*IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*
*ISSN: 2455-264X, Volume 5, Issue 4 (Jul. – Aug. 2019), PP 15-19*
*www.iosrjournals.org*

# Gamma Glutamyl Transpeptidase, De Ritis Ratio, Maddrey Discriminant Function in Chronic Alcoholic Liver Disease and Viral Hepatitis

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***Abstract:*** *Alcohol abuse is a growing health problem in India with a prevalence of 10.4% in men. Alcoholic liver disease (ALD)is the predominant form of chronic liver disease with increasing morbidity and mortality. According to National Integrated Disease Surveillance Programme (IDSP -2011-2013) 8,04,782 seropositive viral hepatitis cases were recorded in India. In the present study Icterus subjects 25 each were selected based on laboratory analysis in Chronic alcoholics and seropositive viral hepatitis. In all subjects liver enzymes were analysed - Serum Transaminases, Gamma Glutamyl Transferase, Alkaline Phosphatase and Prothrombin time. De Ritis Ratio and Maddrey Discriminant Function were calculated. Similrly age and sex matched healthy controls (25) were selected and the same analytes were estimated. GGT and MDF were significantly elevated (P value <0.001) between groups and within the same group. De Ritis Ratio was not statistically significant (p value 0.44) between the groups and within the same group. Incidentally most subjects of CALD(75%) and Viral Hepatitis (50%) displayed De Ritis ratio of >2.0 which was a bad prognostic marker. The study was concluded with an observation that GGT may be included in the group of Liver Function Tests & scoring systems are better than invasive techniques to assess prognosis.*

***Key words:*** *Chronic Alcoholic Liver Disease, Viral Hepatitis, De Ritis Ratio , Discriminant Function, γ Glutamyl Transferase.*

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Date of Submission: 01-07-2019 Date of acceptance: 16-07-2019

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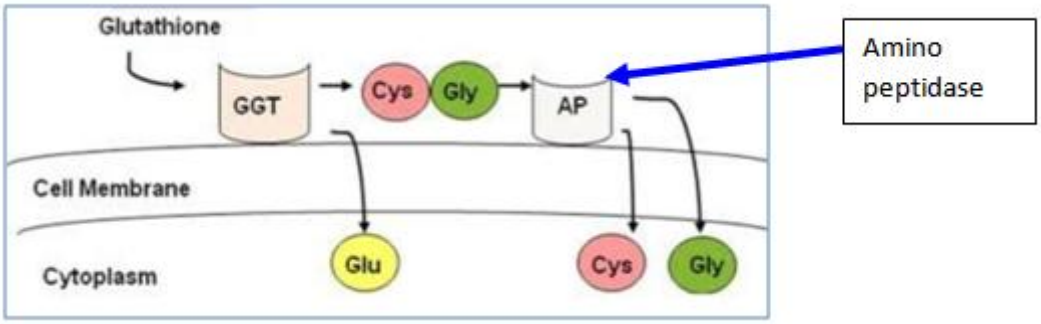
## I. Introduction

Alcohol abuse is a growing health problem in India with a prevalence of 10.4% in men. Alcoholic liver disease (ALD)is the predominant form of chronic liver disease secondary to amount and duration of alcohol consumption. The risk of liver disease increases if more than 60-80gms/day consumed for more than 10 years. Good range of biomarkers of high alcohol consumption were studied like carbohydrate deficient transferring(CDT),Gamma glutamyl transferase(GGT- EC 2.3.2.2), Transaminase ratio (DeRitis ratio) , Glascow alcoholic hepatitis (GAH) score and Maddrey Discriminant Function(MDF). About 40million people fall a prey to viral hepatitis acute/chronic each year in India with a mortality rate of 0.5 -1%. According to National Integrated Disease Surveillance Programme (IDSP -2011-2013) 8,04,782 cases of seropositive viral hepatitis were recorded in all 35 States in India. [1] 74% of these cases were found to be due to HAV. Deaths due to viral hepatitis were more than those due to AIDS/ Tuberculosis. Several studies were conducted to identify biomarkers of viral hepatitis apart from liver biopsies and ultrasonography. With this background we tried to analyze and identify a suitable score to detect liver damage either due to alcohol abuse or any of the viruses.

