

# Evaluation of the Hepatitis C Virus and Classical and Modern Immunoassays Used Nowadays to Diagnose It in Tirana

Stela Papa, Klementina Puto, Migena Pllaha

**Abstract**—HCV is a hepatotropic RNA virus, transmitted primarily via the blood route, which causes progressive disease such as chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma. HCV nowadays is a global healthcare problem. A variety of immunoassays including old and new technologies are being applied to detect HCV in our country. These methods include Immunochromatography assays (ICA), Fluorescence immunoassay (FIA), Enzyme linked fluorescent assay (ELFA), and Enzyme linked immunosorbent assay (ELISA) to detect HCV antibodies in blood serum, which lately is being slowly replaced by more sensitive methods such as rapid automated analyzer chemiluminescence immunoassay (CLIA). The aim of this study is to estimate HCV infection in carriers and chronic acute patients and to evaluate the use of new diagnostic methods. This study was realized from September 2016 to May 2018. During this study period, 2913 patients were analyzed for the presence of HCV by taking samples from their blood serum. The immunoassays performed were ICA, FIA, ELFA, ELISA, and CLIA assays. Concluding, 82% of patients taken in this study, resulted infected with HCV. Diagnostic methods in clinical laboratories are crucial in the early stages of infection, in the management of chronic hepatitis and in the treatment of patients during their disease.

**Keywords**—CLIA, ELISA, hepatitis C virus, immunoassay.

## I. INTRODUCTION

**H**EPATITIS C represents an infectious disease that affects the liver and is caused by the hepatitis C virus (HCV). HCV is now considered a global problem because it is widespread around the world [12]. The data show that about 3% of the world's population is infected with HCV [2]. In Europe alone there are estimated to be about 4 million transmitters of this virus [8]. About 150 million people globally have chronic HCV infection and more than 350 thousand people die every year due to HCV-related liver diseases [9], [15]. Hepatitis viruses are different in genomic type, antigenic patterns, mode of transmission, severity etc. [12].

Usually acute infection by HCV is asymptomatic and is not associated with life-threatening illness, but as soon as it stabilizes, chronic infection progresses very rapidly, causing cirrhosis [8], [19]. About 15-45% of infected patients are cleansed spontaneously from the virus, within 6 months of infection without any treatment [19], [3]. The remaining 55-

85% of them develop chronic hepatitis C. Of those with chronic hepatitis C, the risk of cirrhosis of the liver is 15-30% within 20 years [3], [19].

HCV is transmitted by the bloodstream or its products to the injecting drug users during syringes exchanges with each other, by the sexual contact side, and also from mother to child at birth etc. [2], [4], [20]. Risk factors like intravenous drug abuse, reuse of syringes, dental procedures, unsterile pricks, infected sexual partner and tattooing play an important role in transmission of the HCV infection [18], [20]. The prevalence of HCV infection is very high in patients who have undergone organ transplantation; blood transfusion as well as the use of commercial coagulation factors [4]. In the group of people with high prevalence are also included people who use intravenous drugs as well as those who are subjected to renal dialysis [5], [18].

The HCV genome consists of seven functional regions- the core, the envelope, including the E1 and E2 regions, and the nonstructural region, including NS2, NS3, NS4, and NS5 [4], [12]. Usually hepatitis C is considered to be a curable disease, but in some patients, treatment is not well tolerated [12]. An accurate diagnosis is very important for treating properly patients with HCV infection, especially in acute cases [10]. During last years in Albania there has been an increase in the prevalence of HCV infected individuals, therefore there are needed specialized laboratories and efficient methodologies for diagnostic. Diagnosis of HCV infection in Tirana is mainly based on the detection of anti-HCV IgG antibodies as a screening by immuno-chromatography methods like the Cypress Anti-HCV dipstick, a rapid chromatographic immunoassay for the qualitative detection of antibodies to the HCV in human serum or plasma [4]. Another diagnostic method is the FIA for the quantitative determination of HCV in human serum/plasma [4], as well the VIDAS test which uses the ELFA principle, combining the ELISA with final blue fluorescence detection [10].

