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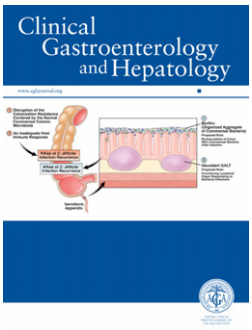

Presentation, Outcomes, and Response to Therapy Among Patients with Acute Exacerbation of Chronic Hepatitis C

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Presentation, Outcomes, and Response to Therapy Among Patients with Acute Exacerbation of Chronic Hepatitis C

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List of abbreviations: ~~ae-CHC: acute exacerbation of chronic hepatitis C~~; LB: liver biopsy; ALT: Alanine aminotransferase; AST: Aspartate-aminotransferase; CHC: Chronic hepatitis C; HAI: Histological Activity Index; HCC: hepatocellular carcinoma; HCV: Hepatitis C Virus; IL: Interleukin; SVR: Sustained Virological Response

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Author Contributions

ES was responsible for the conception and design of the study and wrote the manuscript; MP participated in the conception of the study, performed the analysis of HCV-RNA and ofIL-28B and interpreted the data; MS, VM and CS enrolled and followed-up the patients, LA interpreted and analyzed the data; MS performed the analysis of HCV-RNA and of IL-28B; GP analyzed the histological data; NC was responsible for the conception and design of the study and wrote the manuscript.

ABSTRACT

Background & Aims: The slow asymptomatic progression of chronic Hepatitis C can be interrupted by an acute exacerbation, characterized by increased serum levels of alanine aminotransferase (ALT) and bilirubin and other symptoms of acute hepatitis. We aimed to provide more information about the clinical presentation of acute exacerbation of chronic hepatitis C.

Methods: We identified 82 consecutive patients, from 2 locations in Italy, who had an acute exacerbation of chronic hepatitis C from January 2005 through June 2010; we followed them for a median period of 36 months. These cases were HCV RNA positive, hepatitis B antigen-negative, and had not received anti-HCV therapy. They were matched with 82 subjects with hepatitis C without reactivation for age, sex, and HCV genotype (controls). Sixty-nine cases and 73 controls were followed for at least 2 years. Liver biopsies had been taken from 23 cases and 31 controls—once before enrollment in the study and once during the follow-up period.

Results: HCV genotype 2 was detected in 46.4% of cases genotype 1 in 43.9%. Among cases, the mean level of ALT was 1063±1038 IU/dL and of total bilirubin was 15.87±7.15 mg/dL. A higher percentage of cases carried the *IL-28B CC* genotype than controls (40.2% vs 24.4%; $P<.05$). Among cases, 43.5% had a steady increase in ALT (>2-fold baseline value); for 56.5% of these patients, ALT levels returned to baseline values before the acute exacerbation of chronic hepatitis. Based on comparisons of biopsies, 18 cases (78.3%) and 11 controls (35.5%) had increasing fibrosis, with Ishak scores increasing by >2 ($P<.005$); 14 cases (60.9%) and 3 controls (9.6%) had increases in necro-inflammation >2 points ($P<.005$). Thirty-two cases (46.4%) and 38 controls (52%) received treatment with pegylated interferon and ribavirin; a sustained viral response (SVR) was achieved by 26 cases (81.2%) and 23 controls (60.5%).

Conclusion: Although an acute exacerbation of chronic hepatitis is a serious medical condition, most patients achieve a SVR following treatment with pegylated interferon and ribavirin.

KEY WORDS: hepatic flare, cirrhosis, response to therapy, complication

INTRODUCTION

The clinical presentation of chronic infection by Hepatitis C Virus (HCV) is characterized or moderate histological lesions on liver biopsy (LB) [1-2]. The course of the illness is unpredictable in single cases, but the most frequent outcomes are an indolent persistence of a mild disease or a slow progression to a more severe liver disease, including the development of cirrhosis [1,3,4]. About 3% of cirrhotic patients per year develop hepatocellular carcinoma (HCC) [2].

