American Journal of Immunology 10 (3): 144-155, 2014
ISSN: 1553-619X

doi:10.3844/ajisp.2014.144.155 Published Online 10 (3) 2014 (http://www.thescipub.com/aji.toc)

PREVALENCE OF AUTOANTIBODIES REVEALS A PREDOMINANT SMA AND ANCA-PR3/MPO PATTERN IN HIV INFECTION AND SMA IN HAV-INFECTED CHILDREN

Luz M. Navarta, Carlos A. Espul and Nelson Acosta-Rivero

Laboratorio de Virología, Hospital Central de Mendoza, Mendoza, Argentina

Received 2014-08-08; Revised 2014-09-10; Accepted 2014-09-12

ABSTRACT

Hepatitis A Virus (HAV) and Human Immunodeficiency Virus (HIV) have been associated with development of autoantibodies and autoimmune manifestations in children. Autoimmune Hepatitis (AIH) is particularly aggressive in children/adolescents with a more severe outcome. Thus, studying the mechanisms of virus-related autoimmune disorders in children is a relevant topic of research. We aimed to study the prevalence of autoantibodies in plasma of children infected with either HAV or HIV comparing to healthy children. The relationship between the presence of autoantibodies and biochemical markers of hepatic damage was also investigated. Detection of autoantibodies (SMA) was associated with HAV infection with a prevalence of 35%. Similar levels of hepatic enzymes were observed in sera of HAV-infected patients with reactivity against autoantigens as compared to those without autoantibodies. On the other hand, HIV infection showed broader autoantibodies reactivities than HAV-infected patients and was associated with SMA (18%), ANCA (20%), ANCA-PR3 (15%) and ANCA-MPO (13%). Moreover, either RF or ANA was detected in 8% of HIV-infected children. Prevalence of autoantibodies was not associated with either gender or age of infected children. A high prevalence of SMA was observed in HAV- and HIV-infected patients. As HAV and SMA may persit in some patients and AIH can develop in susceptible children, it is recommended a follow up of virus infected patients. Since ANCA-PR3 and ANCA-MPO have been shown to be pathogenic, proinflammatory and associated with symptomatic HIV infection, further studies are required to determine the role of these autoantibodies in the pathogenesis associated with viral infection in children.

Keywords: HAVM, HIV, SMA, ANCA, Children

1. INTRODUCTION

Hepatitis A Virus (HAV) and Human Immunodeficiency Virus (HIV) infections have been recognized with the ability of breaking tolerance to self-antigens, promoting self-reactivity and autoimmune diseases (Dan and Yaniv, 1990; Roessler, 2007). Children are susceptible to infection with HAV and HIV and to develop autoimmune diseases (Huppertz *et al*., 1995; Skoog *et al*., 2002; Adebajo, 1997). However, little is known about the pathogenesis of these viral infections in promoting self-reactivity and development of autoimmune manifestations in paediatric individuals (Moon *et al*., 2009; Argüello *et al*., 2012). Some of the extrahepatic manifestations and autoimmune diseases associated with HAV infection consist of: Cholestatic hepatitis, cutaneous vasculitis (Dan and Yaniv, 1990), cryoglobulinemia (Ilan *et al*., 1990), several neurologic syndromes (Tabor, 1987), lupus (Segev *et al*., 2001), thrombocytopenic purpura (Cohen *et al*., 1993) and

Corresponding Authors: Luz M. Navarta and Nelson Acosta-Rivero, Laboratorio de Virología, Hospital Central de Mendoza, Mendoza, Argentina. Email: lmnavarta@hotmail.com; n.acostarivero@latinmail.com

Autoimmune Hepatitis (AIH) (Muñoz Bertrán *et al*., 2002). Autoimmune and systemic diseases that have been described in HIV-infected patients (Zandman-Goddard and Shoenfeld, 2002) include: Vasculitis, immune cytopenias, rheumatic diseases, lupus, sarcoidosis, thyroid diseases, hepatic diseases and antiphospholipid syndrome (Iordache *et al*., 2014). Several autoantibodies have been associated with these pathologies including Antinuclear (ANA), Antimitochondrial (AMA), Antismooth muscle (SMA), Antiparietal Cells (APC), anti-Liver-Kidney-Microsomal (LKM), anti-double strand DNA (DNAds), anti-Tyro Globulin (ATG), Rheumatoid Factor (RF) and Anti-Neutrophil Cytoplasmic (ANCA) antibodies (Moon *et al*., 2009; Liberal *et al*., 2013).

HAV has been suggested as a factor contributing to development AIH in both adults and individuals that are susceptible for autoimmune diseases (Muñoz Bertrán *et al*., 2002). AIH is particularly aggressive in children/adolescents with a more severe outcome (Pando *et al*., 1999; Floreani *et al*., 2013). In addition, AIH Type 1 is the major cause of chronic liver disease in Argentinian children (Cuarterolo *et al*., 1995). Moreover, the presence of autoantibodies has been correlated positively with the severity of hepatic lesions in AIH type-1 (Matsuo *et al*., 2000). On the other hand, a small number of studies have reported the presence of autoantibodies and a lower prevalence of autoimmune diseases in HIV-infected children as compared to adults (Argüello *et al*., 2012). Thus, studying the mechanisms of virus-related autoimmune disorders in children is a relevant topic of research.

Detection of autoantibodies is an important criterion for diagnosis and classification of extrahepatic manifestations associated with HAV infection as well as type 1 and 2 AIH (Liberal *et al*., 2013). Besides, it is also relevant to decide optimal treatment options against HAV and HIV. Furthermore, studying the prevalence of autoantibodies in sera of patients with these viral infections is relevant to elucidate the mechanisms of viral infection-related autoimmune manifestations induction. Therefore, the main objective of this study was to study the prevalence of autoantibodies in sera of patients infected with either HAV or HIV as compared to healthy individuals. In addition, the relationship between the presence of autoantibodies and biochemical markers of hepatic damage was investigated.

2. MATERIALS AND METHODS

2.1. Patients

Data of patients studied in this study are shown in **Table 1 to 5**. Control group was negative for: HAV, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), HIV, Human T-Lymphotropic Virus 1 (HTLV-1), chagas disease and toxoplasmosis. Written informed consent was obtained from the parents of each patient and the study was approved by the Ethics Committee of Medical Research from the Hospital Central in accordance with the 1975 Declaration of Helsinki.