Still, immuno-chromatography methods are not the most efficient in regard to the detection of antibodies in the window period. That is why in private laboratories, the most used techniques recently are third-generation tests like ELISA and CLIA for testing anti-HCV, which reduces the time for detection of antibody to an average of 7-8 weeks after infection [8], [12]. This study aims to estimate HCV infection in carriers and unhealthy patients and to evaluate the importance of classic and new diagnostic methods used in Tirana from 2016 to 2019.

Stela Papa and Klementina Puto are with the Department of Biotechnology, FNS, UT (e-mail: stela.papa@fshn.edu.al, klementina.puto@fshn.edu.al).

Migena Pllaha is with the Genius Laboratory, Tirana, Albania (e-mail: migenapllaha@gmail.com)

## II. MATERIALS AND METHODS

The study was done for a period of approximately two years, from September 2016-May 2018. Blood samples received in the laboratory were coded for each patient and the serum was separated after centrifugation. The patients of this study had clinical signs for hepatitis C. They were tested initially with ELFA assay (VIDAS Ultra) and FIA (Ichroma™) for the presence of antibody to the HCV virus. Those cases which were suspected as positive with these methods and those resulted negative were repeated for confirmation of this virus with assays like ELISA and CLIA. The analyses were performed in “Genius” Laboratory in Tirana, Albania.

### A. Immunochromatography Method for HCV Analysis

Mainly rapid test Cypress Diagnostics anti-HCV card is included in the immune-chromatography method. The Cypress Anti-HCV dipstick is a rapid chromatographic immunoassay for the qualitative detection of antibodies to the HCV in human serum or plasma. It can be used for clinical diagnosis of HCV infection and for screening of blood donors at the scene. The recombinant antigens used for the Cypress Anti-HCV dipstick is encoded by genes for both structural (nucleocapsid) and non-structural proteins.

### B. Fluorescence Immunological Methods Enzymatic and not Enzymatic

*Ichroma™* - *Ichroma™* anti-HCV is a FIA for the quantitative determination of HCV in human serum/plasma. The test uses a competitive immune-detection method. In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer to form the complex as sample mixture. The instrument used for FIA assays is *Ichroma™* Reader with the reference number, REF CFPO-143 kit.

*VIDAS Ultra* - The VIDAS anti-HCV assay is an automated qualitative test, used in the VIDAS Ultra Instrument for detecting HCV by IgG antibodies in the serum or plasma of individuals, basically by using the ELFA principle. Detection of specific antibodies, along with other clinical status information, leads us to diagnose the infection in individuals who exhibit hepatitis symptoms and in those individuals who are at risk of being affected by the hepatitis C infection. The test is based on the combination of sandwich method of enzymatic immunological assay concluding with final fluorescence detection (ELFA). This assay can be performed in two protocols: HBL (90 min long protocol) and HBS (60 min short protocol), in the solid phase deposit which also serves as a pipetting device during this phase. All steps are performed automatically by the analyzer, the reagents for analysis are ready to use and the reaction environment is cycled several times, in and out the solid phase. The results were confirmed by VIDAS Ultra Instrument with the reference code REF 30 308.

### C. ELISA

ELISA is based on quantitative/qualitative determination of

IgM antibodies of the HCV in blood serum/plasma with a binding system. The kits for Third-generation anti-HCV ELISA are designed to classify the viral agent and to track the undergoing therapy of chronic patients with HCV infection. Microparticles are coated with HCV immunodominant synthetic antigens (nuclear proteins, recombinant proteins NS3, NS4 and NS5) of the viral genome. In the first incubation, the solid phase is treated with diluted samples, captured by antigens, and HCV IgM antibodies if present. After washing steps of all components, the second incubation binds the complex HCV IgM antibodies- anti-IgM, previously tagged with horseradish peroxidase. The enzyme captured in the solid phase, binds with the substrate/chromogenic mixture, thus generating an optical signal that is proportional to the amount of anti-HCV IgM antibodies present in the sample. The presence of IgM in the sample can be determined by means of a calibration curve capable of reading the antibody content in arbU/ml. The neutralization of anti-HCV IgG is performed directly in the test to block the interference due to the determination of this class of IgM antibodies.

