AnnalsofClinicalandMedical Case Reports

CaseReport ISSN2639-8109Volume7

Relationship Between LDH and Mg in Monitoring of Hematologic and Non-Hematologic Malignant Diseases

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Received: 03 Oct 2021
Accepted: 23 Oct 2021
Published:28Oct2021
JShort Name:ACMCR

Citation:

UdristioiuA,RelationshipBetweenLDHandMgin Monitoring of Hematologic and Non-Hematologic Malignant Diseases. Ann Clin Med Case Rep. 2021;V7(13):1-5

Keywords:

Lactate dehydrogenase; Isocitrate dehydrogenase; CLL-Chronic lymphocytic leukemia; Acute promyelocyticleukemia;Nicotinamidedehydrogenase

1. Abstract

Aim of this study was to evaluate the correlation betweentheserumlevelsoflactatedehydrogenase(LDH)andmagnesium (Mg) in patients with diagnosed malignant diseases.

Method:WereanalyzedLDHandMgparametersonacohort ofpatients(n=75)comprisingmales(n=36)andfemales(n=39) withameanageof57years(SD=12.5).Thebiochemicalparameters were measured using a Vitros 250 dry chemistry analyzer (Johnson & Johnson, USA) using the slides for multi-layer spec- trophotometry measurements.

Results; In the cohort study, 55 patients (73%) who received cancertherapyexhibitednormalserumlevelsofMg(normalvalue =1.60-2.3mg/dL;meanvalue=2.2mg/dL;SD=0.2;p=0.02).In contrast, 12 patients (16%), recently diagnosed with a malignant disease, who had not been treated, displayed high levels of serum Mg,(meanvalue=2.89mg/dL).SerumMglevelswereincreased by the release of Mg^{2+} from malignant tissues in patients with malignant disease prior to treatment with cytostatic drugs. LDH levelsremainedelevatedafterinitialcytostatictreatmentuntilcancerremission.Thenumberofcopiesofchromosomesinmalignant tumors may be correlated with total serum LDH values.

Conclusion: NormalMglevelswithmoderatelyelevated

LDH levels were observed in all patients with regressive cancer aftergoodresponsestospecifictherapy.LowMglevelswithhigh serum LDH levels have also been observed in all patients with poor prognosis and metastases, meaning that Mg and LDH ion levels can be used as markers to monitor treatment responses in patients with or without metastasis.

2. Introduction

Magnesium, which is the second most abundant intracellular cat- ion after potassium, plays a key role in regulating many cellular functions and enzymes, including ion channels, metabolic cycles, and signaling pathways. Magnesium ion (Mg^{2+}) is critical for maintaining the positional integrity of tightly grouped phosphate groups.Thesegroupsoccurinmanydistinctpartsofthecellnucle- us and cytoplasm. Mg^{2+} maintains the integrity of nucleic acids, ribosomesandproteins.Inaddition,thisionactsasatraceelement in the energy catalysis of cells.

Aim of this study was to evaluate the correlation between serum lactate dehydrogenase (LDH) and magnesium (Mg) levels in patientsdiagnosedwithmalignancy,admittedtothehospitaldepartment oncology.

3. Methods

Wasanalyzedacohortofpatients(n=75)comprisingmales(n

= 36) and females (n =39) with a mean age of 57 years (SD = 12.5)whohadcancerdiseasesandwereadmittedtotheoncology department**.** The biochemical parameters were measured using a Vitros 250 dry Chemistry Analyzer (Johnson & Johnson, USA) usingtheslidesformulti-layerspectrophotometry measurements.

In the study were excluded patients with non-neoplastic pathologies or diseases that can induce increased serum levels of Mg and LDH. These diseases included acute or chronic renal failure (CRF), ischemic heart disease, lung infarction, liver cirrhosis, acute or chronic hepatitis, massive muscle injury, megaloblastic anemia and severe syndromes that are associated with respiratory failure.

The CBC with the differential count, biochemistry samples, body radiography, ultrasound and computed tomography (CT) were used for the patient to establish the type of cancer diseases. In different types of leukemia, morphological cells were assessed in stage of differentiation between the pre-B and T cells, mature B cell stages and monocyte blast and myeloid cells.An initial panel ofmonoclonalantibodieswasusedtodeterminetheimmunephenotypes of the subgroups of differentiated T cells and B cells by flow cytometry. Activated B lymphocytes in CLL patients were definedasCD5+/CD19+,CD+_20cellsthatexpressedCD23and/ or CD38 as surface markers.