The stable persistence of an inactive or moderately active stage of the illness and a slow asymptomatic progression of chronic hepatitis may be interrupted by an exacerbation of the disease, named in the present paper "acute exacerbation of chronic hepatitis C", an event characterized by a substantial increase in serum ALT values, at times associated to an increase in bilirubin and/or to other symptoms in common with acute hepatitis [5–8]. Reactivation of chronic hepatitis C (CHC) was first described in 1996 and an annual incidence rate around 10% was observed in 194 patients followed up for more than 5 years [5]. More recently, acute exacerbation of chronic hepatitis C has been described as associated with HCV genotype 2 (HCV-2) [9–12], but its occurrence in patients with other HCV genotypes has also been described [10].

At present, few contributions have been published and more data are needed on clinical presentation of acute exacerbation of chronic hepatitis C. In addition, to our knowledge, there is no information on the clinical course of acute exacerbation of chronic hepatitis C and its impact on the outcome and response to treatment of CHC.

This paper reports the data of a prospective investigation on 82 consecutive patients with symptomatic acute exacerbation of chronic hepatitis C and 82 pair-matched control patients who had not shown signs of acute exacerbation before being enrolled in the present study. Patients were naïve to anti-HCV treatment and most of them observed for at least two years.

PATIENTS AND METHODS

Patients

Two liver units in southern Italy participated in the study, one in Naples and one in Caserta. These two centers have cooperated for 12 years in several clinical investigations using the same clinical approach and the laboratory methods [13,14].

From January 2005 to June 2010, we prospectively enrolled 82 anti-HCV/HCV RNA-positive, HBsAg/anti-HIV-negative patients, naive to anti-HCV therapy (because of no indication, contraindications or refusal), observed for a symptomatic acute exacerbation of chronic hepatitis C (Case Group). In several determinations over the years before reactivation, these 82 patients had been HCV RNA-positive with normal or moderately increased serum ALT levels, suggesting an indolent, slowly progressing course of CHC. A percutaneous LB, performed at least one year before acute exacerbation of chronic hepatitis C, was available for 26 (31.7%) patients in the Case group. The diagnosis of acute exacerbation of chronic hepatitis C was based on an increase in the ALT value of at least five-fold the previous basal values and on the detection of anti-HCV and HCV RNA, in the absence of other viral or iatrogenic factors known to induce liver damage [10-12]. Excluded from the study were patients with a history of alcohol intake, with serological signs of autoimmune hepatitis, those treated in the last 6 months with drugs considered to be hepatotoxic, and those with IgM antibody to hepatitis B core antigen, hepatitis D virus (HDV), hepatitis A virus (HAV), hepatitis E virus (HEV), cytomegalovirus or anti-Epstein Barr virus during the acute phase of the illness. During acute exacerbation of chronic hepatitis C, liver function tests were performed once a week for two months.

Of 479 anti-HCV/HCV RNA-positive CHC patients without HCC observed in the same centers in the same period, we enrolled as a Control group 82 HBsAg-negative patients who never showed signs of symptomatic acute exacerbation of chronic hepatitis C, with steady ALT values in 4 checks per year in the last 5 years and naïve to anti-HCV therapy because of no indication, contraindications or refusal.These patients were pair-matched by age (±5 years), sex and HCV

genotype with the patients in the Case Group. For 35 (42.7%) of the 82 patients in the Control group, a percutaneous LB had been performed before enrolment.

Samples of plasma and whole blood were obtained for each patient in the Case group at the time acute exacerbation of chronic hepatitis C developed and for each patient in the Control Group at enrolment. These samples were fractioned, stored at –80°C and never thawed until used for this investigation.

After a two-month observation during acute exacerbation of chronic hepatitis C, patients in the Case group were followed up for a median period of 36 months (range 24-72), with the exception of 13 patients lost to follow up for lack of compliance.The patients in the Control group were observed for 32 months (range 24-68) after enrolment, with the exception of 9 patients lost to follow up for lack of compliance. During this long-term follow up, all patients were assessed at three-monthly intervals with liver function tests and at six-monthly intervals with abdominal ultrasound scan.

For patients in the Case group, three clinical profiles of long-term biochemical outcome were established: deterioration, stationary status, improvement. Patients were considered as "deteriorated" if a persistent increase in ALT value of at least 2-fold was observed, compared with the values shown before reactivation, as "stationary" if after acute exacerbation of chronic hepatitis C they returned to the ALT values shown before acute exacerbation of chronic hepatitis C, and as "improved" if they showed a stable normalization of ALT values after acute exacerbation of chronic hepatitis C.

