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# Serological and Immunohistochemical Prevalence of Human Cytomegalovirus Co-infection among Hepatitis C Virus Patients Admitted to Kafer El Shiekh Liver and Heart Institute, Egypt

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors HMI, FRAG and REES designed the study. Authors HMI and MAM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HMI, REES and MAM managed the analyses of the study as well as the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Human cytomegaloviruses (CMV) and Hepatitis C virus (HCV) are badly affecting the liver and can lead to hepatitis. Co-infection of CMV and HCV could accelerate the disease pathogenesis and dramatically diminish its treatment.
**Aim:** The current study is aimed to determine the prevalence of CMV diagnosed serologically and immunohistochemically among the HCV patients and to assess the biochemical and haematological alterations in such co-infection.

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**Results:** Overall prevalence of CMV infection was 47.83%, and 4.00%, using ELISA, and immunohistochemistry. No significant difference was detected in the prevalence of CMV among HCV patients based on gender, residence, age and HCV RNA load. Liver cirrhosis at patient with concomitant CMV IgG and chronic HCV infections showed a high percentage compared to those patients with chronic HCV mono-infection. Moreover, a significant reduction in the level of RBCs count, PCV, and Hb concentration was detected in patients with concomitant CMV and chronic HCV infections compared to patients with chronic HCV mono-infection.
**Conclusion:** CMV infection is frequent among HCV patients in Egypt. Obtained data recommend that screening and treating for CMV is of great importance among HCV patients in order to lessen the clinical outcome of chronic HCV infection.

*Keywords: Cytomegalovirus; Hepatitis C; co-infection; ELISA; IHC; Egypt.*

## 1. INTRODUCTION

Human cytomegaloviruses (CMV) have a linear double-stranded DNA and belong to the family of Herpesviridae. CMV can be transmitted vertically and horizontally through close contact with body fluids, such as blood, urine, tears, saliva, semen, vaginal fluids and breast milk [1,2]. Therefore CMV achieved a high global prevalence rates ranged from 60 to 90% of the world's populations [3]. CMV is an opportunistic microbe, results in asymptomatic infection in healthy individuals. After the primary infection, CMV establishes lifelong latency and later in life its reactivation may occur [4]. According to the age in which the patients gain infection and the immune status of the host, the CMV-associated diseases and consequences vary a lot [5]. In neonates, it caused hydrops fetalis and various fetal malformations [5]. In immunocompetent patients, CMV resulted in esophagitis and colitis, pneumonia, pulmonary embolism, hemolytic anaemia, myocarditis, encephalitis, portal vein thrombosis, retinitis, and hepatitis [6-12]. Previous studies reported that CMV can result in direct liver paranchymal damage by efficient cytolytic infection of human liver cells [2,13].

In Egypt, hepatitis C virus (HCV) is a health problem where its prevalence is about 15% of the Egyptian people [14-17]. HCV transmission occurs through dental care and blood transfusions [17-19]. HCV complications such as hepatocellular carcinoma (HCC) and decompensated liver cirrhosis were previously reported [20-22]. *Among the* Arab world, 63% of HCV-associated HCC deaths were recorded in Egypt [23]. Moreover, liver cirrhosis resulted from chronic HCV infection is the main cause for liver transplantation [24]. CMV is not only the common cause of morbidity after liver transplant, but also it may interact and accelerate HCV pathogenesis [25-31]. It was documented that the possibility of achieving sustained virologic response to ribavirin plus pegylated interferon treatment in chronic HCV patients could dramatically diminish during CMV infection [32]. Therefore, the current study is aimed to determine the prevalence of CMV diagnosed by ELISA and Immunohistochemistry (IHC) among the HCV patients and to assess the clinical alterations in CMV co-infection in chronic HCV infected patients.

## 2. MATERIALS AND METHODS

### 2.1 Ethical Statement

The current study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the ethical committee of Faculty of Medicine, Al-Azhar University and Kafer El Shiekh Liver and Heart Institute, Egypt. The purpose and procedures involved in the present study were explained and written informed consent was obtained from all participants.

### 2.2 Study Population

One hundred eighty four patients chronically infected with HCV from Kafer El Shiekh Liver and Heart Institute, Egypt during the period between March 2015 and April 2016 were enrolled in this study. Kafer El Shiekh Liver and Heart Institute serve Egyptian citizens from Gharbiya and Kafer El Shiekh provinces. Both provinces lie in the middle of the Delta of Egypt and north to Cairo. The patients included 84 females and 100 males, with age range 27-56 with a mean of (42.04 ±6.99) years.

