



ORIGINAL RESEARCH ARTICLE

Open Access



Correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis B patients: a systematic review and meta-analysis

Umami Maimunah^{1*} , Puspa Wardhani², Citrawati Dyah Kencono Wungu³, Anisa Firdausi Rahmanda⁴, Andreas Agung Kurniawan⁵ and Andrio Palayukan⁵

Abstract

Background HBV DNA assays have several limitations including being expensive and not widely available. Detection of HBsAg in serum has been the hallmark of HBV infection. However, previous studies regarding the association between HBsAg and HBV DNA revealed contradictory results. This study aims to reassess the correlation between HBsAg and HBV DNA in chronic hepatitis B patients.

Methods Observational studies with naïve chronic hepatitis B patients were included, while studies with other coinfections were excluded. The studies were identified by searching through Google Scholar, PubMed, ScienceDirect, and Springer Link for English and Bahasa articles from 2011 to 2021. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) was followed. Study quality was assessed using the Joanna Briggs Institute (JBI) critical appraisal.

Results A total of 17 studies with 4134 participants met the criteria. The overall analysis revealed a moderate correlation between quantitative HBsAg and quantitative HBV DNA in the total sample of chronic hepatitis B patients ($r=0.57$, 95% *CI* 0.40–0.75, $P<0.00001$). In HBeAg + group, a moderate correlation was indicated while in HBeAg – revealed a weak association ($r=0.55$, 95% *CI* 0.39–0.70, $P<0.00001$ vs $r=0.29$, 95% *CI* 0.20–0.38, $P<0.00001$). The strongest correlation was discovered in HBeAg + chronic HBV infection phase ($r=0.59$, 95% *CI* 0.35–0.82, $P<0.00001$).

Conclusion Serum HBsAg titer supports as a predictor of serum HBV DNA levels in clinical practice with moderate strength of correlation.

Trial registration This review had been registered in PROSPERO (ID: CRD42023421246).

Keywords HBsAg, HBV DNA, Chronic hepatitis B

*Correspondence:

Umami Maimunah
ummima@gmail.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Introduction

Chronic hepatitis B has evolved into a global health crisis. The World Health Organization estimates that 254 million individuals worldwide have chronic hepatitis B in 2022 [1]. This condition is caused by infection with the hepatitis B virus, which leads to liver inflammation. The outcome of acute versus chronic hepatitis B infection varies [2]. If hepatitis B infection becomes chronic and is not appropriately treated, it can lead to deadly consequences such as cirrhosis of the liver and hepatocellular carcinoma (HCC) [3].

The management of chronic hepatitis B necessitates multiple tests, including HBsAg, quantitative HBV DNA, and HBeAg testing. These tests are used to estimate the replication rate of the hepatitis B virus, which is useful for diagnosis, selecting therapeutic methods, and evaluating the therapeutic response [4]. HBsAg is an antigen protein that serves as an early signal when hepatitis B infection is suspected. In order to monitor the patient's status, periodic quantitative HBV DNA testing is required because high blood HBV DNA levels have been observed to dramatically increase the risk of liver cirrhosis [5]. HBeAg + chronic HBV infection, HBeAg + chronic hepatitis B, HBeAg – chronic HBV infection, and HBeAg – chronic hepatitis B [6] are the four phases of chronic hepatitis B that patients may encounter. This phase is characterized based on the HBV DNA test, HBeAg, ALT, and the presence of liver inflammation.

Numerous studies were conducted to evaluate the link between HBsAg and HBV DNA in individuals with chronic hepatitis B, but the results were inconclusive. This systematic review and meta-analysis of quantitative HBsAg in correlation with quantitative HBV DNA in naive chronic hepatitis B patients in various phases is based on controversial results from earlier research regarding the correlation between HBsAg and HBV DNA in chronic hepatitis B patients. This review is conducted to shed light on the nature of the link between these variables and to identify the mechanisms responsible for the differences so that the findings can be used in daily medical practice, particularly in the treatment of chronic hepatitis B infection. Performing quantitative HBV DNA analysis may not be feasible in all healthcare facilities and entails significant expenses. On the other hand, quantitative HBsAg can serve as a substitute indicator for HBV DNA in several capacities, such as assessing the level of viral replication [7]. We hypothesize a link between the quantitative levels of HBsAg and HBV DNA in naive chronic hepatitis B patients.

Materials and methods

Search strategy and identification of studies

We conducted a systematic review and meta-analysis to determine the relationship between HBsAg and HBV DNA in chronic hepatitis B patients with positive or negative HBeAg. The systematic review was registered in PROSPERO and reported using the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) checklist. Using a search keyword comprised of the terms correlation, quantitative HBsAg, HBV DNA, HBeAg, and chronic hepatitis B, literature search strategies were developed. We searched PubMed, Springer Link, ScienceDirect, and Google Scholar. Reviewers J. I. and D. F. screened abstracts based on standard inclusion and exclusion criteria. Two reviewers (A. A. and A. F.) independently assessed all studies identified for full manuscript review against inclusion criteria. The papers were either accepted or rejected, with the reasons for rejection being specified. Disagreements were resolved through dialogue between review authors.

Selection criteria

The following inclusion criteria were in place: Observational studies (cohort, case control, or cross-sectional), studies in English and Indonesian, studies with populations of chronic hepatitis B patients who have not received therapy, studies with a quantitative HBsAg correlation test with HBV DNA, and studies that include the HBeAg status of the patients.

We excluded sources from non-research studies (review articles, conference papers, or book chapters); duplicated studies; studies with chronic hepatitis B patients who have received therapy; studies with chronic hepatitis B patients co-infected with other diseases such as hepatitis C, hepatitis D, HIV, alcoholic liver disease, or NAFLD; studies for which the full text could not be obtained; and studies with insufficient data.

Data extraction and quality assessment

Author's name and publication year, study location and design, total sample size, male-to-female ratio, population age, quantitative HBsAg levels, quantitative HBV DNA levels, HBeAg, *P*-value, and correlation coefficient between HBsAg and HBV-DNA were extracted as variables.

The Joanna Briggs Institute (JBI) critical appraisal was used to evaluate the quality of each article collected to prevent bias [8]. The inclusion criteria included studies that have been assessed and subsequently agreed upon. Low-scoring studies were omitted to prevent bias in the validity of the results (Tables S1–S3).

Data analysis and synthesis

On the basis of the previously mentioned points, data extraction was conducted. The data were organized in a tabular and/or descriptive format to facilitate analysis. This study analyzed the correlation between quantitative HBsAg levels and quantitative HBV-DNA in chronic naive hepatitis B patients with either positive or negative HBeAg by comparing the results of each previous study. We conducted a meta-analysis by combining data using the random-effect method to calculate a pooled correlation coefficient with 95% confidence intervals (CI) and a subgroup analysis to investigate potential sources of heterogeneity. The following is a rough guide to the interpretation of I^2 :

- 0 to 40% suggests that heterogeneity may not be significant.
- 30 to 60% corresponds to moderate heterogeneity.
- 50 to 90% denotes substantial heterogeneity.
- 75 to 100% implies significant heterogeneity [9].

Review Manager software version 5.4.1 was used for data analysis. All statistical tests were two-tailed, and differences with $P < 0.05$ were considered statistically significant. Strong correlation represented by $r \geq 0.67$, moderate correlation by $0.33 < r < 0.67$, and weak moderate by $r \leq 0.33$. Publication bias is assessed through visualization of the funnel plot.