Thesamplestabilitywasmaximalatonehourat15-25°,inconformitywiththeconditionsofthedeliveryofsamplesfortheprimary sample collection, following the instructions of the manufacturer and respecting the Procedures of Collection of Diagnostic Blood Specimens by Venipuncture, NCCLS Document H4-A3 Wayne, PA:NCCLS;1991.Weexcludedsamplesfromthestudybasedon the following criteria: an icteric index > 65 for conjugated bilirubinandanictericindex>37forun-conjugatedbilirubin,hemoly- sis with an H index > 400, turbidity for triglycerides > 300 mg/dl and serum containing para-proteins (multiple myeloma).

ThediagnosisofLAM-3wasmadebasedonbloodsmears,the examination of bone marrow (BM) aspirates, the evaluation of promyeloblasts (greater than 30% in BM), and the presence of a specific immune phenotype. Immunocytochemical detection was performed to confirm the diagnosis of LAM-3 using FAR Leukemia kits and there were positive results for the peroxidase reaction for promyelocytes, myelocytes, granulocytes, and peripheral blood cells (POX+) and negative results for the peroxidase reaction for the blast cells. For evaluation of the neutrophil alkaline phosphatase(NAP)levelsingranulocytes(negativeorlowvalues in LAM-3) using the in vitro NAP test protocol (Code SP 910, ChemicalCompany),withpositiveresults,onthesmearofperipheralbloodsmear,granulocyticlysosomesthatappearasdarkblue or black grains in the cell cytoplasm.

4. Results

Among the patients, 8 patients were diagnosed with lung cancer, 18 patients were diagnosed with breast cancer, 19 patients were diagnosed with genital cancer, 23 patients were diagnosed with colorectal cancer, 5 patients were diagnosed with chronic lymphocytic leukemia (CLL), one patient was diagnosed with acute promyelocytic leukemia (LAM-3) and one patient was diagnosed with chronic monocytic leukemia (CML).

The results were interpreted for each patient based on medical history, clinical and para-clinical examinations and other signs of malignant diseases.Among the patients in this study, 55 patients (73%)exhibitednormalserumlevelsofMg(normalrangevalue = 1.60-2.3 mg/dL; mean value = 2.2 mg/dL; SD = 0.2; p = 0.02) followingcancertherapy.Sixpatients(8%)exhibitedlowlevelsof Mg (range = 0.60-1.50 mg/dL; mean value = 1.05 mg/dL). However,12patients(16%)displayedhighlevelsofserumMg(range =2.6-3.27mg/dL;meanvalue=2.89mg/dL).Thelevelsofserum lacticdehydrogenase(LDH)werealsoevaluatedinpatientsnewly diagnosed withcancer andin patientswith unfavorableresponses to the cancer therapy (range = 240-1330 U/L; mean value = 787 U/L;SD=1.33;p=0.002;normalvalues135-225U/L),(Table1).

Table1: SerumLDHandMglevelsofpatientswithmalignantdiseases<(Normalvalueinhealthypatients:LDH=135-225U/L,Mg=1.6-2.3mg/Dl).

SerumLDHandMglevelsofpatients withnewlydiagnosedmalignant diseases	**Serum LDH and Mg levels of patients in the remission stage of malignant disease following cancer therapy**	**SerumLDHandMglevelsofpatientswith unfavorable responses to cancer therapy**
Lung Cancer Meanvalue: LDH=1270 Mg =2.85	**Lung Cancer** Meanvalue: LDH = 254 Mg =1.60	**Lung Cancer** Meanvalue: LDH=1330 Mg =1.26
Breast Cancer Meanvalue: LDH=1250 Mg =2.55	**Breast Cancer** Meanvalue: LDH =250 Mg =1.80	**Breast Cancer** Meanvalue: LDH=1260 Mg =0.87

