Epidemiological and Virological Study of Hepatitis C Virus Infection in Hemodialysis (Case of Six Centers) in Morocco

Abderrahim Foullous1, 4 Sanaa Mahmal2 Ali Asdadi*3 Naoufal Mtioui2 Fatima Ouaddi2
Selma siham Elkhyat3 Ghislaine Medkouri2 Fatiha Lazaar4 Abdelouahab Bennani2
Abderrahman Hammoumi1

1. Laboratory of Microbiology, Pharmacology, Toxicology and Environmental Ainchok Faculty of Science, University, Hassan II. BP: 5366 Maarif, Casablanca, (MOROCCO)
2. Service of Nephrology, Centre Hospitalier Universitaire Ibn Rochd, 1, Rue des Hôpitaux, Casablanca, (MOROCCO)
3. Laboratory of Plant Biotechnology, team of Planta Sud, Faculty of Science of Agadir, Ibn Zohr University, B.P 28/S, Agadir, Morocco
4. Molecular Biology Laboratory, Department of Medical Biology, Pasteur Institute of Morocco, 1, Place Louis Pasteur, 20360 Casablanca, (MOROCCO)

* E-mail of the corresponding author: asdadiali@gmail.com

Abstract
The purpose of this study was to determine the prevalence of viral RNA and HCV genotypic profile in 6 hemodialysis centers in Casablanca. A total of 630 patients were included in this survey, 194 patients had antibodies antiHCV + ; then the prevalence is 30.79%, indeed search for viral RNA by PCR in this population was detected in 105 patients, also, the prevalence of viral RNA is 54.1%. The study of genotypic profile in this population showed the following distribution: the most prevalent genotype is genotype 1: subtype 1a was found in 19 patients (18.09%); subtype 1b was found in 49 patients either (46.66%); genotype 2a / 2c in 27 patients (25.71%); genotype 2 in 5 patients (4.76%), 4h genotype was detected in one patient with a frequency of 0.95%, genotype 4 was found in 4 patients with a frequency of 3.80%. Indeed, extensive studies and large scale is needed to understand the epidemiology of HCV.

Keywords: Hepatitis C virus, Prevalence, Genotype, Public health, Morocco genotypes in hemodialysis.

1. Introduction
Virus hepatitis c virus (HCV) was identified in 1989 as the causative agent of most of the previously called hepatitis (non-A non-B parenteral transmission) (Choo QL, et al. 1989), the HVC is one of five hepatitis virus (ABCD and E) It is a small virus belonging to the family of Flaviridae wrapped in 55-65 nm the diameter (Kaito, et al. 1994). The HVC genome is a single RNA molecule of 9.6 kb positive-strand (Penin, et al. 2004). The HVC has a high genetic diversity, due to the activity lack of exonuclease 5'-3' (lack of corrective activity) RNA-dependent RNA polymerase (Ogata, et al. 1991).

The 5'UTR (5' Untranslated Region) is one of the most conserved regions of the genome with more than 90% homology between the sequences of different strains (Bukh, et al. 1992). Capsid coding region is also highly conserved from 81 to 88% homology (Simmonds, et al. 1994). The most variable region of the genome is one encoding the envelope proteins E1 and E2. The sequences encoding the hypervariable regions HV1, HV2 and HV3 glycoprotein E2 may vary from 50% of a strain to the other (Choo, et al. 1991, Smith 1999, Troesch, et al. 2006, Weiner, et al. 1991).

To bring order to the overall genetic diversity of HCV, a classification based on molecular phylogenetic analysis of viral sequences was developed, in fact a consensus for a system of nomenclature and classification of variants of HCV genotypes (Simmonds, et al. 2005). Each genotype is phylogenetically subdivided into a number of subtypes named alphabetically in the order of their discovery. Thus variants of HCV are classified into six genotypes (representing 6 genetic groups defined by phylogenetic analysis), 72 subtypes have been identified up to now (Simmonds, et al. 2005).

As HCV is an RNA virus, it circulates in the host as a viral quasispecies, that is to say, a complex mixture and precarious variant genetically distinct but related (Martell, et al. 1992, Farci and Purcell 2000).

In some patients infected with HCV it can be found more than one genotype, this is what we call mixed genotypes infection (Chen, et al. 2003, Antonishyn, et al. 2005).

HCV genotyping is essential and help us to make epidemiological surveys, clinical and treatment management, indeed genotypes and subtypes vary in worldwide geographical distribution (Zein 2000). Genotypes 1a, 1b, 2 and 3 are more common worldwide; genotype 4 is very present in the Middle East. Genetic diversity contributes to the pathogenesis, genotypes 1 and 4 are more resistant than genotypes 2 or 3 to treatment with Interferon α plus Ribavirin. Duration of treatment is tailored to genotype (Hadziyannis, et al. 2004, Fried, et al. 2002).
2. Materials and Methods

The present study is a prospective, cross-sectional, multicenter epidemiological study realized. The purpose of this work is:

- Assess the serological prevalence of HCV.
- Detection of viral RNA by PCR.
- Determine the genotypes and the distribution of types and subtypes in this population by a reverse hybridization assay (LIPA).

