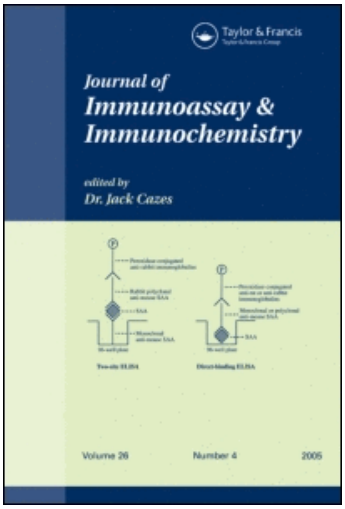

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Distinct Serum Immunoglobulins Pattern in Egyptian Patients with Chronic HCV Infection Analyzed by Nephelometry

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Abstract: Hepatitis C has emerged as a major worldwide public health problem. The host immune response to HCV infection is composed of both a non-specific immune response, including interferon (IFN) production and natural killer (NK) cell activity, and a virus-specific immune response, including humoral and cellular components. Susceptibility to infection has been related to immunological disturbances. Several studies have provided experimental evidence of disorders of both cellular and humoral immunity. The present study was carried out to evaluate the serum immunoglobulins level (IgG, IgM, IgA) and IgG-subclasses (IgG1–4) in chronic hepatitis C patients in comparison with healthy control patients. This study included 50 patients with biochemical, serologic, virologic, and histologic evidence of chronic hepatitis C. Total IgG, IgA, and IgM were assayed by nephelometry. IgG subclasses were assayed using human IgG subclasses enzyme immunoassay. The results showed a significant increase of total serum IgG and IgM levels found in patients with chronic HCV compared with the healthy control patients ($P < 0.001$ for each). There was a statistically significant difference in the IgG subclasses (IgG1 to IgG4) between the patients and controls ($P < 0.001$ for each). On the other hand, no significant difference was

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found between patients and healthy controls in IgA level ($P = 0.4$). The normal total serum immunoglobulins pattern is apparently shifted in chronic hepatitis C infection in the Egyptian patients. This pattern may include an ethnic or biologic background and could be used in the differentiation of the patients with minimal liver disease.

Keywords: Hepatitis C virus (HCV), Immunoglobulins, Nephelometry

INTRODUCTION

Hepatitis C infection is a major worldwide public health problem.[1] Between 70% to 80% of those infected with HCV will develop chronic infection that may progress to cirrhosis, liver failure, or hepatocellular carcinoma.[2] The host immune response to HCV infection is composed of both a non-specific immune response, including interferon (IFN) production and natural killer (NK) cell activity, and a virus-specific immune response, including humoral and cellular components.[3] Susceptibility to infection has been related to immunological disturbances. Several studies have provided experimental evidence of disorders of both cellular and humoral immunity.[4]

The pathogenesis of serum immunoglobulin increase (hyperglobulinemia) is not completely understood, and probably includes additional mechanisms, as the profile of immunoglobulin elevation varies according to the cause of liver cirrhosis. Hyperglobulinemia in patients with liver cirrhosis tends to affect all immunoglobulin subclasses.[5] Hyperglobulinemia affecting the three main immunoglobulin classes was considered a hallmark of chronic active hepatitis,[6] but all studies before 1989 probably contained mixed patients with both autoimmune hepatitis and chronic HCV infection.

