Original Paper

Relationship between Serum Gamma-Glutamyltransferase and Glutathione Antioxidant System in Patients with Liver Cirrhosis of Various Etiology

MIHNEA MARIAN POMACU[1], CAMELIA ELENA STĂNCIULESCU[2], DIANA MARIA TRAȘCĂ[1], VLAD PĂDUREANU[1], LILIANA DALIA GOGA[3], CĂTĂLINA GABRIELA PISOSCHI[2], ANA MARIA BUGĂ[2]

[1]*4th Department of Medical Specialties, University of Medicine and Pharmacy of Craiova, Romania*

[2]*Department of Pharmaceutical Biochemistry, University of Medicine and Pharmacy of Craiova, Romania*

[3]*Clinical Laboratory of the Emergency County Hospital of Craiova, Romania*

ABSTRACT: Oxidative stress involvement in liver diseases has been extensively studied. A direct assessment of the reactive species incriminated is avoided due to their short lifespan and high cost. For these reasons an inexpensive and easy to perform test for whole body oxidative stress is highly desired. This pilot study was conducted to assess the relationship between γ-glutamyl transferase (GGT) activity and markers of oxidative stress: reduced glutathione (GSH), glutathione peroxidase (GPx) activity and lipid peroxidation in patients with liver cirrhosis due to chronic ethanol consumption and viral hepatitis. Forty-eight patients with alcoholic liver cirrhosis and cirrhosis developed after HBV and HCV infection were included in this study.Blood GSH andGPxand serum GGT and MDAwere assayed and the results were statistically analyzed. The activity of serum GGT was significantly higher in the alcoholic group. The relationship between GGT activity, GSH and MDA levels was different between groups.A strong significant positive correlation between GGT and GSH was noticed for the patients from GGT Q3 and Q4 quartiles in the group of viral liver cirrhosis, while for alcoholics the relationship between GGT and GSH showed the trend for a negative correlation.The values of serum MDA differ significantly between groups ($p<0.015$); a very significant variation was observed at low levels of GGT activity ($p<0.006$). Our study demonstrates that the GSH antioxidant defense system is more compromisedin alcoholic cirrhosisand tends to correlate negatively with GGT. Even in its normal range GGT might be an early and sensitive marker of oxidative stress.

KEYWORDS: *Liver cirrhosis, oxidative stress, γ-glutamyl transferase, glutathione antioxidant system.*

Introduction

Liver cirrhosis is the final pathological state that arise from a variety of chronic liver diseaseswith different etiologies among whichchronic alcohol consumption or hepatic viruses infection.

Cirrhosis is the consequence ofan uncontrolled fibrogenesis,and it ischaracterized by continuous accumulation of extracellular matrix resulting in fibrous scars which destroys the normal architecture of the hepatic lobule.

These architectural changeslead to an impaired liver cells'function, vascular alterations and eventual liver failure [1].

The mechanisms involved in liver fibrosis are various, hepatic stellate cells (HSC) activation and their transdifferentiation into collagen producing myofibroblasts being a key event.

This process can be triggered by different factors: chronic inflammation, cytokines, as well as free radicals [2].

The reactive oxygen species (ROS) are defined as atoms or molecules with unpaired electrons, such as hydroxyl radical (HO) or superoxide radicalanion($O_2^{\cdot -}$), species characterized by a high reactivity.

In humans, at physiological levels, ROS has various biological rolesbeing involved inimmune defenceor acting assignalling molecules that controlproinflammatory and profibrotic events, cell proliferation, apoptosis [3].

From the interaction of $O_2^{\cdot -}$ with the nitric oxide (NO) results their derivative, the peroxynitrite ($ONOO^-$), a powerful reactive nitrogen species (RNS) able to damage many biological molecules [4].

However, in many pathological conditions, ROS/RNS level increases leading to oxidative/nitrosative stress.

Oxidative stress arises asa consequence of an imbalance between the intense production of oxidants and a low capacity to neutralizethem.

In this condition, excessive ROS/RNSattack molecules such as proteins, lipids, or DNA, inducing their structural modification (lipid peroxidation, protein nitration, oxidative DNA damage etc.) which leads to cellular dysfunction.

10.12865/CHSJ.48.04.11

Lipid peroxidation is a hallmark of oxidative stress, cellular lipids that contains polyunsaturated acidsbeingvery susceptible to the oxidation induced by ROS.

This is a chain reaction thatproduce initially alipid radical (L) that is transformed in a lipid peroxyl radical (LOO) in the presence of molecular oxygen.

This reactive species could react with antioxidants and transform inlipid hydroperoxide (LOOH), or could attack another molecule of lipid generating new lipid radicals (L) andpropagatingthe lipid peroxidation [5].

The lipid peroxidation cascade generates unstable peroxides and hydroperoxides in its early steps, compounds that further can decompose intovarious end-products such as epoxides, hydrocarbons or aldehydes, such as malondialdehyde (MDA) or 4-hydroxynonenal [6,7].

These aldehydes are toxic because they react with proteins or DNA, changing their structure and biological roles.

The antioxidants are the tools that cells possess in order to annihilate excessive ROS.