A LB was suggested to patients in the Case and Control groups during the long-term follow up, in accordance with the international guidelines [15,16]. For patients in the Case group it was performed at least 8 months after the development of acute exacerbation of chronic hepatitis C. Fifty-four patients, naïve to anti-HCV therapy, agreed to undergo a second LB, 23 in the Case group and 31 in the Control group. The interval between the two biopsies was 5.85 years (IQR 4.2-7.1) for patients in the Case group and 5.05 years (IQR 4.18-6.89) for those in the Control group. A

2nd LB was not performed in 3 patients in the Case group and in 4 in the Controls because it was not indicated or refused. LBs were examined by a pathologist (G.P.) who, unaware of the clinical and laboratory data, compared the serial biopsies of each patient for necroinflammation (HAI) and fibrosis using the Ishak scoring system for both grading and staging [17]. Patients were considered to have deteriorated if they showed an increase of at least 2 degrees in the fibrosis or HAI scores in the 2nd LB, to have a stationary condition if there was no change or only a minimal change (± 1) in liver fibrosis or HAI scores between the two LBs, and to have improved if a reduction of at least two degrees in fibrosis or HAI scores was observed in the 2nd LB.

Of the 69 patients in the Case group and the 73 in the Control group with a 2-year follow up, 32 (46.4%) and 38 (52%), respectively, received pegylated-interferon (peg-INF) plus ribavirin treatment according to international guidelines [15,16]. Thirty-seven (53.6%) Cases and 35 (47.9%) Controls remained untreated because therapy was not indicated, or because of contra-indications to therapy or refusal. The same treatment schedules were applied for patients in both groups. Patients with HCV-1/4 received a 12-month course with peg-INFα-2a (weekly dose of 180μg), or peg-INFα-2b (weekly dose of 1.5 μg/kg of body weight), plus ribavirin at a daily dose of 800-1,200mg according to body weight. Patients with HCV-2/3 received the same treatment schedules for 6 months. The response to treatment was analyzed according to commonly accepted international criteria [15,16].

All the procedures applied in the study were in accordance with the standards on human experimentation of the Ethics Committee of "Azienda Ospedaliera Universitaria of the Second University of Naples" and with the Helsinki Declaration of 1975, revised in 1983. At the first observation, all patients signed an informed consent according to the rules of the same Ethic Committee.

Methods

HCV RNA was sought in the patients' plasma according to the method extensively described in a previous paper [18] with a detection limit estimated at around 40 IU/mL. HCV genotyping was performed by Line-Probe-Assay (INNO-LIPA HCV II, Innogenetics).

The whole blood samples of all patients in the study were tested for interleukin (IL)-28B genotype (Roche Diagnostics, Branchburg, NJ, USA).

HAV, HBV, HDV, HCV, HEV, HIV, Cytomegalovirus and EBV serum markers were sought using the commercial immunoenzymatic assays reported in the supplementary data. Liver function tests were performed applying routine methods.

Statistical analysis

Continuous variables were summarized as mean and standard deviation, and categorical variables as absolute and relative frequencies. Differences in the mean values were evaluated by the Student t-test, and the Chi-squared test was applied to categorical variables. A p value <0.05 was considered to be statistically significant.

RESULTS

Clinical presentation of acute exacerbation of chronic hepatitis C

The demographic, genetic, clinical, biochemical and virological characteristics of the Cases and Controls recorded at enrolment are shown in Table 1. The patients in the Case group had a mean age of 50 years, males predominated (76%) and the most frequent HCV genotypes were HCV-2 (46.4%) and HCV-1 (43.9%) The patients in the Control group showed the same characteristics, reflecting their selection criteria. Patients in the Case and Control groups stated similar risk factors for acquiring HCV infection, surgery without transfusion and a history of injection drug use being the most frequent risk factors in both groups. The patients in the Case group showed a mean AST serum value of 672 ± 788 IU/dL, a mean ALT of 1,063 ± 1,038 IU/dL

and a total bilirubin mean value of 15.87 ± 7.15 mg/dL (Table 1). The patients in the Control group showed AST 51 ± 55 IU/mL, ALT 71 ±68 IU/mL and bilirubin 0.7 ± 0.4 mg/mL.