Either HAV or HIV children coinfected with HBV and HCV were excluded from this study. In addition, children with previous hepatic diseases (including AIH, cholestasis or infectious hepatitis) were excluded from this study.

2.2. Autoantibodies

Autoantibodies reactive for ANA, AMA, SMA, APC, LKM, DNAds and ANCA were assayed by indirect immunofluorecense. ANCA proteinase-3 (ANCA-PR3) and ANCA myeloperoxidase (ANCA-MPO) were assayed by ELISA (Binding site. UK).

TGA and RF were assayed by particle aglutination.

Table 1. Autoantibodies (auto-Abs) prevalence in HAV-infected patients

Auto-Abs	Patients auto-abs+/ total patients HCV+ (%)	Auto-abs titers (Range)	Controls auto-abs+/ total controls (%)	P
RF	1/54 (2%)	1:40	0/44 (0%)	1
SMA	19/54 (35%)	1:50 (1:20-1:160)	0/44 (0%)	<0.0001
ANCA	0/54 (0%)	N/A	0/44 (0%)	N/A
ANA	0/54 (0%)	N/A	0/44 (0%)	N/A
ANTI DNA dc	0/54 (0%)	N/A	0/44 (0%)	N/A
AMA	0/54 (0%)	N/A	0/44 (0%)	N/A
ATG	0/54 (0%)	N/A	0/44 (0%)	N/A
Anti-LKM	0/54 (0%)	N/A	0/44 (0%)	N/A
APC	1/54 (2%)	1:40	0/44 (0%)	1

P: Exact Fisher Test. N/A: Not Applicable

2.3. Liver Function Serum Enzymes

Aspartate Transaminase/Glutamic Oxaloacetic Transaminase (AST/SGOT) and Alanine Transaminase/Glutamic-Pyruvic Transaminase (ALT/SGPT) were assayed using conventional methods.

2.4. Statistics

Graph Prism 5.0 statistical software package was used for data analysis. Data was expressed as Mean ± SD for quantitative measures and both number and percentage for categorized data. Comparison between two independent means for parametric data was done using student test. Comparison between two independent groups for non-parametric data was done using Mann Whitney test. The fisher exact test used to study the association between 2 variables, or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant.

3. RESULTS

Presence of at least one autoantibody was detected in 19 (35%) out of 54 samples of HAV-infected children (p<0.0001, Fisher exact test) (**Table 1 and 3**). In addition, concomitant detection of more than one antibody showed a prevalence of 3.7%. HAV infection was associated with SMA (35%, p<0.0001 fisher exact test) (**Table 1 and 3**). Either RF or APC reactivities was observed in one patient. Moreover, no reactivities were detected for ANA, AMA, TGA, LKM, DNAds and ANCA. Prevalence of autoantibodies (SMA), on the other hand, was not associated with either gender or age of HAV-infected children (**Table 6**). Furthermore, levels of biochemical markers of liver damage (AST and ALT) showed no difference between samples of HAV-infected patients reactive to autoantibodies and those no-reactive (**Table 7**).

It is interesting to note that HIV-infected children showed broader autoantibodies reactivities than HAV-infected patients. The presence of autoantibodies was observed in 18 out of 39 children (46%) and was not associated with either gender or age of HIV-infected children (**Table 2, 4 and 8**). Concomitant detection of more than one antibody was observed in 3 HIV-infected children (7.7%). HIV infection was associated with SMA (18%, p = 0.0037) and ANCA (20%, p = 0.0016) (**Table 2 and 4**). Notably, prevalence of both ANCA-PR3 (15%, p = 0.0086) and ANCA-MPO (13%, p = 0.0198) was associated with HIV-infection. Interestingly, a mixed pattern of ANCA-PR3 and ANCA-MPO was observed in 3 patients (8%) (**Table 4**). Moreover, either RF or ANA was detected in 8% of HIV-infected children. Prevalence of SMA, on the other hand, was not associated with either gender (p = 0.3863) or age (p = 1) of infected children. In addition, prevalence of ANCA was neither associated with gender (p = 1) nor age (p = 0.6857) of patients. No reactivities were detected for TGA, APC, AMA, LKM and DNAds. Finally, similar SMA titers were observed in HAV- and HIV-infected children (p = 0.4119, Mann Whitney test) (**Fig. 1**).

Table 2. Autoantibodies (auto-Abs) prevalence in chronically HIV-infected patients

Auto-abs	HIV+ auto-abs+ patients/total HIV+ patients (%)	Auto-abs titles (range)	Control individuals auto-abs+/total control individuals individuos controls (%)	P
RF	3/39 (8%)	1:67 (1:40-1:80)	0/44 (0%)	0,099
SMA	7/39 (18%)	1:54 (1:20-1:80)	0/44 (0%)	0,0037
ANCA total	8/39 (20%)	N/A	0/44 (0%)	0,0016
ANCA MPO*	5/39 (13%)	15,6* (14,5-16,4)	0/44 (0%)	0,0198
ANCA PR3*	6 /39 (15%)	7,9* (5,1-15)	0/44 (0%)	0,0086
ANA	3/39 (8%)	1:73 (1:20-1:160)	0/44 (0%)	0,099
TGA	0/39 (0%)	N/A-	0/44 (0%)	N/A
APC	0/39 (0%)	N/A	0/44 (0%)	N/A
Anti DNAdc	0/39 (0%)	N/A	0/44 (0%)	N/A
AMA	0/39 (0%)	N/A	0/44 (0%)	N/A
Anti LKM	0/39 (0%)	N/A	0/44 (0%)	N/A