Serum immunoglobulin M (IgM) values are particularly higher in patients with primary biliary cirrhosis.[6] Increased serum immunoglobulin A (IgA) levels may be present in all types of cirrhosis, but are characteristic of those of alcoholic origin.[7] Conversely, selective IgA deficiency was found to be associated with chronic HCV infection in one study.[8] Selectively increased immunoglobulin G1 (IgG1) levels were observed in a group of 76 patients with chronic HCV infection, 29 of them having liver cirrhosis.[5] Assay of immunoglobulins G is a useful method for distinguishing primary HCV infection from chronic or past HCV infection.[9]

As the IgG subclasses are major serum immunoglobulins important in secondary antibody responses, they are responsible for immunological memory and long-term protection against viral and bacterial infections. Each of the four IgG subclasses is encoded by a separate C region gene and endowed with unique biological functions that are important for an efficient humoral response to a given pathogen. In adults, antibody responses to protein antigens are restricted mainly to IgG1, IgG3, or both; IgG2 is generally stimulated by carbohydrate antigens, whereas IgG4 has been associated with chronic antigenic stimulation.[10]

Few and controversial studies are available about the total immunoglobulin pattern in HCV infected patients. In addition, none of these studies, to the best of our knowledge, mentioned the pattern of IgG subclass alterations in those patients. Thus, our rationale was to investigate the effect of the hepatitis C virus infection, as a causative agent of liver disease, on the level of serum immunoglobulins. Different isotypes (IgG, IgM, & IgA) and IgG-subclass levels (IgG1–4) were investigated in patients with chronic hepatitis C infection and compared with healthy control.

EXPERIMENTAL

Patients

This study included 50 patients (40 males and 10 females) with chronic hepatitis C, with median age of 35 years. All of them had positive serum anti-HCV antibodies, positive serum HCV-RNA, and histologically proven chronic hepatitis. The histopathological assessment was carried out separately by two pathologists, and then a consensus between them was made on discordant assessments. The two pathologists were not aware of the clinical picture at the time of assessment. Patients with schistosomiasis, HBV, biliary cirrhosis, autoimmune hepatitis, and other liver-related diseases were excluded during the clinical, laboratory, and pathological investigation. Special attention was given to cases with minimal liver disease, as an approach to evaluate if the causative agent, independently of liver damage, influences the serum immunoglobulin levels. Therefore, all patient groups were without apparent pathological features, i.e., free from cirrhosis (micronodular, macronodular, or mixed), fibrosis, and hepatocellular carcinoma. All these lesions will be tested in a further study. The pathological criteria of these patients ranged between chronic hepatitis with minimal or mild activity to moderate activity with no cirrhosis. All patients were chosen from the out-clinic patients of National Liver Institute, Minufiya University and Gastro-Entrology Surgery Center, Mansoura University. The results of the patient groups were compared with 25 healthy controls away of the patients' neighborhood, with age and gender matching the patients. All patients gave written informed consents. All patients and controls were not alcohol drinkers or tobacco users.

Enzyme Linked Immunosorbant Assay (ELISA) for Detection of Anti-HCV Antibodies

All patients and controls were screened for the presence or absence of anti-HCV antibodies. An ETI-AB-HCV K-3 Kit was used. It is an enzyme immunoassay utilizing the 3rd generation of ELISA (Sorin Biomedica,

Saluggia, Italy). Highly antigenic determinants of both structural (recombinant C22 and C33) and non structural (synthetic NS4 and NS5) regions of HCV genome were coated onto microtitre plates to capture specific antibodies present in serum samples.

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR technique was performed to confirm the obtained results with ELISA and to identify patients with viremia by detection of HCV-RNA. In brief, HCV-RNA was extracted using the phenol/isopropanol method as described by Chomczynski and Sacchi.[11] The synthesis and extension of complementary DNA (cDNA) was performed according to the method of Sullivan and Gerber[12] using dNTPs, primers, and RT enzymes (Promega Corp., Madison, USA), the reaction was run in a programmable DNA thermal cycler (Perkin Elmer, USA) for 35 cycles: denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. Detection of the amplified specific DNA sequences was performed using a DNA enzyme immunoassay kit (Sorin Biomedica). The kit utilizes biotinylated single stranded nucleic acid probes specific for the HCV genome coated onto a microtitre plate. The manufacturer's instructions were followed.