This category includesenzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), and also various non-enzymatic compounds with antioxidant properties, such as glutathione, vitamins E and C.

These antioxidants act alone or synergic to counteract the effects of ROS.$O_2^{\cdot -}$ produced by various reactions is partitioned under SOD actiongenerating O_2 and H_2O_2.

Accumulation of H_2O_2 could generate HO, therefore, to eliminate this possibility, $H_2O_2$is transformed in H_2O and O_2 under CAT action in peroxisomes, or with GPx, which uses glutathione, in mitochondria [8].

The glutathione antioxidant system is considered a key player among the antioxidant mechanisms which counterbalance ROS-generating processes [9].

Glutathione or L-γ-glutamyl-L-cysteinyl-glycine is aubiquitous non-ribosomal tripeptide present in mammals, particularly concentrated in the liver cells [10].

Glutathione exists in the thiol-reduced (GSH) and disulfide-oxidized (GSSG) forms.

In the cytosol of most cells, GSH concentration is about 1-2mM, while in hepatocytes, its concentration can reach about 10mM [11].

In plasma, GSH is in the micromolar range [12].

The sulfhydryl group of cysteine is responsible for GSH biological activity being involved in reduction and conjugation reactions which gives to GSH the role of major defender against oxidative stress [9].

GSH acts as a direct scavenger of ROS and RNS, resulting in the protection of proteins, lipids and DNA.

Under ROS-producing conditions, GSH is oxidized into GSSG by GPx [12,13] and peroxiredoxin Prdx6 [14]; because thisoxidized form is potentially toxic to the cells is necessary to be reconverted to the reduced GSH by GR, a NADPH-dependent enzyme [13].

Thus, GSH:GSSG ratio is often used as a marker of cellular toxicity [15].

In a resting cell, under normal conditions, the GSH: GSSG ratio is around 100:1, but during various types of oxidative stress, thevalues of this ratio has been demonstrated to decrease to 10:1 and even 1:1 (Chai et al, 1994 cited by [16].

Besides its direct involvement in the destruction of ROS and RNS, GSH can also has an indirect contribution to neutralization of oxidative intoxicants in association with vitamins C and Eas part of an interlinking set of redox reactions known as the “antioxidant network” [17].

GSH is synthesized from its precursors, cysteine, glutamic acid and glycine, by the successive action of two enzymes, glutamate-cysteine ligase (GCL) and glutathione synthase (GS), in reactions driven by the hydrolysis of ATP [18].

The synthesis occurs intracellularly, where GSH is very stable.

GSH degradation occurs exclusively extracellularly, under the action of γ-glutamyl transferase or γ-glutamyl transpeptidase (GGT) [19].

GGT is the only enzyme that has the ability to catalyze the degradation of free GSH or from GSH complexes into glutamic acid and a dipeptide, cysteinyl-glycine [20].

It is found on the external surface of specific epithelial cells from kidney, biliary ducts and brain capillaries and allows the recycle of the aminoacids for the intracellular regeneration of GSH in the ”γ-glutamyl cycle”.

Hence, the GSH gradient is dependent on the balance between synthesis, degradation and transport of resulting aminoacids through the cell membrane [18].

Even if the main role of GGT is to hydrolyze extracellular GSH, many research data indicate its involvement in ROS production in the

presence of ferrous or other transition metalsions [21].

ROS/RNS quantification is difficult to perform *in vivo* because these highly reactive molecules have avery low lifespan; for this reason,the evaluation of oxidative stress is based mainly on the quantification of surrogatemarkers, such as individual antioxidants or the evaluation of the total antioxidant capacity, determination of lipid peroxidation end-products, oxidized proteins or products of DNA damage [22].

The aim of the present study was to assess the relation between GGT activity andmarkers of glutathione antioxidant system (GSH and GPx activity) and of lipid peroxidation (MDA) in patients with liver cirrhosis due to chronic ethanol consumption and viral hepatitis.

Material and Methods

Ethical Issues

Forty-eightpatients, with compensated or decompensated liver cirrhosis, hospitalized at the First Clinic of Internal Medicine, Clinical City Hospital "Filantropia" and Second Clinic of Internal Medicine, Emergency County Hospital of Craiova, were included in this pilot studyafter giving their informed consent according to the Declaration of Helsinki and after the study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (no.116/11.11.2019).

Cirrhosis was diagnosticated correlating the clinical examination with the results of laboratory testsand ultrasonographic evaluation.

The following exclusion criteria were taken into account: pregnancy, drug abuse, comorbidities related to the oxidative status (e.g., inflammatory and autoimmune diseases, metabolic syndrome or diabetes mellitus) [23].

Eligible participants in our pilot study were divided as follows: group 1 with alcoholic liver cirrhosis (n=28) and group 2 with non-alcoholic liver cirrhosisdeveloped after HBV and HCV infection (n=20).

For each subject, information regarding the time of disease progression was recorded.

Sample Collection

Blood samples were collected a jeun, in the morning, both in vacuum test tubeswith K_2EDTA and without any anticoagulant (Becton Dickinson, USA).