P: Exact Fisher Test. * ELISA Units. N/A: Not Applicable

Table 3. Demographic and clinical features in HAV-infected children

Patient	Age	Gender	AST	ALT	Auto-Abs (Titers)
1	9	M	136	424	ND
2	8	F	512	879	SMA (40)
3	11	M	1145	1551	ND
4	3	F	831	487	ND
5	5	F	199	371	SMA (40)
6	4	M	750	1280	ND
7	12	M	1219	1318	SMA 40()/APC (40)
8	15	M	841	967	ND
9	10	M	279	315	ND
10	2	F	522	359	ND
11	10	M	175	230	SMA (20)
12	11	M	624	917	SMA (40)
13	7	M	716	881	SMA (80)
14	9	M	1679	928	ND
15	6	F	743	832	SMA (40)
16	4	F	675	734	SMA (40)
17	3	M	452	631	ND
18	5	F	317	570	ND
19	7	M	839	1027	ND
20	14	M	912	728	ND
21	10	M	360	219	ND
22	7	F	570	846	SMA (40)
23	6	F	615	1021	ND
24	9	M	522	783	SMA (160)
25	3	F	411	525	ND
26	12	F	328	697	ND
27	9	F	563	890	ND
28	8	M	987	1203	SMA (40)
29	10	M	182	415	ND
30	6	M	335	572	ND
31	7	M	714	1235	SMA (40)
32	2	F	618	756	SMA (40)
33	5	F	902	1340	ND
34	6	M	240	391	ND
35	15	F	265	344	SMA (40)
36	7	F	554	785	SMA (40)
37	5	M	297	320	ND
38	4	F	976	1013	ND
39	3	M	932	865	ND
40	7	M	136	289	ND
41	10	F	374	445	ND
42	9	M	790	966	ND
43	11	F	825	560	ND
44	7	F	841	1024	ND
45	4	M	417	685	SMA (40)
46	14	M	392	740	SMA (40)
47	13	F	730	1282	ND
48	5	F	649	803	ND
49	6	M	1072	905	ND
50	13	F	568	751	ND
51	4	F	297	376	ND
52	9	F	453	842	ND
53	6	M	692	933	SMA (40)
54	13	M	929	1050	SMA (80)/RF (40)

Auto-Abs: Autoantibodies. ND: No detection

Table 4. Demographic and clinical and virological features in HIV-infected children

Patient	Age	Gender	Auto-abs (titers)
1	0.66	M	ND
2	1.16	M	ND
3	2.25	F	ANCAPR3 (6.7U)/M (16U)
4	5.00	M	ND
5	3.00	F	ND
6	7.00	F	ANCAM (16.4U)
7	5.00	M	ND
8	4.00	F	SMA (80)/RF (80)
9	6.00	F	ND
10	2.00	F	ND
11	8.00	M	ND
12	7.00	M	ANA (40)
13	6.00	F	ND
14	2.00	F	ND
15	3.00	M	ANCAPR3 (5.1U)
16	6.00	F	SMA (20)
17	3.00	F	SMA (40)
18	4.00	F	ND
19	6.00	F	ANA (40)/RF (20)
20	7.00	F	SMA (80)
21	0.25	F	ND
22	3.00	F	ND
23	1.00	F	ND
24	5.00	M	ANCAPR3 (7.4U)/M (15.1U)
25	1.50	M	ND
26	6.00	F	ANCAPR3 (15U)/M (16.2U)
27	2.00	M	ND
28	6.00	F	ND
29	5.00	M	ANCAPR3 (8.2U)
30	2.00	M	ANA (160)
31	4.00	M	ND
32	1.66	F	ANCAM (14.5U)/RF (80)
33	3.00	F	ANCAPR3
34	2.00	F	ND
35	1.50	F	ND
36	0.75	F	SMA (40)
37	3.00	F	ND
38	7.00	M	SMA (80)
39	3.00	F	SMA (40)

Auto-Abs: Autoantibodies. ND: No detection

Table 5. Demographic data of patients include in this study

Samples	Number of children	Average age (range)	Gender (F/M)
Children HAV+	54	7,7 (2-15)	24/30
Children HIV+	39	3,7 (0,25-15)	25/14
Children controls	44	7,3 (0,16-15)	19/25

Table 6. Prevalence of autoantibodies in HAV-infected (HAV+) patients according to gender and age

Prevalence of SMA in HAV+ patients (total)	Gender female (n = 24) p* (%)	Gender male (n = 30) (%)	Age <10 years old (n = 37) p* (%)	Age >/= 10 years old (n = 17) (%)
Positivos (n = 19)	8 (33.33)a	11 (36.66)a	13 (35.13)b	6 (35.29)b
Negativos (n = 35)	16 (66.66)	19 (63.33)	24 (64.86)	11 (64.70)

Gender: *p = 1, Age: *p = 1, Fisher exact test

Table 7. Enzyme levels (AST and ALT) in serum of HAV-infected patients

Auto-abs	SMA+	SMA-	P
AST *	606.5±(263.5)	616.5± (339.2)	0.9111
ALT*	816.9± (285.3)	736.5±(337.6)	0.3824

*: Average title UI/L (Standard deviation), p: Student t test

Table 8. Prevalence of autoantibodies (auto-abs) in HIV-infected patients according to gender and age

Prevalence of auto-abs in HIV+ patients (total)	Genre female (n = 25) p* (%)	Genre male (n = 14) (%)	Age <5 years old (n = 24) p* (%)	Age >/= 5 years old (n = 15) (%)
Positivos (n = 18)	12 (48)a	6 (42.85)	9 (37.5)	9 (60)
Negativos (n = 21)	13 (52)	8 (57.14)	15 (62.5)	6 (40)

Gender: *p = 1, Age: *p = 0.2028, fisher exact test

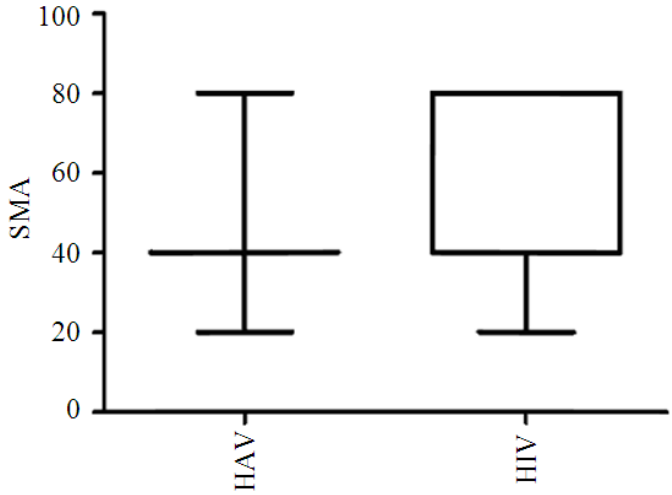

Fig. 1. SMA titers in HAV-and HIV-infected children (p = 0.4119, Mann Whitney test)