In order to evaluate the haematological and biochemical alteration, the study population was

divided into three groups. Group-I: 84 patients with chronic HCV infection without CMV infection. Group-II: 65 patients with concomitant CMV and chronic HCV infections serologically detected. Group-III: 4 patients with concomitant CMV and chronic HCV infections immunohistochemically detected.

### 2.3 Exclusion Criteria

Patients with malignancy, including hepatocellular carcinoma (HCC) or renal, cardiopulmonary or autoimmune disorders and pregnant women were excluded from the study.

### 2.4 Detection of HCV Antibodies and RNA

HCV antibodies were assayed by EIA (COBAS-Amplicore, Germany). Qualitative assessment of HCV-RNA by PCR was done using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.

### 2.5 Serological Analysis of CMV Infection

CMV IgG antibodies were determined by the qualitative EIA test using commercially available kit (Atlas Medical, UK). Tests were done according to the manufacturer's instructions.

### 2.6 Immunohistochemical Analysis of CMV Infection

One hundred out of 184 patients performed a liver biopsy to diagnose or investigate the grading and staging of hepatic disease. Those 100 patients who had the immunohistochemical analysis of CMV infection done were not random sample, but a convenience sample for the current study. The liver sections from this category of patients were deparaffinised and reacted with the CMV specific antibody (Dako, Hamburg, Germany). This step was followed by the addition of a biotinylated secondary antibody that targets the primary antibody (Basic DAB Detection kit; Ventana Medical Systems, Inc., Tucson, Ariz). The complex was then visualised by using a precipitating enzyme generated product [33].

### 2.7 Haematological and Biochemical Analysis

Complete blood count (CBC) was determined using an automated haematology analyser XP series (Sysmex, Japan). Direct, total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), albumin, fasting sugar and creatinine were run on using ABX Pentra400 clinical chemistry analyser (Horiba ABX SAS, Montpellier, France). Antinuclear antibody (*ANA*) and alpha-fetoprotein (*AFP*) were determined using chemiluminescent immunoassay (Liaison, DiaSorin, Germany). *Thyroid-stimulating hormone* (*TSH*) was assayed by a commercial kit (*Teco Diagnostics, CA, USA*). International normalised ratio (INR) was done automatically using a commercial kit (Siemens Healthcare Diagnostic Inc., Germany). In patients, compensated cirrhosis were determined by Fibro-scan™ >12.5 kPa.

### 2.8 Assays Agreement Percentage Calculation

Agreement between ELISA and immunohistochemistry was calculated according to Ibrahim et al. [34-37].

The percentage of agreement between the IHC assay and ELISA = O/T×100.

Where the output (O) that represents the agreement between the two tests (ELISA & IHC) in both the positivity and the negativity = the summation of disagreement between the two assays (S) subtracted from the total No. of tested samples (T).

S = Samples that positive IHC but negative ELISA + Samples that positive ELISA but negative IHC.

### 2.9 Statistical Analysis

For statistical analysis, the SPSS (IBM SPSS statistics for Windows, Armonk, NY) computer program was used. Binary logistic regression was used to assess significant differences of CMV infection rate in HCV-infected patients of different age, localities, and sex. Hematological, biochemical and scan changes were evaluated by using an analysis of variance (ANOVA) test followed by post hoc analysis of group differences that was accomplished by the least significant differences (LSD) test; $p < 0.05$ were considered to be statistically significant.

## 3. RESULTS

There was no difference between the study populations regarding mean weight and height. CMV infection among Egyptian HCV patients

was summarised in Table 1. Overall prevalence was 47.83%, and 4.00%, using ELISA, and immunohistochemistry, respectively. No significant difference was observed among the HCV patients from Gharbiya province 48.10% compared to Kafer El Shiekh province 47.62%. CMV infection among HCV patients was detected only in Kafer El Shiekh province 8.51% using IHC. CMV infection was detected in the liver tissue of HCV patients (Fig. 1).

During the CMV detection among Egyptian HCV patients, the results of the ELISA were cross-tabulated with those of IHC and summarised in Table 2. Among one hundred HCV patients, the agreement percentage between the results of ELISA, and those of IHC was 76%.