Blood collected without anticoagulantwas allowed to clot at RT and then centrifugated at 3500rpm for 10 minutes to obtain the serum used later for the measurement of usual biochemical parameters and of Thiobarbituric Acid Reactive Substances (TBARS).

Blood collected in lavender topped K_2EDTA vacutainers was used to performthe quantitation of GSH level and GPxactivity.

Laboratory Biochemical Measurements

Serum proteins, albumin, ALT and AST transaminases, alkaline phosphatase and γ-glutamiltransferase (GGT) were assessed using an automated analyzer Architect c8000 (Abbott Diagnostics, USA).

Commercial kit RANSEL (Randox, UK) was used to measure blood activity of GPx.

TBARS assay was used to evaluate the lipid peroxidation level as a marker of oxidative stress.

We performed TBARS measurement in serum using a spectrophotometric method, previously described, whichquantifies malondialdehyde (MDA) concentration, a major product of fatty acid peroxidation.

Briefly, human serum was treated with 5% trichloroacetic acid (TCA) and 0.2M Tris-HCl pH=4.7.

After 10 minutes of incubation at RT, the sample was mixed with 0.55M thiobarbituric acid (TBA) in 2M sodium sulphate, heated at 90°C for 45 minutes and cooled on ice [23].

The cold mixture was centrifuged at 15,000xg in a refrigerated centrifuge (Eppendorf 5417R, Eppendorf AG, Germany).

MDA reacts with TBA and forms a pink color product with specific absorption at 532nm [24].

MDA concentration was calculated using the molar extinction coefficient of MDA (1.55×10^5 $M^{-1}cm^{-1}$).

The results were expressed as μmoles/mL MDA.

Statistical Analysis

Data were analyzed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA).

Variables with normal distribution were reported as mean.

Standard deviation (SD) and standard error of the mean (SEM) were also calculated.

Non-normal variables were reported as median (interquartile range-IQR).

The significant differences between studied groups for normally distributed data was performed using One-Way Anova Test.

Pearson correlation coefficient was computed to assess the relationship between blood GSH level and serum GGT activity.

A value of $P < 0.05$ was considered significant.

10.12865/CHSJ.48.04.11

Results

Table 1 and 2 present the baseline findings for the patients included in the groups 1 and 2 based on the GGT quartiles.

The variables in table 1 are expressed as median: Q1, quartile 1 (n=5): GGT<65UI/L; Q2, quartile 2 (n=9): GGT between 65-80UI/L; Q3, quartile 3 (n=7): GGT between 80-138UI/L; Q4, quartile 4 (n=7): GGT≥138UI/L.

Table 1. Baseline characteristics of the group 1 included patients based on their GGT quartiles.

Characteristics	Total Group 1	Group 1 (GGT level)				P value*
		Q1 (<65UI/L)	Q2 (65-80UI/L)	Q3 (80-138UI/L)	Q4 (≥138UI/L)	
		Test median (IQR)				
GGT (UI/L)	80.0 (65.0-138.0)	45.0 (42.5-53.5)	70.0 (65.0-75.5)	106.0 (82.0-129)	223.0 (187-281)	P< 0.05
AST (UI/L)	51.0 (14.0-294.0)	31.0 (18.5-37.5)	34.0 (30.0-61.0)	60.0 (28.0-131)	78.0 (54.0-137.0)	P< 0.05
ALT (UI/L)	29.5 (14.0-119.0)	21.0 (19.0-32.5)	24.0 (19.00-36.5)	41.00 (25.0-59.0)	35.0 (25.0-74.0)	P< 0.05
Total Protein (g/dL)	7.3 (5.2-9.44)	7.2 (6.75-8.82)	7.1 (6.45-7.7)	7.2 (6.3-7.4)	7.3 (6.6-7.4)	P< 0.05
Albumin (g/dL)	2.9 (2.05-5.0)	4.1 (3.43-4.44)	2.6 (2.4-3.15)	3.1 (2.7-4.2)	2.8 (2.5-3.03)	P< 0.05
ALP (UI/L)	150.0 (67-841)	106.0 (73.5-388.5)	135 (98.0-261.0)	228.0 (78.0-427.0)	170 (104-311)	P< 0.05

*p value <0.05-significant difference between group 1 and group 2 median. GGT-γ glutamyl transpeptidase; AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP- alkaline phosphatase.

In the Table 2, the variables are expressed as median as follows: Q1, quartile 1 (n=5): GGT<25UI/L; Q2, quartile 2 (n=5): GGT between 25-38UI/L;Q3, quartile 3 (n=5): GGT between 38-93 UI/L;Q4, quartile 4 (n=7): GGT ≥93UI/L.

Table 2. Baseline characteristics of the group 2 included patients based on their GGT quartiles.

