Metaboloma use in ophthalmology

Uso de metaboloma em oftalmologia: uma revisão narrativa

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ABSTRACT

It is part of the omic sciences to search for an understanding of how the cellular system of organisms works as well as studying their biological changes. As part of the omic sciences, we can highlight the genomics whose function is the study of genes, the transcriptomics that studies the changes in the transcripts, the proteomics responsible for understanding the changes that occur in proteins, and the metabolomics that studies all the metabolic changes that occur in a certain system when it is submitted to different types of stimuli. Metabolomics is the science that studies the endogenous and exogenous metabolites in biological systems, which aims to provide comparative quantitative or semi-quantitative information about all metabolites in the system. This review aims to describe the main applications of metabolomics science in ophthalmology. We searched the literature on main applications of metabolomics science in ophthalmology, using the MEDLINE and LILACS databases, with the keywords "metabolomics" and "ophthalmology", from January 1, 2009, to April 5, 2021. We retrieved 216 references, of which 58 were considered eligible for intensive review and critical analysis. The study of the metabolome allows a better understanding of the metabolism of ocular tissues. The results are important to aid diagnosis and as predictors of the progression of many eye and systemic diseases.

RESUMO

Faz parte das ciências ômicas buscar entender como funciona o sistema celular dos organismos e estudar suas alterações biológicas. Como parte das ciências ômicas, destacam-se a genômica, cuja função é o estudo dos genes; a transcriptômica, que estuda as mudanças nos transcritos; a proteômica, responsável por entender as mudanças que ocorrem nas proteínas, e a metabolômica, que estuda todo o metabolismo das alterações que ocorrem em um determinado sistema quando ele é submetido a diferentes tipos de estímulos. A metabolômica é a ciência que estuda os metabólitos endógenos e exógenos em sistemas biológicos, visando fornecer informações comparativas quantitativas ou semiquantitativas sobre todos os metabólitos do sistema. Esta revisão teve como objetivo descrever as principais aplicações da ciência metabolômica na oftalmologia. Trata-se de revisão narrativa desenvolvida por um grupo de pesquisa da Universidade Federal de São Paulo, em São Paulo (SP). Buscaram-se, na literatura, as principais aplicações da ciência metabolômica em oftalmologia, utilizando as bases de dados Medline® e Lilacs, com as palavras-chave "*metabolomics*" e "oftalmologia", de 1º de janeiro de 2009 a 5 de abril de 2021. Foram recuperadas 216 referências, das quais 58 foram consideradas elegíveis para revisão intensiva e análise crítica. O estudo do metaboloma permite um melhor entendimento do metabolismo dos tecidos oculares. Os resultados são importantes para auxiliar no diagnóstico e como preditores da progressão de muitas doenças oculares e sistêmicas.

INTRODUCTION

We usually define an individual's phenotype as the expression of their genotype associated with changes in their environment. Phenotypes could be exemplified as the aspects of the morphology, physiology, biochemical properties, behavior, and relations with the environment of a certain organism. It is necessary to keep in mind that the phenotypes are dynamic, changing throughout the life of a given organism.[1]

Consequently, the phenotype of a disease can be defined by the interaction between its genotype as the environment in which it is inserted, as well as its life habits. Metabolomics is a science that shows itself as a paradigm break, due to the way it has been approaching different forms of treatment. Metabolites can express the biochemical phenotype of the individual, which allows medical professionals and nutritionists to establish with greater precision how the disease is developing at a given time. While the data from the study of gene expression and proteomic analyses provide information on cellular events, the metabolic profile can provide details about the physiology of a disease at the molecular level.[1]

The metabolome studies endogenous and exogenous metabolites in biological systems. Changes in the metabolome may more accurately reflect the current state of a disease. It is important to note that each organ in the body has its metabolome, however, they are all connected. The metabolome is closer to the molecular phenotype than the genome, transcriptome, or even the proteome, and since it can, for example, be used to detect physiological changes caused by the administration of some medication, the study of metabolites can lead to better forecasting of the resulting phenotype than other omic approaches. Metabolomic science in recent years has been applied in different fields of study such as cancer, chronic kidney disease, and heart disease, in addition to other areas such as clinical analysis, food, and nutrition, sports, environmental, forensic toxicology, or analysis of pathological organisms. Compared to genomics and proteomics, metabolomics is a relatively recent field, but it is becoming an increasingly important tool in medicine.[1]

The study of aspects of the individual's health by analyzing biological fluids began in China, where researchers used ants to assess the presence of glucose in the urine of diabetic patients.[1] The techniques for creating metabolic profiles, currently called metabolomics, were developed in the late 1960s. Linus Pauling and his collaborators proposed the hypothesis that, through the chromatographic profile of the constituents of a body fluid, it was possible to predict or diagnose the onset of the disease and / or make refinements in nutrition to obtain optimal health.[1-3] The study of the metabolome complements the study of gene expression since the levels of RNA transcription do not necessarily reflect the activity of the expressed proteins, since they may not perform their functions and remain inactive. Thus, changes in the transcriptome and proteome may not correspond to phenotypic changes in an individual. In contrast, the study of the metabolome can analyze the phenotype in response to environmental or genetic changes and an individual.[1-3]

