

# Study of Non-organ-specific Antibodies in Children with Genotype 4 Chronic Hepatitis C

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## ABSTRACT

**Background/Aim:** Adult studies established a relationship between hepatitis C virus (HCV) infection and the presence of non-organ-specific antibodies (NOSAs). Most studies were carried out on genotypes 1 and 2. Only a few studies addressed that issue in pediatrics. No studies have been carried out on autoimmunity and genotype 4 in children. We aim to investigate NOSAs in 80 Egyptian children with chronic HCV infection along with studying the underlying genotype of HCV, and correlating autoimmunity with the epidemiological, clinical, biochemical, and virological features. **Materials and Methods:** HCV-RNA was assayed by the polymerase chain reaction and viral genotypes were determined. NOSAs were measured and liver biopsies were taken for histopathological examination. **Results:** Genotype 4 was the only detected genotype in the included 80 patients. Anti-smooth muscle antibodies (ASMA) were the only detected antibodies in 32 (40%) patients, always with V specificity (vessels only) at titers ranging from 1:20 and 1:160. Anti-nuclear antibodies (ANA) and liver-kidney microsomal antibodies-1 (LKMA-1) were not detected in any of our patients. Epidemiologic and clinical features did not significantly differ between autoantibody-positive and -negative patients. Among biochemical features, significantly high levels of total bilirubin, albumin, immunoglobulins, alkaline phosphatase, and gamma-glutamyl transpeptidase were found in the antibody-positive group. **Conclusion:** Genotype 4 HCV is the prevailing genotype in Egyptian children with chronic HCV infection. A consistent proportion of these children with chronic HCV infection circulate non-organ-specific autoantibodies. The prevalence of ASMA and the absence of ANA and LKMA-1 might be related to the unique situation in Egypt with unique prevalence of genotype 4. More studies are warranted on larger pediatric population to validate these findings.

**Key Words:** Children, Egypt, genotype 4, hepatitis C, non-organ-specific antibodies

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Since its discovery in 1989,<sup>[1]</sup> hepatitis C virus (HCV) has been associated with autoimmunity and extrahepatic manifestations.<sup>[2]</sup> In studies among adult patients, the prevalence of autoantibodies in chronic HCV-infected patients varied from 25% to 66%,<sup>[3-6]</sup> Data on these topics in children are scarce, with the prevalence of non-organ-specific autoantibodies (NOSAs) varying from 8% to 65%.<sup>[7]</sup> The most commonly detected autoantibodies

in adults are anti-smooth muscle antibodies (ASMA), followed by anti-nuclear antibodies (ANA), and liver-kidney microsomal antibodies-1 (LKMA-1).<sup>[8]</sup> Same is true for children, with ASMA prevalence varying from 5% to 51%, ANA prevalence from 0% to 10%, and LKMA-1 from 2% to 15%.<sup>[8-11]</sup>

The development of NOSAs is considered part of the natural course of chronic HCV infection in children.<sup>[8-11]</sup> Different mechanisms have been implicated in the development of NOSAs during chronic hepatitis C with clear evidence of altered immune system homeostasis in chronically infected patients.<sup>[12]</sup>

The characteristic lymphotropism of HCV could be one of the basis of the increased production of autoantibodies. It has been hypothesized that HCV interacting with B

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lymphocytes can lower the B-cell activation threshold favoring autoantibodies production, and that HCV triggers autoimmune response via a molecular mimicry mechanism.<sup>[7]</sup>

HCV can induce cellular injury determining the release of self-antigens that are normally protected from the immune system but when released are able to elicit an autoimmune response.<sup>[13,14]</sup>

Egypt has a very heavy burden of liver disease due to chronic HCV infection. According to the Egyptian Demographic Health Survey, 15% among the survey respondents had antibodies to HCV, whereas 10% were found to have active infection<sup>[15]</sup> and 91% of the patients were infected with HCV genotype 4 (HCV-4).<sup>[16]</sup>

Thus Egypt has a unique situation with HCV-4 as the prevailing genotype. We hereby aim to investigate the prevalence of non-organ-specific antibodies in a series of 80 Egyptian children with chronic HCV, along with studying the underlying genotype of HCV, and correlating autoimmunity with the epidemiologic, clinical, biochemical, and virologic features.

## MATERIALS AND METHODS

### Patients

A prospective cohort study was carried out on 80 Egyptian children with chronic HCV infection from those attending the gastroenterology and hepatology outpatient clinic and inpatient ward, Department of Pediatrics, Faculty of Medicine, Zagazig University, Egypt, in the period from April 2012 to March 2013, fulfilling the following inclusion and exclusion criteria.

### Inclusion criteria

1. Children aged 2 to 16 years.
2. Confirmed chronic HCV infection by abnormal alanine aminotransferase (ALT) levels for >6 months, histologic evidence of hepatitis in liver biopsy, serum positivity for anti-HCV, and HCV-RNA.

### Exclusion criteria

1. Patients with serological evidence of co-infection with hepatitis B and delta-virus or with human immunodeficiency virus.
2. Patients with other causes for their chronic liver disease.
3. Patients with de-compensated liver disease.
4. Patients with underlying systemic, metabolic, or autoimmune diseases or with positive family history of these diseases.