IL-28B CC genotype was more frequently detected in the Case group than in the Controls (40.2% vs. 24.4%; $p<0.05$).

Clinical Outcome

Of the 82 patients in the Case group, 13 (15.8%) dropped out because of lack of compliance and 69 were followed up for at least 2 years. On the basis of the ALT profiles during and after acute exacerbation of chronic hepatitis C, 30 (43.5%) of these 69 were considered to have deteriorated, 39 (56.5%) were stationary and none improved.

Changes in the liver fibrosis and HAI scores between the two LBs are shown in Figures 1 and 2, respectively. Deterioration in liver fibrosis of at least 2 scores was observed in 18 (78.3%) of the 23 patients in the Case group and 11 (35.5%) of the 31 in the Control group ($p<0.005$), whereas the fibrosis scores remained stationary in 5 (21.7%) patients in the Case group and 20 (64.5%) in the Control group. Only 1 (3.2%) patient in the Control group improved (Figure 2).

Deterioration of at least 2 HAI scores was observed in 14 (60.9%) patients in the Case group and 3 (9.6%) in the Control group, a difference significant to the statistical analysis ($p<0.005$).An improvement in the HAI of at least 2 scores was found only in 4 (12.9%) patients in the Control group, whereas 9 (39.1%) patients in the Case group and 24 (77.5%) in the Control group remained stationary.

For a more comprehensive understanding of the impact of acute exacerbation of chronic hepatitis C on the clinical course of CHC, the 69 patients with a long-term follow up are presented in Figure 3 and in figures 1-4 of the supplementary data.

Patient number 1 in Figure 3 was a 42-year-old man with HCV-2a, IL28-B CC, who showed a single episode of acute exacerbation and an ALT decline in less than 4 months like another 50 patients (from patient N° 5 to patient N° 54 in Figure 1A-1H of the Supplementary data). In another

6 patients (from patient N° 55 to patient N° 60), after a single episode of acute exacerbation the ALT decline lasted more than 5 months (Figure 2 of the Supplementary data).

Patient number 2 in Figure 3 was a 60-year-old female with HCV- 3, IL28-B CT, who developed an acute exacerbation of chronic hepatitis C in January 2006 with ALT levels increased up to 42-fold and a peak of total bilirubin of 21 mg/dL during reactivation. Serum ALT and bilirubin returned to normal by November 2006, remained so until September 2008 when a second episode of reactivation occurred, followed, after a partial remission, by a third episode in January 2009. The 2nd and 3rd episodes of reactivation were anicteric and of a lesser entity compared to the first.

Patient number 3 (Figure 3) was a 64-year-old male with HCV- 2, IL28-B CC, with a first episode of a-e CHC in August 2008 and two subsequent episodes of reactivation. Total bilirubin ranged from 2-4 mg/dL throughout the observation. Another 6 patients (from patient N° 61 to patient N°66 in Figure 3 of the Supplementary data) showed a course of the disease characterized by two or more episodes of acute exacerbation (Figure 3 of the Supplementary data).

Patient number 4 in Figure 3 was an 87-year-old male with HCV-1, IL28-B CC, who until March 2006 had a clinical, biochemical and ultrasound profile consistent with the diagnosis of CHC, with the ALT value near to normal and normal bilirubin. This patient developed an acute exacerbation of chronic hepatitis C in June 2006 followed by other three episodes of reactivation of a lesser entity up to June 2009 when unequivocal biochemical and ultrasound signs of cirrhosis were documented. Also patients N° 67,68 and 69, showed a rapid transition to liver cirrhosis (Figure 4 of the Supplementary data).