Serum Immunoglobulins Determination

IgG, IgA, and IgM were assayed by nephelometry (BN-II Analyzer, Behring Diagnostics, Marburg, Germany). IgG subclasses were assayed using a Human IgG subclasses enzyme immunoassay kit (The Binding site Ltd., Birmingham, UK). The manufacturer's instructions were applied.

Statistical Analysis

Results were expressed as a median and were analyzed by using Mann-Whitney U test, Kurskal-Wallis test, and Spearman's correlation. $P \leq 0.05$ was considered significant. All statistical procedures were performed by using SPSS software, version 11 for windows.

RESULTS

The demographic and biochemical data for patients and controls groups are listed in Table 1. All HCV patients were positive for HCV-RNA as detected by the RT-PCR, and the controls were confirmed for their negativity in that test and also in an ELISA assay. The median of serum total IgG in the

Table 1. The demographic and biochemical data in the chronic HCV patients and in the healthy controls

Variable	Chronic HCV patients	Healthy controls
Male/Female	40/10	20/5
Age (Years)	35	33
Serum AST (IU/mL)	38	32
Serum ALT (IU/mL)	79	30
Serum albumin (g/dL)	4.2	4.7
Serum bilirubin (mg/dL)	0.9	0.7

Data are expressed as median.

patients group with chronic hepatitis C was 1395 mg/dL demonstrated a significant difference ($P < 0.001$) when compared to that of the healthy control (1111 mg/dL). A significant increase in IgG1, IgG2, IgG3, and IgG4 levels was also found in the patients compared with healthy control ($P < 0.001$ for each) (Figures 1–7) (Table 2).

Serum IgM was increased in patients with HCV infection compared with healthy control ($P < 0.001$). Mean serum total IgA in the patients group was 399.8 mg/dL, which showed no significant difference ($P = 0.4$) when compared with the healthy control (394 mg/dL).

No correlation was found between total IgG and IgA (r = 0.05) and also, between IgM and IgA (r = 0.242). On the other hand, a good correlation was

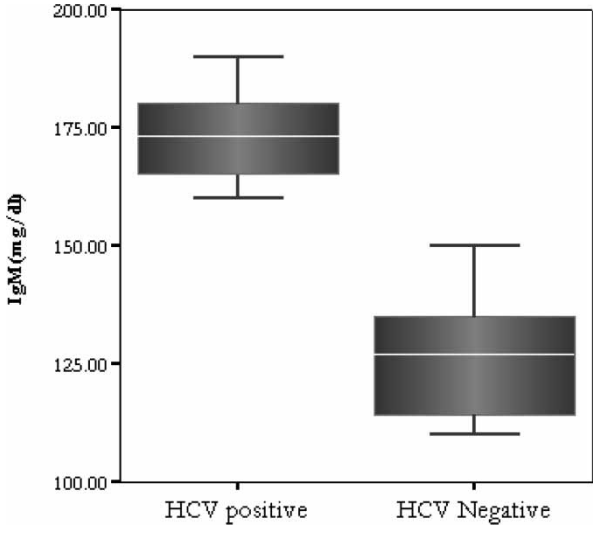

Figure 1. The level of serum total immunoglobulin (IgM) in the patients group (HCV positive) compared to healthy control group (HCV negative).

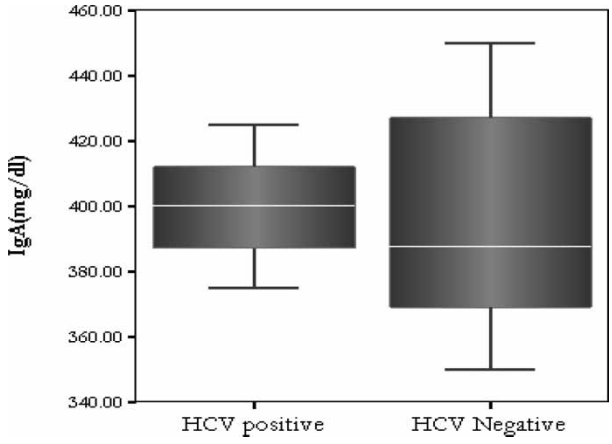

Figure 2. The level of serum total immunoglobulin (IgA) in the patients group (HCV positive) compared to healthy control group (HCV negative).

found between IgM and IgG ($r = 0.87$) (Table 3). No significant correlation was found between the investigated immunoglobulins with the biochemical and demographic data listed in Table 1 and or the pathological criteria of these patients ($P > 0.05$ for each).