Colorectal Cancer Meanvalue: LDH=1250 Mg =2.70	**Colorectal Cancer** Meanvalue: LDH =250 Mg =1.7	**Colorectal Cancer** Meanvalue: LDH=1260 Mg =0.63
AcuteandChronic Leukemia Meanvalue: LDH=1290 Mg =3.75	**AcuteandChronic Leukemia** Mean value: LDH=255 Mg = 2.05	**AcuteandChronic Leukemia** Meanvalue: LDH=1330 Mg =1.6

5. Discussions

CommentsofResults

TheserumMglevelisincreasedviaMg²+releasefrommalignant tissues in patients with malignant disease prior to treatment with cytostaticdrugs.Inthedifferentmalignantdiseases,theserumMg values were high, normal or low, independent of the serum LDH values. The LDH levels remained elevated after initial cytostatic treatment until cancer remission. The number of copies of chromosomesinmalignanttumorsmaybecorrelatedwithtotalserum LDH values. LDH levels in cancer patients are elevated due to highlevelsofLDH-3isoenzymeinpatientswithmalignanciesand high levels of LDH-4 and LDH-5 isoenzymes, elevated patients with cancer of liver, muscle, lung and tissue tissues. conjunctive. High concentrations of serum LDH damage the cell membrane. Thereafter, malignant cells become invasive and metastasizes.

CellularPhysiopathology of Mg^{2+}

Themagnesiumserumlevelsarekeptconstantwithinverynarrow limits(0.65-1.05mmol/dL;1.58-2.25mg/dL),byflowregulation**,** via ascending loop of Henle o kidney [*Walter F and al. 2005,*] *Popescu MP, 2011, Stefano A, 1993*]. Malignant cells use Mg² + ions in metabolic pathways more frequently than normal cells do and absorb magnesium from normal tissues, including bones and muscles.

In cells, the immediate energy sources involve glucose oxidation. In anaerobic metabolism, the donor of the phosphate groupis adenosine triphosphate (ATP), and the reaction is catalyzedvia the hexokinase or glucokinase: Glucose +ATP-Mg^{2+} = Glu- cose-6-phosphate (Δ Go = - 3.4 kcal/mol with hexokinase as the coenzyme for the reaction [*Udristioiu A ,2002]*. Mg^{2+} helps fix ATPintheactivecentersofco-enzymesandotherkinasesthatare ATPdependent.TheenzymeGlucose-6-phosphate,accumulating in the cell follows the path of degradation of anaerobic glycol- ysis. The process of converting glucose-6-phosphate into fruc- tose-6-phosphate is catalyzed via the enzyme phosphoglucomu- tase with the co-factor ATP-Mg^{2+}.

Theconversionofglucose-6-phosphateintofructose-6-phosphate isareversiblereactionbecauseofsmallenergydifference(ΔGo= (- 4 kcal/mol). In the following step, the conversion of G-6-phosphate into F-1-6-bisphosphate is mediated by the enzyme phosphofructokinasewiththeco-factorATP-Mg^{2+}.Thisreactionhasa largenegativefreeenergydifferenceandisirreversibleundernor- mal cellular conditions. Mg² + is essential for maintaining the integrityoftightlygroupedandpositionedphosphategroups.These clusters appear in numerous distinct parts of the cell nucleus and cytoplasm.TheMg^{2+}maintainstheintegrityofnucleicacids,ribosomes and proteins. In addition, this ion acts as an oligo-element with role in energy catalysis [*Black B, 1995*].

Membranes and cell walls have poly-anionic charges on the surface. This has implications for ion transport, especially since different membranes preferentially bind different ions. Both Mg² + and Ca² + regularly stabilize membranes by cross-linking phosphorylated lipid groups. Biological membranes are impermeable to Mg^{2} (and other ions). Therefore, transporter proteins must facilitatetheflowofMg^{2+}intoandoutofcellsorintracellularcompartments. Intracellular calcium induces mitochondrial swelling and aging. The proliferation of osteoclast cells occurs when the intracellular Ca/Mg ratio is 3/2. Mg2+ generally interacts with substrates via the inner coordination sphere, stabilizing anions or reactive intermediates, bindingATPand activating molecules for nucleophilic attack.