Antiviral treatment

Of the 32 patients in the Case group treated with peg-INF+ribavirin, 26 (81.2%) achieved a sustained virological response (SVR) and 6 (18.8%) were non-responders. Of the 38 treated patients in the Control group, 23 (60.5%) obtained a SVR and 12 (39.5%) were non-responders. The data on the response to treatment, according to HCV genotype and IL28-B genotype, are presented in Table 2. The 17 patients with HCV-1/4 in the Case group showed a SVR more frequently (70.6%) than

the 18 with the same HCV genotypes in the Control group (44.4%). Similarly, the 15 patients with HCV-2/3 in the Case group showed a SVR more frequently than the 20 with the same HCV genotypes in the Control group (93.3% vs 75%). Table 2 also shows that SVR was achieved by all 14 patients with IL28-B CC genotype in the Case group, regardless of HCV genotype, and by 8 of the 11 (73%) in the Control group. These differences between small groups or subgroups of treated patients are not significant to the statistical analysis.

CONCLUSIONS

This long-term follow-up study of 82 patients with acute exacerbation of chronic hepatitis C improves the scanty knowledge on the clinical presentation and course of symptomatic acute exacerbation of chronic hepatitis C and on the impact of this clinical event on the outcome and response to antiviral therapy. At the time of the first observation, the mean AST values were as high as 16-fold the normal value, ALT 25-fold and bilirubin 15-fold. There was a marked variability in the clinical presentation, with ALT increased from 6- to 43-fold the normal values and serum bilirubin from 2 to 22 mg/dL. Also variable was the clinical course of acute exacerbation of chronic hepatitis C, usually characterized by a single flare, but in some cases more than one flare can occur. An acute exacerbation of CHC may occur at any age, as shown by the wide range of ages in the study, from 24 to 87 years. After reactivation, the ALT values slowly decreased in all patients and, by the end of a 2-year follow up, about half of the patients returned to their baseline values before acute exacerbation of chronic hepatitis C, whereas for the other half, the ALT values persisted at more than 2-fold.

Nearly half of the patients with an acute exacerbation of chronic hepatitis C in the present study showed HCV-2. This observation confirms the association between this clinical event and HCV-2 shown by studies from Italy [6,9,10], a country where the prevalence of this HCV genotype in patients with CHC is around 20% [19]. The reasons for this association remain unknown and

warrant further investigation. The data from the present study, however, show that is frequent even in patients with HCV-1 and that it can rarely occur also in patients with other HCV genotypes.

Although unexpected, the significantly higher prevalence of IL28-B CC in the Case group may suggest a greater likelihood of developing acute exacerbation of chronic hepatitis C for patients with this genotype, an observation that deserves further consideration in more extensive studies. Most probably acute exacerbation of chronic hepatitis C is a consequence of a reactivation of cell-mediated immune reaction to clear HCV infection [20-22], in some way in line with the well-known propensity of IL28-B CC genotype to a spontaneous or treatment-induced clearance of HCV infection [23,24].

The comparison of liver histology in sequential LB, possible for nearly one third of the patients in each group, suggests that acute exacerbation of chronic hepatitis C frequently causes deterioration both in fibrosis and necroinflammation. In fact, a 2-score deterioration in fibrosis was observed in nearly three quarters of the patients in the Case group and in nearly one third of the Controls.Similarly, a 2-score deterioration in HAI was found in nearly 60% of patients in the Case group and 10% in the Controls. The differences were both statistically significant. The data from previous long-term follow-up studies on the progression of HCV-associated liver disease suggest that the progression of liver fibrosis is indolent for nearly two decades after acute hepatitis C and that morbidity and mortality are more likely to emerge in the third or fourth decade after infection [25]. The rate of acceleration of liver fibrosis consequent to acute exacerbation of chronic hepatitis C shown in the present study is higher than that observed in the Control group of this study and in previous investigations in patients who did not experience this clinical event and frequently showed an indolent course of the disease [25]. This underscores the profound implication of acute exacerbation of chronic hepatitis C on the progression to cirrhosis and risk of HCC.

In the present study, the patients who experienced a symptomatic acute exacerbation of chronic hepatitis C showed a tendency to achieve a SVR to peg-INF+ribavirin treatment, but the high prevalence of SVR (81.2%), although impressive, deserves confirmation in more extensive

multicenter studies.The frequency of patients with HCV 2 and/or IL28-B CC most probably explains the high efficacy of peg-INF+ribavirin treatment in the Case group, but an additional reason might be found in acute exacerbation of chronic hepatitis C itself, which, being immunologically and virologically similar to acute hepatitis, could re-establish the mechanisms that induce the high response rate to interferon observed in acute hepatitis C [26,27].