One hundred-twenty healthy children of matched age and gender served as controls. They were selected from children

who are medically fit and attend the ordinary pediatric outpatient clinic for regular follow-up for growth and nutrition (the Well Child Clinic).

The study was approved by the research and ethical committee, Faculty of Medicine, Zagazig University. The parents of patients and controls signed written consents for the contribution of their children in the current study.

All patients and controls were subjected to full history taking, thorough clinical examination, and analysis of their sera to NOSAs.

### Serological tests for detection of NOSAs

Sera were obtained from the whole blood of patients and controls. The serum samples were frozen at  $-20^{\circ}\text{C}$  until the time of screening. All serological assays were performed by a single investigator under identical experimental conditions in a single laboratory. Patients' serum samples were taken at entry to the study. A strategy was put for those with negative results at initial testing to be repeated once more on follow up visits after 3 and 6 months in order not to miss any positive case, as it is well known that autoantibodies production is fluctuating.<sup>[10]</sup>

Serum ANA, ASMA, and LKMA-1 were detected using indirect immunofluorescence on rat liver/kidney/stomach sections (6 wells) using the kit provided from DiaSorin® (DiaSorin® Deutschland GmbH - Dietzenbach, Germany).<sup>[17]</sup> The FLUORO-KIT Test Systems were based on indirect fluorescent antibody technique. Patient serum samples were diluted (1:20) in phosphate-buffered saline and overlaid onto tissue cryostat sections fixed on a microscope slide. The slides were revealed using a fluorescein-labeled antihuman immunoglobulins. NOSAs were considered positive at titers of dilutions of  $\geq 1:20$ .

ANA was tested by immunofluorescence at 1:20 dilution on HepG2 cells. It was recorded as positive when the nuclei of the liver cells, the nuclei within the distal and proximal kidney tubules, and the nuclei of both the parietal cells and chief cells in the stomach tissue gave apple green fluorescence.

ASMA was recorded positive when the muscularis mucosa and the muscularis externa of the stomach tissue as well as the muscle layer of the arterioles (V) that may be present in any of the tissue sections gave apple green fluorescence. ASMA was considered strongly positive (grade 3) if ( $>1/80$ ),<sup>[18,19]</sup> moderately positive (grade 2) if ( $1/80$ ),<sup>[18,19]</sup> and weakly positive (grade 1) if ( $1/20$  to  $1/40$ ).

LKM-1 was recorded positive when cytoplasm of liver and cytoplasm of kidney tubules gave apple green fluorescence.

## Biochemistry

Serum levels of aspartate aminotransferase (AST), ALT, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and serum gammaglobulins were measured.

Immunoglobulin (Ig) G, IgA, and IgM levels were measured using immunonephelometry using Siemens® reagents on BN Propec (Siemens®, Dortmund, Germany).

## Virologic assays

Serologic evidence of HCV infection was provided by detecting antibodies to HCV by third-generation enzyme-linked immunoassays (Ortho Diagnostic System Raritan, NJ, USA). HCV-RNA levels were obtained for all patients using a commercial quantitative polymerase chain reaction technique (PCR, Amplicor HCV, Roche Mannheim, Germany).

## Hepatitis C virus genotyping

HCV-RNA-positive samples were genotyped using the HCV Real-TM Genotype kit (Sacace Biotechnologies S.r.l., Como, Italy) that detects different HCV genotypes 1a, 1b, 2, 3, and 4, following the manufacturer's instructions. Briefly, 5 µL of a sample of cDNA, 4 µL of Taq DNA Polymerase, and 6 µL of each PCR mix: (PCR-mix-1-FRT HCV 1b/3, PCR-mix-1-FRT HCV 1a/2, and PCR-mix-1-FRT HCV 4/IC) were distributed on a MicroAmp® Optical 96-Well Reaction Plate (Applied Biosystems, Foster City, CA, USA). The PCR reactions were done in a 7500 Real-Time PCR System (Applied Biosystems).

## Liver biopsy

A liver biopsy specimen was obtained from all patients using Menghini technique. A single liver pathologist

reviewed all tissue specimens without prior knowledge of autoantibody results. Liver biopsies were histologically studied and analyzed according to the Metavir and Ishak scores.<sup>[20]</sup>

## Statistical analysis

Data were analyzed using SPSS (version 15.0., SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using the Student's *t* test or Mann-Whitney test, corrected  $\chi^2$  test or Fischer's exact test, where appropriate. The results were expressed as counts and percentages for qualitative variables and as means or medians and ranges for discrete variables. A *P* < 0.05 was considered to be statistically significant.

## RESULTS

### Characteristics of patients

Eighty children with chronic HCV infection were enrolled in the study. Thirty-four (42.5%) were males and 46 were females (57.5%). Age ranged from 2 to 16 years with mean  $\pm$  SD; 7.27  $\pm$  3.60 years. Forty-five patients (56.3%) had a history suggestive of parenteral route of acquiring HCV infection (ie, received blood transfusions for intercurrent diseases or surgery early in life or undergone surgery without transfusions, etc). Thirty (37.5%) had a mother with chronic hepatitis C (vertical transmission not documented). Five (6.2%) had not apparently been exposed to infection. None had a history of acute hepatitis [Table 1].