Although genomic and proteomic studies can provide information about the pathophysiology of a disease, these analyses may show only a limited correlation with the phenotype. On the other hand, the study of metabolites can provide more accurate information about the phenotype of a given system.[1-3]

Metabolites are classified as intermediate products of metabolic reactions, reactions that are catalyzed by a variety of enzymes. They are generally defined as any molecule smaller than 1kDa, which is a product or intermediate of metabolism. They can be classified as endogenous or exogenous. Those that are products of drug degradation are called xenometabolites.[4]

Studies in the metabolome area may include animal models, in vitro tissue culture and clinical epidemiological studies to investigate the pathogenesis of diseases, biomarkers, drug efficacy, and toxicity. Longitudinal studies can be carried out where multiple collection points are performed on the same research subjects.[4]

Metaboma studies are generally not performed using biological fluids such as blood, urine, tear, cerebrospinal fluid (CSF), as well as solid tissues and cells. The ophthalmologic metabolomic study usually involves the use of tissues or fluids, among them we can mention corneas, crystalline, retina, vitreous, and aqueous humor. As the metabolites exhibit a high diversity of chemical composition, ranging from sugars to lipids, it is very difficult to track all metabolites using a single extraction technique, so methanol and formaldehyde extraction methods can be adapted to screen most existing metabolites in the sample.[4]

Currently, there are several different databases used in the study of the metabolome. Among the databases used in magnetic resonance (MR) spectroscopy, we can mention the Human Metabolome Database (HMDB; www.hmdb.ca), Metlin (metlin.scripps.edu) and Biological Magnetic Resonance Database (BMRB; www.bmrb.wisc.edu/metabolomics).

Databases commonly used for gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS) include the US National Institute of Science and Technology Database (NIST; (https://data.nist.gov/sdp) the Golm Metabolite Database (GMD; http://gmd.mpimp-golm.mpg.de/ MassBank (http://www.massbank.jp), Metlin, the Madison Metabolomics Consortium database (MMCD; https://www.g6g-softwaredirectory.com/bio/metabolomics/dbs-kbs/20670-Univ-Madison-WI-MMCD.php, and the MetaboAnalyst (https://www.metaboanalyst.ca/).

The Metlin database, developed in 2005 for the study of human metabolites, continued until 2011, when it included more than 40 thousand registered metabolites, being the largest database of mass spectrometry (MS) in the area of metabolomics.[5] Although the metabolome databases are expanding each year, there are still significant numbers of unidentified metabolites in biological systems. Initiatives were made to create a central reporting database to allow the sharing of methodologies and results between laboratories.

Analytical technologies commonly used in metabolomic analysis include MR spectroscopy and MS. Magnetic resonance spectroscopy works by evaluating the magnetic property of certain atomic nuclei, which can be used to determine the physical and chemical properties of atoms or molecules in which they are contained. Currently, the instruments that use frequencies of 500 and 600MHz are the most widely used because they have the best cost-benefit.[6] Magnetic resonance spectroscopy can be used to analyze liquid, solid, and gas samples. One of its main advantages is the use of small amounts of samples for analysis, in addition to offering a quantitative analysis. Its main disadvantage is related to its lower sensitivity to MS.[7]

Mass spectrometry analysis, on the other hand, allows molecular information obtained in two or three spatial dimensions, thus allowing the determination of the distribution of small molecules within a tissue and virtual tissue dissection. One of the greatest advantages of this technique is to correlate metabolite information with histological data. In general, it has a much higher sensitivity than MR, thus allowing the measurement of a wider range of metabolites.[7]

Direct MS involves the analysis of metabolites without chromatographic separation. However, the analysis of the metabolites can be performed using hyphenated MS techniques, coupled with some separation technique, and the most used are liquid and gas chromatographies, which allows for the separation of the metabolites in different types of chromatographic columns. The great advantage of this type of analysis is the possibility of separating the compounds, their final identification being made not only by molecular mass but associated with their retention time. Mass spectrometry coupled with chromatographic techniques also allow for the quantification of metabolites with that of both internal and external standards.[8] Another advantage of MS and the use of the magnetic resonance (MR) spectroscopy analysis method is to select a specific ion for each metabolite, making its identification even more accurate.