ELISA was done as per manufacturer's recommendations (Bio-Rad ELISA).

### D. CLIA

CLIA was performed on Snibe MAGLUMI Immunodiagnostic equipment. The test kit used in the equipment was a third generation anti-HCV kit, IVD MAGLUMI Anti-HCV. The kit is designed for the qualitative determination of hepatitis C antibodies in the serum of individuals. It is an immunological test performed in a single step. The anti-HCV (calibrator/control) sample binds to biotinylated and FITC antigens, forming a sandwich complex. After incubation at 37°C, the complex binds to ABEI substrate and magnetic microglia through the interaction of biotin and streptavidin. After washing steps, the initiators are added in order to start the chemiluminescent reaction. Incubation time of CLIA was 45 minutes and the light signal was captured by a photomultiplier in 3 seconds, which is proportional to the concentration of anti-HCV present in the samples.

## III. RESULTS AND DISCUSSIONS

### A. Diagnostic Methods Used during the Study Period to Detect HCV

HCV is a typical example of an illness that, in order to give a precise diagnosis, it is more important to detect directly the virus. The HCV window period, is still a major problem in the world to have a safe blood [17]. Various studies indicate the need for the use of accurate and standardized HCV identification techniques [1]. The methods used during this study are: ICA, FIA, ELFA, ELISA to detect HCV antibodies in blood serum, and recently more sensitive methods as automated analyzer CLIA. Immunological methods and serological techniques for the detection of HCV antibodies, like VIDAS (ELFA) or *Ichroma™* (FIA), are highly susceptible to HCV identification after the window of infection [17]. Serological methods cannot determine whether

there is an active infection or whether the virus is self-cleaned [1]. It is therefore imperative to use methods that directly identify the virus [1], [20].

It must be emphasized that Rapid Tests (ICA) cannot detect HCV because anti-HCV levels are almost undetectable in the early stages of infection, or in cases where the individual is a carrier, the infection is not in its active stage. Therefore, the techniques used in this study are the other four mentioned above. One of the objectives of our study consisted in identifying HCV cases by immunological and immunochromatography methods in private clinics in Albania and to evaluate the most sensitive methods in this regard. For this reason, ELISA-3 (third generation) and CLIA methods have been used in most of the cases during the two year period of study to determine the infected patients for HCV diagnosis.

Table I shows the diagnostic methods used during the study period to detect HCV. In this study, 2913 cases were analyzed, which were initially tested by VIDAS (ELFA) and Ichroma (FIA) for the determination of anti-HCV, then with other methods. VIDAS Anti-HCV is a technique used earlier in time, based mainly on the binding of HCV antigen via monoclonal or polyclonal antibodies, and the detection by binding both of them, using fluorescence conjugate [7], [9]. According to Table I, this technique is used a lot in 2016 and 2017 but we cannot say the same for 2018, mostly because of the introduction of more sensitive methods.

TABLE I  
DIAGNOSTIC METHODS USED DURING THE STUDY PERIOD TO DETECT HCV

Years	Rapid Tests/ICA	Vidas/ELFA	Ichroma/FIA	ELISA	Snibe Maglumi /CLIA	Total
2016	0	147	10	78	0	235
2017	0	217	47	554	876	1694
2018	0	87	45	390	462	984

During the period of study, 2016-2018 there was no case analyzed with rapid tests. Meanwhile, until 2016 there was not an immunoassay such as CLIA introduced in Albanian Laboratories. Therefore, the 235 cases analyzed in 2016 were detected with ELFA method, FIA and ELISA.