In hepatocytes Alanine Transaminase(ALT # EC No.2.6.1.2) is found in cytoplasm only whereas isoenzymes of Aspartate transaminase(AST# EC No.2.6.1.1)exist in mitochondria and cytoplasm**.** [2,3]. Cytosolic isoenzyme form of AST enters circulation. In liver disease , mitochondrial AST is elevated with long half life.[4] Alkaline phosphatase (ALKP# EC No. 3.1.3.1) arises from bone, intestine, liver and placenta.. Gamma glutamyl transferase(GGT- EC 2.3.2.2),is a hepatic microsomal enzyme and membrane bound glycoprotein.; Catalyzes transfer of γ-glutamyl moiety from Glutathione (GSH) to various peptide acceptors.

**Fig 1:** Hydrolysis of Glutathione by GGT




GGT is induced by alcohol. Measurement of serum GGT showed sudden elevation with recent bout of alcohol.[5]. Elevation of serm GGT and ALKP simultaneously indicates hepatic origin of ALKP.

AST/ALT ratio (De Ritis Ratio) was found to be a useful parameter to differentiate alcoholic liver disease from Nonalcoholic Liver Disease in previous studies [6]. De Ritis ratio of >2.0 indicates hepatic damage due to alcohol abuse. Scoring systems based on biomarkers have been developed for assessing liver damage and progression to fibrogenesis. Algorithms like Model for End Stage Liver Disease (MELD), Combined Clinical and Laboratory Index (CCLI),Maddrey Discriminant Function (MDF) are few such indices. MDF is calculated based on Total Bilirubin (mgs/dl) and Prothrombin time(PT). MDF of >32 indicates poor prognosis and higher fatality rate.

**Aim and Objectives**

In the present study we tried to determine

a)Different biochemical parameters to assess severity of Liver Disease.

b) Utility of GGT as a routine biochemical parameter in Liver Function Tests.(LFT)

c) Biomarker based scoring system to differentiate ALD from Viral Hepatitis.

## II. Material and Methods

This is an institution based retrospective study. Indiviuals admitted in medical wards of Government General Hospital , Guntur,Andhra Pradesh were selected for this study from January to April 2019. Subjects with history of alcohol consumption for more than 15yrs, admitted with complications of chronic liver disease (N=25) were selected - Group I. Subjects seropositive for Viral Hepatitis (N=25) were selected as Group - II. Age and sex matched healthy controls (N=25) were selected as Group-III. Individuals positive for Retroviral Disease were eliminated from study.5ml Venous blood samples were collected from all subjects under strict aseptic conditions. Serum was separated by centrifugation.

The following biochemical parameters were analysed in Beckman Coultier Au 480.

1.Total Bilirubin - Diazo method of Ehrlisch

2.AST & ALT - Kinetic method

3.ALKP – Method of Bower's and McComb (Conversion of pNitro Phenyl Phosphate to pNitro Phenol in the presence of AMP)

4.GGT - Analyzed on Erba chem. 7 –semiautoanalyzer –Carboxy substrate method – (kits were used from Coral clinical systems)

5. Prothrombin time (PT) - Coagulation method

Following calculations were made

1. AST/ALT ratio (De Ritis ratio)
2. Maddrey Discriminant Function (MDF) -=4.6(Patient's PT - Control PT)+ T.Bil.(mg/dl)

Mean and Standard Deviation (SD) was calculated for all analytes. Statistical analysis was performed by student's 't' test and one-way ANOVA of means at a significance level of <0.05.

## III. Results

All observations made in the study were shown in Tables 1 as mean and Standard Deviation(SD).The values of Total Bilirubin , transaminases, ALKP & GGT were significantly well above normal range and more so in CALD. More than 75% subjects were having 3-fold increase of GGT , De Ritis ratio of >2.0 and MDF of >32 in CALD. Similarly less than 50% subjects of Viral Hepatitis showed elevated GGT, De Ritis ratio of >2.0 and DF >32.