The ocular system has its metabolome due to the hematoretinal barrier, creating a barrier for vitreous and aqueous humor. The tear can also be analyzed, showing eye health results. Therefore, it is possible to identify biomarkers for screening, diagnosis, and prognosis of diseases. Animal species may have different results from enzymatic activities. Thus, the results found in animal models should be carefully extrapolated to results in humans.[8]

The study of the metabolome allows for an answer to how biological environments respond to drugs. The studies can be used for drug development, investigating the mechanism of action, and toxicity. In this study, the potential for metabolome application in the diagnosis and monitoring of ophthalmological changes is reviewed.

The objective of the current narrative review was to describe the main applications of metabolomics science in ophthalmology.

We searched the literature on main applications of metabolomics science in ophthalmology, with the keywords "metabolomics" and "ophthalmology", from January 1st, 2009, to April 5, 2021. We used the Medline® database (via PubMed®) and *Literatura Latino-Americana e do Caribe em Ciências da Saúde* (Lilacs), via Virtual Health Library (BVS, acronym in Portuguese), to identify relevant articles. The articles on the potential applications of the metabolome in ophthalmic diseases have been revised. A summary of the selected articles was produced.

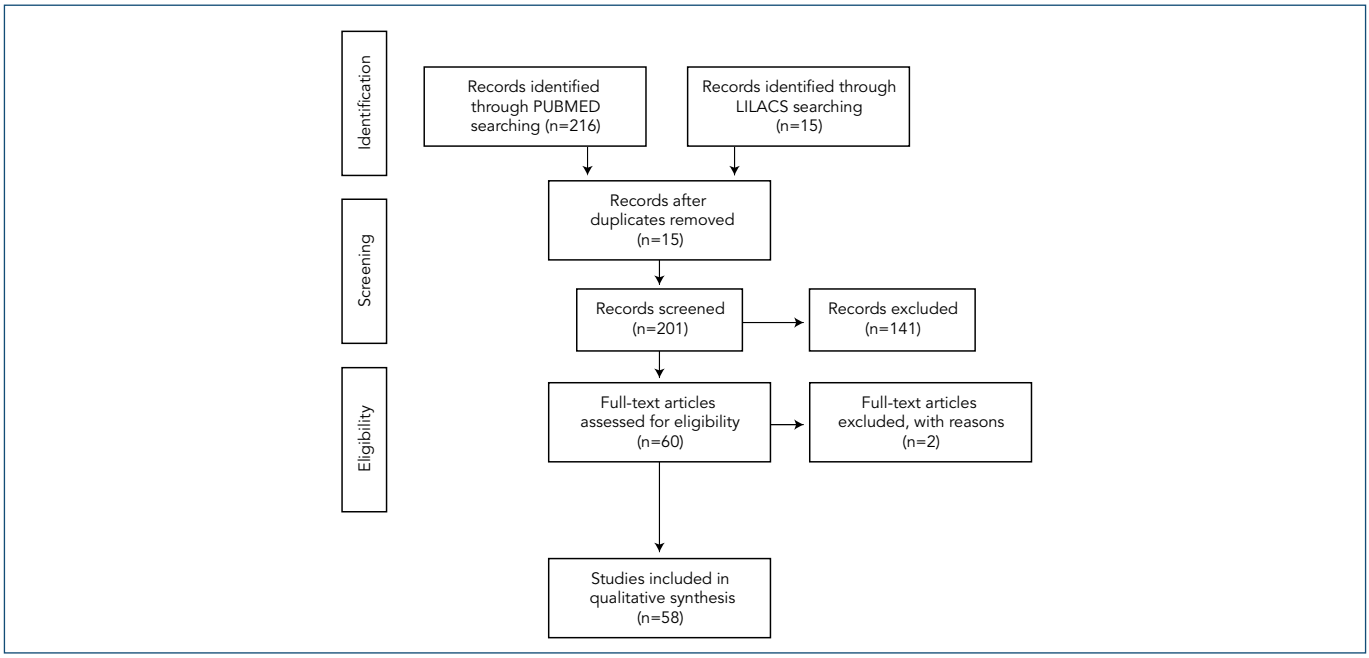
The details of the search strategy are shown in table 1.

Table 1. The details of the search strategy

Database	Search strategies	Papers found
Medline® (via PubMed®)	("ophthalmology") AND ("metabolomics")	216
Lilacs (via Virtual Health Library)	("ophthalmology") AND ("metabolomics")	15

Lilacs: *Literatura Latino-Americana e do Caribe em Ciências da Saúde.*

From the search in the databases, 1 clinical trial, 1 randomized controlled trial and 21 reviews were identified. After screening the titles and abstracts, removing



Lilacs: *Literatura Latino-Americana e do Caribe em Ciências da Saúde.*

Figure 1. Flow diagram of the study selection process.

duplicates and screening the citations, 58 studies were considered eligible for critical analysis. The article selection process is detailed in figure 1.

