# LACTATE DEHYDROGENASE AND OXIDATIVE STRESS ACTIVITY IN PRIMARY OPEN-ANGLE GLAUCOMA AQUEOUS HUMOUR

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## Abstract

Lactate dehydrogenase (LDH) and lactate are some of the hypoxy biochemical parameters. Extracellular activity of this enzyme increases under the condition of oxidative stress, since the cell integrity can be disrupted during the lipid peroxidation process. Subsequently that leads to the increase level of the lactic acid and lactic acid salts. The objective of this investigation is establishing the level of LDH, LDH1 (HBDH) and the lactate concentration in aqueous humour in patients with primary open-angle glaucoma.

Biochemical analysis have been made by enzymatic-colometric method (lactate) and UV-kinetic method (LDH and HBDH) in aqueous humour of 30 patients (42 eyes) with primary open-angle glaucoma (POAG) and 30 patients (40 eyes) with cataract (the control group).

The increased values of lactate and the activity of LDH and HBDH enzyme in aqueous humour of POAG patients in correlation with the control group are the results not only of oxidative stress but also of hypoxy and the mitochondry oxidative function ($p<0,001$).

The increased activity of the examined biochemical parameters in the aqueous humour of the POAG patients points to the fact that other mechanisms, besides IOP, have a role in glaucoma pathogenesis.

KEY WORDS: glaucoma, oxidative stress, ischaemia, lactate dehydrogenase, lactates.

## Introduction

Glaucoma is a progressive chronic optical neuropathy. For a long time, this term has been equalized to increased intraocular pressure. Modern ophthalmology rejects this attitude owing to the investigations that have made stress on significance and role of other pathogenic mechanism in developing glaucoma (1-7). Recent scientific knowledge about glaucoma shows that we cannot seek for the explanation of some dilemmas exclusively in ophthalmology and related medicine disciplines, but also in the fundamental science disciplines, genetics and biochemistry, which leads us to the genetic and molecular definition of etiology of the disease. That is the reason why in recent years, besides others, intensive investigations of possible role of oxidative stress in primary open-angle glaucoma pathogenesis have been done (8-11). Under physiological conditions oxidative stress exists in all the cells that breath. Free radicals, which are produced in this process, participate in the homeostatic and protective mechanisms of the cells. But, the radicals are produced in numerous pathogenic processes and are responsible for various damages at the level of almost all biomolecules (12). Owing to the free radicals toxic effect, simultaneously with the adaptation to aerobic living conditions, some antioxidative mechanisms have been developed during the evolution (13). Under normal conditions, the balance between oxidative processes and antioxidative capability keeps the stability of structure and functions of all the living cells. The condition of oxidative stress occurs if the balance is disturbed because of the increased production of the reactive oxygen metabolites or decreased capacity of antioxidant protection, or because of both processes at the same time (14-17). The results of an oxidative stress are lipid peroxidation of cell membrane, the enzyme inactivation through sulph-hydrile groups, creating the proteins aggregation, polysaccharides polymerisation and the damage of nucleic acids. It occurs in the chain of acute processes, such as in some inflammatory reactions. However, the effect of oxidative stress can also be chronic, gradually damaging, with cumulative effect and changes that are demonstrated with the course of time, which happens in glaucoma (18). Lactate dehydrogenase-LDH is an oxidoreductase which takes part in the process of the carbon hydrates glycolisis. It catalyzes the reaction of transforming piruvic into lactic acid (under anaerobic conditions), and vice versa, transforming lactic into piruvic acid under aerobic conditions. Coenzyme of this ferment is NAD. This enzyme is found in many human tissues, and the greatest concentration of it is found in kidneys, miocard, sceletal muscles and liver. There are not data about its quality or quantity presence in the eye. Within the cells it is located in citoplasm. Its concentration in serum and other tissue fluids is smaller than in body tissues. There are 5 LDH isoenzymes (LDH1-(LDH5). α -hydroxybutirate dehydrogenase (HBDH) is an isoenzymes of LDH1 (19). Extracellular activity of this intracellular enzyme increases under the condition of oxidative stress, due to the integrity of cell membrane destroying in the course of lipid peroxidation. This leads to an increase of lactic acid concentration, that is to say, of lactic acid salts.

