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ORIGINAL ARTICLE

Association of Human Cytomegalovirus with Hepatitis C Virus Infections and Its Impact on Antiviral Therapy

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ABSTRACT

Background: *CMV infection enhances HCV pathogenesis by preventing the normal mechanisms responsible for HCV clearance, thus playing vital role in HCV persistence and pathogenicity.* ***Objective:*** *To examine the effect of HCV - CMV coinfection on outcome of treatment with pegylated interferon alpha and Ribavirin in chronic HCV patients.* ***Methodology:*** *The study included of 50 cases selected from Outpatient Clinic of The National Hepatology and Tropical Medicine Research Institute (NHTMRI). Two groups were classified group 1 (positive HCV PCR and negative CMV Ig G) and included 15 patients versus group 2 (positive HCV PCR and positive CMV Ig G) and included 35 patients.* ***Results:*** *The non responders to treatment were higher in group 2 (65.7 %) than in group 1 (53.3 %) but with no statistical significance.* ***Conclusion:*** *CMV co-infection may influence the HCV treatment outcome, despite it failed to have statistical significance.*

INTRODUCTION

More than 170 million patients worldwide are chronically infected with HCV. Prevalence rates range from 0.5% in Northern European countries to 28% in some areas of Egypt[1]. Chronic hepatitis C (CHC) is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC)[2].

The aim of therapeutic intervention for CHC patients is to halt disease evolution to cirrhosis, or if already established, to prevent its complications[3]. The recommended treatment for CHC patients was a combination therapy with pegylated interferon plus ribavirin for 48 weeks[4]. Since a significant number of patients will fail to respond to treatment, it is important to identify risk factors for non-responding patients as early as possible both for patient welfare and for cost-effectiveness [5].

Cytomegalovirus (CMV) is a ubiquitous herpes virus that, depending on the population studied, infects 60%-100% of humans. The outcome of primary CMV infection is latency in various cells, which ensures persistence throughout the life of the host[6]. CMV infection is common in CHC patients, who could be regarded as patients at high risk for CMV disease[7]. CMV infection not only causes direct effects in target organs (e.g., hepatitis), but it has also a number of indirect effects that may affect HCV infection prognosis and response to treatment, including a general immunosuppressive syndrome that may lead to poor immunologic control of HCV[8]. CMV may result in cytokine dysregulation and cross-reactive immunologic responses, which may also lead to more severe forms of HCV which is resistant to treatment [9].

METHODOLOGY

Subjects

The present study was conducted on seventy five Egyptian patients with HCV infection. All of them were selected from patients attending the outpatient clinic of The National Hepatology and Tropical Medicine Research Institute (NHTMRI). These patients did not start treatment. Patients with other causes of chronic liver disease other than HCV were excluded. These included patients with other viral hepatitis B, A (HBV, HAV), metabolic hepatitis, alcohol induced hepatitis (history of alcohol consumption), and drug induced hepatitis (history of hepato toxic drug use). All of these patients were decided to start antiviral treatment (pegylated interferon plus ribavirin) based on laboratory and clinical decision.

Specimen Collection and Preperation

Sera were collected from September 2010 till July 2011. Laboratory tests were done in the Microbiology department of the institute and in the department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University. The laboratory tests were performed on serum. A volume of 10 ml (milliliter) of blood was withdrawn from all patients. A volume of 2 ml of Serum was separated from blood by centrifugation and divided into five alliquots to prevent repeated

freezing & thawing of the same alliquot which may alter the results. All patients included in the study were hepatitis B surface antigen (HBsAg) and HIV antibodies negative by enzyme linked immune sorbent assay (ELISA). All patients were subjected to detection of the HCV-RNA by Real Time PCR to determine HCV viral load at baseline before the start of treatment (primary HCV PCR test). All of these patients were tested for CMV IGg by quantitative ELISA at baseline before the start of treatment (primary CMV Ig G test).