DEGENERATION OF MACULA

Macular degeneration (AMD) is characterized by macular degenerative changes in the neurosensorial retina and retinal pigment epithelium (RPE), such as drusen, RPE atrophy, usually in patients over 50 years old. There may be neovascularization of the choroid, which characterizes the exudative form. Some studies have already been carried out to evaluate the profile of macular degeneration, using transcriptomes, proteomes, and metabolomas. High levels of proteins from the complement pathways and extracellular matrix were found in different donor tissues of patients with macular degeneration. The study of metabolomics allows for a better understanding of the pathophysiology of macular degeneration, identification of possible new treatments, as well as possible biomarkers in the development of more personalized treatment.[9-11]

A study demonstrated an increase in glycerophospholipids and platelet activation factor in patients with choroidal vasculopathy.[12] Modifications of oxidative proteins were found in the proteomic analysis of drusen, demonstrating the role of oxidative stress in the pathophysiology of macular degeneration.[13] The phospholipid transfer protein (PLTP) and lectin serine protease 1 have been studied as possible plasma biomarkers for AMD.[14]

In addition to proteins, mRNAs are candidates for AMD biomarkers.[15] The combination of genomic information with environmental factors could improve the prediction of susceptibility to AMD. Plasma measurements of complement components combined with genetic factors increase diagnostic accuracy.[16] A study comparing the plasma sample of AMD patients with controls identified an increase in di- and tripeptides, covalently modified amino acids, and a decrease in bile acids and vitamin D in patients with neovascular AMD.[17] Another study compared patients with AMD with a control group over 50 years of age and with no changes in the macula. In the study with 396 participants, 243 were followed up in Coimbra (42 controls and 201 patients with AMD) and the rest of the patients were followed up in Boston (43 controls and 113 patients with AMD). It was possible to observe a difference between patients in both cities. Patients in both countries showed similar variations in the level of histidine, unsaturated fatty acids, and protein levels. Other components that showed differences may represent differences in diet and lifestyle between countries.[18]

Metabolomics study can also assess the pathophysiology of the formation of drusen, which revealed the deposition of hydroxypatite and cholesterol beads in the RPE.[19] Some studies have detected an increase in oxidation products such as malondialdehyde, polyunsaturated fatty acid, and nitric oxide in patients with AMD in dry form.[20]

With aging, there is a reduction in glycolysis in the external retina, which can lead to an interruption in the

metabolic ecosystem of the retina, contributing to the pathophysiology of macular degeneration. The RPE becomes less dependent on glycolysis over time, leaving less glucose for the photoreceptors, leading to changes in the RPE mitochondria.[21] The reduced oxygen level due to the thickening of the Bruch's membrane in patients with AMD affects the glucose and lipid metabolism in the RPE, leading to photoreceptor degeneration, structural changes in the RPE, with reduced lipid oxidation.[22]

Lipofuscin is a pigmented granule containing lipids, which accumulates in various tissues of the human body due to aging and can be found in retinal pigment epithelial (RPE) as a risk factor for the development of AMD. This substance could contribute to the interruption of mitochondrial function and oxidative stress in AMD.[23] In the retina, zinc is necessary for the metabolism of the outer segments of the photoreceptors ingested by the RPE and protects against oxidative stress. For this reason, it has been suggested that zinc deficiency is linked to AMD, with oral supplementation providing a protective effect.[24]

The molecular composition of the drusen demonstrates the presence of apolipoprotein E, amyloid components, and vitronectin. Sub-EPR deposits may also contain hydroxyapatite beads (HAP or Ca5 (PO4) 3OH). These studies suggest metabolic changes associated with mineral drusen formation.[19,25,26]

Iron is another element related to AMD, due to the induction of oxidative stress. Within the RPE, the accumulation of iron contributes to the accumulation of lipofuscin, interfering with the complement system.[27] The accumulation of high concentrations of calcium phosphate in the sub-EPR space is related to the progression of sub-EPR deposits.[28]

Blood plasma samples from AMD patients were compared with control patients for cysteine (Cys), cystine (CySS), glutathione (GSH), isofurans (IsoFs), and F2-isoprostanes (F2-IsoPs), demonstrating an increase in the oxidation of these metabolites as a risk factor for AMD.[29] Luo et al evaluated the plasma metabolomic profile of a Chinese cohort of patients with AMD and healthy controls. In this study, ten metabolites analyzed differed significantly between the two groups, most of which were acidic amino acids, and the most significant finding was an increase in L-phenylalanine in patients with AMD.[30]

One study evaluated the plasma of 60 healthy individuals with AMD and patients diagnosed with polypoid choroidal vasculopathy. Glycerophospholipids, amino acids, di/tripeptides and several carnitine species have been shown to be elevated in patients with AMD and polypoid vasculopathy.[31]

One study evaluated the plasma metabolites of patients with different stages of AMD progression and age-matched controls. Eighty-seven metabolites were identified as different between AMD and controls. Most of the metabolites were members of the lipid pathway (82.8%), followed by amino acids (5.7%).[32]

Therefore, metabolomic investigations can provide important information to distinguish AMD from other diseases. Further studies in this area could identify new biomarkers that could be applied in the future of the treatment of AMD's precision medicine.