4. DISCUSSION

HAV infection is a common cause of acute hepatitis worldwide (Moon *et al*., 2009). In Argentina, HAV occurrence has been reported to vary from low to high endemicity in different regions (Gonzalez *et al*., 1997). Although HAV is mostly a self-limiting disease, prolonged infection has been reported in both adults and children (Vento *et al*., 1991; Fainboim *et al*., 2001; Elfaramawy *et al*., 2010). HAV infection has been associated with development of extrahepatic manifestations and autoimmune diseases that are related with the production of autoantibodies in susceptible individuals (Dan and Yaniv, 1990; Huppertz *et al*., 1995; Skoog *et al*., 2002; Moon *et al*., 2009; Ilan *et al*., 1990; Tabor, 1987; Segev *et al*., 2001; Cohen *et al*., 1993; Muñoz Bertrán *et al*., 2002; Fainboim *et al*., 2001; Elfaramawy *et al*., 2010; Singh *et al*., 2007). Transient increased levels of autoantibodies have been documented in acute HAV infection and after vaccination (Fainboim *et al*., 2001; Karali *et al*., 2011; Gutierrez *et al*., 1996; Vento *et al*., 1988; Holborow *et al*., 1971; Seok *et al*., 2011). However, persistent autoantibodies have been detected in prolonged HAV infection in children (Fainboim *et al*., 2001; Elfaramawy *et al*., 2010). Particularly, SMA, ANA and antiasialoglycoprotein receptor antibodies have been associated with HAV infection (Moon *et al*., 2009; Fainboim *et al*., 2001; Elfaramawy *et al*., 2010; Karali *et al*., 2011; Gutierrez *et al*., 1996; Vento *et al*., 1988; Holborow *et al*., 1971; Seok *et al*., 2011).

Several mechanisms have been proposed to explain HAV-related induction of autoantibodies and autoimmune diseases including impaired function of regulatory T cells. HAV have been suggested to impair CD4+/CD25+ T regulatory lymphocytes in self-limited infection (Perrella *et al*., 2008). Interestingly, it has been shown that interaction of HAV with its Cellular Receptor

1 (HAVCR1) inhibited regulatory T cell functions and reduced production of Transforming Growth Factor-β (TGF-β) and IL-10 (Manangeeswaran *et al*., 2012). Moreover, individuals with a defect in suppressor-inducer T lymphocytes (that control immune responses to the asialoglycoprotein receptor) have been suggested to develop AIH type 1 after HAV infection (Vento *et al*., 1991). In addition, antibody-dependent citotoxicity has been involved in AIH (Vergani and Mieli-Vergani, 2008).

In this study, high prevalence of SMA (35%) was associated with HAV+ patients. This is in agreement with previous reports showing similar prevalence of SMA in HAV+ children (Fainboim *et al*., 2001; Elfaramawy *et al*., 2010). Although transient SMA reactivities have been observed in self-limited HAV infection, persistence of SMA has been associated with proctacted forms of paediatric infection (Fainboim *et al*., 2001; Elfaramawy *et al*., 2010). Interestingly, HLA DRB1*1301 haplotype has been associated with a higher susceptibility to develop prolonged HAV infection in children (Fainboim *et al*., 2001). Notably, HLA DRB1*13 haplotype has shown a strong association with paediatric type 1 AIH in high endemicity regions for HAV infection (Elfaramawy *et al*., 2010; Bittencourt *et al*., 2008; Fainboim *et al*., 1994; Bittencourt *et al*., 1999). In addition, prevalence of AIH type 1 with a pattern SMA/ANA reactivities is higher than AIH type 2 (LKM-1 reactivity) in children (Vergani and Mieli-Vergani, 2008). On the other hand, HAV vaccination has been associated with development of autoantibodies and rarely with autoimme hepatitis (Karali *et al*., 2011; Berry and Smith-Laing, 2007; Perumalswami *et al*., 2009). These experimental evidences suggest that AIH type 1 may develop after prolonged HAV infection in susceptible individuals (Huppertz *et al*., 1995; Skoog *et al*., 2002; Vento *et al*., 1991; Singh *et al*., 2007). Patients in this study were studied in the acute phase of infection and not at later times after infection. So, neither the prolonged nature of detected autoantibodies nor its association with viral presistance could be assessed. As both HAV and autoantibodies (SMA) may persit in some patients and AIH can develop in susceptible children, a follow up of virus infected patients is important.

Some studies have shown SMA associated with biochemical and histological features of disease activity in adult HAV infection and AIH (Dan and Yaniv, 1990; Couto *et al*., 2014). However, in the current study SMA was not related to the levels of biochemical markers of liver damage in infected children. Different experimental conditions and susceptibilities between children and adults may explain these discrepancies. In fact, different gene susceptibilities have been shown in children with AIH (associated with HLA DRB1*13 haplotype) as compared to adults (associated with HLA-DRB1*0405 haplotype) (Pando *et al*., 1999). Different autoantibody profiles have also been reported, with higher prevalence of SMA in paediatric AIH and of ANA in adult AIH (Pando *et al*., 1999). In addition, HLA-DRB1*12 haplotype has been associated with paediatric HAV-related AIH type 1 (Elfaramawy *et al*., 2010).