In conclusion, acute exacerbation of chronic hepatitis C is a clinical event frequently associated to HCV-2 and to IL28-B CC genotype and is responsible for an unfavorable outcome in patients with chronic hepatitis C. However, the majority of patients with acute exacerbation of chronic hepatitis C obtained a SVR, most probably because of the high frequency of HCV genotype 2 and IL28-B CC genotypes in the Case group, and possibly because the reactivation of a cell-mediated immune response may favor HCV clearance.The more rapid progression to liver cirrhosis and the risk of HCC strongly warrant the early initiation of anti-HCV therapy for acute exacerbation of chronic hepatitis C patients, who in this study showed an impressive rate of SVR to peg-IFN+ribavirin.

Table 1: Initial clinical, epidemiological, biochemical, virological and genetic characteristics of the patients in the Case and Control groups

	Case group (N°=82)	**Control group (N°=82)**
Median age (range)	53 (23-87)	51 (21-85)
Males, N°(%)	62 (76%)	62 (76%)
Females, N°(%)	20 (24%)	20 (24%)
Risk factors, N° (%):		
Surgery without blood transfusion	37 (45.2%)	30 (36.6%)
HCV infection in the household	1 (1.2%)	2 (2.4%)
injection drug use	31 (37.8%)	29 (35.4%)
blood transfusion	2 (2.4%)	2 (2.4%)
not determined	11 (13.4%)	19 (23.2%)
Years of HCV infection, M ± SD	9.06 ± 7.9	8.7 ± 8.1
AST IU/mL, M ± SD (n.v.10-40)	672 ± 788	51 ± 55
ALT IU/mL, M ± SD (n.v.10-40)	1063 ± 1038	71 ± 68
Bilirubin, M ± SD (n.v 0.4-1.0)	15.87 ± 7,15	0.7 ± 0,4
Bilirubin, N°(%):		
> 2.5 mg/dL	31 (37.8%)	0
≤ 2.5 mg/dL	51 (62.2%)	82 (100%)
HCV RNA IU/ml, M ± SD	1,289,827 ± 1,175,515	2,141,200 ± 1,384,745
Genotype, N°(%):		
2, 2a, 2a/2c, 2b	38 (46.4%)	38 (46.4%)
1a, 1b	36 (43.9%)	36 (43.9%)
3	6 (7.3%)	6 (7.3%)
4	2 (2.4%)	2 (2.4%)
IL28-B genotype, N°(%):		
CC	33 (40.2)	20 (24.4)
CT	33 (40.2)	48 (58.5)
TT	16 (19.6)	14 (17.1)

M: mean; SD: standard deviation; AST: aspartate-aminotransferase; ALT: alanine-aminotransferase; IL28-B: interleukin-28B

Differences significant to the statistical analysis: CC versus CT+TT, $p < 0.05$

Table 2. Sustained virological response (SVR) to Peg-INF+ribavirin treatment in 32 patients in the Case group and 38 in the Control group

	Case group		Control Group	
	N° of patients	**with SVR N° (%)**	**N° of patients**	**with SVR N° (%)**
HCV genotype 1:				
IL28-B CC	8	8 (100)	6	4 (66.7)
IL28-B CT/TT	9	4 (44.4)	12	4 (33.3)
All HCV genotype1 cases	17	12 (70.6)	18	8 (44.4)
HCV genotype non-1:				
IL28-B CC	6	6 (100)	5	4 (80)
IL28-B CT/TT	9	8 (88.9)	15	11 (73.3)
All HCV genotype non-1 cases	15	14 (93.3)	20	15 (75)
Total cases	32	26 (81.25)	38	23 (60.5)

SVR: Sustained Virological Response; IL28-B: interleukin-28B

Figure legends:

Figure 1. Changes in fibrosis score between the first and second liver biopsy of patients in the Case and Control groups

Fibrosis score: ▨ Stationary, ■ Increased ≥ 2, □ Decreased ≥ 2.

Footnotes: with increased fibrosis score ≥ 2, Case group versus Control group, $p < 0.005$

Figure 2. Changes in necroinflammation (HAI) score between the first and second liver biopsy of patients in the Case and Control groups

HAI score in Case and Control groups: ▨ Stationary, ■ Increased ≥ 2, □ Decreased ≥ 2.