Study Design

Out of these 75 patients, 15 patients were negative for CMV IGg (group 1) (HCV positive and CMV negative). The remaining 60 patients were HCV positive, and CMV positive, 35 patients were selected randomly from them to be (group 2).

All 50 patients, group 1 (15 patients) and group 2 (35 patients) received antiviral treatment (pegylated interferon plus ribavirin) for 12 weeks.

Patients of both groups were subjected to detection of CMV Ig G by quantitative ELISA after 12 weeks of treatment (secondary CMV Ig G test). Patients of both groups were subjected to detection of the HCV-RNA by Real Time PCR to determine HCV viral load after 12 weeks of treatment (secondary HCV PCR test).

Statistical Analysis

Data was analyzed using PASW statistics 18. Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables.

For quantitative data normally distributed, comparison between two groups was done using paired t test (parametric t-test). Comparison between 2 groups was done using willcoxon signed rank test (non-parametric paired data). A p-value < 0.05 was considered significant.

RESULTS

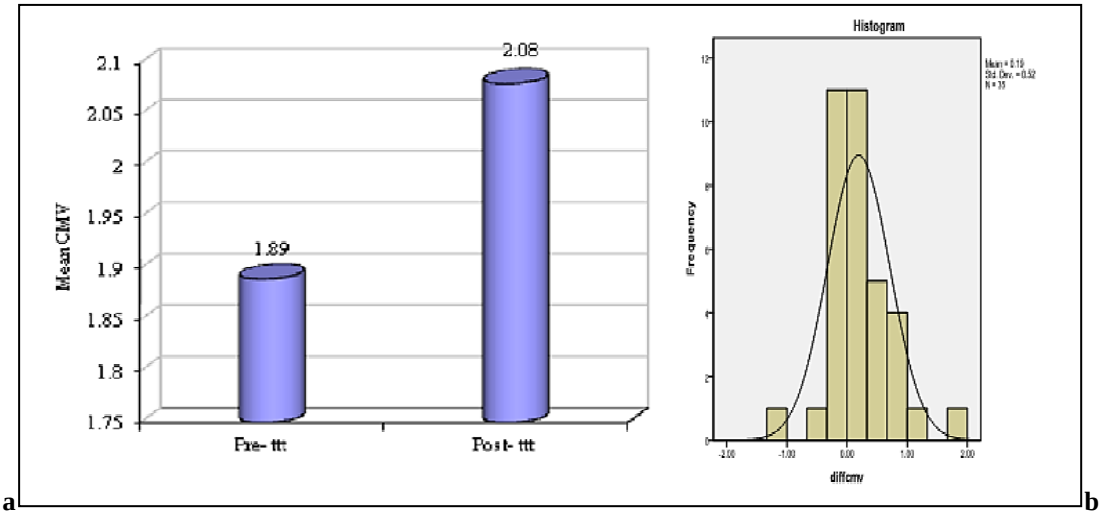
The study included 50 patients, 22 of them were below the age of 40 years and 28 of them were above the age of 40 years with the mean age of 41.36± 10.8 years. Out of 50 patients included in the study 31 patients were males and 19 were females.

In group 2; the mean value of anti CMV-IgG levels post HCV treatment was higher than that in pre HCV treatment and the difference between them was of statistical significance.

Table 1: The mean value of anti CMV- IgG levels in group 2 patients' pre and post HCV treatment:

CMV-IgG	Number	CMV-	Difference	Significance
		Mean ± SD	Mean ± SD	
Pre- ttt	35	1.89 ± 0.68	0.19 ± 0.52	.035 (S)
Post- ttt	35	2.08 ± 0.68		

* paired t test



a b

Fig. 1 a,b: The mean value of anti CMV- IgG levels in group 2 patients' pre and post HCV treatment

On comparing the effect of presence of CMV- on response to combined anti HCV treatment regimen in the 2 studied groups, the non responders were higher in group 2 (65.7 %) than in group 1 (53.3 %) but with no statistical significance (Table 2 & Fig. 2)

