Microbes and Infectious Diseases 2021; 2(3): 451-457

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

# Hepatitis B virus infection among patients with rheumatoid arthritis in Suez Canal University Hospital

*Nashaat Soliman [1], Tasneem Fahmey [1], Mohamed Hefny [2], Rania Kishk [3], Ahmed Gaber [*1]*

1- *Department of Endemic and Infectious Diseases, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*
2- *Department of Rheumatology & Physical Medicine Faculty of Medicine Suez Canal University, Ismailia, Egypt..*
3- *Department of Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*

**ARTICLE INFO**

*Article history:*
Received 5 April 2021
Received in revised form 30 May 2021
Accepted 3 June 2021

**Keywords:**
Hepatitis B virus
Rheumatoid arthritis
HBV DNA
HBV vaccine

**ABSTRACT**

**Background:** About 350 million individuals are considered having hepatitis B virus (HBV) infections worldwide, and 6.8% of cases have been observed of a reactivation of HBV triggered by immunosuppressive medications. Due to HBV reactivation, biologic or non-biological Synthetic antirheumatic disease-modifying drugs may be involved. For rheumatologists, HBV infection is a big concern. **Methods:** A descriptive cross-sectional study was carried out at Rheumatology outpatient clinic at Suez Canal University Hospital, Ismailia, during the period from 22 of January to 30 of July 2020 and included 200 patients with a history of Rheumatoid Arthritis. Patients were asked about their demographics and possible risk factors, and blood samples were collected for detection of HBV markers. **Results:** Evidence of present or past HBV infection with positive anti-HBc was found in 12% of patients (n=24). Among them, 4 patients had Overt HBV infection with positive HBV surface antigen (HBsAg) (2%) and another 4 patients had Occult hepatitis B, with negative HBsAg and positive HBV DNA (2%). Regarding HBV vaccination status, we found that 4 patients (16.7%) of HBV core antibody (HBc Ab) positive patients were vaccinated and 69 patients (39.2%) of HBc Ab negative patients were vaccinated. **Conclusion:** This research indicates that HBV among rheumatoid arthritis patients is comparatively prevalent. Moreover, HBV carriers experience rheumatological symptoms. Risk factors for HBV infection in patients with Rheumatoid Arthritis included history of needle stick injury, dental visits, and prior surgical procedures.

## Introduction

About 350 million individuals are considered having hepatitis B virus (HBV) infections worldwide and 1/3 of the world population either has an infection or history of HBV infection in the past. HBV-related end-stage liver disease accounts for more than 1 million deaths annually [1].

Hepatitis B virus reactivation (HBvr) is an emerging problem for patients with weak or suppressed immune systems and a possible life-threatening complication of rapid serum rise in HBV levels that is most associated with hepatitis flare many weeks later [2].

Rheumatoid arthritis (RA) is an autoimmune rheumatic disease (ARD) which causes progressive joint inflammation and destruction; in some patients, RA may include multiple tissues or organsaa. The incidence in developed countries is about 0.5% – 1.5% of the overall population, an average 1.5 males and 3.6 females per 10,000 people annually [3].

DOI: 10.21608/MID.2021.71083.1141
* *Corresponding author:* Ahmed Gaber
E-mail address: agaber2006@yahoo.com

Glucocorticoid (GC) play a major role in RA management and has been shown to increase HBV viral replication by attaching to the GC-responsive component. Synthetic antirheumatic disease-modifying drugs are also combined to reduce long term sensitivity to high-dose GC, based on their disease activity [4]. Several case reports have identified the possibility of raising the risk of HBVr in synthetic antirheumatic disease-modifying drugs, especially methotrexate [5].

HBV reactivation related to immunosuppressant therapy has been reported in 6.8 percent , of fulminant hepatitis patients. Since reactivation of the HBV may be due to biological or non-biological Disease-modifying antirheumatic drugs (DMARDs) [6].