Since 2017 in private diagnostic laboratories in Tirana, most of the immunoassays were done by ELISA (third generation) and CLIA as a very efficient method in detecting HCV. In most of the cases, ELFA method is used to monitor the progress of cases of patients who are chronically infected, in order to see the rate of viral infection [11], [15]. It is important to mention that the methodology used in this study is modern and really accurate and is used only in few private laboratories in Tirana. On the other side, Ichroma<sup>TM</sup> (FIA), as it can be seen from Table I, even though a modern technique, it is not used with efficiency for diagnosing HCV patients because of the limitations it has as an assay.

#### B. Evaluation of Positive and Negative Cases Based on Diagnostic Immunoassays for HCV during September 2016-May 2019

There have been used various methods to detect HCV presence in Albania for years, but as it is a small country, the

introduction of new immunological methods is a key part to the epidemiology of this disease. In the early stages of HCV infection, there is a window period in which the antibodies have not yet reached the detection level since they require a time to produce (8 to 16 weeks) from the moment of exposure to at the time of detection (period of seroconservation) [12], [14]. Because of the window period and the HCV genome, it increases the need in diagnostic laboratories to use more sensitive methods, especially third generation tests which reduce the time for detection of antibody to an average of 7-8 weeks after infection [10] due to the addition of more antigens [17].

Table II shows the results taken from the evaluation of positive and negative cases based on diagnostic immunoassays for HCV during the study period September 2016 – May 2019. According to Table II, out of 159 cases resulted positive for 2016, the most used methods were ELFA (VIDAS) and ELISA, with respectively 78 and 74 of positive cases diagnosed with these assays. We can see an increase in the use of ELISA and CLIA methods in 2017 and 2018, based on the number of positive cases for HCV resulted, respectively, 495 and 696 in 2017 and 364 and 349 in 2018. ELFA assays (VIDAS Ultra) is used less in 2017 and 2018, also the positive cases diagnosed are less in number, as a result of the introduction of new modern methods mentioned above.

TABLE II  
EVALUATION OF POSITIVE AND NEGATIVE CASES BASED ON DIAGNOSTIC IMMUNOASSAYS FOR HCV PER EACH YEAR 2016-2018

Year	Rapid Tests		Vidas/ELFA		Ichromaa (FIA)		ELISA		Snibe Maglumi /CLIA		Total
	P	N	P	N	P	N	P	N	P	N	
2016	0	0	78	69	7	3	74	4	0	0	159/235
2017	0	0	185	32	36	11	495	59	696	180	1412/1694
2018	0	0	71	16	38	7	364	26	349	113	822/984

Based on the results of Table II, it is obvious that FIA assays (Ichroma) is the less used and one of the methods with 81 positive cases during the whole study period. Most of the results taken from this method are false positives due to the cross reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies. The test may also yield false negative result. Results for false negativity of immuno-chromatography assays are related even with blood donors, dialysis patients or sometimes with patients infected with HIV virus, chronic immune disease patients [18], [4]. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies [12], [19].

The positive and negative cases detected for HCV presence with the immunoassays taken in study from September 2016-May 2019, is presented in Figs. 1-3. According to Figs. 1-3, during 2016, the most used technique to detect HCV presence is ELFA with 33.3% of all assays, during 2017, 41.09% of the positive results were detected with CLIA assay and in 2018, both third generation assays ELISA and CLIA, were used to detect respectively 36.95% and 35.2% of the positive HCV

cases.