Footnotes: with increased HAI score ≥ 2, Case group versus Control group, $p < 0.005$

Figure 3. Serum ALT and bilirubin values throughout the observation in four patients with acute exacerbation of chronic hepatitis C.

Footnotes: LB: liver biopsy; S: staging; G: grading; IL28-B: interleukin-28B; ALT: alanine-aminotransferase; UNV: upper normal value

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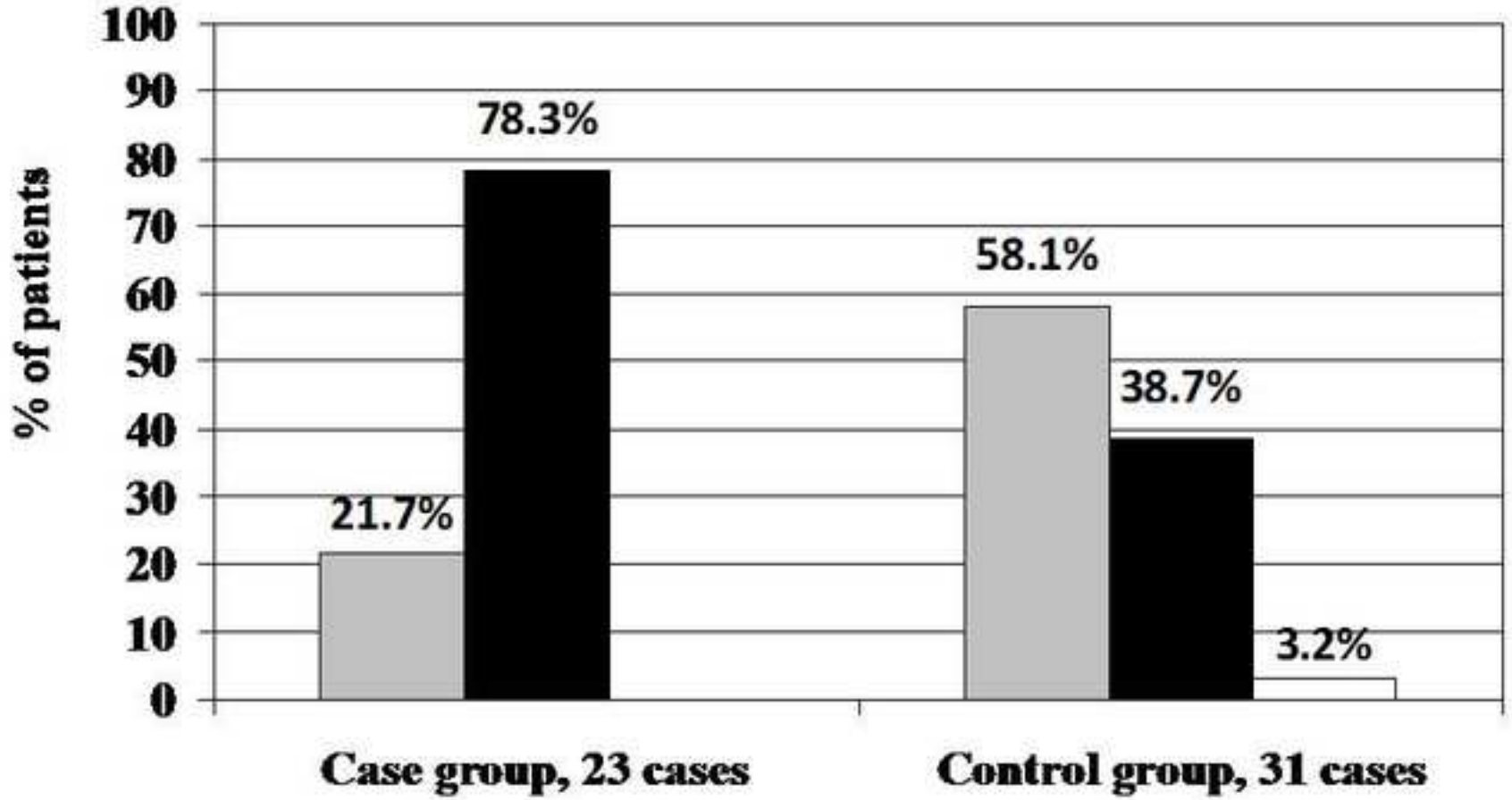