Diagnosis of HBV infection is dependent on serologic markers such as HBV surface antigen (HBsAg) and HBV core antibodies (HBcAbs) which be positive before and about one month after clinical symptoms. When HBsAg was negative in window time, the only diagnostic viral marker is anti-HBc IgM antibody assay [7].

Many of the remedies used with rheumatoid arthritis have induced immunodeficiency and latent hepatitis reactivation. In a hepatitis patient, rituximab and anti-TNFa, should be cautiously used as a result of increasing risk of reactivation [8].

Hepatitis B virus reactivation happens in two forms: first of all, the harmful proliferation of HBsAg, stable carriers or patients with chronic HBV hepatitis, and, secondly, patients with HBsAg negative and anti-HBcAb positive and occult HBV virus infection [9]. This research was also intended to determine the prevalence of hepatitis B virus infection in RA patients.

## Patients and Methods

A descriptive cross-sectional study was carried out at Rheumatology outpatient clinic at Suez Canal University Hospital, Ismailia, during the period from 22 of January to 30 of July 2020 and included 200 patients with a history of RA.

*Inclusion criteria:*

- Gender: both sexes were included.
- Age: > 18 years.
- Patients have a confirmed diagnosis of RA.

*Exclusion criteria:*

- Other Rheumatologic disorders such as SLE, Sjogren syndrome, Osteoarthritis, Dermatomyositis, polymyositis, Scleroderma, Vasculitis.

*Study procedures:*

All patients were subjected to full history taking to detect cases with RA. An informed consent was taken from confirmed cases after explanation of the whole procedure. Patients were asked about their demographics and possible risk factors, and then blood samples collected for detection of HBV markers.

A. Interview-based questionnaire including:
   - Demographic data included gender, age, education, marital status and occupation.
   - Risk factors for HBV infection included HBV vaccination status, past history of HBV or HCV infection, blood transfusions and their number, exposure to blood of infected person, needle stick injury, history of previous operations, previous dental procedure and family history of HBV infection.

B. Laboratory testing:

Aseptically, 5 ml venous blood was collected in sterilized plain vial from each study participant. Serum was separated and stored at – 20º c till starting the work. All samples were screened for HBsAg, hepatitis B core antibody (total anti-HBc) and Quantitative HBsAb by commercial Enzyme linked immune sorbent assay (ELISA), using (Bioneovan CO.LTD., Beijing, China) kits. HBV DNA by PCR was done for patients whose HBc IgG Ab was positive.

### PCR technique

#### *DNA extraction protocol*

This protocol is for purification of viral nucleic acids from 200 µl of plasma using the QIAamp MinElute Virus Spin Kit and a microcentrifuge (QIAGEN®, QIAamp®, QIAcube®, BioRobot®, EZ1TM MinElute® (QIAGEN Group); Corex® (Corning, Inc.); Eppendorf® (Eppendorf- Netheler-Hinz GmbH). It was done by using universal primer pairs P1 (sense) ….5'-TCA CCA TAT TCT TGG GAA CAA GA-3' (2823–2845 nt) 1063bp , and S1-2(antisense)……5'CGA ACC ACT GAA CAA ATG GC-3' (704-685 nt). The reaction mixture contains 5 ul of extracted DNA, 25 ul of master mix, 5 Pmol of each primer completed to 50ul with distilled water. The thermal cycler was programmed at 95 ˚C for 10 minutes, followed by 40 cycles at 92 ˚C for 20 sec (denaturation), 56 ˚C for 20 sec (annealing), and 72 ˚C for 1 min (extension) then at

72 ˚C for 10 minutes. Hepatitis B virus genomic DNA and a negative sample were used as positive and negative controls, respectively from our microbiology lab. For the analysis of the PCR amplification, 10 µL of the amplified DNA were run on 3% agarose gel after addition of 4 µL of loading buffer. The presence of a1063-bp fragment indicated a positive result. In parallel with samples, a 100-bp DNA ladder was also run on the gels to estimate the molecular weight of DNA fragments in the gel.