### HCV-RNA and genotyping

Patients exhibited mean  $\pm$  SD HCV-RNA levels of

**Table 1: Epidemiological, clinical, and histological features of chronic HCV patients at entry into the study**

Parameters of comparison	NOSAs negative N=48	NOSAs positive N=32	<i>P</i> value	Total HCV patients N=80	Controls N=120	<i>P</i> value
Age (mean $\pm$ SD), years	7.6 $\pm$ 3.87	6.8 $\pm$ 3.1	>0.05	7.27 $\pm$ 3.6	6.6 $\pm$ 3.1	>0.05
Males, <i>n</i> (%)	20 (41.7)	14 (43.7)	>0.05	34 (42.5)	56 (46.7)	>0.05
Female, <i>n</i> (%)	28 (58.3)	18 (56.3)		46 (57.5)	64 (53.3)	
Mode of transmission, <i>n</i> (%)						
Parenteral	20 (41.7)	25 (78.1)	<0.001	45 (56.3)	0 (0)	<0.004
Vertical	24 (50)	6 (18.8)		30 (37.5)	0 (0)	
Unknown	4 (8.3)	1 (3.1)		5 (6.2)	0 (0)	
Clinical examination, <i>n</i> (%)						
Pallor	36 (75)	14 (43.7)	<0.05	50 (62.5)	0 (0)	<0.05
Jaundice	12 (25)	14 (43.7)	>0.05	26 (32.5)	0 (0)	>0.05
Fever	0 (0)	2 (6.3)		2 (2.5)	0 (0)	>0.05
Hepatomegaly, <i>n</i> (%)	12 (25)	14 (43.7)	>0.05	26 (32.5)	0 (0)	>0.05
Splenomegally, <i>n</i> (%)	12 (25)	17 (53.1)	<0.05	29 (36.3)	0 (0)	<0.05
Liver histopathology, <i>n</i> (%)						
Mild-to-moderate chronic hepatitis	6 (12.5)	25 (78.1)	<0.0001	35 (43.8)	NA	<0.0001
Minimal chronic hepatitis	42 (87.5)	7 (21.9)		45 (56.3)	NA	

NOSAs: Non-organ-specific antibodies, HCV: Hepatitis C virus, NA: Not applicable

**Table 2: Correlation of autoimmunity and biochemical characteristics of chronic HCV patients**

Biochemical parameter Mean±SD Range 95% CI	Patients		t	P
	NOSAs negative N=48	NOSAs positive N=32		
Total bilirubin (mg/dL)	1.86±0.4 (1.2-2.8) 1.74-1.98	2.1±0.4 7 (1.5-2.9) 1.93-2.27	2.37	<0.01*
Direct bilirubin (mg/dL)	0.76±0.3 (0.3-1.5) 0.67-0.85	0.87±0.4 (0.4-1.4) 0.73-1.01	1.33	0.09
ALT (U/L)	51.2±27.9 (10-95) 43.1-59.3	52.2±19.7 (12-103) 45.1-59.3	0.14	0.42
AST (U/L)	41.3±18.6 (11-88) 35.9-46.7	47±23.2 (17-89) 38.6-55.4	1.16	0.4
ALP (KAU/dL)	9.9±8.7 (4-43) 7.37-12.43	17±7.3 (4.5-33) 14.37-19.63	MW 7.5	0.0005*
GGT (U/L)	47.4±16 (29.5-95.5) 42.75-52.02	77.3±19.3 (30-97.4) 70.34-84.26	7.25	<0.0001*
Total protein (g/dL)	6.2±0.6 (6-9) 6.03-6.37	6.36±0.67 (5.0-7.3) 6.12-6.6	1.1	0.14
Albumin (g/dL)	3.4±0.5 (3.3-4.9) 3.3-3.5	4.1±0.45 (3.5-4.9) 3.9-4.3	6.5	<0.0001*
Globulin (g/dL)	2.8±0.5 (2.1-4.7) 2.66-2.95	2.7±0.5 (2.1-3.9) 2.52-2.88	0.88	0.4
PT (s)	13.9±1.3 (12-18) 13.52-14.28	13.66±1.3 (12-17) 13.19-14.13	0.81	0.42
PTT	38.4±1.6 (37-44) 38.02-38.78	38.6±4.3 (28-46) 37.05-40.15	0.25	0.86
IgG (mg/dL)	1229.5±649.3 (680-2850) 1040.8-1418.2	1812.0±567.4 (845-3040) 1607.4-2016.6	4.24	0.0001*
HCV-RNA (×10 <sup>3</sup> copy/mL)	96.7±52.7 (23-210) 84.97-108.43	99.8±51.0 (15-210) 90.58-109	0.18	0.85

\*Highly significant, SD: Standard deviation, N: Number, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, MW: Mann-Whitney test, GGT: Gamma-glutamyl transpeptidase, PT: Prothrombin time, PTT: Partial thromboplastin time, IgG: Immunoglobulin G, HCV-RNA: Hepatitis C virus ribonucleic acid, NOSAs: Non-organ-specific antibodies

97.85 ± 51.4 × 10<sup>3</sup> (range 15–210 × 10<sup>3</sup>) copies/mL. Serum viral levels of HCV-RNA did not vary significantly between NOSA-positive and -negative groups [Table 2].