Results

Study selection and characteristics

A total of 909 citations were identified, and 47 full-text articles with matching populations and variables were examined, yielding a total of 17 studies that met the inclusion and exclusion criteria (Fig. 1). Fifteen out of 17 studies employed a cross-sectional methodology. One study utilized a cohort design and the other a case-control design. The studies included between 62 and 645 individuals as samples. The age of the samples ranged from 1 to 80 years old, with a mean age of between 33.50 and 49.30 years. Patients with chronic hepatitis B who have never received anti-HBV treatment make up all of the samples (Table 1).

Correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis B

The correlation between quantitative HBsAg and quantitative HBV DNA in the total sample of chronic hepatitis B patients was examined in 10 studies (Table 2). All studies discovered significant correlations. There is a strong correlation between both variables, according to three studies. Five additional studies demonstrated a modest correlation. Two studies have identified a weak

correlation between HBsAg and HBV DNA levels in patients with chronic hepatitis B.

There was substantial heterogeneity among the included studies ($I^2 = 84\%$, $P 0.00001$). Thus, a model with random effects was employed. Figure 2 illustrates the compiled results. The pooled correlation between quantitative HBsAg and quantitative HBV DNA in patients with chronic hepatitis B was 0.57 (95% confidence interval: 0.40–0.75, $P 0.00001$), indicating a moderate strength.

Correlation between quantitative HBsAg and quantitative HBV DNA in HBeAg + chronic hepatitis B

In addition, a subgroup analysis was conducted for the association between HBeAg+ and HBeAg- (Fig. 3) patients. The correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis B patients with HBeAg+ was reported in 10 studies (Table 3). One study found a moderate correlation, seven studies found a moderate correlation, and the remaining studies found a strong correlation. A meta-analysis of 10 studies utilizing a random-effects model revealed a moderate correlation ($r = 0.55$, 95% CI: 0.39–0.70, $P 0.00001$) between quantitative HBsAg and quantitative HBV DNA in HBeAg+.

Correlation between quantitative HBsAg and quantitative HBV DNA in HBeAg – chronic hepatitis B

Two included studies found no significant correlation between quantifiable HBsAg and quantifiable HBV DNA. Three studies indicated a weak correlation, while four studies demonstrated a moderate one. None of the included studies found a strong association. In the HBeAg- group, the pooled analysis revealed a weak correlation ($r = 0.29$, 95% CI: 0.20–0.38, $P 0.00001$).

Correlation between quantitative HBsAg and quantitative HBV DNA according to the phase of chronic hepatitis B

Additionally, we performed a subgroup analysis of the association based on the stage of chronic hepatitis B infection (Fig. 4). Chronic HBV infection is the initial phase of chronic hepatitis B. In this phase, four studies reported the correlation. One study found no correlation, one study found a moderate correlation, and two studies found a strong correlation. This group's pooled analysis revealed a moderate correlation ($r = 0.59$, 95% confidence interval: 0.35–0.82, $P 0.00001$). The relationship between quantitative HBsAg and quantitative HBV DNA in the second phase of HBeAg+ chronic hepatitis B was discovered by three studies. These studies found, respectively, a strong, moderate, and insignificant association. This group's pooled analysis revealed a moderate correlation ($r = 0.51$; 95% CI: 0.15–0.87; $P 0.00001$).

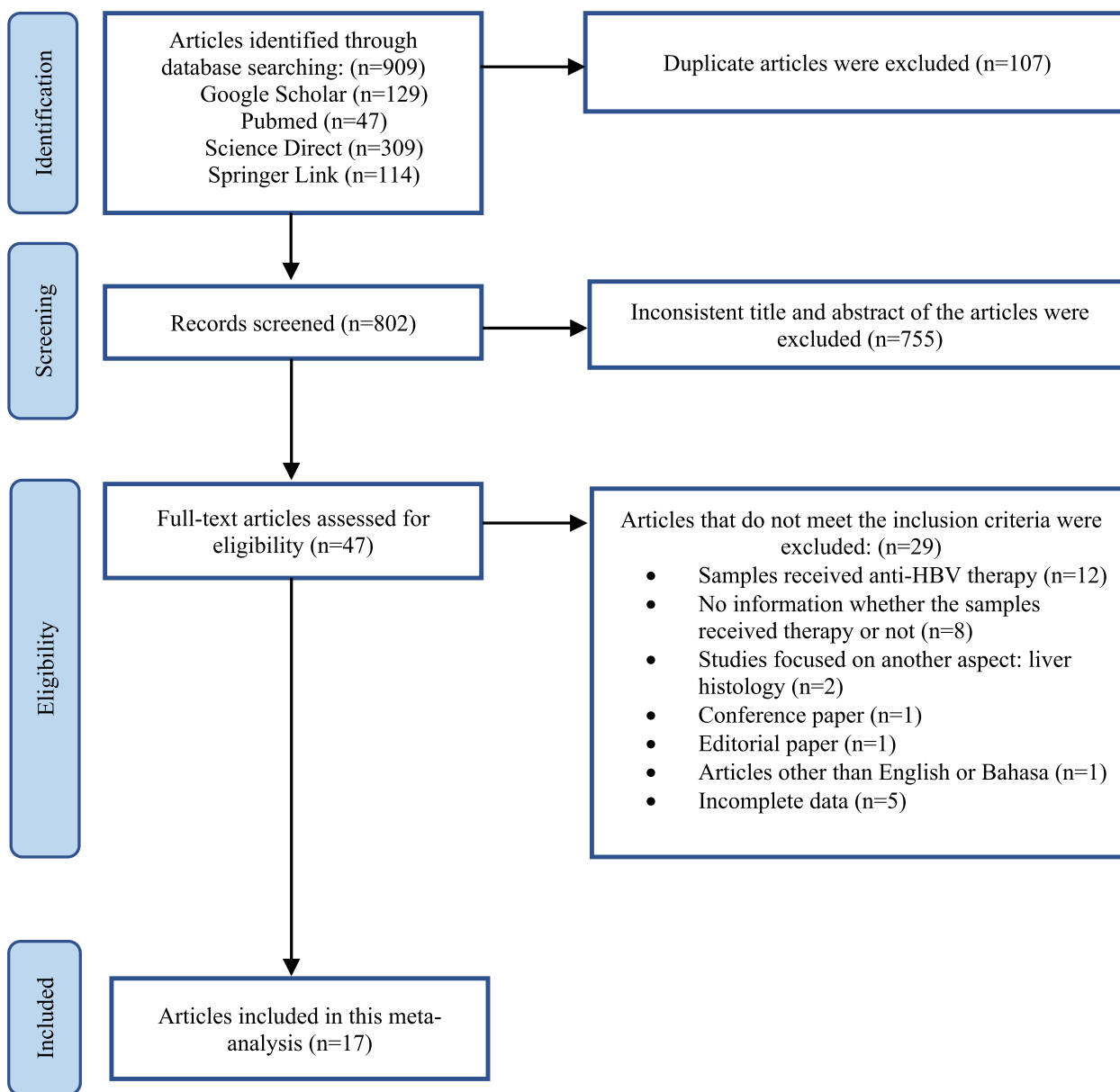


Fig. 1 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) study flowchart. *HBV*, hepatitis B virus

In the third phase, HBeAg-chronic HBV infection, the majority of the five studies that examined the correlation between quantitative HBsAg and quantitative HBV DNA, revealed no significant association, with the exception of one study by Alghamdi et al. There was a weak correlation discovered by Alghamdi et al. [14]. The pooled analysis of this group revealed no significant correlation ($r = 0.13$, 95% *CI*: -0.02 to 0.27 , $P = 0.08$). All of the studies included in this review that were conducted during the fourth phase of HBeAg-chronic hepatitis B found a moderate correlation

between quantitative HBsAg and quantitative HBV DNA. This group's pooled analysis revealed a moderate correlation ($r = 0.40$, 95% *CI*: 0.27 – 0.53 , $P 0.00001$) (Table 4).

