

IMUNOENZYMATIC INVESTIGATIONS IN VIRAL INFECTIONS HEPATIC B AND C

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INTRODUCTION

Viral hepatitis B and C affect a large percentage of the population of Romania (over 1 million infected with the C virus and one million infected with the B virus). Viral hepatitis is currently being detected using viral markers. When analyzing markers that detect hepatitis viruses, either the presence of antigens (portions of the virus or the whole virus) or the antibodies that the organism generates against the virus are searched.

Statistical data from the European Center for Disease Prevention and Control (ECDC) indicate about 4.7 million people with chronic hepatitis B and 3.9 million with liver C infection in Europe. Most are not aware of their infection because they have not been tested and diagnosed.

The general trend in Europe indicates a 26% increase in diagnosed cases, but this evolution does not apply to all countries, which ECDC interprets as being due to the deficiencies in the national testing programs.

Romania ranks 25th in Europe in the treatment of hepatitis C.

There are very effective drugs available to treat people infected with hepatitis B and C, but the main difficulty we see in Europe is the real detection of cases: too many viral hepatitis infections remain undiagnosed.

The situation is similar on a global level. The World Health Organization (WHO) claims in its reports that 257 million people are living with hepatitis B and 71 million people are living with hepatitis C.

Moreover, according to the WHO, viral hepatitis killed 1.34 million people in 2015. This figure is close to that of the number of TB deaths and higher than the number of HIV deaths.

The present paper aims to implement and understand the mechanisms by which the hepatic viruses B and C intervene in the pathogenesis of the liver, as well as the knowledge of their diagnostic methods.

The studies are channeled towards an evaluation of the incidence of hepatitis (B and C) infections by immunoenzymatic methods, using

commercial kits, according to the following algorithm: all samples were tested for the detection of antigen (Ag) HBs and for the presence of anti-HCV antibodies (Ac).

The analysis of biological parameters that express liver dysfunction in viral hepatitis was based on the simple reasoning that between the types of viral hepatitis there are pathogenic and evolutionary differences (which can be found in the correlation of these parameters). Thus, in our study we followed the activity of the main enzymes with diagnostic value in viral hepatitis (alanine aminotransferase and aspartate aminotransferase).

The research activity was carried out and the results obtained were performed in the Medical Analysis Laboratory of the Bacău County Emergency Hospital between January and February 2019.

MATERIAL AND METHODS

The biological materials studied in this work are represented by samples of peripheral venous blood (for the immunological test and for determining the activity of ALAT (TGP) and ASAT (TGO) enzymes. As additional material we used observation sheets from the Infectious Diseases section of the County Emergency Hospital. The results records from the Hospital's Medical Analysis Laboratory were also searched. The study was performed on all cases registered in January and February in 2019 (1.342 patients).

The following markers were used: hepatitis B virus surface antigen (AgHBs) for hepatitis B virus infection and hepatitis C virus anti - antigen antibodies (anti-HCV Ac) for hepatitis C virus infection and the enzymatic activity of transaminases was determined.

Determination of the markers of viral hepatitis - antigens and antibodies - was performed by immunodiagnostic tests based on the antigen-antibody reaction and highlighting the complex by ELISA immunoassay tests (Figs. 1, 2, 3). The determination of the activity of ALAT (TGP) transaminase enzymes, ASAT (TGO) was performed using the COBAS 6000 analyzer, by the spectrophotometric method (Fig. 4).



Fig. 1. The Adaltis Personal Lab automatic analyzer for the determination of antigen HBs and anti-HCV antibody



Fig. 4. "COBAS 6000" automatic analyzer

RESULTS AND DISCUSSIONS

In January and February 2019, 1.342 patients were investigated, of which there were positive responses to the markers of AgHBs and Anti-HCV 125 patients. The situation of the response to the markers is represented in graphs (Figures 5,6,7,8,9,10).

Of the patients with a positive response to hepatitis B and C markers (125), 100 patients were selected and the transaminase enzyme values - TGP = ALAT (alanine aminotransferase) and TGO = ASAT (aspartate aminotransferase) were also determined. The results are graphically represented in Figures 11, 12, 13, 14, 15, 16, 17).