Table 2: Relation between CMV- presence and response to combined anti HCV treatment in the 2 studied groups:

CMV	Response		P. value	Significance
	Responder	Non responder		
G1(CMV- negative group) n =15	7 (46.7%)	8 (53.3%)	0.683	.303 (NS)
G2(CMV- positive group) n =35	12 (34.3%)	23 (65.7%)		
Total	19	31		

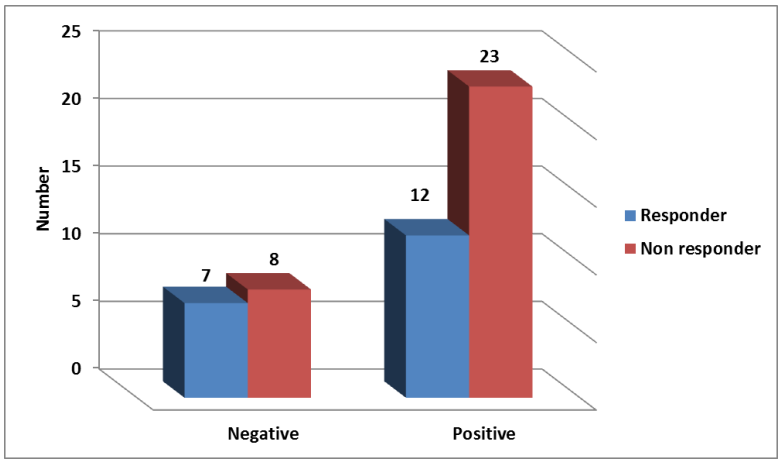


Fig. 2: Relation between CMV- presence and response to combined anti HCV treatment in the 2 studied groups

There was high statistical significant correlation between HCV - PCR viral load in the studied two groups pre and post treatment in the non responders. (Table 3 & Fig. 3)

Table 3: Relation between HCV - PCR pre and post antiviral treatment in the non-responders cases in both groups:

PCR n = 31	PCR Mean ± SD	Difference Mean ± SD	Significance
Pre- ttt	4.48E5 ± 2.42E5	3.34E5 ±2.74E5	.0000 (HS)
Post- ttt	1.14E5 ± 1.78E5		

