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Tear and Aqueous Humor Biomarkers in Primary Open Angle Glaucoma

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Abstract

Background: Glaucoma are structural and functional landmarks of optic nerve degeneration (OND), a kind of progressive ocular disease. OND presents itself in the ocular fundus as optic disc cupping as a result of retinal ganglion cell (RGC) loss, and the corresponding visual field (VF) defects (1-3). One of the leading causes of permanent blindness on a worldwide scale, glaucoma is a neurological illness that impacts millions of people. Age is associated with an increased risk of primary open-angle glaucoma (POAG) in Europe. As the European population ages, the likelihood of POAG will increase due to the increased strength of the elderly population. Additional research, particularly focussing on biomarkers, is needed to clarify the function of inflammation in the pathophysiology of POAG. One of the most convenient and non-invasive ways to test for glaucoma biomarkers is using tears. The pathogenesis of the illness and the impact of topical treatments on inflammatory processes may be better understood if inflammatory biomarkers in tears could be identified and correlated with cytokines in the aqueous humour. Goal: The purpose of this review is to assess the efficacy of inflammatory cytokines in aqueous humour and tears as indicators of primary open angle glaucoma. Final thoughts: Biomarkers have attracted a lot of attention from scientists and clinicians looking for solutions to these problems, and there are a lot of substances that might be used as biomarkers. We categorise biomarkers as either non-invasive, slightly invasive, or invasive based on the method used to get fluid samples, and we summarise what is currently known about the most robust molecular biomarkers suggested in POAG. Many obstacles and restrictions, largely arising from structural and scientific considerations, characterise the transition of biomarkers from discovery to clinical practice in glaucoma and other areas of ophthalmology and medicine. Most importantly, there is a lack of biomarker characterisation and validation methodologies, insufficiently rigorous analytic methods used in clinical trials, and a failure to make diverse decisions before beginning the discovery phase.

Keywords: Tear Diagnostic Tools; Aqueous Serum Biomarkers for primary open angle glaucoma.

Introduction

Glaucoma a diverse set of degenerative eye illnesses that gradually impair vision by damaging the optic nerve and causing the gradual death of retinal ganglion cells (RGC). The majority of cases of primary open angle glaucoma (POAG) do not manifest any symptoms until the disease has progressed further. Some subgroups of POAG patients continue to advance over time despite therapy, however the majority of individuals have stability for a long period. Timely therapy is essential for individuals with increasing POAG, but it is difficult to detect these people in the early stages of the illness [1]

Recent years have shown that biomarkers provide a fresh viewpoint on illness diagnosis. Researchers have looked for biomarkers in ocular samples including vitreous, aqueous humour, and tears to see whether they might reveal normal or abnormal processes. Aqueous humour (AH) is a readily available fluid that comes into direct touch with the trabecular meshwork, an important location in the development of glaucoma. Maintaining a constant intraocular pressure (IOP) requires AH production and outflow. Hence, AH might be a biomarker-type for glaucoma that is both sensitive and specific [2].

We set out to assess the efficacy of proinflammatory cytokines in aqueous humour and tears as diagnostic indicators of primary open angle glaucoma.

Primary Open Angle Glaucoma

The Pathophysiology and Treatment of Glaucoma: A Review - PMC

The Glaucomas are a class of eye diseases called optic neuropathies that cause the ganglion cells in the retina to gradually deteriorate. Neurones of this kind are located in the optic nerve and have their cell bodies in the inner retina. Visual loss and the optic disc's distinctive cup shape are symptoms of degeneration of these nerves. [3] noted that the variables that contribute to the advancement of glaucoma and our understanding of its biological basis are both lacking.

Glaucoma is the most common cause of permanent blindness, affecting about 70 million people globally. Roughly 10% of those affected are blind in both eyes. Since glaucoma often doesn't cause any noticeable symptoms until the disease has progressed enough, the actual number of people impacted is probably much greater than the number of cases

reported. Only 10% to 50% of glaucoma patients are aware of their condition, according to population-level assessments. In general, there are two types of glaucoma: open-angle and angle-closure. Although open-angle glaucoma accounts for over 80% of occurrences in the US, a disproportionate percentage of individuals with significant visual loss are affected by angle-closure glaucoma. Primary cases of either open-angle or angle-closure glaucoma are possible. Trauma, inflammation, tumours, certain drugs (like eye corticosteroids), and disorders (such pigment dispersion or pseudo-exfoliation) may all lead to secondary glaucoma [4].

Disease mechanisms

Intraocular pressure is associated with the loss of retinal ganglion cells, albeit the exact mechanism by which glaucoma develops is still a mystery. The intra-ocular pressure is determined by the equilibrium between the ciliary body's production of aqueous humour and its drainage via two separate channels, the trabecular meshwork and the uveoscleral outflow pathway. The trabecular meshwork becomes more resistant to aqueous outflow in open-angle glaucoma patients. Patients with angle-closure glaucoma, on the other hand, often have their drainage channels blocked (Figure 1) According to [5].

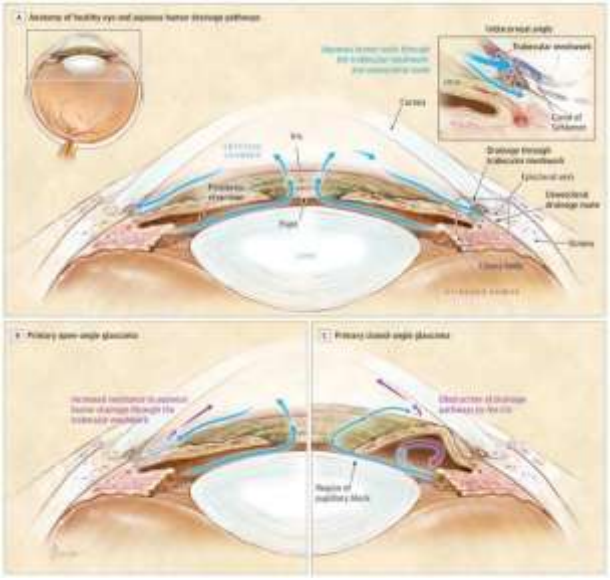

Fig. 1: Aqueous Humor Drainage Pathways of Healthy and Glaucomatous Eyes [5].

Intraocular The eye's posterior components, especially the lamina cribrosa and surrounding tissues, are vulnerable to mechanical stress and strain when subjected to pressure. Axons from retinal ganglion cells, which make up the optic nerve, escape the eye via a perforation in the sclera at the lamina. The pressurised eye's lamina is its weakest wall component. As a consequence of mechanical axonal damage and disruption of axonal transport, the retrograde delivery of essential trophic factors to retinal ganglion cells from their brainstem target, the relay neurones of the lateral geniculate nucleus, can be interrupted when intraocular pressure induces stress and strain, which can lead to compression, deformation, and remodelling of the lamina cribrosa [6].