The variability of the transaminases in the patients investigated with the present AgHBs and the present anti HCV Ac is shown in Figures 18,19,20,21.



Fig. 2. Reagent kit MONOLISA Ag HBs ULTRA



Fig. 3. MONOLISA anti-HCV Plus reagent kit

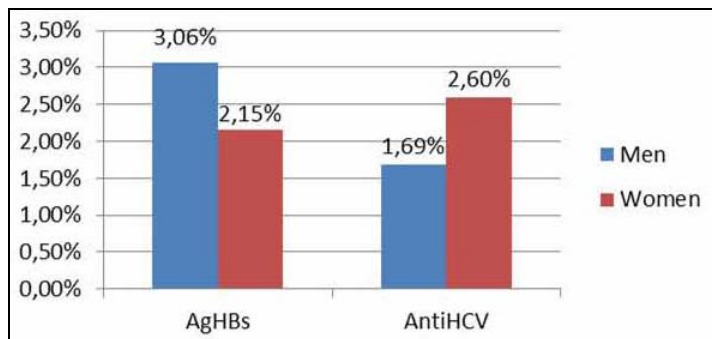


Fig. 5. Percentage distribution by sex of persons with AgHBs and anti-HCV Ac present in January 2019

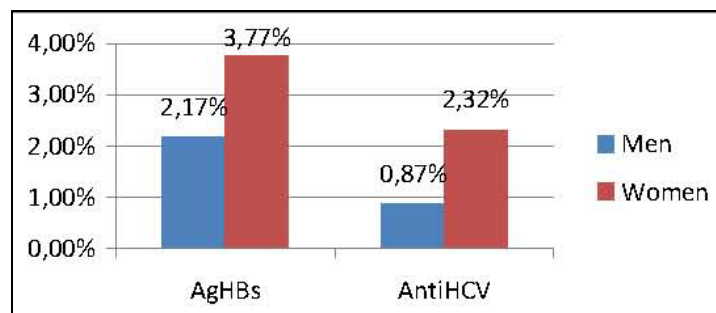


Fig. 6. Percentage distribution by sex of persons with AgHBs and anti-HCV Ac present in February 2019

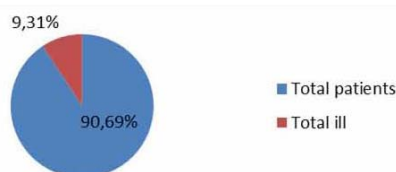


Fig. 7. Percentage distribution of persons with AgHBs and anti-HCV Ac present
125 patients (9.31%)

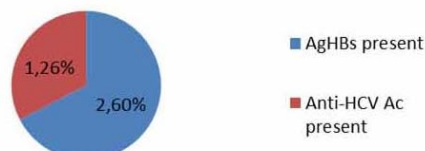


Fig. 8. Percentage distribution of male persons with AgHBs and anti-HCV Ac present
52 patients (3.86%)

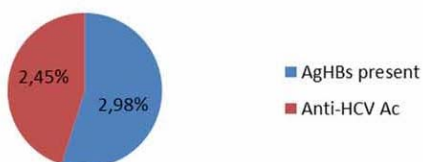


Fig. 9. Percentage distribution of female persons with AgHBs and anti-HCV Ac present
73 patients (5.43%)

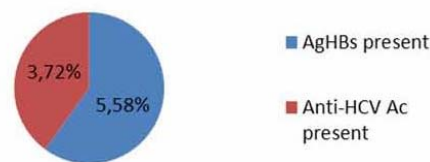


Fig. 10. Percentage distribution of persons with AgHBs and anti-HCV Ac present

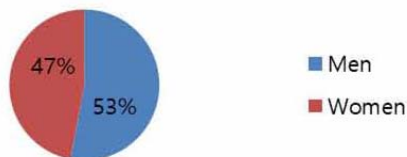


Fig. 11. Percentage distribution by sex of the 100 patients (53 men and 47 women) selected for markers and determination of TGO (AST) and TGP (ALT) transaminases