Characteristics	Total Group 2	Group 2 (GGT level)				P value
		Q1 (<25UI/L)	Q2 (25-38UI/L)	Q3 (38-93UI/L)	Q4 (≥93UI/L)	
		Test median (IQR)				
GGT (UI/L)	38.00 (24.5-93.25)	21,0 (21.0-22.0)	33.0 (32.0-33.0)	59.5 (43.0-76.00)	157.5 (99.0-216.0)	P< 0.05
AST (UI/L)	39.5 (26.0-94.0)	48.0 (37.0-58.0)	39.0 (35.0-42.0)	29.0 (26.0-32.0)	81.0 (67.0-94.0)	P< 0.05
ALT (UI/L)	30,5 (25.0-48.0)	30.5 (29.0-32.0)	37.5 (29.00-46.00)	25.0 (24.75-25.10)	44.5 (41.0-48.0)	P< 0.05
Total Protein (g/dL)	7.1 (4.7-7.7)	6.9 (6.6-7.2)	6.65 (6.10-7.20)	7.45 (7.2-7.7)	5.85 (4.7-7.0)	P< 0.05
Albumin (g/dL)	3.25 (2.5-3.8)	3.2 (2.6-3.8)	3.0 (2.5-3.5)	2.85 (2.6-3.1)	3.4 (3.15-3.5)	P< 0.05
ALP (UI/L)	246.5 (68-468)	214.5 (68.0-361.0)	269 (70.0-468.0)	204.5 (70.0-339.0)	257 (154-360)	P< 0.05

*p value <0.05-significant difference between group 1 and group 2 median GGT-γ glutamyl transpeptidase; AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP-alkaline phosphatase.

As we expected, the activity of GGT was higher in patients with alcoholic cirrhosis.

There was a significant difference between the median values of all laboratory tests for the patients divided in the four GGT quartiles for both etiological groups of cirrhosis.

A correlation between serum levels of GGT activity and blood GSH based on the GGT quartiles for both groups of study is presented in the Table 3 and the Figure 1.

Table 3. Correlation between the serum GGT activity and blood GSH level in group 1 vs. group 2 and Q1-Q4 subgroups according with GGT level.

	GGT (level)	Group 1 Pearson r (95% CI)	P value	Group 2 Pearson r (95% CI)	P value*
GSH (mg/dL)	**Q1**	-0.11(-0.91-0.85)	0.852	-0.25(-0.928-0.811)	0.685
	Q2	-0.011(-0.67-0.66)	0.978	-0.75(-0.981-0.400)	0.148
	Q3	0.139(-0.686-0.808)	0.767	**1.00(0.999-1.000)**	**<0.0001**
	Q4	-0.684(-0.948-0.144)	0.091	**1.00(1.000-1.000)**	**<0.0001**
	Total	-0.27 (-0.59-0.12)	0.167	**0.65(0.20-0.87)**	**0.0091**

*p value < 0.05-significant correlation; p value > 0.05-unsignificant correlation

Data revealed a significant correlation between GSH and GGT for patients in group 2 (liver cirrhosis with viral etiology); the values of Pearson r coefficient and P-values for the patients from the Q3 and Q4 of GGT quartiles in this group indicated a strong significant positive correlation.

For the patients in group 1 (alcoholic cirrhosis), we observed the trend for a negative correlation, almost significant for GGT Q4 quartile.

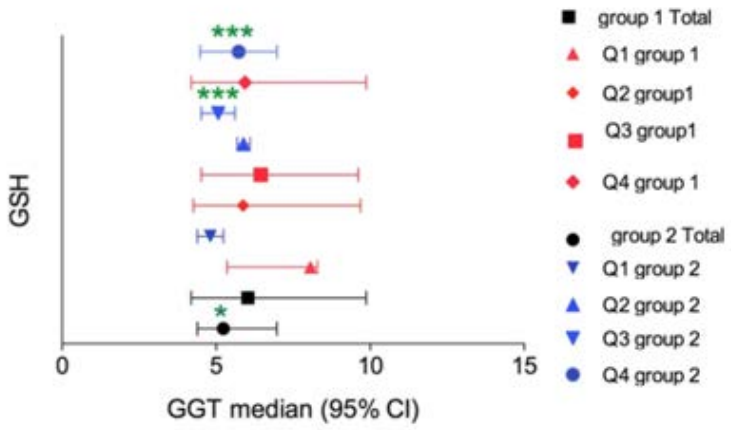

Figure 1. Correlation between serum GGT activity and blood GSH level in group 1 (alcoholic liver cirrhosis) vs. group 2 (non-alcoholic liver cirrhosis) and between Q1-Q4 subgroups.

Regarding the activity of GPx, we didn't notice a significant difference between the patients from the two groups divided based on GGT quartiles (Table 4 and Figure 2A-C).

Table 4. Values of GPx activity in the patients included in the two groups (total group 1 vs. group 2) and intra and inter groups based on GGT quartiles.

GGT (level)	Group 1 **GPx** (mean ±SEM)	Group 2 **GPx** (mean±SEM)	P value
Q1	642.7±233.0	485.8±25.39	0.522
Q2	533.1±105.8	460.6±23.51	0.879
Q3	352.7±61.41	454.2±48.91	0.228
Q4	524.0±120.5	588.8±109.1	0.699
Total	488.7±55.18	497.4±34.14	0.237

*p value <0.05-significant difference between group 1 and group 2 means.

