



SEROPREVALENCE OF VIRAL HEPATITIS B, C AND E IN TB PATIENTS FROM THE REPUBLIC OF MOLDOVA

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Introduction. For the first time, in the Republic of Moldova, there was established the seroprevalence of viral hepatitis B, C and E markers in patients with tuberculosis depending on gender, age and geographical areas.

Material and methods. 200 blood samples were collected from patients with tuberculosis and tested by the immuno-fermentative method for presence of viral hepatitis B, C and E markers.

Results. Following the investigations, it was established that the seroprevalence of the anti-HEV Ig marker in patients with tuberculosis is $12.0 \pm 2.3\%$. The seroprevalence of viral hepatitis B marker HBsAg in patients with tuberculosis was $13.5 \pm 2.4\%$, and that of viral hepatitis C anti-HCV – $9.0 \pm 2.0\%$. The study of investigations results for the presence of nominated markers depending on the geographical areas showed a significant statistically difference in the seroprevalence of the anti-HEV IgG marker in TB patients in the Central area of the country compared to the Southern area ($p < 0.05$).

Conclusions. Patients with tuberculosis showed a high level of seroprevalence of viral hepatitis marker viz. HBsAg - $13.5 \pm 2.4\%$; anti-HVC - $9.0 \pm 2.0\%$ and anti-HVE IgG - $12.0 \pm 2.3\%$. People aged 40-49 and > 60 years were the most affected, including males.

Cuvinte cheie: seroprevalența, investigații de laborator, hepatitele virale B, C și E, bolnavii cu tuberculoză.

SEROPREVALENȚA HEPATITELOR VIRALE B, C ȘI E LA BOLNAVII CU TUBERCULOZĂ DIN REPUBLICA MOLDOVA

Introducere. Pentru prima dată în Republica Moldova a fost stabilit nivelul seroprevalenței markerilor hepatitelor virale B, C și E la bolnavii cu tuberculoză în dependență de gen, vârstă și zonă geografică.

Material și metode. Au fost colectate 200 probe de sânge de la bolnavii cu tuberculoză și testate prin metoda imuno-fermentativă la prezența markerilor hepatitelor virale B, C și E.

Rezultate. Urmare a investigațiilor a fost stabilit că seroprevalența markerului anti-HEV Ig la bolnavii cu tuberculoză constituie $12,0 \pm 2,3\%$. Seroprevalența markerului hepatitei virale B - AgHBs la bolnavii cu tuberculoză a constituit $13,5 \pm 2,4\%$, iar a hepatitei virale C anti-HCV – $9,0 \pm 2,0\%$. Studiarea rezultatelor investigațiilor la prezența markerilor nominalizați în dependență de zonele geografice a demonstrat diferență statistică semnificativă a seroprevalenței markerului anti-HEV IgG la bolnavii cu tuberculoză din zona de Centru a țării comparativ cu cei din zona de Sud ($p < 0,05$).

Concluzii. S-a stabilit un nivel înalt al seroprevalența markerilor hepatitelor virale la bolnavii cu tuberculoză: AgHBs – $13,5 \pm 2,4\%$; anti-HVC – $9,0 \pm 2,0\%$ și anti-HVE IgG – $12,0 \pm 2,3\%$. Preponderent fiind afectate persoanele cu vârsta cuprinsă între 40-49 ani și >60 ani, inclusiv cele de gen masculin.

INTRODUCTION

Both viral hepatitis and tuberculosis are major public health issues. Although, literature data highlight on tuberculosis and viral hepatitis co-infection, their convergence is less studied yet. On the other hand, there are almost no studies on the seroprevalence of the hepatitis E virus in patients with tuberculosis, and even fewer studies on the impact of viral hepatitis E on the TB course and treatment.

The most recent and relevant studies in this area have pointed out that the prevalence of chronic hepatitis B (HBV) among TB patients ranges from 0.5% to 44% (1), whereas the prevalence of viral hepatitis C among TB patients is estimated at 3.4-44.6% (2). Co-infection of tuberculosis and viral hepatitis B and C increases the risk of treatment failure (3), activates latent tuberculosis (4), and increases the risk of death (5) and drug-induced injury (6). Hepatitis B infection increases the TB severity by 59.5%, and hepatitis C increases the TB severity by 34.5% (7). It's due to the fact that hepatitis virus reactivates tuberculosis, leading to severe clinical manifestations (8).