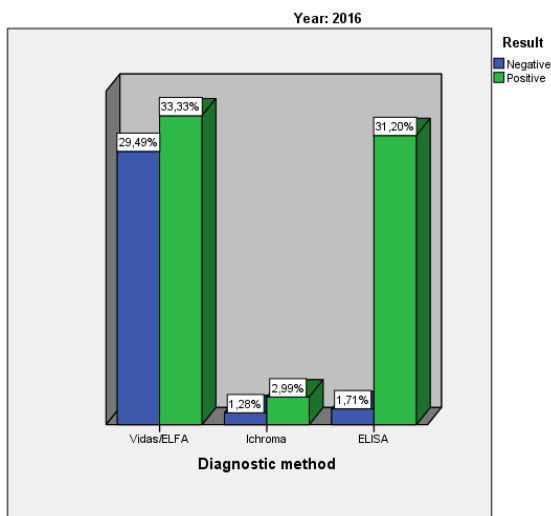


Fig. 1 Evaluation of positive and negative cases based on diagnostic immunoassays for HCV for 2016

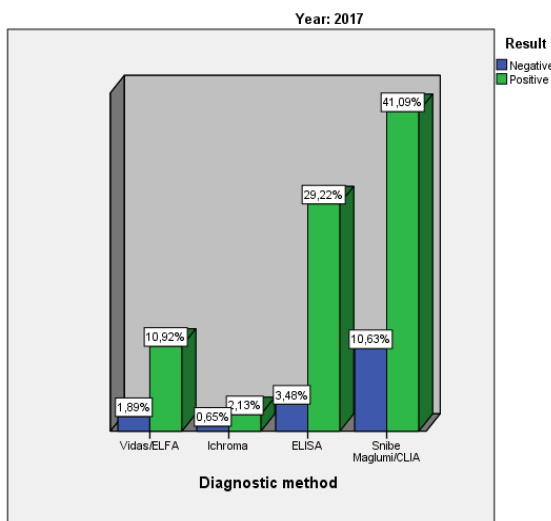


Fig. 2 Evaluation of positive and negative cases based on diagnostic immunoassays for HCV for 2017

ELISA and CLIA methods are the most sensitive used techniques in our study. ELISA is able to identify the presence of specific antibodies produced as a result of the immune response after exposure to the HCV virus [10], [4]. Antibodies can be undetected during the first weeks of infection due to the “window period”. In order to solve this problem, is used the new technique CLIA during 2017 and 2018. One of the advantages of CLIA is the early detection and catching the acute phase at very early stages. Also, performing sample dilutions from the apparatus itself to a higher concentration than normal increases the efficiency of this method [11], [12].

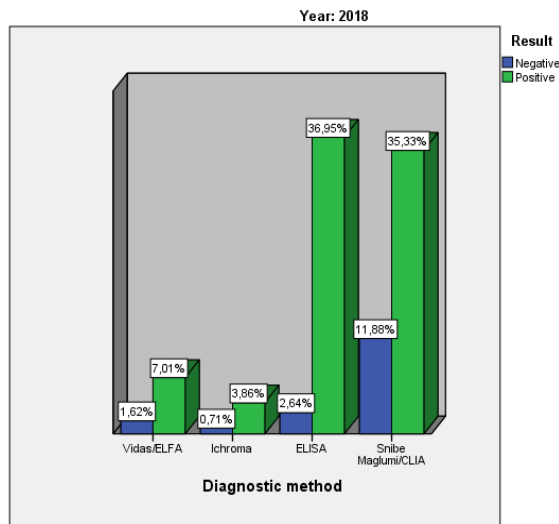


Fig. 3 Evaluation of positive and negative cases based on diagnostic immunoassays for HCV for 2018

*C. Dynamic of Positive and Negative Cases for HCV*

This study analyzes a limited number of patients, 2913, who did not represent the general population. Based on our results, 2393 patients were infected with HCV and 520 of them were negative, from the diagnostication with the mentioned immunoassays.

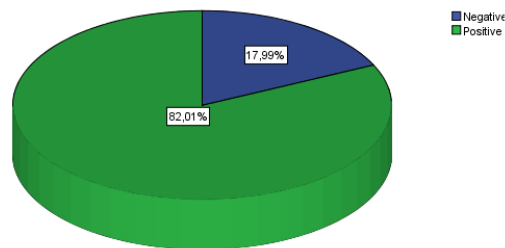


Fig. 4 Dynamic of positive and negative cases for HCV

Fig. 4 shows that 82.01% of the patients analyzed resulted positive and only 17.99% resulted negative. These percentages actually show an increase of the infected individuals with this virus in Tirana, especially when we consider that these cases were not the whole Albanian population.

