

**ANRS RESEARCH SITE ON VIRAL HEPATITIS IN CAIRO, EGYPT  
JANUARY 2010**

**EXECUTIVE SUMMARY**

Since 2001, a Franco-Egyptian network combining several research institutions and universities has been collaborating on hepatitis C virus (HCV) research in Egypt. The strength of the network is its multi-disciplinarity, combining epidemiologists, clinicians, virologists, immunologists, geneticists, pathologists, and health economists from the two countries. This informal network became in 2007 an official research site on viral hepatitis for the French Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS).

The main research objectives of the network are the assessment of the burden of HCV in Egypt, the study of factors associated with HCV transmission for better prevention programs, the optimization of drug regimens for the treatment of acute and chronic hepatitis C, and the study of factors associated with spontaneous HCV clearance in acute hepatitis C for vaccine development. The project has been successful in attracting fifteen international research grants (from the EC, ANRS, and Wellcome Trust), and has already led to more than 20 publications in international peer-reviewed journals.

The main findings of the network are:

- A predicted doubling of HCV-related deaths between 2000 and 2020, reaching 20000 deaths per year in 2020.
- The identification of medical injections, and particularly intravenous injections and infusions, as the main cause of new infections in Egypt.
- The role of intra-familial transmission of HCV in 5 to 10% of all new infections, involving shared predisposing genetic factors when infections occur in children.
- A 61% efficacy of 48 weeks of combined pegylated interferon and ribavirin in treating genotype 4 chronic hepatitis C.
- A 41% rate of spontaneous clearance of HCV among patients with symptomatic acute hepatitis C. In patients still infected 3 to 6 months after onset of symptoms, 12 weeks of pegylated interferon achieve 88% of cure.

The project has closely collaborated with Egyptian Ministry of Health officials in elaborating the National Control Strategy for Viral Hepatitis that was adopted in April 2008. One of the main achievements of the National Control Strategy has been the opening of 20 centres which have already initiated treatment for more than 60000 patients with chronic hepatitis C.

Our aim for the next four years is 1) to get a better understanding on the circumstances associated with HCV iatrogenic transmission, and particularly intravenous medical injections, to suggest specific targets for prevention programs; 2) to improve patient's management by validating non invasive markers of liver fibrosis, evaluating new drugs for hepatitis C treatment, and assessing the cost-effectiveness of various treatment schemes; and 3) to continue our search for molecular determinants of HCV spontaneous healing following acute infection for the development of vaccine and better drugs.

Project's website: <http://www.hepegypt.org/>

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# 1. INTRODUCTION: HEPATITIS C IN EGYPT

The epidemic of hepatitis C started more than 50 years ago (1,2). However, its causal agent, the hepatitis C virus (HCV), was identified only in 1989 (3), and several assays are now available for the detection of viral RNA or antibodies in the blood of infected individuals (4). Epidemiological studies using these tests have revealed large discrepancies in the spread of HCV around the world (5). Egypt is the country with the highest HCV prevalence worldwide: among adults, the proportion infected is estimated at 20% in rural areas, and 10% in urban areas (Figure 1 & 2) (6). By comparison, only 1% of adults in Western countries are infected.

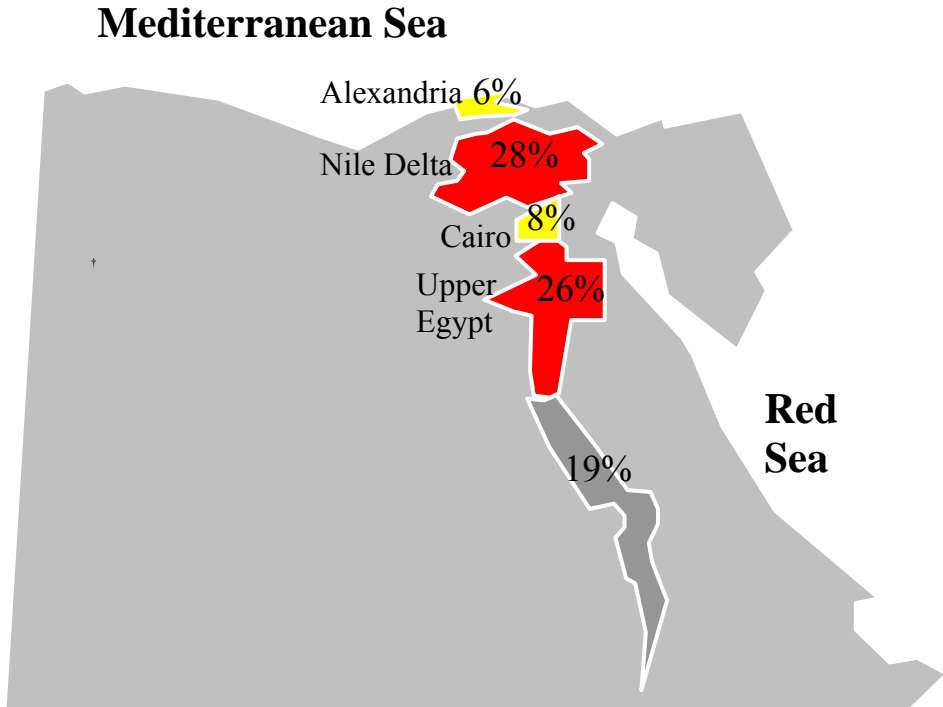
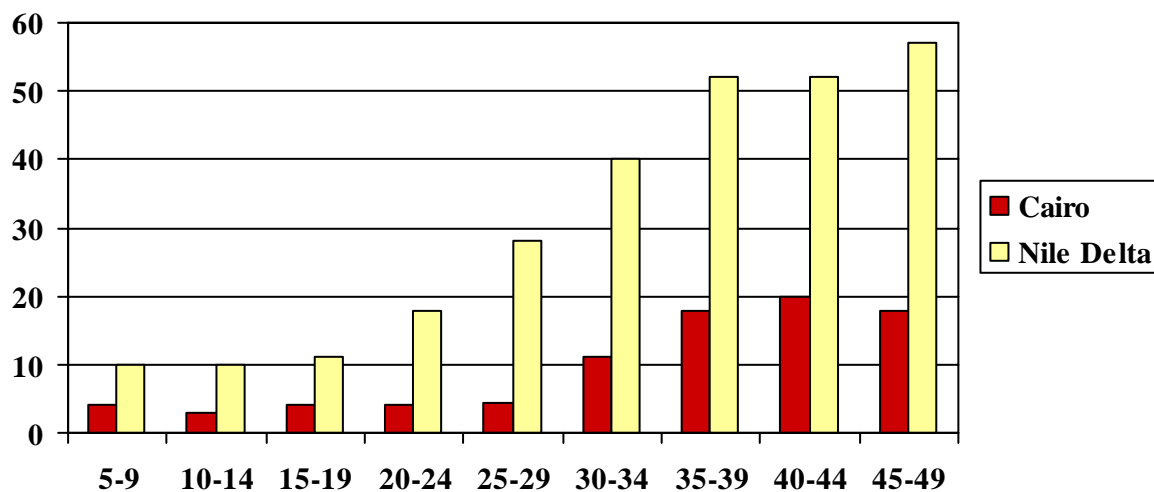


Figure 1. Proportion with HCV antibodies among 10-50 years by geographical area, national survey, Ministry of Health and Population, 1996.

### HCV antibody prevalence (%)



National survey, Ministry of Health and Population, 1996  
Adapted from Frank et al., Lancet, 2000, 355:887-891

Figure 2. HCV antibody prevalence in Cairo and the Nile Delta, national survey, 1996.

HCV infection is important due to its long-term complications (Figure 3): as many as 20% of infected individuals will develop liver cirrhosis, on average twenty to thirty years after infection (7). Liver cirrhosis is fatal in itself, or can lead to liver cancer.

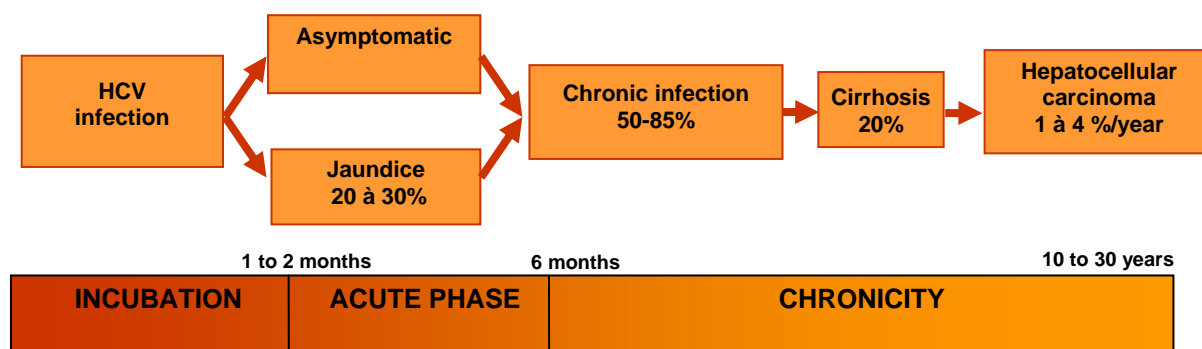


Figure 3. Natural history of HCV infection

HCV is mainly transmitted through contact with infected blood (8). Research by Egyptian members of the group and the MOHP suggested that the origin of the HCV epidemic in Egypt is linked to the mass campaigns of anti-schistosomiasis (bilharziasis) treatment carried out in the 1960s-1970s (6). During these field campaigns, intravenous injections were given to more than 7 million individuals above the age of 6 years. Due to insufficient sterilization of injection equipment, the HCV, unknown at that time, spread among individuals being treated.

This mode of transmission ended in 1982, when oral praziquantel was introduced for the treatment of schistosomiasis. However, this did not stop the transmission of HCV, as shown by the presence of infection in children born after 1982, and by the continued diagnosis of acute hepatitis C among hospital patients in Egypt (9). Risk factors for current infections in Egypt are not well known. Blood transfusion may explain some of past HCV infections, but should only play a limited role today since routine screening for HCV antibodies was established in blood banks in the 1990s.

Treatment for hepatitis C exists, and current drug regimens (pegylated interferon alfa combined with ribavirin) cure about 40% to 80% of patients, depending on the type of genetic variants of the virus (10). Few data exist about the efficacy of combined regimens against genotype 4 (11), the genotype most prevalent in Egypt (12). In any case, treatment is long (one year), poorly tolerated (many side-effects), difficult to administer (through injections, requiring experienced physicians and sophisticated laboratories for patients monitoring), and very expensive (several thousand of \$US per patient depending on drug regimens).

## **2. ESTABLISHMENT OF A FRANCO-EGYPTIAN RESEARCH NETWORK ON HEPATITIS C**

Given the severity of the HCV epidemic, the Egyptian Government encouraged international collaborations, leading to the creation of a research network bringing together the Egyptian Ministry of Health (MOH), the National Hepatology and Tropical Medicine Research Institute (NHTMRI), Ain Shams, Cairo, Minia and Mansoura Universities, and, in France, the Institut Pasteur, the INSERM, and several teaching hospitals. External funding to support this research has been made available through the European Commission, the French ANRS, and the Wellcome Trust. This network has since expanded and combines several research institutes. In 2007, it was awarded the status of “ANRS research site on viral hepatitis” (a detailed description of the partners of this network is available in Annex 2). The list presented below has been updated to include on-going collaborations only.

In Egypt:

- Ain Shams University, Cairo: epidemiology (Prof Mostafa K Mohamed) and immunology (Prof Mona Rafik),
- National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo: virology (Prof Mohamed Abdel Hamid) and clinical expertise (Prof Gamal Esmat),
- University of Mansoura: pathology (Prof Khaled Zalata).

In France:

- Institut Pasteur, Paris: epidemiology (Dr Arnaud Fontanet and Dr Elisabeth Delarocque-Astagneau), and immunology (Dr Matthew Albert),
- Laboratory attached to the National Reference Centre for Viral Hepatitis B, C and delta, Villejuif: virology (Dr Valérie Thiers),
- Cochin Hospital, Paris: clinical expertise (Prof Stanislas Pol),
- Tenon Hospital, Paris: clinical expertise (Dr Philippe Bonnard)
- INSERM U550, Paris: genetic epidemiology (Dr Laurent Abel and Dr Sabine Plancoulaine).
- EA 2694 – CERIM, Lille: cost-effectiveness studies (Prof Yazdan Yazdanpanah and Dr Sylvie Deuffic-Burban).
- INSERM U758-EVIR, Lyon: virology (Dr François-Loïc Cosset and Dr Ophélie Granot)
- Beaujon Hospital, Paris: pathology (Prof Pierre Bedossa).
- INSERM U563, Toulouse : virology (Prof Jacques Izopet and Dr Florence Abravanel)



**Network meeting, Cairo,  
January 2003**

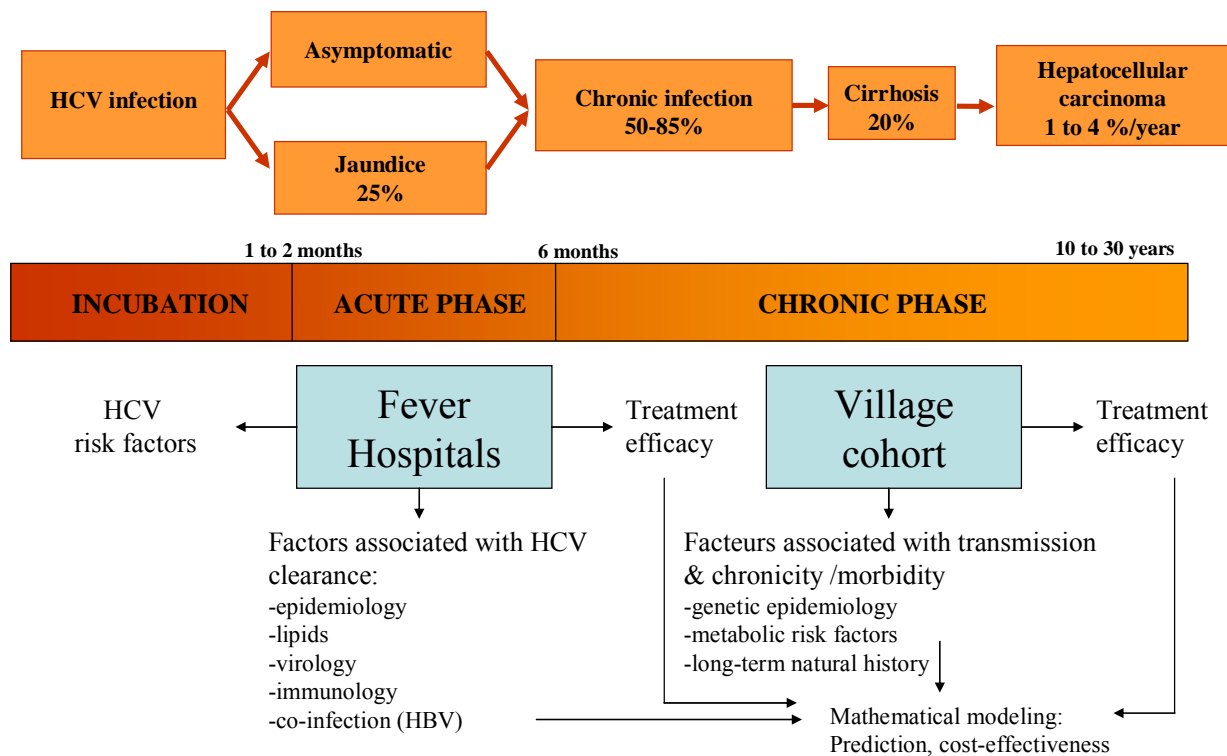
### 3. RESEARCH PROGRAM OF THE NETWORK

#### 3.1. Research objectives

The overall aim of the research work is **to gain better control of the HCV epidemic in Egypt**. Its specific objectives are:

- 1) To estimate the **magnitude** of the epidemic and its morbidity / mortality impact.
- 2) To identify the **risk factors** and routes of HCV transmission for better prevention programs.
- 3) To estimate the **efficacy of treatment regimens**, and elaborate treatment protocols adapted to the local health care system, and
- 4) To understand the **mechanisms of HCV clearance** in acute infection for future drugs and vaccine development.

Given these objectives, the research program has been organised around two study sites: a village cohort in the Nile Delta, and two “Fever Hospitals” in Cairo.



### **3.2. Establishment of two study sites.**

#### ***3.2.1. Village cohort on HCV incidence and progression.***

This cohort has been established in Zwyat Razin, a Nile Delta village north-west of Cairo in an area endemic for schistosomiasis. More than 4000 individuals were screened for HCV infection, allowing studies of risk factors for HCV infection and HCV-related morbidity. Patients in need of treatment were enrolled in a clinical trial estimating the efficacy of pegylated interferon and ribavirin for the treatment of chronic hepatitis C. The village cohort site has several specificities which make it uniquely attractive: community-based study, avoiding selection biases associated with hospital-based recruitment; familial clustering of infections rarely seen in non-endemic countries, allowing studies of intra-familial transmission and genetic studies; and long-term follow-up in a stable population for natural history studies.