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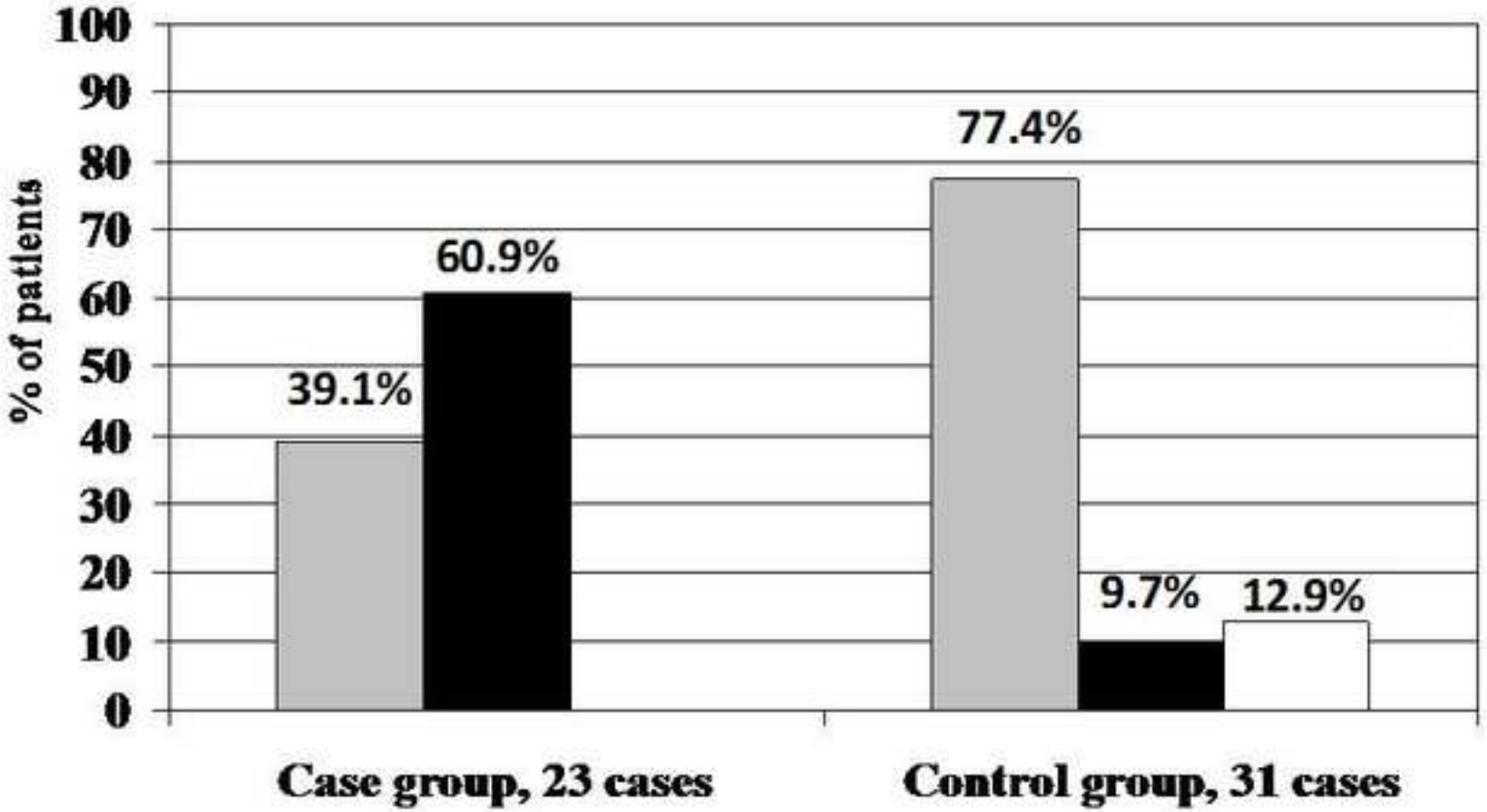

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