10.12865/CHSJ.48.04.11

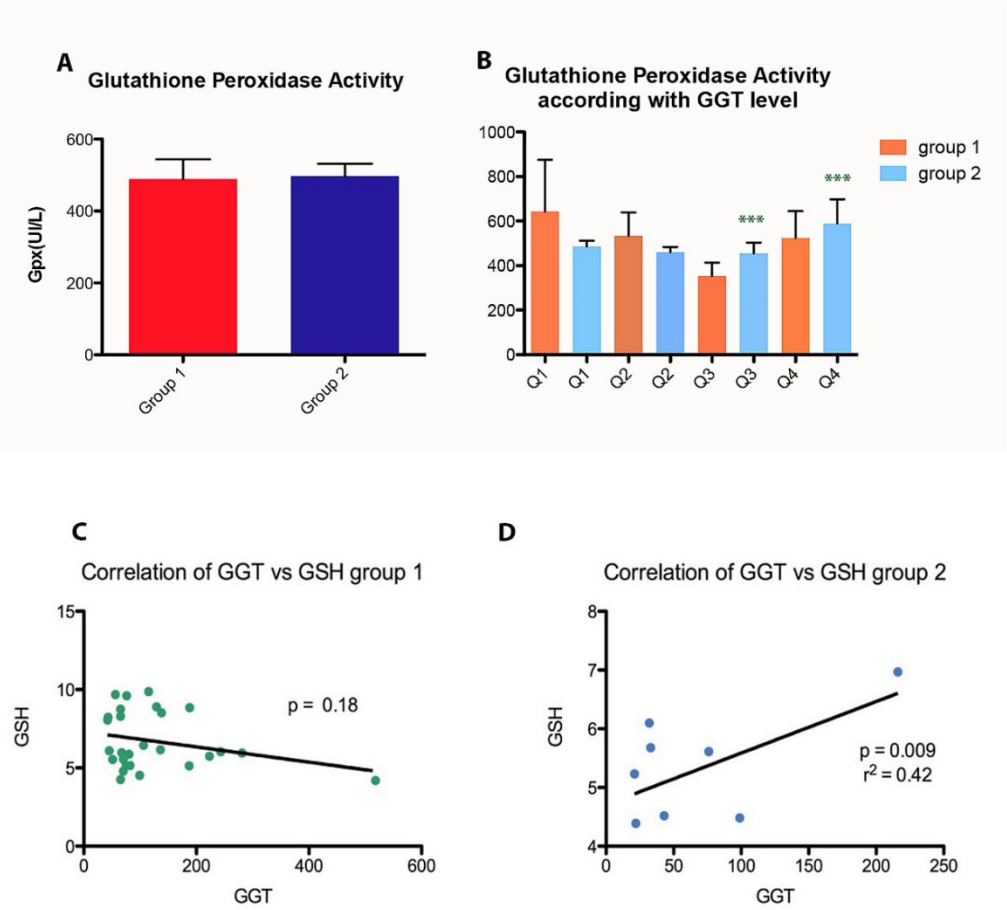

Figure 2. A. Variation of glutathione peroxidase (GPx) activity group 1 (alcoholic liver cirrhosis) vs. group 2 (non-alcoholic liver cirrhosis); B. Intra and intergroup comparison of GPx activity according to GGT level; C. Correlation of GGT vs. GSH level in group 1 (p>0.05); D. Correlation of GGT vs. GSH level in group 2 (p=0.009).***

Results regarding the levels of serum MDA as a marker of lipid peroxidation are presented in Table 5 and Figure 3.

These revealed a significant difference of lipid peroxides levels in patients from group 1 vs. patients from group 2 being highly significant from those in the GGT Q1 quartile.

This means that GGT within its normal range might be an early and sensitive marker related to oxidative stress.

Table 5. Values of MDA, marker of lipid peroxides, in the two groups (total group 1 vs. group 2) and intra and inter groups based on GGT quartiles.

GGT (level)	**Group 1**	**Group 2**	
	MDA (mean±SEM)	**MDA**(mean±SEM)	**P value**
Q1	0.607±0.04	0.442±0.01	**0.006***
Q2	0.750±0.105	0.468±0.007	0.07
Q3	0.893±0.173	0.584±0.004	0.166
Q4	0.674±0.09	0.613±0.014	0.599
Total	0.751±0.06	0.527±0.02	**0.015***