*D. Evaluation of Positive and Negative Cases of HCV Presence Based On Gender*

HCV has a widespread disruption to both male and female tested patients.

Among the 2393 samples that resulted positive for Anti-HCV antibody with all methods diagnosed, most of the positive cases were females (Table III).

It is noted (see Fig. 5) that out of 1899 women tested, 1553 or 53.28% of them are positive for the presence of HCV, while 346 or 9.82% of them are negative. Fig. 5 shows that out of 1014 tested males, 841 or 28.73% of them are positive for

HCV presence, while 173 or 8.17% of them are negative.

TABLE III  
DISTRIBUTION OF POSITIVE AND NEGATIVE CASES OF HCV PRESENCE BASED ON GENDER

	Female	Male
Positive	1553	841
Negative	346	173

Distribution of positive and negative cases of the HCV virus expressed in percentage is shown in Fig. 5. In this study, from 2393 positive resulted cases for the presence of HCV virus, 53.28% of them were females (1553) and 28.73% of them were males (841). It must be taken into consideration also the fact that the number of males analyzed is lower than females analyzed but still, there is a high dynamics of females infected with the HCV.

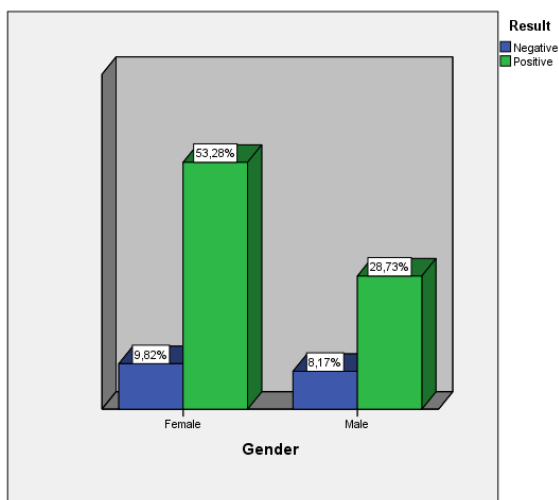


Fig. 5 Distribution of positive and negative cases of HCV presence based on gender

There is evidence that the high level of estrogen hormone affects the purification of the virus in females [7]. This is one of the reasons that in some studies men are more affected than women, but we cannot say the same for our results. Meanwhile, Gheorghe and his associates, in a study conducted in the adult population of Romania for the period 2006-2008, concluded in a high prevalence for females compared to male ones [5]. As a result, gender distribution of HCV infected patients is not a significant factor in determining a connection between gender and infected possibility.

*E. Evaluation of Positive and Negative Cases of HCV Presence Based on Group Age*

Individuals of this study are categorized in six age groups, beginning with age 0 to 15, as the youngest category, up to >75 years, as the oldest category. A very large number of positive cases are observed in the 15-30 age groups but also in the 45-60 age groups, as shown in Table IV. According to Table IV, 751 positive cases resulted in the group age 15-30 years old patients and with a slight difference, 625 positive

cases in the group age 30-45 years old patients.

It is thought that the reason of the greatest positive number in this age group, compared to other age groups analyzed, may be related with the fact that this age group coincides with the age of people who are sexually active and as well those who may be injecting drug users.

Fig. 6 represents the positive cases for each age group expressed in percentage. The percentage of positivity of infected patients with HCV by age groups resulted as below:

In the first age group 0-15 years, 5.8% of the cases; in the age group 15-30 years, 25.78% of the cases; in the age group 30-45 years, 21.46% of the cases; in the age group 45-60, 19.81% of the cases; in the age group 60-75 years old, 6.49% of cases; and, in the age group > 75 years old, 2.81% of the cases.