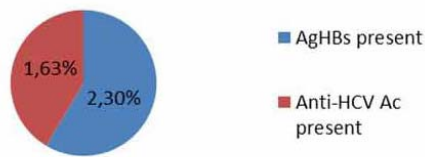


Fig. 12. Percentage distribution of male persons with present AgHBs - 31 patients (2.30%) and anti-HCV Ac present - 22 patients (1.63%)

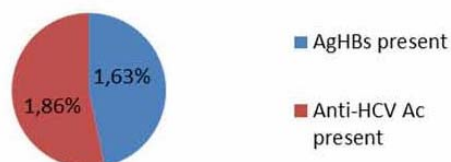


Fig. 13. Percentage distribution of female individuals with present AgHBs and anti-HCV Ac present
22 patients (1.63%) and anti-HCV Ac present - 25 patients (1.86%)

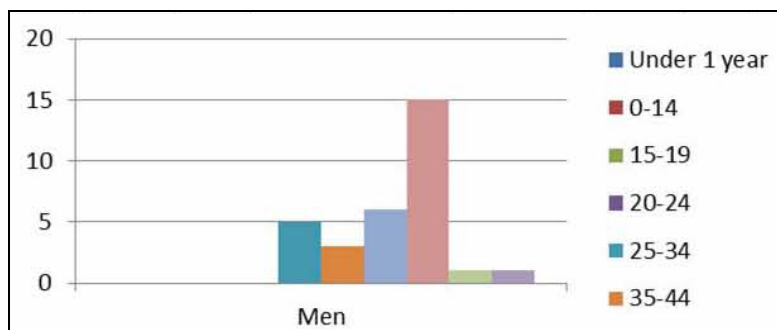


Fig. 14. Age distribution of male persons with present AgHBs (31 patients)

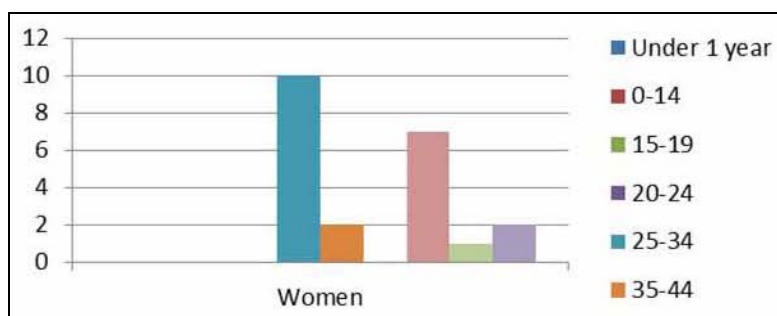


Fig. 15. Distribution by age group of female persons with present AgHBs (22 patients)

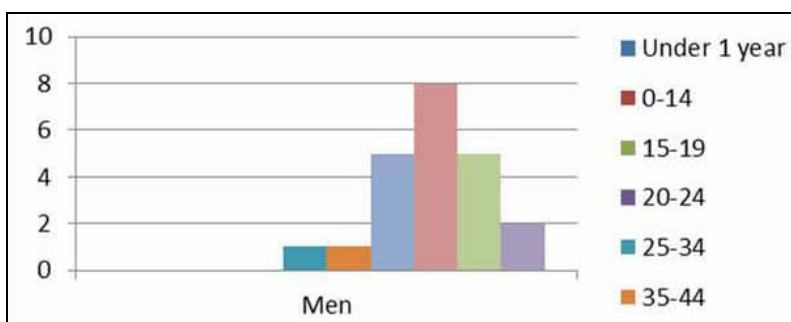


Fig. 16. Age distribution of male persons with antibodies anti-HCV present (22 patients)