On the other hand, HIV-infected individuals with various levels of immunological control have been associated with different autoimmune diseases (Iordache *et al*., 2014). However, few studies have reported the presence of autoimmune diseases in HIV-infected children. These studies have shown a lower prevalence of autoimmune diseases in children as compared to adults (Adebajo, 1997; Nguyen and Reveille, 2009; Schuval *et al*., 2001; Martínez-Rojano *et al*., 2001; Rodríguez-Mahou *et al*., 1994; Jarvis *et al*., 1993; Rodriguez *et al*., 1993; González *et al*., 1998; Stricker *et al*., 1998; Eley *et al*., 1999). Long term HIV infection leading to chronic activation and exhaustion of the host immune system and increased expression of autoantigens has been proposed to promote development of autoimmune diseases (Gaddi *et al*., 2000). Several autoantibodies have been associated with HIV infection in children including ANA, SMA, DNAds and RF. It has been suggested that polyclonal B cell activation, increased levels of total immunoglubulins and dysfunction of CD4+ T regulatory cells contribute to development of autoantibodies in HIV-infected children in the absence of clinical autoimmune disease (Argüello *et al*., 2012; Rodriguez *et al*., 1993; González *et al*., 1998; Stricker *et al*., 1998; Eley *et al*., 1999). Interestingly, low absolute levels of CD4+FoxP3+T cells and enhanced frequencies of a subset of dysfunctional regulatory T cells (CD4+FoxP3+CD25- T cells) have been associated with a higher prevalence of autoantibodies and hypergammaglobulinaemia in HIV-infected children with severe immunosuppression (Argüello *et al*., 2012).

In this study, the presence of autoantibodies was associated with HIV infection (46%). Prevalence of SMA (18%), ANA and RF (8%) in HIV-infected children is in agreement with previous reports

(Argüello *et al*., 2012; Schuval *et al*., 2001; Rodríguez-Mahou *et al*., 1994; Jarvis *et al*., 1993). Interestingly, a previous study showed that SMA and RF were detected more frequently in HIV-infected children with low CD4+ T cell counts (Argüello *et al*., 2012). Detection of SMA in adult HIV-patients with low CD4+ T cells but not in patients with high CD4+ T cells has also been reported (Chretien *et al*., 2003). In the present study, CD4+ T cells levels were not assayed because of constrains in the samples available. Therefore, the relationship between autoantibodies reactivities and CD4+ T cells was not determined. Another limitation of this study was the lack of information on clinic manifestations. So, the relationship between autoantibodies reactivities and clinic manifestations could not be evaluated.

Interestingly, ANCA (20%) was associated with HIV infection and ANCA-PR3/ANCA-MPO were observed in 6 (15%) and 5 (13%) patients, respectively. Moreover, a mixed pattern of ANCA-PR3 and ANCA-MPO were detected in 3 patients. ANCA have been associated with the pathogenesis of systemic vasculitis (Hu *et al*., 2009). Several experimental evidences support a pathogenic role for ANCA in the pathogenesis of ANCA-Associated Vasculitis (AAV). It has been shown that stimulation of neutrophils with pro-inflammatory cytokines (ex. TNF-α) induced translocation of ANCA antigens (PR3 and MPO) to the neutrophil surface. Binding of ANCA IgG to its antigens and Fc receptors activate neutrophils and release free oxygen radicals and various proteases contributing to pathogenesis in vasculitic lesions (Chen and Kallenberg, 2010). In addition, a crucial role for the alternative pathway of complement activation in ANCA-mediated neutrophil activation and AAV has been reported (Schreiber *et al*., 2009). Moreover, it has been observed in mice models that administration of anti-MPO induced leukocytes recruitment to sites affected in human ANCA-associated vasculitis such as kidney and lung (Nolan *et al*., 2008). It is interesting to note that PR3-specific Th17 cells have also been involved in ANCA-mediated autoimmune diseases pathogenesis (Abdulahad *et al*., 2008).

On the other hand, ANCA have been associated with symptomatic HIV infection in adults (prevalence 18%-41.9%) (Habegger de Sorrentino *et al*., 1997; Kamat *et al*., 2010; Cornely *et al*., 1999; Klaassen *et al*., 1992; Savige *et al*., 1994; Nikolova *et al*., 2002). Interestingly, ANCA have been positively correlated with pulmonary infection and Mycobacterium tuberculosis in HIV-infected patients (Habegger de Sorrentino *et al*., 1997). Nevertheless, prevalence of ANCA is low in asymptomatic HIV infection and ANCA-associated vasculitides are rare in infected individuals (Iordache *et al*., 2014; Habegger de Sorrentino *et al*., 1997; Cornely *et al*., 1999). In addition, most of reported studies have shown low prevalence of ANCA in children infected with HIV (Argüello *et al*., 2012). A recent study showed ANCA reactivities in 3 out of 15 (20%) immunosupressed HIV-infected children (CD4+ T cells<15%) but no reactivity in children with no evidence of immunosuppression (CD4+ T cells >25%) (Argüello *et al*., 2012). Immunosupressed children showed low absolute levels of regulatory T cells and enhanced frequencies of a subset of dysfunctional regulatory T cells (CD4+FoxP3+CD25- T cells) in the absence of autoimmune disease. It is interesting to note that increased levels of the alternative complement pathway (complement activation fragments C3a and Bb), that is involved in ANCA pathogenesis, have been observed in HIV-infected children (Jarvis *et al*., 1993). Thus, ANCA (including ANCA-PR3, ANCA-MPO and a mixed pattern ANCA-PR3/MPO in infected children) could contribute to pathogenesis of HIV-related diseases including autoimmunity.

HIV-infected children showed broader autoantibodies reactivities (SMA (18%), ANCA (20%), FR (8%), ANA (8%)) than HAV-infected patients (SMA (35%)). Higher reactivity against autoantigens in HIV-infected patients could be related with chronic infection, the viral ability to stimulate B lymphocytes persistently and virus antigenic mimicry with autoantigens (Zandman-Goddard and Shoenfeld, 2002). In addition, HAV mainly targets hepatocytes while HIV targets several immune cells directly affecting immune regulatory mechanisms (Cuarterolo *et al*., 1995).

5. CONCLUSION

In summary, detection of autoantibodies was associated with both HAV and HIV infection in children. HIV-infected patients showed broader reactivities for autoantibodies than HAV-infected children. HAV infection was associated with SMA while HIV infection was associated with SMA, ANCA-PR3 and ANCA-MPO. Similar levels of biochemical markers of liver damage were observed in HAV-infected patients with or without reactivity against autoantibodies. As HAV and SMA may persit in some patients and AIH can develop

in susceptible children, it is recommended a follow up of virus infected patients. Children infected with HIV are an especially susceptible population. Since ANCA-PR3 and ANCA-MPO have been shown to be pathogenic, proinflammatory and associated with symptomatic HIV infection, further studies are required to determine the role of these autoantibodies in the pathogenesis associated with HIV infection in children.