RETINITIS PIGMENTOSA

This disease is characterized by a dysfunction, cell loss and even atrophy of the retinal tissue of a hereditary and progressive character. It is a dystrophy that affects the patients' retinal cones. The most frequently found defects are mutations in rhodopsin and other components of the phototransduction cascade. The signaling pathway with insulin/mTOR acts in the synthesis of proteins and ribosomes, which altered lead to a picture of cone autophagy, suggesting that nutrient deficiency may be a fundamental factor in the degeneration of the cones. Insulin depletion led to cell death in experiments carried out with mice. Besides the identification of mutations in a third gene of the pre-mRNA, binding factor may be related to a new mechanism of photoreceptor degeneration. Another factor that may be related to the pathophysiology of this disease is the change in the metabolism of Muller cells, which can be documented with the change in local levels of taurine, glutamate, glutamine, GSH, glutamine synthetase.[33-35]

MACULAR TELANGIECTASIA

It is an idiopathic disorder of the development of the retinal vasculature, characterized by ectasia of retinal capillaries in the region adjacent to the fovea. This disease has been linked to systemic diseases such as diabetes, obesity, and systemic arterial hypertension. Studies in the field of metabolomics have found increased levels of serine, glycine and teronin in the plasma of these patients. The change in Müller cells was observed in a proteomic study of the eye. It revealed a reduction in proteins involved in the glycolytic pathway that occurs in Müller cells and glial cell markers. These changes were validated by Western blotting and immunohistochemical studies. The proteomic analysis of the vitreous revealed specific protein

changes in the retina associated with the pathophysiology of telangiectasis.[36,37]

DIABETES

Diabetic retinopathy is a medical condition in which damage occurs to the retina due to diabetes mellitus. Diabetic retinopathy is the leading cause of blindness among people aged 16 to 64 years old. The risk of visual loss and blindness is reduced with early detection of the disease and rapid access to treatment. Metabolic disorders are related to the pathophysiology of diabetes, so the metabolomic analysis of human samples can be useful to better understand the pathophysiology of this disease. Studies in this area can evaluate possible biomarkers that can be used as therapeutic targets in the diagnosis and treatment of diabetic retinopathy.[38-39]

Hyperglycemia alters cellular metabolic homeostasis. Patients with diabetic retinopathy may experience an increase in lactate in glucose metabolism and an increase in the activity of the enzyme arginine-troponin. A study analyzing samples of vitreous humor from 22 type 1 diabetic patients and 22 controls with a macular hole found that levels of glucose, sorbitol, and mannitol were elevated in the vitreous of patients with proliferative diabetic retinopathy. Galactitol and ascorbic acid levels were significantly lower in the vitreous of patients with proliferative diabetic retinopathy compared to controls.[38,39]

Increase in L-aspartic acid and linoleic acid has been studied to distinguish different stages of diabetic retinopathy. A study using plasma from diabetic patients analyzed 89 patients with type 2 diabetes with or without diabetic retinopathy and 30 non-diabetic patients as controls. The authors identify metabolites as possible biomarkers for diabetic retinopathy including β hydroxybutyric acid, trans-oleic acid, lineoleic acid and arachidonic acid.[40]

Hyperglycemia has been associated with epigenetic changes that may persist due to metabolic memory.[41] Apolipoprotein A1 may be associated with diabetic retinopathy and be present in the vitreous, aqueous humor, and tears.[42] A retinal study of diabetic rats found elevated glucose levels and reduced lactate levels. These data suggest that diabetes causes decreased retinal glycolysis.[43]

One of the limitations of the study of vitreous metabolomics is the volume of the sample, which is usually small (1mL). Thus, studies with plasma may have a higher volume collection. A case-control study conducted in Singapore analyzed the plasma sample of patients with type 2 diabetes, 40 participants with moderate non-proliferative diabetic retinopathy, and 40 without signs of diabetic retinopathy. Patients with diabetic retinopathy had reduced levels of 1.5 anhydroglucitol and increased levels of 1.5 gluconolactone, 2-deoxyribonic acid, 3,4-hydroxybutyric acid, gluconic acid, lactose/cellobiosis, maltose, mannose, ribose, and urea.[44]

RETINAL DETACHMENT

It is the entity characterized by the anatomical separation between the RPE and the neurosensorial retina. The study of the metabolomic profile of the humor vitreous of patients with retinal detachment and other patients with recurrent retinal detachment due to the formation of proliferative vitreoretinopathy (PVR) and control patients, identified inflammation-related metabolites in patients with PVR, such as L-carnitine, which was decreased in patients with retinal detachment compared to patients with retinal detachment and retinal vitreous proliferation. L-carnitine is responsible for inhibiting inflammation. In patients with PVR, an increase in ascorbate and valine was found, which are usually related to fibroblast proliferation. The increase in citrate and d-glucoronalactone was observed in patients with retinal detachment and PVR, suggesting abnormalities in energy metabolism. The study had a small sample (eight patients with retinal detachment, seven with PVR, and six normal eyes), requiring future studies with cohorts to confirm the findings.[45]