Genotype 4, was the only detected genotype in the studied

patients.

### Prevalence of non-organ-specific autoantibodies

ASMA was positive in 32/80 patients (40%); 18 females (56.3%) and 14 males (43.7%). ASMA titers

ranged from 1:20 to 1:160, with V pattern in 100% of the ASMA-positive patients with no G (glomeruli) or T (tubules) patterns; ( $P < 0.001$ ).

ASMA was strongly positive in 10 patients (20%), moderately positive in 14 patients (28%), and weak in the rest of ASMA positive patients. None of the patients were positive to ANA or LKMA-I. All controls were negative to NOSAs.

### Autoantibodies and features of liver disease

The relationship between NOSAs and the main epidemiological and clinical aspects of chronic HCV patients on entry are shown in Table 1. There were no statistically significant differences in most of the parameters explored. Pallor was significantly high in NOSA-positive children, which may be explained by the statistically significant higher incidence of splenomegaly in these children.

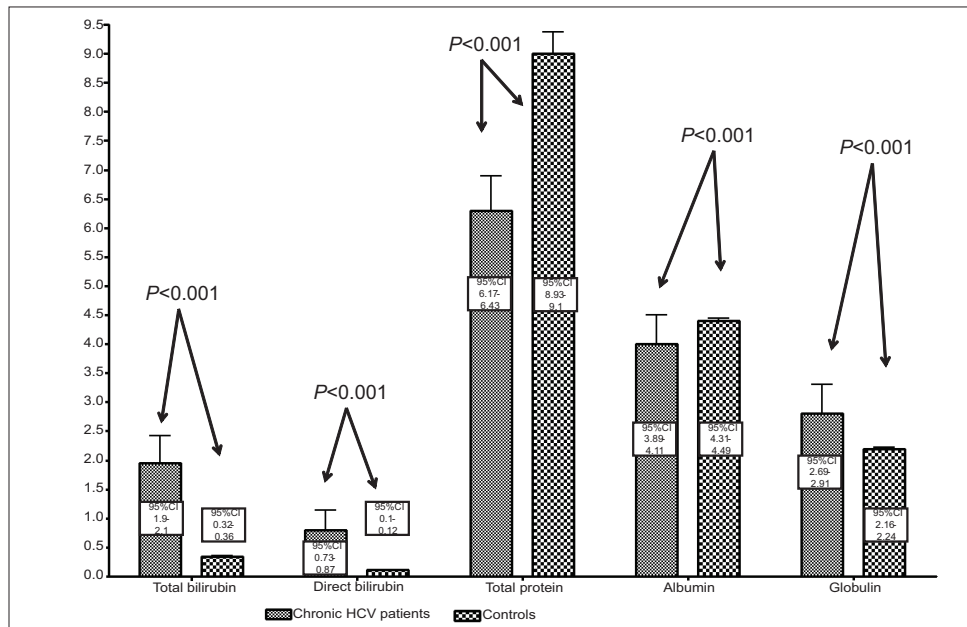


Figure 1a: Comparison of mean values of bilirubin (total and direct) and proteins (total, albumin and globulin) in chronic HCV cases and controls