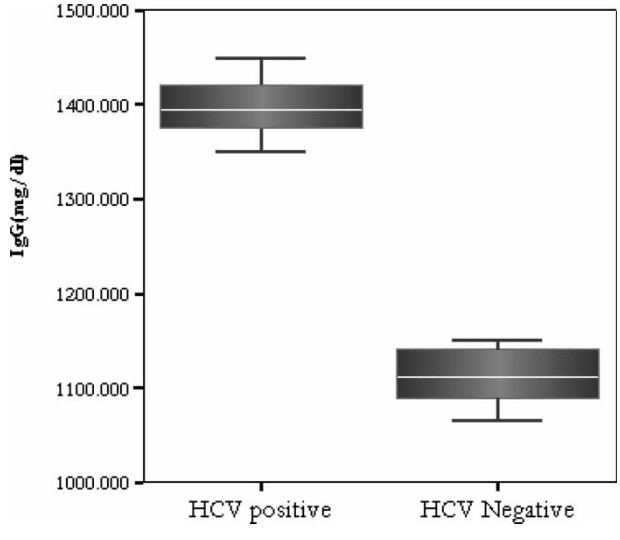

Figure 3. The level of serum total immunoglobulin (IgG) in the patients group (HCV positive) compared to healthy control group (HCV negative).

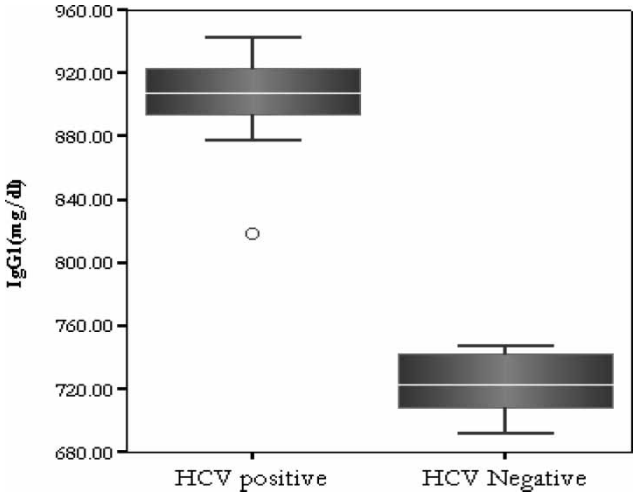

Figure 4. The level of serum total immunoglobulin (IgG1) in the patients group (HCV positive) compared to healthy control group (HCV negative).

DISCUSSION

It is well known that a number of viral infections profoundly change the immune state.[13] In the present study, the data showed that the chronic HCV infection is associated with a distinct pattern of serum immunoglobulin.

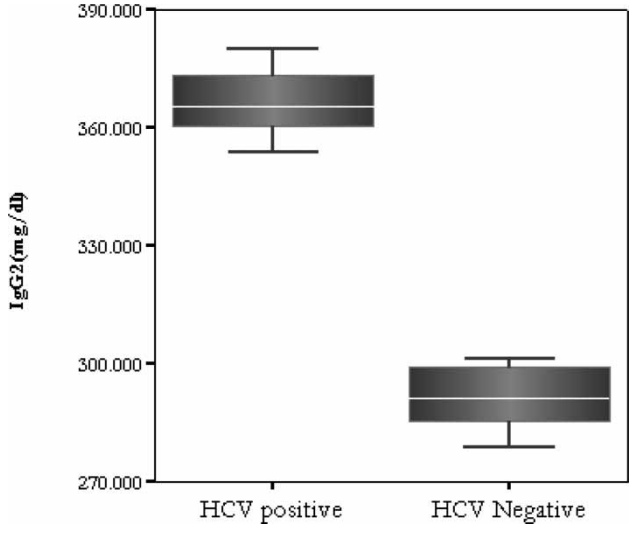

Figure 5. The level of serum total immunoglobulin (IgG2) in the patients group (HCV positive) compared to healthy control group (HCV negative).