Zwyat Razin  
Village,  
Nile Delta.

Field  
Hospital



#### ***3.2.2. Fever hospitals in Cairo***

In these hospitals serving disadvantaged populations of Cairo, patients with fever and jaundice are screened for acute hepatitis C. Such recruitment, hardly feasible in the industrialized world due to low HCV incidence, has proven effective in Cairo. This site is used to study risk factors for acute hepatitis C, to identify factors associated with spontaneous HCV clearance, and, for those who do not clear the virus after three months, to study the efficacy of pegylated interferon for the treatment of acute hepatitis C.

In addition, studies are performed in specific places according to the needs. For instance, the “Prick Injury Study” (ANRS 12171) is taking place in Ain Shams University hospital, and population-based surveys were performed in Cairo to study the attitude of the Egyptian public towards hepatitis C and their willingness-to-pay on HCV-related morbidity.

### **3.3. Research findings of the network.**

#### ***3.3.1. Estimation of the magnitude of the epidemic and its morbidity / mortality impact.***

- **HCV-related morbidity in the village cohort** [Mohamed MK et al., J Med Virol, 2006] (*EC contract (ICA3-CT2000-30011) & ANRS 1211*).

While data are available on HCV prevalence, little is known about the morbidity impact of HCV. Only a proportion of subjects with antibodies develop chronic hepatitis, and we have assessed the HCV-related morbidity in the village cohort by testing all cohort participants plus volunteers for HCV antibodies, and by providing clinical examination, measurement of liver enzymes, and detection of HCV RNA in all anti-HCV positive study subjects. The study sample included 2425 village residents aged 18 to 65 years recruited through home-based visits. Overall HCV antibody prevalence was 448/2425 (18.5%), reaching 50% in males over 40 years of age, and 30% in females over 50 years of age. Of those with HCV antibodies, 284/448 (63.4%) had chronic HCV infection, among which 107/266 (40.2%) had elevated alanine aminotransferase (ALT) (see Figure 3). Overall, of all patients with HCV antibodies, 10% had a treatment indication. Considering the estimated 6.7 million adult Egyptians with HCV antibodies, more than 600,000 individuals would currently need treatment in Egypt if the same percentage (10%) was to be applied nationwide.

In the village, around 20% of adults had anti-HCV antibodies. Of these, 10% had a treatment indication. If the same percentage (10%) applies nationwide, around 600,000 individuals currently need treatment in Egypt.

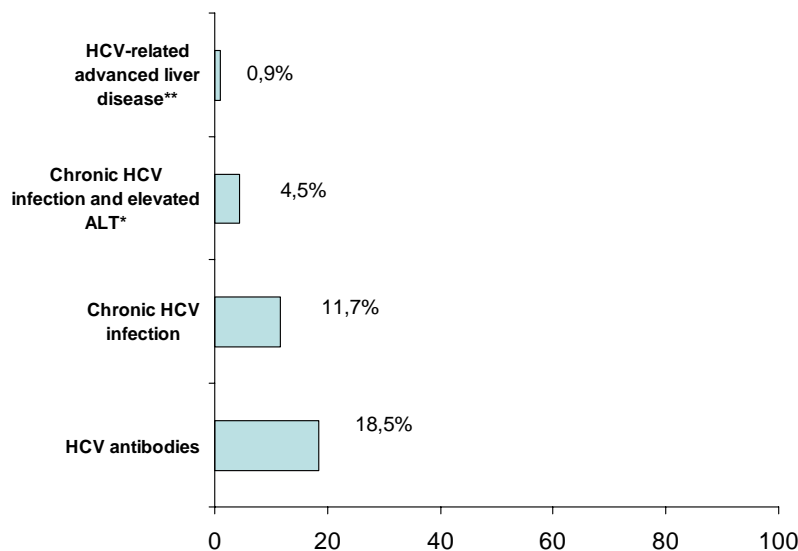


Figure 4. HCV-related morbidity among home-based adult participants (n = 2425). Zawyat Razin, 2002.

- **Assessment of health-related quality-of-life (HRQOL) among villagers with chronic HCV infection** [Schwarzinger et al, Hepatology, 2004] (ANRS 1279).

Previous Western studies showed a consistent and marked reduction in health-related quality of life (HRQOL) in patients chronically infected with hepatitis C virus (HCV). However, these studies were conducted on patients whose knowledge of their serological status may have affected their HRQOL. The same study was carried out in the cohort village, before participants knew about their HCV infection status. HRQOL was assessed by an Arabic translation of the Short-Form 12, and a visual analogue scale (VAS) of the relative severity of one's health status. HCV chronic infection was defined by positive tests for anti-HCV antibody and RNA-HCV. One hundred and forty-six Egyptians chronically infected with HCV had similar Short-Form 12 and VAS scores compared to 1140 uninfected controls from the same rural community. In individuals chronically infected with HCV, serum aminotransferase levels did not correlate with HRQOL. In conclusion, this study did not find a significant reduction of HRQOL in patients chronically infected with HCV.

HCV-infected subjects do not have alteration of quality of life before development of symptoms, a rather late event in the natural history of infection. Only systematic screening may identify infected individuals.

- **Prediction of HCV-related mortality** [Deuffic-Burban et al., J Hepatol, 2006] (EC contract (ICA3-CT2000-30011)).

A previously published back calculation model was adapted to the Egypt case. It combines a general model of the natural history of HCV infections with available epidemiological data to back calculate the annual HCV incidence in the past from observed 1980-1999 hepatocellular carcinoma (HCC) mortality. In turn, the current and future burdens of HCV-related mortality due to pre-2000 infections are projected in the future. Compared with 1999, the model predicts a 3.5 fold increase for HCC mortality, and a 2.4 fold increase for HCV-related mortality. These figures do not take into account the mortality related to infections posterior to the year 2000, or the impact that antiviral therapy may have now that it has become available for a small fraction of the infected population.

HCV-related mortality is predicted to more than double between 2000 and 2020 and may reach 20000 deaths per year in 2020.

### 3.3.2. Identification of HCV risk factors and routes of transmission.

- **Risk factors for HCV infection at the cohort site** [Arafa et al, J Hepatol, 2005] (EC contract (ICA3-CT2000-30011) & ANRS 1211).

Risk factors for HCV infection were studied at the intake of the cohort study: 475/4022 (11.8%) study subjects aged 5-65 years had anti-HCV antibodies (Figure 5). The prevalence of HCV antibodies increased from 2.7% in those <20 years of age to more than 40% in males aged 40-54 years. The peak in HCV prevalence in the 40-54 year age group corresponds to the aging of the cohort of children infected through schistosomiasis intravenous treatments in the 1960s-70s (accounting for 12.4% of all HCV infections observed today among adults). From that initial founding event, the infection spread to other villagers through intravenous injections (40% of the population attributable fraction). Other known risk factors (surgery, blood transfusions, complicated deliveries, endoscopies...) accounted for a limited amount of infections. Among children (< 20 years old), although 47 of them were infected, only few infections could be explained (complicated deliveries in young married girls). Children had been exposed to injections, blood transfusion, or surgery in the past 20 years, but with no apparent increased risk for HCV infection.

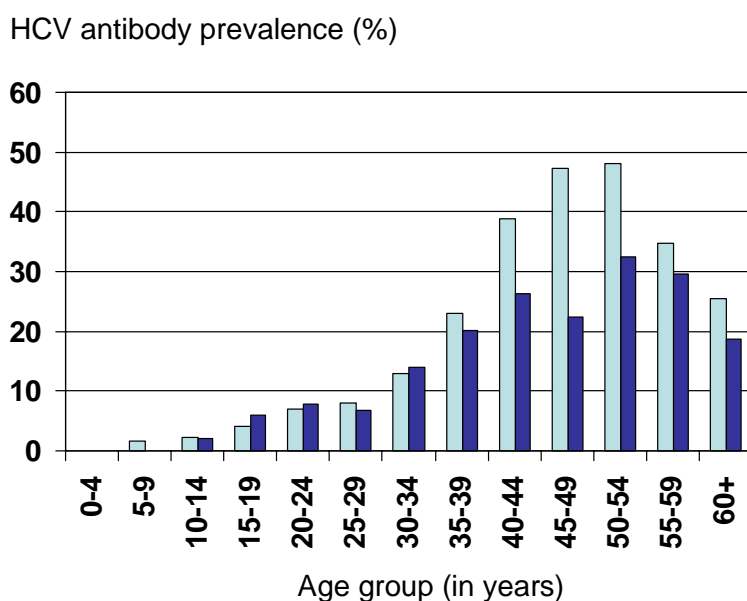


Figure 5. HCV antibody prevalence (%) by age and sex (light grey: males; dark grey: females) (n = 4020). Zawiat Razin, 2002.

Past exposure to mass anti-schistosomiasis treatment explains 12% of all infections seen in 2002 in the village. From the pool of infected individuals, the epidemic spread primarily through injections to the rest of the village population (40% of all infections). There was no explanation for infections seen in children suggesting a possible role for intrafamilial transmission (see below).

- **Study on intra-familial transmission of HCV at the village cohort, and genetic susceptibility to infection and disease** [Plancoulaine et al., Gut, 2008] [Laouénan C, Human Genetics, 2009] (*ANRS grant 12107*).

Previous analyses showed that no risk factor could explain the infections among children aged 5-15 years in the village (16). Another unusual feature of the epidemic was the important clustering of infections at the household level, suggesting either, common at-risk behaviours, intra-familial transmission, or genetic susceptibility to infection or disease. Overall HCV seroprevalence was 12.3%, increasing with age. After adjustment for relevant risk factors, highly significant intrafamilial resemblances in HCV seroprevalence were obtained between father-offspring (odds ratio =3.4[95%CI 1.8-6.2]), mother-offspring (3.8[2.5-5.8]), and sib-sib (9.3[4.9-17.6]), while a weaker dependence between spouses (2.2[1.3-3.7]) was observed. Phylogenetic analysis showed greater HCV strain similarity between family members than between unrelated subjects indicating that correlations can be explained in part by familial sources of virus transmission. In addition, refined dissection of correlations between first-degree relatives supported the role of host genes predisposing to HCV infection.

To test this hypothesis, we performed a segregation analysis for HCV infection, defined as seropositive/seronegative HCV status, in the same population. We used the regressive logistic model, which allows taking into account simultaneously in addition to the genetic effect, the familial correlations (father-mother, father-offspring, mother-offspring and sib-sib) and the relevant associated risk factors. In this analysis of 312 pedigrees (3703 subjects), we found evidence for a dominant major gene predisposing to HCV infection. The predisposing allele frequency was 0.013, indicating that 2.6% of the subjects, in particular those younger than 20 years old, were predisposed to HCV infection. Linkage analysis is now ongoing to locate this major gene within the whole genome.

This study suggests that intra-familial transmission of HCV may occur, particularly among siblings sharing common genetic predisposition to HCV. The predisposing allele would be present in an estimated 2.6% of the subjects living in the studied village.

- **Study of HCV incidence and risk factors for transmission at the village cohort site** [Mostafa A. et al., Liver Int, in press] (*ANRS 1211 & ANRS 12107*).

HCV incidence was estimated during the follow-up of the 3580 subjects who tested negative at baseline. 25 participants (11 females) seroconverted in 10578 person years of follow-up (PY), [Incidence rate of 2.4/1000 person-years; 95% CI: 1.6–3.5]. The median age at seroconversion was 26 years [Interquartile range (IQR) 19 to 35] among males and 20 years (IQR 13 to 24) among females. The only significant risk factor identified for these cases was receiving injections [Adjusted odds ratio (OR<sub>adj</sub>) =3.3; 95% CI: 1.1–9.8]. Two of the 17 viremic seroconvertors were infected with the same strain as at least one of their family members, giving a 12% estimate for intra-familial transmission of HCV. No more than one seroconversion per household was observed during the follow-up period, suggesting that the primary infection period is not particularly infectious to close contacts.

The HCV incidence rate was 2.4 per 1000 person-years in this rural cohort study, with medical injections being the only significant risk factor for infection that could be identified. Intra-familial transmission accounted for an estimated 12% of all new infections.

- **Study on risk factors associated with acute hepatitis C in Cairo** [Paez A. et al, PLoS One, 2009] (*EC contract (ICA3-CT2000-30011) & ANRS 1213 & 12122*).

The identification of incident cases of acute hepatitis C in Cairo offers a unique opportunity to identify current risk factors for infection through a case-control study design. For each patient with definite acute hepatitis C (i.e., negative serology and positive PCR), two age-matched controls were selected among patients with acute hepatitis A and family members. Parenteral exposures in the one to six months prior to onset of symptoms were compared between cases and controls. In addition, family members were asked to provide blood, and HCV sequences between index cases and chronically infected family members were compared to study HCV intra-familial transmission.

From 2002 to 2007, 94 definite acute symptomatic HCV cases and 188 controls were enrolled in the study. In multivariate analysis, intravenous injections (OR=5.0; 95% CI=1.2-20.2), medical stitches (OR=4.2; 95% CI=1.6-11.3), injection drug use (IDU) (OR=7.9; 95% CI=1.4-43.5), recent marriage (OR=3.3; 95% CI=1.1-9.9) and illiteracy (OR=3.9; 95% CI=1.8-8.5) were independently associated with an increased HCV risk. Out of 100 acute HCV patients participating in the study, 18 had viremic HCV-infected household members. Sequencing of the viral isolates and phylogenetic analysis were possible for 12 of these households. Three married couples were infected with virtually identical sequences. They were all long-married (>15 years) and none of the three recently infected index patients reported any exposure at risk.

As for studies performed in rural areas, medical injections came out as the main risk factor for HCV infection. In this study, the increased risk was observed for intravenous injections, including IV infusions, but not for intramuscular or subcutaneous injections. Of interest, intravenous drug use, practised by 2% of controls (i.e., representative of the population attending the fever hospitals), was also associated with increased risk of HCV infection.

- **Study on risk factors associated with acute hepatitis B in Cairo** [Paez et al., International Journal of Epidemiology, 2009] (*EC contract (ICA3-CT2000-30011)*).

The same design as the one proposed for the identification of risk factors for acute hepatitis C was applied to acute hepatitis B cases recruited in the same fever hospitals. Patients with acute hepatitis A and family members served as controls. Between April 2002 and June 2006, 233 cases and 233 controls were recruited in the study. In multivariate analysis, factors associated with an increased HBV risk in males were illiteracy, shaving at barbers, and injecting drug use (IDU). In females, factors associated with an increased HBV risk were illiteracy, recent (< 1 year) marriage (when compared to singles), and birth giving.

It is noteworthy that in this study, HBV transmission took primarily place in the community, whether related to recent marriage (presumably first sexual intercourse), shaving at barber, or IDU, and was more common among illiterates. By contrast, HCV transmission was mostly related to iatrogenic factors except for intravenous drug use.

**3.3.3. To estimate the efficacy of treatment regimens, and elaborate treatment protocols adapted to the local health care system.**

- **Safety and efficacy of the combined pegylated interferon and ribavirin in the treatment of chronic hepatitis C** [Elmakhzangy H. et al, J Med Virol, 2009] (*ANRS 1211*).

The combination of pegylated interferon and ribavirin has proven to be the most effective, with sustained virological response (SVR) rate of 42% in patients infected with genotype 1, and 80% in patients infected with genotype 2-3 (10). However, few data are available from patients infected with genotype 4, the genotype prevalent in Egypt. In our study, 100 patients (47 from the village cohort and 53 from local recruitment in Cairo) were treated with 48 weeks of the combined pegylated interferon alfa-2a 180 µg/week SC (Pegasys®) and ribavirin 11 mg/kg/d per os. The SVR was 58/95 = 61.1%, 95% CI (50.1%, 70.9%) among patients infected with genotype 4, i.e. intermediate between that observed with genotype 1 and 2-3. Of interest, none of the 17 patients who did not have a two-log decline in viral load by 8 weeks of treatment had SVR.

The cure rate was 61% among patients with genotype 4 chronic hepatitis C treated with 48 weeks of pegylated interferon alfa-2a and ribavirin..

- **Serum alpha-fetoprotein (AFP) level predicts treatment outcome in chronic hepatitis** [Males et al, Antiviral Therapy, 2007; Gad et al, Liver International, 2008] (*ANRS 1211*).

Among these 100 patients, median serum AFP level was 4.5 ng/mL with AFP values ranging from 1.2 to 49.8 ng/mL. In multivariate analysis, higher fibrosis stage and higher steatosis score were independently associated with higher serum AFP level. SVR rate was 61.0% (61/100), and was lower for patients with AFP levels above versus under the median value (40.8% versus 80.4%, respectively,  $p < 0.001$ ). In multivariate analysis, including adjustment for age, gender, body mass index, steatosis score, fibrosis stage, ALT level, hemoglobin level, clotting time, HCV RNA viral load, and treatment dose received, a baseline serum AFP level above the median value was associated with a lower SVR rate (OR [95% CI] = 0.10 [0.03-0.42],  $p < 0.001$ ). None of the seven patients with elevated (above 15 ng/mL) pretreatment AFP achieved a SVR.