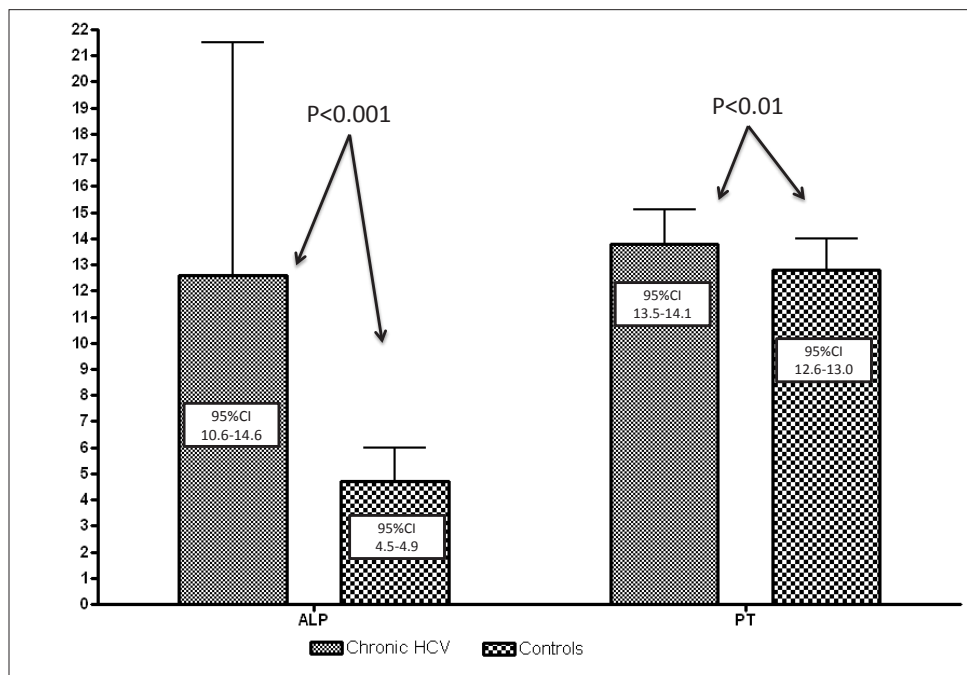
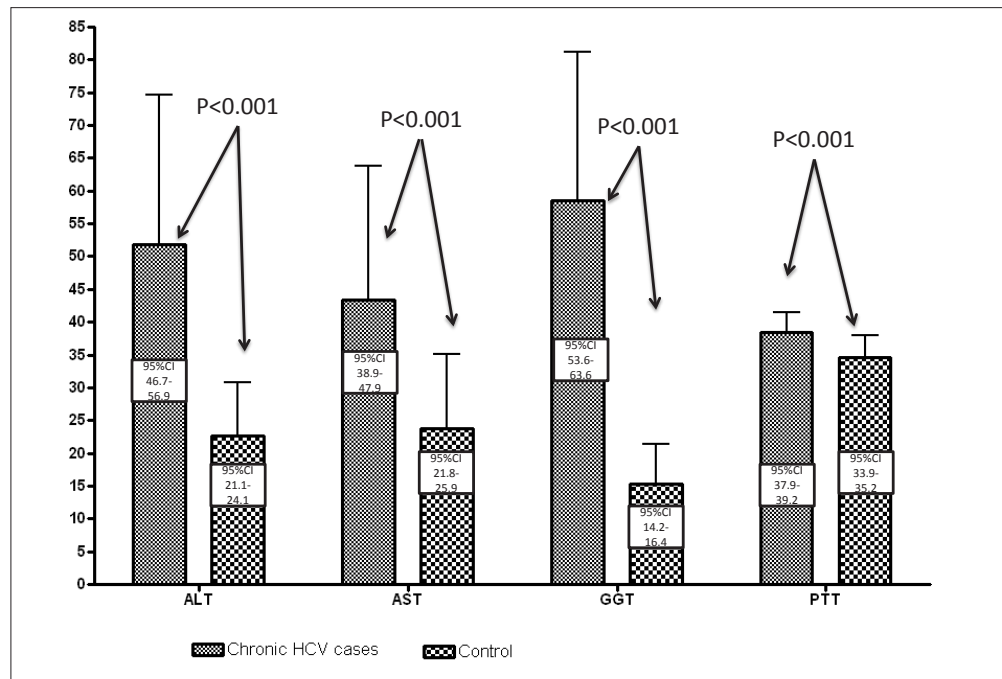


Figure 1b: Comparison of ALP and PT in chronic HCV cases and controls



**Figure 1c:** Comparison of ALT, AST, GGT, and PTT in chronic HCV cases and controls. HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; PT, prothrombin time; PTT, partial thromboplastin time

### Autoantibodies and biochemistry

The details of correlations between autoantibodies and biochemistry are shown in Table 2 and Figures 1a-c. Patients with positive NOSAs had significantly higher total bilirubin, ALP, GGT, albumin, and IgG compared with NOSA-negative patients [Table 2 and Figures 1a-c].

Figure 2a shows the receiver operating curve (ROC), which separated successfully cases of NOSA-positive cases from NOSA-negative cases by a cutoff threshold of 1773 mg/dL for IgG with sensitivity 94% and specificity 93%. In addition, Figure 2b showed very strong correlation between ASMA grading and IgG level with  $r = 0.83$ ,  $r^2 = 0.7$  (which means that one grade increase in ASMA will increase IgG level by 70%) ( $P < 0.0001$ ).

### Autoantibodies and liver biopsy

Liver biopsy showed evidence of chronic hepatitis ranging from minimal chronic hepatitis that prevailed mainly in NOSA-negative cases to mild-to-moderate hepatitis cases that was mainly present in NOSAs positive, highlighting that inflammation, hepatocellular necrosis, and fibrosis are more significantly pronounced in NOSA-positive HCV patients as compared with negatives. None of the patients had cirrhosis [Table 1].

