

**Table 3**  
HCV Genotypes in Cuban patients\*

GENOTYPE	CASES	%**
I	3	10.7
II	26	92.9
III	0	0
IV	4	14.3
V	11	39.3
VI	0	0
MULTIPLE *	16	57.1
NON CLASSIFIED	5	
TOTAL	33	

\* Classification of genotypes after Okamoto *et al*, 1992

\*\* On the basis of 28 classified cases

## REFERENCES

1. OKAMOTO *et al.* (1992) *Virology*, 188:331-341
2. PADRON *et al.* (1994) *Biotechnología Aplicada*, 11 (in press)
3. ARÚS *et al.* (1994) *Hepatology* 19: 40Y (abstract)

## THE HEPATITIS C VIRUS INFECTION IN CUBA: PREVALENCE, ANTIBODY AND RISK FACTORS

Guillermo J. Padrón<sup>1</sup>, Enrique Arús<sup>2</sup>, Luis Rivera<sup>3</sup>, Ariel Viña<sup>1</sup>, Jorge Bacallao<sup>4</sup>.

<sup>1</sup>Center for Genetic Engineering and Biotechnology, <sup>2</sup>Hospital "Hermanos Ameijeiras". <sup>3</sup>Havana Hospital "Carlos J. Finlay", and <sup>4</sup>Havana Medicine School, Havana, Cuba

### INTRODUCTION

The infection by hepatitis C virus (HCV) has been characterized based on data mainly derived from developed countries. Consequently, there is a lack of information relative to the prevalence in general population and the associated risk factors in developing countries. The present paper reports the distribution of antibodies to HCV observed in different regions and groups of the Cuban population, and the risk factors linked to this infection.

### MATERIALS AND METHODS

Anti-HCV antibodies were tested in samples from 470 patients with liver diseases, 2 463 blood donors and 560 pregnant women, from 10 out of 14 Cuban provinces. Furthermore, 1 141 samples of general population were studied in Cienfuegos City. Three EIA systems were employed: BioSCREEN anti-HCV (Heber Biotec, Havana, Cuba). UBI HCV EIA (United Biomedical Inc., New York, USA) and Ortho HCV ELISA 2nd. generation (Ortho Diagnostics, Beerse, Belgium). The relative risk associated with the HCV infection was assessed by an epidemiological questionnaire.

### RESULTS AND DISCUSSIONS

The overall prevalence among blood donors (0.8%) and pregnant women (1.1%) does not differ ( $p=0.45$ ), in spite of a tendency of a higher prevalence in Havana than in the rest of the country (table 1). This difference is statistically significant for blood donors (1.6% vs. 0.4%,  $p=0.01$ ).

**Table 1**

Anti-HCV prevalence in low risk groups. Cuba, 1990 - 1993

GROUP	STUDIED POPULAT.	ANTI-HCV +	PREV., %
BLOOD DONORS, CUBA	2 478	20	0.8
HAVANA*	1 744	27	1.5
OTHER PROVINCES**	1 603	6	0.37
HIGH ALAT***	170	22	12.9
PREGNANT WOMEN, CUBA	1 236	13	1.1
HAVANA****	556	7	1.3
OTHER PROVINCES**	680	6	0.8

\* Includes 3 studies in 5 blood banks of Havana, 1991 - 1992.

\*\* Study in 10 out of 14 Cuban province, September, 1992.

\*\*\* Blood Bank of Marianao, Havana, September, 1990; March, 1991.

\*\*\*\* All pregnant women, 8 health areas, Havana, January-May, 1992.