Publication bias

The funnel plot revealed a possible publication bias in the pooled analysis of the correlation between quantitative HBsAg and quantitative HBV DNA in patients with chronic hepatitis B (Figs. S1–S3).

Table 1 Study characteristics

No	Study	Study design	Country	Total sample	Age	Genotype
1	Balkan et al. (2016) [10]	Cross-sectional	Turkey	204	33.54 ± 11.74	No data
2	Tan et al. (2014) [11]	Cross-sectional	China	233	37 ± 12	B, C
3	Cheng et al. (2013) [12]	Cross-sectional	Taiwan	198	36.4 ± 10.5	B, C
4	Keshvari et al. (2015) [13]	Cross-sectional	Iran	151	40.9 ± 14.2	D
5	Alghamdi et al. (2013) [14]	Cross-sectional	Saudi Arabia	106	39.3 (21–75)	D
6	Primadharsini et al. (2013) [15]	Cross sectional	Indonesia	62	42.34 ± 13.07	No data
7	Hong et al. (2014) [16]	Cross sectional	China	362	35.56 ± 9.9	B, C
8	Goyal et al. (2014) [17]	Cross-sectional	India	481	31 (14–65)	A, D
9	Antaki et al. (2012) [18]	Cross-sectional	Syria	272	33 (1–76)	D
10	Turyadi et al. (2013) [19]	Cross-sectional	Indonesia	152	44 (14–80)	B, C
11	Martinot-Peignoux et al. (2013) [20]	Cross-sectional	France	406	40 ± 12	A, B, C, D, E
12	Bathaix et al. (2015) [21]	Retrospective cross-sectional	West Africa	105	39.01 ± 9.72	E
13	Nasser et al. (2021) [22]	Cross-sectional	Egypt	92	36.1 ± 10.5	D
14	Puspitasari et al. (2021) [23]	Case control	Indonesia	70	36.86 ± 12.732	No data
15	Zhang et al. (2021) [24]	Retrospective cohort	China	472	(28–51)	No data
16	Tatar et al. (2020) [25]	Retrospective cross-sectional	Turkey	123	48 ± 11.2	D
17	Kim et al. (2011) [26]	Retrospective cross-sectional	Korea	645	49.38 ± 11.85	C

Table 2 Correlation between quantitative HBsAg and quantitative HBV DNA in total sample of chronic hepatitis patients

Study	Sample (n)	HBsAg ^a	HBV DNA ^a	r	P	Strength of correlation
Antaki et al. (2012) [18]	272	3.67 (3.45–3.62) log IU/mL	3.83 (4.16–4.69) log IU/mL	0.830	<0.008	Strong
Balkan et al. (2016) [10]	100	5150.78 ± 8473.16 IU/mL	59,900.47 ± 140,555.35 IU/mL	0.533	<0.001	Moderate
Hong et al. (2014) [16]	362	3.80 ± 0.58 log IU/mL	6.05 ± 2.08 log IU/mL	0.305	<0.0001	Weak
Kim et al. (2011) [26]	645	2.92 ± 1.26 log IU/mL	4.41 ± 2.51 log IU/mL	0.693	<0.001	Moderate
Nasser et al. (2021) [22]	92	No data	No data	0.750	<0.001	Strong
Primadharsini et al. (2013) [15]	62	No data	No data	0.737	0	Strong
Puspitasari et al. (2021) [23]	70	No data	No data	0.599	<0.001	Moderate
Tan et al. (2014) [11]	233	3.61 ± 0.68 log IU/mL	No data	0.637	<0.001	Moderate
Tatar et al. (2020) [25]	123	4625.9 ± 5614.9 IU/mL	11,377.6 ± 25,598.4 IU/mL	0.303	0.001	Weak
Turyadi et al. (2013) [19]	152	3.25 (1.30–4.99) log IU/mL	4.24 (0.04–8.14) log IU/mL	0.659	<0.001	Moderate

^a Data is presented in mean ± standard deviation or median (range)

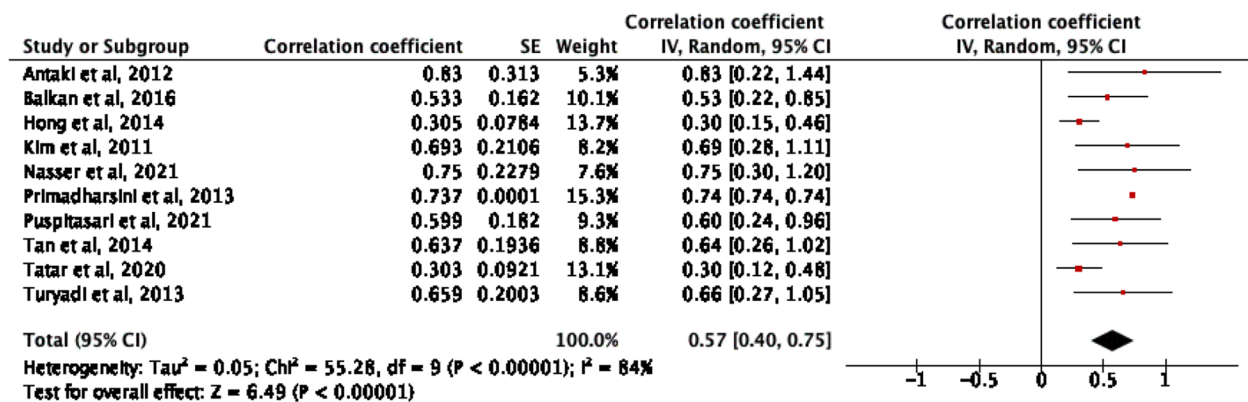


Fig. 2 Forest plot of the correlation between quantitative HBsAg and quantitative HBV DNA in the total sample