*p value <0.05-significant difference between group 1 and group 2 means

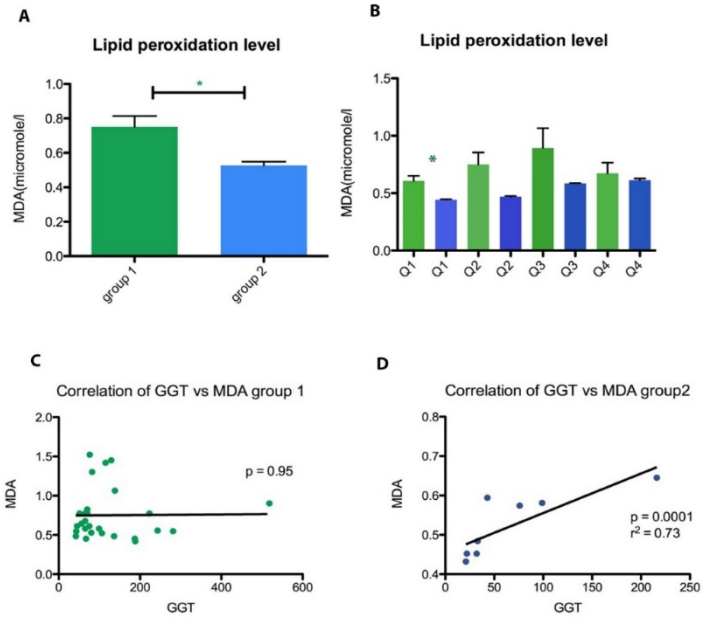

Figure 3. A. Variation of lipid peroxidation marker (MDA) in group 1 (alcoholic liver cirrhosis) vs. group 2 (non-alcoholic liver cirrhosis), p<0.05(*); B. Intra and intergroup comparison of MDA level according to GGT level; C. Correlation of GGT vs. GSH level in group 1 (p>0.05); D. Correlation of GGT vs GSH level in group 2 (p=0.0001).***

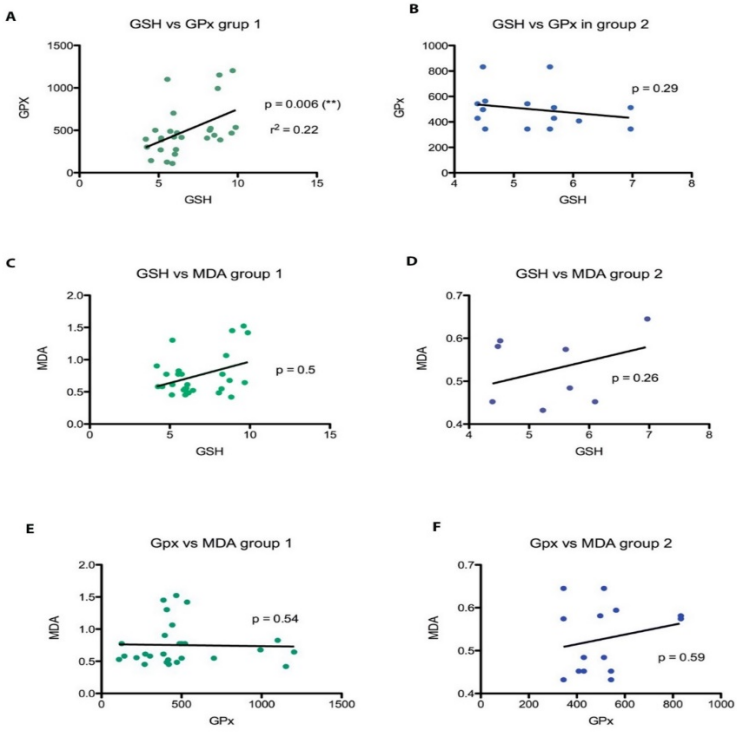

Figure 4. A. Correlation of GPx with GSH level in group 1 (alcoholic liver cirrhosis), p<0.05 (**); B. Correlation of GPx with GSH level in group 2 (non-alcoholic liver cirrhosis), p>0.05 (ns); C. Correlation of GSH with lipid peroxidation marker (MDA) in in group 1 (alcoholic liver cirrhosis); D. Correlation of GSH with lipid peroxidation marker (MDA) in in group2 (non-alcoholic liver cirrhosis), p>0.05 (ns); E. Correlation of GPx with lipid peroxidation marker (MDA) level in group 1 (alcoholic liver cirrhosis), p>0.05 (ns); F. Correlation of GPx with GSH level in group 2 (non-alcoholic liver cirrhosis), p>0.05 (ns).

10.12865/CHSJ.48.04.11

Discussion

Oxidative stress involvement in liver diseases has been extensively studied and the impact of ROS/RNS as a pathogenic trigger of various liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease, viral hepatitis and hepatocellular carcinoma has been reported [25].

Biochemical markers used to assess the oxidative stress in liver diseases and not only are numerous, such as lipid peroxides, F2 isoprostanes, 8-hydroxydeoxyguanosine and protein carbonyls [26].

These biomarkers describe the oxidative damage of lipids, DNA and proteins, but the tests are avoided because, on one hand, they are expensive and, on the other hand, are difficult to perform in good conditions due to the short lifespan of the markers assessed.

That's why an inexpensive and easy to perform test for whole body oxidative stress is highly desired by those involved in studies related to chronic liver diseases.

As it is known since the 1960s, GGT is very sensitive for the diagnosis of liver injury, as a marker of cholestasis, but it has poor specificity for a certain etiology.

Further research data have suggested that it also has a key role in the antioxidant protection and xenobiotic metabolism.

Various medications and ethanol abuse are able to induce the rise of GGT level.

This and its rise in other metabolic diseases (diabetes mellitus, obesity, pancreatitis) sustain the lack of specificity in GGT [27].

The conditions that increase GGT activity (chronic ethanol consumption, obstructive liver disease)led to an increased ROS production and the threat of a GSH depletion [28].