**Statistical analysis**

All statistical analyses were performed using the *SPSS* statistical package for social science version 22. Descriptive statistics were applied in numerical form (mean, SD or percentages) to describe the quantitative variables. Diagrammatic and tabular forms were used to describe the qualitative variables. T-test had been used to compare quantitative data (expressed as mean ± standard deviation), with a confidence interval of 95%. Chi square test or Fishers exact test will be used to evaluate the association between seropositivity for HBV and categorical variables (risk factors) (expressed as number and percentage). Two-sided *p-values <0.05* were considered statistically significant.

**Results**

In the current study, 200 RA patients were included, 172 of them(86%) were female, their ages ranged between 27 to 70 years with a mean of about 40 years old, 63% are working, 72% are highly educated and 65% are married.

The prevalence of HBsAg and anti-HBc were 2%, and 12% respectively. Hepatitis B virus immunity achieved by past HBV infection was found among 8.5%, while immunity after vaccination was found in 35.5%. Those still susceptible to HBV infection comprised 52.5% of the patients. We found that 3 patients (1.5 %) had indeterminate results with isolated anti-HBc positivity caused by either long-standing resolved infections with low anti-HBs titres or current infections with low HBsAg titres or window phase **(Table 1)**.

Hepatitis B virus DNA by PCR was done for patients with positive HBcAb (n=24), only one third of them (8 patients) had positive PCR results; 4 patients had overt hepatitis with positive HBsAg and the other 4 had occult hepatitis with negative HBsAg **(Table 2)**.

Patients with HBV infection were older (45.0±6.9) than those without (39.6±8.9), and that was statistically significant. No significant difference between both groups regarding gender and marital status. Most patients with HBV infection (87.5%) were not working and that was statistically significant. Lastly HBV infection was more common among lower educational level than people who are highly educated and that was statistically significant **(Table 3)**.

Risk of HBV infection was assessed in relation to different risk factors, it was observed that HBV infection was more common among those with history of previous operation, previous blood transfusion, dentist visiting, positive family history and history of needle stick injury with significant relation between multiple variables and acquiring Hepatitis B infection **(Table 4)**.

**Table 1.** Interpretation of serologic markers: HBV infection status of patients and corresponding percentages (n= 200).

| **Serologic markers** | | | **Interpretation** | **N = 200 (%)** |
|---|---|---|---|---|
| **HBsAg** | **HBsAb** | **HBcAb** | | |
| **Negative** | **Negative** | **Negative** | Susceptible | 105 (52.5%) |
| **Negative** | **Positive** | **Positive** | Immune after infection, | 17 (8.5%) |
| **Negative** | **Positive** | **Negative** | Immune after vaccination | 71 (35.5%) |
| **Positive** | **Negative** | **Positive** | Current infection | 4 (2%) |
| **Negative** | **Negative** | **Positive** | Indeterminate: *possibilities:*<br>*i) Window phase*<br>*ii) Remote resolved infection with low anti-HBs*<br>*iii) Chronic infection with low levels of HBsAg* | 3 (1.5%) |

**Table 2.** Results of HBV DNA among cases with positive HBcAb.

| | No. | % |
|---|---|---|
| **Negative** | 16 | 66.7 |
| **Positive** | 8 | 33.3 |
| Overt | 4 | 16.7 |
| Occult | 4 | 16.7 |
| **Total** | 24 | 100 |