Since chronic hepatitis B and C cause liver damage, the treatment of TB patients co-infected with hepatitis viruses is still a health problem in the Republic of Moldova, due to the common anti-TB drug-induced hepatotoxicity (9). Currently, isoniazid, rifampicin, pyrazinamide, and ethambutol are considered first-line drugs, however, they are associated with hepatotoxicity. The incidence of hepatotoxicity and other side effects ranges from 3% to 28% (10).

Regarding viral hepatitis E, recent studies conducted in Europe show a heterogeneity of its spread from 0.6% to 52.5% among the population, where anti-HEV IgG traces have been found. The anti-HEV IgG seroprevalence varies, depending on the geographical region, the type of test used and population under study (11).

On the other hand, as it has been already mentioned above, there are almost no literature data on the prevalence of viral hepatitis E in patients with tuberculosis. Although hepatitis E is usually a self-limiting disease in immunocompetent individuals, it can cause serious complications in risk groups such as pregnant women (12) and organ transplant recipients (13). In cases where viral hepatitis E is associated with another pre-existing

viral hepatitis (B or C), it quickly changes from chronic into a fulminant condition, followed by severe liver damage and high mortality rates (14).

The treatment options for viral hepatitis E remain limited so far, and only one vaccine has been developed, which, unfortunately, is still not used in our country.

This research paper is aimed at studying the seroprevalence of viral hepatitis B, C and E in TB patients from the Republic of Moldova.

MATERIAL AND METHODS

A cross-sectional descriptive-epidemiological study was conducted on TB patients admitted to the IMPH Clinical Hospital of Phthisiopulmonology in Chisinau during the period from January to February 2021. 200 blood samples were collected, including 7 samples – from the northern, 178 – from the central and 15 – from the southern districts of the country. The men-women ratio was 131 (65.5%) and 69 (34.5%), correspondingly.

All the samples were studied for the presence viral hepatitis B (HBsAg), viral hepatitis C (anti-HVC) and viral hepatitis E (anti-HEV IgG) markers via the enzyme immunoassay (ELISA). Tests were performed using Dia. Pro Diagnostic Bioprobes kit that has a sensitivity of 99.9% and a specificity of 99.9%. A total of 672 laboratory studies were performed (including repeated studies with doubtful results). Statistical data processing was carried out using Microsoft Excel and EpiInfo programs.

RESULTS

The laboratory findings of TB patients showed that the seroprevalence of the surface antigen HBsAg was $13.5 \pm 2.4\%$, the anti-HCV marker of hepatitis C was $9.0 \pm 2.0\%$ and the anti-HEV IgG was $12.0 \pm 2.3\%$. In females, HBsAg seroprevalence was $5.8 \pm 2.8\%$, and in males – $17.6 \pm 3.3\%$, which indicated a significant statistical difference between the sexes ($p < 0.05$). The anti-HCV marker was detected almost to the same extent both in females – $8.6 \pm 3.4\%$ and in males – $9.2 \pm 2.5\%$. The predominant anti-HEV IgG marker was found in $17.4 \pm 4.6\%$ of females and in $9.2 \pm 2.5\%$ of males. Thus, the obtained results reveal that TB patients are at higher risk of developing viral hepatitis B, C

and E (tab. 1).

The analysis and assessment of the laboratory findings of the examined TB patients, depending on their geographical areas of origin, revealed that the seroprevalence of HBsAg in the Northern region was 14.3±13.2% and anti-HEV IgG – 14.3±13.2%. There were no cases of anti-HCV

marker positivity among 7 patients from this area. At the same time, the seroprevalence of HBsAg was 13.5±2.6%, anti-HCV – 8.9±2.1%, and anti-HEV IgG – 10.1±2.3% within the central part of the country. The seroprevalence of TB patients in the Southern regions was 33.3±12.1%, and 13.3±8.8% for both viral hepatitis E and for HBsAg and anti-HCV markers (tab. 2).

Table 1. The test results on viral hepatitis B, C and E markers of TB patients according to their gender distribution.

Gender	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
Males	131	23	17.6±3.3	12	9.2±2.5	12	9.2±2.5
Females	69	4	5.8±2.8	6	8.6±3.4	12	17.4±4.6
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

Table 2. The test results on viral hepatitis B, C and E markers of TB patients according to their geographical origin distribution.