The analysis of factors associated with treatment failure was extended by adding patients from another trial comparing combined standard interferon to combined pegylated interferon alfa-2b. In total, 250 patients with genotype 4 chronic hepatitis C were included in the analysis. Factors associated with treatment failure were higher serum AFP levels, severe fibrosis on liver biopsy, presence of steatosis on liver biopsy, and treatment with standard interferon (when compared to pegylated interferon).

Of note, two other studies have since confirmed the predictive value of AFP in patients infected with genotype 1 (Abdoul et al. PLoS One, 2008) and in HIV-co-infected patients (Carrat et al., AIDS, 2008).

Higher baseline serum AFP levels, severe fibrosis, presence of steatosis, and treatment with standard interferon (when compared to pegylated interferon) independently predicted a lower treatment response rate among patients with chronic hepatitis C.

- **Safety and efficacy of pegylated interferon in the treatment of acute hepatitis C** [Sharaf et al., PLoS One, 2008] (*ANRS 1213*).

Patients with acute hepatitis C and no viral clearance three months after the onset of symptoms were offered treatment with once-weekly pegylated interferon alfa-2a (Pegasys®) for twelve weeks. Between May 2003 and February 2006, 17 patients were enrolled: 12 were males; the median (IQR) age was 31 (27-38) years; and the median (IQR) viral load at treatment initiation was 74,400 (7,900- 444,000) IU/mL. By the end of the 12-week treatment, 15/17 (88.2%) had cleared the virus. Treatment was continued for another 12 weeks for the 2 patients who tested HCV RNA positive at 12 weeks; one of them remained HCV RNA positive for the rest of the follow-up while the other cleared the virus. However, one patient who had cleared the virus by week 12 became HCV RNA positive again by week 24. The overall SVR rate was 15/17 = 88.2% (95%CI = 63.5% - 98.5%). No adverse events requiring hospitalization were observed.

The cure rate was 88.2% with 12 weeks of pegylated interferon alfa-2a in acute hepatitis C patients still viremic four to six months after onset of symptoms.
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### 3.3.4. *To understand the mechanisms of HCV clearance in acute infection for future drugs and vaccine development.*

- **Estimation of the spontaneous HCV clearance rate in acute symptomatic infection** [N. Sharaf et al., PLoS One, 2008] (*ANRS 1213*).

Following the pilot study on acute hepatitis C identification, recruitment of patients with acute hepatitis C was continued. Follow-up was proposed to patients with acute hepatitis C. Between May 2002 and February 2006, 2243 adult patients with acute hepatitis were enrolled in the study. Of these, 647 (28.8%) were diagnosed with acute hepatitis A, 609 (27.2%) were diagnosed with acute hepatitis B, and 160 (7.1%) were diagnosed with acute hepatitis C. The spontaneous viral clearance rate among 117 patients with acute hepatitis C and follow-up was 33.8% (95%CI [25.9% – 43.2%]) at three months and 41.5% (95%CI [33.0% – 51.2%]) at six months. No factors were found associated with viral clearance.

About 7% of all patients with acute hepatitis seen in the Fever Hospitals had acute hepatitis C. Among patients with acute hepatitis C, the spontaneous viral clearance rate was 34% at three months and 42% at six months.

- **Comparison of HCV clearance among males and females at the village cohort** [Bakr et al., Gut, 2006] (*EC contract (ICA3-CT2000-30011) & ANRS 1211*).

According to studies, 14% to 46% of subjects clear HCV from the blood after infection. Controversial results exist about gender differences in HCV clearance rate. We took the opportunity of the village cohort study to compare HCV clearance in males and females. Definitions used were: cleared HCV infection (positive HCV antibody and negative HCV RNA test results); and chronic HCV infection (positive HCV antibody and positive HCV RNA test results). The study sample included 4720 village residents aged 18 to 65 years recruited through home-based visits (n=2425) or voluntary screening (n=2295). Overall HCV antibody prevalence was 19.3%, reaching 52.3% in males over 40 years of age, and 30.6% in females over 50 years of age. Of those with HCV antibodies (n=910), 61.5% had chronic HCV infection. Compared to males, females were more likely to have cleared the virus (44.6% versus 33.7%, respectively,  $P = 0.001$ ). Control for age, schistosomiasis history, iatrogenic exposures, and sexual exposure to HCV did not alter the positive association between female gender and viral clearance.

HCV spontaneous clearance rate was one third higher among females compared to males.

- **Role of lipids in HCV clearance** [Marzouk et al., Gut, 2007] (*EC contract (ICA3-CT2000-30011) & ANRS 1211*).

A large literature exists on the interaction between lipids and HCV entry into hepatocytes (13-15). As part of a study on the interaction between HCV and lipid / glucose metabolism, we found that village cohort participants who cleared HCV infection had higher triglyceride levels compared to those never infected (age- and gender- adjusted differences [95% CI] was +16.0 [0.03 to 31.9] mg/dl), suggesting that elevated triglycerides at the time of acute infection may facilitate viral clearance. This hypothesis will be tested further in the study on HCV clearance after acute hepatitis C.

High serum triglycerides at the time of infection may be associated with spontaneous HCV clearance.

- **The role of plasmacytoid dendritic cells (pDCs) in the endogenous production of type I interferons during acute HCV** [Mansour et al., submitted] (*ANRS 12135*).

In this study, we investigated if plasmacytoid dendritic cells (pDCs) were activated as a result of HCV infection. We demonstrated that even during acute infection, circulating pDCs maintained a similar precursor frequency and resting phenotype as compared to healthy individuals. Moreover, stimulation with a TLR9 agonist resulted in an intact inflammatory response.

These data support the growing consensus that pDCs are not directly activated by HCV and therefore are viable targets for immunotherapy throughout HCV infection.

- **The inflammatory signature of acute HCV genotype 4 infection and identification of biomarkers of spontaneous viral clearance** [Decalf et al., submitted] (*ANRS 12135*).

We performed a medium-throughput, high-quality proteomic screen for immune and metabolic analytes present in the plasma of acute HCV genotype 4 patients who spontaneously cleared the virus and patients who developed persistent infection. This permitted the evaluation of surrogate markers for spontaneous clearance of HCV. Additionally, we monitored the role of liver inflammation during acute hepatitis, by comparing our HCV cohort to patients with acute HBV. Using clinically validated tests, we quantified the concentration of 85 serum analytes, and identified 6 molecules that discriminate cleared *vs* non-cleared HCV patients. These observations offer, for the first time, biological markers associated with HCV spontaneous clearance. In addition, we defined HBV- and HCV-specific inflammatory signatures. We report 7 molecules that serve as a core panel for viral hepatitis. Interestingly, there exists a disease-specific relationship when evaluating the correlation between inflammatory analytes and liver injury (as measured by ALT, AST and bilirubin levels).

These data offer new information on how the liver responds to different liver-tropic viruses and establishes a foundation for identifying mechanisms responsible for the failure to spontaneously clear acute HCV infection.

### **3.4. On-going and planned research work.**

In this section of the document, we will present our views and plans for the next four years. These have been greatly influenced by our better understanding of the dynamics of the HCV epidemic in Egypt, by recent developments in patient management worldwide (e.g., introduction of non invasive markers of fibrosis and new drugs), and by new hypotheses concerning molecular determinants of the early phases of HCV infection. As for previous years, we want our research work to be closely linked to public health action. As a result, our aims target key elements of the National Control Strategy for Viral Hepatitis that was launched in April 2008, in the four areas covered by the National Control Strategy, i.e., surveillance and monitoring; prevention; patient management; and research. The presentation of our aims will therefore combine an update on recent knowledge with proposed projects and integration in the National Control Strategy.

#### ***3.4.1. Studies on HCV transmission and prevention.***

It is now widely accepted that the mass treatment campaigns for schistosomiasis in the 1960s-1980s have been responsible for the burst of the HCV epidemic in Egypt. What is more difficult to comprehend is the way the HCV epidemic continues to spread. Most studies published so far were based on cross-sectional surveys, and were therefore prone to biases related to investigation of prevalent cases of infection, cases who might have been infected anytime in the past decades. The possibility of investigating incident infections through cohort studies in the Nile Delta village, and case-control studies in Cairo, gave us for the first time an understanding of the factors associated with current HCV transmission, be it in a rural area or in Greater Cairo. Such findings are important for two reasons: 1) they have a high level of validity, since they are based on infections for which the time period at risk is recent and well-defined, allowing precise investigation of potential risk factors; 2) they tell us about on-going transmission, responsible for new infections, and not infections of the past. These are the risk factors that prevention programs should target to stop on-going transmission.

Of all studies performed by us and others, the key risk factor that came out as consistently associated with on-going HCV transmission, both in urban and rural areas, was **medical injections**. Since medical injections are largely used, they may account for more than 50% of all new infections. We have some evidence, at least in Greater Cairo, that intravenous, rather than intramuscular or subcutaneous, injections were involved in HCV transmission. Intravenous infusions and use of catheters also came as risk factors. Unfortunately, our epidemiological questionnaires do not provide more specific details around the circumstances of HCV transmission through these exposures. Other risk factors that came out in Greater Cairo were medical stitches, dental gum treatment, and intravenous illicit drug use. Intra-familial transmission does also play a role, although it may concern only 5-10% of new infections. When intra-familial transmission involves children, a genetic component of predisposition to HCV infection is highly suggested by our findings in household studies..

*ANRS 12210: Acute hepatitis C cohort study (Prof Mostafa K Mohamed and Dr Arnaud Fontanet)*

With the renewal of the funding of the acute hepatitis C cohort study for the period 2009-2011, we will continue the recruitment of cases and controls for the study on risk factors associated with acute hepatitis C in Greater Cairo. We estimate our recruitment rate at 50 per

year, of which 50% can be followed-up over time. If the recruitment rate is not as expected, we planned to expand our recruitment to new sites, in Alexandria and in Aswan. For the three main risk factors identified in the initial study (medical injections, medical stitches, intravenous drug use), we will add to the existing questionnaire several questions that examine in greater depth these modes of transmission and the specific circumstances in which transmission took place. Other exposures with increased risk, but not reaching statistical significance in the previous study, such as obstetrical exposures, may become significant with more patients. Indeed, with an additional 150 patients, we will be able to detect as significant ORs of 2 or more for exposures present in 5% of the control population (so far, only ORs greater than 3.5 were statistically significant for most exposures).

At this stage we believe that traditional analytical epidemiological methods for the investigation of HCV transmission through medical procedures and household exposures would require alternative approaches utilizing tools in social sciences. We think that these alternative approaches from social sciences are required if we want to get more in-depth information about the circumstances of HCV transmission to plan for effective prevention programs. This will be done in collaboration with local and foreign social scientists. Our aim will be to conduct ethnographic investigations of the contexts in which selected Greater Cairo populations engage in certain practices that are associated with increased risk factors identified in the team's epidemiological studies. Such investigations would examine medical practices offered at pharmacies and the local uses of pharmacy services; undertake collaboration with local non-governmental organizations to understand better injection drug use practices and their implications for drug users and their families; investigate the working environment processes and conditions that nurses face in hospitals to comprehend how and why patients face an increased risk of acute hepatitis following medical injections and stitching. In all cases, ethnographic study would seek to identify ways to frame more effective public health interventions so as to reduce HCV transmission.

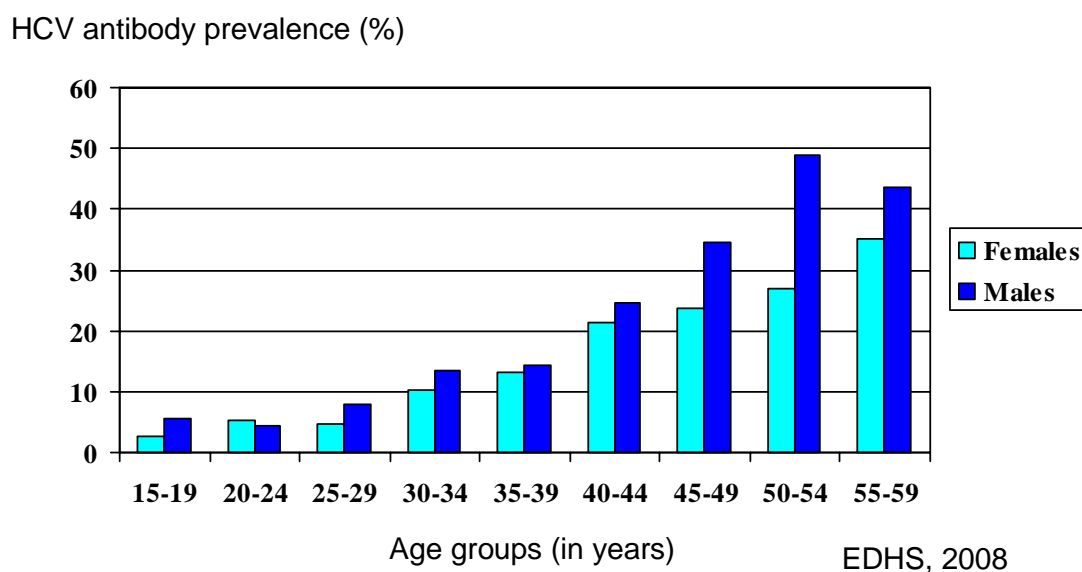
All these results will be shared with the Department of Communicable Diseases of the MOHP, responsible for the implementation of the Infection Control Program (ICP) since 2002. If, as we believe, medical injections and stitches are responsible for the bulk of new infections, infection control should go beyond MOHP facilities to include University Hospitals (under Ministry of Higher Education and Scientific Research), Health Insurance Organisation (HIO) facilities, and private hospitals and clinics. A newly created Infection Control Scientific Advisory Board is currently working on a scheme to expand ICP to these sectors by inviting representatives of the MOHE and HIO to write their ICP guidelines, with reporting for every sector. Including the private sector will require a legislative plan, which would require private clinics to receive training from a certified ICP organization in order to renew their licenses every 3-5 years.

#### ***3.4.2. Estimation of the magnitude of the epidemic and its morbidity / mortality impact.***

In 2008, HCV testing was included in the Demographic and Health Survey (DHS), as recommended by the National Control Strategy document. A three-stage random sampling of the population of Egypt was performed, and included 12,780 adults aged 15-59 years old. Of these, 6,702 (52.4%) were female. Blood samples were available for 84.1% and 90.6% of men and women, respectively. Reasons for not providing blood samples were refusal (6.2% of all individuals approached), absence (5.4%), and others (0.8%).

HCV antibody prevalence in this 15-59 years old group was 14.7% overall, being higher in men compared to women (17.4% versus 12.2%, respectively). Chronic infection, as defined by the presence of HCV RNA, was present in 12.1% and 7.8% of men and women, respectively. As expected, HCV antibody prevalence was higher in rural compared to urban areas (18.0% versus 10.3%), respectively.

## HCV antibody prevalence by age and by sex, Egypt Demographic Health Survey, 2008



The 2008 DHS data suggest that the HCV epidemic is still active. Direct comparison with the 1996 national survey reveals very similar levels for all age groups above 30 years. Rates between 15 and 29 years old in 2008 were 50% lower than 1996. This could be explained by reduced transmission in the past 10 years for this age group but could be also due to the use of less specific ELISA assay in 1996 without confirmation resulting in more false positive samples in the earlier national survey

Romulus Breban, working at Institut Pasteur, has developed a model of HCV transmission in the village of Zwyat Razin, in which HCV prevalence is 12%. This model includes iatrogenic and community transmission of HCV. This model will be extended to two other villages displaying higher (24%), and lower (8%), HCV antibody prevalence. If validated, this model may be used 1) to determine whether the epidemic is self-sustained in Egypt by estimating the  $R_0$  in the three epidemiological contexts; 2) to test the impact on HCV spread of specific control measures such as the use of sterilised material in the health care system; and 3) to test the impact on HCV spread of large scale HCV treatment in the population. This project will be submitted through a Young research Grant initiative at the ANR, but may be proposed to ANRS if unsuccessful under that scheme.

### ***3.4.3. Improved management of chronic hepatitis C in Egypt.***

One of the biggest achievements of the National Control Strategy for Viral Hepatitis has been the opening of 20 National Treatment Reference Centers for patient management. By December 2009, 100000 patients had initiated treatment with pegylated interferon and ribavirin in these centers (treatment is available free of charge to most patients through various subsidized schemes, although its cost is estimated around 3,000 euros for drugs only).