The objective of the investigation is to establish the activity of LDH, LDH1 (HBDH) the concentration of the lactate in aqueous humour in patients with primary open-angle glaucoma, in the light of explaining the metabolic disorders which occur in glaucoma patients.

## Materials and Methods

The aqueous humour specimens were obtained from 30 patients (42 eyes) with primary open-angle glaucoma and 30 age and sex matched patients (40 eyes) with age-related cataract, as the control group. All study participants have had no other ophthalmologic diseases except two mentioned. The study was performed with informed consent of every participant and is approved by Ethic Committee of Clinical centre Niš. The specimen of aqueous humour has been taken by paracenthesis, immediately before the start of fistulising antiglaucomatous surgery and before phacoemulsification surgery in relevant patients. We have established activity of LDH, HBDH by UV-kinetic method and the concentration of the lactates by enzymatic-colorimetric method (20, 21, 22). The Wilcoxon signed ranks test has been used for establishing statistic significance of the results.

## Results

Glaucoma patients were of age 62,17±6,67 years (ranged 52-73), whereas cataract patients were 62,57±6,68 years (52-74 years). Males were more numerous in both groups (18 male and 12 female patients respectively). There were no significant difference in the tested parameters between men and

women inside both groups (p>0,05, Mann-Whitney U test), except higher lactate values in cataract female patients, compared to the male ones (p<0,05). Although the mean values of the all investigated parameters increased in older patients glaucoma and cataract subgroups (divided on 51-60, 61-70 and 70 years and older ones), there were no differences between different ages inside two main groups. The activity of LDH and HBDH enzymes, as well as the level of lactate in the aqueous humour of patients with primary open-angle glaucoma, are shown in Table 1. In patients with glaucoma the values of the tested parameters were increased with a high statistic significance.

| X±SD(U/L) | Glaucoma | Cataract | P* |
|---|---|---|---|
| LDH | 435,46±102,61 | 112,97±30,81 | <0,001 |
| HBDH | 107,3±30,06 | 40,08±10,28 | <0,001 |
| lactates | 0,042±0,04 | 0,022±0,03 | <0,05 |

TABLE 1. LDH and LDH isoenzyme and lactates values in aqueous humour of primary open angle glaucoma patients compared to the age-related cataract patients.

LDH-lactate dehydrogenase
HBDH-α -hydroxybutirate dehydrogenase
*Wilcoxon Signed Ranks Test