6. ACKNOWLEDGEMENT

We would like to thanks Prof Virginia Rivero for her contribution to this study.

7. REFERENCES

Abdulahad, W.H., C.A. Stegeman, P.C. Limburg and C.G. Kallenberg, 2008. Skewed distribution of Th17 lymphocytes in patients with wegener's granulomatosis in remission. Arthritis Rheumatol., 58: 2196-2205. DOI: 10.1002/ART.23557

Adebajo, A.O., 1997. Rheumatic manifestations of infectious diseases in children. Current Opin. Rheumatol., 9: 68-74. PMID: 9110137.

Argüello, R.J., J. Balbaryski, G. Barboni, M. Candi and S. Laucella *et al*., 2012. Altered frequency and phenotype of CD4+ forkhead box protein 3+ T cells and its association with autoantibody production in human immunodeficiency virus-infected paediatric patients. Clin. Exp. Immunol., 168: 224-233. DOI: 10.1111/j.1365-2249.2012.04569.x

Berry, P.A. and G. Smith-Laing, 2007. Hepatitis a vaccine associated with autoimmune hepatitis. World J. Gastroenterol., 13: 2238-2239. DOI: 10.3748/WJG.V13.I15.2238

Bittencourt, P. L., A.Q. Farias, G. Porta, E.L.R. Cançado and F.J. Carrilho *et al*., 2008. Frequency of concurrent autoimmune disorders in patients with autoimmune hepatitis: Effect of age, gender and genetic background. J. Clin. Gastroenterol., 42: 300-305. DOI: 10.1097/MCG.0B013E31802DBDFC

Bittencourt, P.L., A.C. Goldberg, E.L.R. Cançado, G. Porta and J. Kalil *et al*., 1999. Genetic heterogeneity in susceptibility to autoimmune hepatitis types 1 and 2susceptibility to AIH types 1 and 2. Am. J. Gastroenterol., 94: 1906-1913. DOI: 10.1111/J.1572-0241.1999.01229.X

Chen, M. and C.G.M. Kallenberg, 2010. Anca-associated vasculitides-advances in pathogenesis and treatment. Nature Rev. Rheumatol., 6: 653-64. DOI: 10.1038/NRRHEUM.2010.158

Chretien, P., J.C. Monier, F. Oksman, M. San Marco and J. Sibilia *et al*., 2003. Autoantibodies and human immunodeficiency viruses infection: A case-control study. Clin. Exp. Rheumatol., 21: 210-212. PMID: 12747276.

Cohen, O., D. Mevorach, Z. Ackerman and R. Oren, 1993. Thrombocytopenic purpura as a manifestation of acute hepatitis A. J. Clin. Gastroenterol., 17: 166-167. PMID: 8409322.

Cornely, O.A., S. Hauschild, C. Weise, E. Csernok and M. Schrappe *et al*., 1999. Seroprevalence and disease association of antineutrophil cytoplasmic autoantibodies and antigens in HIV infection. Infection, 27: 92-6. DOI: 10.1007/BF02560504

Couto, C.A., P.L. Bittencourt, G. Porta, C.P. Abrantes-Lemos and E.L. Cançado *et al*., 2014. Antismooth muscle and antiactin antibodies are indirect markers of histological and biochemical activity of autoimmune hepatitis. Hepatology, 59: 592-600. DOI: 10.1002/hep.26666

Cuarterolo, M., M. Ciocca and F. Álvarez, 1995. Hepatitis autoinmune en niños. Perspectivas actuales. Arch. Argent. Pediatr., 112 169-175. DOI: 10.5546/aap.2014.169

Dan, M. and M. Yaniv, 1990. Cholestatic hepatitis, cutaneous vasculitis and vascular deposits of immunoglobulin M and complement associated with hepatitis A virus infection. Am. J. Med., 89: 103-107. DOI: 10.1016/0002-9343(90)90107-O

Eley, B.S., J. Hughes, S. Potgieter, M. Keraan and G.D. Hussey *et al*., 1999. Immunological manifestations of HIV-infected children. Annals Tropical Paediatr., 19: 3-7. DOI: 10.1080/02724939992572

Elfaramawy, A.A., R.M. Elhossiny, A.A. Abbas and H.M. Aziz, 2010. HLA-DRB1 as a risk factor in children with autoimmune hepatitis and its relation to hepatitis A infection. Italian J. Pediatr., 36: 73-73. DOI: 10.1186/1824-7288-36-73

Fainboim, L., M.C.C. Velasco, C.Y. Marcos, M. Ciocca and M. Zelazko, 2001. Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB1*1301, a marker for pediatric autoimmune hepatitis. Hepatology, 33: 1512-1517. DOI: 10.1053/jhep.2001.24562

Fainboim, L., Y. Marcos, M. Pando, M. Capucchio and M.L. Satz *et al*., 1994. Chronic active autoimmune hepatitis in children: Strong association with a particular HLA-DR6 (DRB1∗1301) haplotype. Human Immunol., 41: 146-150. DOI: 10.1016/0198-8859(94)90008-6

Floreani, A., R. Liberal, D. Vergani and G. Mieli-Vergani, 2013. Autoimmune hepatitis: Contrasts and comparisons in children and adults-a comprehensive review. J. Autoimmunity, 46: 7-160. DOI: 10.1016/j.jaut.2013.08.004

Gaddi, E., S. Laucella, J. Balbaryski, C. Cantisano and V. Giraudi *et al*., 2000. Prognostic value of soluble intercellular adhesion molecule-1 (s-ICAM-1) in HIV-infected children. Scandinavian J. Immunol., 52: 628-33. DOI: 10.1111/j.1365-3083.2000.00820.x

González, C.M., F.J. López-Longo, J. Samson, I. Monteagudo and L. Carreño *et al*., 1998. Antiribonucleoprotein antibodies in children with HIV infection: A comparative study with childhood-onset systemic lupus erythematosus. AIDS Patient Care STDS, 12: 21-8. DOI: 10.1089/apc.1998.12.21

Gonzalez, J., O. Fay, M.C. Canero-Velasco, E. Fernandez and E. Cisaruk *et al*., 1997. Hepatitis a virus infection in children in Argentina: A pilot study. Acta Gastroenterol. Latinoamericana, 27: 331-334. PMID: 9460514.