*ANRS 12184: liver fibrosis evaluation in chronic hepatitis C (Prof Gamal Esmat and Dr Philippe Bonnard)*

With the generalization of subsidized treatment across the country, we feel it is important to provide health authorities with up-to-date information about the best available management practices. In recent years, non invasive markers for liver fibrosis have been evaluated, including serum markers or elastometry. Similar evaluation is required in Egypt, where there exist several unique features of the disease: (i) HCV genotype distribution (mostly genotype 4) is different from Europe, (ii) co-infection with hepatitis B virus or *S.mansoni* may be present in up to 5% of HCV-infected patients, (iii) liver steatosis is common, and (iv) patients have an elevated body mass index. Although elastometry and some serum markers may seem too costly or impractical for most treatment facilities in Egypt, their use may be considered within the National Treatment Reference centres. Moreover, cost-benefit analysis may demonstrate that such implementation would provide a cost savings by treating only those who would really evolve towards long term disease complications (i.e., cirrhosis, hepatocarcinoma). In this study, 400 patients coming for pre-treatment evaluation at the National Treatment Reference Centres in Cairo will undergo a liver biopsy, a liver stiffness evaluation by Fibroscan<sup>®</sup>, and a blood sampling for the calculation of the Fibrotest<sup>®</sup>, the Fibrometre<sup>®</sup>, the Fib-4 score and the APRI score. Based on the results of this study, guidelines for the use of elastometry and serum fibrosis markers in Egypt will be developed.

*ANRS (approved for funding): Nitazoxanide efficacy trial (Prof Gamal Esmat and Dr Arnaud Fontanet)*

Treatment options are also expanding. In 2009, telaprevir, a protease inhibitor, was shown to increase sustained virological response (SVR) rate to over 60% in patients with genotype 1 chronic hepatitis C, despite reduction of the combined pegylated interferon and ribavirin therapy to 24 weeks only (telaprevir was limited to the first twelve weeks because of side effects such as anemia, nausea, diarrhea, pruritus and rash) (see McHutchison et al., and Hézode et al., NEJM, 2009). Similarly, other protease inhibitors, and helicase or polymerase inhibitors may become available in the near future. Unfortunately, these products were shown efficacious on genotype 1 replicons only, and their cost may be prohibitive (at least in the near future) for use in Egypt. We were therefore more eager to evaluate another molecule that was recently shown to be effective against genotype 4 chronic hepatitis C, in combination with pegylated interferon and ribavirin. This molecule, named nitazoxanide (NTZ), is a thiazolide with known efficacy against cryptosporidiosis and giardiasis. Its antiviral effect was incidentally discovered when given to AIDS patients treated for cryptosporidiosis, and co-infected with HCV. A trial recently published in Gastroenterology suggests that genotype 4 Egyptian patients treated with a 4-week leading phase of NTZ, and then 36 weeks of combined NTZ + pegylated interferon + ribavirin, may achieve 79% SVR (Rossignol et al., Gastroenterology, 2009), better than the 60% commonly accepted for genotype 4 patients. Considering the limited cost of NTZ (200 euros per treatment) and its availability in Egypt,

we propose to evaluate its efficacy in a randomised double blind trial comparing the standard 48 weeks of combined pegylated interferon and ribavirin to a 4-week leading phase of NTZ followed by 48 weeks of combined NTZ + pegylated interferon + ribavirin. This project has been submitted to ANRS at the September 2009 session (scientific coordination: Prof Gamal Esmat and Dr Arnaud Fontanet).

*ANRS 12215: Cost-effectiveness of treatment strategies (Prof Mostafa K Mohamed and Prof Yazdan Yazdanpanah)*

In addition to evaluating new drugs and means of predicting response to therapy, we will assess the cost-effectiveness of various treatment strategies, based on different starting and stopping rules. This project will evaluate when it is most cost-effective to start treatment (based on ALT and fibrosis thresholds), and to stop treatment (based on results of qualitative, and quantitative HCV RNA at 12 and 24 weeks following initiation of treatment). This project will also contribute to the modelling of HCV infection natural history in Egypt (fibrosis, complications and mortality); the assessment of combined pegylated interferon and ribavirin on HCV natural history; the estimation of direct medical costs associated with each HCV infection stage; and the estimation of quality-adjusted life years (QALYs) associated with each HCV infection stage.

The most important factor in expanding access to treatment will be reducing the price of pegylated interferon, as the Egyptian government will have difficulty sustaining this expense in the long term. Full treatments are now available in Egypt for approximately 3000 euros, but purchasing generic pegylated interferon from producers in India, China, or possibly Brazil or Thailand may provide cheaper alternatives. It may also be possible to override the current patent, as the Egyptian situation constitutes a public health emergency. One Chinese company already produces pegylated interferon for approximately €6.82 a dose, which, with ribavirin, would equate to a total cost of about 500 euros. Given these perspectives, treatment on a large scale is desirable as well as feasible in Egypt, particularly if international financiers can be convinced of the gravity of the epidemic. As has been the case for HIV, the cost of drugs for chronic HCV treatment will likely continue to drop in the near future, and very large purchases, such as those made in the context of a national treatment program, would further bring down the cost. In addition, the limited duration of treatment (one year) would allow funders to commit to a complete course of therapy, as opposed to the open-ended commitment represented by HIV (for which treatment is life-long). Ideally, locally-produced generic drugs would be purchased in large quantity and distributed through an efficient network of national treatment centers. As previously described, the political commitment for such an endeavor is already extant. Given this context, support of health economists who have extensive experience in dealing with similar issues in the HIV field should be seriously considered. The ANRS AC 27, headed by Benjamin Coriat, has been alerted about this situation.

#### ***3.4.4. Study of factors associated with HCV clearance during acute infection***

Deciphering the mechanisms of HCV clearance following infection may open the door to the development of new drugs and vaccines. Several factors may contribute to spontaneous HCV clearance following acute infection (estimated at 41% among patients with acute symptomatic hepatitis C), and these include host factors (young age, female sex, genes such as the recently evidenced IL28 B gene variation, and immune response) and viral factors (sequence diversity, co-infection with HBV). We plan to study these factors among the patients recruited at the

two fever hospitals in Cairo as part of the extension of the acute hepatitis C cohort study (ANRS 12210). We also address this issue among health care workers exposed through occupational exposure to HCV RNA positive blood (ANRS 12171; Prof Mostafa K Mohamed and Dr Arnaud Fontanet).

The projects will rely on a biobank of serum/plasma and cells obtained from patients with acute hepatitis C and followed prospectively for two years after onset of symptoms. Two ANRS-funded projects have already relied on this biobank for obtaining samples (ANRS 12135 and ANRS 12188). A third ANRS project has recently been approved for funding with the aim of studying inflammatory signatures of viral hepatitis (ANRS 12199, Prof Mona Rafik and Dr Matthew Albert). A fourth project is currently under evaluation at the EC with the aim to utilize an “-omics” approach to determine which plasma analytes and cellular immune system activation parameters correlate with viral clearance; and to identify genetic determinants of viral clearance using candidate-gene approaches and genome-wide association studies.

*ANRS 12199: inflammatory signature of viral hepatitis (Prof Mona Rafik and Dr Matthew Albert)*

Cellular immune responses are thought to play an important role in the immunopathogenesis of HCV infection. Viral clearance is associated with vigorous and maintained T cell responses that target multiple HCV epitopes during acute infection. By contrast, patients developing chronic hepatitis C appear to display weak, narrowly focused, and often dysfunctional antiviral cell-mediated immunity. Early interactions between the virus and the host immune response seem to be critical in determining outcome of the disease. In this respect, patients with acute HCV infection provide an ideal opportunity to understand the correlates for successful or failed immune response to hepatitis C virus. The aim of this study would then be to identify immunological determinants of HCV clearance or persistence in patients with acute hepatitis C. We will investigate possible predictors of spontaneous resolution as well as immunological factors responsible for viral clearance or persistence.

The primary focus of this project will be the pathogenesis of hepatitis C, but we propose to gain insight into the unique aspects of the host response to HCV by comparing it to other agents of viral hepatitis. We will conduct proteomic analysis on plasma samples taken from patients infected with each of the four types of viral hepatitis (A,B,C,E), thus offering a unique look at the core inflammatory response of viral hepatitis as well as the pathogen-specific signatures characteristic of the four viruses. In addition, we aim to extend our preliminary studies on acute HBV and HCV and conduct proteomic analysis of the entire kinetic (24 months for acute hepatitis C and six months for acute hepatitis B).

In addition to the proposed proteomic screen, we will continue our evaluation of circulating immune cells with the aim of identifying a correlation between immune activation and spontaneous clearance of HCV. In our prior studies (ANRS 12135), we evaluated the role of type I interferon and the involvement of plasmacytoid DCs (pDCs). While we found evidence of interferon production, there was no apparent role for pDCs in the pathogenesis of acute HCV. We propose to sort different cell populations (e.g. NKs, cDCs, monocytes) in order to identify the cell source of IFN $\alpha/\beta$ ; and we will continue to probe the hematopoietic system for evidence of immune activation.

*ANRS 12188: neutralising antibodies in acute hepatitis C (Prof Mohamed Abdel-Hamid and Dr François-Loïc Cosset).*

This project focuses on another aspect of immune response to HCV, i.e., the humoral immune response. It has been extensively reported in the literature that the quasispecies nature of HCV is involved in viral persistence. Quasispecies are HCV variants that by mutating dominant epitopes escape the host's immune response and thus gain selective advantage to spread in their host. Studies focusing on innate and cellular immune responses have shown that a sufficiently large HCV inoculum is able to circumvent the defenses of the host. Spontaneous HCV clearance is associated with a strong, early, broad and long lasting cellular immune response. In contrast, the role of humoral immunity at the acute stage of HCV infection remains poorly characterized. The recent development of infection assays has confirmed that sera from HCV-infected patients neutralise infection *in vitro*, that antibody-mediated neutralisation occurs during HCV infection *in vivo* and that polyclonal antibodies to HCV can be protective. Using well-defined nosocomial or single-source HCV outbreaks and using matching HCVpp in neutralisation assays, we have revealed that Nt-antibodies are induced in the early phase of infection and could play a role in controlling viral infection or viral clearance. In further studies, we have highlighted the presence of a serum component, the high-density lipoprotein (HDL), that attenuates the impact of Nt-antibodies and, more particularly, cross-Nt-antibodies during acute and chronic infection. Finally, we have shown that serum amyloid A (SAA), an acute phase apolipoprotein mainly produced by the liver immediately after infection, tissue damage or inflammation inhibits HCV entry in cell culture. Since it is produced during the acute phase of infections, it is likely that SAA will have an effect on HCV infectivity during that time period.

Thus, it is worth noting that in addition to HCV-specific antibodies, several serum factors can also affect HCV entry and infectivity and may contribute to the control of HCV infection in acutely infected patients. In order to investigate the relevance of our findings *in vivo*, here, we would like to correlate a) neutralisation, b) effects of HDL on viral infectivity using sera and HCV (quasi)sequences derived from patients at various stages of disease and c) SAA inhibition. These investigations should provide new insights into the mechanisms underlying chronicity of hepatitis C and will be addressed on samples obtained from the Egypt acute hepatitis C cohort.

#### 4. TRAINING

The project is based around two training centers in Egypt:

- in epidemiology and public health: the Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University (Prof Mostafa Mohamed).
- in virology and clinical medicine: the NHTMRI (Prof Mohamed-Abdel Hamid, University of Minia, in microbiology; and Prof Gamal Esmat, University of Cairo, in hepatology).

This specific location provides an academic environment to students, both Egyptian and French, for getting their master and doctoral degrees.

The project has input in the training programs:

- by providing research data of high quality for thesis work (eight master thesis and two doctoral thesis completed so far),
- by offering local and foreign supervision to students in master and doctoral degrees,
- by providing lectures and seminars to academic researchers and students from the Department of Community Medicine at the faculty of Medicine.
- by organizing training courses in epidemiology and biostatistics in Cairo (two-week course on “introduction to biostatistics”, “logistic regression”, and “survival analysis” using Stata given to 20 master students in July 2005, July 2007, February 2009).
- by inviting Egyptian students to Summer Courses in Europe (fourteen so far), with visit extension to French laboratories for data analysis and paper writing (ten so far).



Teaching at the Faculty of  
Medicine, Ain Shams University



Since the beginning of the project, several students have been enrolled in the program.

In Egypt:

- Master degree in Public Health and Epidemiology:
  - Dr Sherine Khalil (2004): sentinel surveillance of HCV infection through blood banks.
  - Dr Noha Sharaf (2005): estimation of the clearance rate in acute hepatitis C.
  - Dr Aya Kamal (2007): HCV incidence at the cohort site.
- Doctoral degree in Public Health:
  - Dr Naglaa Arafa (2005): factors associated with HCV transmission in a rural area of Egypt).
  - Dr Rita Arafat (2008): cost-effectiveness of chronic hepatitis C treatment in Egypt.
- Doctoral degree in Immunology:
  - Ms Hala Mansour (2009): immunological factors associated with HCV clearance during the acute phase of the infection

In France:

- Master of Science in epidemiology:
  - Dr Françoise Colombani (Bordeaux II, 2003) : epidemiology of acute hepatitis in Cairo.
  - Dr Justine Sass (Paris VI, 2004): hepatitis C and metabolic liver disease.
  - Dr Sylvia Males (Paris XI, 2005): efficacy of the combination pegylated interferon and ribavirin for the treatment of chronic hepatitis C in genotype 4-infected patients.
  - Dr Nathalie Nicolay (Paris XI, 2006): intra-familial transmission of HCV at the cohort site.
  - Dr Hendy Abdoul (Paris XI, 2007): fibrosis markers among patients treated for chronic hepatitis C.
  - Dr Nicolas Vignier (Paris XI, 2009); reliability of elastometry in evaluating liver fibrosis.
- Doctoral degree in epidemiology:
  - Ms Adela Paez (Paris VI University, on-going): risk factors for acute viral hepatitis in Cairo.
  - Dr Noha Sharaf (Paris VI University, on-going): factors associated with HCV clearance during acute hepatitis C.

Mai El Daly, working in the virology laboratory of Prof Mohamed Abdel-Hamid, has already performed several visits to virology laboratories in France to learn on molecular biology techniques and viral phylogenetic analysis. Among these laboratories are those of Valérie Thiers, François Simon, and Vincent Thibault. Sherif El Kafrawy, also from Prof Mohamed Abdel-Hamid laboratory, visited Matthew Albert's laboratory in immunology.

## 5. GRANTS

**2001: EC contract (ICA3-CT2000-30011):** village cohort study of HCV incidence and progression in the Nile Delta (500 000 euros) (included in a larger project involving five Mediterranean countries; scientific coordination: Dr Bernard Larouzé, INSERM U707)

**2001: ANRS 1211:** efficacy and safety of treatment of chronic hepatitis C (genotype 4) with pegylated interferon and ribavirin (520 000 euros) (scientific coordination: Prof Mostafa Mohamed and Dr Arnaud Fontanet). Drugs provided free of charge by Roche laboratories.

**2001: ANRS 1213:** efficacy and safety of treatment of acute hepatitis C with pegylated interferon alone (431 000 euros) (scientific coordination: Prof Mostafa Mohamed and Dr Arnaud Fontanet). Drugs provided free of charge by Roche laboratories.

**2002: ANRS 1279:** cost-effectiveness of treatment strategies for hepatitis C in Egypt (scientific coordination: Prof Mostafa Mohamed and Dr Michaël Schwarzingger) (109000 euros).

**2005: PAI (French MFA and Egyptian Ministry of Higher Education) Imhotep:** mobility grant (2005-6) (30 000 euros).

**2005 : ANRS 12107 :** Etude des modes de transmission intra-familiale du VHC en zone endémique et recherche des facteurs génétiques humains prédisposant à l'infection par le VHC » (scientific coordination: Prof Mostafa Mohamed and Dr Laurent Abel) (293 000 euros).

**2005: Wellcome Trust:** Hepatitis C infection and clearance: associations with atherosclerosis and the metabolic syndrome (scientific coordination: Prof Mostafa Mohamed and Prof Nish Chaturvedi) (£284 000).

**2005: ANRS 12122:** Acute hepatitis C: risk factors, spontaneous clearance, and response to treatment (scientific coordination: Prof Mostafa Mohamed and Dr Arnaud Fontanet) (331 000 euros).

**2006: ANRS 12135:** Immunology of acute hepatitis C (scientific coordination: Prof Mona Rafik and Dr Matthew Albert) (250 000 euros).

**2007: ANRS 12171:** HCV transmission and transient HCV infection among healthcare workers in Cairo, Egypt C (scientific coordination: Prof Mostafa Mohamed and Dr Sylvia Taylor) (320 000 euros).

**2008: ANRS 12184:** Liver fibrosis evaluation among HCV genotype 4-infected patients in Egypt. Comparison of elastometry, histology and serum markers. (scientific coordination: Prof Gamal Esmat and Dr Philippe Bonnard) (200 000 euros).

**2008 : ANRS 12175:** Indicateurs de qualité et de respect des bonnes pratiques cliniques dans les essais thérapeutiques sur les sites ANRS : spécificités, pertinence, choix, définition, et validation (scientific coordination : Dr Mina Hana and Dr Brigitte Bazin).