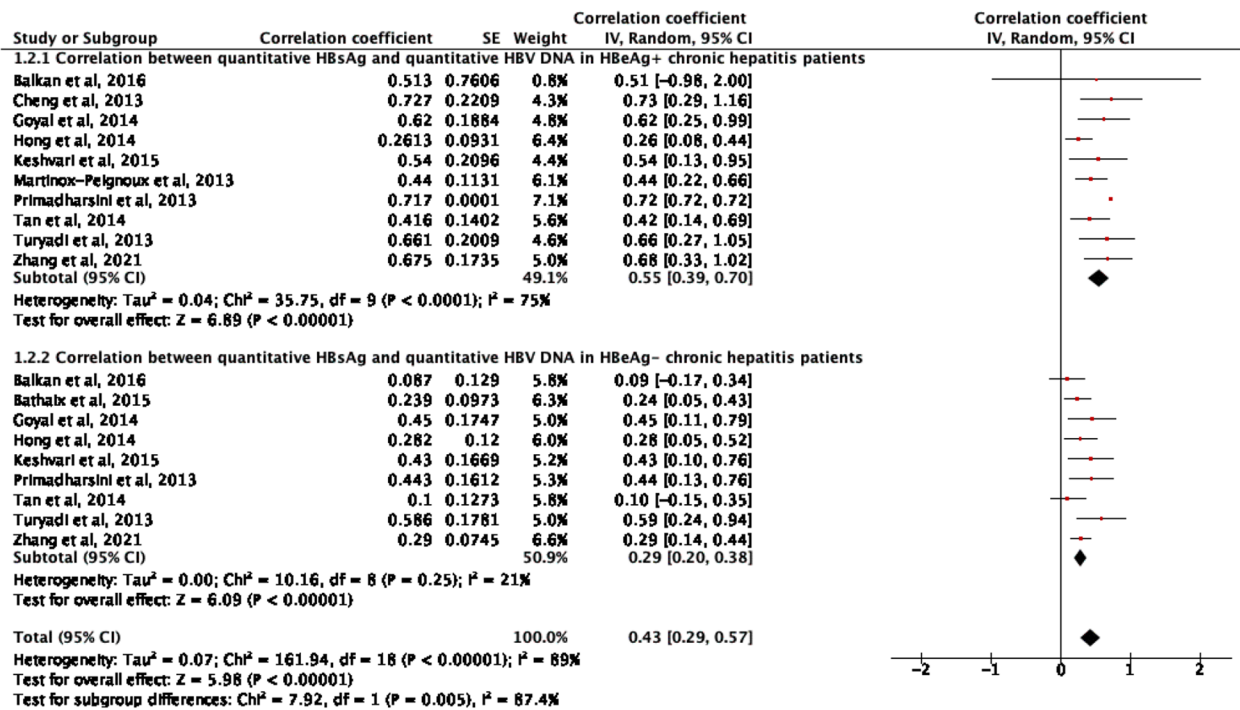


Fig. 3 Forest plot of the correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis patients based on HBeAg status

Table 3 Correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis patients based on HBeAg status

Study	Sample (n)	HBsAg ^a	HBV DNA ^a	r	P	Strength of correlation
HBeAg +						
Balkan et al. (2016) [10]	38	No data	No data	0.513	0.001	Moderate
Cheng et al. (2013) [12]	198	4.3 (1.7–5.4) log IU/mL	8.7 (4.8–10.0) log copies/mL	0.727	< 0.001	Strong
Goyal et al. (2014) [17]	126	4.60 (1.26–6.26) log IU/mL	8.39 (2.10–11.56) log IU/mL	0.62	< 0.001	Moderate
Hong et al. (2014) [16]	210	3.87 ± 0.64 log IU/mL	7.17 ± 1.45 log IU/mL	0.2613	0.005	Weak
Keshvari et al. (2015) [13]	30	4.6 (UR) log IU/mL	9.0 (UR) log IU/mL	0.54	< 0.01	Moderate
Martinox-Peignoux et al. (2013) [20]	101	4.24 ± 0.90 log IU/mL	7.06 ± 1.71 log IU/mL	0.44	< 0.0001	Moderate
Primadharsini et al. (2013) [15]	25	2.81 × 10 ⁵ ± 1.3 × 10 ⁶ log IU/mL	5.9 × 10 ⁷ ± 5.45 × 10 ⁷ copies/mL	0.717	0	Strong
Tan et al. (2014) [11]	49	4.17 ± 0.66 log IU/mL	7.0 ± 0.8 log IU/mL	0.416	0.003	Moderate
Turyadi et al. (2013) [19]	65	No data	No data	0.661	< 0.001	Moderate
Zhang et al. (2021) [24]	241	No data	No data	0.675	< 0.0001	Moderate
HBeAg -						
Balkan et al. (2016) [10]	62	No data	No data	0.087	0.5	Not significant
Bathaix et al. (2015) [21]	105	12,111.2 ± 10,617.4 IU/mL	4.4 e7 ± 7.5 e7 IU/mL	0.239	0.014	Weak
Goyal et al. (2014) [17]	355	3.47 (1.11–4.66) log IU/mL	3.40 (0.30–7.94) log IU/mL	0.45	< 0.01	Moderate
Hong et al. (2014) [16]	152	3.68 ± 0.44 log IU/mL	4.20 ± 1.58 log IU/mL	0.282	0.0188	Weak
Keshvari et al. (2015) [13]	121	3.6 log IU/ml	5.1 log IU/ml	0.43	< 0.01	Moderate
Primadharsini et al. (2013) [15]	37	4.9 × 10 ³ ± 2.05 × 10 ⁴ IU/mL	7.53 × 10 ⁶ ± 2.55 × 10 ⁷ copies/mL	0.443	0.006	Moderate
Tan et al. (2014) [11]	64	3.23 ± 0.40 log IU/mL	4.8 ± 1.0 log IU/mL	0.1	0.432	Not significant
Turyadi et al. (2013) [19]	87	No data	No data	0.586	< 0.001	Moderate
Zhang et al. (2021) [24]	106	No data	No data	0.29	< 0.0001	Weak

^a Data is presented in mean ± standard deviation or median (range)