* paired t test

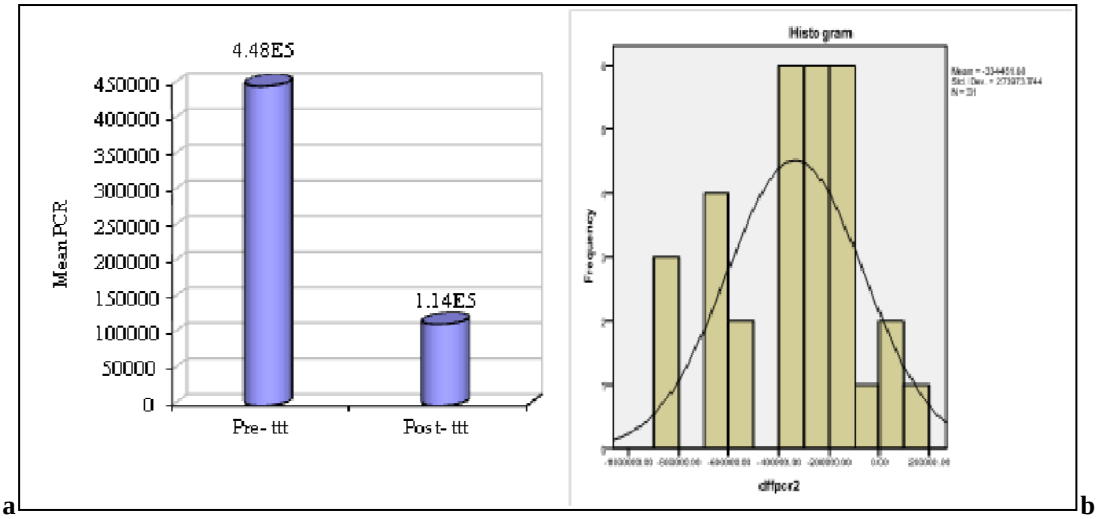


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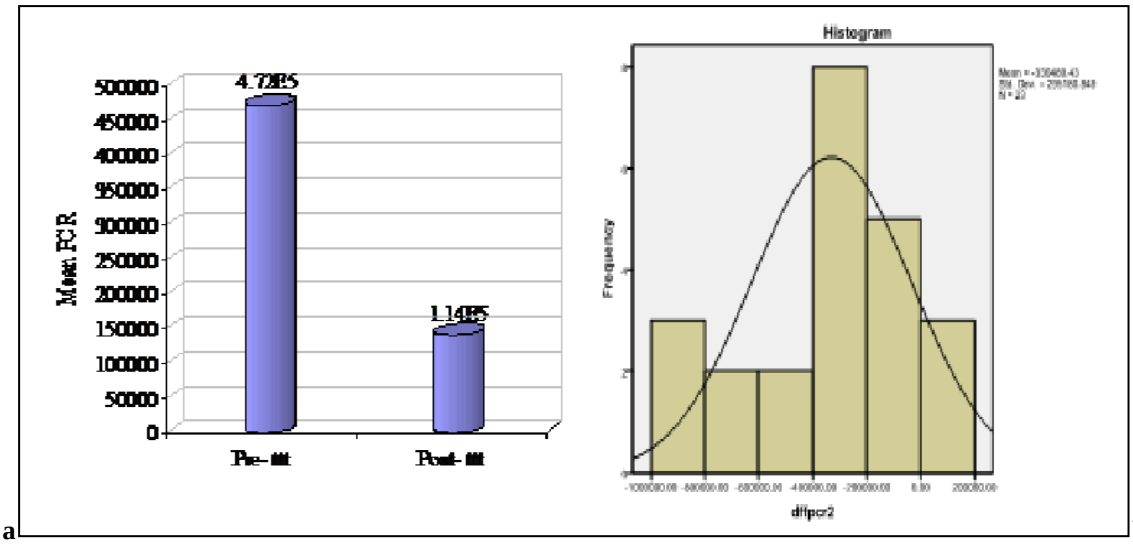
Fig. 3a,b: Correlation between HCV - PCR pre and post antiviral treatment in the non-responders cases in both groups

There was a highly significant statistical correlation between HCV PCR viral load pre and post treatment among non responders of group 2. (Table 4 & Fig. 4)

Table 4: Comparison between HCV - PCR pre and post anti viral treatment in the non responders in group 2:

PCR n = 23	PCR Mean ± SD	Difference Mean ± SD	Significance
Pre- ttt	4.72E5 ± 2.53E5	1.46E6 ±1.99E6	0.001 (HS)
Post- ttt	1.14E5 ± 1.98E5		



a **b**

Fig. 4a,b: Comparison between HCV - PCR pre and post anti viral treatment in the non responders in group 2

DISCUSSION

HCV is a globally prevalent pathogen and a leading cause of death and morbidity [10]. The most recent estimates of disease burden show an increase in seroprevalence over the last 15 years to 2.8%, equating to >185 million infections worldwide [11]. Persistent HCV infection is associated with the development of liver cirrhosis, HCC, liver failure, and death [12]. While the incidence rate of HCV infection is apparently decreasing in the developed world, deaths from liver disease secondary to HCV infection will continue to increase over the next 20 years [13].

Egypt has the highest recorded prevalence of HCV in the world, reaching 14.7% for HCV-antibody positivity among 15- to 59-year-olds in 2008 [14]. The viremic population of Egypt was estimated at over 6 million in 2008 [15].

Pegylated interferon and ribavirin combined therapy was the standard approved treatment for HCV and is only effective in around 50% of patients [16]. Several factors have been attributed with therapeutic response of chronic HCV patients including host factors, viral factors, metabolic factors, histological factors, type of regimen used and duration of infection [17].