Ocular hypertension in cats and monkeys has been shown to inhibit axonal transport in both directions at the level of the lamina cribrosa, according to studies. Early in the pathophysiology of glaucoma, in experimental systems, axonal transport is disrupted, leading to vesicle collections and postlaminar and prelaminar microtubule and neurofilament disorganisation. Postmortem examinations of eyes affected by glaucoma reveal comparable ultrastructural alterations in the fibres of the optic nerve.13 During times of metabolic stress caused by intraocular pressure, it may be challenging to supply the high energy demands of retinal ganglion cells and astrocytes due to mitochondrial malfunction [7].

Even in those whose intraocular pressures are within the normal range, glaucomatous optic neuropathy may develop. A significant pressure gradient across the lamina may develop in these individuals due to an unusually low pressure of cerebrospinal fluid in the subarachnoid region of the optic nerve. Other potential causes of glaucoma include changes in immunity, excitotoxicity, oxidative stress, and impaired microcirculation. Secondary neurodegeneration of other retinal neurones and cells in the central visual pathway may be caused by primary neural degenerative

processes, which modify their environment and increase their sensitivity to injury [8].

Diagnosis and Clinical Presentation

Multiple population-based studies have shown that 25% to 50% of glaucomatous people had intraocular pressures below 22 mm Hg, despite the fact that increased IOP is a very reliable risk factor for glaucoma.Despite the high correlation between IOP and glaucoma, many individuals with IOP never have glaucoma, even after several years of monitoring [9].

Until glaucoma has progressed to a point where it has caused significant neurological damage, symptoms will not appear. When symptoms do manifest, the condition causes blindness, which in turn lowers quality of life and makes it harder to accomplish things like drive. To reduce the disease's course, early intervention is vital. Patients who are at risk of developing glaucoma should be referred to an ophthalmologist [10].

Changes in the appearance of the optic nerve head and retinal nerve fibre layer are diagnostic of glaucoma, which is characterised by the death of retinal ganglion cells and optic nerve fibre loss.1Figure 2 shows that these alterations, which are the most crucial part of a glaucoma diagnosis, may be seen during an ophthalmoscopic examination of the optic nerve head. When it comes to glaucoma identification, the significance of a proper ophthalmologic examination of the eye cannot be described. Loss of retinal ganglion cells leads to a gradual worsening of visual fields, which often starts in the middle of the field and may move centripetally until only the centre or the periphery of the image is left [11].

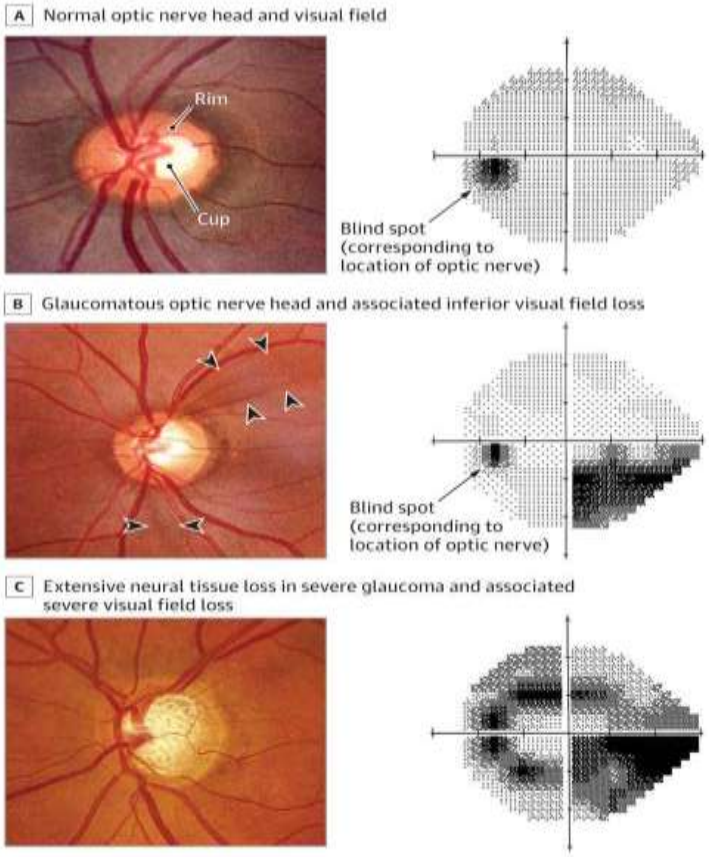

Fig. (2) Normal, Glaucomatous, and Severe Glaucomatous Optic Nerve Heads and Visual Field Test Results [11].

Because Due to the lack of a universally accepted diagnostic tool, glaucoma may be difficult to detect in its early stages. Signs of neuronal loss may be shown by examining the optic nerve head, although early damage diagnosis is difficult due to the large variety in how it appears in healthy individuals. Conventional visual field testing may confirm the diagnosis if distinctive flaws are present, however it can take up to half a million cells to die out of the retinal ganglion cells before the faults become apparent [12].

Therefore, a crucial part of diagnosing the condition is doing longitudinal

evaluations and documenting any structural damage to the optic nerve.28 Taking pictures of the optic nerve head or looking at it with an ophthalmoscope are two ways to do this kind of examination. Nevertheless, glaucoma optic disc damage may be difficult to subjectively identify; even glaucoma experts dispute on how severe the damage is [13].

Quantitative and objective information on the quantity of optic nerve fibre (retinal ganglion cell axon) loss may be obtained by a number of newly developed laser scanning imaging methods. Optical coherence tomography, scanning laser polarimetry, and confocal scanning laser ophthalmoscopy are a few of the methods that have made it easier to detect infections at an early stage and to track the gradual atrophy of the optic nerve [14].

Patients with a personal or familial history of glaucoma should be referred to a comprehensive ophthalmologic examination by their primary care physician for an accurate diagnosis. It is recommended that everyone with a history of the condition in their family have a dilated funduscopic examination of the optic nerve head within the last two years. Direct ophthalmoscopy examinations of the optic nerve conducted by primary care doctors as part of standard clinical visits may also detect symptoms suggestive of optic nerve injury, necessitating referral to an ophthalmologist [15].

Medical Care

Treatment objectives for glaucoma primarily include reducing disease progression and maintaining quality of life. Early diagnosis and treatment are crucial since the glaucoma-related decline in quality of life may start sooner than expected. The one effective treatment for glaucoma is lowering intraocular pressure. Several randomised controlled studies have shown that reducing intraocular pressure may delay or even stop the evolution of several eye diseases [16].