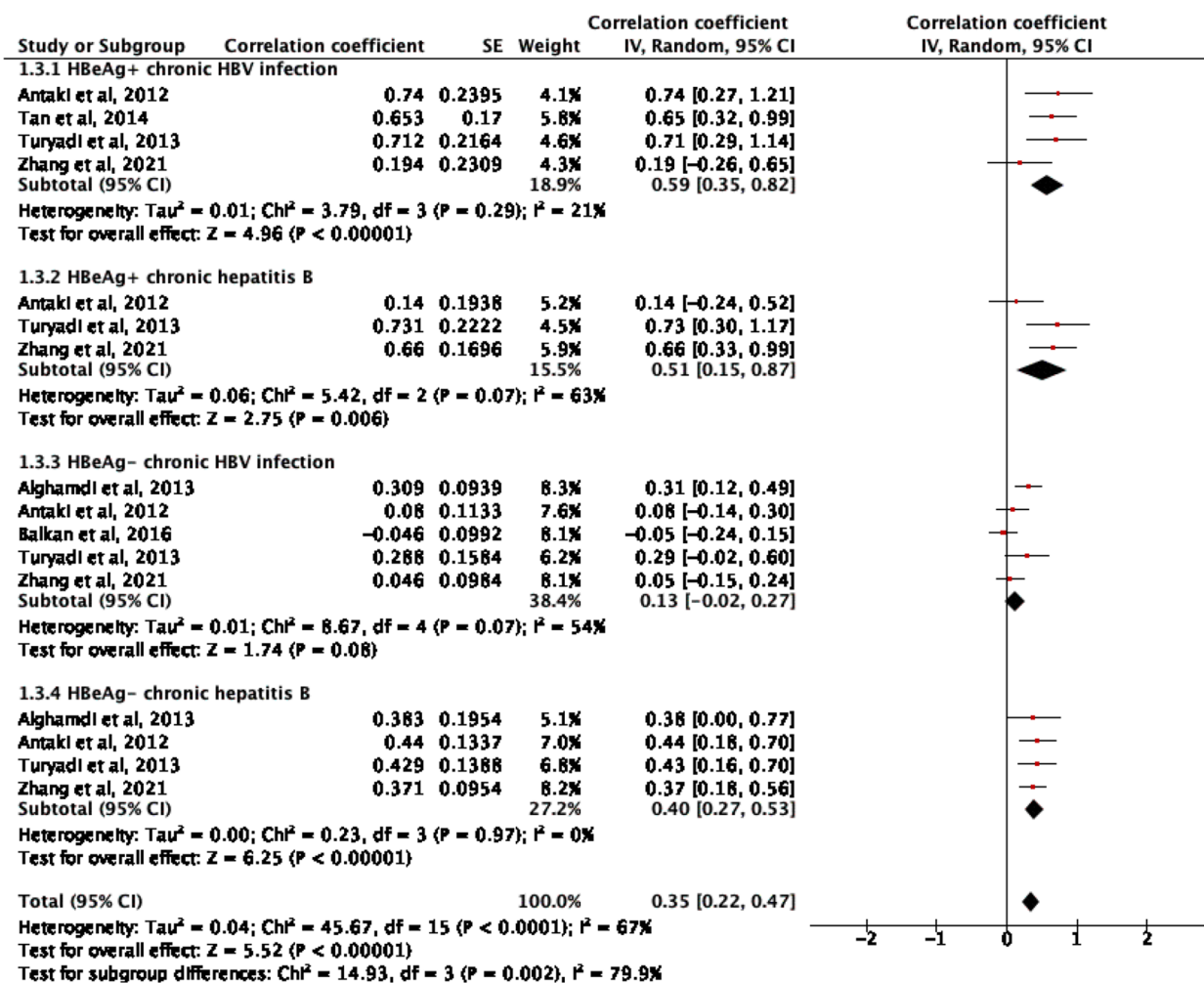


Fig. 4 Forest plot of the correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis patients according to the phase of chronic hepatitis B infection course

Discussion

This systematic review and meta-analysis sought to determine the relationship between HBsAg and HBV DNA levels in chronic hepatitis B patients. The correlation analysis could depict the dynamic of HBV DNA that reflected active replication and quantitative HBsAg that demonstrated immune control and viral transcription activity in hepatocytes (intrahepatic cccDNA). A significant correlation between the two variables would permit the substitution of quantitative HBsAg.

According to 10 studies included in this review, there is a correlation between the level of quantitative HBsAg and HBV DNA in the general population of hepatitis B patients with chronic infection. Five of 10 studies demonstrated a moderate correlation, whereas 3 and 2 studies, respectively, demonstrated a strong and weak correlation. Several factors may account for the disparities in the strength of correlation among these studies.

All studies that discovered this correlation in the total sample of patients with chronic hepatitis B had mean and median ages ranging from 30 to 50 years old. Tan et al. [11] found that age has a significant negative correlation with HBsAg level. Patients with hepatitis B who are younger are primarily in the HBeAg+ chronic HBV infection phase. This phase lasts longer in vertically infected patients than in horizontally infected patients. In addition, the immune system has not yet been activated to combat the hepatitis B virus during this phase. It is manifested by an increase in HBV DNA levels with normal ALT levels and nonspecific histological changes [27]. The immune system, which is continuously active against the hepatitis B virus, will suppress the production of HBsAg as a person ages.

The variation in phenotype may influence the production of HBsAg and HBV DNA. The levels of HBeAg and HBV DNA are higher in genotypes B and C than in

Table 4 Correlation between quantitative HBsAg and quantitative HBV DNA according to the phase of chronic hepatitis B infection course

Study	Sample (n)	HBsAg ^a	HBV DNA ^a	r	P	Strength of correlation
HBeAg + chronic HBV infection						
Antaki et al. (2012) [18]	9	20,781 IU/mL	7.6×10^7 IU/mL	0.74	0.002	Strong
Tan et al. (2014) [11]	29	4.50 ± 0.43 log IU/mL	7.1 ± 0.7 log IU/mL	0.653	0.000123	Moderate
Turyadi et al. (2013) [19]	33	$4.22 (-1.3-4.99)$ log IU/mL	$4.55 (0.78-7.82)$ log IU/mL	0.712	<0.001	Strong
Zhang et al. (2021) [24]	21	4.779 log IU/mL	7.878 log IU/mL	0.194	0.4007	Not significant
HBeAg + chronic hepatitis B						
Antaki et al. (2012) [18]	26	26,774 IU/mL	4.1×10^7 IU/mL	0.14	0.47	Not significant
Turyadi et al. (2013) [19]	32	$3.34 (-1.3-4.99)$ log IU/mL	$5.13 (0.44-8.04)$ log IU/mL	0.731	<0.001	Strong
Zhang et al. (2021) [24]	220	3.943 log IU/mL	7.167 log IU/mL	0.66	<0.0001	Moderate
HBeAg – chronic HBV infection						
Alghamdi et al. (2013) [14]	78	1235.6 (0.1–34,985) log IU/mL	371 (undetected–2123) log IU/mL	0.309	<0.001	Weak
Antaki et al. (2012) [18]	131	2112 IU/mL	50 IU/mL	0.08	0.48	Not significant
Balkan et al. (2016) [10]	104	5150.78 ± 8473.16 IU/mL	$0.640 \times 10^3 \pm 0.584$ IU/mL	-0.046	0.643	Not significant
Turyadi et al. (2013) [19]	34	$2.52 (-1.3-3.93)$ log IU/mL	$1.73 (0.04-3.87)$ log IU/mL	0.288	0.069	Not significant
Zhang et al. (2021) [24]	106	3.059 log IU/mL	2.699 log IU/mL	0.046	0.64	Not significant
HBeAg – chronic hepatitis B						
Alghamdi et al. (2013) [14]	28	$3.09 (-1-4.4)$ log IU/mL	$20,958 (2.3 \times 10^3-1.9 \times 10^8)$ log IU/mL	0.383	<0.05	Moderate
Antaki et al. (2012) [18]	106	5184 IU/mL	19,500 IU/mL	0.44	<0.001	Moderate
Turyadi et al. (2013) [19]	53	$3.37 (-1.3-4.65)$ log IU/mL	$4.83 (0.71-8.14)$ log IU/mL	0.429	0.002	Moderate
Zhang et al. (2021) [24]	125	3.409 log IU/mL	5.057 log IU/mL	0.371	<0.0001	Moderate

^a Data is presented in mean \pm standard deviation or median (range)

genotypes A and D [28]. How genotype variation contributes to these clinical markers remains unknown. Cheng et al. [12] found that genotype B correlates with HBsAg secretion and HBV DNA replication more strongly than genotype C. While Tuaillon et al. [29] demonstrated that HBsAg tends to correlate with HBV DNA in genotype A but not in genotype D, HBsAg does not tend to correlate with HBV DNA in genotype D.

Aside from the aforementioned variables, mutations in the hepatitis B virus may also be responsible for the difference in correlation. No studies on mutations are included in this review, but mutations can influence the production of HBsAg and HBV DNA. Zafrullah et al. [30] found that mutations of the S gene affect HBsAg expression, with the exception of genotype A2. There are mutations in all open reading frames (ORFs) of the hepatitis B virus, including preS/S, polymerase, precore/core, and X. The preS/S open reading frame (ORF) encodes three different molecules that will form HBsAg, so mutations in this ORF will result in distinct HBsAg production [31].

In chronic hepatitis B, HBsAg levels are significantly higher in patients with positive HBeAg, as are HBV DNA levels [17, 22]. According to the pooled analysis, the correlation between the two variables in HBeAg-positive individuals was significant and moderately strong,

whereas in HBeAg-negative individuals, the correlation was weak.