**Table 1** – Mean and SD of analytes in all 3 Groups

| **Group I Chr.Alcoholic Liver Disease (CALD)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **N=25** | T.Bil. | SGOT (AST) | SGPT (ALT) | ALKP | GGT | PT | Deritis ratio | DF |
| Normal | 0.4-1.0 mg/dl | 13-35 IU/L | 15-47 IU/L | 85-105 IU/L | 10-35 IU/L | <14 Seconds | <2.0 | <32 |
| Mean | 11.58 | 198.56 | 107.56 | 186.24 | 91.64 | 20.57 | 2.48 | 44.22 |
| SD (±) | 7.126 | 220.12 | 138.6 | 98.172 | 110.7011 | 8.36 | 1.01 | 40.97 |
| **N = 25** | **–Group – II Viral Hepatitis (VH)** | | | | | | | |
| Mean | 10.96 | 222.64 | 134.04 | 198.6 | 61.16 | 18.56 | 2.599 | 32.3 |
| SD ( ±) | 5.82 | 210.47 | 169.22 | 100.13 | 31.59 | 3.99 | 2.53 | 20.27 |
| **N= 25** | **Group - III - Controls** | | | | | | | |
| Mean | 0.7 | 41.92 | 31.16 | 114 | 25.16 | 17.08 | 1.97 | 14.87 |
| SD (±) | 0.185 | 31.064 | 28.165 | 55.17 | 10.03 | 3.377 | 1.65 | 15.542 |

Student's 't' test of GGT and MDF scores of CALD & VH compared to controls were statistically significant with p-value of <0.001 and <0.00001 respectively(Table 2).De Ritis ratio is not significant amongst cases as well as compared to controls. Similarly one way ANOVA of GGT and MDF was statistically significant with p-value of <0.003 & <0.00001 respectively.(Table3 &5) .Whereas one way ANOVA of means of De Ritis ratio was not significant. (Table 4; p-value o 0.44). Another significant finding was elevated AST levels in both CALD & VH subjects compared to ALT.Though in both groups, I & II, AST & ALT levels were 6-8 times Upper level Normal(ULN), we could not establish any significance relating to prognosis.

**Table -2** Student's 't' test

| Parameter | Groups | P value | Significance |
|---|---|---|---|
| GGT | I Vs III | <0.002 | Significant |
| | II Vs III | <0.00001 | Highly significant |
| | I Vs II | 0.09 | NOT significant |
| De Ritis Ratio | I Vs III | 0.09 | NOT Significant |
| | II Vs III | 0.15 | NOT Significant |
| | I Vs II | 0.41 | NOT Significant |
| MDF | I Vs III | <0.0007 | Significant |
| | II Vs III | <0.00001 | Highly significant |
| | I Vs II | 0.09 | NOT Significant |

**Table 3 -** ONE WAY ANOVA of GGT

| | **CALD (Gr-I)** | **VH (Gr-II)** | **Controls (Gr-III)** | **p- value** |
|---|---|---|---|---|
| **N** | **25** | **25** | **25** | |
| **Mean** | 61.16 | 91.64 | 25.16 | |
| **SD (±)** | 31.5907 | 110.7011 | 10.0319 | |
| | *F* ratio = 6.21999 | | | <0.003 |

**Statistically significant**

**Table – 4** One way of De Ritis Ratio

| | **CALD (Gr-I)** | **VH (Gr-II)** | **Controls (Gr-III)** | **p- value** |
|---|---|---|---|---|
| **N** | **25** | **25** | **25** | |
| **Mean** | 2.4832 | 2.5948 | 1.9732 | |
| **SD (±)** | 1.0144 | 2.5322 | 1.6508 | |
| **F ratio = 0.8102** | | | | **0.4487** |

**Statistically not significant**

**Table 5 -** ONE WAY ANOVA of MDF ( *Maddrey Discriminant Function)*

| | **CALD (Gr-I)** | **VH (Gr-II)** | **Controls (Gr-III)** | **p- value** |
|---|---|---|---|---|
| **N** | **25** | **25** | **25** | |
| **Mean** | 44.228 | 32.3 | 14.872 | |
| **SD (±)** | 40.9753 | 20.2732 | 15.5429 | |
| *F ratio* = 7.01133 | | | | <0.001 |

**Statistically significant**

**Table 6 -** ONE WAY ANOVA of analytes in Group – I (N=25)

| | **GGT** | **ALKP** | **Deritis ratio** | **MDF** | **p-value** |
|---|---|---|---|---|---|
| **Mean** | 91.64 | 186.24 | 2.4832 | 44.228 | |
| **SD (±)** | 110.7011 | 98.1726 | 1.0144 | 40.9753 | |
| *F ratio* = 26.45111 **Highly significant** | | | | | <0.00001 |