GLAUCOMA

Glaucoma is a chronic and slowly progressive optic neuropathy characterized by atrophy of the neural rhyme and associated with characteristic patterns of visual field loss. Studies carrying out a proteomic analysis of aqueous humor and serum found proteins related to inflammation and oxidative stress in patients with primary open-angle glaucoma and exfoliative glaucoma.[46,47]

Recent studies have characterized specific proteomes for tear glaucoma. The proteomic study demonstrated 27 different proteins in the tear of patients with glaucoma compared with controls, 16 of which were associated with the inflammatory response, elimination of free radicals, cell signaling, and cell interaction. Overall, the protein modulation shown in the tears of glaucoma patients is involved with biochemical networks linked to inflammation, such as lysozyme C, lactotransferrin, proline-rich protein 4, prolactin-inducible protein, zinc alpha-2-glycoprotein, the receptor for polymeric immunoglobulin, cystatin S, region C of the kappa Ig chain, region C of the kappa Ig chain, region C of the alpha-2 Ig chain,

immunoglobulin J region C of the alpha alpha-1 chain of the Ig.[48,49]

A reduced glucose level has been shown in eyes with glaucoma compared to healthy eyes. The increase in intraocular pressure could lead to a reduction in the energy supply in the anterior chamber. Glutamate has also been found at high levels in the vitreous in patients with glaucoma. This substance has a toxic potential for retinal ganglion cells.[50] In a study with mice with chronic glaucoma, an increase in sphingolipid, ceramide, ketoacetate, citrate, and several amino acids, including alanine, lysine, and valine, was detected, as well as a decrease in glucose.[51,52]

Thus, some biomarkers for glaucoma have already been suggested after proteomic analysis, but studies with larger cohorts are still required for this confirmation. Studies with biomarkers are very important in the initial evaluation of patients with glaucoma since it is a disease that usually causes symptoms in the patient after irreversible damage to the optic nerve.

CATARACT

A cataract is characterized by an opacity of the lens that, by absorbing or dispersing the light rays, leads to a decrease in visual acuity, both quantitatively and qualitatively. The presence of oxidation of Cys residues has been observed in the lens of patients with cataracts.[53] A study using MR and LC-MS demonstrated a profound difference between the normal lens metabolome and that of patients with cataracts, who have a higher concentration of antioxidants. The dysfunction of the lens epithelium, which synthesizes most of the metabolites, may be responsible for the development of cataracts.[54] Corticosteroid treatment has been related to the depletion of taurine and corneal ascorbate which could explain the accelerated process of cataract formation of patients on topical or systemic steroids.[55]

DRY EYE

Tears are a potential source of biomarkers. Although it is relatively small in the volume of tears analyzed, the technologies made it possible to study proteomics, lipidomics, and metabolomics composition. The tear has been studied to identify several biomarkers of eye diseases, such as dry eye, keratoconus, trachoma and diabetic retinopathy. Most tear analysis studies have focused on the proteome, as the relative amount of protein is greater than the other metabolites.[56]

The tear sample is safer compared to samples of the conjunctiva and aqueous, and vitreous humor. The composition of the tears provides a good reflection of the health of the ocular surface including water, inorganic salts (e.g., Na +, K +, Cl– and Ca2 +), carbohydrates (for example, N-acetylneuramic acid and mucins), lipids (e.g., triglycerides, cholesterol, and monounsaturated fatty acids), proteins (e.g., lysozyme, lactoferrin, lipocalin, and immunoglobulin A), and countless metabolites of all types that can be evaluated. Tear sampling is performed by direct methods (microcapillary tube and micropipette) and indirect methods (Schirmer test strip, filter paper disc, and cellulose sponge).[56]

Compared to the microcapillary tube, the average protein concentration in a tear sample collected by the Schirmer test strip is higher. Once the tear sample is collected, it is essential to assess metabolic activity quickly. Otherwise, the metabolite is renewed (metabolic flow), making the fluid chemically unstable and less representative of in vivo status. The evaluation of ex vivo metabolic activity can be done by an immediate drop in temperature to <0°C, followed by storage at -80°C or enzymatic denaturation by increasing the temperature or by applying organic solvents.[57]

Patients with dry eye have a change in the composition of the tear film, so the analysis of the tear film proteome is important in understanding this disease and in identifying biomarkers. Potential biomarkers would be proline-rich protein 4, prolactin-inducible protein, lipocalin-1, lysozyme, and proteins from the S100 family.[58]

The lipid layer of the tear film is important to maintain the homeostasis of the ocular surface and limit the evaporation of tears, which makes lipidomycin an important study in dry eye research. The study of biomarkers can be an important indicator of disease progression.

