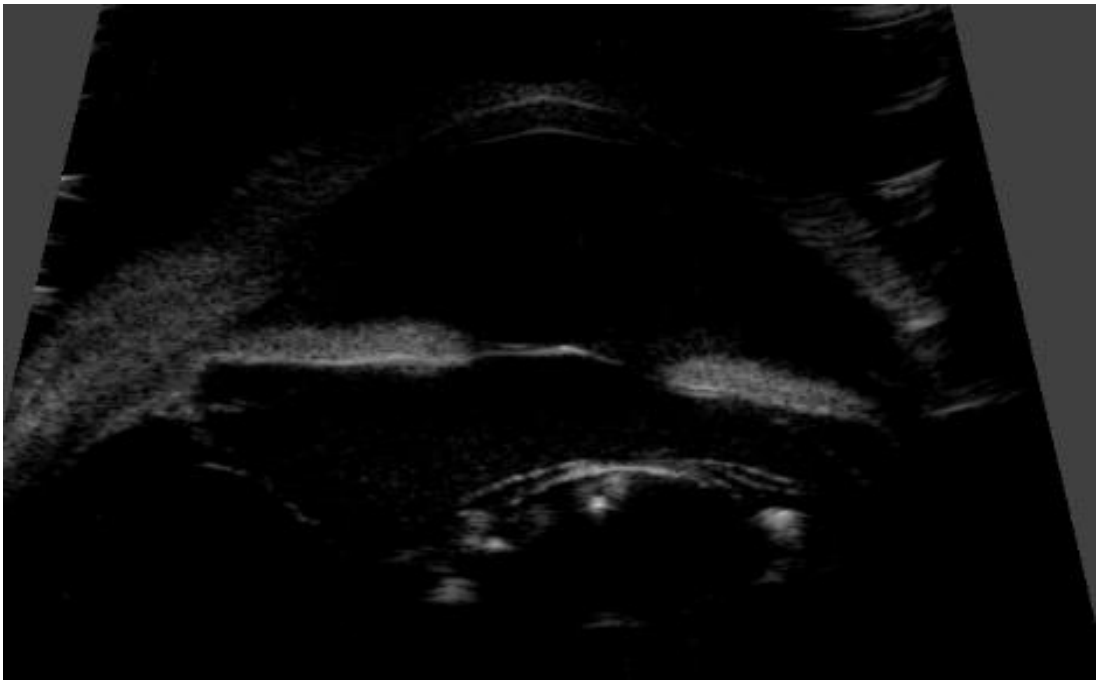


Fig 15 –HISTOPATHOLOGY OF AQUEOUS HUMOR IN A PATIENT WITH PHACOLYTIC GLAUCOMA SHOWING BOTH MACROPHAGE AND NEUTROPHIL

Fig :16 – UBM PICTURE SHOWING MINIMAL DISTANCE BETWEEN ANTERIOR CAPSULE AND IRIS IN IMMATURE CATARACT (a) AND SIGNIFICANT DISTANCE BETWEEN CAPSULE AND IRIS IN PHACOLYTIC GLAUCOMA (b)