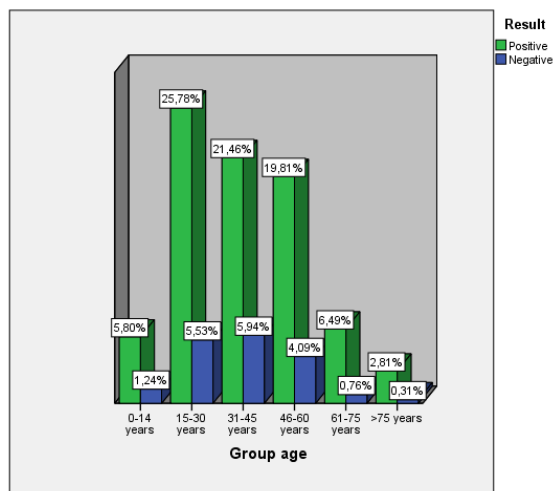


Fig. 6 Distribution of positive and negative cases of HCV presence based on group age

TABLE IV  
DISTRIBUTION OF POSITIVE AND NEGATIVE CASES OF HCV PRESENCE BASED ON GROUP AGE

	Positive	Negative	Total
0-15 years	169	36	205
15-30 years	751	161	912
30-45 years	625	173	798
45-60 years	577	119	696
60-75 years	189	22	211
>75	82	9	91
Total	2393	520	2913

Numerous studies in the region such as Italy [2], Slovenia [16], Serbia and Montenegro [13], Macedonia [14] present different trends of prevalence of this virus in different age groups. In these studies, a significant association between infection and age group has been observed.

In some studies conducted in different regions of southern and northern Italy, a high prevalence rate has been reported in <30 years old age group and 45-60 years old age groups [6]. Some of the causes that the 45-60 years old group age has a high positive may be related to:

- a) Life before the 1990s, when most of the syringes used were multipurpose and made of glass, undergone in an inefficient "sterilization" process.
- b) The ineffective "sterilization" of medical devices in the surgical rooms and in the dentist's cabinets have increased the number of new infected cases.
- c) Failure to carry out blood testing for the presence of anti-HCV before the 1990s in Blood Transfusion Centers has led to unsafe blood collection.

#### IV. CONCLUSIONS

- This study analyzes a limited number of 2913 patients, where 2393 resulted positive for the presence of the HCV virus.
- Among the 2393 samples that resulted positive for Anti-HCV antibody with all methods diagnosed, most of the positive cases were females. Out of 1899 women tested, 1553 or 53.28% of them are positive for the presence of HCV, while 346 or 9.82% of them are negative.
- A very large number of positive cases infected with HCV are observed in the 15-30 years old age groups and with a slightly difference in the 45-60 years old age groups.
- The used immunoassays to detect HCV presence in this study were FIA, ELFA, ELISA and CLIA.
- Rapid Tests (ICA) cannot detect HCV because anti-HCV levels are almost undetectable in the early stages of infection, or in cases where the individual is a carrier.
- FIA assays (Ichroma™) is the less used because the test may yield false negative or false positive results.
- ELISA and CLIA methods are the most sensitive techniques because they are able to identify the presence of specific antibodies produced as a result of the immune response right after the exposure to the HCV virus (in the window period).