A magnesium ion progressively removes nearly all of the water via a selective pore before the magnesium ion is released on the far side of the membrane. The changes occur in low percentages ofligandexchangeinthecoordinationcomplexcomprisingwater andtheMg2+ion[*LuninVV,2006,DalmasO,2017*].Thetrans- portmechanismdependsonthe3-Dstructureofthecomplexthat arises by hydrating the Mg2 + ion in the aqueous medium. The innershellofthecomplexcomprises6watermolecules,relatively closely related, and the outer shell comprises 12-14 water molecules [*Kehres DG, 2002]*. The pore is a funnel-shaped pentamer with two transmembrane spirals on each monomer composed of chainsofatomschainedincarbohydratesandlipids.Theionchan- nel consists of an inner group of 5 spirals and closes through the voluminous hydrophobic residues**,** (Figure 1).

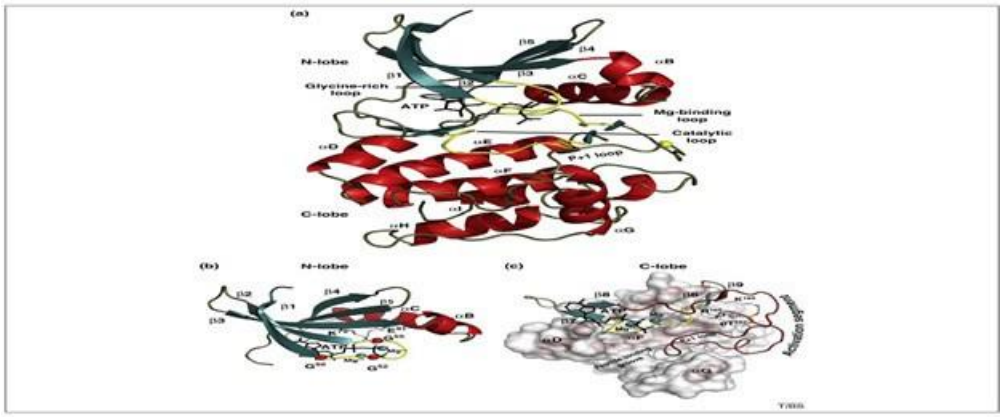

Figure 1: The structure of the conserved protein kinase core: alpha Protein kinases have a characteristic bi-lobal fold. *[The N Terminal lobe contains fivebetawiresandaconserveduniversalaChelixheC-lobeismostlyhelical(coloredred).(a),AnATPmoleculeisboundtoadeepcleftbetweenthe lobes.Thecatalyticallyimportantloopsarecoloredyellow.(b)N-lobestructure.TheGly-richloopcoordinatesthephosphateswithinATP.Threeconservedglycineresiduesareshownasredspheres.Lys72fromthebeta3strandcouplesthephosphatesandthealphaC-helix.Catalyticandregulatory machinerybindstherigidhelicalcoreoftheC-lobe.Theextendedactivationsegment(coloreddarkred)containsaphosphorylationsitethatisbound to b9 (K189) and the HRD-arginine (R165), [Dalmas O, 2010]*.

CellularPhysiopathologyofIsoformsLDH

The LDH enzyme, presented in serum as a tetramer, is composed oftwomonomers,LDH-AandLDH-B,whichcanbegroupedinto 5isoenzymes:LDH-1(B4),LDH-2(B3-A1),LDH-3(B2-A2), LDH-4(B1-A3)andLDH-5(A4)andconvertanaerobictolactate indifferentcells.TotalLDH,whichisderivedfromprocesses.The LDH-Ageneislocatedonchromosome11,whiletheLDH-Bgene islocatedonchromosome12.LDHisusedasamarkertomonitor the response to chemotherapy in patients with neoplasm with or without metastases. [Harrison, 2018].

TheLDHlevelsremainedelevatedafterinitialcytostatictreatment untilcancerremission.Inthemalignantcellsthetransformationof pyruvicacidintolacticacidalteredtheprocessofglycolysisfrom theaerobictotheanaerobicpathway.TheLDHenzymecatalyzes the reversible reduction of pyruvate in lactate by using the cofac- tor NADH as a co-enzyme. Neoplastic conditions promote high intracellular LDH production and increased use of Mg^{2} + during multiple molecular syntheses with the reaction, Pyruvate acid > LDH/NADH > Lactate acid + NAD.