Geographical areas	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
North	7	1	14.3±13.2	0	0	1	14.3±13.2
Centre	178	24	13.5±2.6	16	8.9±2.1	18	10.1±2.3
South	15	2	13.3±8.8	2	13.3±8.8	5	33.3±12.1
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

Subsequently, all cases were assessed by age. Thus, the highest level of HBsAg seroprevalence was found in the age group of 40-49 years old viz. 24.5±6.1%, whereas the other age groups revealed as following: 20-29 years old – 7.7±7.4%, 30-39 years old – 7.5±4.2%, 50-59 years old – 10.0±4.7%, 60 and over – 11.3±4.3%.

As regarding the anti-HCV marker, it was mainly determined in the 60 age group and older, showing a seroprevalence of 13.2±4.6%, followed by the age group of 50-59 years, where the same marker was determined in 10.0±4.7% of cases, the age group of 40-49 years exhibited the smallest distribution of 5.6±3.1%, the age group of 30-39 years was found in 7.5±4.2% cases and, finally, only one positive case was determined in TB patients aged 20-29, the marker being of 7.7±7.4% (tab. 3).

The studies related to the age-dependent presence of viral hepatitis E marker in TB revealed that the seroprevalence of anti-HEV IgG in the group aged 20-29 years old was 7.7±7.4%, 7.5±4.2% in patients aged 30-39 years,

14.8±4.8% in those aged 40-49 years, 10.0±4.7% in 50-59 year-old group, and 15.1±4.9% in TB patient older than 60 years old.

Therefore, the obtained results show that the highest seroprevalence of surface antigen (HBsAg) was recorded in TB patients aged 40-49, whereas the anti-HCV and anti-HEV IgG markers showed a higher prevalence in patients aged over 60.

The last task performed in our study was to analyze and evaluate the seroprevalence of viral hepatitis associated with TB patients. Thus, the seroprevalence of the anti-HEV IgG marker associated with HBsAg was 25.9±5.1% of cases (7 people out of 27). The same association of markers was found in male patients in 22.2±8.0% of cases (6 people out of 27), and in females in 3.7±3.6% of cases (1 person out of 27). There is a statistical difference in the prevalence of these associated markers in men compared to women (p<0.05). Depending on the age, the highest level of association of anti-HEV IgG and HBsAg markers was found in TB patients aged 40-49 years viz. 8±8.8%

(4 people out of 27). The seroprevalence of patients under study revealed the anti-HEV IgG associated with HBsAg, which was $20.8 \pm 8.3\%$ (5 patients out of 24) in the Central region and 100%

in the Southern region (2 patients out of 2). No cases of these associations have been registered in the Northern regions.

Table 3. The test results on viral hepatitis B, C and E markers of TB patients according to their age distribution.

Age (years)	Total	Markers					
		Ag HBs		Anti-HCV		Anti-HEV IgG	
		positive	P±ES	positive	P±ES	positive	P±ES
20-29	13	1	7.7±7.4	1	7.7±7.4	1	7.7±7.4
30-39	40	3	7.5±4.2	3	7.5±4.2	3	7.5±4.2
40-49	54	13	24.0±5.8	3	5.6±3.1	8	14.8±4.8
50-59	40	4	10.0±4.7	4	10.0±4.7	4	10.0±4.7
≥60	53	6	11.3±4.3	7	13.2±4.6	8	15.1±4.9
Total	200	27	13.5±2.4	18	9.0±2.0	24	12.0±2.3

The anti-HEV IgG marker associated with anti-HCV was detected in $22.2 \pm 9.8\%$ of cases (4 out of 18 people). Depending on gender, this association showed a seroprevalence of $16.7 \pm 9.6\%$ (3 out of 18 people) men, and $5.6 \pm 5.4\%$ (1 out of 18 people) in women. The highest age-related seroprevalence was registered in patients aged 30-39 years old – $11.2 \pm 7.4\%$ (2 people out of 18). Depending on the geographical distribution, the association of these markers was detected in $18.8 \pm 9.8\%$ of cases (3 people out of 16) from the Central area of the country, in $50.0 \pm 35.3\%$ of cases (1 person out of 2) from the Southern area, whereas no cases have been reported in the northern area.