Other studies demonstrated serum GGT augmentation following a decrease in liver GSH as a consequence of drug induction [29] which can sustain the involvement of GGT in liver GSH homeostasis.

Although, it is not clear to what extent intracellular and extracellular GGT level reflects oxidative stress, however, assessment of serum GGT is inexpensive and easy to perform in any laboratory.

This is the reason why we intended to assess the relationship between GGT activity, GSH, GPx and lipid peroxides as biomarkers of oxidative stress in this pilot study.

Our results revealed a different trend for the interdependence between GGT activity and GSH level in various cirrhosis conditions: for the patients with cirrhosis of viral etiology, we observed a strong positive correlation between GSH level and GGT activity, while for those with alcoholic cirrhosis, these markers tend to negatively correlate.

These findings are related tothe different pathogenic pathways Chronic ethanol consumption stimulates the proliferation of the smooth endoplasmic reticulum and the release of GGT [28].

Ethanol oxidationgenerates acetaldehyde, its toxic metabolite, and induce a prooxidant status in the liver cells [30].

GSH plays a key role in the maintenance of the redox status and its decline is indicative for an increased oxidative stress.

Oxidative conditions induce apoptosis of the lining epithelial cells of the bile ducts [31] and thereby favor the release of GGT.

GGT serves as both an antioxidant and a prooxidant because outside the cells its products, cysteine-glycine and cysteine are able to reduce ferric to ferrous ions producing ROS as coproducts of this reduction [32].

An easily detectable marker of ROS effects is MDA, generated as one of the products of the oxidative stress-induced lipid peroxidation.

In our study, MDA level was higher in the group of alcoholic cirrhosis;when comparing the values between groups stratified according GGT its variation was highly significant only for the GGTQ1 quartile.

This suggests that even in its normal range GGT might be a sensitive marker related to oxidative stress.

Also, we found that GGT level is significant correlated with GSH level in non-alcoholic liver cirrhosis ($p=0.009$) (Figure 2C).

In alcoholic liver diseases the correlation between GGT level and GSH level did not reach the statistical significance ($p=0.18$), Figure 2D.

One possible explanation for this is due to the limited number of patients included in this pilot study.

In contrast, we did not find any significant correlation between lipid peroxidation biomarker, MDA and GGT (Figure 3 C,D).

The low correlation between all the markers assessed with GGT activity could be related to the small number of patients included, a limit of our study, and also to the fact that several other factors affect GGT expression.

Besides ethanol and xenobiotic metabolism, GGT activity has been found to be influenced by

sex, aging, smoking, weight, diet and drinking of boiled coffee [33-36].

That's why is important to increase the number of patients selected in order to refine the interdependence of serum GGT activity level and the various biomarkers of oxidative stress.

Correlation of different oxidative stress biomarkers in non-alcoholic and alcoholic liver cirrhosis showed that, here is a significant correlation between GSH and GPx level in alcoholic liver cirrhosis, but not in non-alcoholic liver cirrhosis.

Conclusions

This study demonstrates that GSH antioxidant defense system is more compromisedin alcoholic cirrhosisand tends to correlate negatively with GGT.

Even in its normal range GGT might be an early and sensitive marker related to oxidative stress.

Refining the interference GGT-oxidative stress as a pathogenic mechanism of liver cirrhosis is important for a better management of the disease.

Also, here we report that GSH and GPx can be potential biomarkers of oxidative distress in alcoholic liver lesions.