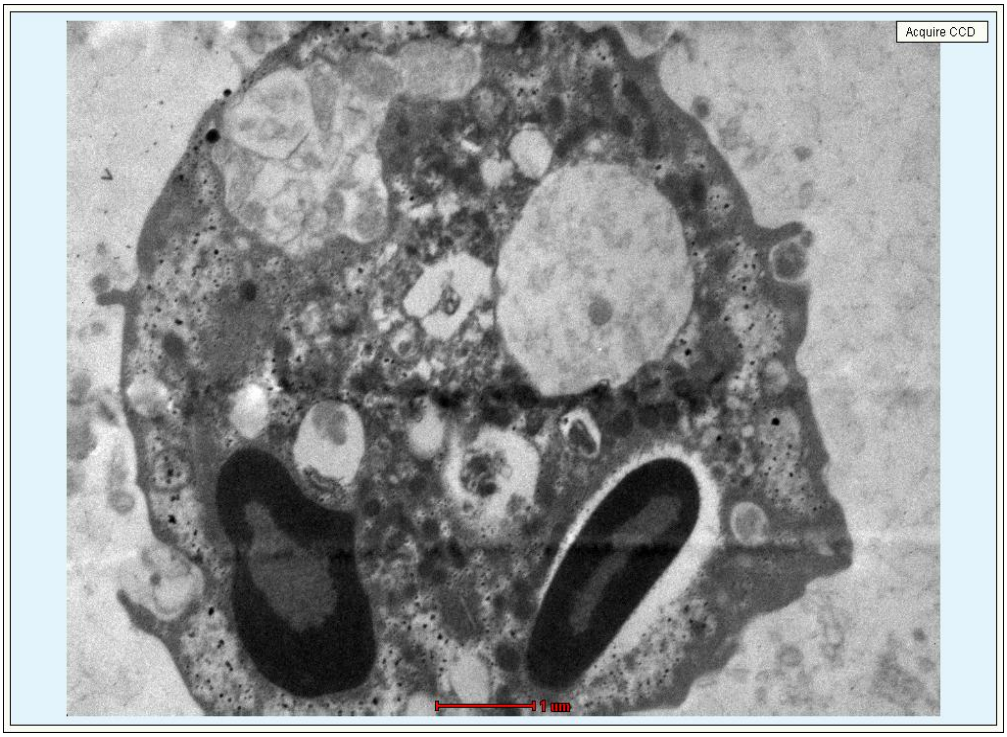


16(a)



16(b)

Fig : 17 - NEUTROPHIL WITH ENGULFED LENS PROTEINS



INSTITUTIONAL REVIEW BOARD (IRB)
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Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

April 5, 2013

Dr. Reetha.B.T
PG Registrar
Department of Ophthalmology
Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project NEW PROPOSAL:**
Comparative histopathological and biochemical analysis of aqueous in phacolytic glaucoma and immature cataracts.
Dr. Reetha.B.T, P.G.Registrar, Ophthalmology, Dr. Thomas Kuriakose
Dr. ArathiSimha R, Ophthalmology, Dr. SukeshChandran Nair,
Transfusion Medicine, Dr. Anna Pulimood, Gastrointestinal Sciences
Dr. Joseph Fleming, Clinical Biochemistry.

Ref: IRB Min. No. 8164 dated 09.01.2013

Dear Dr. Reetha.B.T,

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Thomas Kuriakose, Department of Ophthalmology, CMC

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

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MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

A sum of Rs. 40,000/- (Rupees Forty Thousand only) will be granted for 1 year.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Thomas Kuriakose, Department of Ophthalmology, CMC

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The following Institutional Review Board (Research & Ethics Committee) members were present at the meeting held on January 9, 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. Susanne Abraham	MBBS, MD	Professor, Dermatology, Venerlogy & Leprosy, CMC.	Internal, Clinician
Dr. Benjamin Perakath	MBBS, MS, FRCS	Professor, Surgery (Colorectal), CMC.	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS MCh	Professor, Neurological Sciences, CMC	Internal, Clinician
Dr. P. Prasanna Samuel	B.Sc, M.Sc, PhD	Professor Dept. of Biostatistics, CMC	Internal, Statistician
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Dept. of Pulmonary Medicine, CMC.	Internal, Clinician
Dr. Simon Rajaratnam	MBBS, MD, DNB (Endo), MNAMS (Endo), PhD (Endo), FRACP	Professor, Endocrinology, CMC	Internal, Clinician
Dr. Anup Ramachandran	PhD	The Wellcome Trust Research Laboratory Gastrointestinal Sciences	Internal
Dr. Chandrasingh	MS, MCH, DMB	Urology, CMC	Internal, Clinician
Dr. Paul Ravindran	PhD, Dip RP, FCCPM	Professor, Radiotherapy, CMC	Internal

