Indian Journal of Biochemistry & Biophysics
Vol. 57, December 2020, pp. 681-686

Evaluation of Gamma glutamyl-transferase (GGT) levels in COVID-19: A retrospective analysis in tertiary care centre

Neha Saini[1], Ram Krishan Saini[1], Mohana Kumari[1], Dev Kumar[1], Manish Bhardwaj[1], Shiv Soni[2], Vikas Suri[3], Pankaj Malhotra[3], Sant Ram[1]* & Deepy Zohmangaihi[1]*

[1]Department of Biochemistry, [2]Department of Anaesthesia and Intensive Care & [3]Department Internal Medicine

Postgraduate Institute of Medical Education and Research, Chandigarh-160 012, India

Received 09 October 2020; *revised 11 October 2020*

Many recent studies have reported that patients infected with novel coronavirus 2019 or SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) might have a liver injury. However, few studies have focussed on the levels of Gamma glutamyl-transferase (GGT) alone and the variations associated with it. We retrospectively analysed the GGT levels of 476 admitted patients with confirmed COVID-19 in a tertiary care centre, PGIMER (Post Graduate Institute of Medical Education and Research), Chandigarh. Out of the total 476 COVID-19 patients studied, 35% had elevated GGT levels. ICU care was required for 51.19% (P <0.0001) of these patients and their hospital stay was of longer duration as compared to the patients with normal GGT levels. The incidence of GGT elevation was found to be more pronounced in males and elderly patients. The male population displayed higher GGT levels with 52% having raised levels compared to females where only 21.6% had elevated GGT levels. Although the number of COVID-19 cases was majorly from young age groups, the elevation in GGT levels has been reported more in elderly patients. GGT levels can therefore serve as a predictor for the extent of liver injury and severity in COVID-19 patients.

Keywords: Cholangiocytes, Gamma-glutamyl transferase, Hepatocytes, Hospitalisation, Squamous epithelium

The current Coronavirus disease 2019 (COVID-19) pandemic, triggered by SARS-CoV-2 virus has spread rapidly throughout the world with high rates of transmission and substantial mortality and has severely impacted the living and working conditions of billions of people. As of 9 October 2020, there have been 36, 361, 054 confirmed cases of COVID-19, including 1, 056, 186 deaths, reported to WHO[1]. This emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causal agent of COVID-19, although primarily targets the upper respiratory tract, many studies have indicated that the liver, among other organs, may also be affected by the virus[2,3]. Similarly, liver damage was reported to occur during the infection of another pathogenic coronavirus; the severe acute respiratory syndrome coronavirus (SARS-CoV), and the Middle East respiratory syndrome coronavirus (MERS-CoV)[4,5].

*Correspondence:
Phone: +91-8414863196, 7087008274, 7508925668, 7087008287 (Mob)
E-mail: drsantram2016@gmail.com (SR); drdeepyz14@gmail.com (DZ)

Abbreviations: ACE2, angiotensin-converting enzyme-2; ALP, alkaline phosphatase; COVID-19, Coronavirus disease 2019; GGT, Gamma glutamyl-transferase; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus

As with SARS-CoV, angiotensin-converting enzyme 2 (ACE2) appears to be the susceptible receptor for SARS-CoV-2 and is expressed in more than 80% of alveolar cells in the lungs[6]. ACE2 receptors expression is also observed in smooth muscles of the gastrointestinal tract[7] and squamous epithelium of the nasal, oral and nasopharyngeal mucosa[8]. Hepatic distribution of the ACE2 receptor is unique as it is mostly expressed in cholangiocytes (59.7%) than hepatocytes (2.6%)[9]. Markers for hepatic cholangiocytic activity which are primarily assessed by measurements of serum alkaline phosphatase (ALP) and Gamma glutamyl-transferase (GGT) are hence important.

Infection with SARS-CoV-2 and its association with an increased risk for an abnormal liver function could either be a transient biochemical abnormality or a significant indicator of liver injury observed during the course of infection. Few authors consider this feature not to be clinically significant[10-14], while others have highlighted this association with adverse outcomes[15-17] or reduced survival[15-20]. This controversy can be explained in part due to the lack of a consensus on the definition of COVID-19 associated liver injury[21]. Therefore, generating valid evidence for potential variability in COVID-19 related liver injury particularly about the hepatic cholangiocytic activity as measured by GGT levels is relevant and paramount.