Gutierrez, A., V. Chinchilla, A. Espasa and M. Perez-Mateo, 1996. Transient positivity of antinuclear and anti-smooth muscle antibodies in hepatitis a virus infection. J. Hepatol., 24: 123-128. DOI: 10.1016/S0168-8278(96)80197-8

Habegger de Sorrentino, A., P. Motta, E. Iliovich and A. P. Sorrentino, 1997. Anti-Neutrophil Cytoplasmic Antibodies (ANCA) in patients with symptomatic and asymptomatic HIV infection. Med. B. Aires, 57: 294-298. PMID: 9640762.

Holborow, E.J., G.D. Johnson, L.J. Farrow, A.J. Zuckerman and P.E. Taylor *et al*., 1971. Immunofluorescent detection of autoantibodies in the sera of acute infective hepatitis patients. Ann. N. Y. Acad. Sci., 177: 214-217. DOI: 10.1111/j.1749-6632.1971.tb35049.x

Hu, N., J. Westra, M.G. Huitema, M. Bijl and C.G. Kallenberg *et al*., 2009. Coexpression of CD177 and membrane proteinase 3 on neutrophils in antineutrophil cytoplasmic autoantibody-associated systemic vasculitis: Anti-proteinase 3-mediated neutrophil activation is independent of the role of CD177-expressing neutrophils. Arthritis Rheumatism, 60: 1548-1557. DOI: 10.1002/art.24442

Huppertz, H.I., U. Triechel, A.M. Gassel, R. Jeschke and K.H. Meyer zum Büschenfelde, 1995. Autoimmune hepatitis following hepatitis a virus infection. J. Hepatol., 23: 204-208. DOI: 10.1016/0168-8278(95)80336-X

Ilan, Y., M. Hillman, R. Oren, A. Zlotogorski and D. Shouval, 1990. Vasculitis and cryoglobulinemia associated with persisting cholestatic hepatitis a virus infection. Am. J. Gastroenterol., 85: 586-591. PMID: 2337062.

Iordache, L., O. Launay, O. Bouchaud, V. Jeantils and O. Fain *et al*., 2014. Autoimmune diseases in HIV-infected patients: 52 cases and literature review. Autoimmunity Rev., 13: 850-857. DOI: 10.1016/j.autrev.2014.04.005.

Jarvis, J.N., H. Taylor, M. Iobidze, J. Dejonge and F. Cohen *et al*., 1993. Rheumatoid factor expression and complement activation in children congenitally infected with human immunodeficiency virus. Clin. Immunol. Immunopathol., 67: 50-54. DOI: 10.1006/clin.1993.1044

Kamat, A., P. Ancuta, R.S. Blumberg and D. Gabuzda, 2010. Serological Markers for Inflammatory Bowel Disease in AIDS Patients with Evidence of Microbial Translocation. PLoS One, 5: 15533-15533. DOI: 10.1371/journal.pone.0015533

Karali, Z., S.T. Basaranoglu, Y. Karali, B. Oral and S.S. Kilic, 2011. Autoimmunity and hepatitis a vaccine in children. J. Investigat. Allergol. Clin. Immunol., 21: 389-393. PMID: 21905502.

Klaassen, R.J., R. Goldschmeding, K.M. Dolman, A.B. Vlekke and A.E. Von Dem Borne *et al*., 1992. Anti-neutrophil cytoplasmic autoantibodies in patients with symptomatic HIV infection. Clin. Exp. Immunol., 87: 24-30. PMID: 1733634.

Liberal, R., G. Mieli-Vergani and D. Vergani, 2013. Clinical significance of autoantibodies in autoimmune hepatitis. J. Autoimmun., 46: 17-24. DOI: 10.1016/j.jaut.2013.08.001

Manangeeswaran, M., J. Jacques, C. Tami, K. Konduru and G.G. Kaplan *et al*., 2012. Binding of Hepatitis a virus to its cellular receptor 1 inhibits T-regulatory cell functions in humans. Gastroenterology, 142: 1516-1525. DOI: 10.1053/j.gastro.2012.02.039

Martínez-Rojano, H., H.E. Juárez, D.G. Ladrón and M.D.C. Gorbea-Robles, 2001. Rheumatologic manifestations of pediatric HIV infection. AIDS Patient Care STDS, 15: 519-526. DOI: 10.1089/108729101753205685

Matsuo, I., N. Ikuno, K. Omagari, H. Kinoshita and I.R. Mackay *et al*., 2000. Autoimmune reactivity of sera to hepatocyte plasma membrane in type 1 autoimmune hepatitis. J. Gastroenterol., 35: 226-234. DOI: 10.1007/s005350050335

Moon, H.W., J.K. Noh, M. Hur, Y.M. Yun and S.Y. Kwon *et al*., 2009. High prevalence of autoantibodies in hepatitis a infection: The impact on laboratory profiles. J. Clin. Pathol., 62: 786-788. DOI: 10.1136/jcp.2009.064410

Muñoz Bertrán, E., V.R. Salazar, F.H. Robles, J.A.C. Estañ and E.M. Ramírez *et al*., 2002. Autoimmune hepatitis caused by acute hepatitis due to hepatitis a virus. Gastroenterol. Y Hepatol., 25: 501-504. PMID: 12361532.

Nguyen, B.Y. and J.D. Reveille, 2009. Rheumatic manifestations associated with HIV in the highly active antiretroviral therapy era. Current Opin. Rheumatol., 21: 404-10. DOI: 10.1097/BOR.0b013e32832c9d04

Nikolova, M., M. Liubomirova, A. Iliev, R. Krasteva and I.D. Vlahov *et al*., 2002. Clinical significance of antinuclear antibodies, anti-neutrophil cytoplasmic antibodies and anticardiolipin antibodies in heroin abusers. Israel Med. Associat. J., 4: 908-910. PMID: 12455177.