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Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Anand Zachariah	MBBS, MD, DNB	Professor, Dept. of Medicine, CMC	Internal, Clinician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Mr. Sampath	BSc, BL	Advocate	External, Legal Expert
Mr. Harikrishnan	BL	Lawyer, Vellore	External, Legal Expert
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M.Phil, BL	Legal Advisor, CMC.	Internal, Legal Expert
Mr. Joseph Devaraj	BSc, BD	Chaplain, CMC	Internal, Social Scientist
Dr. B. J. Prashantham (Chairperson), IRB Blue Internal	MA (Counseling), MA (Theology), Dr Min(Clinical	Chairperson(IRB)& Director, Christian Counselling Centre	External, Scientist
Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, DrPH(Epid), DMHC	Retired Professor, Vellore	External, Scientist
Dr. Nihal Thomas	MD MNAMS DNB(Endo) FRACP(Endo) FRCP(Edin)	Secretary IRB (EC)& Dy. Chairperson (IRB), Professor of Endocrinology & Addl. Vice Principal (Research), CMC.	Internal, Clinician

We approve the project to be conducted as presented.

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

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April 5, 2013

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PG Registrar
Department of Ophthalmology
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Comparative histopathological and biochemical analysis of aqueous in phacolytic glaucoma and immature cataracts.
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Transfusion Medicine, Dr. Anna Pulimood, Gastrointestinal Sciences
Dr. Joseph Fleming, Clinical Biochemistry.

Ref: IRB Min. No. 8164 dated 09.01.2013

Dear Dr. Reetha.B.T,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Comparative histopathological and biochemical analysis of aqueous in phacolytic glaucoma and immature cataracts." on January 09, 2013.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Information Sheet, Informed Consent Form and Consent for Ultrasound Biomicroscopy (English and Tamil)
3. Proforma
4. Cvs of Drs. Thomas Kuriakose, ArathiSimha, SukeshChandran Nair, Anna B Pulimood, Fleming Jude Joseph
5. A CD Containing documents 1- 4

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இந்த ஆய்வின் நோக்கம் பேக்கோலைட்டிக் க்ளாக்கோமாவில் (Phacolytic Glaucoma) கண் உள்விழி அழுத்தம் அதிகரிப்பு காரணமாக லென்சின் மீதுள்ள திரவம் வெளியேற்றப்பட்டு, உள்விழி அழுத்தத்தை குறைத்த பிறகு செய்யும் புரை அறுவை சிகிச்சை பற்றியது.

எனவே வெளியேற்றப்படும் திரவத்தை கொண்டு சில ஆய்வுகள் மூலமாக (Histopatho Logical. Bio-Chemical and Electron Microscopy) சோதித்து பார்த்து அதன் ரசாயன தன்மையில் எதாவது மாற்றம் உள்ளதா என்று அறிந்து கொள்வதற்காக இந்த ஆய்வு நடத்தப் படுகிறது. இந்த ஆய்வில் பங்குபெறும் பட்சத்தில் மேற்கண்ட சோதனைகளுக்கு எந்த தொகையும் வசூலிக்கப்படமாட்டாது. ஆனால் புரை நீக்க அறுவை சிகிச்சைக்கு வேண்டிய தொகையை செலுத்த வேண்டும்.

இந்த ஆய்வில் பங்கேற்பவர்களுக்கு எந்தவித பாதிப்பும் ஏற்படாது என்பதை உறுதி படுத்துகிறோம். ஒருவேளை ஏதாவது சிறு பாதிப்பு ஏற்படும் பட்சத்தில் அதற்கு இலவசமாக சிகிச்சை அளிக்கப்படும்.

இந்த ஆய்வை குறித்த குறிப்புகளை மருத்துவ இதழில் பதிவேற்றப்படும். ஆனால் உங்களை குறித்த அடையாளங்களை வெளிப்படுத்தப்படமாட்டாது. உங்களின் ஆய்வு குறிப்புகளை மேற்கூறிய ஆய்வோடு தொடர்புடைய மற்ற ஆய்வுகளுக்கு பயன்படுத்தலாம் என்று உங்களுக்க தெரிவிக்கப்படுகிறது. எனவே நீங்கள் ஆய்வில் சேர்ந்து பயனடைய கேட்டுக் கொள்கிறோம்.