Methods

Study Design and participants

We retrospectively evaluated and analysed the GGT levels of 494 patients of both genders with confirmed COVID-19 from March 15, 2020, up to August 15, 2020, admitted in Nehru Hospital Extension (NHE), designated COVID Care Centre at Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. Out of these 494 patients, 18 patients suffering either from chronic liver diseases, alcoholic patients or hepatitis positive patients were excluded from the study, hence, 476 patients were included in the study. The study was approved by the Institutional Ethics Committee (INT/IEC/2020/000765).

Laboratory Examination

The diagnosis for COVID-19 was confirmed by RT-PCR analysis of nasopharyngeal and oropharyngeal swab samples in the Department of Virology, PGIMER, Chandigarh, India as per guidelines of Indian Council of Medical Research (ICMR), New Delhi, India.

Following standard operating procedures, the blood samples were handled by a designated technician with proper safety gear. The sample tubes were decontaminated by wiping and spraying with 0.1% hypochlorite solution, which was followed by placing the vacutainers under UV-C Biosafety Cabinet for 15 min. The samples were then centrifuged for 10 min at 3500 RPM; the lid of the centrifuge was kept closed for the next 15 min for the aerosols to settle down. The serum samples for GGT tests were analysed after maintaining a proper quality control check in Roche Cobas 8000 Autoanalyzer. All tests were performed according to standard protocols and procedures.

Abnormality in GGT levels was defined as levels >61 U/L on the day of admission[22].We have taken the requirement of the Intensive Care Unit as a primary outcome and median hospital stay as a secondary outcome along with the GGT level in the patients.

Statistical Analysis

GraphPad Prism 8.0 software was used for statistical analysis. Descriptive analysis and Fischer exact test were performed.

Results

Recruited patients for the study were 476. Out of these, raised levels of GGT were found in 168 (35%, Fig. 1) with a median GGT value of 106 and a maximum GGT value of 682 (11× upper limit of normal, Table 1).

Out of these 168 patients with raised GGT levels, ICU care was required for 51.19% of the patients, while only 22.07% of patients with normal GGT levels required ICU care. This finding was found to be statistically significant with a *P* value <0.0001 (Fig. 2A). Also, median days of hospital stay in patients with elevated GGT levels were 6 days as compared to 4 days in patients with normal GGT levels (Fig. 2B).

This increase was more prevalent in males with 127 out of 287 (44%) as compared to females, where elevated GGT levels were found only in 41 out of 189 (21.6%) (Fig. 3A). Although no significant difference was found in the median values of GGT between these groups, *i.e.* 107 and 102 in males and females with increased enzyme levels, respectively, (Fig. 3B and Table 2).

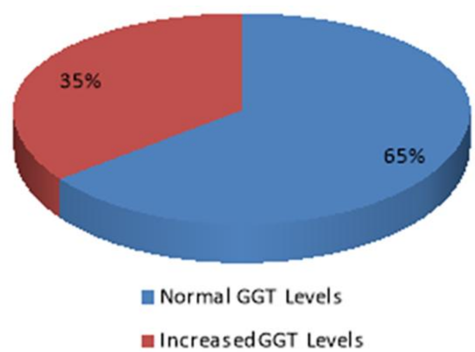

Fig. 1 — Pie chart showing the percentage of patients with normal and increased GGT levels

Table 1 — Table showing the GGT values in the different groups of total subjects

	Total number of patients	Patients with normal GGT	Patients with increased GGT
Number of values	476	308	168
Minimum GGT value (U/L)	4.420	4.420	62.00
25% Percentile (U/L)	20.00	15.00	81.25
Median GGT value (U/L)	37.00	23.00	106.0
75% Percentile (U/L)	84.00	35.50	188.0
Maximum GGT value (U/L)	682.0	61.00	682.0

Also, the incidence of GGT surge is reported to be age-dependent as we found a concomitant increase in the percentage of patients with abnormally high GGT levels with increasing age. Out of the total patients falling under age groups, 13-39 years, 40-59 years and >60 years, the percentage of patients with elevated GGT levels was found to be 24.51%, 42.59%, and 49.45%, respectively, (Fig. 4A). The median GGT values were found to be