Nolan, S.L., N. Kalia, G.B. Nash, D. Kamel and C.O. Savage *et al*., 2008. Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions in vivo. J. Am. Society Nephrol., 19: 973-84. DOI: 10.1681/ASN.2007111166

Pando, M., J. Larriba, G.C. Fernandez, H. Fainboim and L. Fainboim *et al*., 1999. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: Evidence for differential genetic predisposition. Hepatology, 20: 1374-1380. DOI: 10.1002/hep.510300611

Perrella, A., L. Vitiello, L. Atripaldi, C. Sbreglia and O. Perrella *et al*., 2008. Impaired function of CD4+/CD25+ T regulatory lymphocytes characterizes the self-limited hepatitis a virus infection. J. Gastroenterol. Hepatol., 23: 105-110. DOI: 10.1111/j.1440-1746.2007.05008.x

Perumalswami, P., L. Peng and J.A. Odin, 2009. Vaccination as a triggering event for autoimmune hepatitis. Seminars Liver Dis., 29: 331-334. DOI: 10.1055/s-0029-1233537

Rodriguez, C., E.R. Stiehm and S. Plaeger-Marshall, 1993. Peripheral B-cell activation and immaturity in HIV-infected children. Annals N. Y. Acad. Sci., 693: 291-294. DOI: 10.1111/j.1749-6632.1993.tb26287.x

Rodríguez-Mahou, M., J. López-Longo, N. Lapointe, L. Carreño and E. Fernández-Cruz *et al*., 1994. Autoimmune phenomena in children with human immunodeficiency virus infection and acquired immunodeficiency syndrome. Acta Paediatr. Supplement, 400: 31-34. DOI: 10.1111/j.1651-2227.1994.tb13331.x

Roessler, V.P., 2007. Manifestaciones Reumatológicas en Pacientes con Síndrome de Inmunodeficiencia Adquirida (VIH/SIDA). Reumatología, 23: 151-155.

Savige, J.A., L. Chang, S. Horn and S.M. Crowe, 1994. Anti-nuclear, anti-neutrophil cytoplasmic and anti-glomerular basement membrane antibodies in hiv-infected individuals. Autoimmunity, 18: 205-211. DOI: 10.3109/08916939409007997

Schreiber, A., H. Xiao, J.C. Jennette, W. Schneider and R. Kettritz *et al*., 2009. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. J. Am. Society Nephrol., 20: 289-98. DOI: 10.1681/ASN.2008050497

Schuval, S.J., V.R. Bonagura and N.T. Ilowite, 2001. Rheumatologic manifestations of pediatric HIV infection. J. Rheumatol., 20: 1578-82. DOI: 10.1089/108729101753205685

Segev, A., R. Hadari, T. Zehavi, M. Schneider and Y.A. Mekori *et al*., 2001. Lupus-like syndrome with submassive hepatic necrosis associated with hepatitis a. J. Gastroenterol. Hepatol., 16: 112-114. DOI: 10.1046/j.1440-1746.2001.02314.x

Seok, Y.S., K.G. Lee, E.S. Jung, H. An and H.S. Ryu *et al*., 2011. Clinical significance of the detection of antinuclear antibodies in patients with acute hepatitis a. Gut Liver, 5: 340-347. DOI: 10.5009/gnl.2011.5.3.340

Singh, G., S. Palaniappan, O. Rotimi and P.J. Hamlin, 2007. Autoimmune hepatitis triggered by hepatitis a. Gut, 56: 304-304. DOI: 10.1136/gut.2006.111864

Skoog, S.M., R.E. Rivard, K.P. Batts and C.I. Smith, 2002. Autoimmune hepatitis preceded by acute hepatitis a infection. Am. J. Gastroenterol., 97: 1568-1569. DOI: 10.1111/j.1572-0241.2002.05751.x

Stricker, K., E. Knipping, T. Böhler, A. Benner and K.M. Debatin *et al*., 1998. Anti-CD95 (APO-1/Fas) autoantibodies and T cell depletion in Human Immunodeficiency Virus type 1 (HIV-1)-infected children. Cell Death Differentiat., 5: 222-230. DOI: 10.1038/sj.cdd.4400332

Tabor, E., 1987. Guillain-barré syndrome and other neurologic syndromes in hepatitis A, B and non-A, non-B. J. Med. Virol., 21: 207-212. DOI: 10.1002/jmv.1890210303

Vento, S., B.M. McFarlane, C.G. McSorley, S. Ranieri and I.G. McFarlane *et al*., 1988. Liver autoreactivity in acute virus A, B and non-A, non-B hepatitis. J. Clin. Laboratory Immunol., 25: 1-7. PMID: 3133482.

Vento, S., T. Garofano, G. Di Perri, L. Dolci and D. Bassetti *et al*., 1991. Identification of hepatitis a virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. Lancet, 337: 1183-1187. DOI: 10.1016/0140-6736(91)92858-Y

Vergani, D. and G. Mieli-Vergani, 2008. Autoimmune Hepatitis. Textbook Hepatol. Basic Sci. Clin. Practice, 1: 1089-10101. DOI: 10.1002/9780470691861.ch11b

Zandman-Goddard, G. and Y. Shoenfeld, 2002. HIV and autoimmunity. Autoimmunity Rev., 1: 329-337. DOI: 10.1016/S1568-9972(02)00086-1

8. LIST OF ABBREVIATIONS

ANA: Anti-nuclear antibodies
AMA: Anti-mitochondrial antibodies
SMA: Anti-smooth muscle antibodies
APC: Anti-parietal cells antibodies
LKM: Anti-liver-kidney-microsomal antibodies
DNAds: Anti-double strand DNA antibodies
ATG: Anti-tyroglobulin antibodies
RF: Rheumatoid factor
ANCA: Anti-neutrophil cytoplasmic antibodies
ANCA-PR3: ANCA proteinase-3
ANCA-MPO: ANCA myeloperoxidase
HAV: Hepatitis A virus
HIV: Human Immunodeficiency Virus
AIH: Autoimmune hepatitis
HBV: Hepatitis B virus
HCV: Hepatitis C virus
HTLV-1: Human T-lymphotropic virus 1
AST/SGOT: Aspartate transaminase/Glutamic oxaloacetic transaminase
ALT/SGPT: Alanine transaminase/Glutamic-pyruvic transaminase
AAV: ANCA-associated vasculitis
HAVCR1: Hepatitis A virus cellular receptor 1
TGF-β: Transforming growth factor-β