RESEARCH ARTICLE

Glutamate Dehydrogenase Applicability in Clinical Practice

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Abstract

Glutamate dehydrogenase (GLDH) catalyzes reversible deamination of glutamate into alpha-ketoglutarate and ammonium ion. In the metabolism of a cell, GLDH has the key role of acting as an interface between carbohydrates and amino acids in the vicinity of the citric acid cycle and urea cycle. In the brain, GLDH appears in astrocytic processes associated with glutamatergic terminals, but it is also involved in glia metabolic processes. GLDH probably protects postsynaptic membranes against the neuroexcitotoxic glutamate effect.
GLDH is an equally accurate marker of alcoholism in comparison to others, if its significantly faster decrease is taken into consideration. Watching changes in the activity of laboratory markers of alcoholism, after cessation of drinking, is an effective yet overlooked aid in diagnostics.
The fast increase of leukocytes GLDH activity is specific for alcohol addiction. Alcohol consumption reduces GLDH activity to some extent and consecutively it could lead to increased protein production and strengthen of diminished leukocyte protective ability.
The gradual decrease in GLDH activity may be one of key factors for neurodegenerative ageing processes. The decrease in GLDH activity in the cerebrospinal fluid of patients with neurodegenerative disorders may be one of the reasons for the neuroexcitotoxic glutamate effect.
The probable decrease in GLDH activity in the cerebrospinal fluid of patients with neurodegenerative disorders and of patients with nerve, nerve root and plexus disorders needs further investigations to be adequately understood.

Keywords: glutamate dehydrogenase, isoform, leukocyte, alcohol, addiction, neurodegeneration, ageing, decision trees, diagnostics, cerebrospinal fluid.

Introduction

Glutamate dehydrogenase (GLDH) (EC. 1.4.1.3.) consists only of polypeptide chains (1). GLDH catalyzes reversible deamination of glutamate into alpha-ketoglutarate and ammonium ion. Its catabolic function is of major importance (2). In the metabolism of a cell, GLDH has the key role of acting as an interface between carbohydrates and amino acids in the vicinity of the citric acid cycle and urea cycle (2).

GLDH can almost exclusively be found in mitochondrial matrix but it also in the rough endoplasmic reticulum (3). The distribution among the major organs is very uneven, with the liver being the outstanding one (3,4). Even in 21.99% of healthy subjects there is any measurable GLDH activity (5).

The GLUD1 gene is localized on human chromosome 10 and is mainly present in hepatocytes, whereas GLUD2 is localized on human chromosome X and is in nerve tissues, testicles, retina and probably in leukocytes (2,6).

In the brain, GLDH appears in astrocytic processes associated with glutamatergic terminals, but it is also involved in glia metabolic processes (7). GLDH probably protects postsynaptic membranes against the neuroexcitotoxic glutamate effect (8).

Alcoholism and serum GLDH

Alcohol dependence with its variety of symptoms is a disease similar to other mental disorders. It is an interaction between various psychosocial, genetic, biological and behavioural patterns (9).

There are still no reliable biochemical markers for alcohol consumption and dependence whose sensitivity and specificity would be high enough to be relied on (10,11). So far, IFCC has not accepted the reference procedure for determining serum GLDH activity. Dialab, the producer of equipment and reagents, recommends reference activities up to 117 nkat/L (men) and 83.3 nkat/L (women). However, Tietz's Laboratory medicine recommends reference activities up to 133 nkat/L (men) and 100 nkat/L (women) (12). However in their study Kravos and Malešič calculated the reference interval of up to 124.0 nkat/L (men) respectively 64.5 nkat/L (women) (13).

In their study Kravos and Malešič discovered significantly higher GLDH activity in alcohol dependents than in healthy subjects. Furthermore, GLDH activity in moderate drinkers was lower than in alcohol dependents. They showed a 65.5 % mean sensitivity, which increased to 72.2 % with those who had drunk alcohol within 48 hours before first test (13). However, after one week of cessation, GLDH activity in men decreases to the upper reference level (13).

A 24-hour interval of alcohol abstention is sufficient enough for a reliable evaluation of the fall in GLDH activity. The rapid dynamic of fall in serum GLDH activity is a response to a break in drinking and proceeds for a relatively long time, for at least 10 days. The normalisation of GLDH activity after 24 hours is faster than that of GGT and the kinetics of GLDH is more applicable than GGT kinetics after a week's cessation of drinking. GLDH had the best specificity of all liver enzymes (13).

The determination of thermo-stable and labile GLDH isoforms could also be applied as an additional marker for alcohol dependency, possible liver injury or even as prognostic factor for the possibly (ir)reversible damage. The GLDH activity in serum persists at liver necrosis but it decreases very quickly after cessation of drinking with reversible damage of mitochondria and endoplasmic reticulum. The kinetics and determination of two different GLDH isoforms give us important data about alcohol toxicity on liver tissues (13).