இந்த ஆய்வை குறித்த மேற்படி தகவல்களுக்கு டாக்கர் ரீதா என்பவரை 0416–3071201 என்ற தொலைபேசியிலும் drreethabt@cmcvellore.ac.in என்ற மின்னஞ்சல் விலாசத்திலும் அனுகலாம்.

ஒப்புதல் படிவம் (UBM Test)

கண்ணில் உள்ள புரை முற்றியதால் ஏற்படும் கண் உள்விழி அழுத்த நோயால் பாதிக்கப்பட்டவர்களுக்கு லென்சில் கசியும் திரவத்தை குறித்த ஆய்வு.

எனவே இந்த ஆய்வில் பங்குபெறும் மேற்கூறிய பிரச்சனை உள்ளவர்களுக்கு UBM என்னப்படும் அல்ட்ரா சவுண்டு பையோ மைக்ராஸ்கோபி சோதனை செய்யப்படும். இப்படி சோதனை செய்வதின் மூலம் வெளியேறும் திரவத்தின் தன்மை குறித்த ரசாயன மாற்றங்களை அறிந்து கொள்ளலாம்.

இந்த ஆய்வில் பங்கேற்பவர்களுக்கு கண்ணில் சொட்டு மருந்து விட்டு சோதனையை மேற்கொள்ளப்படும்.

இந்த ஆய்வினால் எந்தவித பாதிப்பும் ஏற்படாது.

ஆய்வு எண். :

பங்கேற்பாளரின் பெயர் :

பிறந்த தேதி / வயது :

_____ என்பவரின் மகன் / மகள் ஆகிய எனக்கு இந்த ஆய்வை குறித்தும் எனக்குள் எழுந்த சந்தேகங்கள் குறித்தும் அறிந்து கொண்டேன்.

இந்த ஆய்வில் என்னுடைய சுய விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும் இந்த எந்த நிபந்தனையும் இல்லாமல் விலக்கி கொள்ளவும் எனக்கு அதிகாரம் உண்டென்றும் இதனால் கொடுக்கப்படுகின்ற சிகிச்சையில் எந்த பாதிப்பும் இருக்காது என்றும் அறிந்திருக்கிறேன்.

பரிசோதகர் வெண்படல ரசாயன பரிசோதனையில் Phacolytic வெண்படல நோயாளியின் மாதிரி எது என்றும், முதிர்ச்சி பெறாத புரை நோயாளியின் மாதிரி எது என்றும் அறிவார் என்பதும் அறிந்திருக்கிறேன்.

இந்த ஆய்வில் எடுக்கப்படும் எல்லா பரிசோதனைகளும் இலவசம் என்றும், புரை நீக்க அறுவை சிகிச்சைக்கு மட்டும் பணம் செலுத்த வேண்டும் என்றும் அறிந்திருக்கிறேன்.

இந்த ஆய்வின் போது ஏதாவது சிறிய காயமோ அல்லது பக்க விளைவினால் ஏற்படும் சிறு பிரச்சனைகளுக்கு இலவசமாக சிகிச்சை அளிக்கப்படும் என்றும் பண உதவி எதுவும் அளிக்கப்படமாட்டாது என்றும் அறிந்திருக்கிறேன்.

இந்த ஆய்வில் இருந்து நான் விலகினாலும் என்னுடைய அனுமதியின்றி என்னுடைய ஆய்வு குறிப்புகளை பயன்படுத்தி கொள்ளலாம் என்றும் என்னுடைய அடையாளங்களை யாருக்கும் தெரிவிக்கப்படமாட்டாது என்றும் அறிந்திருக்கிறேன்.

நான் முழு சம்மதத்துடன் இந்த ஆய்வில் பங்கேற்க சம்மதிக்கிறேன்.

பெயர் :

கையொப்பம் :

தேதி :

சாட்சியின் கையொப்பம் :

பங்கேற்பாளருக்கு என்ன உறவு

தேதி :

COMPARATIVE HISTOPATHOLOGICAL AND BIOCHEMICAL ANALYSIS OF AQUEOUS HUMOR IN PHACOLYTIC GLAUCOMA AND IMMATURE CATARACT

Clinical Research Form

Hospital No.