DISCUSSIONS

Viral hepatitis remains a challenging public health problem. Many studies show that TB patients are at higher risk of becoming infected with viral hepatitis viruses, including viral hepatitis E (3, 15).

Patients suffering from pulmonary tuberculosis on the underlying viral hepatitis are becoming increasingly sensitive to the hepatotoxic effect of anti-tuberculosis drugs. This is mostly due to the fact that hepatitis infection leads to liver damage, making the organ more susceptible to drug damage (6, 10).

A study conducted by Kim et al. reported that 13.7% of the studied patients treated with anti-TB drugs developed drug-induced liver injury (6). In patients with liver disease, TB treatment may increase the chance of developing liver failure. This means a delayed treatment of tuberculosis in patients suffering from acute hepatitis. However,

one study found that patients who received anti-viral drugs shortly after being diagnosed with TB had a lower risk of liver damage caused by anti-TB drug hepatotoxicity (3). Based on the risks listed above, a series of worldwide studies set out similar tasks as in the present study to establish the prevalence rates of viral hepatitis in TB patients in order to assess their potential risk (1, 2, 11, 16-19).

In this context, the results obtained by Feleke B.E. et al. (18) found a prevalence of viral hepatitis B of 15.1% (95% CI: 13.92-16.28%) in a sample of 3537 TB patients, which is similar to the present study, which found an HBsAg seroprevalence of $13.5 \pm 2.4\%$.

As regarding the prevalence of hepatitis C virus infection in TB patients, a reference meta-analysis study including 21 studies found a prevalence of 2% to 27%. Based on a random effect model, the overall prevalence was 7% (95% CI: 6-9%) (17). The result of $9.0 \pm 2.0\%$ HCV marker seroprevalence obtained in the present study falls within this range. On the other hand, the above-mentioned meta-analysis (17) found that the prevalence of hepatitis C virus in men with tuberculosis is about 10% (95% CI: 14-16%) compared to women, where this rate was significantly lower, namely by 2% (95% CI: 1-4%), indicating a statistically significant difference ($p=0.0672$). This finding showed that men have a higher risk of developing HCV than women (odds ratio, OR = 2.02 (95% CI: 1.28–3.18)). The present study also found higher levels of anti-HCV marker seroprevalence in men compared to women viz. $9.2 \pm 2.5\%$

vs. $8.6 \pm 3.4\%$, though not to the same extent.

Moreover, the seroprevalence of the anti-HVE IgG marker found in the present study, namely, $12.0 \pm 2.3\%$ is almost similar to the results obtained in a study, which was conducted in Italy (2013) showing an anti-HEV prevalence of 11.6% (113/973) (16).

Our results suggest that the levels of seropreva-

lence of viral hepatitis B, C and E markers in patients with tuberculosis from our country are quite high, however, they fit into the global trend related to this area. Thus, special attention should be given to testing and carrying out preventive measures against these infections in order to avoid liver injury during treatment with anti-tuberculosis drugs, as well as their possible association with viral hepatitis.

CONCLUSIONS

1. The TB patients are at higher risk of developing viral hepatitis, being proved by the high seroprevalence of the following markers: HBsAg – $13.5 \pm 2.4\%$; anti-HVC – $9.0 \pm 2.0\%$ and anti-HVE IgG – $12.0 \pm 2.3\%$.
2. The simultaneous presence of the anti-HVE IgG marker associated with anti-HCV was detected in $22.2 \pm 9.8\%$ of cases, predominantly in men – $16.7 \pm 9.6\%$ and among patients aged 30-39 years old in $11.2 \pm 7.4\%$ of cases.
3. The seroprevalence of the anti-HVE IgG marker associated with HBsAg was found in $25.9 \pm 5.1\%$ of cases, predominantly in men – $22.2 \pm 8.0\%$ and among patients aged 40-49 years in $24.0 \pm 5.8\%$ of cases.

CONFLICT OF INTERESTS

No conflicts of interest.

ETHICAL APPROVAL

Study Protocol was approved by Medical Ethics Committee of National Center of Disease Control and Public Health (N2018-055; 24.12.2018).