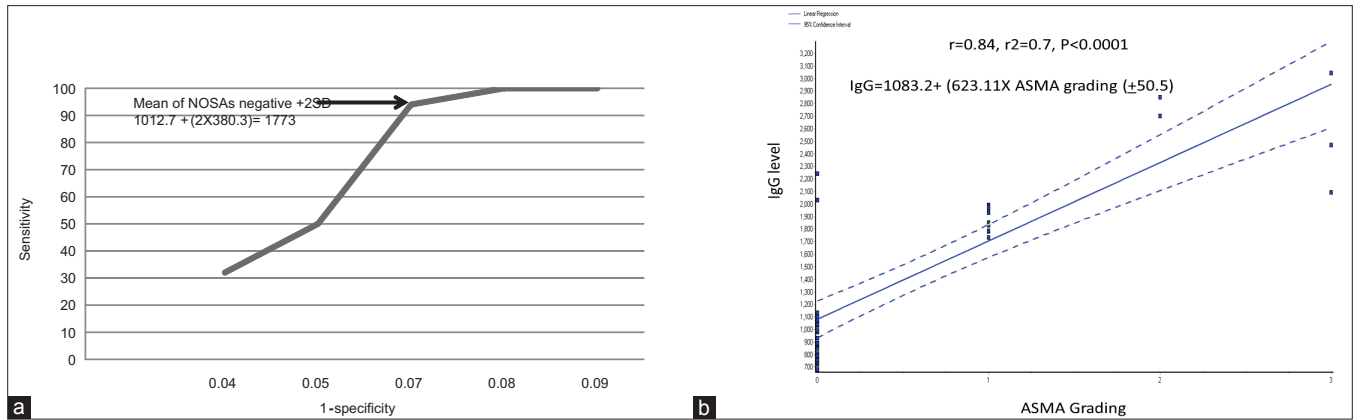
### DISCUSSION

The current study revealed that HCV-4 is the prevailing genotype in Egyptian children with ASMA as the only NOSA detected in these patients. ANA and LKMA-1 were detected in none of the included patients.

It is known that chronic HCV infection is associated with autoimmunity. Recent experimental studies suggest hypothetical pathogenic explanations for the appearance of NOSAs in patients with HCV infection. The E2 envelope protein of HCV binds to CD81 on the surface of B lymphocytes, and this interaction promotes not only B-cell proliferation and clonal expansion, but it also lowers the B-cell activation threshold, thus favoring antibody production.<sup>[13,21]</sup> In addition, the identification of several significant homology motifs between HCV polyprotein and autoantigens,<sup>[14]</sup> which may play a role in developing particular autoantibodies in patients with chronic HCV infection.<sup>[11]</sup>

The few studies that have investigated NOSAs in chronic HCV adults. Studies in children are even much scarcer. To our knowledge, no adult or pediatric studies have been carried out on NOSAs in HCV-4 patients. The current study is the first study so far.

The prevalence of NOSA-positive chronic HCV patients in



**Figure 2:** (a) Receiver operating curve for IgG in NOSAs positive and negative patients; (b) correlation between IgG and ASMA grading in NOSAs positive and negative patients. IgG, immunoglobulin; ASMA, anti-smooth muscle antibodies; NOSAs, non-organ-specific antibodies

**Table 3: Studies investigating the prevalence of non-organ-specific autoantibodies in children with chronic hepatitis C**

Study	Patients' number	NOSAs (%)	ASMA (%)	ANA (%)	LKMA-1 (%)	Dilution threshold of positivity	Genotype
Bortolotti <i>et al.</i> 1996 <sup>[9]</sup>	40	32.5	17.5	7.5	10	1:20	1a, 2
Gregorio <i>et al.</i> 1998 <sup>[10]</sup>	51	65	51	10	8	1:10	Not mentioned
Muratori <i>et al.</i> 2003 <sup>[11]</sup>	47	34	17	9	15	1:10	1a, b, 2c, 3a
Gehring <i>et al.</i> 2006 <sup>[8]</sup>	39	8	5	-	2	1:40	Not mentioned
Current study	80	40	40	-	-	1:20	4

ANA: Anti-nuclear antibodies, NOSAs: Non-organ-specific antibodies, ASMA: Anti-smooth muscle antibodies, LKMA-1: Liver-kidney microsomal antibodies-1

previous pediatric studies ranged from 8% to 65%.<sup>[8-11]</sup> In the current study, 32 patients (40%) had circulating NOSAs, a figure similar to that reported by Bortolotti *et al.*<sup>[9]</sup> and Muratori *et al.*,<sup>[11]</sup> whereas Gregorio *et al.*<sup>[10]</sup> reported the highest prevalence, which might be partly explained by the low dilution used (1:10). However, Gehring *et al.*<sup>[8]</sup> reported the lowest prevalence, which may be partly attributed to the higher dilutions used (1:40) and the geographic differences and, therefore, differences in genetic predisposition as Gehring's study is largely from Germany, whereas most of the other studies are from Italy.

In concordance with Bortolotti *et al.*<sup>[9]</sup> and Muratori *et al.*,<sup>[11]</sup> we used 1:20 screening dilution, which in our view is the most acceptable screening dilution in pediatric population.

ASMA was the most prevalent NOSA detected in previous pediatric studies (5-51%) followed by ANA (0-10%), and LKMA-1 (2-10%),<sup>[8-11]</sup> as shown in Table 3. ASMA-V was the only detected in our cohort; none had G or T types. ANA and LKMA-1 were not detected in any patient. These findings were unique as compared with other studies.