This study includes 630 patients chronically infected by HCV and undergoing hemodialysis, in six hemodialysis centers in Casablanca. The epidemiological and clinical Collection has been given by the establishment of a questionnaire: A detailed clinical history and a survey on risk factors have been undertaken.

The study procedure was approved by the Ethics Committee at Pasteur Institute of Morocco, patients involved were informed of the purpose of this survey and a consent form was signed. After the collection of blood in hemodialysis patients (approximately 10 ml were collected), the samples were placed in EDTA tubes, which are transported to the laboratory.

Molecular biology at Pasteur Institute storage, packaging has been sterilized in bags. These samples were centrifuged to obtain plasma which is the biological material to be used. The aliquot serum is divided into three tubes, one for serological tests, the other 2 for molecular analysis (PCR and Genotyping) plasmas that are not used immediately and were stored at -20 degrees Celsius.

Serotyping: characterization of antibodies specific for different genotypes:
The anti-HCV antibodies in serum search are carried out by ELISA (enzyme-linked immunosorbent assay) of the third generation.
The subject is considered positive if the antiHCV + ELISA test is positive.

The molecular tests based on the analysis of a region of the viral genome by PCR:
Detection of HCV RNA in the serum is performed by a standard method of reverse transcription followed by a chain reaction amplification of DNA polymerase (RT-PCR).

Our molecular biology laboratory is equipped with Cobas TaqMan HCV Test (Roche) [Cobas Monitor HCV 2.0 (Roche Diagnostics)], which allows the Detection and Quantification of HCV RNA in the serum of patients.

The serum of all patients with positive serology is collected to extract RNA VIRAL according to manufacturer's instructions (Cobas Monitor HCV 2.0 (Roche Diagnostics)), samples of which were not revealed viral RNA are considered as a patients who eliminated HCV: a negative PCR.

The genomic typing: characterization of polymorphism of a genome fragment
The method most widely used to determine the genotype of HCV is the line probe assay commercially available (INNO-LIPA), based on specific oligonucleotides of the 5'-untranslated region (5 'untranslated region) that are immobilized on a nitrocellulose strip, the oligonucleotides are then probed with a 5 ' UTR amplicon labeled with biotin, which binds to a specific bands genotype manner; the oligonucleotides are then visualized by using a reaction with streptavidin-peroxidase. The genotype is then identified with an algorithm model.(Stuyver, et al. 1993) (Sandres-Saune, et al. 2003)

In our study, genotyping was performed on all serum from HCV-RNA positive samples.

3. Results

We conducted this study with all patients (630 patients) in 6 centers hemodialysis. Median age is 48.33 +/- 15.5 years, the range ages is 8-90 years, while the mean age was 48 years, sex male / female ratio of 0.91. No patients included in this study are infected with HIV; two patients are co-infected with HBV.

Statistical analysis showed that the prevalence increases with age, but this is not significant findings. Then, there's no correlation between HCV infection and sex.

Among the 630 patients seeking hemodialysis antiHCV antibodies is positive for all 194 of them, prevalence is 30.79%.
4. Discussion

Search for antiHCV by ELISA in 630 patients in 6 centers antibody was positive in 194 patients a prevalence of 30.79%, these results are similar to an earlier study conducted by our research team (Foulous A., et al. 2014). Then; the prevalence of antibodies antiHCV is dramatically high; which can be explained by nosocomial transmission associated with risk factors: duration of dialysis and blood transfusion before 1994 (Foulous A., et al. 2014, Sekkat, et al. 2008, Caramelo, et al. 1994).

Among the 194 patients; Research From viral HCV RNA revealed the detection of the viral genome in 105 patients then the prevalence is 54.1%.

The prevalence varies considerably from one center to another, indeed centers 1, 2 and 3 have a high prevalence (table 1) explained by a deficiency in structures and lack of personal manager , Centers 1 ,2 and 3 start working before 1994 (blood transfusion problem), other centers 4, 5 and 6 are newer. These results are comparable to one’s found in several studies; the prevalence of Viral RNA varies from 58 to 92% of hemodialysis patients chronically infected with HCV antibody (Roth 1995, Stehman-Breen, et al. 1998, Espinosa, et al. 2001, Natov, et al. 1998, Salama, et al. 2000).