Patients in the Ocular Hypertension Treatment Study were randomly assigned to either get treatment or receive no treatment at all if they had ocular hypertension, defined as high intraocular pressure without any obvious symptoms of glaucomatous damage to the optic nerve or visual field. The percentage of patients showing symptoms of glaucoma at the conclusion of the 5-year follow-up period was 4.4% in the treatment group and 9.5% in the untreated group [17].

According to [18], current management guidelines from the American Academy of Ophthalmology Preferred Practice Pattern suggest lowering the intraocular pressure towards a target level. This target level is the level, or range of values, at which the clinician believes the disease progression will be slowed down enough to avoid functional impairment.

The goal intraocular pressure levels for a specific eye are determined by taking into account the following: the amount of retinal damage, the severity of the damage, the risk factors for its advancement, the expected life expectancy, and the possibility of treatment-related side effects. While a 20% to 50% drop in pressure is often the starting point, it is important to continually reevaluate the goal pressure throughout patient follow-up in order to account for how the illness is progressing. As an example, it may be necessary to reduce the goal if the illness development (optic nerve alterations or visual field loss) persists at pressure levels equal to or higher than the original value [19].

Achieving the desired intraocular pressure should be done with little medication and side effects. There are a number of groups of drugs that may reduce blood pressure. Factors such as medication cost, side effects, and dose regimens might impact medication choice. The first line of defence in medical treatment is often prostaglandin analogues. These medications lower IOP by increasing the flow of aqueous humour via the uveoscleral route and decreasing the outflow resistance. The systemic side effects of these medications are minimal to nonexistent, and they are taken only evening. On the other hand, they have the potential to produce local side effects such increased blood pressure in the eyes, longer and darker eyelashes, a condition known as prostaglandin-associated periorbitopathy, where the orbital fat is lost, induced iris darkening, and pigmentation of the skin around the eyes [20].

When it comes to reducing intraocular pressure, prostaglandin analogues are head and shoulders above other groups of topical medicines. They are used as a secondary line of defence or in cases when prostaglandin analogues are not suitable or are to be tolerated. [18] found that carbonic anhydrase inhibitors and prostaglandin analogues lowered intraocular pressure day and night.

Some medications, including β-adrenergic blockers and α-adrenergic agonists, work best during the day and not when you're sleeping.Patients who have a history of chronic pulmonary obstructive disease, asthma, or bradycardia should not use these medications, including β-adrenergic blockers, since they may have serious systemic side effects. It is recommended that patients gently close their

eyes for 2 minutes after topical pharmaceutical instillation or utilise punctal occlusion to reduce systemic absorption of the drug. Topical glaucoma drugs, such as β-blockers, may have serious or even fatal side effects, which general practitioners and internists should be cognisant of. Persistence in following the prescribed treatment plan is key to a positive treatment outcome [19].

Research on neuroprotective glaucoma therapies that shield the optic nerve from optic nerve injury has received a lot of attention. It is unfortunate that there is now no solid proof that these medications can stop glaucoma from becoming worse in patients. Inadequate knowledge of the pathophysiological pathways linked to optic nerve damage, a dearth of medications that can medicate these pathways, and an unworkable regulatory system for drug approval are all reasons why neuroprotection has not been successful [21].

Surgery with a laser or an incision is necessary when medical methods fail to reduce intraocular pressure to an acceptable level while causing unacceptable side effects. There are an estimated 274 incisional glaucoma procedures done per 100,000 individuals in the US each year. As a first-line treatment, surgery may be considered in cases when the patient is not adhering well or when the condition is very severe [22].

By causing biological changes in the trabecular meshwork, which lead to enhanced aqueous outflow, laser trabeculoplasty decreases intraocular pressure. The technique is done during an office visit and has a very good safety profile. Most patients do indeed see significant drops in intraocular pressure, but this impact wears off with time, and around 10% of patients have failure each year [23].

One of the most typical incisional surgical procedures to reduce intraocular pressure is trabeculectomy. In order to provide a pathway for aqueous humour to be absorbed under the conjunctiva, a little piece of the trabecular meshwork and/or neighbouring corneoscleral tissue is surgically removed. In order to improve the surgery's success rate and lower the fibroproliferative response, antiscarring drugs are often administered to the surgical site. However, these medicines might raise the risk of problems including infection and damage caused by very low intraocular pressure. An alternative to trabeculectomy that is just as successful in decreasing intraocular pressure is a device that drains aqueous humour to an external reservoir [24].

A number of potential substitutes for these treatments are now under study. Less sight-threatening consequences may be associated with these so-called less invasive glaucoma operations. Although the effectiveness of these treatments in reducing intraocular pressure has not been as shown as with trabeculectomy, they might be warranted in some circumstances when the risks and benefits outweigh those of trabeculectomy. [25] found that trabeculectomy had a greater risk of complications but was more successful in decreasing pressure compared to nonpenetrating operations such as deep sclerectomy, viscocanalostomy, and canaloplasty.

Possible Dangers

Female gender, advanced age, and Asian ancestry (e.g., Chinese) are risk factors for angle closure. There are several biometric traits that are shared by eyes with angle closure. Having a tiny eye with a packed anterior segment, shallow central anterior chamber depth, a thicker lens positioned anteriorly, and a short axial length is the major ocular risk factor for angle closure. Other anatomical risk factors for angle closure have been recently revealed using anterior segment optical coherence tomography. These include a smaller anterior chamber width, area, and volume, thicker irides with larger iris curvature, and a bigger lens vault [26].

Information on Aqueous Humour Physiology and Circulation - StatPearls - NCBI Bookshelf

Two fluid-like fluids called humours fill the human eye and keep the eyeball in place and at the proper pressure. The fluid in front of the lens is called aqueous humour, and it looks like water. Beside the retina and situated behind the lens is vitreous humour, a gel-like material. The intraocular fluid's volume and pressure are controlled by the constant secretion and reabsorption of aqueous humour, a low-viscosity fluid.When secretion and reabsorption are out of whack, normal functioning is disrupted, and illness states might manifest [27].

The nonpigmented cells of the ciliary epithelium create aqueous humour, a colourless, clear fluid that fills the anterior chamber of the eye. Its nutritional, optical, and mechanical roles in eye physiology are crucial. Many people thought that aqueous humour was only a stagnant fluid when the 1900s

started. Later discoveries on the anatomy and physiology of aqueous drainage led to the revocation of this theory, which had been the subject of several investigations. The traditional (pressure-dependent) route for aqueous humour secretion begins in the posterior chamber, travels via the anterior chamber's pupil, and ultimately empties into the veins. The components of this system include aqueous and episcleral veins, scleral collector channels, trabecular meshwork, and Schlemm's canal. [28] describe the uveo-scleral (non-conventional, pressure independent conduit) as the remainder's route into the orbit. This pathway consists of the ciliary body lymphatics, suprachoroidal space, sclera, and interstices of the ciliary muscle.