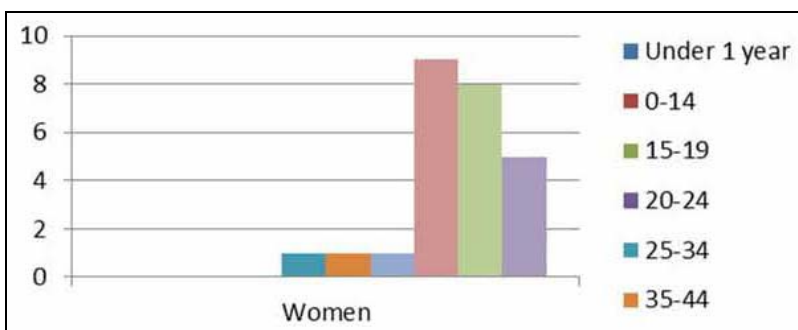


Fig. 17. Distribution by age group of female with antibodies anti-HCV present (25 patients)

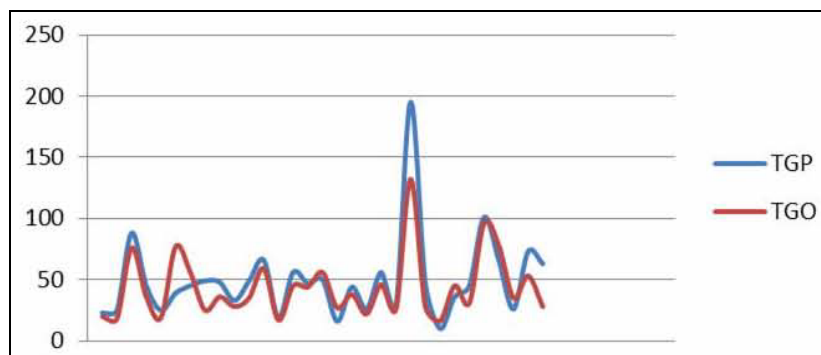


Fig. 18. Transaminase variability in male patients with AgHBs present (31 patients)

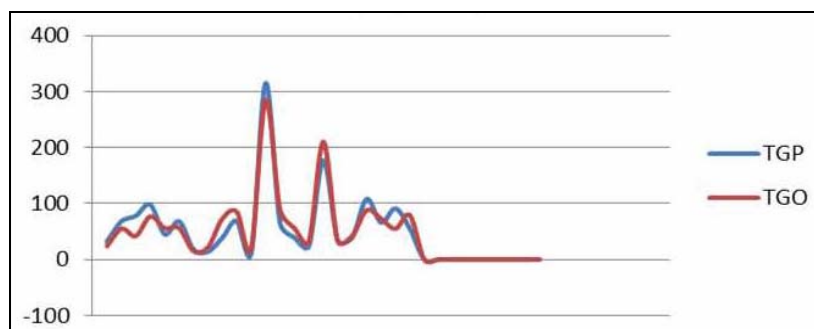


Fig. 19. Transaminase variability in male patients with antibody anti HCV present (22 patients)

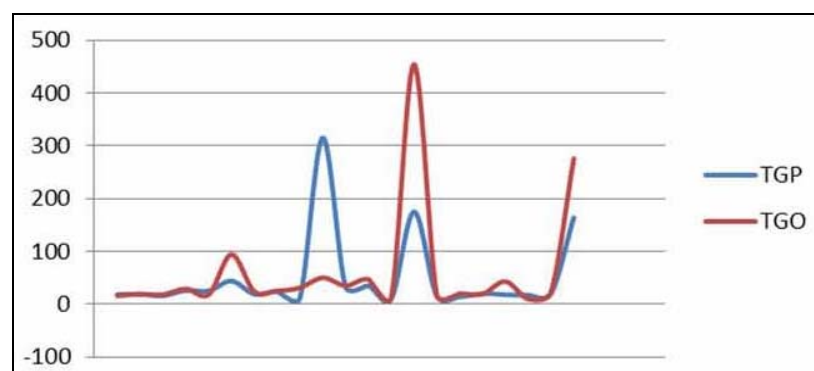


Fig. 20. Transaminase variability in female individuals with present AgHBs HBs antigen (22 patients)