**2008: ANRS 12188:** An investigation of serum factors that modulate HCV infectivity in an Egyptian cohort of acutely infected patients: role of quasispecies evolution, neutralising antibodies, serum amyloid A and lipoproteins. (scientific coordination: Prof Mohamed Abdel-Hamid and Dr François-Loïc Cosset) (200 000 euros).

**2009: ANRS 12199:** The inflammatory signature of viral hepatitis (scientific coordination: Prof Mona Rafik and Dr Matthew Albert) (308 000 euros).

**2009: ANRS 12210:** Symptomatic acute hepatitis C in Egypt (scientific coordination: Prof Mostafa K Mohamed and Dr Arnaud Fontanet) (765 000 euros).

**2009: ANRS 12215:** Cost-effectiveness of different antiviral treatment strategies in HCV-infected patients in Egypt: defining best starting and stopping criteria (scientific coordination: Prof Mostafa K Mohamed and Prof Yazdan Yazdanpanah) (150 000 euros).

**2009: ANRS 12216** (“contrat d’initiation”): Preclinical study for the evaluation of chemokine antagonism in chronic hepatitis C genotype 4 infection (scientific coordination: Prof Mona Rafik and Dr Matthew Albert) (15 000 euros).

**2010: ANRS 12XXX:** Efficacy and safety of the combination of nitazoxanide (NTZ) with pegylated interferon alpha 2a (PEG-IFN) and ribavirin (RBV) in Egyptian patients with untreated chronic hepatitis C genotype 4. A phase III randomized, double-blind, clinical trial (scientific coordination: Prof Gamal Esmat and Dr Arnaud Fontanet).

**Submitted:**

**EC: FP7-HEALTH-2010-single-stage:** Spontaneous clearance in Patients acutely infected with HCV: Immune profiling, Novel biomarkers and X-omics approaches (scientific coordination: Dr Matthew Albert).

## **6. SCIENTIFIC PUBLICATIONS IN INTERNATIONAL PEER-REVIEWED JOURNALS (SEE ABSTRACTS IN ANNEX 1).**

1. Mostafa A, Saeed M, Abubakr, Fontanet A, Godsland I, Coady E, El-Hoseiny M, Abdel-Hamid M, Hughes A, Mohamed MK, Chaturvedi N. Hepatitis C infection and clearance: impact on atherosclerosis and the metabolic syndrome (submitted).
2. Decalf J, Mansour H, Laird M, Lefouler L, El-Daly M, Sharaf Eldin N, Mapes J, Rafik M, Abdel Hamid M, Mohamed MK, Fontanet A, Albert ML. The inflammatory signature of acute HCV genotype 4 infection and identification of biomarkers of spontaneous viral clearance (submitted).
3. Mansour H, Laird ME, Saleh R, Sharaf Eldin N, El Kafrawy S, Hamdy M, Decalf J, Rosenberg BR, Fontanet A, Abdel-Hamid M, Mohamed MK, Albert ML and Rafik M. Circulating plasmacytoid dendritic cells in acutely infected patients with hepatitis C virus genotype 4 are normal in number and phenotype (submitted).
4. Paez Jimenez A, Sharaf Eldin N, Rimlinger F, El-Daly M, El-Hariri H, El-Hoseiny M, Mohsen A, Abdel-Hamid M, Saeed N, Delarocque-Astagneau E, Fontanet A, Mohamed MK, Thiers V HCV intrafamilial transmission in Greater Cairo, Egypt (submitted).
5. Schwarzinger M, Raafat R, Dewedar S, Fontanet A, Carrat F, Luchini S, Mohamed MK. Hepatitis C virus and major environmental risks: risk perception and priority setting of the Cairo community (submitted).
6. Mostafa A, Taylor SM, El-Daly M, El-Hoseiny M, Bakr I, Arafa N, Thiers V, Rimlinger F, Abdel-Hamid M, Fontanet A, Mohamed MK Is the Hepatitis C Virus epidemic over in Egypt? : Incidence and risk factors of new HCV infections. *Liver International*, in press.
7. Jimenez AP, Mohamed MK, Eldin NS, Seif HA, El Aidi S, Sultan Y, Elsaid N, Rekacewicz C, El-Hoseiny M, El-Daly M, Abdel-Hamid M, Fontanet A. Injection drug use is a risk factor for HCV infection in urban Egypt. *PLoS One*. 2009 Sep 28;4(9):e7193.
8. Laouénan C, Plancoulaine S, Mohamed MK, Arafa N, Bakr I, Abdel-Hamid M, Rekacewicz C, Obach D, Fontanet A, Abel L. Evidence for a dominant major gene conferring predisposition to hepatitis C virus infection in endemic conditions. *Hum Genet*. 2009 Jul 23. [Epub ahead of print]
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## **7. ETHICAL COMMITTEES.**

The two ethics committees of the institutions related to the project have recently received international accreditation:

- Ethics committee of Ain Shams University (President: Dr Adel M Gad; FWA assurance number: FWA00002980).
- Ethics Committee of the National Hepatology and Tropical Medicine Research Institute (Accreditation number: IORG0003280).

In addition, all clinical trials need approval from the Institutional Review Board of the Ministry of Health and Population.

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# Injection Drug Use Is a Risk Factor for HCV Infection in Urban Egypt

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## Abstract

**Objective:** To identify current risk factors for hepatitis C virus (HCV) transmission in Greater Cairo.

**Design and Setting:** A 1:1 matched case-control study was conducted comparing incident acute symptomatic hepatitis C patients in two "fever" hospitals of Greater Cairo with two control groups: household members of the cases and acute hepatitis A patients diagnosed at the same hospitals. Controls were matched on the same age and sex to cases and were all anti-HCV antibody negative. Iatrogenic, community and household exposures to HCV in the one to six months before symptoms onset for cases, and date of interview for controls, were exhaustively assessed.

**Results:** From 2002 to 2007, 94 definite acute symptomatic HCV cases and 188 controls were enrolled in the study. In multivariate analysis, intravenous injections (OR=5.0; 95% CI=1.2–20.2), medical stitches (OR=4.2; 95% CI=1.6–11.3), injection drug use (IDU) (OR=7.9; 95% CI=1.4–43.5), recent marriage (OR=3.3; 95% CI=1.1–9.9) and illiteracy (OR=3.9; 95% CI=1.8–8.5) were independently associated with an increased HCV risk.

**Conclusion:** In urban Cairo, invasive health care procedures remain a source of HCV transmission and IDU is an emerging risk factor. Strict application of standard precautions during health care is a priority. Implementation of comprehensive infection prevention programs for IDU should be considered.

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## Introduction

The highest HCV prevalence in the world occurs in Egypt at an estimated 12% [1], i.e. 10 to 20 fold higher than in Northern Europe [2] or in the United States [3]. The bulk of chronic infection is age-related [4] and occurs among persons of rural origin. Cohort studies have estimated a 9% prevalence and 0.8/1000 person-years incidence in Upper Egypt, and a 24% prevalence and 6.8/1000 incidence in the Nile Delta [5,6].

The widespread schistosomiasis treatment campaigns with intravenous tartar emetic, carried out in the countryside in the 60's- early 80's, ignited this epidemic through reuse of insufficiently sterilised needles and syringes [7]. Since then, cross-sectional studies have shown unsafe injection practices, history of blood transfusion, invasive medical procedures, and instrument-assisted birth deliveries as associated with HCV infection [8–10]. Intra-familial transmission may also have played an important role, as evidenced in two recent cohort studies [6,11].

However, HCV transmission has been studied almost exclusively in rural areas, with only two uncontrolled studies reporting on urban hepatitis C patients [12,13]. After last decades of large rural exodus leading to the suburbs of Cairo, 45% of the Egyptian population is urban (Source CAPMAS, 2000). In order to identify current risk factors for HCV infection in urban Egypt, we have conducted a case-control study recruiting incident HCV case patients, i.e. recently acquired infections, in two hospitals serving Greater Cairo (Cairo and its suburbs).

## Methods

### Participants' recruitment and questionnaire

From April 2002 to June 2007, a 1:1 matched case-control study with two control groups was conducted. Incident acute symptomatic hepatitis C patients were enrolled as cases either (i) before seroconversion, with negative anti-HCV antibody and positive HCV RNA laboratory results or (ii) with rapid seroconversion: positive anti-HCV antibody and positive HCV RNA associated

## Evidence for a dominant major gene conferring predisposition to hepatitis C virus infection in endemic conditions

Cédric Laouénan · Sabine Plancoullaine · Mostafa Kamal Mohamed · Naglaa Arafa · Iman Bakr · Mohamed Abdel-Hamid · Claire Rekacewicz · Dorothée Obach · Arnaud Fontanet · Laurent Abel

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**Abstract** Hepatitis C virus (HCV), infecting 170 million people worldwide, is a major public health problem. In developing countries, unsafe injections and blood transfusions are thought to be the major routes of transmission. However, our previous work in a population from Egypt, endemic for HCV, revealed highly significant familial correlations, strongly suggesting the existence of both familial transmission of the virus and genetic predisposition to HCV infection. We investigated the hypothesis of genetic predisposition by carrying out a segregation analysis of HCV infection in the same population. We used a logistic regression model simultaneously taking into

account a major gene effect, familial correlations and relevant risk factors. We analyzed 312 pedigrees (3,703 subjects). Overall HCV seroprevalence was 11.8% and increased with age. The main associated risk factors were previous parenteral treatment for schistosomiasis and blood transfusions. We found strong evidence for a dominant major gene conferring a predisposition to HCV infection. The frequency of the predisposing allele was 0.013, reflecting a strong predisposition to HCV infection in 2.6% of the subjects, particularly those under the age of 20. This study provides evidence for the involvement of host genetic factors in susceptibility/resistance to HCV infection in endemic conditions.

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### Introduction

Hepatitis C virus (HCV) infects 170 million people worldwide and is thus a major public health problem (Lauer and Walker 2001; Thomson and Finch 2005; WHO 1999). In developing countries, blood transfusions and unsafe therapeutic injections are thought to be the major routes of transmission (Shepard et al. 2005). Unsafe injections are thought to have been the origin of the epidemic in Egypt, which has the highest prevalence of HCV infection of any country in the world, with an estimated 8 million infected inhabitants in 1999 (Egyptian Ministry of Health Annual Report 2007), and a seroprevalence ranging from 10% in children to 45% in adults (Frank et al. 2000). The origins of this HCV epidemic have been attributed, more precisely, to the parenteral treatment of schistosomiasis with antimony salts between 1960 and 1982 (Frank et al. 2000). Due to the inadequate sterilization of injection equipment, the virus, which had not yet been identified, spread between treated individuals. The

## Response to Pegylated Interferon Alfa-2a and Ribavirin in Chronic Hepatitis C Genotype 4

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The safety and efficacy of pegylated interferon (PEG-IFN) alfa-2a and ribavirin were studied among patients treated for genotype 4 chronic hepatitis C. Ninety-five patients with chronic hepatitis C genotype 4 were treated with PEG-IFN alfa-2a (180 µg/week) plus ribavirin (≥11 mg/kg/day) for 48 weeks. The primary end point was sustained virological response, defined as non-detectable levels of HCV RNA at the end of follow up (week 72). The proportion with sustained virological response was 58/95 = 61.1% (95% CI = 50.5–70.9%). Side effects were generally mild, well managed by dose reductions (in 62% of patients); in only two patients were side effects sufficiently severe to require treatment interruption. Ninety percent of patients adhered to treatment up to week 12, and their sustained virological response rate was higher compared to non-adherent (65% vs. 22%, respectively,  $P=0.012$ ). None of the patients who failed to achieve 1 log reduction of viral load by week 8 ( $n=15$ ), or 2 log reduction by week 12 ( $n=17$ ), had a sustained virological response. In conclusion, sustained virological response in genotype 4 Egyptian patients treated with PEG-IFN alfa-2a and ribavirin was estimated around 60%, intermediate between sustained virological response observed in genotype 1 and genotype 2–3 patients in Western countries. The early virological response (week 4 or week 8) should be investigated as a criterion to decide whether the patient may benefit from a shorter duration of therapy. **J. Med. Virol.** 81:1576–1583, 2009. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** chronic hepatitis C; genotype 4; pegylated interferon; sustained virological response; treatment response predictors

### INTRODUCTION

Hepatitis C virus (HCV) affects 3% of the world population (170 million people) and is a major cause of chronic liver disease and liver transplantation [WHO, 1997; Lauer and Walker, 2001]. Marked geographical variations in the prevalence of HCV exist. Egypt has an exceptionally high prevalence of HCV antibodies in the general adult population (15–20%), which has been attributed to the mass parenteral antischistosomal therapy used in the 1960s and 1970s [Frank et al., 2000]. HCV has six major genotypes and over 76 subtypes described according to variations in the nucleotide sequence of the virus [Simmonds, 1999; Hoofnagle, 2002; Simmonds et al., 2005]. Genotype 4 is the predominant genotype in Egypt, and has been isolated

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# Community transmission of hepatitis B virus in Egypt: results from a case–control study in Greater Cairo

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**Background** To identify current risk factors for hepatitis B virus (HBV) transmission in Greater Cairo.

**Methods** A 1:1 matched case–control study was conducted in two ‘fever’ hospitals in Cairo. Acute hepatitis B cases were patients with acute hepatitis, positive HBs antigen, and high anti-HBc IgM titres. Control subjects were acute hepatitis A patients (positive anti-HAV IgM) or relatives of patients diagnosed with acute hepatitis C, identified at the same hospitals, with no past HBV infection (negative anti-HBc) and matched to cases on the same age and sex. Conditional logistic regression was used to identify factors associated with acute hepatitis B.

**Results** Between April 2002 and June 2006, 233 cases and 233 controls were recruited to the study. In multivariate analysis, factors associated with an increased HBV risk in males were illiteracy [odds ratio (OR)=6.1, 95% confidence interval (CI)=2.8–13.1], shaving at barbers (OR=2.1, 95% CI=1.1–3.9) and injecting drug use (IDU) (OR=3.4, 95% CI=1.0–11.4). In females, factors associated with an increased HBV risk were illiteracy (OR=2.2, 95% CI=1.0–5.0), recent (<1 year) marriage (OR=42.0, 95% CI=3.8–463.9 compared with single women) and giving birth (OR=3.7, 95% CI=1.0–13.9).

**Conclusion** In this study, HBV transmission took place primarily in the community, whether as a result of recent marriage (presumably first sexual intercourse), shaving at barbershops or IDU, and was more common among illiterates. Health promotion campaigns should be carried out to increase awareness about community transmission of HBV. In addition to routine immunization for infants and other populations, premarital screening might be useful to identify at-risk spouses in order to propose targeted immunization.

**Keywords** Acute hepatitis, risk factors, hepatitis B infection, epidemiology, Egypt

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# Symptomatic Acute Hepatitis C in Egypt: Diagnosis, Spontaneous Viral Clearance, and Delayed Treatment with 12 Weeks of Pegylated Interferon Alfa-2a

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## Abstract

**Background and Objectives:** The aim of this study was to estimate the proportion of spontaneous viral clearance (SVC) after symptomatic acute hepatitis C and to evaluate the efficacy of 12 weeks of pegylated interferon alfa-2a in patients who did not clear the virus spontaneously.

**Methods:** Patients with symptomatic acute hepatitis C were recruited from two "fever hospitals" in Cairo, Egypt. Patients still viremic three months after the onset of symptoms were considered for treatment with 12 weeks of pegylated interferon alfa-2a (180 µg/week).

**Results:** Between May 2002 and February 2006, 2243 adult patients with acute hepatitis were enrolled in the study. The SVC rate among 117 patients with acute hepatitis C was 33.8% (95%CI [25.9%–43.2%]) at three months and 41.5% (95%CI [33.0%–51.2%]) at six months. The sustained virological response (SVR) rate among the 17 patients who started treatment 4–6 months after onset of symptoms was 15/17 = 88.2% (95%CI [63.6%–98.5%]).

**Conclusion:** Spontaneous viral clearance was high (41.5% six months after the onset of symptoms) in this population with symptomatic acute hepatitis C. Allowing time for spontaneous clearance should be considered before treatment is initiated for symptomatic acute hepatitis C.

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**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Management of acute hepatitis C is a complex issue. While studies have shown that treatment during the acute phase can achieve high (72%–98%) success rates [1–9], the optimal regimen and timing of treatment are still a matter of debate [10–13]. One crucial issue that remains to be resolved is whether physicians should treat all patients diagnosed with acute hepatitis C, or should wait and treat only those who failed to clear the virus in the first few months after infection. Among recently published studies (2006–7) [5–9], the trend has been to treat early (i.e., within three months of infection or one month of onset of symptoms), and with simplified regimens (12 or 24 weeks of monotherapy with pegylated interferon). In these studies, intravenous drug use and occupational exposure were the main risk factors for infection, and the majority of patients were a- or pauci-symptomatic, except for the German HEP-NET study, where

recruitment was more diverse and patients with jaundice represented 62% of all cases [6].