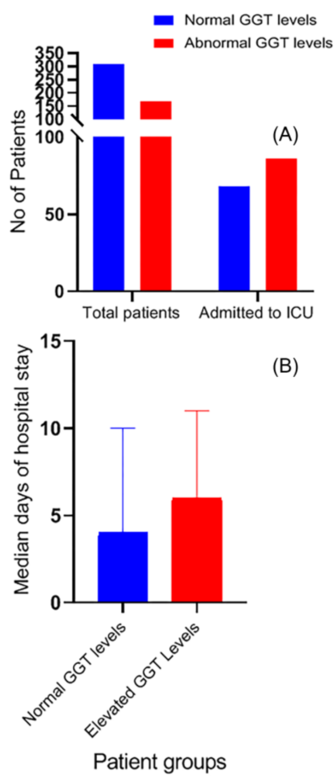

Fig. 2 — (A) The comparison of the number of patients admitted in ICU in normal, and elevated GGT groups (P <0.0001); and (B) Median days of hospital stay in normal and elevated GGT groups (P = ns)

Table 2 — Table showing the GGT values in female and male populations with raised levels of enzymes

	Female	Male
Number of values	41	127
Minimum GGT value (U/L)	62.00	62.00
25% Percentile (U/L)	81.00	81.00
Median GGT value (U/L)	102.0	107.0
75% Percentile (U/L)	228.0	180.0
Maximum GGT value (U/L)	519.0	682.0

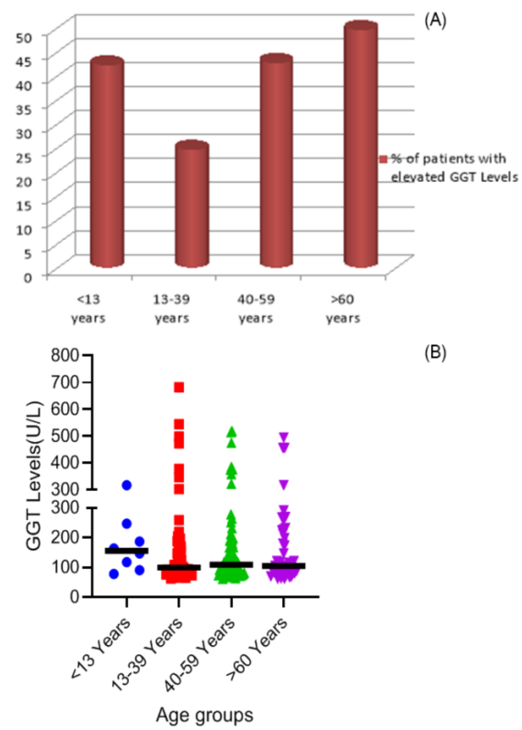

Fig. 4 — (A) The percentage of patients with increased GGT values under different age groups; and (B) The levels of GGT in patients with increased enzyme levels falling under different age groups

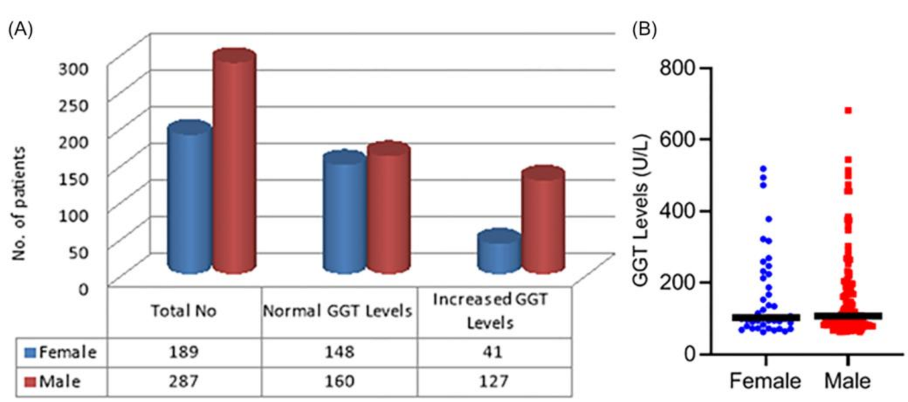

Fig. 3 — (A) The gender-wise distribution of the total number of patients, patients with normal GGT levels, and patients with increased GGT levels; and (B) The levels of GGT in females and males with increased enzyme levels with median values

Table 3 — Table showing the GGT values in different age groups with increased levels of enzymes