## Discussion

The physiology of aqueous humour production and filtration and especially its chemical composition have always drawn attention of the researchers (23). It occurs in the processes of active transport (70-75%), ultrafiltration (20-25%), and to a smaller extent, in pinocytosis. The flow of all the blood plasma substances through the haemato-aqueous barrier, as a selective active transport membrane, is not identical: the flow of liposolubile substances is facilitated, the flow of urea is moderate and the flow of proteins is very difficult. It is thought that Na-pump has a key role in aqueous humour formation, its action potential being used for ATP-ase activation and production of energy necessary for those processes. That is the reason why there is the difference between the composition of aqueous humour and the composition of blood plasm. The concentration of glucose, urea and proteins in aqueous humour is lower than the concentration of ascorbic acid (3 times higher), pyruvates (25 times higher) and lactates (almost 2 times higher). The concentration of other chemical substances shows fewer variations (24-26). Hydroperoxide is a regular component of aqueous humour. It is quite understandable that its concentration in this eye compartment is very low. It is supposed that it is formed in aqueous humour during the process of ascorbic acid oxidation, in the presence of oxygen molecule. Cilliar epithel actively secrets ascorbate in aqueous humour, the ascorbate concentration being 20 times higher than the one in blood plasma (15). The concentration of glutathione in aqueous humour is micromolar (27). Approximately 99% of total content is its reduced form. Aqueous humour also contains low concentrations of albumins, transferin and urates (28-31). By investigating biochemical composition of aqueous humour in various ophthalmologic diseases we get useful data pointing to the pathogenic mechanisms of some diseases. However, there are few investigations of biochemical composition of the aqueous humour in glaucoma optical neuropathy, especially investigations of ischaemy indicators such as LDH enzyme and its isoenzymes, whose extracellular activity increases under the condition of oxidative stress. Oxidative stress is one of depicted pathogenic mechanisms occurring in primary open-angle glaucoma (32). Free radicals do not show their toxic effects due to their antioxidative protection system under the physiological conditions. In the conditions of imbalance between the production and free radicals elimination (firstly, in ischaemia occurring in glaucoma), there is an increase in free radicals concentration which intensifies the lipid peroxidation processes. The lipid peroxidation exists under physiological conditions, regulating membrane lipids metabolism, the change of their physical and chemical features and permeability. When there are higher concentrations of free radicals in aqueous humour and the eye tissues, the lipid peroxidation process proceeds in an undesirable direction and unsaturated fatty acids oxidation occurs in the cell membrane phospholipids, endangering integrity and function of the cell (33-35). Lipid peroxidation is autocatalytic, progressive and most often irreversible process. It represents the most distinct negative phenomenon in free radicals activity and has two phases. In the first phase, the hydrogen in the double-bond unsaturated fats is eliminated and alkyl radicals and other primary products of lipid peroxidation are formed (conjugated dienes, peroxide radical and lipid hydroperoxides). In the second phase, these unstable primary products are dissolved in secondary products: short-chained carbohydrates, aldehydes and ketones, as well as malondyaldelhide (MDA), as the ultimate product of lipid peroxidation. Parallel with that process, there is the formation of free radicals which can reactivate these reactions leading to recurrence of cyclic lipid peroxidation (36). As a result of Ca-pump inactivation and increased