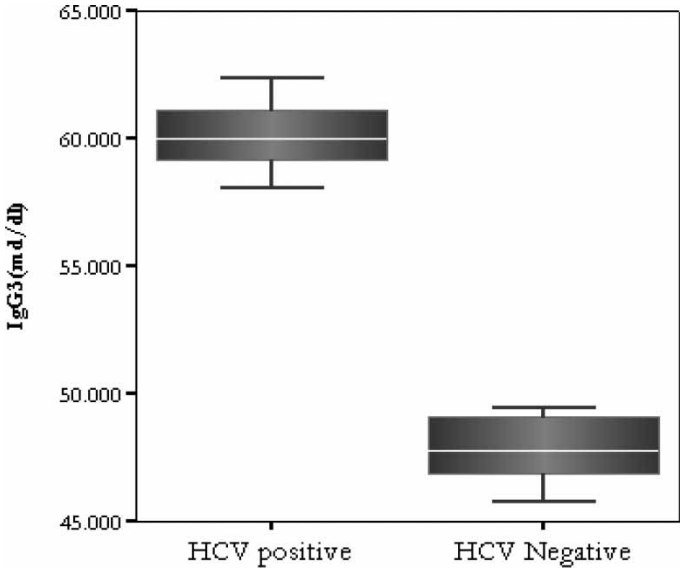

Figure 6. The level of serum total immunoglobulin (IgG3) in the patients group (HCV positive) compared to healthy control group (HCV negative).

Total IgG, IgG1, IgG2, IgG3, and IgG4 levels were increased in patients with HCV infection when compared with the controls.

Chronic HCV infection is associated with many immunological aberrations, which include alterations of the Th1/Th2 cytokine balance.[14,15] A specific cytokine imbalance could prompt B cells to increase IgG production in patients with HCV infection, independently from liver damage. HCV infection is also associated with many cases of cryoglobulinemia and some cases of B-cell lymphoproliferative disorders.[16–18] Andre and McQuilkan[19] reported that the increase in gamma globulin is due to stimulations of numerous plasma cell clones by either exogenous or endogenous antigens.

Some investigators[20,21] demonstrated that elevated antibodies of a particular IgG subclass in chronic diseases suggest that the immunoregulation of these antibodies would reflect fundamental humoral abnormalities in these disorders. The elevation of IgG1 and IgG2 level may be due to higher humoral response and the lymphocytotoxic activity has been found mainly in the IgG subclasses IgG1 and IgG2. These findings are similar with models of generalized enhanced B-cell responses. In many of these models elevated serum IgG subclass levels have been noticed to exist in addition to elevated specific antibody levels.[20]

Our results agree with Monos et al.[22] and Buelow et al.[23] who stated that the elevated serum IgG is associated with most of the lymphocytotoxic

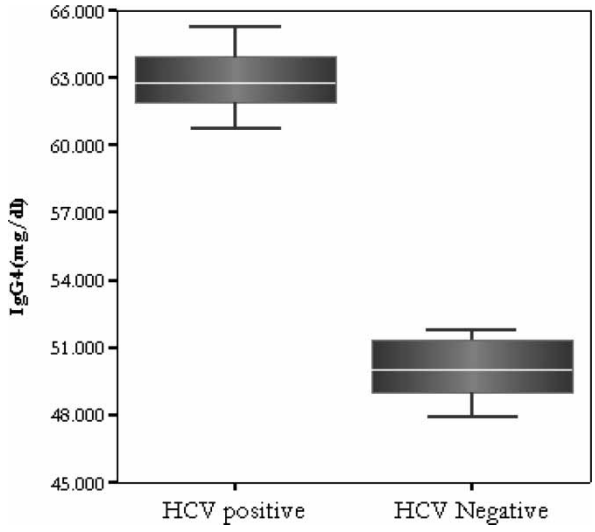

Figure 7. The level of serum total immunoglobulin (IgG4) in the patients group (HCV positive) compared to healthy control group (HCV negative).