	<13 Years	13-39 Years	40-59 Years	>60 Years
Number of values	6	50	69	43
Minimum GGT value (U/L)	78.00	63.00	62.00	62.00
25% Percentile (U/L)	97.75	78.75	82.00	82.50
Median GGT value (U/L)	155.5	99.50	109.0	105.0
75% Percentile (U/L)	232.0	174.5	184.0	218.5
Maximum GGT value (U/L)	317.0	682.0	519.0	494.0

Table 4 — Table showing the age-related distribution in different groups of subjects

	Total number of Patients	Patients with normal GGT	Patients with increased GGT
Number of values	476	308	168
Minimum Age (years)	<1	<1	<1
25% Percentile (years)	29	30	35
Median Age (years)	40	42	48
75% Percentile (years)	55	56	60
Maximum Age (years)	94	87	94

155.5, 99.5, 109 and 105 in age groups, <13 years, 13-39 years, 40-59 years and >60 years, respectively, (Fig. 4B, & Table 3). The median age with maximum GGT levels was found to be 48 years (Table 4).

Although the number of patients admitted in the hospital was more from the young age group *i.e.* 204 belonging to age group 13-39 years, followed by 162 and 91 from age groups 40-59 years and >60 years, respectively, the incidence of GGT increase was found to be more in later groups. As patients with age <13 years were less than 20 in number and were therefore, not considered to avoid misleading interpretations.

Discussion

The study was retrospectively carried out in 476 COVID-19 positive patients. Out of the total admitted patients, 168 (35%) had elevated GGT levels. The increase in GGT was more prevalent in males with 127 out of 287 (44%) as compared to females with 41 out of 189 (21.6%). Elevated GGT levels were observed more in the elderly age group as compared to the younger patients with COVID-19 which is consistent with the previous reports, that patients of older age had more severe illness and the incidence rate was higher in men[23].

The median GGT value in patients with increased GGT was 106 and the maximum GGT value observed was of 682 U/L (11× upper limit of normal). The median age of patients with elevated GGT levels was 48 years.

Another vital observation in our study was that the majority of the COVID-19 positive patients with raised GGT levels required admission in the intensive care units (ICU). It was observed that only 22.07% of patients with normal GGT levels required ICU care whereas 51.19% of patients with raised GGT levels required ICU admission. This finding was found to be statistically significant with $P < 0.0001$. The average hospital stay duration in patients with elevated GGT levels was 6 days whereas in patients with normal GGT levels were 4 days after admission. These observations clearly indicate that elevated GGT was more marked in patients with severe manifestations of SARS-CoV-2 infection, thereby requiring ICU admission.

There are studies reporting similar findings that patients in intensive care units and critical states are more likely to have their liver biochemical markers deranged denoting the severity of infection[24]. Elevated levels of GGT reported to be highest at the time of admission corresponding with the outcomes that have been presented in a number of studies[25-26]. In a recently conducted study, it was found that at intervals of admission into isolation wards, the elevation of GGT was highest. Our study showed the association of elevated GGT levels with an increased number of ICU admissions.

We also observed in our study that the maximum patients with COVID-19, 204 out of 476 were in the age group of 13-39 years as compared to the other age groups. This age group reflects the mobile and active group in a population. In a study that examined the relationship between COVID-19 transmissions with mobility, a statistically significant correlation was seen between social distancing, measured by mobility patterns and subsequent reduction in COVID-19 cases. This could explain the increased incidence of COVID-19 infection in the particular age group.

Our study also has its limitations. It is a retrospective study carried out using the data collected from a single centre. Medication and clinical history of the patients were not included. We examined exclusively the role of GGT in COVID-19 and have not considered the role of other liver enzymes and inflammatory markers. Therefore, more studies documenting the derangements of liver enzymes during hospitalization and their relation to clinical outcomes of the patients are required. Liver injury COVID-19, as a result of ACE2 receptor-associated bile duct endothelial disruption and derangements of cholestatic liver enzymes needs more research to extrapolate the mechanism of COVID-19 induced morbidity.

Conclusion

COVID-19 can cause derangements in liver profile. The abnormal liver function could be associated with raised GGT levels. Abnormal liver profile is found to be more pronounced in males and elderly patients, thereby requiring extensive treatment. Markers of liver function could therefore serve as an independent prognostic factor for COVID-19 induced morbidity.