Aqueous humour production and secretion

Cells of the non-pigmented ciliary epithelium constantly produce aqueous humour. The creation of aqueous humour is a result of three physiological processes: active secretion, ultrafiltration, and diffusion. The first two do not include any active cellular involvement and are considered passive processes. A concentration gradient is the path that diffusion follows. It's a great method for compounds with a high lipid solubility to cross biological membranes. The variations in fluid pressure inside the eye's many chambers allow for the ultrafiltration process to occur. Hydrostatic pressure drives this mechanism, which regulates the flow of blood plasma across the ciliary capillary endothelia that have been fenestrated. As a result of its development, the stroma stores plasma ultra filtrate, which is actively secreted as aqueous humour [29]. Intraocular pressure (IOP) is mostly unaffected by systemic blood pressure, and when IOP rises, aqueous inflow falls, indicating that aqueous inflow is sensitive to changes in hydrostatic pressure gradients. Compared to ultrafiltration, the process of cellular secretion is more well known. The ciliary epithelium has particular transport mechanisms that maintain the concentration of aqueous humour, which contains organic molecules, bicarbonate, glucose, some amino acids, and sodium, potassium, and chloride. Hydrolysis of ATP provides the energy necessary for active secretion. Against a concentration gradient, this energy is used for the secretion of chemicals. The production of total aqueous humour is influenced by active secretion to the tune of 80 to 90%. Substances are transported in the opposite direction from what would be anticipated by passive processes alone by active secretion. Active secretion causes aqueous humour to have higher quantities of lactate, ascorbate, and certain amino acids than plasma. Na+K+ -2Cl- symport, carbonic anhidrase, parallel Cl-/HCO3 - and Na+ /H+ antiports, and amino acid membrane transporters are among the membrane active transport systems discovered in the ciliary epithelium [30].

The fact that inhibitors of cellular enzymes may block the entry of aqueous humour provides proof that the ciliary epithelium is actively secreting something. Experimental topical and intravitreal injection of ouabain and vanadate, two Na+K +ATP-ase inhibitors, results in a decrease of intraocular pressure (IOP). While vanadodate ions do decrease intraocular pressure (IOP) in rabbits, they have no such effect in people with ocular hypertension [31].

Beyond a certain point, adding more substrate does not enhance transport any more; this is the defining feature of an active transport system. The system is considered saturated once this limit is reached. Take the saturability of the ascorbate transport system in the eye as an example. Isolated ciliary epithelium electrophysiological investigations have shown that Na+ and HCO3-are required for the upkeep of transepithelial potential difference and short-circuit current, two markers of ion transport or secretion across membranes. The anterior uvea's anionic transport systems are highly interdependent on the kidney's and liver's systems. Similarities between the processes of aqueous humour and cerebrospinal fluid production have been noted [30].

Aqueous humoral mixture Compared to the cornea, the refractive index of aqueous humour is lower, at 1.336. Light beams somewhat diverge when they reach the cornea-aqueous contact because to this feature. Aqueous humour has a slightly greater osmolality than plasma but a lower density and viscosity than water. The human anterior chamber has a volume of around 200 µL, whereas the posterior chamber has a capacity of about 60 µL. The protein concentration of aqueous is much lower than that of plasma, at around 0.5% [32]. This is the primary distinction between the two.

Aqueous and plasma protein compositions are distinct from one another. Beta lipoproteins, immunoglobulin, and other high molecular weight proteins are far less abundant in water than in plasma. The concentration of IgG in aqueous is 3mg per 100ml, however there is no presence of IgM, IgA, or IgD [33] When uveitis is present in the eye, not only does the IgG level rise, but IgA and IgM also show up. Patients with fixed myopia and an axial length larger than 26 mm

have been shown to have higher levels of aqueous matrix metalloproteinase (MMP-2) and tissue inhibitors of MMP (TIMP-1, TIMP-2, and TIMP-3), according to a study by [34].

Even while plasminogen and plasminogen proactivator are present at far higher concentrations in blood, there are also small amounts of complement proteins and components of the fibrinolytic and coagulation systems in aqueous humour. The aqueous outflow channel is kept clear of fibrin because only minimal amounts of plasminogen activator inhibitors are present. A minor amount of mono- and dinucleotide is also present in aqueous humour, and it controls the transit of ions via the corneal endothelium. The levels of α and γ-lens crystallins, which are present in healthy eyes in modest amounts, rise as cataracts form. In many cases, the concentration of amino acids in water is greater than in plasma. Three independent systems for neutral amino acids and three distinct mechanisms for basic amino acids, acidic amino acids, and urea have been proposed as amino acid transporters in the ciliary epithelium. According to [35], 676 unique proteins were identified by proteomic study of human aqueous humour.

A better understanding of the aqueous proteome will pave the way for future research into protein function and the discovery of differentially expressed markers linked to anterior segment disorders. In comparison to cataract patients, those with primary or pseudoexfoliative open angle glaucoma had higher concentrations of several Alzheimer disease biomarkers in their aqueous humour. Complement factor H, complement C3, α2 macroglobulin, transthyretin (TTR), apolipoprotein Al, and Apolipoprotein CIII levels are all higher in these individuals [36].

The levels of ascorbate and lactate in aqueous humour are very high. The existence of an ATP and Na+ gradient is necessary for the active secretion of ascorbate into the aqueous humour. Protecting lens epithelial DNA from UV-induced damage, ascorbate is mostly concentrated by this tissue. Ascorbate controls the sol-gel equilibrium of mucopolysaccharides in the trabecular meshwork, acts as an antioxidant, and partly absorbs ultraviolet light. The ciliary body and retina create lactate, which builds up in the anterior chamber. There is a much larger concentration of lactate in the anterior chamber compared to plasma. Hydrogen peroxide is another component of aqueous humour that is produced when trace metals combine with ascorbic acid. Researchers have shown that when human trabecular cells are exposed to 1 mM hydrogen peroxide, their adhesiveness to fibronectin, laminin, and collagen types I and IV is diminished [37].

A key histopathologic alteration in glaucoma is cell loss, which may occur when trabecular meshwork cells adhere less strongly to one another as a consequence of repeated oxidative stress in vivo. There is a little decrease in glucose concentration from plasma to aqueous humour. Glucose permeates both the cornea and the surrounding water. According to [38], its concentration in the corneal endothelium is half of what it is in water.

Aqueous humour contains oxygen and the transforming growth factor β2, which might be involved in the development of glaucoma. The immune deviation associated with the anterior chamber is maintained in part by the intrinsic action of TGF β2. The release of aqueous humour is affected by many physiological systems, including the circulatory, endocrine, and central neurological systems, as well as changes in metabolic activity. Nighttime sees a 50% drop in aqueous output, which follows a diurnal pattern [39].