Because ASMA is the serological marker of type-1 autoimmune hepatitis (AIH), one of the major diagnostic

dilemmas that researchers face is to distinguish children with true AIH infected by HCV from patients with chronic HCV and associated HCV-induced autoimmunity. We based our differentiation between these two entities on clinical, serological, and antigenic specificity of ASMA with application of the revised AIH scoring.<sup>[22]</sup> All included autoantibody-positive HCV patients had a mild asymptomatic disease, without a history of acute onset. In AIH severe liver disease is often associated.<sup>[23]</sup> Another important point was that ASMA levels were lower than those usually associated with AIH-1, never exceeding 1:160. ASMA always reacted only with vessels rather than actin, sparing glomerular and tubular structures, which are additional targets in AIH type 1.<sup>[24]</sup> On application of the revised AIH scoring<sup>[22]</sup> at entry to the study, eight patients were in the probable category. On follow-up visits, none proved to be definite. Splenomegaly and increased gammaglobulins, typical of autoimmune hepatitis, were more common in autoantibody-positive than -negative cases.

As shown in Table 3, there is heterogeneity of the prevalence of NOSAs among different studies. This could probably be due to technical differences in the laboratory methods used. Higher percentage was observed in studies using lower dilution

thresholds of positivity<sup>[10,11]</sup> and more sensitive laboratory methods.<sup>[10]</sup> We used 1:20 dilution similar to that used by Bortolotti *et al.*,<sup>[9]</sup> which was intermediate between those who used lower (1:10)<sup>[10,11]</sup> and higher (1:40)<sup>[8]</sup> dilutions.

The high incidence and the detection of ASMA alone with the absence of ANA and LKMA-1 in the current study can be probably attributed to ethnicity, immunogenetics, or to the viral genotype prevailing in Egypt, HCV-4, which was not studied in any of the published studies in the literature in English.

In the studies of Gregorio *et al.*<sup>[10]</sup> and Muratori *et al.*,<sup>[11]</sup> children with chronic hepatitis C were enrolled together with children with chronic hepatitis B and with other chronic liver diseases as controls. Overall, NOSAs were more common in children with chronic hepatitis C than in children with other liver disorders of similar severity, suggesting that the presence of autoantibodies is not just a consequence of the chronic liver damage and that HCV infection plays a pivotal role.

We did not find any significant difference in epidemiological data of NOSA-positive and -negative patients. Among clinical data, pallor and splenomegaly were significantly higher in autoantibody-positive group as compared with the negatives, which are two findings associated with most of the autoimmune phenomena.

Interestingly, comparing biochemical parameters revealed highly significant correlation between autoantibody positivity and total bilirubin, albumin, ALP, GGT, and IgG as compared with the negative group [Table 2 and Figures 1a-c]. In 2008, Hennes *et al.*<sup>[25]</sup> reported that an IgG level of 1.44-fold greater than normal was found to be the best diagnostic predictor of AIH and can differentiate it from infectious hepatitis. This means that IgG is highly linked to autoimmunity based on that, the current study added the cutoff level between NOSA-negative and -positive cases, in such a way cases with IgG level above 1773 mg/dL is a good predictor for detection of NOSA-positive cases with sensitivity 94% and specificity 93% [Figure 2a], which was very similar to Fallatah and Akbar<sup>[26]</sup> data. In addition, we found that IgG level could predict grading of ASMA positivity [Figure 2b].

The clinical significance of NOSAs in the course of chronic hepatitis C is still debated.<sup>[14]</sup> The real challenge for clinicians and scientists is to understand whether and to what extent HCV-induced autoimmunity contributes to liver damage. Some hypothesized that NOSAs could be considered a simple consequence of hepatocellular damage without pathogenic significance. Others believed that they could have pathogenic implications in liver damage.<sup>[27,28]</sup> We agree with the latter opinion, supported by the significantly high

IgG, ALP, and GGT in the positive group associated with the presence of mild-to-moderate hepatitis on histopathological assessment in 78% of NOSA-positive patients compared with 12.5% in the negative group.

## CONCLUSION

Chronic HCV is highly immunogenic in children with variable NOSAs. HCV-4 is the prevailing genotype in Egyptian children with chronic HCV infection. The prevalence of ASMA-V and the absence of ANA and LKMA-1 might be related to the unique situation in Egypt with the unique prevalence of genotype 4. A cutoff threshold of 1773 mg/dL for IgG can predict ASMA positivity in HCV patients with very high sensitivity and specificity. There is a very strong correlation between ASMA grading and IgG level. One grade increase in ASMA will increase IgG level by 70%. This could suggest the use of IgG which is an easy to measure cost-effective parameter, as a screening and predictor of autoimmunity with HCV.

More studies on a larger population are needed to validate our findings. The current study will open the field for more studies on genotype-4-HCV, which is still understudied due to its prevalence in developing countries where research is still struggling.

Finally, one question remains unanswered, which is whether the Egyptian race plays a hidden role in these findings, or is it completely attributable to HCV genotype 4?