In Egypt, the epidemiological situation differs from that of Western countries. HCV prevalence is very high (estimated among adults at 10 and 20% in urban and rural areas respectively) [14]. The origin of the epidemic has been attributed to mass campaigns of parenteral anti-schistosomiasis treatment in rural areas in the 1960s–70s. Since the virus has continued to spread, mainly through intravenous injections and other medical procedures [15], acute hepatitis C is commonly diagnosed among patients presenting with jaundice [16].

Among these patients, waiting for spontaneous clearance and treating only those still viremic three months after the onset of symptoms makes sense for two reasons: the higher rate of spontaneous clearance expected from patients with symptomatic compared to asymptomatic forms of acute hepatitis C (estimated at

## Dissection of familial correlations in hepatitis C virus (HCV) seroprevalence suggests intrafamilial viral transmission and genetic predisposition to infection

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► Supplementary information concerning the epidemiological factors investigated is published online only at <http://gut.bmj.com/content/vol57/issue9>

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### ABSTRACT

**Objective:** Unsafe injections and transfusions used during treatments are considered to be responsible for many cases of transmission of hepatitis C virus (HCV) in developing countries, but cannot account for a substantial proportion of present infections. The aim of the present work was to investigate familial clustering of HCV infection in a population living in a highly endemic area.

**Design, setting and participants:** A large seroepidemiological survey was conducted on 3994 subjects (age range, 2–88 years) from 475 familial clusters in an Egyptian rural area. Epidemiological methods appropriate for the analysis of correlated data were used to estimate risk factors and familial dependences for HCV infection. A phylogenetic analysis was conducted to investigate HCV strain similarities within and among families.

**Main outcome measures:** HCV familial correlations adjusted for known risk factors, similarities between viral strains.

**Results:** Overall HCV seroprevalence was 12.3%, increasing with age. After adjustment for relevant risk factors, highly significant intrafamilial resemblances in HCV seroprevalence were obtained between father–offspring (odds ratio (OR) = 3.4 (95% confidence interval (CI), 1.8 to 6.2)), mother–offspring (OR = 3.8 (95% CI, 2.5 to 5.8)), and sibling–sibling (OR = 9.3 (95% CI, 4.9 to 17.6)), while a weaker dependence between spouses (OR = 2.2 (95% CI, 1.3 to 3.7)) was observed.

Phylogenetic analysis showed greater HCV strain similarity between family members than between unrelated subjects, indicating that correlations can be explained, in part, by familial sources of virus transmission. In addition, refined dissection of correlations between first-degree relatives supported the role of host genes predisposing to HCV infection.

**Conclusions:** Current HCV infection in endemic countries has a strong familial component explained, at least partly, by specific modes of intrafamilial viral transmission and by genetic predisposition to infection.

Hepatitis C virus (HCV) infection is a major public health problem worldwide.<sup>1–3</sup> In developing countries, blood transfusions and unsafe injections used during treatments are thought to be the major routes of transmission.<sup>1,4</sup> However, recent studies in highly endemic areas have shown that a substantial proportion of HCV infections, particularly in children, cannot be accounted for by iatrogenic factors, strongly suggesting the involvement of other modes of transmission.<sup>5</sup> Perinatal mother-to-child HCV transmission remains limited, accounting for less than 5% of paediatric cases

in the absence of HIV co-infection in the mother.<sup>6</sup> While sexual transmission of HCV has been suggested,<sup>4,7,8</sup> it is much less efficient for HCV than for other sexually transmitted viruses.<sup>4</sup> Finally, several studies have reported that HCV infection may cluster in families or households, based on the higher prevalence of HCV infection among family members of infected cases (mainly patients with chronic liver diseases, haemophilia, or on haemodialysis) than in controls.<sup>9–14</sup> However, none of these studies investigated specific familial correlations (eg, parent–offspring, sibling–sibling (sib–sib)) in the context of a global familial study, as previously carried out for other viral infections, such as human herpes virus-8 (HHV-8),<sup>15</sup> to demonstrate intrafamilial resemblance.

Egypt has the highest prevalence of HCV infection of any country in the world, with an estimated 8 million infected inhabitants in 1999.<sup>16</sup> In rural areas, HCV prevalence ranges from 10% in children to 45% in adults. The origins of this HCV epidemic have been attributed to the parenteral treatment of schistosomiasis by antimony salts between 1960 and 1982.<sup>16</sup> The introduction of oral praziquantel for the treatment of schistosomiasis in 1982 halted this mode of transmission, and the current prevalence of *Schistosoma mansoni* infection in our Egyptian cohort is extremely low (2.4%).<sup>17</sup> A recent epidemiological study in this cohort showed that although the parenteral treatment of schistosomiasis may have been at the origin of the HCV epidemic in this population, this and other iatrogenic factors account for only half of all current infections in adults.<sup>5</sup> Moreover, no iatrogenic factor was identified for infected individuals under the age of 20 years, for whom HCV seroprevalence was 3%. In the present work, we searched for other mechanisms of HCV infection. To do so, we dissected the patterns of intrafamilial resemblance of HCV infection in a cohort of 4000 subjects living in a highly endemic area, using both epidemiological and phylogenetic analyses. Taking advantage of the high HCV seroprevalence in this population, we also investigated familial resemblance in chronic HCV infection.

### MATERIALS AND METHODS

#### Study population

We carried out a large epidemiological study between May and November 2002 in Zawiat Razin, a village of around 20 000 inhabitants, about 85 km northwest of Cairo in the Menoufia Governorate (Nile Delta). The practical details of

## CLINICAL STUDIES

**Predictors of a sustained virological response in patients with genotype 4 chronic hepatitis C**

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**Keywords**

adherence to treatment –  $\alpha$ -fetoprotein – chronic hepatitis C – pegylated interferon – sustained virological response – treatment response predictors

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**Abstract**

**Objectives:** To determine the clinical, biological, virological and histological predictive factors associated with a sustained virological response (SVR) to combined interferon therapy among Egyptian patients infected by genotype 4 hepatitis C virus (HCV). **Patients and Methods:** Individual data from 250 patients with genotype 4 chronic hepatitis C, treated with different regimens of combined interferon, were analysed. The primary end point was SVR defined as undetectable HCV RNA by polymerase chain reaction (PCR) 24 weeks after the end of treatment. Multivariate logistic regression analysis was performed to select the independent prognostic parameters associated with SVR. **Results:** A sustained virological response was achieved among 137/250 (54.8%) patients. Baseline factors independently and negatively associated with SVR were serum  $\alpha$ -fetoprotein (AFP) level (above 0.3 upper limit of normal) [odds ratio (OR) = 0.5, 95% confidence interval (CI): 0.2–0.8], severe fibrosis (Metavir score > F2) (OR = 0.4, 95% CI: 0.2–0.8), presence of steatosis (OR = 0.5, 95% CI: 0.3–0.97) and standard interferon treatment (OR = 0.4, 95% CI: 0.2–0.8). **Conclusions:** Among genotype 4 chronic hepatitis C patients, severe fibrosis, severe steatosis, treatment with standard interferon and a high serum AFP level were all negatively associated with SVR. Pretreatment serum AFP level should be considered in the routine assessment of factors predictive of a treatment response.

The hepatitis C virus (HCV) infects about 170 million people worldwide and is responsible for approximately 20% of acute hepatitis and 70% of chronic hepatitis cases (1). The morbidity and mortality related to chronic hepatitis C is a major public health issue in Egypt, where HCV prevalence nationwide is estimated to be around 12%. Interestingly, genotype 4 represents over 90% of the cases in Egypt (2). Interferon  $\alpha$ /ribavirin combination therapy is the standard treatment for patients with chronic hepatitis C. Estimates of sustained virological response (SVR) range from 40% among patients infected by HCV genotype 1 to 80% among patients infected by HCV genotype 2–3 (3). Less data are available for patients infected with

genotype 4. Recent studies suggest that the SVR for patients infected with genotype 4 is around 60% (4, 5).

Various factors have been associated with response to treatment of chronic hepatitis C including viral factors (including pretreatment viral load and viral genotype), individual patient characteristics [including age, gender, race, alanine aminotransferase (ALT) levels,  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT) levels, liver fibrosis and steatosis, HLA alleles and body mass index (BMI)] and interferon regimen (including type, dose, frequency and duration of treatment and combination of interferon with other anti-HCV agents) (6–8). Because treatment response varies significantly by viral genotype, it is plausible that predictors of SVR differ

# Serum $\alpha$ -foetoprotein level predicts treatment outcome in chronic hepatitis C

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**Objectives:** To analyse the association between serum  $\alpha$ -foetoprotein (AFP) levels and sustained virological response (SVR) in treated patients.

**Methods:** One-hundred patients with chronic hepatitis C were treated with pegylated interferon  $\alpha$ -2a plus ribavirin for 48 weeks. The primary endpoint was SVR. Linear regression analysis was performed to identify clinical, biological, and histological factors affecting baseline AFP levels. The association between pretreatment serum AFP and SVR was assessed by multivariate logistic regression analysis.

**Results:** Of 100 patients, 95 were infected with genotype 4, one with genotype 1, and four with undetermined genotype. The median serum AFP level was 4.5 ng/ml and AFP values ranged from 1.2 to 49.8 ng/ml. In multivariate analysis, higher fibrosis stage and higher steatosis score were independently associated with higher serum AFP

levels. SVR rate was 61.0% (61/100), and was lower for patients with AFP levels above rather than below the median value (40.8% versus 80.4%, respectively,  $P<0.001$ ). In multivariate analysis, including adjustment for age, gender, body mass index, steatosis score, fibrosis stage, ALT level, haemoglobin level, clotting time, HCV RNA viral load, and treatment dose received, a baseline serum AFP level above the median value was associated with a lower SVR rate (OR [95% CI]=0.10 [0.03–0.42],  $P<0.001$ ). None of the seven patients with increased (above 15 ng/ml) pretreatment AFP achieved SVR.

**Conclusions:** In this study, higher baseline serum AFP levels independently predicted a lower SVR rate among patients with chronic hepatitis C. If confirmed with genotypes other than 4, these findings would suggest adding serum AFP to the list of factors predictive of treatment response.

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## Introduction

Serum  $\alpha$ -foetoprotein (AFP) is a foetal glycoprotein produced by the yolk sac and foetal liver [1]. Following birth, AFP levels decrease rapidly to less than 20 ng/ml and increase significantly in certain pathological conditions. Serum AFP is a debated, but routinely used, marker for hepatocellular carcinoma (HCC) in patients with chronic liver disease [2–5]. Yet, significant elevations of AFP are commonly seen in non-hepatic malignancies and benign conditions, such as acute and chronic viral hepatitis [6–13]. Studies reported that the prevalence of increased serum AFP varies from 10% to 43% in patients with chronic hepatitis C [9–13] and

suggested an association between increased serum AFP and advanced fibrosis or cirrhosis [11,12]. No study has yet, to our knowledge, looked at the association between serum AFP and response to treatment during chronic hepatitis C.

The aims of this study were to determine the clinical, biological, virological and histological factors associated with AFP levels among Egyptian patients infected by hepatitis C virus (HCV) genotype 4 and to analyse the association between serum AFP and sustained virological response (SVR) to pegylated interferon plus ribavirin therapy.

## PAPER

# Metabolic and cardiovascular risk profiles and hepatitis C virus infection in rural Egypt

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**Background and aim:** To investigate the relationship between lipid profiles and diabetes with past and chronic hepatitis C virus (HCV) infection among village residents of Egypt.

**Patients and methods:** Fasting lipids and glucose profiles were compared among adults never infected with HCV (negative HCV antibodies), infected in the past (positive HCV antibodies and negative HCV RNA) and chronically infected (positive HCV antibodies and HCV RNA).

**Results:** Of the 765 participants, 456 (59.6%) were female, and median age was 40 (range 25-88) years. Chronic HCV infection was present in 113 (14.8%) and past infection in 67 (8.8%). After adjustment for age and sex, participants with chronic HCV infection had lower plasma low density lipoproteins (LDL) cholesterol and triglyceride levels compared with those never infected (age and sex adjusted differences (95% CI) were -19.0 (-26.3 to -11.7) mg/dl and -26.2 (-39.0 to -13.3) mg/dl, respectively). In contrast, participants with cleared HCV infection had higher triglyceride levels compared with those never infected (age and sex adjusted difference (95% CI) was +16.0 (0.03 to 31.9) mg/dl). In multivariate analysis, participants with chronic HCV infection were more likely to have diabetes (OR 3.05, 95% CI 1.19 to 7.81) compared with those never infected, independent of LDL cholesterol levels.

**Conclusion:** In conclusion, this community based study has shown that in a single population, chronic HCV infection is associated with glucose intolerance and, despite that, a favourable lipid pattern. An intriguing finding was the high triglyceride levels observed among participants with past infection, suggesting that elevated triglycerides at the time of acute infection may facilitate viral clearance.

Infection with hepatitis C virus (HCV) has been associated with alterations in lipid metabolism in some studies<sup>1-3</sup> and type 2 diabetes in others.<sup>4-8</sup> Lipid changes are characterised by hypobetalipoproteinaemia, and may be more common among patients infected with HCV genotype 3 who develop liver steatosis.<sup>1-3</sup> Type 2 diabetes was initially documented among patients with HCV related cirrhosis,<sup>4</sup> although subsequent studies have demonstrated its occurrence at all stages of HCV infection.<sup>5-8</sup> This combination of favourable lipids and diabetes is unusual, as the conventional metabolic syndrome, a constellation of risk factors for atherosclerosis, includes, among others, an atherogenic lipid profile, glucose intolerance and insulin resistance.<sup>9</sup> Whether the protective effect of hypobetalipoproteinaemia will counterbalance the effect of diabetes in the pathogenesis of atherosclerosis among HCV infected individuals is not known.

Egypt has the highest HCV prevalence in the world (overall prevalence of HCV antibody is 12% among the general population and reaches 40% in persons 40 years of age and above in rural areas).<sup>10-12</sup> The origin of the HCV epidemic in Egypt has been attributed to intravenous schistosomiasis treatment in rural areas in the 1960s-70s.<sup>13</sup> As treatment was targeted at children and young adults, those infected at that time are now 40-65 years old and will be at risk of cardiovascular disease. We therefore investigated the association between HCV infection and atherosclerosis risk factors in one rural area of Egypt subjected to schistosomiasis treatment campaigns in the past.

## SUBJECTS AND METHODS

The study took place at Zwyat Razin village in the lower Nile Delta region of Egypt. Between March and November 2002, all residents over 5 years of age and living in one sector of the

village (representing 25% of the total village population) were invited to participate in a cohort study of the incidence and progression of HCV infection.<sup>14 15</sup> After informed consent was obtained (from the head of household for children less than 18 years of age), participants were administered a questionnaire on sociodemographic characteristics, clinical history and risk factors for HCV infection. The informed consent form was written in Arabic and read to participants who were illiterate. In each study team, there was a medical doctor able to provide answers to queries from study participants regarding the natural history of HCV infection and cardiovascular disease, the importance of the study and the risks associated with participation in the study (blood drawing). Questionnaires were close-ended and administered by trained interviewers. Venous blood (10 ml) was drawn and transported on the same day for centrifugation and freezing of serum (-70°C) at the National Hepatology and Tropical Medicine Research Institute (NHTMRI) in Cairo. Serological status was determined according to an algorithm validated locally on Egyptian sera<sup>16</sup>: sera were first tested for HCV antibodies using Innotech HCV Ab IV (Innogenetics, Ghent, Belgium) (lower 95% CI of specificity reported at 98.1% during the evaluation of hepatitis C assays by the Blood Safety and Clinical Technology of the World Health Organisation<sup>17</sup>), samples positive for HCV antibodies were tested again using Abbott HCV EIA 3.0 (Abbott Laboratories, Diagnostics Division, Illinois, USA) and those testing positive by the two serological tests were considered positive for HCV antibodies. Samples with discordant results were considered to

**Abbreviations:** BMI, body mass index; EIA, enzyme immunoassay; HCV, hepatitis C virus; HDL, high density lipoprotein; LDL, low density lipoprotein; NHTMRI, National Hepatology and Tropical Medicine Research Institute; VLDL, very low density lipoprotein; WHR, waist hip ratio

## HCV-Related Morbidity in a Rural Community of Egypt

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The origin of the hepatitis C virus (HCV) epidemic in Egypt has been attributed to intravenous schistosomiasis treatment in rural areas in the 1960s to 70s. The objective of this study was to estimate the HCV-related morbidity in a rural area where mass schistosomiasis treatment campaigns took place 20–40 years before. The study sample included 2,425 village residents aged 18–65 years recruited through home-based visits. Overall, HCV antibody prevalence was 448/2,425 = 18.5% (95% CI = 16.9–20.1%), reaching 45% in males over 40 years, and 30% in females over 50 years. Of those with HCV antibodies, 284/448 (63.4%, 95% CI = 58.7–67.9%) had chronic HCV infection, among which 107/266 (40.2%, 95% CI = 34.3–46.4%) had elevated alanine aminotransferase (ALT). As part of pre-treatment screening, 26 consenting patients had a liver biopsy: 13 (50.0%) had a treatment indication. Thus, of all patients with HCV antibodies, 13 (2.9%) were eligible for treatment and willing to be treated. The relatively low level of morbidity observed in this study is discussed in view of co-factors of HCV infection progression, such as young age at infection, absence of alcohol intake, the prevalence of *Schistosoma mansoni* infection, and the prevalence of chronic hepatitis B. **J. Med. Virol.** 78:1185–1189, 2006.