The disconnection between quantitative HBsAg levels and HBV DNA in HBeAg–patients could be due to a number of factors. First, the dynamic interaction between HBV and host immunity may stimulate HBV replication during the HBeAg–chronic HBV infection phase and vice versa. Second, the HBsAg synthesis pathway is distinct from the HBV DNA replication pathway as a result of distinct immune control mechanisms [14]. Mutations in the pre-core promoter may impair the secretion of HBV virions, the primary source of HBV DNA. Also, reducing HBsAg secretion is mutations in the pre-S region. As these mutations do not always occur simultaneously, the production of HBsAg and HBV DNA becomes unbalanced, thereby reducing the correlation between HBsAg and HBV DNA. The correlation between HBsAg and HBV DNA was observed in individuals with wild-type PreS/S sequences but not in subgroups with BCP (basal core promoter) double mutations or PreC mutations [32].

The natural progression of chronic hepatitis B in the absence of HBeAg can be highly variable and frequently unpredictable. HBsAg titers should be correlated with HBV DNA concentrations. HBsAg is an HBV replication

product [33]. This correlation may be invalidated, however, because HBV gene expression is governed by distinct mechanisms and the inhibition of HBV DNA replication. Mutations may affect the secretion of HBV virion and HBsAg, but mutations typically occur at different times, resulting in unequal production and a weakened correlation between the variables [32].

Separate dynamics govern the replication of HBV DNA and the production of HBsAg, which is an additional consideration. In the absence of viral replication, a nonessential aspect of HBV's life cycle produces HBsAg, causing the number of HBsAg to exceed that of virions. HBsAg transcription and secretion could be spared if viral replication is controlled posttranscriptionally [34].

HBsAg levels are highest during the first phase, HBeAg+ chronic HBV infection; then decrease during the second phase, HBeAg+ chronic hepatitis B; and continue to decline when entering the third phase, HBeAg– chronic HBV infection. When entering the fourth phase of HBeAg– chronic hepatitis B, HBsAg levels will rise again. Quantitative HBV DNA levels also exhibit a similar pattern of increase and decrease. Because HBV virions and antigens undergo minimal or no immune response at the onset of infection, their levels will be elevated. As the phase progresses, the immune system will remain active, resulting in a decline in HBsAg, which can eventually lead to seroconversion in the third phase, HBeAg-negative chronic HBV infection. In the fourth phase, the re-increase of HBsAg indicates the reactivation of the disease, which causes liver damage.

Due to differences in HBV transcription that are minimally influenced by the host immune response, quantitative HBsAg and HBV DNA may not be significantly correlated. HBsAg can also be produced not only from intrahepatic cccDNA but also from HBV sequences that have been integrated [24]. In the HBeAg– chronic HBV infection phase, the host immune system is active, suppressing HBV DNA levels; however, this phase has the potential to reactivate and transition to the HBeAg– chronic hepatitis B infection phase and vice versa. In these instances, regardless of HBsAg levels, HBV DNA levels decrease to undetectable levels. Numerous patients in this phase have undetectable HBV DNA and elevated levels of HBsAg. Antaki et al. discovered that the HBsAg/HBV DNA ratio was highest during the third phase. This suggests that immune system regulation does not always inhibit HBsAg production [18].

This investigation yielded significant heterogeneity. One possible explanation for these findings is the variation in clinical characteristics among the studies that were included. Significant disparities in age might contribute to a high level of heterogeneity. The inclusion of various research designs, such as cross-sectional,

case–control, and cohort studies, in this analysis further contributes to the heterogeneity seen. Geographical variations can also exert an influence. Funnel plot asymmetry in the meta-analysis can be attributed to several variables, resulting in the majority of studies being situated near the tip of the funnel plot. These issues encompass publication bias, heterogeneity, and methodological quality in research characterized by smaller sample sizes [35].

We acknowledge that this study contains several flaws. In order to assess the maintenance of the relationship between HBsAg and HBV DNA in this context, we exclude patients receiving antiviral therapy. The correlation found in a few studies reviewed in this article was not established as the primary result. The majority of study designs were cross-sectional, so there was no sample follow-up. A further drawback is that some studies omitted variable data, which may have an impact on the analysis of the final result. To clarify this association, additional research with a larger number of samples and studies is necessary.

In this meta-analysis, studies included have various sample sizes where most of them had small sample size. This could lead to small study effect, which is a phenomenon where smaller studies may show different, often larger effects than larger ones. Smaller studies may be more susceptible to biases because they were more likely to report larger beneficial effects than larger studies [36]. Smaller studies may also overestimate the effect size, which could lead to erroneous conclusions [37].

Conclusion

In conclusion, our study established a moderate correlation between HBsAg and HBV DNA in the entire cohort of patients with chronic hepatitis B. These findings require confirmation in larger studies with more comprehensive methods, characteristics, and criterion to reduce bias. The result of this study must be interpreted cautiously due to the possibility of publication bias and small study effect.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43066-024-00336-5>.

Additional file 1: Supplementary figures. **Fig. S1.** Funnel plot Correlation between quantitative HBsAg and quantitative HBV DNA in total sample of chronic hepatitis B patients. **Fig. S2.** Funnel plot Correlation between quantitative HBsAg and quantitative HBV DNA in chronic hepatitis B patients according to the HBeAg status. **Fig. S3.** Funnel plot Correlation between quantitative HBsAg and quantitative HBV DNA based on chronic HBV infection course. **Supplementary tables:** **Table S1.** Cross-sectional study critical appraisal. **Table S2.** Case-control study critical appraisal. **Table S3.** Cohort study critical appraisal.

Acknowledgements

None

Authors' contributions

UM: Conceptualization, project design, supervision, materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted. PW: Conceptualization, project design, supervision, materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted. CDKW: Conceptualization, project design, supervision, materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted. AFR: Materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted. AAK: Materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted. AP: Materials, literature search, data collection and/or processing, data analysis and/or interpretation, writing and/or review the manuscript, and final approval of the version to be submitted.

Funding

No funding for this study.

Availability of data and materials

The authors confirm that the data supporting the findings of this review are available within the article and/or its supplementary materials.

Declarations**Ethics approval and consent to participate**

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Division of Gastroenterohepatology, Faculty of Medicine, Airlangga University, Surabaya 60286, Indonesia. ²Department of Clinical Pathology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ³Department of Physiology and Medical Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ⁴Faculty of Medicine, Airlangga University, Surabaya, Indonesia. ⁵Department of Internal Medicine, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