passive cell membrane permeability to Ca ions, there is an increased concentration of Ca ions in cytosole, which activates A 2 and C phospholipases. Phospholipases hydrolyze the membrane phospholipids releasing arachidonic acid. In that way, it enables the metabolic cascade of arachidonic acid, followed by free radicals release and further recycling of lipid peroxidation. Using electron microscope, the first changes of the eye tissue cell based on the depicted mechanism are investigated in the chamber angle (37, 38). It is expected since the trabeculum and the inner side of Schlemm's canal are completely avascular and their metabolism is totally provided by aqueous humour (39, 40). It causes descvamation and lysis of corneoscleral trabeculum endothelial cells in chamber angle. The collagen structure is disturbed since the intermolecular bonds (cross-intersections) are formed among adjoining collagen fibres. It results in collagen fibres disintegration and intertrabecular space obliteration, leaving the residues of tissue disintegration products in trabecular meshwork and Schlemm's canal. Simultaneously, there is an interaction between free radicals and highly reactive secondary products of lipid peroxidation (MDA), on one side, and groups of free SH and NH 2 amino acids, peptides, proteins, nucleotides and phospholipids in the endothelial cells of corneoscleral trabeculum and Schlemm's canal, on the other, which induces further disturbance of their functional characteristics and consequent decreased facility of aqueous humour outflow and increased intraocular pressure. Higher intraocular pressure causes the damage of retinal ganglion cells, which is depicted in well-known mechanical theory of glaucoma (41-44). Endothelial cells of small blood vessels (arterioles) in optic disc and retina neural fibrae beds can be damaged by very reactive and toxic hydroxyl radicals. They occur when stimulated polimorphonuclear leukocytes, monocites and macrophages form a significant quantity of superoxide union radicals and hydroperoxide, in the presence of NADPH oxidase enzyme. Afterwards, the reaction continues in the presence of unstable valence ions ( Fe 2+ into Cu2+). In these cells there is a mieloperoxidase enzyme (MPO) which enables the occurrence of strong hypochloric acid oxidant (HOCL). Hypochloric acid can produce even more reactive metabolite (hydroxyl radical and singlet oxygen). These oxidants have an important role in phagocytosis, but they damage blood vessels endothelial cells and adjoining neural tissue, disturbing the structure and function of numerous biomolecules and cellular organeles. It leads to ischemic damages of retina and its ganglion cells, which should be investigated in the future, since other free radicals, such as xanthine oxidase and NO, could also impact these damages (45-48). In the blood of patients with advanced glaucoma, secondary products of lipid peroxidation (MDA) can be found, causing further deterioration of microcirculation in the eye. They selectively inhibite the capacity of blood vessels endothelial cells to synthetize prostacyclin, whose level significantly defines aggregation and adhesive features of trombocytes. In that manner, the toxicity of the ischemic component in glaucoma disease is intensified. Activated autocatalytic reactions induce weakening of the cell adaptive capability and consequently the death of retinal ganglion cells (apoptosys) occurs as a result of exhaustion, cessation of functions or insufficient quantity of factors which are protective mechanisms against free radicals harmful effects (49, 50). Under the condition of oxidative stress and retinal ischaemy, there is a metabolic change in the eye tissue leading to the aqueous humour chemical change. Thus the increased activity of LDH is expected, since under anaerobic conditions LDH catalyzes the reaction of transforming piruvic into lactic acid and increased concentration of lactates (51). The glaucoma patients in this study were on local beta-blocker and carbonic anhydrase inhibitors therapy previously, but decompensated at the time of surgery, despite of therapy. Although those medication influence aqueous flow, they do not interfere with investigated biochemical parameters. Moreover, the results of the tests on human specimens are completely congruous with prior experimental investigations. After inducing alpha-chymotripsin experimental glaucoma in rabbits (52), we have investigated the LDH and HBDH activity as well as the concentration of lactate in aqueous humour of rabbits with experimental glaucoma, and have obtained manifold increase of all the parameters (4 to 6 times) comparing to the aqueous humour control group results of the rabbits without glaucoma - $p<0,001$ (unpublished results). Increased lactates values and increased LDH and HBDH activities in the group with glaucoma are the caused not only by oxidative stress but also by distinct hypoxy and the disturbed mitochondry oxidative function. Under the conditions of hypoxy, the only possibility for NADH + reoxidation is the lactate production. In that way, glucose could be metabolized in glycolithic process, in absence of oxygen or in decreased oxygen concentrations in the cell. The renewal of NAD is attained with assistence of LDH enzyme, "lending" a reduced coenzyme from 3 -phosphoglyceraldehide dehydrogenase.

The study performed on LDH activity in cataract patients showed that significant decrease in lens LDH did not bear any relationship to LDH in serum and aqueous humour in cases of senile cataract (53).Yet, the investigation of these enzymes and other biochemical parameters of hypoxy in aqueous humour and retina tissues can point to their possible role in pathogenesis of primary open-angle glaucoma.

## Conclusion

The increased activity of the investigated parameters (LDH, HBDH and lactates) in aqueous humour in patients with primary open-angle glaucoma points to the fact that other mechanisms, besides increased intraocular pressure, such as oxidative stress and ischemic conditions can have a role in glaucoma pathogenesis.
Increased extracellular activity of LDH, which is an intracellular enzyme, is the result of distrupted cell membrane integrity, which occurs during the lipid peroxidation, under the influence of free radicals and in the conditions of oxidative stress and ischaemy in the eye affected by glaucoma. That is the reason why the investigation of metabolic disturbances in glaucoma, and more important their correction, is a promising way in curing this chronic disease leading to blindness.

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## Conflicts of interests

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