activity. The highest lymphocytotoxic activity was present mainly in the IgG1 and 2 subclasses. Kanno and Kazuyana[9] reported that an assay of IgG is a useful method for distinguishing primary HCV infection from chronic or past HCV infection.

There was a significant difference ($P < 0.001$) found in serum immunoglobulins level (IgG and IgM) between patients with chronic hepatitis C and the healthy control. These results may indicate that HCV, per se, may induce such immunoglobulin alteration independently from liver pathology itself. This is in agreement with Gonzalez-Quintela *et al.*[24] in regard to the IgG

Table 2. Serum total immunoglobulins concentrations (expressed in mg/dL) in healthy subjects and in chronic HCV patients

Variable	Chronic HCV patients		Healthy controls
	Median (5th, 95th percentiles)		*P* value
IgA	400 (375, 421)	387.5 (350, 450)	$P = 0.4$
IgM	173 (160, 190)	127 (110, 150)	$P < 0.001$
IgG	1395 (1350,1448.5)	1111 (1065, 1150)	$P < 0.001$
IgG1	906.8 (836.7, 941.5)	722 (692, 747.5)	$P < 0.001$
IgG2	365.5 (353.7, 379.5)	291 (279, 301)	$P < 0.001$
IgG3	59.99 (58.1, 62.3)	47.8 (45.8, 49.5)	$P < 0.001$
IgG4	62.8 (60.8, 65.2)	50 (47.9, 51.8)	$P < 0.001$

Table 3. Correlations between different serum immunoglobulins concentration

IgG × IgA	0.05	$P = 0.77$
IgM × IgA	0.242	$P = 0.161$
IgG × IgM	0.87	$P < 0.001$

elevation in those patients. They suggested that the causative agent of liver disease may induce an imbalance in the regulation of immunoglobulin classes.

In regard to the serum IgM, it was found to be increased in patients with HCV infection compared with control. The elevated serum IgM levels may be related to chronic antigenic stimulation and this is in agreement with previous studies in patients with other liver diseases, such as primary biliary cirrhosis.[6] The elevated levels of IgM and IgG in HCV infection, described by Sarin and his collaborators,[25] could be due to the dysfunction of the T cells, currently regarded as responsible for the virus persistence.[26] The elevated levels of IgM found in the HCV group in our study corroborate Sarin's *et al.* findings[25] and Caly *et al.*[27]

Similar IgA was found in patients and controls. Previous reports showed that the IgA is increased in patients with liver cirrhosis and IgA values correlate with liver dysfunction.[7,28] The elevation of IgA was found in alcoholic liver disease but not in chronic HCV infection as reported in many studies.[24,25,27] These studies in agreement with our study and contradict the results of Watt *et al.*[29] who reported that the IgA was elevated in chronic HCV infection.

In conclusion, our data revealed an elevation of humoral immune response in chronic hepatitis C as indicated by increasing serum immunoglobulin isotypes IgG, IgM, and IgG subclasses (IgG1–4). In addition, it showed that the HCV infection is apparently associated with alteration of serum immunoglobulins in cases with minimal liver disease. Finally, these findings may provide some new insights about antibody response to HCV. Further studies are needed to elucidate both the mechanisms and the consequences of such immunoglobulins elevation. In addition, further study is needed to explore whether or not the total immunoglobulins pattern affects the different modalities of therapy, chronicity, persistence, and clearing of HCV.

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