Table 3 demonstrated the relation between CMV positivity and age, gender, and residence among Egyptian HCV patients. According to gender, the virus prevalence was lower in female patients, 45.24%, 0.00% than males, 50.00%, and 6.67% using ELISA and IHC, respectively. Although, higher prevalence was recorded in younger patients and urban area residents compared to older patients and rural area residents, no significant difference was detected in the prevalence of CMV among HCV patients based on gender, residence and age, using ELISA, or IHC.

Characteristics of cirrhosis, HCV load and some biochemical data of the study population were shown in Table 4. Liver cirrhosis at patient with concomitant CMV and chronic HCV infections serologically detected showed a higher percentage compared to those patients with chronic HCV mono-infection; cirrhosis was determined using Fibro-scan test. Although the obvious reduction in the HCV RNA load in the patients with concomitant CMV and chronic HCV infections immunohistochemically detected when compared to those patients with chronic HCV mono-infection, no significant difference was demonstrated at the level of HCV RNA load among the study populations. Moreover, similar patterns were detected at the levels of TSH, glucose, INR, and ANA among the study populations. AFP of patients with concomitant CMV and chronic HCV infections immunohistochemically detected showed lower levels than those patients with concomitant CMV and chronic HCV infections serologically detected or those patients with chronic HCV mono-infection.

**Table 1. Prevalence and diagnosis of CMV infection among HCV infected patients from Egypt**

| Regions | Total | ELISA | Total | IHC |
|---|---|---|---|---|
| Kafer El Shiekh | 105 | 50 (47.62%) | 47 | 4 (8.51%)* |
| Gharbiya | 79 | 38 (48.10%) | 53 | 0 (0.00%) |
| Total | 184 | 88 (47.83%) | 100 | 4 (4.00%) |

** Prevalence of CMV is significantly different ($p<0.05$, logistic regression test)*

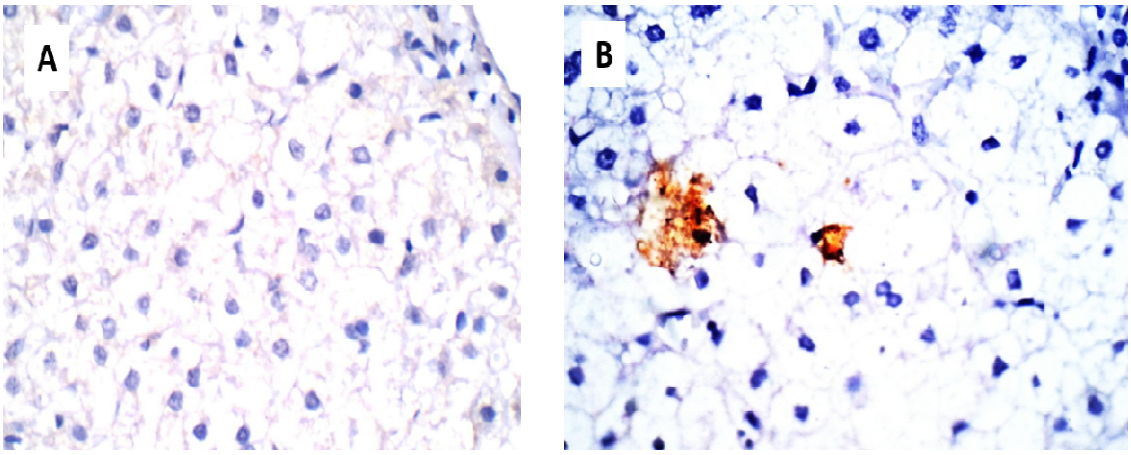


**Fig. 1. Identification of cytomegalovirus in the liver from HCV infected patient**

*(A) Immunohistochemical section of the liver from HCV infected patient without any detection of cytomegalovirus (B) Specific detection of cytomegalovirus with anti- cytomegalovirus antibody immuno-stained as a dark brown color, original magnification, ×400*

**Table 2. Summary on the detection of CMV infections in ELISA and IHC**

| | | ELISA [a] | IHC [b] | |
|---|---|---|---|---|
| | | | (+) | (-) |
| CMV | (+) | 28 | 4 | 24 |
| | (-) | 72 | 0 | 72 |
| | Total | 100 | 4 | 96 |

[a] *The frequencies of positive and negative samples as results of ELISA*
[b] *The frequencies of positive and negative samples as results of IHC cross-tabulated with ELISA results*