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**KEY WORDS:** hepatitis C; natural history; epidemiology

### INTRODUCTION

Hepatitis C virus (HCV) antibody prevalence in Egypt was estimated around 12% nationwide in 1999

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(Egyptian Ministry of Health and Population, 1999). The origin of the epidemic has been attributed to mass campaigns of intravenous schistosomiasis treatment in rural areas in the 1960s to 70s [Frank et al., 2000]. Treatment consisted of several (up to 12) intravenous injections of tartar emetic administered in weekly doses to anyone living in schistosomiasis-endemic areas, and particularly children and young boys treated while at school. Evidence suggests that HCV transmission occurred due to insufficient sterilization of injection equipment between patients. This mode of HCV transmission was cut after 1982 when oral praziquantel was introduced for schistosomiasis treatment. In 2002, individuals infected 20–40 years previously may have begun to develop HCV-related liver disease. The objective of this study was to estimate HCV-related morbidity in a rural area of Egypt where mass campaigns for schistosomiasis treatment took place in the 1960s to 70s.

### PARTICIPANTS AND METHODS

Participants were inhabitants of Zawyat Razin, a village one hour-and-a-half driving distance north-west of Cairo in Menofia Governorate (Nile Delta). From May to November 2002, all residents older than 5 years of age

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# Higher clearance of hepatitis C virus infection in females compared with males

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**Background and aims:** According to the literature, 14–46% of subjects clear hepatitis C virus (HCV) from blood after infection. Controversy exists about sex differences in HCV clearance rates.

**Patients and methods:** We compared HCV clearance in males and females using data from a large population based study on HCV infection in Egypt. Definitions used in the paper were: cleared HCV infection (positive HCV antibody and negative HCV RNA test results) and chronic HCV infection (positive HCV antibody and positive HCV RNA test results). The study sample included 4720 village residents aged 18–65 years recruited through home based visits (n=2425) or voluntary screening (n=2295).

**Results:** Overall, HCV antibody prevalence was 910/4720 (19.3% (95% confidence interval 18.2–20.4)). Of those with HCV antibodies (n=910), 61.5% had chronic HCV infection. Compared with males, females were more likely to have cleared the virus (44.6% v 33.7%, respectively; p=0.001). Control for age, schistosomiasis history, iatrogenic exposures, and sexual exposure to HCV did not alter the positive association between female sex and viral clearance.

**Conclusion:** This study provides strong evidence in favour of a higher HCV clearance rate in females compared with males.

Progression of hepatitis C virus (HCV) infection is known to be worse in males than in females.<sup>1,2</sup> Independent of alcohol intake, males have a twofold greater progression rate to fibrosis compared with females.<sup>3</sup> Less is known about sex differences in HCV clearance rates, defined in cross sectional surveys as the proportion of non-viraemic subjects among those with HCV antibodies. Viral clearance rates vary from 14% to 46% according to the literature.<sup>1</sup> Two studies showing the highest clearance rates (45%) were of Irish and German women who received HCV contaminated Rh immune globulin in late pregnancy or early post partum.<sup>4,5</sup> Based on the results of these two studies, it is commonly believed that women clear HCV well, and most likely better than males. However, the studies had no male comparison group, included women who were pregnant or in early post partum when infected with HCV (unknown influence on HCV clearance rate), and women who were young when infected with HCV (known factor of higher clearance rate<sup>6,7</sup>).

Moreover, three of the largest population based studies, NHANES in the USA, the Trent Study in the UK, and Dionysos in Italy, gave controversial results. In the NHANES study, a sex difference in HCV clearance was seen only in the subgroup of non-Hispanic Blacks while no sex difference was observed in the other groups (non-Hispanic Whites and Mexican Americans).<sup>8</sup> In the Trent and Dionysos studies, no sex difference in HCV clearance was seen.<sup>9–11</sup> We took the opportunity of using data from a large population based study of HCV infection in a village in Egypt to compare viral clearance rates between males and females.

## SUBJECTS AND METHODS

Participants were inhabitants of the village Zawyat Razin, a 90 minute drive north west of Cairo in Menofia Governorate (Nile Delta). All residents older than five years of age and living in one sector of the village (25% of the village population, estimated at approximately 20 000) were invited to participate in the study.<sup>12</sup> Invitations took place during home visits, which were repeated outside of working hours

when adult residents were not found at the initial visit. In addition, all village residents over 18 years of age and living outside of the study sector who were willing to be tested for HCV antibodies were invited to participate through a voluntary screening programme. After informed consent was obtained (from the head of household for children less than 18 years of age), participants were given a questionnaire on sociodemographic characteristics, clinical history, and risk factors for HCV infection. Participants were asked to provide blood and stool samples. Stool samples were examined at a local laboratory for *S mansoni* eggs using the Kato test. Blood samples were transported the same day for centrifugation and freezing of serum (–80°C) at the National Hepatology and Tropical Medicine Reference Institute in Cairo.

Sera were tested for HCV antibodies using Innostest HCV Ab IV (Innogenetics, Ghent, Belgium) (lower 95% confidence limit of specificity was reported as 98.1% during evaluation of hepatitis C assays by the Blood Safety and Clinical Technology of the World Health Organisation).<sup>13</sup> Samples positive for HCV antibodies were tested again using Abbott HCV EIA 3.0 (Abbott Laboratories, Diagnostics Division, Illinois, USA), and those testing positive by the two serological tests were considered positive for HCV antibodies.<sup>14</sup> Samples with discordant results (n=35) were considered to be negative. All HCV antibody positive samples were tested for HCV-RNA using a one step reverse transcriptase-polymerase chain reaction in house assay, using 5'UTR primers with modifications,<sup>15</sup> and for serum alanine aminotransferase (ALT). Genotyping was performed by sequencing the NS5B and E1 regions of 135 HCV-RNA positive sera included in a substudy of intrafamilial transmission of HCV. Participants with positive HCV-RNA and elevated ALT were invited to attend the study clinic at Ismail Sallam Hospital for an eligibility screening for treatment with pegylated interferon and ribavirin. The study

**Abbreviations:** HCV, hepatitis C virus; ALT, alanine aminotransferase; OR, odds ratio; HIV, human immunodeficiency virus

## Expected increase in hepatitis C-related mortality in Egypt due to pre-2000 infections

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See Editorial, pages 441–443

**Background/Aims:** Egypt has the highest prevalence of Hepatitis C Virus (HCV) in the world, apparently due to mass parenteral antischistosomal therapy. Estimating the future burden of HCV in Egypt is important to support health policies to combat the epidemic.

**Methods:** A previous back calculation model was adapted to the situation in Egypt. It combines a model of the natural history of HCV infections with available epidemiological data to back calculate the past HCV incidence from observed 1980–1999 hepatocellular carcinoma (HCC) mortality. In turn, the HCV-related mortality burden is projected in the future due to pre-2000 infections.

**Results:** Compared with the observed number of HCC deaths in 1999, the model predicts a 3.5-fold increase in this mortality in the next 20 years. Globally, the model predicts a 2.4-fold increase in the HCV-related mortality. These predictions do not take into account the new infections that may occur after 2000, which would still increase the estimated future mortality burden.

**Conclusions:** HCV-related mortality is expected at least to double in the next 20 years. The use of antiviral therapies can lower these predictions. Efficient prevention policies are needed to avoid these predictions being exceeded.

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**Keywords:** Back calculation; HCV; Hepatocellular carcinoma; Liver disease; Markov model; Mortality; Parenteral antischistosomal therapy; Predictions

### 1. Introduction

The World Health Organization has declared hepatitis C a global health problem, with approximately 3% of the world's population infected with the hepatitis C virus (HCV). There are more than 170 million HCV chronic carriers at risk of developing liver cirrhosis and/or hepatocellular carcinoma (HCC) [1,2]. Egypt has the

highest prevalence of hepatitis C virus (HCV) in the world, ranging from 6 to 28% [3–6] with an average of approximately 13.8% in the general population. These estimates lead roughly to 9 million persons who have acquired HCV infection and 7 million who have HCV chronic liver disease in 1996.

In Egypt, the major route of exposure to HCV appears to be the mass parenteral antischistosomal treatment (PAT) [3], with more than 35 million injections for more than 6 million Egyptians given over a 20-year period (1960–1980). Although schistosomiasis was the major public health problem in the past, HCV has become the most important problem in Egypt [3]. After termination of the PAT program (mid-1980s), transmission of HCV continued through other

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## Changing pattern of hepatitis C virus spread in rural areas of Egypt

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**Background/Aims:** To identify patterns of HCV spread in the Nile Delta of Egypt.

**Methods:** Residents in a Nile Delta village were invited to participate in a cohort study of HCV infection. Risk factors for past or current infection were identified at cohort intake using generalized estimated equations models. Attributable fractions were calculated for all independent risk factors.

**Results:** The prevalence of HCV antibodies increased from 2.7% in those <20 years of age to more than 40% in males aged 40–54 years. The peak in HCV prevalence in the 40–54 year age group corresponds to the aging of the cohort of children infected through schistosomiasis intravenous treatments in the 1960s–70s (accounting for 12.4% of all HCV infections observed today among adults). Following this initial founding event, the HCV epidemic has spread in the community through iatrogenic factors, and particularly injections (37.9% of the overall attributable fraction in adults). In children, however, no iatrogenic factors were associated with increased risk of infection, suggesting a change in the pattern of HCV spread.

**Conclusions:** While HCV infections in adults could be attributed to iatrogenic factors, and particularly injections, infections in children could not be explained by similar routes of transmission.

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**Keywords:** HCV; Risk factors; Iatrogenic; Epidemiology

### 1. Introduction

Hepatitis C virus (HCV) antibody prevalence in Egypt was estimated around 12% nationwide in 1999 (Egypt Ministry of Health and Population, 1999). The origin of the epidemic has been attributed to mass campaigns of parenteral anti-schistosomiasis treatment (PAT) in rural areas in the 1960s–70s [1]. Treatment consisted of several (up to 12) intravenous (IV) injections of tartar emetic spaced by one week and it is postulated that

transmission occurred due to insufficient sterilisation of injection equipment between subjects. During these treatment campaigns, the target population was anyone living in schistosomiasis-endemic areas, and particularly children and young boys who were treated while at school. This mode of HCV transmission stopped after 1982 when oral praziquantel was introduced for schistosomiasis treatment. HCV spread continued however, and it would be useful for on-going prevention programs to have data on current risk factors for transmission. To study HCV incidence rate and related risk factors, we initiated a cohort study in a rural area of Egypt where schistosomiasis treatment campaigns took place in the 1960s–70s. HCV antibody prevalence and risk factors at the cohort intake are presented here.

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## Surveillance of Acute Hepatitis C in Cairo, Egypt

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Surveillance of acute hepatitis has been set up in two fever hospitals in Cairo to diagnose acute hepatitis C. Patients were categorized as definite acute hepatitis C with positive hepatitis C virus (HCV) RNA and without anti-HCV antibody, or probable acute hepatitis C with positive HCV RNA, positive anti-HCV antibody, alanine aminotransferase  $\geq 4$  times the upper limit of normal (ULN), and high risk parenteral exposure in the 1–3 months prior to the beginning of symptoms. From May to November 2002, 315 patients were recruited in the study. Of these, 115 (36.5%) had acute hepatitis A, 89 (28.3%) had acute hepatitis B, and 111 (35.2%) had non-A non-B acute hepatitis. Of the total with complete data ( $n = 309$ ), 12 (3.9%, 95% CI = 2.0%–6.7%) had definite acute hepatitis C, and 11 (3.6%, 95% CI = 1.8%–6.3%) had probable acute hepatitis C. In patients with definite acute hepatitis C, dental exposure ( $n = 5$ ) and intravenous drug use ( $n = 2$ ), were the only high risk procedures found in the 6 months prior to diagnosis. Five patients had no identifiable parenteral exposure. In conclusion, results from this study suggest that acute hepatitis C can be diagnosed by surveillance of acute hepatitis in hospital settings in Cairo and that minor community exposures contribute substantially to local HCV transmission. *J. Med. Virol.* 76:520–525, 2005. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** HCV; risk factors; dental care; genotypes; surveillance

### INTRODUCTION

Egypt is the country with the largest hepatitis C virus (HCV) prevalence in the world. HCV antibody prevalence is estimated at around 12% in the general population, and exceeds 30% among adults older than

age 30. The origin of the HCV epidemic has been attributed to mass campaigns of intravenous treatment for schistosomiasis in the 1970s [Frank et al., 2000]. However, little is known about current HCV transmission now that oral praziquantel has replaced intravenous treatment for schistosomiasis and anti-HCV antibody testing has been generalized in Egyptian blood banks. Most recently published studies on HCV risk factors rely on interviews of HCV antibody carriers, whose infection might be several decades old [Habib et al., 2001; Medhat et al., 2002].

Since 2002, an acute hepatitis surveillance project was initiated in two hospitals in Cairo. The aim of this project was to estimate the proportion of HCV infections among acute hepatitis; to identify risk factors for acute hepatitis C; to study the efficacy of pegylated interferon among acute hepatitis C patients who have not cleared the virus by three months; and to study the natural history of HCV infection in the remaining cases. The results of the first two objectives during the feasibility study are described.

### PATIENTS AND METHODS

The study took place from May to November 2002 in Abassaia and Imbaba hospitals, two “fever” hospitals of Cairo. Fever hospitals are large public and non-paying hospitals whose patient population derives mostly from

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# Chronic Hepatitis C Virus Infection: Does It Really Impact Health-Related Quality of Life? A Study in Rural Egypt

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Arnaud Fontanet,<sup>3</sup> Fabrice Carrat,<sup>1</sup> and Mostafa Kamal Mohamed<sup>2</sup>

Previous Western studies showed a consistent and marked reduction in health-related quality of life (HRQOL) in patients chronically infected with hepatitis C virus (HCV). However, these studies were conducted on patients whose knowledge of their serological status may have affected their HRQOL. This HRQOL survey conducted in the Egyptian rural population provides a unique opportunity to clarify this issue among a population whose serological status is unknown. HRQOL was assessed by an Arabic translation of the Short-Form 12, and a visual analog scale of the relative severity of one's health status. HCV chronic infection was defined by positive tests for anti-HCV antibody and HCV-RNA. HRQOL was compared according to HCV chronic infection status in linear mixed models adjusted for potential confounding factors, such as age, sex, education, and health care-related risk factors, and adjusted for interviewer as a random effect. One hundred forty-six Egyptians chronically infected with HCV had similar Short-Form 12 and visual analog scale scores, compared with 1,140 uninfected controls from the same rural community. In individuals chronically infected with HCV, serum aminotransferase levels did not correlate with HRQOL. **In conclusion**, this study did not find a significant reduction of HRQOL in patients chronically infected with HCV compared with uninfected, contemporaneous controls. This may be explained in part by a lower morbidity amongst patients chronically infected with HCV in rural Egypt and a higher morbidity amongst uninfected controls as compared with those of Western studies, as well as a lack of awareness of hepatitis C serological status. (HEPATOLOGY 2004;40:1434–1441.)

Approximately 15% of 59 million Egyptians in 1996 were estimated to have positive test results for anti-hepatitis C virus (HCV) antibody, and based on 60% viremia, more than five million Egyptians

are chronically infected with HCV.<sup>1</sup> The treatment of chronic hepatitis C (CHC) patients is considered a public health priority in Egypt to reduce both the burden of liver disease and the transmission of HCV. However, dramatic health care budget constraints limit access to the costly treatment recommended in Western countries.<sup>2–4</sup> Decision to treat should depend on the expected benefits from treatment of CHC patients, who are mostly infected with the genotype 4, living in the Nile Delta rural areas, and generally unaware of their HCV serological status in the absence of systematic screening.<sup>1</sup>

Previous Western studies have reported a consistent and marked reduction in health-related quality of life (HRQOL) among CHC patients as compared with nationally representative samples of adults, particularly in physical health-related domains.<sup>5–16</sup> The HRQOL of CHC patients declines even more during the 6 to 12 months of treatment, but it returns to the pretreatment level during the 6 months after treatment because of significant improvements in HRQOL of sustained virologi-

*Abbreviations:* HCV, hepatitis C virus; CHC, chronic hepatitis C; HRQOL, health-related quality of life; SF-12, Short-Form 12; VAS, visual analog scale; SF-36, Short-Form 36; PCS, physical health summary score; MCS, mental health summary score; ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase.