REFERENCES

1. Iglecias LM, Puga MA, Pompilio MA, Teles SA, Croda J, Lima LA, et al. Epidemiological study of hepatitis B virus among prisoners with active tuberculosis in Central Brazil. *Int J Tuberc Lung Dis*. 2016;20(11):1509-1515.
2. Araujo-Mariz C, Lopes EP, Ximenes RA, Lacerda HR, Miranda-Filho DB, Montarroyos UR, et al. Serological markers of hepatitis B and C in patients with HIV/AIDS and active tuberculosis. *J Med Virol*. 2016;88(6):996-1002.
3. Chen L, Bao D, Gu L, Gu Y, Zhou L, Gao Z, et al. Co-infection with hepatitis B virus among tuberculosis patients is associated with poor outcomes during anti-tuberculosis treatment. *BMC Infect Dis*. 2018;18(1):295.
4. Pedrosa M, Nogales S, Vergara M, Miquel M, Casas M, Dalmau B, et al. Reactivation of peritoneal and pleural tuberculosis during hepatitis C treatment with direct-acting antivirals. *Gastroenterol Hepatol*. 2019;42(3):174-175.
5. Ladep NG, Agbaji OO, Agaba PA, Muazu A, Ugoagwu P, Imade G, et al. Hepatitis B Co-Infection is Associated with Poorer Survival of HIV-Infected Patients on Highly Active Antiretroviral Therapy in West Africa. *J AIDS Clin Res*. 2013;2013(Suppl 3):1-7.
6. Kim WS, Lee SS, Lee CM, Kim HJ, Ha CY, Kim HJ, et al. Hepatitis C and not hepatitis B virus is a risk factor for anti-tuberculosis drug induced liver injury. *BMC Infect Dis*. 2016;16:50.
7. Al-Khazraji A, Alkhawam H, Garrido B. *Hepatitis B virus reactivation in an inactive carrier of chronic HBV after the initiation of treatment for tuberculosis*. Great Britain: BMJ Publishing Group Limited; 2016.
8. Pillai AA, Anania FA, Pearlman BL. Caution: reactivation of hepatitis B during hepatitis C treatment with direct-acting antiviral therapy. *Am J Gastroenterol*. 2016;111(12):1854.
9. Sharma SK, Balamurugan A, Saha PK, Pandey RM, Mehra NK. Evaluation of clinical and immunogenetic risk factors for the development of hepatotoxicity during antituberculosis treatment. *Am J Respir Crit Care Med*. 2002;166:916-919.

10. Van Hest R, Baars H, Kik S, et al. Hepatotoxicity of rifampin-pyrazinamide and isoniazid preventive therapy and tuberculosis treatment. *Clin Infect Dis*. 2004;39:488-496.
11. Hartl J, Otto B, Madden RG, et al. Hepatitis E Seroprevalence in Europe: A Meta-Analysis. *Viruses*. 2016;8(8):211. doi:10.3390/v8080211
12. Patra S, Kumar A, Trivedi SS, Puri M, Sarin SK. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann. Intern. Med.* 2007;147:28-33.
13. Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N. Engl. J. Med.* 2008;358:811-817.
14. Kumar Acharya S, Kumar Sharma P, Singh R, Kumar Mohanty S, Madan K, Kumar Jha J, et al. Hepatitis E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death. *Journal of Hepatology*. 2007;46(3):387-94.
15. Perlman DC, Jordan AE, Nash D. Conceptualizing care continua: lessons from HIV, hepatitis C virus, tuberculosis and implications for the development of improved care and prevention continua. *Frontiers in public health*. 2017;4:296.
16. Rapicetta M, Monarca R, Kondili LA, Chionne P, Madonna E, Madeddu G, et al. Hepatitis E virus and hepatitis A virus exposures in an apparently healthy high-risk population in Italy. *Infection*. 2013;41(1):69-76.
17. Behzadifar M, Heydarvand S, Behzadifar M, Bragazzi NL. Prevalence of Hepatitis C Virus in Tuberculosis Patients: A Systematic Review and Meta-Analysis. *Ethiop J Health Sci*. 2019;29(1):945-956.
18. Feleke BE, Feleke TE, Adane WG. et al. Impacts of hepatitis B and hepatitis C co-infection with tuberculosis, a prospective cohort study. *Virology*. 2020;17(113).
19. Khan AF, Sajjad A, Mian DA, et al. Co-infection With Hepatitis B in Tuberculosis Patients on Anti-tuberculosis Treatment and the Final Outcome. *Cureus*. 2021;13(4):e14433. doi:10.7759/cureus.14433

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