**Table 2**

Anti-HCV in general population.  
Cienfuegos, May 29-July 17, 1992

SEX	AGE	SAMPLES	HCV +	PREV., %
MALE	15-34	133	2	1.5
	35-54	185	4	2.2
	>55	208	2	1.0
	TOTAL	526	8	1.6
FEMALE	15-34	164	3	1.8
	35-54	243	5	2.1
	>55	208	8	3.8
	TOTAL	615	16	2.3
TOTAL		1 141	24	1.9

\* Non significance difference

**Table 3**

Anti-HCV prevalence in high risk groups. Cuba, 1990-1993

GROUP	ANTI-HCV+	PREV., %
HEMOPHILIACS *	8	44.4
HEMODIALYSIS, CUBA (1992)	137	46.7
PERITONEAL DIALYSIS, CUBA (1991)	6	9.1
HIV SEROPOSITIVES **	6	2.8
PLASMAPHERESIS BLOOD DONORS *	43	47.3
MALE HOMOSEXUALS	3	5.3
HIGH RISK PROFESSIONALS ***	0	0.0

\* City of Havana, September, 1991

\*\* All seropositives from Havana City and Pinar del Rio province

\*\*\* All risk personnel of a blood bank and an hemodialysis unit.

Regarding the general population (table 2), a slightly higher prevalence was found in women and in adults over 35 years old. The antecedents of blood transfusions (RR=4.25, CI 1.26; 14.37) and the history of two or

more surgical operations (RR=4.95, CI 1.8; 13.56) were the only risk factors found to be associated with HCV infection, without linkage among them ( $p=0.45$ ).

The main risk groups for HCV infection were those associated with the hematic infection route (table 3).

## A RECOMBINANT *Treponema Pallidum* Ag AND ITS EVALUATION BY ELISA FOR THE DIAGNOSIS OF SYPHILIS

María del C. Domínguez, Alina Miranda, Maida Candelario

Center for Genetic Engineering and Biotechnology. P.O. Box 6162, Havana 10600, Cuba

### INTRODUCTION

In recent years different groups have developed ELISA-type diagnostic kits for syphilis, based on the use of recombinant proteins from *Treponema pallidum* (1). The sensitivity and specificity of the ELISAs for the detection of syphilis is comparable to those of the most widely used methods (2). This paper describes the cloning of a 42 kDa *T. pallidum* inner membrane Ag (TnpA) (3), and its expression in *E. coli*, under the tryptophan promoter. High expression levels were achieved using a 58 aa sequence of human interleukin-2 as stabilizer. A study of this recombinant TnpA by ELISA against a panel of VDRL+ and VDRL- sera revealed high sensitivity and specificity, indicating that this antigen is a good option for the diagnosis of syphilis.

### MATERIALS AND METHODS

The gene in question was obtained through PCR, using as substrate DNA from *T. pallidum* isolated from the lymphatic fluid of a patient. The gene was inserted in an expression vector controlled by the tryptophan promoter, using a 174 bp human IL-2 stabilizer and the T4 phage transcription terminator (4). Induction of expression was performed in M9 salts minimum medium. Protein purification was done by electroelution and purity was calculated by densitometry of SDS-PAGE. The purified recombinant TnpA was evaluated against a panel of 92 VDRL+ sera, 31 sera weakly reactive to VDRL, and 274 VDRL- sera. ELISA plates were coated

with 100 mL of antigen at a concentration of 5 mg/mL. A protein A-HRPO conjugate was used. OPD was employed as substrate. Sera that did not match by ELISA and VDRL were studied using a hemagglutination confirmation kit (Fujirebio Inc.).

### RESULTS AND DISCUSSION

The protein was highly expressed soluble and cytoplasmic (ca. 30% of total bacterial protein). Previously, all the proteins expressed with this vector had been produced insoluble and forming inclusion bodies (4). We hypothesized that being TnpA a bacterial protein, its structure is more prone to adopt a correct conformation in the *E. coli* cytoplasmic environment, without insolubilization. The protein obtained by electroelution was 95% pure. The ELISA developed with this electroeluted antigen showed 99.2% of specificity for the VDRL-samples and 100% sensitivity with VDRL+ samples. These results indicate that the recombinant TnpA is a good option for the diagnosis of syphilis by ELISA.

### REFERENCES

1. YOUNG, J. (1992). *International Journal of STD and AIDS*, 3: 391-413
2. VAN EMBDEN, J. D. A. (1989). *Journal of Clinical Microbiology*, 1: 152157
3. VAN EMBDEN, J. D. A. (1985) *Journal of Bacteriology*, 3: 1227-1237
4. NOVOA, L. I. et al. (1991). European Patent Application No. 90202108.8