**Table 3. Socio-demographic characteristics and prevalence of CMV infection among HCV infected patients using ELISA and IHC**

| Characteristics | Total | ELISA | Total | IHC |
|---|---|---|---|---|
| **Age** | | | | |
| Less than(45) | 111 | 55 (49.55%) | 64 | 4 (6.25%) |
| 45 and More | 73 | 33 (45.21%) | 36 | 0 (0.00%) |
| **Sex** | | | | |
| Male | 100 | 50 (50.00%) | 60 | 4 (6.67%) |
| Female | 84 | 38(45.24%) | 40 | 0 (0.00%) |
| **Residence** | | | | |
| Urban | 42 | 21(50.00%) | 69 | 4(5.80%) |
| Rural | 142 | 67(47.18%) | 31 | 0(0.00%) |

**Table 4. Characteristics of cirrhosis, HCV RNA load and biochemical data of the study population**

| Parameter | Group I (HCV) | Group II (HCV+ CMV) ELISA | Group III (HCV+ CMV) IHC |
|---|---|---|---|
| Cirrhotic Liver | 6 (7.14%) | 7 (10.77%) | 0 (0.00%) |
| HCV RNA ($10^5$ IU/ml) | 18.31±2.72 | 20.30±3.28 | 11.62±2.70 |
| AFP (ng/dl) | 4.51±0.46 | 4.72±0.69 | 2.48±0.65 |
| TSH (µlU/ml) | 1.64±0.12 | 1.57±0.11 | 0.74±0.21 |
| Glucose (mg/dl) | 99.29±3.68 | 113.25±7.04 | 81.5±3.40 |
| INR | 1.06±0.01 | 1.07±0.16 | 1.28±0.12 |
| ANA (Negative/Positive) | 184(100%)/0(0%) | 184(100%)/0(0%) | 100(100%)/0(0%) |

*Data are expressed as: mean ± standard error (STE) or number (% among study population)*

**Table 5. Liver & kidney function findings of different groups**

| | Study population | | |
|---|---|---|---|
| | Group I (HCV) | Group II (HCV+ CMV) ELISA | Group III (HCV+ CMV) IHC |
| Creatinine (mg/dl) | 0.74±0.02 | 0.75±0.02 | 0.68±0.05 |
| Direct-Bil (mg/dl) | 0.38±0.04 | 0.30±0.03 | 0.20±0.00 |
| T-Bil (mg/dl) | 1.05±0.07 | 0.86±0.05 | 0.63±0.03 |
| *Albumin* (g/dl) | 3.79±0.06 | 3.85±0.06 | 3.50±0.18 |
| ALT (U/L) | 62.92±4.77 | 54.14±4.35 | 58.50±21.65 |
| AST (U/L) | 56.39±4.63 | 49.28±3.85 | 41.75±11.21 |

*Data are expressed as: mean ± standard error (STE)*

Liver, kidney function and hematological findings of the study population were illustrated in Tables 5 and 6. No significant differences were determined on the levels of albumin, *ALT*, *AST*, direct bilirubin, total bilirubin or creatinine (Table 5). On the level of hematological findings, a slight significant decrease ($p < 0.05$) was detected at the level of RBCs count, PCV, and Hb concentration in patients with concomitant CMV chronic HCV infections serologically or immunohistochemically detected compared to those patients with chronic HCV mono-infection. No significant alterations were determined on the levels of the other examined hematological parameters (Table 6).

**Table 6. Hematological findings of different groups**

| | Study population | | |
|---|---|---|---|
| | **Group I (HCV)** | **Group II (HCV+ CMV) ELISA** | **Group III (HCV+ CMV) IHC** |
| RBCs $\times 10^6$ | 5.11±0.06 | 4.83±0.07* | 4.58±0.15 |
| PCV (%) | 42.65±0.46 | 40.67±0.59* | 37.5±1.56* |
| Hb (g/dl) | 14.03±0.18 | 13.54±0.22 | 11.98±0.31* |
| MCV (%) | 83.58±0.70 | 84.72±0.64 | 82.3±2.50 |
| MCH (%) | 27.64±0.36 | 28.22±0.29 | 26.25±0.83 |
| MCHC (%) | 33.02±0.26 | 33.28±0.19 | 31.93±0.81 |
| Platelets $\times 10^3$ | 170.65±8.00 | 185.55±8.32 | 194±23.33 |
| WBCs $\times 10^3$ | 6.59±0.23 | 6.43±0.22 | 6.43±0.68 |
| Lym (%) | 38.37±0.95 | 39.16±0.98 | 35.95±1.92 |
| Neu (%) | 50.87±1.06 | 49.56±1.12 | 52.35±4.39 |
| Mon (%) | 10.76±0.36 | 11.43±0.36 | 10.54±1.67 |