Circulating cathecolamines, adrenaline, and norepinephrine, as well as the subject's activities, impact the aqueous diurnal cycle. There is currently little evidence that ciliary epithelial cell loss is age-dependent, yet aqueous output does decrease somewhat with age. Reduced production of aqueous humour due to hypothermia is indicative of metabolic pathways that are essential for active secretion becoming inactive. Aqueous formation's ultrafiltration component is pressure sensitive; as intraocular pressure (IOP) rises, it becomes less effective. Pseudofacility is the scientific name for this measurable phenomena. Reducing plasma osmolality also decreases uveitis, particularly iridocyclitis, and aqueous formation. Aqueous secretion and intraocular pressure (IOP) are both affected by hormones, and many pharmaceuticals work by lowering IOP. These medications, which include βadrenoceptor antagonists like timolol and betaxolol among others, carbonic anhydrase inhibitors, and α2 -adrenoceptor agonists like brimonidine, are often used to treat glaucoma. There may be a correlation between a thinner cornea and reduced uveo-scleral outflow and aqueous production [37].

Vascular lining of the blood

Despite having a high concentration in plasma, large molecules like protein are only found in trace amounts in water. An average human plasma total protein level is 6 g/100 mL, but the amount of protein in aqueous humour is less than 20 mg/100 mL, or less than 0.5 % of the quantity in plasma. Reason being, there is a blood-aqueous barrier, which is an epithelial barrier made up of the endothelium of the iridial arteries, the nonpigmented ciliary epithelium, and the posterior iridial epithelium. The "leaky" sort of tight connections are present in both of these compounds. Climatic epithelial tight junctions may contain fewer sealing strands than less tight, slightly more "leaky" epithelia, which would explain their low transepithelial resistance [30].That being said, the blood-aqueous barrier is not impenetrable. A molecule's capacity to cross the barrier and enter the posterior chamber is directly proportional to its lipid solubility. Although they move more slowly than across capillary walls, chemicals including urea, creatinine, and some carbohydrates are able to traverse the blood-aqueous barrier. Many things may damage the blood-aqueous barrier, including corneal abrasions, inflammation of the uvea, infections inside the eye, paracentesis, surgery within the eye, and topical medications (anticholinesterase medicines). The end product is a highly concentrated form of water known as secondary aqueous, which contains much more protein. Pharmacological agents that lower intraocular pressure (IOP) include mannitol, which has a low affinity for the blood-water barrier. A decrease in intraocular pressure (IOP) results from their accumulation in the body's extracellular spaces, where they generate a high osmotic pressure that sucks water out of cells and ocular fluids [40].

The release of aqueous humour

The ciliary body's output fluid travels from the posterior chamber to the anterior chamber via the pupil, the trabecular meshwork, Schlemm's canal, and aqueous veins. A change in pressure guides the flow of the fluid. A normal intraocular pressure (IOP) is 15 mm Hg, but in subconjunctival (SC) and aqueous veins (AV) pressures decrease to 9 mm Hg and 7-8 mm Hg, respectively [41].

The trabecular meshwork and Schlemm's canal are involved in mechanosensing, which is the process by which cells convert mechanical changes into biological signals. Though there is no hard evidence of baroreceptor activity in the eye, there is some evidence for a "ocular baroreflex" due to the fact that intraocular pressure (IOP) is closely controlled throughout life and that eyes that are stretched or have increased fluid flow revert to their initial IOP values. the contact between the juxtacanalicular portion of the trabecular meshwork and Schlemm's canal seems to be the most probable location for this baroreceptor activity in the traditional outflow channel.

In glaucoma, the outflow resistance is thought to be located in these cells and the extracellular matrix. In contrast to one another, the inner wall of Schlemm's canal serves two purposes. To begin, it has to be permeable from the base to the top so that water may enter the canal. Second, the ciliary epithelium, posterior iris epithelium, iris vascular endothelium, and Schlemm's canal are all components of the blood aqueous barrier [42].

When the raised episcleral venous pressure surpasses the intraocular pressure (IOP), blood products are unable to enter the eye due to the barrier that forms at the tight connections of the endothelial cells that line the inner wall of the canal. No one really knows what controls the blood-water barrier at the Schlemm's canal level. In reaction to external forces, the endothelium lining Schlemm's canal contracts and enlarges in size. As a result of the increased pressure, the canalicular endothelial cells produce more large vacuoles and enlarged pores. Like the cells in big arteries, those in Schlemm's canal are subject to shear stress and respond by lining up with the flow. It seems that the outflow system's pressure is affected by pulse, blinking, and head movement. This suggests that there could be systems in place to react quickly or slowly to changes in pressure. The permeability of Schlemm's canal and trabecular meshwork to pressure, shear stress, and fluid flow may be affected by cell and tissue stiffness. Changing the substrate stiffness causes trabecular meshwork cells to undergo changes in cytoskeleton, shape, protein, and gene expression [24].

In primitive open angle glaucoma, the stiffness of the trabecular meshwork rises with regional heterogeneity, as is well documented. Evidence suggests that pharmacological therapies may alter the rigidity of Schlemm's canal as well. There would be more resistance to outflow if the medication therapy made Schlemm's canal stiffer. Cellular relaxation in response to pharmacological therapy reduced resistance. The development of vacuoles and pores in Schlemm's canal cells is affected by stiffness and is reliant on pressure. Glaucoma tissue has a decreased number of holes. Outflow

resistance may be more pronounced in older patients or those with primitive open angle glaucoma due to stiffer trabecular and canal walls, which may decrease baroreceptor activity and pore development. As a result, latrunculins and Rho Kinase inhibitors are two examples of medications that may lessen cell stiffness and outflow resistance by altering the cytoskeleton in some way. Novel treatment possibilities for primitive open angle glaucoma may be revealed by elucidating the mechanosensing activity of the traditional outflow channel. When tested on live cynomolgus monkeys, latrunculins A and B consistently decreased intraocular pressure (IOP) and increased facility. Latrunculins' ability to actively disrupt the actin cytoskeleton in the trabecular meshwork suggests they might be effective in the treatment of glaucoma. A possible safety concern is the impact on the endothelium or ciliary epithelium of the cornea, which may lead to pseudoguttata or an increase in central corneal thickness [32].