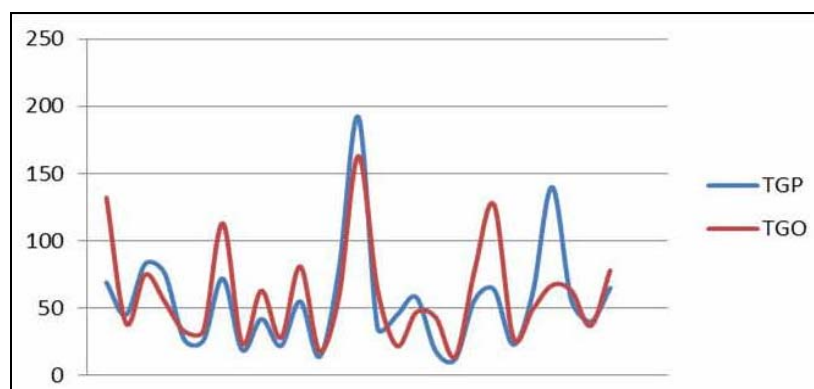


Fig. 21. Transaminase variability in females with antibodies anti-HCV present

For hepatitis B there are several types of markers that detect the presence of the virus or antibodies. There is a certain order to make these markers. Each of the markers plays an important role in establishing the treatment and is done only at the advice of the specialist doctor.

The first recommended marker is HBs antigen (AgHBs). If this test is positive, it means that the B virus infection exists in the body.

For hepatitis C, the first-line test is to detect anti-virus C (anti-HCV) antibodies. The presence of anti-HCV antibodies means the presence of viral C infection in the body.

For the year 2019, in the two months of study - January and February - 1342 people were investigated, of which 125 were positive for both viral markers, AgHBs and AntiHCV.

AgHBs positive were 75 (5.58%), men 35 (2.60%), women 40 (2.98%); (Figs. 70, 71, 72, 73).

In 2019, 50 patients with HCV antibodies were present (4.72%), 25 men (1.86%) and 25 women (1.86%) in January and February. The age group with the highest incidence was 55-64 years in both men (36.36%) and women (36%).

The 100 patients selected for comparison of transaminase values with hepatitis type also show a high incidence in the 55-64 age group in men (48.39%), (fig. 79). In women, the age group 25-34 years has the highest number of patients infected with AgHBs (45.45%), followed by the group 55-64 years (31.83%), (fig. 80). The monthly average of infected was 2.79%.

During the same period of 2019, 50 patients with anti-HCV antibodies were present (4.72%), 25 men (1.86%) and 25 women (1.86%). The age group with the highest incidence was 55-64 years in both men (36.36%) and women (36%).

Transaminase enzymes are predominantly found in the liver (TGP), but are also found in other organs (TGO). In the hepatic cell they contribute to the detoxification of the various substances that reach the blood and they also have a metabolic role.

Even in healthy people, some of these enzymes cross the liver cell membrane and enter the bloodstream. If the liver is overloaded, the hepatocyte membrane cracks, and a large amount of transaminases reaches the bloodstream.

TGP and TGO transaminase enzymes typically have increases associated with inflammatory alteration and / or hepatocyte injury. TGP (ALAT) is the indicator of liver cytolysis, detecting even minimal hepatic injury, being more specific than TGO (ASAT). Increases in TGO are not always an expression of hepatic impairment, as a normal value does not always mean a state of hepatic normality. Increased TGO may also be due to damage to other organs or even to drugs. As a result, associated examinations and other enzymes and parameters were required to determine the exact

nature of the hepatic impairment that led to the increased value of transaminases.

Of the 125 patients who were positive for markers for hepatitis B and C, 100 of them were selected and transaminase values were determined to correlate the change in the value of transaminases with the type of liver viral infection.

In acute viral hepatitis B and C, a characteristic cytolytic syndrome, sometimes very intense, is marked by increased transaminases (TGP more than TGO). The growth of these enzymes takes place early, before other biochemical parameters change.

Normal transaminase values are for TGO $<37\text{U/L}$ and for TGP $<42\text{U/L}$.

Determination of TGP in blood serum in persons with AgHBs present resulted in values ranging from 16 to 195U/l in men (Fig. 83) and between 12 and 315U/l in women.