Peral et al analyzed the levels of diadenosine polyphosphates, diadenosine tetraphosphate (Ap4A), and diadenosine pentaphosphate (Ap5A) in tears samples from patients with dry eye, both symptomatic and asymptomatic, and from control subjects. The symptomatic group showed higher levels of these dinucleotides, particularly in individuals with low lacrimal secretion compared to the control group. Additionally, there was a significant gender-specific difference in the levels of Ap4A and Ap5A of symptomatic individuals. Regardless of the levels of tear secretion, asymptomatic and symptomatic women had higher concentrations than symptomatic men. Due to the low volume of samples, the analysis was not possible in the group of individuals who had very low tear secretion. The study suggested these two substances, mainly Ap4A, as objective biomarkers in the diagnosis of

dry eye. Diadenosine polyphosphates are naturally occurring compounds, whose mechanism of action is not fully understood. They demonstrated modular action in intraocular pressure and accelerated the healing of wounds in the cornea acting through P2 receptors.[59,60]

Pescosolido et al investigated the presence of carnitine and its derivatives, l-acetylcarnitine, and l-propionylcarnitine, in tears and compared their levels in patients with dry eye and control subjects. The analysis demonstrated the presence of significantly lower concentrations of carnitine, l-acetylcarnitine and l-propionylcarnitine in the tear fluids of patients with dry eye than in controls ($p<0.05$). Therefore, the authors proposed a possible protective effect of carnitine, avoiding the harmful impacts of a hypertonic tear film.[61]

Galbis-Estrada et al examined the tear metabolism of patients with dry eye. Comparing with a group of healthy patients, differences in the levels of cholesterol, N-acetylglucosamine, glutamate, creatine, amino-n-butyrate, choline, acetylcholine, arginine, phosphoethanolamine, glucose and phenylalanine, glucose, phenylalanine and format were observed. The group extended its investigation by prescribing to participants an oral supplement (containing antioxidants and essential polyunsaturated fatty acids) of three capsules a day for three months. The patients analyzed had different baseline profiles of tear metabolism and the authors were able to identify ~50 metabolites of cholesterol, N-acetylglucosamine, glutamate, amino-n-butyrate, choline, glucose, and shape before supplementation and choline/acetylcholine after supplementation. The authors identified that the metabolic profile of patients' tears can be modified with adequate oral supplementation containing antioxidants and essential fatty acids.[62,63]

Pieragostino et al developed a study to measure steroid levels in tear samples. The identification of androgen, estrogen, progesterone, and prolactin receptors in various ocular tissues provided further evidence of the involvement of sex hormones in the pathophysiology of dry eye. They simultaneously quantified cortisol, corticosterone, 11-deoxycortisol, 4-androstene-3,17-dione, testosterone, 17α-hydroxyprogesterone, and progesterone in patients with dry eye. Concerning the tear film, the normal function of the tear gland and its structural organization has been associated with androgen levels.[64]

Chen et al evaluated the tear metabolism profile of patients with dry eye compared to healthy patients. They identified a total of 156 metabolites, of which 32 were significantly altered ($p<0.05$) in patients with dry eye. These specific metabolites for dry eye belonged to eight super classes including benzenoids; hydrocarbons; lipids and lipid-like molecules; nucleosides, nucleotides and the like; organic acids and derivatives; organic nitrogenous compounds; and phenylpropanoids and polyketides. Study findings suggested potential biomarkers for dry eye and revealed that metabolic processes related to glycolysis/gluconeogenesis, amino acid metabolism, complement, and coagulation cascades were involved in the pathophysiology of this disease.[65]

Androgen levels have been shown to influence the formation of the tear film. Reduced androgen levels are related to reduced tear volume, hyperosmolarity, and reduced tear film stability.[66] Metabolomic analyses of human conjunctiva epithelial cells in response to hyperosmotic stress have shown that levels of 21 metabolites have changed significantly. Glycerophosphocholine increased more under hyperosmotic stress, and the authors concluded that this metabolite could act as an important osmoprotector.[67] Androgens and possibly epiandrosterone can act as a biomarker for dry eye.[68]

EYE BURN

Burns are usually related to accidents at work or domestic accidents. Chemical burns can be caused by acids or alkalis, being the most common bases and causing more serious injuries. Ocular changes are proportional to toxicity, concentration, volume, and penetration during exposure. A decrease in the concentration of ascorbate in aqueous humor was observed in patients with alkali eye burn and the use of exogenous ascorbate was related to the improvement of corneal healing due to the promotion of collagen secretion by fibroblasts, and high doses of vitamin C may be recommended after a chemical eye injury.[69]

ARTIFICIAL INTELLIGENCE

The continued growth of metabolomics research has demanded improvements in instrumentation, bioinformatics algorithms, data science methods, and access to computational resources. Artificial Intelligence (AI) is a branch of computer science that deals with the development of algorithms that seek to simulate human intelligence.