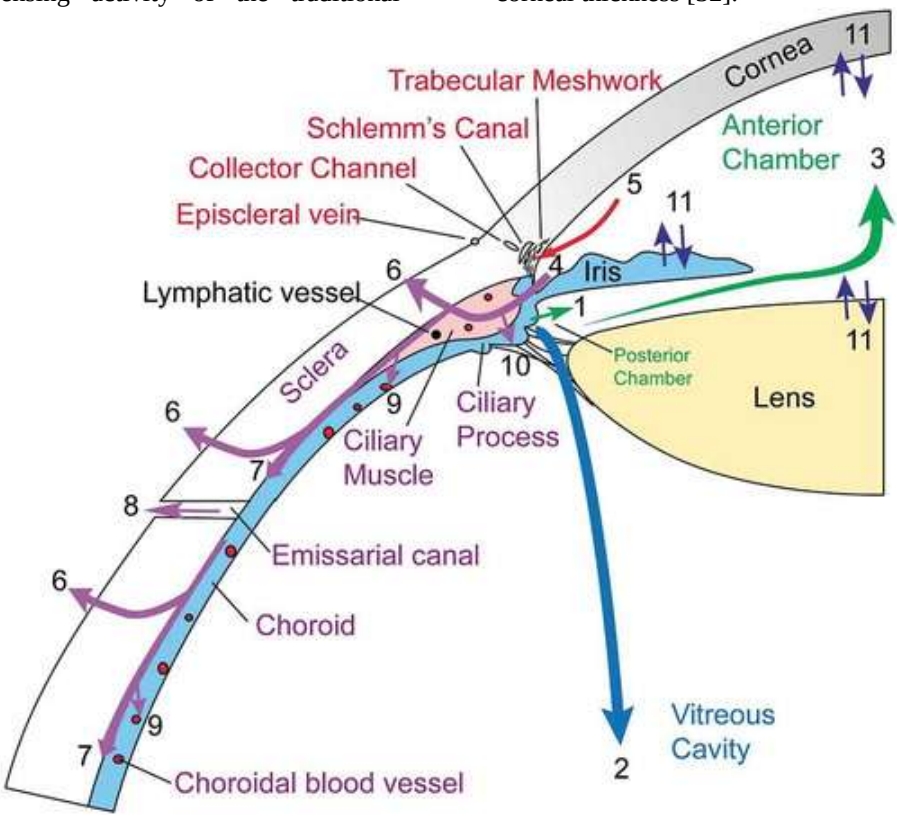

Fig. (3) Outline of the generation and drainage of aqueous humour **[43]**

Tear Film Restored

The epithelium lining the surface of the eye meets the outside world via the tear film. Water, electrolytes, mucins, and a variety of proteins and lipids make up the precorneal tear's very complex makeup, despite its estimated thickness of 3 microns. Using liquid chromatography-mass spectroscopy (LC-MS), researchers were able to identify more than 1500 proteins in human tear fluid. Tear film structure is still being determined, but what we know so far points to a secretory mucus layer—a layer of hydrated mucus—covered in lipid that glides over the glycocalyx on the surface epithelium **(Figure 4) [44].**

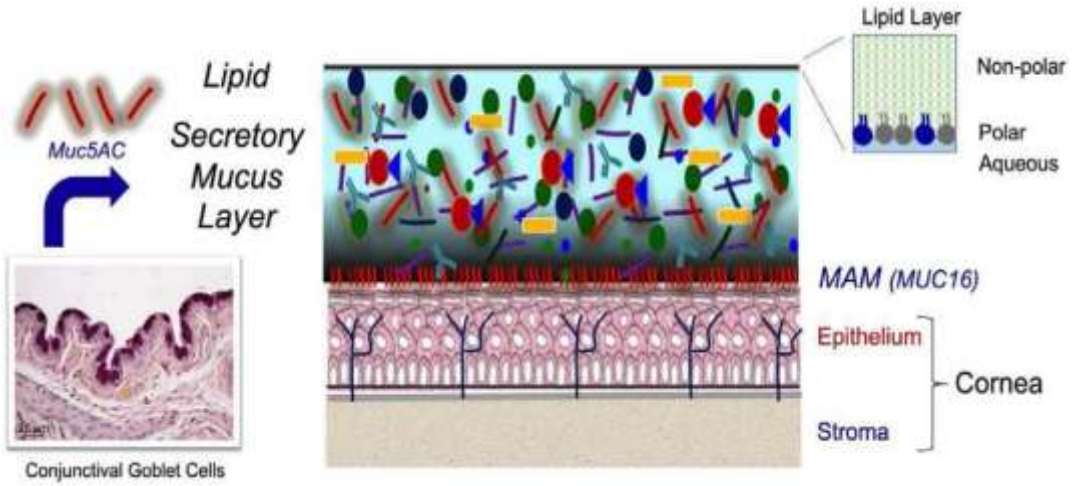

Fig. (4) Tear film structure [44].

❖ **Regulation of Tear production**

The The typical tear film is able to perform its essential duties due to its well regulated composition of ions, proteins, and lipids. The eye's principal optical surface is perhaps its most crucial function. As it moves over the surface of the eye while blinking, the tear film provides comfort by acting as a lubricant and reducing shear pressures from the edge of the lid. Tear production decreases and tear film composition changes in DED can raise shear force levels, which can cause corneal epithelial disease, pain, nociceptor stimulation, and epitheliopathy of the lid marginal conjunctiva, which wipes the ocular surface when blinking (called lid wiper epitheliopathy). The typical tear film also shields the surface epithelium of the eye from the environmental stresses that it experiences every day. Microbes, contaminants, allergies, and unfavourable climatic factors including low humidity and fast air movement caused by wind or HVAC systems are all part of the problem. Aqueous secretion of antimicrobials (such as IgA, lactoferrin, lysozyme, and defensins) and hydrating glycoproteins (such as lactoferrin) is responsible for this. The surface epithelial tissues of the eye are provided with a trophic environment by the tear film. The epithelium's integrity and secretory function are crucial for it to continue acting as an intrinsic barrier and "seal" over the vast network of free nerve terminals that are epithelial [45].

In order to keep the surface of the eye in a constant, homeostatic state, the lacrimal functional unit (LFU) controls the ocular surface's tear production, distribution, and clearing. The anatomical components of the lacrimal gland unit (LFU) comprise the glands responsible for secreting tears (primary and auxiliary glands lacrimal glands, Meibomian glands, conjunctival goblet cells), the epithelium lining the surface of the eye, the eyelids, the drainage system for tears, the immunological system involving glands and mucosa, and the innervation that connects these systems. According to [46], the LFU's neural component is a reflexive loop that begins at the highly innervated cornea and sends afferent traffic to various parts of the central nervous system, such as the brainstem and cerebral cortex.

Blinking and secretory tear production are controlled by efferent neurones that originate in the brain's affective centres and secretory and motor systems. A normal homeostatic tear composition is maintained by strictly controlling the secretion of the major components of the tear film. This is shown by the fact that the efferent routes end inside the main and auxiliary lacrimal glands, conjunctival goblet cells, and the meibomian glands. Carlos Belmonte and colleagues mapped out the many kinds of ocular surface nociceptors and found, among other things, that proper tear production is driven by the TRPM8 "cold receptor"—a protein that is activated when the corneal surface is cooled in the time between blinks [47].