**Table 3.** Relation between HBcAb positivity and sociodemographic data of study participants.

| Sociodemographic data | HBcAb | | *p value* |
|---|---|---|---|
| | Negative (176) | Positive (24) | |
| **Age (Mean ± SD)** | 39.6±8.9 | 45.0±6.9 | 0.002*§ |
| **Gender** | | | |
| Male | 27(15.3%) | 1(4.2%) | 0.210¥ |
| Female | 149(84.7%) | 23(95.8%) | |
| **Marital status** | | | |
| Single | 25(14.2%) | 0(0.0%) | 0.226¶ |
| Married | 111 (63.1%) | 19(79.2%) | |
| Divorced | 17(9.7%) | 2(8.3%) | |
| Widow | 23(13.1%) | 3 (12.5%) | |
| **Occupational status** | | | |
| Working | 123(69.9%) | 3(12.5%) | <0.001*¶ |
| Not working | 53(30.1%) | 21(87.5%) | |
| **Educational level** | | | |
| Illiterate/ Primary education | 10(5.7%) | 6(25.0%) | <0.001*¶ |
| Secondary / technical education | 16(9.1%) | 12(50.0%) | |
| Higher education | 139(79.0%) | 6(25.0%) | |
| Postgraduate | 11(6.3%) | 0(0.0%) | |

§ Mann-Whitney test, ¥ Fisher's Exact test, ¶ Chi-Square test

**Table 4.** Relation between HBcAb positivity and hepatitis B infection risk factors among study participants

| Hepatitis B infection risk factors | HBcAb | | *p value* |
|---|---|---|---|
| | Negative (176) | Positive (24) | |
| **Needle prick** | | | |
| Yes | 2(1.1%) | 9(37.5%) | <0.001*¥ |
| No | 174(98.9%) | 15(62.5%) | |
| **Surgical operation** | | | |
| Yes | 20(11.4%) | 11(45.8%) | <0.001*¥ |
| No | 156(88.6%) | 13(54.2%) | |
| **Blood transfusion** | | | |
| Yes | 16(9.1%) | 15(62.5%) | <0.001*¥ |
| No | 160(90.9%) | 9(37.5%) | |
| **Dentist visiting** | | | |
| Yes | 27(15.3%) | 8(33.3%) | <0.043*¥ |
| No | 149(84.7%) | 16(66.7%) | |
| **Family history of HBV infection** | | | |
| Yes | 10(5.7%) | 9(37.5%) | <0.001*¥ |
| No | 166(94.3%) | 15(62.5%) | |

* Statistically significant at *p <0.005*, ¥ Fisher's Exact test ,¶ Chi-Square test.

**Discussion**

Hepatitis B virus infection is a challenging problem among RA patients; however, its prevalence, and contributing risk factors are controversial. Over the past few decades, scientists have been furiously investigating the prevalence of HBV among the risky groups such as patients with RA. What is more debatable is their infection status; are they more susceptible to new HBV infection, or it is the treatment what causes HBV reactivation? Therefore, we conducted this study to assess the prevalence of HBV infection among patients with RA, and to identify potential risk factors.

The results of this study are outstanding, as it revealed reassuring; yet, surprising findings about HBV infection among RA patients. We were able to retrieve invaluable data about the association between HBV infection and RA.

Approximately 47% of our patients had one or more positive HBV seromarker. On the bright side, few of them had current infection. However, more than half of them were not immunized. Whether HBV provoked RA or the opposite will remain a confusing question.

In our study, the prevalence of current infection was low (2%). This was in line with previous national and international studies. Similar to our results, findings from a Brazilian study where RA existed in 2.6% of patients with current HBV infection [10]. On the national level, a study conducted at Assiut University Hospital revealed similar findings; a 2% prevalence of HBV infection among RA patients [11].

Regarding past HBV infection, 8.5% of our patients were positive for HBsAb and HBcAb. This is similar to a study by **Harigai et al.** who found that past infection presented in 8.8% of patients with RA [12]. On the other hand, this prevalence considered low in comparison to **Mori's** study where he found that 25.1% of patients with RA had past HBV infection. Of notice, most of those patients had received or were current users of antirheumatic drugs by the time of investigation [13].