Received: 11 September 2023 Accepted: 24 April 2024

Published online: 21 May 2024

References

- WHO. Hepatitis B 2024. <https://www.who.int/news-room/fact-sheets/detail/hepatitis-b> (accessed 21 Apr 2024)
- Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z et al (2022) Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol* 7:796–829. [https://doi.org/10.1016/S2468-1253\(22\)00124-8](https://doi.org/10.1016/S2468-1253(22)00124-8)
- Tripathi N, Mousa OY (2022) Hepatitis B
- Liang TJ (2009) Hepatitis B: The virus and disease. *Hepatology* 49:513–21. <https://doi.org/10.1002/hep.22881>
- Qu C, Huang X, Liu K, Li K, Tan B, Qu L et al (2019) Effect of hepatitis B virus DNA replication level and anti-HBV therapy on microvascular invasion of hepatocellular carcinoma. *Infect Agent Cancer* 14:2. <https://doi.org/10.1186/s13027-019-0219-8>
- European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver (2017) EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 67:370–398. <https://doi.org/10.1016/j.jhep.2017.03.021>
- Ozaras R, Tabak F, Tahan V, Ozturk R, Akin H, Mert A et al (2008) Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. *Dig Dis Sci* 53:2995–2998. <https://doi.org/10.1007/s10620-008-0263-5>
- Barker TH, Stone JC, Sears K, Klugar M, Leonardi-Bee J, Tufanaru C et al (2023) Revising the JBI quantitative critical appraisal tools to improve their applicability: an overview of methods and the development process. *JBI Evid Synth* 21:478–93. <https://doi.org/10.11124/JBIES-22-00125>
- Higgins JPT, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* 21:1539–1558. <https://doi.org/10.1002/sim.1186>
- Balkan A, Namiduru M, Balkan Y, Mete A, Karaoglan I, Bosnak V (2016) Are serum quantitative hepatitis b surface antigen levels, liver histopathology and viral loads related in chronic hepatitis b-infected patients? *Saudi J Gastroenterol* 22:208. <https://doi.org/10.4103/1319-3767.182454>
- Tan Z, Li M, Kuang X, Tang Y, Fan Y, Deng G et al (2014) Clinical implications of hepatitis B surface antigen quantitation in the natural history of chronic hepatitis B virus infection. *J Clin Virol* 59:228–234. <https://doi.org/10.1016/j.jcv.2014.01.013>
- Cheng P-N, Tsai H-W, Chiu Y-C, Ho C-H, Wu I-C, Chang T-T (2013) Clinical significance of serum HBsAg levels and association with liver histology in HBeAg positive chronic hepatitis B. *J Clin Virol* 57:323–330. <https://doi.org/10.1016/j.jcv.2013.04.012>
- Keshvari M, Alaviani SM, Sharafi H (2015) Comparison of serum hepatitis B virus DNA and HBsAg levels between HBeAg-negative and HBeAg-positive chronic hepatitis B patients. *Jundishapur J Microbiol* 8:e21444. <https://doi.org/10.5812/jjm.21444>
- Alghamdi A, Aref N, El-Hazmi M, Al-Hamoudi W, Alswat K, Helmy A et al (2013) Correlation between hepatitis B surface antigen titers and HBV DNA levels. *Saudi J Gastroenterol* 19:252. <https://doi.org/10.4103/1319-3767.121035>
- Primadharsini PP, Wibawa IDN (2013) Correlation between quantitative HBsAg and HBV-DNA in chronic hepatitis B infection. *Indones J Gastroenterol, Hepatol Dig Endosc* 14:9–12. <https://doi.org/10.24871/14120139-12>
- Hong M-Z, Huang W-Q, Min F, Xu J-C, Lin Z, Fang K-N et al (2014) Enhanced HBsAg synthesis correlates with increased severity of fibrosis in chronic hepatitis B patients. *PLoS ONE* 9:e87344. <https://doi.org/10.1371/journal.pone.0087344>
- Goyal SK, Jain AK, Dixit VK, Shukla SK, Kumar M, Ghosh J et al (2015) HBsAg level as predictor of liver fibrosis in HBeAg positive patients with chronic hepatitis B virus infection. *J Clin Exp Hepatol* 5:213–220. <https://doi.org/10.1016/j.jceh.2015.04.008>
- Antaki N, Zeidane N, Alhaj N, Hadad M, Baroudi O, Antaki F et al (2012) HBsAg titers in the different phases of hepatitis B infection in Syrian patients. *J Clin Virol* 53:60–64. <https://doi.org/10.1016/j.jcv.2011.10.004>
- Turyadi, Thedja MD, Ie SI, Harahap AR, Elkhobar KE, Roni M et al (2013) HBsAg, HBeAg and HBV DNA level changes and precore/basal core promoter mutations in the natural history of chronic hepatitis B in Indonesian patients. *Hepatol Int* 7:969–80. <https://doi.org/10.1007/s12072-013-9438-z>
- Martinot-Peignoux M, Carvalho-Filho R, Lapalus M, Netto-Cardoso ACF, Lada O, Batrla R et al (2013) Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naïve, e antigen-positive patients. *J Hepatol* 58:1089–1095. <https://doi.org/10.1016/j.jhep.2013.01.028>
- Bathaix MFY, Soro D, Bangoura AD, Doffou AS, Koné S, Kissy YH et al (2015) Hepatitis B surface antigen serum level is correlated with fibrosis severity in treatment-naïve, chronic hepatitis B patients in Côte d'Ivoire (West Africa)? *Open J Gastroenterol* 05:164–172. <https://doi.org/10.4236/ojgas.2015.511026>
- Nasser M, Zayed N, Gamal Eldeen H, Abdo M, Kabara Y, Elserafy M (2021) Inter-method variability of hepatitis B surface antigen quantification in a cohort of Egyptian patients with chronic hepatitis B virus. *Arab J Gastroenterol* 22:151–157. <https://doi.org/10.1016/j.ajg.2021.05.003>
- Puspitasari Y, Wardhani P, Fitriyah M, Hasudungan E, Atika, Maimunah U, et al (2021) Profile quantitative hepatitis B surface antigen (qHBsAg) of chronic naïve hepatitis B patients in Dr. Soetomo Hospital, Surabaya, Indonesia. *Indian J Forensic Med Toxicol*. <https://doi.org/10.37506/ijfimt.v15i2.14941>

24. Zhang Z, Shi B, Lu W, Huang D, Wang Y, Feng Y (2021) Quantitative serum HBV markers in predicting phases of natural history of chronic HBV infection. *J Virol Methods* 296:114226. <https://doi.org/10.1016/j.jviromet.2021.114226>
25. Tatar B, Acar A, Adar P, Kose S (2020) Role of quantitative hepatitis B surface antigen levels in predicting liver biopsy time in treatment-naive chronic hepatitis B patients. *Clin Exp Hepatol* 6:55–59. <https://doi.org/10.5114/ceh.2020.93058>
26. Kim YJ, Cho HC, Choi MS, Lee JH, Koh KC, Yoo BC et al (2011) The change of the quantitative HBsAg level during the natural course of chronic hepatitis B. *Liver Int* 31:817–823. <https://doi.org/10.1111/j.1478-3231.2011.02516.x>
27. Croagh CM, Lubel J (2014) Natural history of chronic hepatitis B: phases in a complex relationship. *World J Gastroenterol* 20:10395. <https://doi.org/10.3748/wjg.v20.i30.10395>
28. Sunbul M (2014) Hepatitis B virus genotypes: global distribution and clinical importance. *World J Gastroenterol* 20:5427. <https://doi.org/10.3748/wjg.v20.i18.5427>
29. Tuaillon E, Mondain A-M, Nagot N, Ottomani L, Kania D, Nogue E et al (2012) Comparison of serum HBsAg quantitation by four immunoassays, and relationships of HBsAg level with HBV replication and HBV genotypes. *PLoS ONE* 7:e32143. <https://doi.org/10.1371/journal.pone.0032143>
30. Zafarullah M, Vazquez C, Mixson-Hayden T, Purdy MA (2021) In vitro characterization of six hepatitis B virus genotypes from clinical isolates using transfecting linear HBV genomes. *J Gen Virol* 102. <https://doi.org/10.1099/jgv.0.001675>
31. Caligiuri P (2016) Overview of hepatitis B virus mutations and their implications in the management of infection. *World J Gastroenterol* 22:145. <https://doi.org/10.3748/wjg.v22.i1.145>
32. Liu M-H, Chen Q-Y, Harrison TJ, Li G-J, Li H, Wang X-Y et al (2015) The correlation between serum HBsAg levels and viral loads depends upon wild-type and mutated HBV sequences rather than the HBeAg/anti-HBe status. *J Med Virol* 87:1351–1360. <https://doi.org/10.1002/jmv.24186>
33. Locarnini S, Bowden S (2012) Hepatitis B surface antigen quantification: not what it seems on the surface. *Hepatology* 56:411–414. <https://doi.org/10.1002/hep.25732>
34. Seto W-K, Wong D-K, Fung J, Hung IF-N, Yuen JC-H, Tong T et al (2013) Serum hepatitis B surface antigen (HBsAg) kinetics in hepatitis B e antigen (HBeAg)-negative chronic hepatitis B. *Hepatol Int* 7:119–26. <https://doi.org/10.1007/s12072-012-9373-4>
35. Sterne JAC, Harbord RM (2004) Funnel plots in meta-analysis. *SJ* 4:127–141. <https://doi.org/10.1177/1536867X0400400204>
36. Zhang Z, Xu X, Ni H (2013) Small studies may overestimate the effect sizes in critical care meta-analyses: a meta-epidemiological study. *Crit Care* 17:R2. <https://doi.org/10.1186/cc11919>
37. Hong C, Salanti G, Morton SC, Riley RD, Chu H, Kimmel SE et al (2020) Testing small study effects in multivariate meta-analysis. *Biometrics* 76:1240–1250. <https://doi.org/10.1111/biom.13342>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