Continued reliability.

Preserving comfort and quality of vision requires constant attention to tear stability. The main tear components, which are detailed below, must interact dynamically in order for tears to remain stable. A significant objective of treatment is to maintain stability of the tear film, which is an unstable film that is a characteristic of tear dysfunction or deficit [48].

In tears, mucin glycoproteins and water work together to keep the hydrophobic surface epithelial cell membranes hydrated and wettable, to act as a matrix for components released by the lacrimal gland, and to reduce blinking-induced friction. One of the main

components of the glycocalyx is membrane associated mucins (MAM), which are produced by surface epithelial cells on the cornea and conjunctiva. These cells include MUC1, MUC4, and MUC16 [49].

Mucin granules on human conjunctival goblet cells may help evacuate gel-forming mucin, and MUC16 is expressed not only on the apical corneal and conjunctival epithelia but also on these cells. A subset of goblet cells also expresses MUC5B, whereas the majority of cells express MUC2—genes that code for a mucin that forms a gel. A little amount of mucins linked with the surface epithelium and the gel-forming mucin MUC5AC make up tear mucus [50]..An contact between the tear film's aqueous layer and the air is provided by the surface lipid layer, which is mostly produced by the Meibomian glands. At the air-tear film lipid interface, there is a larger layer of non-polar lipids, and on the other side, there is a thin layer of polar lipids that interact with the secretory mucus layer below. As stated by [65], the lipid layer serves many purposes, including providing a smooth visual surface, lowering the surface tension of the tear film, preventing the anterior migration of aqueous tears onto the lid border, and delaying evaporation.

When we blink, the lipid layer compresses towards the bottom of the eyelid and then extends upward when we open our eyes. Tear instability is a symptom of Meibomian gland dysfunction, which is characterised by altered spreading and localised weakening of the lipid layer. It has been suggested that regions with lipid thinning may experience increased tear evaporation and osmolarity, which might further destabilise the tears [45].

Based on models, the osmolarity of tears in tear-breakup regions might be as high as 800-900 mOsM, which is much higher than the usual range of 290-340 mOsm and the DED range of 305-360 mOsm recorded in the inferior tear meniscus. The corneal nociceptors' pain-inducing tear osmolarity threshold is about 450 mOsm, and applying hypertonic solutions topically in the 800-900 mOsm range caused discomfort comparable to that experienced during tear breakup. The pain felt during tear splitting may be due to the localised increase in osmolarity, according to these studies [51].

Display presentation

The tear film plays an essential role in the eye's optical system. About 80% of the eye's refractive power is comprised of tears and the front surface of the cornea. [47] point out that the tear film is functionally important for preserving good quality vision because it prevents the cornea from becoming less smooth, reduces contrast sensitivity, and increases optical aberrations that diminish the quality of retinal images.

Reflections from the central cornea/tear film are more irregular in DED, according to studies employing the topographic surface regularity index (SRI) created by Wilson and Klyce. Additionally, DED was shown to have a greater time-dependent rise in SRI from 0 to 10 seconds after a blink. Optical distortions and optical scattering are both made worse by DED. When the tear film breaks apart, variations in thickness cause higher-order optical aberrations to become more pronounced. Reduced low contrast and functional visual acuity in DED may be caused by these alterations in optical characteristics. According to [52], these changes may lead to symptoms including visual fatigue and photophobia, as well as an increase in the blink rate.

Aqueous Humour and Tear Film Biomarkers

Glaucoma, dry eye syndrome, and other eye ailments and diabetic retinopathy, have posed serious risks to the well-being and vision of humans. In order to stop the evolution of eye illnesses and their negative clinical effects, early diagnosis is key, as is constant monitoring of the relevant biomarkers. Nevertheless, traditional methods for identifying and treating eye disorders are not only unpleasant and tedious, but they also need specialised medical facilities, severely limiting their practical use. Even worse, certain eye disorders are hard to differentiate, and physicians need a lot of skill to diagnose them. A new approach is desperately needed that is both more accessible and more accurate [53].

A potential new method for the detection and tracking of ocular and some systemic disorders has arisen in recent years: biomarker sensing based on tear fluid. The lacrimal gland secretes tears as a protective fluid; these tears include many indicators, including as proteins, lipids, electrolytes, and metabolites. When it comes to clinical information, these indicators may tell you a lot, and not only about your eyes. For example, according to [54], tears might be used as a

glucose monitor for diabetes since their fluctuations are similar to those in blood.

Wearable eye contact devices have emerged as a promising new field of medical technology in recent years, allowing for the real-time reflection of biomarker information via the measurement and monitoring of physiological parameters or biomarker levels. Thanks to its non-invasive nature and capacity to continuously monitor tear biomarkers, wearable contact lens sensors have recently emerged as a new kind of point-of-care testing (POCT). Contact lenses enable patients to gather real-time data without disrupting their everyday lives, unlike traditional tear sample techniques like capillary tubes and test strips. Because of all the benefits listed above, they are perfect instruments for accurately monitoring eye illnesses over the long term [55].

Intraocular pressure (IOP) monitoring was the original goal of the revolutionary idea of contact lens biosensors, which was first presented in 1967. Greene et al., who developed the first noninvasive method for monitoring intraocular pressure (IOP), were the driving forces behind this groundbreaking concept. Their groundbreaking idea included creating a soft contact lens with a strain gauge cleverly built in, so that intraocular pressure (IOP) could be monitored invisibly and without discomfort. A lot has changed in this industry since then. Researchers analyse a variety of tear biomarkers, including glucose, electrolytes, and cytokines, by using colorimetric, optical, and electrochemical detection techniques. New opportunities for contact lens biosensors in illness diagnosis and treatment have arisen as a result of developments in flexible materials and sensor technology. This study seeks to provide a thorough synopsis of tear-based biomarkers pertinent to the detection of ocular disorders and to emphasise new developments in contact lens biosensors for POCT applications, considering the significant risk of ocular diseases [56].

Research into biomarker finding has recently focused on biofluids such tear fluid and aqueous humour (AH). Biomarkers have the ability to predict, diagnose, and evaluate treatment responses; yet, there is a great deal of inter-individual heterogeneity in these markers, according to advances in high-throughput proteomics studies. 'Personalised' medicine has emerged as a response to this diversity; it seeks to optimise treatment and prevention of illness in each individual rather than focussing on results at the population level [57].