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## **ANNEX 2. Description of the research groups of the network.**

### **A2.1 Research institutions in Egypt.**

#### **A2.1.1 Ain Shams University, Cairo.**

##### Institution and research expertise

Ain Shams University is one of the two largest universities in Cairo. The epidemiology of viral hepatitis in Egypt is currently one of the major research activities of the department of Community Medicine. The department conducted two national surveys on HCV prevalence in collaboration with the Egyptian Ministry of Health. It has been involved in studies of HCV transmission, progression, and treatment in several international collaborations. The immunology laboratory was recently renovated and is now fully dedicated to hepatitis C research.



Department of Community,  
Environmental and Occupational  
Medicine,  
Faculty of Medicine,  
Ain Shams University,  
Cairo

##### Staff

Professor Mostafa K. Mohamed is an epidemiologist trained in Egypt and in the United-States (PhD in Public Health in Washington University, Seattle). He has been the Principal Investigator in several epidemiological studies and clinical trials on Hepatitis C in Egypt and has an expertise in conducting nationwide research projects. He is leading the team of 10 senior researchers, 25 junior researchers, PhD and MSc students and 3 data managers and technical staff working on the viral hepatitis projects in the department. The department has a large team of researchers composed of university staff, professors, assistant professors, teachers and young researchers. Graduate students, are involved in many of the planned research studies as part of the requirement for their thesis preparation.

Prof Mona Rafik is an immunologist with long-standing experience in viral hepatitis studies. She was among the first to publish on non-A non-B hepatitis in Egypt. She coordinates a team of postdoc and doctoral students in immunology in the newly renovated laboratory at Ain Shams University.

##### Role in the collaboration

Coordinator “South” for the ANRS research site. Epidemiological and immunological studies.

## **A2.1.2 National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.**

### Institution and research expertise

The National Hepatology and Tropical Medicine Research Institute (NHTMRI) in Cairo is under the Ministry of Health and Population and performs research on hepatitis and tropical diseases. It hosts the Viral Hepatitis Research Laboratory (VHRL) which was established in 1998 to improve the infrastructure and research capabilities on hepatitis C in Egypt. It also has a Department of Tropical Medicine for in- and outpatients, with clinicians specialised in hepatology and tropical diseases. The laboratory is currently involved in both clinical and fundamental research studies related to different viral hepatitis infections in Egypt such as : prevalence, incidence and risk factors of HCV and HEV ; treatment trials of HCV ; immunology, genetics, proteomic analysis of HCV infections ; malignant complications of HCV. The laboratory also performs routine testing for all chemical and blood investigations necessary for the patient management enrolled in the different studies (biochemistry, haematology, virological markers...) and is well equipped to allow management of frozen sample repository at -80°C freezer or in liquid nitrogen.



Clinical and laboratory activities at the National Hepatology and Tropical Medicine Research Institute, Cairo



### Staff

Dr. Mohamed Abdel-Hamid MD PhD directs the VHRL and holds a faculty appointment at the University of Maryland in Baltimore. There are 2 supervisors, and 6 technicians working in the lab in addition to several master degree and PhD thesis candidates currently working at

the VHRL who are from Egyptian Institutes and Universities. Currently, a doctoral candidate in Pathology/Hematology is also working in the laboratory.

The clinical team is headed by Prof Gamal Esmat, a senior hepatologist from the University of Cairo. Prof Gamal Esmat and his team have been involved in several clinical trials for the treatment of acute and chronic hepatitis C.

#### Role in the collaboration

Virological research on HBV and HCV, with special expertise in the area of HCV molecular epidemiology and genotyping. Routine testing for HCV markers: viral load and serological diagnosis. Clinical research on HCV morbidity and treatment.

#### **A2.1.3 Department of Pathology, Faculty of Medicine, University of Mansoura, Mansoura.**

#### Staff

Dr Khaled Zalata is a medical doctor specialised in pathology and trained at the University of Mansoura where he is teaching now. He spent two years in the Department of Pathology and Laboratory Medicine at Temple University, Philadelphia (U.S.A.), where he worked on oncogenesis in colon cancer with intestinal bilharziasis.

#### Role in the collaboration

Histo-pathological studies based on liver biopsy examinations of patients enrolled in the clinical trials.

### **A2.2 Research institutions in France.**

#### **A2.2.1 Emerging Diseases Epidemiology Unit. Institut Pasteur, Paris.**

#### Institution and research expertise

The Emerging Diseases Epidemiology Unit has been created in 2001 and has collaborative projects with developing countries on viral emerging diseases (HIV, HCV, SARS, dengue). The group is composed by five senior researchers, one biostatistician, one doctoral student, three MPH students, one public health resident, one data manager, and one secretary.

#### Staff

Dr Arnaud Fontanet leads this group. He was trained as a medical doctor in France, and got his PhD in the epidemiology of infectious diseases at the Harvard School of Public Health. He has since worked in the field of infectious and tropical diseases in Asia and Africa, and has followed several cohort studies on HIV and HCV. From 1994 to 1999, he has been the expatriate Project Manager of the Ethio-Netherlands AIDS Research Project, a multidisciplinary research project on AIDS in Addis Ababa, Ethiopia. Since 2000, he is the P.I. and scientific coordinator of international and multidisciplinary research projects on HCV in Egypt and SARS in China.

Dr Elisabeth Delarocque-Astagneau is epidemiologist. She was trained as a medical doctor (Hepatology) in France. She worked for several years at the Institut de Veille Sanitaire (French Public Health Surveillance Institute), in the Infectious Diseases Department and received her PhD in Epidemiology. She joined the team in 2008 to work on hepatitis C projects in Egypt. Tamara Giles-Vernick is a social scientist who joined the team in 2008. She was previously a tenured faculty member at the University of Minnesota, having received her PhD in African history with a focus on anthropology and qualitative research methods, from the Johns Hopkins University. Her current research addresses malaria, influenza and hepatitis B and C in Africa. Dr Sylvia Taylor is trained in epidemiology at Columbia University (New York). She joined the project in October 2006 and provides support in the area of epidemiology and biostatistics. Dr Aline Munier is a postdoctoral fellow in epidemiology and joined the team in November 2009 to work on hepatitis C projects in Egypt. Dr Adela Paez is a doctoral student. She graduated from the University of Madrid with an MPH in 2001, and has followed several summer courses at Johns Hopkins University in infectious diseases epidemiology. She will focus on the epidemiology of acute hepatitis C. Lénaig Le Fouler is trained in public health at Paris XI University (Paris). She joined the team in March 2008 and provides support in data management and biostatistics.

#### Role in the collaboration

Coordinator “North” for the ANRS site. Expertise in epidemiology and biostatistics.

### **A2.2.2 Laboratory of Dendritic Cell Immunobiology. Institut Pasteur, Paris & INSERM U818**

#### Institution and research expertise

The Laboratory of Dendritic Cell Immunobiology was created in 2003 and is focused on defining the role of dendritic cells in the pathogenesis of viral infection and cancer. In particular, the lab concentrates on HCV infection and bladder cancer. The group is composed by six scientists (three students, two post-docs and one medical fellow), one engineer, one technician and one clinical coordinator / projects manager. In addition, we co-sponsor one student (Hala Mansour) who works principally in Cairo on the ANRS sponsored study concerning the role of DCs in HCV pathogenesis.

#### Staff

Dr Matthew Albert leads this group. He was trained as a medical doctor in the USA, and received his PhD in immunology at The Rockefeller University. He has since worked in the fields of tumor immunity and infectious disease and has led several clinical studies including one interventional trial and four observational studies. Since 2003, he has been the P.I. of the Laboratory of Dendritic Cell Immunobiology.

Jérémie Decalf is a doctoral student who joined the project in early 2005. He has participated in developing key technologies and has played in active role in the training of Hala Mansour and the establishment of a close partnership with colleagues in Egypt. Armanda Casrouge is an INSERM engineer and has been developing reagents for monitoring IP-10 in HCV patients. Stéphanie Thomas is the projects manager and has been coordinating interactions with investigators in Cairo and Paris. In addition, she is working to develop clinical studies in

Paris in collaboration with Dr. Stanislas Pol. These trials will parallel the work in Egypt and validate our ideas, in parallel, in cohorts of genotype 1 HCV patients. Melissa Laird is a postdoctoral fellow. Her two main projects focus on day-to-day coordination of the Immunology research in Cairo, and the development of an experimental mouse model of chemokine antagonism and cell trafficking in Paris.

#### Role in the collaboration

Dr Matthew Albert will be the French coordinator of the effort on immunology and the defining of immune correlates of HCV disease pathogenesis.

#### **A2.2.3 Centre Hépatobiliaire, INSERM Unit U785, Paul Brousse Hospital. Laboratoire associé au CNR Hépatites virales B, C et Delta, CHU Henri Mondor- INSERM U635.**

#### Institution and research expertise

The molecular analysis of the hepatitis viruses is a major topic of this group. Previous studies have led to dissect the mechanisms of HBV- and HCV-related chronic hepatitis and liver carcinomas. A study has been conducted, in collaboration with other partners of this project on HCV genetic variability in Egypt (INCO-MED program). This group has accumulated considerable experience in hepatitis B and C viruses and has available all the necessary facilities for molecular biology and cellular biology techniques described in the proposal. This group acts also as a laboratory officially associated with the National Reference Centre on hepatitis B, C and Delta viruses (appointed by the French Ministry of Health).

#### Staff

Valérie Thiers is the Director of the laboratory associated with the National Reference Centre on Viral Hepatitis B, C and Delta viruses since 2002. This group has internationally recognised expertise in virology and molecular epidemiology of HBV and HCV, and has been involved in the investigation of HCV nosocomial transmission episodes. It also has special expertise in the area of liver carcinogenesis. François Rimlinger is in charge of the laboratory analyses and training aspects as part of the collaboration.

#### Role in the collaboration

HCV molecular epidemiology and transmission. Expertise in liver carcinogenesis.

#### **A2.2.4 Hepatology Department – Cochin Hospital, Paris.**

#### Institution and research expertise

The Hepatology team led by Prof Stanislas Pol, formerly based at Necker Hospital and now at Cochin Hospital has been conducting clinical research in hepatology for several decades in close connection with the INSERM. Their major expertise is in HBV and HCV clinical research, with participations in many international multicentric clinical trials.

### Staff

Prof Stanislas Pol is an internationally recognised expert in the area of hepatology clinical research. Prof Stanislas Pol has been for the past five years the clinical consultant on the French side for the two clinical trials undertaken as part of the collaboration: treatment of chronic hepatitis C with the combination pegylated interferon and ribavirin, and treatment of acute hepatitis C by pegylated interferon alone.

### Role in the collaboration

Clinical expert.

## **A2.2.5 Human genetics of infectious diseases - Institut National des Sciences et de la Recherche Médicale, Paris, France (INSERM)**

### Institution and research expertise

The general objective of INSERM Unit 550, 'Human genetics of infectious diseases', is to identify the human genes that are involved in the predisposition and/or the resistance to infectious agents, mainly bacteria and virus. The originality and the strength of the laboratory lie in the synergic combination of two complementary groups (genetic epidemiology and genetic immunology) which allows addressing this question from the perspectives of both Mendelian predisposition to rare severe infections and complex predisposition to common infections. This strategy has already been successful in the study of mycobacterial infections with the identification of several genes responsible of severe infections by poorly virulent mycobacteria (such as BCG vaccine), and the mapping of a major gene in leprosy. This strategy is also currently applied to the study of several viral infections, rare and severe (such as herpetic encephalitis, fulminant hepatitis) and common (such as chronic infection by HTLV-1, HHV-8, HCV).

### Staff

The group of genetic epidemiology headed by Laurent Abel has been working in genetic epidemiology of infectious diseases for more than 15 years with the goal to identify the main genes involved in the determinism of infectious diseases and to specify the role of environmental factors interacting with these genes. In the last years their research has focused on the study of common mycobacterial diseases and chronic infection by some oncogenic viruses. The main findings include the identification of a major gene in susceptibility to leprosy, and the detection of genes predisposing to infection by HTLV-1 and HHV-8 in children living in regions endemic for those viruses. Thier group has a long experience for analyzing human genetic data, and has also developed powerful genetic epidemiology methods for such analyses. In the present project, they are involved in the design and the analysis of the studies aimed at identifying the human genes that could influence the response to SARS virus. The principal participating persons are Sabine Plancoulaine (post-doctoral fellow) and Laurent Abel (director of research).

### Role in the collaboration:

Expertise in genetic epidemiology of infectious diseases.

### **A2.2.6 Department of Pathology, Beaujon Hospital and Liver Physiopathology Research Unit (CNRS UMR 8149), University Paris 5**

#### Institution and research expertise

The Department of Pathology of Hospital Beaujon has a long time experience on liver biopsy specifically in chronic viral hepatitis. Members of the department participate to several national and international clinical trials as central pathologists. The Pathology laboratory works in close coordination with the CNRS 8149 Research Unit and is the leader of several multicentric research projects on liver fibrogenesis and carcinogenesis. The group has a recognized expertise in molecular pathology.

#### Staff:

Prof Pierre Bedossa is the chairman of the pathology department and director of the liver physiopathology research unit (CNRS 8149). He is the leader of the METAVIR group that developed an internationally validated classification of chronic viral hepatitis. He is central pathologist for international clinical trials including EPIC3. Valerie Paradis is a MD, PhD working also in both departments and has a well known expertise in proteomic approaches. The research group focuses on mechanisms of liver fibrogenesis.

#### Role in the collaboration:

Expertise in pathology.

### **A2.2.7 Department of Infectious Diseases, Hôpital Tenon, University Paris 6**

#### Staff

Dr Philippe Bonnard is an expert in the area of infectious diseases. He conducted studies on liver fibrosis in Senegal on patients infected with *S.mansoni*. He recently carried out a study in Burkina Faso comparing elastometry with liver biopsy and serum biomarkers in patients infected with hepatitis B virus.

#### Role in the collaboration:

He is the principal investigator (North) in the ARNS 12184 study: "Liver fibrosis evaluation among HCV genotype 4 infected patients in Egypt. Comparison of elastometry, histology and serum markers."

### **A2.2.8 EA2694-CERIM, Lille**

#### Institution

The EA 2694 is a research unit on public health focusing on epidemiology and modelling of chronic diseases, and in particular infectious diseases.

#### Staff

Prof Yazdan Yazdanpanah, M.D., Ph.D, is a Professor of infectious diseases at Tourcoing Hospital, and Lille Medical School EA 2694. He is mainly involved in the epidemiology and modelling of HIV and HCV, as well as in cost-effectiveness of infectious diseases.

Sylvie Deuffic-Burban, Ph.D, is a researcher in Biomathematics at EA2694 and INSERM U995, a research unit devoted to gut and hepatic inflammation (Lille Medical School). She is

mainly involved in the modelling of infectious and chronic diseases, like HCV and HIV. Dorothée Obach, M.Sc, an epidemiologist at EA2694, works on both HCV and HIV projects.

#### Role in the collaboration

Prof Yazdan Yazdanpanah is the principal investigator (North) of the study ANRS 12215: “Cost-effectiveness of treatment strategies”.

### **A2.2.9 INSERM U758- ENS Lyon, Human Virology Department: Virology**

#### Institution

The Human Virology Department is a joint laboratory of the national institute of health and medical research (INSERM) and the Ecole Normale Supérieure de Lyon (ENSL). It pursues basic research on the molecular and cellular biology of pathogenic human viruses, their interactions with the cellular factors that govern most aspects of their viral cycle, and the characterization of pathologies associated with infection, such as chronic infections (e.g., HIV and HCV).

#### Staff

François-Loïc Cosset is the head of the Human Virology Department, group leader of the “Viral Envelopes and Retrovirus Engineering” team and research director at the CNRS. Ophélie Grano is a postdoctoral fellow.

#### Role in the collaboration

François-Loïc Cosset is the principal investigator (North) of the study ANRS 12188 “An investigation of serum factors that modulate HCV infectivity in an Egyptian cohort of acutely infected patients: role of quasispecies evolution, neutralising antibodies, serum amyloid A and lipoproteins”.

### **A2.2.10 INSERM Unit U563, Toulouse: Virology**

#### Institution

The Inserm Unit 563 or The Physiopathology Centre of Toulouse Purpan (CPTP) is a mixed Research Centre Inserm – University of Paul Sabatier. The collaboration between the diverse research teams is organised around 4 themes with a continuous flow of fundamental research into clinical research: mechanisms of oncogenesis and research for new therapeutic targets; signalling pathway and role of the lipid mediators; Immunopathology and immuno-virology; Genetics and gene therapy

#### Staff

Prof Jacques Izopet and Florence Abravanel (Inserm Unit U563, “Viral infections: persistence, host response and pathophysiology”) study HIV pathophysiology under potent antiretroviral therapy and the HEV persistence in immunocompromised patients. They are also working on the variability of hepatitis viruses including HCV and HEV and its influence on the virus clearance.

#### Role in the collaboration

Prof Jacques Izopet is the reference virologist responsible for the quality control of PCR and other virologic tests.