The current study revealed positive HBcAb (24) only eight of them (23.4%) had positive HBV DNA. This agrees with the findings of **Gutiérrez-García et al.** who found that only 20% of patients with positive HBcAb had positive HBV DNA [14]. While higher sensitivity (69.8%) of HBcAb was revealed by **Honarmand et al.** [15]. This controversy may be due to many patients have occult hepatitis B infection where they express negative HBs and/or HBc antibodies, while they have positive HBV DNA [16].

We found that positive HBsAg was prevalent among unemployed people, with primary or intermediate education. Moreover, those patients were characterized by having more than one source of infection transmission including previous needle stick injury, previous blood transfusion, operations, and visits to dentist. While patients with HBS Ab positive were more likely of educated and employed people. Less number of those patients had previous operations, visits to dentists or family history of HBV infection, otherwise they share the other characteristics the latter group had.

One might think that it is logical to have an infective disease via the common sources of HBV infection, but what makes RA patients at higher risk of infection more than non-RA? That is exactly why further studies were keen on identifying the association between RA and HBV infection.

In essence, patients with RA must be exposed to needlestick injuries in the process of extracting the blood because they are permanent healthcare seekers. In addition, RA patients may have a blood transfusion for anaemia, and thus introduce another cause of infection [11].

In our study, the prevalence of current infection in RA patients was low (2%). This was in line with previous national and international studies. Similar to our results, findings from a Brazilian study where RA existed in 2.6% of patients with current HBV infection [10]. On the national level, a study conducted at Assiut University Hospital revealed similar findings; a 2% prevalence of HBV infection among RA patients [17].

**Hsu et al.** in a nation-based large case control study in Taiwan they revealed that the incidence of HBV infection is 13% higher among patients with RA than non RA patients [18].

Unfortunately, more than half of our participants were unimmunized, and Egypt is endemic for HBV. The prevalence of HBsAg in Egypt is ranges between 2%–8%, and approximately, 2 to 3 million Egyptians are chronic carriers [19]. Adding to this, receiving immunosuppressive drug would be a huge risk for patients with RA to tolerate. Thus, rheumatologist should consider all these risk factors while managing patients with RA as this could be the earlier manifestations of a silent hepatic disorder.

Rheumatoid arthritis with HBV is dangerous since certain synthetic antirheumatic disease-modifying drugs widely used to treat RA, such as methotrexate or leflunomide, could lead to hepatotoxicity. Rheumatoid arthritis also leads to risky RA treatment. Moreover, certain medicines that influence immune reactivation can contribute to HBV reactivation. Rheumatoid arthritis medication dependent on the state of hepatitis B should also be tailored [20].

**Conclusion**

HBV infection and RA are two common concurrent diseases. Hepatitis B virus is relatively prevalent among patients with rheumatoid arthritis. Besides, rheumatological symptoms are common among HBV carriers. Illiteracy, poor education, and unemployment, in addition to history of needle stick injuries, previous operations, visits to dentist are all risk factors for active HBV infection among patients with rheumatoid RA.

**Ethical approval**

Consent for an interview was taken from each participant, who was assured about the confidentiality of his information. The faculty of medicine Suez Canal University research ethics committee approved the study.

**Funding:** None

**Conflicts of interest:** There are no conflicts of interest.

**References**

1-**European Association For The Study Of The Liver.** EASL clinical practice guidelines: management of chronic hepatitis B virus infection. Journal of hepatology 2012 ;57(1):167-85.

2-**McMahon BJ.** Chronic hepatitis B virus infection. Medical Clinics 2014 ;98(1):39-54.

3-**Akhter E, Bilal S, Haque U.** Prevalence of rheumatoid arthritis: a review. Rheumatology international 2011;31(7):849-55.

4-**Tur-Kaspa R, Burk RD, Shaul Y, Shafritz DA.** Hepatitis B virus DNA contains a glucocorticoid-responsive element. Proceedings of the National Academy of Sciences 1986;83(6):1627-31.

5-**Oshima Y, Tsukamoto H, Tojo A.** Association of hepatitis B with antirheumatic drugs: a case–control study. Modern rheumatology 2013;23(4):694-704.