Acknowledgement

We would like to acknowledge the contribution of all faculty members of the Department of Biochemistry, PGIMER, Chandigarh, India with special thanks to Dr Arnab Pal. We also acknowledge all the technical staff of the department.

Conflict of interest

All authors declare no conflict of interest.

References

1 WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. [cited 2020 Oct 9]. Available from: https://covid19.who.int/.

2 Pandit K, Gupta S & Sharma AG, Clinico-Pathogenesis of COVID-19 in children. *Indian J Biochem Biophys*, 57 (2020) 264.

3 Ghosh S & Ghosh S, Air quality during COVID-19 lockdown: Blessing in disguise. *Indian J Biochem Biophys*, 57 (2020) 420.

4 Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY & Yan Y, The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Mil Med Res*, 7 (2020)1.

5 Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GV & van Goor H, Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*, 203 (2004) 631.

6 Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A, Zhou J, Shi G, Fang N, Fan J & Cai J, Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. *Biorxiv*, (2020) (in press).

7 Bloom PP, Pasricha TS & Viveiros K, We know liver biochemistries are elevated in COVID-19, but should we be concerned? *Clin Gastroenterol Hepatol*, 18 (2020) 2384.

8 Zhang Y, Zheng L, Liu L, Zhao M, Xiao J & Zhao Q, Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single centre in Wuhan city, China. *Liver Int*, (2020) (in press).

9 Zippi M, Fiorino S, Occhigrossi G & Hong W, Hypertransaminasemia in the course of infection with SARS- CoV-2: Incidence and pathogenetic hypothesis. *World J Clin Cases*, 8 (2020) 1385.

10 Cai Q, Xu L & Chen J, Reply to “Liver tests abnormalities in COVID-19: trick or treat?” *J Hepatol*, (2020) (in press).

11 Fan Z, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z & Cheng J, Clinical features of COVID-19 related liver damage. *Clin Gastroenterol Hepatol*, (2020) (in press).

12 Xie H, Zhao J, Lian N, Lin S, Xie Q & Zhuo H, Clinical characteristics of non-ICU hospitalized patients with coronavirus disease 2019 and liver injury: a retrospective study. *Liver Int*, (2020) (in press).

13 Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J & Sun Y, COVID-19: abnormal liver function tests. *J Hepatol*, (2020) (in press).

14 Deng Y, Liu W, Liu K, Fang YY, Shang J, Zhou L, Wang K, Leng F, Wei S, Chen L & Liu HG, Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 in Wuhan, China: a retrospective study. *Chin Med J*, 133 (2020) 1261.

15 Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X & Guan L, Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*, (2020) (in press).

16 Phipps MM, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J & Verna EC, Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large US Cohort. *Hepatology*, (2020) (in press).

17 Lei F, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S & Wang X, Longitudinal association between markers of liver injury and mortality in COVID-19 in China. *Hepatology*, (2020) (in press).

18 Ye Z & Song B, COVID-19 related liver injury: call for international consensus. *Clin Gastroenterol Hepatol*, (2020) (in press).

19 Liver Function tests, https://www.mayoclinic.org/tests-procedures/liver-function-tests/about/pac-20394595.

20 Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y & Yu T, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, 395 (2020) 507.

21 Bangash MN, Patel J & Parekh D, COVID-19 and the liver: little cause for concern. *Lancet Gastroenterol Hepatol*, 5 (2020) 529.

22 Zhang C, Shi L & Wang FS, Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol*, 5 (2020) 428.

23 Xu L, Liu J, Lu M, Yang D & Zheng X, Liver injury during highly pathogenic human coronavirus infections. *Liver Int*, 40 (2020) 998.

24 Li J & Fan JG, Characteristics and mechanism of liver injury in 2019 coronavirus disease. *J Clin Trans Hepatol*, 8 (2020) 13.

25 Asghar MS, Akram M, Rasheed U, Hassan M, Iqbal Z, Fayaz B, Hayat H, Ather A, Hussain H & Syyed E, Derangements of Liver enzymes in COVID-19 positive patients of Pakistan: A retrospective comparative analysis with other populations. *Arch Microbiol Immunol*, 4 (2020) 110.

26 Badr HS, Du H, Marshall M, Dong E, Squire MM & Gardner LM, Association between mobility patterns and COVID-19 transmission in the USA: a mathematical modelling study. *Lancet Infect Dis*, (2020) (in press).