## REFERENCES

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;244:359-62.
2. Zignego AL, Ferri C, Pileri SA, Caimi P, Bianchi FB; Italian Association of the Study of Liver Commission on Extrahepatic Manifestations of HCV infection. Extrahepatic manifestations of hepatitis C virus infection: A general overview and guidelines for a clinical approach. *Dig Liver Dis* 2007;39:2-17.
3. Lorenzen J, Matern S, Lammert F. The presence of non-organ-specific autoantibodies is associated with a negative response to combination therapy with interferon and ribavirin for chronic hepatitis C. *BMC Infect Dis* 2004;4:4.
4. Lenzi M, Bellentani S, Saccoccio G, Muratori P, Masutti F, Muratori L, *et al.* Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: A nested case-control study of the Dionysos cohort. *Gut* 1999;45:435-41.
5. Cassani F, Cataleta M, Valentini P, Muratori P, Giostra F, Francesconi R, *et al.* Serum autoantibodies in chronic hepatitis C: Comparison with autoimmune hepatitis and impact on the disease profile. *Hepatology* 1997;26:561-6.
6. Clifford BD, Donahue D, Smith L, Cable E, Luttig B, Manns M, *et al.* High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C. *Hepatology* 1995;21:613-9.
7. Indolfi G, Bartolini E, Olivito B, Azzari C, Resti M.



- Autoimmunity and extrahepatic manifestations in treatment naïve children with chronic hepatitis C virus infection. *Clin Dev Immunol* 2012;2012:785627.
8. Gehring S, Kullmer U, Koepplmann S, Gerner P, Wintermeyer P, Wirth S. Prevalence of autoantibodies and the risk of autoimmune thyroid disease in children with chronic hepatitis C virus infection treated with interferon-alpha. *World J Gastroenterol* 2006;12:5787-92.
  9. Bortolotti F, Vajro P, Balli F, Giacchino R, Crivellaro C, Barbera C, *et al.* Non-organ-specific autoantibodies in children with chronic hepatitis C. *J Hepatol* 1996;25:614-20.
  10. Gregorio GV, Pensati P, Iorio R, Vegnente A, Mieli-Vergani G, Vergani D. Autoantibody prevalence in children with liver disease due to chronic hepatitis C virus (HCV) infection. *Clin Exp Immunol* 1998;112:471-6.
  11. Muratori P, Muratori L, Verucchi G, Attard L, Bianchi FB, Lenzi M. Non-organ-specific autoantibodies in children with chronic hepatitis C: Clinical significance and impact on interferon treatment. *Clin Infect Dis* 2003;37:1320-6.
  12. Bogdanos DP, Mieli-Vergani G, Vergani D. Non-organ-specific autoantibodies in hepatitis C virus infection: Do they matter? *Clin Infect Dis* 2005;40:508-10.
  13. Maecker HT, Do MS, Levy S. CD81 on B cells promotes interleukin 4 secretion and antibody production during T helper type 2 immune responses. *Proc Natl Acad Sci U S A* 1998;95:2458-62.
  14. Bogdanos DP, Mieli-Vergani G, Vergani D. Virus, liver and autoimmunity. *Dig Liver Dis* 2000;32:440-6.
  15. El-Zanaty F, Way A. Egypt demographic and health survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International; 2009.
  16. Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, *et al.* Hepatitis C virus (HCV) infection in a community in the Nile Delta: Population description and HCV prevalence. *Hepatology* 2000;32:111-5.
  17. Cavallaro J, Palmer DF, Bigazzi PE. Immunofluorescence detection of autoimmune diseases, Atlanta, GA: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Center for Disease Control; 1977.
  18. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, *et al.* International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929-38.
  19. Medscape [homepage on the Internet]. Anti-smooth –muscle antibody. Available from: <http://emedicine.medscape.com/article/2086774-overview#showall>. [Last accessed on 2013 Apr 15].
  20. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-9.
  21. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, *et al.* Binding of hepatitis C virus to CD81. *Science* 1998;282:938-41.
  22. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, *et al.* International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929-38.
  23. Mieli-Vergani G, Vergani D. Progress in pediatric autoimmune hepatitis. *Semin Liver Dis* 1994;14:282-8.
  24. Abuaf N, Johanet C, Homberg JC. Autoantibodies in autoimmune chronic active hepatitis. In: Krawitt L, Wiesner RH, editors. *Autoimmune Liver Disease*. New York: Raven Press; 1991. p. 93-109.
  25. Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, *et al.* Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008;48:169-76.
  26. Fallatah HI, Akbar HO. Elevated serum immunoglobulin G levels in patients with chronic liver disease in comparison to patients with autoimmune hepatitis. *Libyan J Med* 2010;5:4857-61.
  27. Stroffolini T, Colloredo G, Gaeta GB, Sonzogni A, Angeletti S, Marignani M, *et al.* Does an 'autoimmune' profile affect the clinical profile of chronic hepatitis C? An Italian multicentre survey. *J Viral Hepat* 2004;11:257-62.
  28. Muratori P, Muratori L, Guidi M, Granito A, Susca M, Lenzi M, *et al.* Clinical impact of non-organ-specific autoantibodies on the response to combined antiviral treatment in patients with hepatitis C. *Clin Infect Dis* 2005;40:501-7.

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