Conflict of interests

None to declare

References

1. Osna NA, Donohue TM, Jr, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. Alcohol research: current reviews, 2017, 38(2):147-161.
2. Jung YK, Yim HJ. Reversal of liver cirrhosis: current evidence and expectations. The Korean journal of internal medicine, 2017, 32(2):213-228.
3. Checa J, Aran JM. Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. Journal of inflammation research, 2020, 13:1057-1073.
4. Radi R. Peroxynitrite, a stealthy biological oxidant. The Journal of biological chemistry, 2013, 288(37):26464-26472.
5. El-Aal HAHMA, editor. Lipid Peroxidation End-Products as a Key of Oxidative Stress: Effect of Antioxidant on Their Production and Transfer of Free Radicals2012.
6. Petrovic S, Arsic A, Ristic-Medic D, Cvetkovic Z, Vucic V. Lipid Peroxidation and Antioxidant Supplementation in Neurodegenerative Diseases: A Review of Human Studies. Antioxidants, 2020, 9(11)
7. Ilie M, Margină D. Trends in the Evaluation of Lipid Peroxidation Processes. In: Catala A (Eds): 111-130, IntechOpen Limited, 2012, London,
8. Fuloria S, Subramaniyan V, Karupiah S, Kumari U, Sathasivam K, Meenakshi DU, Wu YS, Sekar M, Chitranshi N, Malviya R, Sudhakar K, Bajaj S, Fuloria NK. Comprehensive Review of Methodology to Detect Reactive Oxygen Species (ROS) in Mammalian Species and Establish Its Relationship with Antioxidants and Cancer. Antioxidants, 2021, 10(1)
9. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C. Changes in Glutathione Content in Liver Diseases: An Update. Antioxidants, 2021, 10(3)
10. Lu SC. Glutathione synthesis. Biochimica et biophysica acta, 2013, 1830(5):3143-3153.
11. Meister A. Glutathione metabolism and its selective modification. The Journal of biological chemistry, 1988, 263(33):17205-17208.
12. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Molecular aspects of medicine, 2009, 30(1-2):1-12.
13. Csiszár J, Horváth E, Bela K, Gallé Á, editors. Glutathione-Related Enzyme System: Glutathione Reductase (GR), Glutathione Transferases (GSTs) and Glutathione Peroxidases (GPXs)2016.
14. Ralat LA, Manevich Y, Fisher AB, Colman RF. Direct evidence for the formation of a complex between 1-cysteine peroxiredoxin and glutathione S-transferase pi with activity changes in both enzymes. Biochemistry, 2006, 45(2):360-372.
15. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2003, 57(3-4):145-155.
16. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J, Trnkova L, Kruseova J, Eckschlager T, Kizek R. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncology letters, 2012, 4(6):1247-1253.
17. Pangrazzi L, Balasco L, Bozzi Y. Natural Antioxidants: A Novel Therapeutic Approach to Autism Spectrum Disorders? Antioxidants, 2020, 9(12)
18. Aquilano K, Baldelli S, Ciriolo MR. Glutathione: new roles in redox signaling for an old antioxidant. Frontiers in pharmacology, 2014, 5:196.
19. Baudouin-Cornu P, Lagniel G, Kumar C, Huang ME, Labarre J. Glutathione degradation is a key determinant of glutathione homeostasis. The Journal of biological chemistry, 2012, 287(7):4552-4561.
20. Pompella A, Corti A, Paolicchi A, Giommarelli C, Zunino F. Gamma-glutamyltransferase, redox regulation and cancer drug resistance. Current opinion in pharmacology, 2007, 7(4):360-366.
21. Lee DH, Blomhoff R, Jacobs DR, Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? Free radical research, 2004, 38(6):535-539.
22. Arauz J, Ramos-Tovar E, Muriel P. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Annals of hepatology, 2016, 15(2):160-173.
23. Pomacu MM, Trasca MD, Padureanu V, Buga AM, Andrei AM, Stanciulescu EC, Banita IM, Radulescu D, Pisoschi CG. Interrelation of inflammation and oxidative stress in liver cirrhosis. Experimental and therapeutic medicine, 2021, 21(6):602.

 10.12865/CHSJ.48.04.11

24. Yagi K. Assay for blood plasma or serum. Methods in enzymology, 1984, 105:328-331.
25. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International journal of molecular sciences, 2015, 16(11):26087-26124.
26. Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. The Journal of nutrition, 2003, 133 Suppl 3:933S-940S.
27. Brennan PN, Dillon JF, Tapper EB. Gamma-Glutamyl Transferase (gamma-GT)-an old dog with new tricks? Liver international : official journal of the International Association for the Study of the Liver, 2022, 42(1):9-15.
28. Singh M, Gupta S, Singhal U, Pandey R, Aggarwal SK. Evaluation of the oxidative stress in chronic alcoholics. Journal of clinical and diagnostic research : JCDR, 2013, 7(8):1568-1571.
29. Whitfield JB. Gamma glutamyl transferase. Critical reviews in clinical laboratory sciences, 2001, 38(4):263-355.
30. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism, 2003, 27(4):277-284.
31. Salunga TL, Cui ZG, Shimoda S, Zheng HC, Nomoto K, Kondo T, Takano Y, Selmi C, Alpini G, Gershwin ME, Tsuneyama K. Oxidative stress-induced apoptosis of bile duct cells in primary biliary cirrhosis. Journal of autoimmunity, 2007, 29(2-3):78-86.
32. Corti A, Belcastro E, Dominici S, Maellaro E, Pompella A. The dark side of gamma-glutamyltransferase (GGT): Pathogenic effects of an 'antioxidant' enzyme. Free radical biology & medicine, 2020, 160:807-819.
33. Whitehead TP, Robinson D, Allaway SL. The effects of cigarette smoking and alcohol consumption on serum liver enzyme activities: a dose-related study in men. Annals of clinical biochemistry, 1996, 33 (Pt 6):530-535.
34. Skurtveit S, Tverdal A. Erratum to "Sex differences in gamma-glutamyltransferase in people aged 40-42 years in two Norwegian counties". Drug and alcohol dependence, 2003, 69(1):103-106.
35. Danielsson J, Kangastupa P, Laatikainen T, Aalto M, Niemelä O. Dose- and Gender-dependent Interactions between Coffee Consumption and Serum GGT Activity in Alcohol Consumers. Alcohol and Alcoholism, 2013, 48(3):303-307.
36. Lee D-H, Steffen LM, Jacobs DR, Jr. Association between serum γ-glutamyltransferase and dietary factors: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. The American Journal of Clinical Nutrition, 2004, 79(4):600-605.

Corresponding Author: Cătălina Gabriela Pisoschi, Department of Pharmaceutical Biochemistry, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania
e-mail: catalina.pisoschi@gmail.com