In the 31 men, 12 cases with normal TGP value $<42\text{U/l}$ were identified, 17 cases of moderate increases in values (below 90U/l) and two cases of more significant increases (with 195U/l value and with the value 101U/L).

A similar dynamic is also observed for TGO concentration. TGO values among men with positive AgHBs show normal values ($<37\text{U/l}$) in 17 patients, moderately increased values in 13 patients ($<96\text{U/l}$) and only one case with a significantly increased value with 132U/l .

In women with AgHBs present, investigated for TGP value (22 women) the results were as follows: 17 women had normal TGP values ($<42\text{U/l}$), only one woman had moderately increased value (with 44U/l) and four cases had higher values (with 315U/l ; 192U/l ; 175U/l and with 164U/l). TGO values at AgHBs women were positive with similar values: 15 women had normal values, four had moderate increases ($<50\text{U/l}$) and three had more significant increases (with 454U/L ; with 276U/l and with 185U/l).

In the 22 men with Anti-HCV antibodies present the TGP situation was as follows: 9 patients had normal values ($<42\text{U/l}$), 10 patients had moderately increased values ($<98\text{U/L}$) and only three patients had values significantly high (with 315U/l ; with 177U/l and with 108U/l). In women with anti-HCV antibodies present (25 women), the situation of TGP values is as follows: in 8 women there were normal values, 16 cases had moderately increased values ($<83\text{U/l}$) and only one case had a value significantly high (with 140U/l).

In men with anti-HCV antibodies present TGO values were normal in 6 of them, 14 had moderately increased values ($<89\text{U/l}$) and only two cases had high values (with 285U/l and with 210U/l).

In women with anti-HCV antibodies present (25 women), TGO values were normal in 10 patients, moderately increased in 12 patients ($<81\text{U/l}$), and

three patients had higher values (with 132U / l; with 113U / l and with 127 U / l).

Interesting is the case of a 67-year-old woman with present AgHBs and antiHCV antibodies present, showing normal transaminases, TGP and TGO.

In viral chronic hepatitis B and C, transaminases have a moderate increase (especially type B) or may be within normal limits (type C). TGP tends to have a higher value than TGO, but with the onset of cirrhosis, the situation can be reversed.

In chronic hepatitis, as in hepatic cirrhosis, hepatocytolysis syndrome is associated with mesenchymal inflammation syndrome, that is, growth of serum γ -globulins and hepatopriv syndrome, expressed by altered coagulation function, decreased serum albumin, hypoglycemia, increased ammonia and cholestasis enzymes. In acute hepatitis the values of transaminases can exceed more than 100 times the normal values.

CONCLUSIONS

- The presented results allow us to affirm that viral hepatitis B and C are still a major public health problem, affecting all age categories of the population (small children and infants, young, adults and elderly).
- In our study, the total number of men infected with viruses is insignificantly higher than the total number of women (3.93% men and 3.49% women), the risks of illness being the same, regardless of gender.
- In January and February 2019, the age group most affected by AgHBs was 55-64 years old in men, in women, the age group 25-34 years old, followed by the 55-64 years old group.
- The other age categories where fewer but significant cases were identified are not neglected, thus cases of children under one year, up to 14 years, young people up to 19 years were identified, especially in the case of infection with hepatitis C.
- In the case of the evolution of the TGP and TGO transaminases determined in the two months of 2019, on the two types of infection, B or C, there is generally a moderate increase in both cases, between 100-300U / L, sometimes normal values. The association of moderate or normal values of transaminases with the affected age groups after 25-34 years, predominantly 55-64 years, indicates the chronic installation of hepatitis B or C.
- Higher values of the TGO enzyme relative to TGP, if other associated conditions are not involved, may indicate cirrhosis or even liver cancer.
- Although visible progress has been made - the introduction of rapid screening tests, new effective treatments for HCV (90-95% cure rate), including the possibility of liver

transplantation for patients with cirrhosis / hepatocarcinoma, are not available in many states with limited financial resources.