In aerobic glucose metabolism, the oxidation of citric acid requiresADP and Mg^{2+}, which will increase the speed of the reaction: Iso-citric acid + NADP (NAD) --- isocitrate dehydrogenase (IDH)=alpha-ketoglutaricacid.IntheKrebscycle,theIDH1and IDH2 isoenzymes are dependent on the NADP + cofactor which catalyzestheinter-conversionoftheaminoacidD-isocitratetoal- phaketoglutarate.

TheIDH1andIDH2genesaremutatedin>75%ofdifferentma- lignant diseases.Two distinct alterations are caused by tumor-de- rived mutations in IDH1 or IDH2: the loss of normal catalytic activity in the production of α-ketoglutarate (α-KG) and the gain of catalytic activity to produce 2-hydroxyglutarate (2-HG) [*Hartmannc, 2009*].

Thelastproductofthereactionisacompetitiveinhibitorofmulti- pleα-KG-dependentdioxygenaseenzymes,includingdemethylases,prolyl-4-hydroxylase,andTETenzymes(Ten-Eleven-2Translocation),andcausesgenome-widealternationsinhistoneproteins and methylation. DNA [*Raymakers RA, et al., 2009*]. IDH1 and IDH2 mutations are found in primary and secondary leukemias and in malignancies of the pre-leukemic clone, including myelodysplastic syndrome and myeloproliferative neoplasm, [*Wagner K, 2010.*].

The energetic sum of anaerobic glycolysis isΔGo = -34.64 kcal/ mol**.**However,aglucosemoleculecontains686kcal/mol,andthe energy difference (654.51 kcal) allows the potential for un-controlledreactionsduringcarcinogenesis.ThereactionADP^{3}+P^{2-} + H^{2}- ATP + H_2O is reversible. The terminal oxygen from ADP bindstheP^{2-}byforminganintermediatepenta-covalentcomplex, resulting in the formation ofATPand H_2O.This reaction requires Mg^{2+}andanATP-synthetase,whichisknownastheH+-ATPaseor the Fo-F1-ATPase complex. Intracellular calcium induces mitochondrial swelling and aging.The proliferation of osteoclast cells occurs when the intracellular Ca/Mg ratio is 3/2. Mg^{2+} generally interacts with substrates via the inner coordination sphere, stabilizinganionsorreactiveintermediates,bindingATPandactivating molecules for nucleophilic attack [*Kehres, DG, et al, 2012*].

The LDH enzyme, presented in serum as a tetramer, is composed oftwomonomers,LDH-AandLDH-B,whichcanbegroupedinto 5isoenzymes:LDH-1(B4),LDH-2(B3-A1),LDH-3(B2-A2), LDH-4(B1-A3)andLDH-5(A4)andconvertanaerobictolactate indifferentcells.TotalLDH,whichisderivedfromprocesses.The LDH-Ageneislocatedonchromosome11,whiletheLDH-Bgene islocatedonchromosome12.LDHisusedasamarkertomonitor

the response to chemotherapy in patients with neoplasm with or without metastases [8].

The number of chromosome copies in malignant tumors can be correlatedwiththetotalserumLDHvalues.LDHlevelsincancer patients are elevated due to high levels of LDH-3 isoenzyme in patients with malignancies and high levels of LDH-4 and LDH-5 isoenzymes, elevated patients with cancer of liver, muscle, lung andtissuetissues.conjunctive.HighconcentrationsofserumLDH damage the cell membrane.

Normally, cells in the body communicate via intra-cytoplasmic channels and maintain the energetic potential across cell membranes,whichis1-2.5µmolofATPintheformofATP-ADP/ATP-ADP-IMP.Iftheintra-cellularandextra-cellularlevelsof$Mg^{2+}$are high, the extra-cellular charges of the cells will not be uniformly distributed.Thischangeindistributioninducesahighnetpositive charge for the cell and induces a loss of contact inhibition via the electromagneticinductionofoscillation,[*KehresDG*,etal.,2010] *ChienMM*etal.1999,MilionisHJ,1999].Thereafter,malignant cells become invasive and metastasize.