*Data are expressed as: mean ± standard error (STE). * P<0.05 indicate significant difference compared to the patients with chronic HCV mono-infection.*

## 4. DISCUSSION

In the present study, the CMV prevalence in 184 HCV infected patients from Gharbiya and Kafer El Shiekh provinces was examined by ELISA, and from those patients 100 HCV infected patients was demonstrated using IHC. CMV overall prevalence was 47.83%, and 4.00% using ELISA, and IHC, respectively. According to the area, no significant differences were detected among patients using ELISA. While CMV infection among HCV patients was demonstrated only in Kafer El Shiekh province using IHC. Altogether, high prevalence of CMV was recorded among HCV infected patients. Consistent with the current study, Tabll et al. detected higher CMV positivity 87% and 25% for IgG and IgM antibodies, respectively, among HCV patients from Mansoura city (North Delta of Egypt) [38]. The same study detected 38% CMV positivity among HCV patients using nested-PCR [38]. Molecular prevalence of CMV was demonstrated in 36% of chronic HCV patients in Turkey [2]. Prevalence of CMV among HCV patients using IHC was 70% from Liver and Digestive System Technical Hospital in Baghdad, Iraq [39]. Moreover, CMV IgG levels were highly detected in HCV/HIV co-infected women compared to HIV mono-infected women [40].

In general, pathogen detection using IHC or PCR showed lower prevalence than serological assays. Many sampling issues, such as randomised distribution of viral units, small sample size of the collected tissue, and maybe low numbers of the viruses in the tested human tissues, are perhaps the reason for the weak reliability of the IHC assays. Although a high concordance among the results of IHC, and ELISA was observed in the current study, ELISA recorded higher prevalence than IHC. Here, the difficult detection by the microscopic examination could be attributed to low viremia and previously mentioned sampling issues. Furthermore, the antibody response is always independent of viral burden. Many pervious reports recorded that ELISA and serology are more sensitive than nested PCR, real-time PCR for CMV detection [38,41].

Although no significant relation was demonstrated between the CMV positivity and age, gender and residence among HCV infected patients using ELISA and IHC, the data of IHC was demonstrated only in males, younger patients and urban area residents. There was no statistical difference between different ages, marital status, and salary in the prevalence of CMV antibodies among blood donors at the National Blood Transfusion Centre, Nairobi [42]. Moreover, there was no significant difference between seroprevalence rates of CMV and gender among patients with hematologic disorders in Bahia State, Brazil [43]. Glory et al. reported that although no significant relation of CMV IgG seropositivity with age, occupation, residence, stage of pregnancy and parity, high prevalence was detected among the urban pregnant women from Nigeria [44].

In the current study, liver cirrhosis at patient with concomitant CMV IgG and chronic HCV infections showed a high percentage compared to those patients with chronic HCV mono-infection. Previous study demonstrated an intimate relationship between CMV and the

progression of liver injuries such as steatosis and fibrosis in HCV patients [45]. Moreover, several reports illustrated augmented severity of hepatitis activity index and fibrosis status during CMV/ HCV co-infection in patients undergoing liver transplantation [46-48]. In this study, a slight reduction in the HCV RNA load was detected in concomitant CMV and chronic HCV infections using IHC when compared to patients with chronic HCV mono-infection. Bayram et al. demonstrated reduction in the HCV RNA loads in CMV-infected patients that determined in liver tissue biopsies by real-time quantitative polymerase chain reaction method [2].

Moreover, in the current study, a significant reduction in the level of RBCs count, PCV, and Hb concentration was detected in patients with concomitant CMV and chronic HCV infections compared to patients with chronic HCV mono-infection. In general, The CMV syndrome is characterised by fever, malaise, and leukopenia, monocytosis, and anaemia [49]. Furthermore, patients with *chronic kidney disease* who are CMV seropositive require higher erythrocyte stimulating agent doses to treat anaemia than patients without CMV antibodies [50].

## 5. CONCLUSION

The present study indicated that CMV infection is frequent in Egypt, with noticeable prevalence among HCV patients. Further studies are required to understand the association of HCV with CMV antibodies in Egypt. Obtained data provide critical information regarding the co-infection of HCV and CMV in Egypt and recommend screening and treating for CMV is of great importance among HCV patients in order to lessen the clinical outcome of chronic HCV infection.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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