Study No.

Initials of the patient:

Age:

Sex:

Address:

Socioeconomic status:

Reasons for delay in surgery

1. Socioeconomic cause
2. Ignorance
3. Fear of surgery
4. Social

Chief complaints

- Pain
- Redness

History:

- Use of topical medications
- Trauma
- Uveitis
- Pre-existing glaucoma
- Previous surgery

Examination

	Right eye	**Left eye**
Circumciliary congestion		
Corneal edema		
Deposits on back of cornea		
Anterior chamber a)Depth b)Flare (2+ and above) c)Iridescent particles d)Hypopyon		
RAPD		
Lens a)Type b)Calcific spots c)Soft spots d)Phacodonesis		
Intraocular pressure		

Ultrasound Biomicroscopy a)Central anterior chamber depth b)Angle opening distance c)Central lens thickness d)Anterior chamber echoes e)Vitreous echoes		
Vision		

ANALYSIS OF AQUEOUS HUMOR

1)Date of 1st paracentesis :

a)Sample stored and sent :

b)Histopathology report

Number of cells /high power field:

Macrophages present:

2)Date of second paracentesis:

Biochemical analysis of sample:

Protein concentration in sample:

3)Electron microscopy study:

a)Date of collection of sample:

b)Electron microscopy report:

Macrophage type:

Material engulfed by macrophages:

ABBREVIATIONS

1)PHACOLYTIC GLAUCOMA	P.G
2)UTRASOUND BIOMICROSCOPY	UBM
3)ELECTRON MICROSCOPY	EM
4)INTRAOCULAR PRESSURE	IOP
5)LENS PROTEINS	LP
6)MELANIN PIGMENT	MP
7)HISTOPATHOLOGY	HPE
8)BIOCHEMISTRY	BIOCHEM
9)DALTONS	Da
10)ASPARAGINE	Asn
11)ASPARTIC ACID	Asp

DATA SHEET

COMPARATIVE HISTOPATHOLOGY AND BIOCHEMICAL ANALYSIS OF AQUEOUS HUMOR IN PHACOLYTIC GLAUCOMA AND IMMATURE CATARACT

A) AGE- 1……50-60yrs

2……60-70yrs

3…….>70yrs

B) SEX----------MALE/FEMALE

C)SOCIOECONOMIC STATUS---------

D) REASONS FOR DELAY IN SURGERY---------None-0

Socioeconomic cause-1

Ignorance-2

Fear of surgery-3

Social- 4

E) CHIEF COMPLAINTS------- Pain------Yes-1, No-2

Redness----------Yes-1 ,no-2

F) ON EXAMINATION------Circumcilliary congestion----------yes-1/no-2

Corneal edema----------yes-1/no-2

Deposits on back of cornea----------yes-1/no-2

Anterior chamber------

a)- depth----- >1/2-----1 <1/2-----2

b) flare---------- yes=1/no-2

c) iridescent particle----------yes-1/no-2

d) hypopyon----------yes-1/no-2

pupil RAPD----------yes-1/no-2

lens-------a) type of cataract--------normal=1/IMC-2/MC-3/HMC-4

b) calcific spots----------yes=1/no-2

c) phacodonesis----------yes-1/no-2

INTRAOCULAR PRESSURE (mm of Hg)----------exact values

ULTRASOUND BIOMICROSCOPY

a)-Central ACD----VALUES

b) angulation – values

c) central lens thickness- values

d)AC echoes- yes-1/no-2

e) vitreous echoes---yes-1/ no-2

HISTOPATHOLOGY REPORTS--no cells-0/macrophages-1/neutrophils-2/macrophages and neutrophil-3