Table 1: prevalence of viral RNA in the HCV in hemodialysis patients

<table>
<thead>
<tr>
<th>CENTRE</th>
<th>PCR+</th>
<th>PCR-</th>
<th>TOTAL PATIENT</th>
<th>PREVALECE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENTRE1</td>
<td>56</td>
<td>34</td>
<td>90</td>
<td>62.22%</td>
</tr>
<tr>
<td>CENTRE2</td>
<td>23</td>
<td>16</td>
<td>39</td>
<td>58.97%</td>
</tr>
<tr>
<td>CENTRE3</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>75%</td>
</tr>
<tr>
<td>CENTRE4</td>
<td>8</td>
<td>22</td>
<td>30</td>
<td>26.6%</td>
</tr>
<tr>
<td>CENTRE5</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>28.57%</td>
</tr>
<tr>
<td>CENTRE6</td>
<td>7</td>
<td>9</td>
<td>16</td>
<td>43.75%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>105</td>
<td>89</td>
<td>194</td>
<td>54.1%</td>
</tr>
</tbody>
</table>

Our sample was 194 patients; we have detected RNA viral in 105 patients: prevalence is 54.1%.

In this study the results of the genotyping technique by LIPA found that genotype 1b was the most common with a frequency of 46.66% and the genotype 1a/1b (18.09%), genotype 2a/2c (25.71%), genotype 2 (4.76%), genotype 4h (0.95%) and genotype 4 (3.8%).

In Morocco, the first study operated in 1997 found that genotype 1b was predominant in the general population with a frequency 47.6%, followed by genotype 2a / 2c (37.1%) and genotype 1a (2, 8%), while in hemodialysis patients, only one genotype was found with a prevalence of 68.4% for subtype 1b and 15.8% for subtype 1a(Benani, et al. 1997).

In Moroccan general population a recent study, showed that genotypic distribution is comparable to ours: The results of sequencing the 5'UTR showed the presence of three genotypes 1, 2 and 4. On 174 infected
patients; 122 presented genotype 1b (70.1%); 49 presented genotype 2 (28.2%) and (0.6%) non classified (Brahim, et al. 2012).

Another study conducted by Rabat team in 2000 found that the distribution of genotypes of HCV genotype 1 (21.47%), 2a/2c (13.29%), 1a (6, 13%) and 3 (1.2%,) (Cacoub, et al. 2000). Genotype 4 was found in 2 centers, which is not common in MOROCCO, it was found in 5 patients of 105 hemodialysis patients with a frequency of 4.75%, after an investigation; these patients were infected during their trip to Mecca (pilgrimage).

It seems that the prevalence and incidence of different genotypes of HCV depends on geographic regions and modes of transmission (Wasley and Alter 2000). Genotypes, 1, 2 and 3 are ubiquitous genotypes and they are most common in the world (Bhattacherjee, et al. 1995, McOmish, et al. 1994, Nousbaum, et al. 1995), genotype 4 is very common in Central Africa, Egypt and Middle East(Ray, et al. 2000). Genotype 5 is predominant in South Africa (Smuts and Kanneveyer 1995). While genotype 6 is present in South-East of Asia (Zeiin 2000, Pawloulsky 2003).

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5. Conclusion
The study of viral diversity provides a better understanding of the epidemiology of HCV infection, the origin and dynamics of viral infections. Epidemiological studies all over the world show that the distribution of HCV genotype is characteristic of each region.

In our series, the prevalence varies between 66.1% and 4.2% depending on the center, with an average of 30.79% . This high seroprevalence can be explained by risk factors in hemodialysis: vascular, the immunodepresion hemodialysis patients, blood transfusion and duration of dialysis. The detection of viral ARN showed an average prevalence of 54.1%. While the Results from the genotype analysis shows that the genotype 1b is most common with a 46.66% of frequency; genotypes 1a / 1b (18.09%), genotypes 2a / 2c (25.71%), genotypes 2 (4.76%), genotypes 4h (0.95%) and genotypes 4 (3.8%). The HCV have a high genetic diversity. This one is caused by non attendance of exonuclease activity resulting from 5'-3' (lack of corrective activity) of ARN polymérase dependent RNA, but also the high level of replication (10x12000 new HCV virions per day) then a genetic variability is estimated to 1.9x10000 per site per year(Ogata, et al. 1991, Smith, et al. 1997, Neumann, et al. 1998).
This variability is involved in treatment response, it’s one main obstacles development of a vaccine, determining HCV genotype is very important in order to understand the transmission way. Genotyping is essential since genotypes 1 and 4 are more resistant to treatment than genotypes 2 or 3 with treatment combination pegylated Interferon α (pegIFNα) plus Ribavirin (RBV) and also the duration of treatment is tailored to each genotype.

Other studies should be undertaken to study the dynamics of change over time and to identify mutations in the protein coding region of the capsid in order to understand the mechanisms of viral persistence and resistance to antiviral drugs and to study the geographical distribution of genotypes and subtypes circulating HCV in different hemodialysis centers.

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