#### REFERENCES

- [1] Damen M, Cuypers H T M, Zaaier H W, Reesink H W, Schaasberg W P, Gerlich W H, Niesters H G M, Lelie P N. International collaborative study on the second EUROHEP HCV-RNA reference panel. *J Virol Methods*. 1996; 58:175-185 (PubMed).
- [2] Fabris P, Baldo V, Baldovin T, et al. Changing epidemiology of HCV and HBV infections in Northern Italy; a survey in the general population. *J Clin Gastroenterol* 2008; 42: 527-532.
- [3] Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. *Gastroenterology*. 2003;125(1):80-8.
- [4] Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49:1335-74.
- [5] Gheorghe L, Csiki I. E, Iacob S, Gheorghe C, Smira G, Regep L. The Prevalence and Risk Factors of Hepatitis C Virus Infection in Adult Population in Romania: A Nationwide Survey 2006 - 2008. *J Gastrointestin Liver Dis* December 2010 Vol.19 No 4, 373-379
- [6] Guadagnino V, Stroffolini T, Rapicetta M, et al. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. *Hepatology* 1997; 26: 1006-1011.
- [7] Hayashi J, Kishihara Y, Ueno K, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. *Intern Med*. 1998; 158: 177-181.
- [8] Hepatitis C (Internet). World Health Organization. 2017 (cited 22 August 2017). Available from: <http://www.who.int/mediacentre/factsheets/fs164/en/>.
- [9] Hyun J, Ko DH, Kang HJ, Whang DH, Cha YJ, Kim HS. Evaluation of the VIDAS Anti-HCV Assay for Detection of Hepatitis C Virus Infection. *Ann Lab Med*. 2016;36(6):550-554. doi:10.3343/alm.2016.36.6.550
- [10] Kalem F, Yükksekaya S, Türk D H., et al. Comparative evaluation of automated chemiluminescence tests and RIBA assay used in HCV diagnosis. *Biomedical Research*. April 2016;27(4):1261-1264.
- [11] Kim S, Kim J, Yoon S., et al. Clinical Performance Evaluation of Four Automated Chemiluminescence Immunoassay for Hepatitis C Virus Detection. *J. Clin. Microbiol*. December 2008;46:3919-3923.
- [12] Majumder P, Shetty A. K. Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody. *Indian J Microbiol Res* 2017;4(4):353-357. DOI: 10.18231/2394-5478.2017.0078
- [13] Neda S, Delic D, Simonovic J, Jevtovic D, Dokic L, Gvozdenovic E, Boricic I, Terzic D, Pavic S, Neskovic G, Sonja Zerjav S, Urban V. Hepatitis C virus genotypes in Serbia and Montenegro: The prevalence and clinical significance. *World J Gastroenterol* 2007 January 21; 13(3): 355-360.
- [14] P. Dzekova, A. Slavkovska, L. Simjanovska, I. Nikolov, A. et al. Prevalence of hepatitis C virus infection in dialysis patients. *BANTAO Journal* 2006; 4 (1): 6.
- [15] Park Y, Lee J, Kim B., et al. New Automated Hepatitis C Virus Core Antigen Assay as an Alternative to Real-Time PCR for HCV RNA Quantification. *J. Clin Microbiol*. June 2010;48: 2253-2245.
- [16] Seme K, Vrhovac M, Mocilnik T, Maticic M, Lesnicar G, Baklan Z, Volkar JM, Rajter M, Stepec S, Lunar M, Poljak M. Hepatitis C virus genotypes in 1,504 patients in Slovenia, 1993-2007. *J Med Virol*. 2009 Apr; 81(4):634-9.
- [17] Shang G, Seed CR, Wang F, Nie D, Farrugia A. Residual risk of transfusion transmitted viral infections in Shenzhen, China, 2001 through 2004. *Transfusion* 2007; 47:529-39.
- [18] Tashkandy, M. A., Khodari, Y. A., Ibrahim, A. M., Dhafar, K. O., Gazzaz, Z. J., and Azab, B. A. (2007) Evaluation of the available anti-HCV antibody detection tests and RT-PCR assay in the diagnosis of hepatitis C virus infection. *Saudi J. Kidney Dis. Transpl.* 18, 523-531.
- [19] Thomson EC, Fleming VM, Main J, Klenerman P, Weber J, Eliahoo J, et al. Predicting spontaneous clearance of acute hepatitis C virus in a large cohort of HIV-1-infected men. *Gut*. 2011; 60 (6):837-45.
- [20] Zameer M, Shazad F, Saeed M., et al. Comparison between ELISA and ICT techniques for the detection of Anti HCV antibody among blood donors. *Biomedica* 2016;32:4.