6. Conclusions

NormallevelsofMgwithmoderatelyincreasedLDHlevelswere observed in all patients who had cancer that was in the regression phasefollowinggoodresponsestoaspecificcancertherapy.Low levels of Mg with high levels of serum LDH were observed in all patientswithpoorprognosisandmetastases.Thetotalserumlevel of LDH, which is released by cytolytic cells during the progressionofmalignantdiseases,andtheserumMglevelcanbeusedas markers for monitoring treatment responses in patients with neoplasm with or without metastasis.

References

1. Black CB, Cowan JA. Magnesium-dependent enzymes in nucleic acid biochemistry. The Biological Chemistry of Magnesium. 1995; p: 735-39.
2. Chien MM, Zahradka CE, Newel MK, Fred JH. Fas induced in Bcellsapoptosisrequireanincreaseinfreecytosolicmagnesiumasinearly event. J Biol Chem. 1999; 274: 7059-66.
3. DalmasO,CuelloLG,JoginiV,CortesMD,RouxB,PerozoE.Sruc-tural dynamics of the magnesium-bound conformation of CorAin alipid bilayer. Structure. 2010; 18: 868-78.
4. Harrison, Principles of Internal Medicine, 14 Editions, Ed Theora, Bucharest, Online; Chapter Nutrition-17th, rewritten in year 2018.
5. Hartmann C, Meyer J, Balss J. Capper D, Mueller W, ChirstiansA,et al., Type and frequency of IDH1 and IDH2 mutations are relatedtoastrocyticandoligodendroglialdifferentiationandage:astudy of1,010 diffuse gliomas. Acta Neuropathol. 2009; 118: 464-74.
6. Kehres DG, Maguire ME. Structure, properties and regulation ofmagnesium transport proteins". Bio Metals 2002; 15: 261–70.
7. Lunin VV, Dobrovetsky E, Khutoreskaya G, Zhang R, JoachimiakA,DoyleDA,etal.,CrystalstructureoftheCorAMg2+transporter.Nature. 2006; 440: 833-7.
8. MilionisHJ,BourantasCL,SiamopoulosCK,ElisafMS.Acidbasesand electrolytes abnormalities in Acute Leukemia. Am J Hematol.1999; 62: 201-07.
9. Nadler MJS, Hermosura MC, Inabe K, PerraudAL, Zhu Q, StokesAJ,etal.,LTRPC7isaMg.ATP-regulateddivalentcationchannelrequired for cell viability. Natur. 2001; 411: 590–5.
10. PopescuMP.Cellularmagnesiumhomeostasis(Review).ArchBiochem and Biophysic.s. 2011; 5: 10
11. Raymakers RA, Langemeijer SM, Kuiper RP, Berends M, Jansen JH,vanderReijdenBA.AcquiredmutationsinTET2arecommonin myelodysplastic syndromes. Nat Genet. 2009; 41:838–42.
12. StefanoA,RoinelN,RouffignacC,WittnerM.TransepithelialCa2+and Mg2+ transport in the cortical thick ascending limb of Henle'sloopofthemouseisavoltage-dependentprocess.RenPhysiolBio-chem. 1993; 164: 157–66.
13. SeyfriedTN,SheltonLM.CancerasaMetabolicDisease.NutrMe-tab 2010; 7: 7.
14. Udristioiu A, Bioenergetica celulara normala si maligna, Tratat, ISBN:973-85342-6-7,Chpt.Biofizica,Metabolismenergetic,Indici declasificare,577.23/.38,BibliotecaCentralaUniversitaraBucuresti,Edit.AcademicaBrâncusiTarguJiu,TipografiaEverst,Bucuresti, 2002.
15. WagnerK,DammF,GohringG,GorlichK,HeuserM,KrauterJ,etal. Impact of IDH1 R132 mutations and an IDH1 single nucleotidepolymorphism in cytogenetically normal acute myeloid leukemia:SNPrs11554137isanadverseprognosticfactor.JClinOncol.2010;28: 2356–64.
16. WalderRY,LandauD,MeyerP,ShalevH,TsoliaM,BorochowitzZ.MutationofTRPM6causesfamilialhypomagnesemiawithsecond-ary hypocalcemia. Nat Genet. 2002; 31(2): 171–4.
17. Walter F, Boron P. Medical Physiology: A Cellular and MolecularMg Approach. Elsevier/Saunders, Medical Physiology. Elsevier.2005; p: 871-75.