When abstinence has been restored, GLDH activity starts to decrease almost immediately after the cessation of drinking

(13), whereas that of GGT not earlier than four to five days later (14) [33]. CDT returns to normal values after two to three weeks, GGT after six to eight weeks and MCV within three months (14,15). The speed of restoring to reference levels is most rapid and explicit in GLDH but slightly less so in AST, ALT and GGT (16). The differences of pathological markers values are not so expressive that they could be used as a predictor for abstention symptoms (17).

Combinations of alcohol markers increase sensitivity and diminish specificity. The GGT and CDT combination is the most important (18).When combining two markers, by taking kinetics into consideration, the best results were reached with combinations of MCV (with slow decrease kinetics) and GLDH kinetics (16).

The diagnostic applicability of GLDH could broaden also with the decision tree intelligent data analysis (19). The decision tree should be used by exclusion of every ascertainable person from the next step (figures 1 and 2). MCV rarely plays an important role in diagnostic of alcohol dependence syndrome, as it is primarily increased in many hematological diseases, which must be taken into consideration also at the valuation of our decision tree for alcohol dependents (19).

Figure 1. The decision tree (c) for diagnosing alcohol dependency by GGT and GLDH (19).

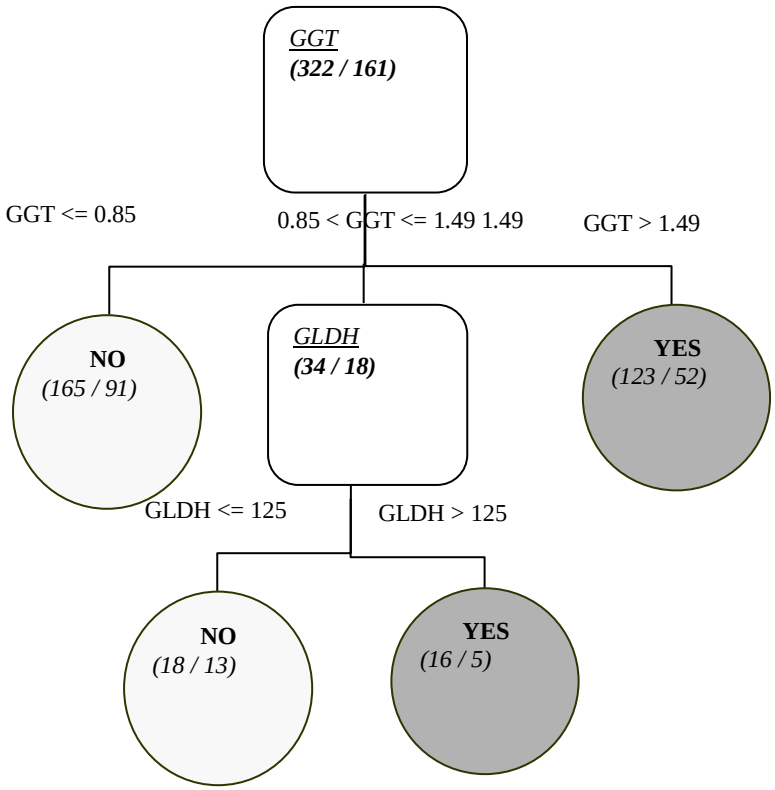
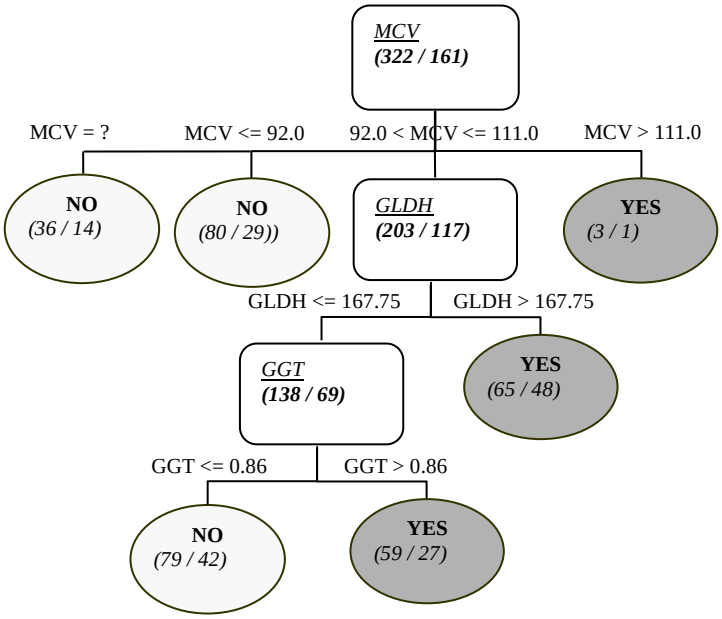


Figure 2. The decision tree (b) for diagnosing alcohol dependency by MCV, GLDH and GGT (19).



Direct biochemical supervision over the maintenance of abstinence from last drink could be performed by the normalization of the activity of particular markers: from the 1st- 2nd day by determining alcohol in blood, from the 1st-7th day by GLDH, from the 5th-10th day by GGT, from the 7th- 28th day by CDT and up to three months by MCV. Pathological values return to reference values if the alcohol dependent person abstains (16).