- Therefore, emphasis should be placed on prophylaxis, especially as there is specific prevention for HBV and HCV, through effective and safe vaccine products (Engerix B, Recombivax HB against hepatitis B virus). A first HCV vaccine is also produced by a British company, which is under study, but so far it is well tolerated and generates a strong immunological reaction.
- Non-specific preventive measures - improving sanitation conditions, ensuring water potability, food hygiene, observing the rules of personal and collective hygiene, implementing safety strategies for parenteral treatment, blood transfusions and sexual behavior, reducing the impact of viral hepatitis on public health.
- To achieve these desires, sustained activities are needed to promote health in both the general population and at the risk groups.

ABSTRACT

In the present paper, the studies are channeled towards an evaluation of the incidence of infections with hepatic viruses (B and C) by immunoenzymatic methods, using commercial kits. The study was performed on all cases registered in January and February in 2019 (1342 patients). The following markers were used: hepatitis B virus surface antigen (AgHBs) for hepatitis B virus infection and hepatitis C virus antigen antibody (anti-HCV Ac) antibody for hepatitis C virus infection and enzyme transaminases activity was determined (TGO and TGP). The presented results allow us to state that viral hepatitis B and C are still a major public health problem, affecting all age categories of the population (small children and infants, young, adults and elderly). In January and February 2019, the age group most affected by AgHBs was 55-64 years old in men, the age group 25-34 years old being the dominant age group. 50 patients with HCV antibodies were present (4.72%), 25 men (1.86%) and 25 women (1.86%) in January and February. The age group with the highest incidence was 55-64 years in both men (36.36%) and women (36%). For 2019, in the two months of study - January and February - of the 1342 people investigated, 125 were positive for both viral markers, AgHBs and AntiHCV.

REFERENCES

1. BOCĂRNEA, C., 1999 - Boli infecțioase și epidemiologice (Infectious and epidemiological diseases), Info Team Ed., Bucharest.
2. BRAD, C., 1996 - Actualități în hepatologie (News in hepatology), Medical Ed., Bucharest.

3. BUDD, J. et al., 2005 - Hepatita C și medicina de familie: rolul esențial al asistenței medicale primare în stoparea epidemiei (Hepatitis C and family medicine: the essential role of primary health care in stopping the epidemic). Br J Gen Pract.
4. CHIOTAN, M., 1999 - Hepatite acute virale, în Boli infecțioase (Acute viral hepatitis, in Infectious diseases) - National Ed., Bucharest.
5. CERNESCU COSTIN, 2014 - Actualități în tratamentul hepatitelor virale B și C (News in the treatment of viral hepatitis B and C), Medical Ed., Bucharest.
6. GHEORGHE, C. Gheorghe, 2006 - Actualități în hepatita C (News in hepatitis C), Medical Editing celsius, Bucharest.
7. HOPE, V.D., ERAMOVA, I., CAPURRO, D., DONOGHOE, M.C., 2014 - Prevalence and estimation of hepatitis B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association, Epidemiology and infection.
8. NELSON et al., 2011 - Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. The Lancet.
9. QUARANTA, J.F., 2003 - Hepatite (Hepatitis), Ed Corint, Bucharest.
10. REBEDEA I., 2000 - Hepatite acute virale, în Boli Infecțioase (Acute viral hepatitis, in Infectious Diseases), Medical Publishing House, Bucharest.
11. STREINU-CERCEL, A., 2009 - Totul despre hepatite și virusurile hepatice (All about hepatitis and liver viruses), Ed Coreus Publishing.
12. TĂNĂSESCU, C., 1999 - Boli ale ficatului și pancreasului (Diseases of the liver and pancreas), 2nd edition-Themis Cart Edition.
13. VOICULESCU, M., 1977 - Hepatita virală (Viral hepatitis), Medical Ed., Bucharest.
14. WALLACH JACQUES, 2001 - Analize de sânge. In Interpretarea testelor de diagnostic (Blood tests. In Interpreting diagnostic tests), Medical Sciences Publishing House, Romania, 7th ed.
15. YVES, Hecht, 2002 - Ficatul și bolile sale (The Liver and Its Diseases), Corinth Ed., Bucharest.

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