Immune suppression and aggravation of other viral diseases is a consequence of CMV infection. CMV infection enhances HCV pathogenesis by preventing the normal mechanisms responsible for HCV clearance, thus playing vital role in HCV persistence and pathogenicity [18].

There are few studies on CMV-HCV coinfection. A study examined the association of HCV infection with measures of the immune response against CMV. The results of that study showed that both CMV seroprevalence and CMV IgG levels differed by HCV infection status, and concluded that co-infection of CMV and HCV may affect the prognosis of HCV-infected patients. Our study results come in line with this study that showed that 87% out of HCV- RNA positive patients were positive for CMV IgG [19]. Another study was conducted to examine the role of CMV reactivation in determining the response rate to treatment with interferon and ribavirin therapy in chronic HCV patients, and concluded that besides the staging of liver fibrosis, CMV co-infection should be considered as an extremely important factor when designing predictive models for HCV response to interferon treatment. Whether HCV predisposes patients to CMV infection or CMV predisposes patients to HCV is not clear [20].

Chronic persistent HCV infection leads to exhaustion and death of HCV-specific T-cells, but also causes defects in the overall immune defense. This notion is supported by the fact that peripheral dendritic and naive CD4+ T-cells are reduced both in number and function in individuals with chronic HCV [21], Peripheral CD4+ T-cell numbers are also reduced in patients with

HCV-associated cirrhosis. Therefore, chronic HCV infection will affect the immune response against CMV [22]. Notably, in our study the mean of CMV Ig G levels after treatment were higher than the mean of CMV Ig G levels before treatment with slight statistical significance.

In comparing the response to treatment of HCV in the two groups of our study, the percent of non responders was higher in group 2 (HCV CMV coinfection) with 65.7 % than in group 1 (HCV alone) with 53.3 %, but there are no statistical significant difference. In a study conducted to test whether the status of positive CMV - DNA detection adds to the predictive value of IL28B in response to treatment in egyptian HCV type 4 patients, the results showed that co-infection with CMV seriously diminished the response to IFN therapy, with SVR rates in CHC genotypes 87.5% in CMV-negative patients and 12.5% in CMV-positive patients. These data indicate that a supplemental assay for CMV viremia adds to the prognostic value of IL28B genotyping [23].

Infection with CMV has evolved multiple mechanisms for disrupting the IFN-stimulated JAK/STAT signal transduction. It appears to inhibit IFN-α responsiveness by decreasing JAK1 protein, which is an essential component of IFN-α signaling [24]. It was reported that CMV blocks IFN-stimulated gene factor 3 (ISGF3)-dependent (MHC class I, OAS) and ISGF3-independent gene expression in infected cells. Moreover, the essential component of ISGF3, protein 48 (p48), is significantly decreased by CMV. Therefore decreased JAK1 and p48 protein would inhibit IFN-α stimulated signal transduction, transcription factor activation, and gene expression, thus it is likely to globally block IFN-stimulated responses in CMV-infected patients [25].

Small sample size, assessment of CMV- DNA, and no detection of HCV genotype were the limitations in our study.

HCV drug therapy has depended on interferon-α (administered by injection) and ribavirin over many months. The resources required for treating HCV patients with such drugs have been a considerable barrier for healthcare systems in many low and/or lower-middle income countries, despite treatment outcomes that are comparable to those in well-resourced settings[26]. That said, the first generation of new direct-acting antivirals were given in combination with interferon and ribavirin, and thus added to the burden of side effects [27], second-generation DAA therapies with minimal side effects and shortened courses of therapy are associated with cure rates of more than 90% in Phase II or III studies[28]. Moreover, multiple DAA therapies targeting distinct HCV proteins have been developed and, when given in combination, will obviate the need for interferon treatment [29].

In conclusion our study found that CMV – HCV coinfection is predominant in Egypt, and CMV co-infection may influence the HCV treatment outcome, despite it failed to have statistical significance.

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