Alcoholism and leukocyte GLDH

GLDH activity was partly deficient in the brain and particularly in the leukocytes of patients with heterogeneous neurological disorders and degeneration of multiple neuronal systems (3). Reference values for GLDH activity in leukocytes were determined from 0.08 – 1.21μkat/g (16). Kravos and Malešič have found lower leukocyte GLDH activity in alcohol dependents than in healthy subjects but not statistically significant. However, regarding the time elapsed since last alcohol intake there is a strong correlation between the increase in leukocyte GLDH activity and the cessation of drinking in alcohol dependent persons. The shorter the interval since last drink, the faster is the increase in leukocyte GLDH activity. The decrease in leukocyte GLDH activity is probably directly caused by alcohol, most likely because of inhibition. Thus the discontinuity in drinking could restore the process by increasing leukocyte GLDH activity (16).

Neurodegeneration and leukocyte GLDH

In the brain, GLDH probably protects postsynaptic membranes against neuroexcitotoxic glutamate effects (7,8). GLDH deficiency in leukocytes and brain in patients with neurodegenerative diseases (cerebellum, basal ganglia) leads to accumulation of glutamate and degeneration of postsynaptic neurons (20).

GLDH activity declines almost evenly through the ages in men; however, in women it declines faster in the first three decades of lifespan. Afterwards in the years of menopause, GLDH activity declines slower. Overall GLDH activity decreases more

slowly in the age-group from 30 to 60 years, yet evidently more rapidly afterwards, particularly in men where activity drops to 0.333 μkat/g; in women to 0.414 μkat/g (21). The highest leukocyte GLDH activity is in healthy subjects of both sexes younger than 30 years. In fact, leukocyte GLDH activity decreases over time (21).

The gradually decreased enzyme activity over life has probably some contribution in the process of aging. Nevertheless, in the cases of neurodegenerative disorders is the rapidity of gradual GLDH activity decline faster (21).

Neurodegeneration and cerebrospinal fluid GLDH

The damage to the cells of the central nervous system leads to enzyme release and to a rise of enzyme activities in the extracellular fluid in a similar manner to damage in other tissues (4).

Astrocyte extensions contain most of the GLDH (7). The enzyme probably provides crucial protection for postsynaptic membranes against the neurotoxic effects of glutamate neurotransmitter (8). The diminished GLDH activity is displayed as brain disease because of high glutamate concentrations in the brain (22). However, the GLDH activity of patients with degenerative disorders is lower than in persons without neurological diseases. The GLDH activity in the brain decreases over lifespan, and the elimination of glutamate as a neurotransmiter consequently forward the neuroexcitotoxic process, presumably leading to neurodegeneration (23).

Conclusions

Time aspect considered serum GLDH activity is an ideal marker of alcoholism since it is elevated in alcohol abuse but its activity declines promptly after the last alcohol intake in alcohol dependent subjects and the change lasts long enough to be able to be evaluated. The application value is in combination with other markers. Direct biochemical supervision over the maintenance of abstinence from last drink could be performed by the normalization of the activity of particular markers: from the 1^{st}- 2^{nd} day by determining alcohol in blood, from the 1^{st}-7^{th} day by GLDH, from the 5^{th}-10^{th} day by GGT, from the 7^{th}- 28^{th} day by CDT and up to three months by MCV. Pathological values return to reference values if the alcohol dependent person abstains. Above all, determining serum GLDH is helpful in distinguishing drunken alcohol addicts from drunken regular drinkers. For confirmation of alcohol addiction, the kinetics of serum GLDH and AST activities after cessation of drinking are particularly important.

The kinetics of leukocyte GLDH activity is a specific marker of alcohol dependence as well as of the cessation of drinking alcohol. The diagnostic value of leukocyte GLDH activity is almost irrelevant, because of overly complicated tests and its sensitivity to parallel drug induction except in indistinct cases.

Somehow lower GLDH activity in leukocytes may play an indirect role in the development and course of neurodegenerative alcohol induced diseases. Like the alcohol dementia as an outcome of alcohol abuse or the greater exposure to infection because of diminished antibody protein production.

In the brain and in the leukocytes the metabolism of proteins and possibly of carbohydrates diminish with ageing. The gradual decrease in GLDH activity may be one of key factors for neurodegenerative ageing processes and may be one of the reasons for the neuroexcitotoxic glutamate effect. But the role of decreased GLDH activity in patients with nerve, nerve root and plexus disorders needs further investigations to be adequately understood.

First of thing the GLDH serum determination is a useful add on marker in

diagnosing alcohol dependence and secondly the leukocyte determination could be an indirect marker in determining neurodegenerative processes in the brain.
However, there are still questions that need to be answered: what is the short-term and long-term influence of alcohol on GLDH activity, what is the mechanism of increased GLDH activity after cessation of drinking, what is the process of normalization, what is the role of GLDH in normal ageing and in neurodegenerative disorders, metabolic consequences and other factors affecting GLDH activity and low GLDH activity in leukocytes and in the brain in some healthy persons.

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