BIOCHEMICAL REPORTS----- protein content(mg/.dl)----------values

MASTER CHART

serial no	hospital n	initials of	age	sex	socioeco	reasons f	pain	redness	circumcill	corneal e	deposits	depth	flare(2+ a	iridescent	hypopyon	RAPD	type	calcific spots	soft spots	phacodonesis	intraocular pressure	central anterio	angle opening dist	central lens thickness	anterior chamb	vitreous echoes	histopathology	biochemistry reports
1	3849965	KA	68	M	1	3	1	1	1	1	2	1	1	1	2	1	4	1	1	1	34	3.08	0.58	3.79	1	2	1	95
2	4084025	MM	56	M	2	2	1	1	1	1	2	1	1	1	2	1	4	1	1	2	62	2.62	0.56	3.93	2	2	0	315
3	4085435	KG	73	M	2	1	1	1	1	1	2	1	1	1	2	1	4	1	2	2	30	3.05	0.71	3.61	2	2	2	698
4	4089005	CC	63	M	1	1	1	1	1	1	2	1	1	2	2	1	4	1	1	1	50	3.1	0.66	3.98	1	2	3	158
5	4116345	FV	65	M	2	2	1	1	1	1	2	1	1	2	2	1	4	1	1	2	52	3.21	0.78	3.88	2	2	0	288
6	4121335	KM	72	M	1	1	1	1	1	1	1	1	1	1	2	1	4	1	1	1	38	2.45	0.72	4.76	2	2	2	481
7	4068725	SN	60	F	2	1	1	1	1	1	2	1	1	1	2	1	4	1	1	2	52	3.42	0.83	3.61	2	2	0	673
8	4140265	KP	70	M	2	2	1	1	1	1	2	1	1	2	2	1	4	1	1	2	22	2.77	0.6	3.75	2	2	1	341
9	4078835	SK	78	F	2	1	1	1	1	1	2	1	1	1	1	1	4	1	1	2	44	3.24	0.65	4.12	2	2	2	332
10	2417215	NV	79	M	2	2	1	1	1	1	2	1	1	1	2	1	4	1	1	1	53	2.41	0.72	4.81	2	2	3	224
11	938938E	KG	85	F	1	1	1	1	1	1	2	1	1	1	2	1	4	1	1	1	40	2.64	0.66	4.35	2	2	3	147
12	4233335	KV	62	F	2	1	1	1	1	1	2	1	1	1	2	1	4	1	1	1	32	3.06	0.62	3.96	2	2	3	570
CONTROL	HISTOPATHOLOGY																											
1	3256235	MG	62	F	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	12	3.27	0.54	3.49	2	2	0	
2	4057545	RP	59	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	12	3.01	0.51	3.38	2	2	0	
3	4043455	KS	76	F	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	13	2.51	0.65	4.3	2	2	0	
4	4053945	MI	63	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	18	2.82	0.55	3.7	2	2	0	
5	4088955	VR	64	F	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	8	2.82	0.52	3.84	2	2	0	
6	4077265	SG	63	F	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	16	2.22	0.54	3.96	2	2	0	
7	4072655	SA	51	M	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	18	2.59	0.56	4.16	2	2	0	
8	4168945	G	52	F	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	14	2.9	0.6	3.65	2	2	0	
9	4168885	MR	57	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	14	3.01	0.69	3.79	2	2	0	
10	4169885	RM	60	F	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	8	2.31	0.52	3.84	2	2	0	
CONTROL	BIOCHEMISTRY																											
1	4108215	GK	65	M	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	18	2.68	0.56	3.75	2	2		16
2	4108175	RS	58	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	15	2.64	0.69	3.65	2	2		12
3	4107625	SV	63	M	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	14	3.28	0.55	3.24	2	2		9
4	4123965	SM	60	F	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	16	3.05	0.6	3.38	2	2		10
5	4089245	AM	78	M	3	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	12	3.14	0.62	3.66	2	2		111.16
6	8086858	KK	63	F	3	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	12	3.1	0.55	3.93	2	2		10
7	3067105	S	67	F	3	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	10	2.68	0.54	3.98	2	2		16
8	4196285	KK	80	M	1	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	16	2.64	0.5	3.75	2	2		24
9	3904805	MA	73	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	10	3.24	0.62	3.7	2	2		12
10	4194715	SA	64	M	2	0	2	2	2	2	2	1	2	2	2	2	2	2	2	2	14	2.17	0.56	3.1	2	2		19