6-**Oketani M, Ido A, Nakayama N, Takikawa Y, Naiki T, Yamagishi Y, et al.** Intractable Hepato-Biliary Diseases Study Group of Japan. Etiology and prognosis of fulminant hepatitis and late-onset hepatic failure in J apan: Summary of the annual nationwide survey between 2004 and 2009. Hepatology Research 2013;43(2):97-105.

7-**Ramezani A, Banifazl M, Eslamifar A, Aghakhani A.** Serological pattern of anti-HBc alone infers occult hepatitis B virus infection in high-risk individuals in Iran. The Journal of Infection in Developing Countries 2010;4(10):658-61.

8-**Vassilopoulos D, Calabrese LH.** Management of rheumatic disease with comorbid HBV or HCV infection. Nature Reviews Rheumatology 2012 ;8(6):348.

9-**Harigai M, Mochida S, Mimura T.** A proposal for management of rheumatic disease patients with hepatitis B virus infection receiving immunosuppressive therapy. Mod Rheumatol 2014;24:1–7.

10-**de Oliveira Í, da Silva R.** Rheumatological Manifestations Associated with Viral Hepatitis B or C. Journal of the Brazilian Society of Tropical Medicine 2019;52:1–7.

11-**Yılmaz N, Karadağ Ö, Kimyon G, Yazıcı A, Yılmaz S, Kalyoncu U, et al.** Prevalence of hepatitis B and C infections in rheumatoid arthritis and ankylosing spondylitis: A multicenter countrywide study. Eur J Rheumatol 2014;1(2):51-54.

12-**Harigai M, Winthrop K, Takeuchi T, Hsieh TY, Chen YM, Smolen JS, et al.** Evaluation of

hepatitis B virus in clinical trials of baricitinib in rheumatoid arthritis. RMD Open 2020;6(1):e001095.

13-**Mori S.** Past hepatitis B virus infection in rheumatoid arthritis patients receiving biological and/or nonbiological disease-modifying antirheumatic drugs. Modern rheumatology 2011;21(6):621-7.

14-**Gutiérrez-García ML,Fernandez-Rodriguez CM, Lledo-Navarro JL, Buhigas-Garcia I.** Prevalence of occult hepatitis B virus infection. World Journal of Gastroenterology: WJG 2011;17(12):1538.

15-**Honarmand A, Pourahmad M, Solhjoo K, Kohan M, Davami MH, Zadeh SK.** Occult hepatitis B demonstrated by anti-HBc and HBV DNA in HIV-positive patients. Reports of biochemistry & molecular biology 2014;3(1):38.

16-**Lledó JL, Fernández C, Gutiérrez ML, Ocaña S.** Management of occult hepatitis B virus infection: an update for the clinician. World journal of gastroenterology: WJG 2011;17(12):1563.

17-**Abdel-metaal MGE, Doma MA.** Prevalence of hepatitis B and C in rheumatoid arthritis.AAMJ 2003;1(2):112-6

18-**Hsu CS, Lang HC, Huang KY, Lin HH, Chen CL.** Association of rheumatoid arthritis and hepatitis B infection: a nationwide nested case-control study from 1999 to 2009 in Taiwan. Medicine 2016;95(18).

19-**Mansour AK, Aly RM, Abdelrazek SY, Elghannam DM, Abdelaziz SM, Shahine DA, et al.** Prevalence of HBV and HCV infection among multi-transfused Egyptian thalassemic patients. Hematology/oncology and stem cell therapy 2012 ;5(1):54-9.

20-**Tamori A, Koike T, Goto H, Wakitani S, Tada M, Morikawa H, et al.** Prospective study of reactivation of hepatitis B virus in patients with rheumatoid arthritis who received immunosuppressive therapy: evaluation of both HBsAg-positive and HBsAg-negative cohorts. Journal of gastroenterology 2011 ;46(4):556-64.