**Table 7 -** ONE WAY ANOVA of analytes in Group – II (N=25)

| | GGT | ALKP | Deritis ratio | MDF | p-value |
|---|---|---|---|---|---|
| **Mean** | 61.16 | 198.6 | 2.5948 | 32.3 | |
| **SD (±)** | 31.5907 | 100.1328 | 2.5322 | 20.2732 | |
| *F* ratio = 65.62729 **Highly significant** | | | | | <0.00001 |

ANOVA of means of GGT, ALKP, AST/ALT ratio & MDF within the same group was statistically significant(p- <0.00001) in Gr.I & II (Table 6&7) .The results in our study suggested that GGT, ALKP and MDF can differentiate ALD from VH.

## IV. Discussion

Alcoholism is diagnosed on the basis of history, socio-economic conditions of the individual and questionare about alcohol consumption, which is sometimes unreliable. Alcohol abuse is increasing in India with altered socio-economic conditions and cultural habits.Hazardous drinking habits were being observed even in younger age groups (±15yrs). Seasonal variations and malnutrition are basis for different viral infections including Hepatitis, as preventive immunization not included in primary schedule.

Prevoius studies indicated an improvement in diagnosis and management could be achieved by combining two or more biomarkers[7]. Scoring sytems like MELD, MDF based on selected combinations of biomarkers were developed to assess liver damage irrespective of primary cause .[8].Similarly an attempt was made in our present study to compare scoring sytems and liver enzymes among established cases of CALD and VH. Drawback in our study was the bulk of study group. All the subjects of CALD & VH have elevated serum Total Bilirubin levels(Table 1) . AST is elevated 6-8 times above norrmal but Deritis ratio in both groups I & II was not statistically significant(Table2). This finding could be due to increased ALT levels detectable in extrahepatic health risks like Type 2 DM, Metabolic Syndrome . This observation is in accordance with Kim HC et al.[9]

GGT , a liver microsomal enzyme , found to be elevated in both CALD & VH ,moreso in groupI and statistically significant within the same group(Table 6 &7) and amongst groups (Table 3) [10;11] Previous studies showed elevated GGT levels in ageing, cancer, metabolic syndrome and increased oxidative stress like cardiovascular disease.

In our study AST, ALT ,ALKP, PT and MDF were marginally elevated in CALD compared to Viral Hepatitis and controls in spite of the history of alcohol consumption of >15yrs.(Table1) GGT elevated 3-times ULN(Upper level Normal) in CALD compared to VH , where the levels were <double ULN.(Table1). Hepatocyte damage coupled with oxidative stress could be the underlying reason for high GGT levels.As observed by Fernando De Ritis [12] in 1957 Serum AST/ALT ratio should possibly differentiate aetiology of hepatic damage. But in this study we failed to prove any thing potively on this issue , even though we selected laboratory proven seropositive subjects of VH and CALD subjects with withdrawal symptoms. ANOVA of means of MDF between groups was highly significant (p<0.001) (Table 5). Alcoholics in general are malnourished , may be having B6 deficiency; which could be the reason for lowered AST/ALT values. In our study we did not differentiate acute and chronic cases. But in both CALD and VH De Ritis ratio in our study was **2.5:1**(table 1) which indicates poor prognosis and the same was confirmed by MDF score , 44.2± 40.97 and 32.3 ± 20.27 respectively.(Table 5)

The present study was concluded with a confirmed opinion of including GGT in LFT as a routine test as well the importance of scoring systems was proven beyond doubt in assessing prognosis of liver diseases.

## Acknowledgements

My sincere thanks to Sri Bhaskar, Balu, Krishna and Mrs. Rama for all the help extended for analysis of samples. My humble thanks to the subjects and their relatives for the cooperation during study period.

**Conflict of Interest :** None

**Abbreviations**

**CALD** - Chronic Alcoholic Liver Disease
**VH** - Viral Hepatitis
**GGT** - Gamma Glutamyl Transferase
**AST** - Aspartate Transaminase
**ALT** - Alanine Transaminase
**PT** - Prothrombin Time.
**ULN** - Upper Level Normal

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IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Dr. V. Aruna " Gamma Glutamyl Transpeptidase, De Ritis Ratio, Maddrey Discriminant Function in Chronic Alcoholic Liver Disease and Viral Hepatitis." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 5.4 (2019): 15-19.