A number of ocular disease conditions, including pseudoexfoliation syndrome, uveal melanoma, age-related macular degeneration (AMD), diabetic retinopathy (DR), and primary open angle glaucoma (POAG), have been studied in the proteome of AH. Not long ago, our group used state-of-the-art mass spectrometry equipment and data analytic techniques to sensitivityly identify the constitutive proteome of human AH. According to [58], AH has shown potential in these trials to be a valuable tool in the diagnosis and treatment of eye problems.

Tear fluid collection offers a non-invasive alternative to invasive anterior chamber AH collection and has shown promise as a source of biomarkers with translational potential to clinical practice. Research on the tear fluid proteome has included both healthy individuals and those with various eye illnesses, such as dry eye disease (DED), glaucoma, age-related macular degeneration (AMD), and others. The employment of different sampling strategies and analytical technology in these investigations makes it very challenging to draw meaningful comparisons between them. The goal of the collection, the required sample size, and the simplicity of collection all play a role in determining the best technique for collecting tears. Microcapillary tubes and Schirmer strips are the two most used ways to collect tears. Factors such as research or diagnostic needs, patient comfort, and the required tear volume or quality for analysis determine the choice of procedure, which has both benefits and drawbacks [59].

The second interleukin:

A tiny cytokine having pleiotropic effects on the immune system, IL-2 has a molecular weight of 15 kDa. The trimeric IL-2 receptor (IL-2R), which is mostly expressed on immunosuppressive regulatory T (Treg) cells, is the preferred binding site for low amounts of IL-2. This receptor is composed of IL-2Rα (CD25), IL-2Rβ (CD122), and the common gamma chain (CD132). According to [60], trimeric IL-2Rs have an affinity for IL-2 that is about 10-100 times more than that of dimeric IL-2Rs. This makes them known as high-affinity IL-2Rs.

Dimeric IL-2Rs, mostly found on resting antigen-experienced (memory) cells, are stimulated by IL-2 after the limited levels of trimeric IL-2Rs on these cells have been used up. the natural killer (NK) cells and effector T (Teff) cells.Experiments with high-dose IL-2 were driven by its stimulatory effect on Teff and NK cells; in 1992, recombinant human IL-2 (Aldesleukin) was approved as the

first immunotherapy by the US Food and Drug Administration for the treatment of metastatic renal cell carcinoma (RCC), and in 1998, for metastatic melanoma. Nevertheless, the effectiveness of high-dose IL-2 in cancer was hindered by its stimulatory effects on Treg cells, which reduce immune responses to self-antigens, including specific tumour antigens. Additionally, the substantial adverse effects of IL-2 at high dosages, caused by vascular leak syndrome, further reduced its efficiency. Furthermore, IL-2 has a very short in vivo half-life—minutes—so it has to be administered often [61].

Consequently, complete responses (CR) on high-dose IL-2 monotherapy were only attained by 9.3% of patients with RCC and 4.0% with metastatic melanoma. In response to these limitations of high-dose IL-2 therapy, IL-2-based biologic medicines with enhanced selectivity for effector immune cell subsets, decreased toxicity, and extended half-life have been developed. Additionally, the pharmaceutical industry's interest in IL-2 immunotherapy was revived with the development of immune checkpoint inhibitors, such as mAbs directed against programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). This is where the synergistic effects of IL-2 immunotherapy and checkpoint inhibitors come into play. In cancers that are not immunogenic or have a low immune cell infiltration, checkpoint inhibitors are ineffective. However, IL-2 has the potential to make these tumours immunogenic by indirect stimulation of dendritic cell infiltration, making them more susceptible to treatment with checkpoint inhibitors.According to [62], combining techniques that target both axes might be very appealing.

The fourth interleukin

The pleiotropic cytokine IL-4, which was first identified in the mid-1980s and has since served several purposes, is still the subject of intense study. While activated T cells are the primary producers of this mediator, basophils, eosinophils, and mast cells all contribute. Sharing sequence homology, cell surface receptors, intracellular signalling, and partial functional effects on cells with IL-13, IL-4 is a typical cytokine structurally, with a molecular weight ranging from 12 to 20 kDa due to varied natural glycosylation. According to [63], IL-4 is an important cytokine that controls cell growth, death, and gene expression in a wide range of cell types, including lymphocytes, macrophages, fibroblasts, endothelial cells, and lymphocytes.

Even without taking into account important relevant aspects, such as recent advances concerning functionally related Th2 mediators like thymic stromal lymphopoietin, IL-33, IL-21, or IL-25, it is extremely difficult to present a systematic review of this pivotal regulator in a journal article given the already staggering and still rapidly growing volume of information available about IL-4. With the growth of IL-4 research, it is now clear that the simplistic view of IL-4 function drawn in the '80s and '90s requires updating to account for the many complexities surrounding IL-4 production, receptor use, and cellular impacts. In particular, non-linear situations have been shown to abound in the realm of IL-4 biology, as opposed to linear ones. New information on other alternative branchings linked to IL-4 has come to light, in addition to the well-known and now textbook-described role of IL-4 in deciding the various fates of Th cells, namely in supporting Th2 and blocking Th1 and Th17 development.

When first reported, vascular endothelial growth factor (VEGF) was a mitogen that targeted just endothelial cells. Its other name is vascular permeability factor (VPF). Kidney mesangial cells, tumour cells, platelets, keratinocytes, and macrophages are among the several cell types that make VEFG. Normal physiological processes involving VEFG include development, bone formation, haematopoiesis, wound healing, and vascular system activation [64].

To combat cancer, anti-VEFG techniques were developed to block neovascularisation by targeting VEFG's pro-angiogenic activity. Because more and more data points to the presence of paracrine and autocrine VEFG loops inside tumours, anti-VEFG treatments may have a double impact. Some research suggests that VEFG may shield tumour cells from apoptosis and make them more resistant to traditional radiation and chemotherapy if applied directly to the cells. Evidence suggests that VEFG levels inside tumours rise in response to chemotherapy and radiation, and that these elevated levels may actually shield tumour cells from the harmful effects of these treatments. Therefore, it is probable that anti-VEFG treatments will aim at both VEFG's pro-angiogenic and anti-apoptotic/pro-survival properties [64].

Final thoughts:

Biomarkers have attracted a lot of attention from scientists and clinicians looking for solutions to these problems, and there are a lot of substances that might be used as biomarkers. We categorise biomarkers as either non-invasive, slightly invasive, or invasive based on the method used to get fluid samples, and we summarise what is currently known about the most robust molecular biomarkers suggested in POAG. Many obstacles and restrictions, largely arising from structural and scientific considerations, characterise the transition of biomarkers from discovery to clinical practice in glaucoma and other areas of ophthalmology and medicine. Most importantly, there is a lack of biomarker characterisation and validation methodologies, insufficiently rigorous analytic methods used in clinical trials, and a failure to make diverse decisions before beginning the discovery phase.

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