Machine learning tends to focus on a subset of problems within statistics, emphasizing, in particular, the analysis of large heterogeneous data sets, such as calibration of probability estimates, estimation of statistical confidence, and power calculations. The use of machine

learning allows for the establishment of correlations between different metabolites and the pathophysiology of the disease.[70]

Success in applying a machine learning method depends on the extent to which the method successfully encodes various types of prior knowledge on the subject studied. There is no ideal machine learning algorithm that works for all problems, so the researcher's approach and prior knowledge is crucial to the success of the analysis.

Machine learning is effective in situations where you want to understand the relationship between large data sets. Thus, machine learning methods are likely to become increasingly important for metabolomics as larger sets become available. On the other hand, even in the presence of large amounts of data, machine learning techniques generally cannot be applied in a completely arbitrary manner. Theoretical and practical knowledge of the machine learning methodology and the specific application area is necessary to achieve good performance in data analysis. In this sense, machine learning and machine learning researchers are likely to become increasingly important for metabolomics. This machine learning strategy has already been used in studies to effectively integrate genomics, proteomics, metabolomics, and phenotypic data.[71]

Continuous advances in the ease of use of the implementation of the deep learning model are helping to democratize researchers' access to these powerful research tools, with the use of big data organization software that makes it easier for researchers to train deep learning models on a large scale. Cooperative studies between different research centers worldwide can be useful for the development of algorithms with better accuracy and clinical applicability. Currently, there are large databases (big data) of electronic medical records, which allow the recognition of patterns in large volumes of data in a short period of time, reducing errors in diagnostics and therapeutics and creating personalized medicine. It is, therefore, the use of technology in addition to human expertise.

DISCUSSION

Metabolomics provide a sensitive information about the phenotype of a biological system, acting in a complementary way to the genomic, transcriptomic, and proteomic approach. The metabolome is defined as a set of metabolites found in a biological sample, thus being the final product of gene expression. Changes in the metabolome, occurring in seconds to minutes, may reflect the current state of the disease with greater precision. Since metabolites are the products or intermediates of metabolism, any altered concentration of metabolites reveals clues to dysfunctional metabolic pathways that may be related to the disease studied. With knowledge of the variation in the level of metabolites between healthy and diseased tissues, we can better understand the diagnosis of diseases and improve possible treatments.[71]

Unlike genetic analyses, metabolomic profiles vary depending on the biofluid being evaluated. Thus, the collection and proper processing of samples are important to avoid errors of analysis. Studies in the area of the proteome, metabolome, transcriptome, and lipidome were carried out with small sample sizes, but findings identified possible biomarkers, which can be confirmed in cohorts with a larger size of participants.[71]

Research in this area is important in classifying patients and predicting their response to treatment. The cataloging of metabolic profiles will allow us to validate and interpret genomic variants in a physiologically relevant context.

Although the eye is theoretically a good organ for studies in the area of the metabolome, due to its own metabolome, it offers samples in low quantities. One of the major problems to be solved in this area is the large set of data generated from experiences. However, the use of algorithms in the field of artificial intelligence can help the development of new predictors of disease progression in these areas. The clinical applicability of the study in the metabolome area depends on the accuracy of the data acquisition, collection, selection, and storage of the samples.[71]

Continuous improvements in databases and metabolite analysis software will reveal new and relevant metabolites in the future. As the number of participants evaluated in many studies is relatively low, increasing the number of participants in future studies can also help to improve the power of the results obtained.

FUTURE PERSPECTIVES, STRENGTHS, AND LIMITATIONS

One of the biggest problems in data analysis is the large set of data generated from the experiments. Studies in the field of artificial intelligence and big data analysis can be useful in this field of study, which can help in the integration of different layers of information, such as genomics, proteomics, transcriptomics and metabolomics. These data can be important in the development of personalized and precision medicine, which identifies biomarkers for the monitoring and diagnosis of eye diseases.

The collection of biofluids from patients before and after treatment can better monitor the effectiveness and toxicity of treatment for eye diseases.

The articles included in this review generated heterogeneous data because of the diversity in the design of the studies. The main limitation of this review was the lack of tools for methodological assessment of the reviews. The narrative review does not provide quantitative answers to specific questions about the study of the metabolome.

CONCLUSION

The study of the metabolome allows a better understanding of the metabolism of ocular tissues. The eye is a good organ for studies in this area due to its unique metabolome, however, it is difficult to obtain samples. Unique biological markers can be identified using this technique. The results are important to aid diagnosis and may function as predictors of the progression of many eye and systemic diseases. This would allow a better understanding of eye pathologies and the development of personalized therapies.

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