KARYA AKHIR

**HUBUNGAN ANTARA HBsAg DENGAN DNA VHB PADA PASIEN
HBeAg POSITIF DAN HBeAg NEGATIF HEPATITIS B KRONIK YANG
MENDAPAT TERAPI NA**



Oleh:
Rennilia Nita, dr
011828156302

Pembimbing:

Prof. Dr. Aryati, dr., MS., Sp.PK(K)
Dr. Puspa Wardhani, dr., Sp.PK(K)
Ummi Maimunah, dr., Sp.PD, K-GEH, FINASIM
Dr. Ir. Mahmudah, M.Kes

PROGRAM STUDI
PENDIDIKAN DOKTER SPESIALIS PATOLOGI KLINIK
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2023

LEMBAR PENGESAHAN

KARYA AKHIR

" Hubungan antara HbxAg dengan DNA VHB pada pasien HbeAg positif dan HbeAg negatif Hepatitis B Kronik yang mendapat Terapi NA"

Peneliti:
Rennilia Nita, dr

Karya akhir ini telah diuji oleh Tim Penguji dalam rangka memenuhi persyaratan Program Pendidikan Dokter Spesialis Patologi Klinik Fakultas Kedokteran Universitas Airlangga/ RSUD Dr. Soetomo Surabaya
Tanggal 12 April 2023

Pembimbing I



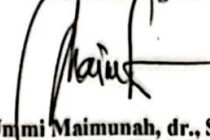
Prof. Dr. Aryati, dr., MS.,
Sp.PK(K)
NIP. 19630815 199002 2 001

Pembimbing II



Dr. Puspa Wardhani, dr.,
Sp.PK(K)
NIP19750225 200604 2 001

Pembimbing III



Unmi Maimunah, dr., Sp.PD,
K-GEH, FINASIM
NIP19640110 198903 2 012

**Ketua Program Studi
Departemen Patologi Klinik
FK UNAIR/RSUD DR SOETOMO**



Dr. Puspa Wardhani, dr., Sp.PK(K)
NIP19750225 200604 2 001

**Kepala Urusan Litbang
Departemen Patologi Klinik
FK UNAIR/RSUD DR SOETOMO**



Prof. Dr. Jusak Nugraha, dr., MS., Sp.PK(K)
NIP 19560214 198502 1 001

**Ketua Departemen Patologi Klinik
FK UNAIR/RSUD Dr. SOETOMO Surabaya**



**Dr. Yetti Hernaningsih, dr., Sp.PK(K)
NIP: 19731220 200501 2 001**

ABSTRAK

HUBUNGAN ANTARA HBxAg DENGAN DNA VHB PADA PASIEN HBeAg POSITIF DAN HBeAg NEGATIF HEPATITIS B KRONIK YANG MENDAPAT TERAPI NA

Pendahuluan: Infeksi Hepatitis B kronik (HBK) merupakan suatu proses dinamis yang menggambarkan interaksi antara replikasi virus Hepatitis B (VHB) dan respon imun host. Infeksi HBK dibagi menjadi 4 fase. DNA VHB merupakan baku emas penentu diagnosis dan terapi pada infeksi VHB. DNA VHB mengindikasikan banyaknya virus dalam tubuh. HbxAg adalah salah satu gen yang disandi oleh DNA VHB, suatu protein regulator non struktural yang diperlukan untuk replikasi virus. Tujuan penelitian ini menganalisis hubungan nilai HBxAg dengan DNA VHB pada fase Hepatitis kronik dengan HBeAg positif dan HBeAg negatif pasien infeksi VHB kronik

Metode: Penelitian analisis observasional dengan rancang bangun *cross sectional* ini dilakukan di IRJ Poli Hepato RSUD Dr Soetomo Surabaya dan instalasi Patologi klinik periode Januari sampai Maret 2023. Pengambilan sampel dilakukan dengan teknik *consecutive*. Uji Hubungan HBxAg dengan DNA VHB pada data yang tidak terdistribusi normal menggunakan uji korelasi Spearman, uji beda HBxAg pada kelompok HBeAg positif dan kelompok HBeAg negatif pada data yang terdistribusi normal menggunakan uji T sampel bebas, dan sensitivitas diagnostik nilai HBxAg dihitung dengan menggunakan rumus sensitivitas diagnostik

popu
sasa

ABSTRACT

CORRELATION BETWEEN HBxAg AND HBV DNA IN POSITIVE HBeAg AND NEGATIVE HBeAg CHRONIC HEPATITIS B PATIENTS RECEIVING NA THERAPY

Introduction: Chronic Hepatitis B (CHB) infection is a dynamic process that describes the interaction between Hepatitis B virus (HBV) replication and the host's immune response. CHB infection is divided into 4 phases. HBV DNA is the gold standard for diagnosing and treating HBV infection. HBV DNA indicates the amount of virus in the body. HbxAg is one of the genes encoded by HBV DNA, a non-structural regulatory protein required for viral replication. The purpose of this study was to analyze the relationship between HBxAg and HBV DNA in the chronic hepatitis phase with positive HBeAg and negative HBeAg in patients with chronic CHB infection.

Methods: This ^{study was} observational analytic study with a cross-sectional design, ^{the study} was carried out at the Clinical Pathology Installation and Outward patient Hepato Clinical Pathology Department RSUD Dr Soetomo Surabaya from January to March 2023. The samples were taken using a consecutive technique. Spearman's correlation test was used to analyze the correlation between HBxAg and HBV DNA of non-normally distributed data, free sample T test was used to analyze difference in positive HBeAg group and HBeAg negative group of chronic hepatitis phase of

*the study
Kriteri-?
Indeks?*



**KOMITE ETIK PENELITIAN KESEHATAN
RSUD Dr. SOETOMO SURABAYA**

**KETERANGAN KELAIKAN ETIK
(* ETHICAL CLEARANCE *)**

0562/KEPK/I/2023

**KOMITE ETIK RSUD Dr. SOETOMO SURABAYA TELAH MEMPELAJARI
SECARA SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, MAKA
DENGAN INI MENYATAKAN BAHWA PENELITIAN DENGAN JUDUL :**

*** HUBUNGAN ANTARA HBxAg DENGAN HBV DNA PADA PASIEN HBeAg
POSITIF DAN HBeAg NEGATIF HEPATITIS B KRONIK YANG MENDAPAT
TERAPI NA ***

PENELITI UTAMA : Prof. Dr. Aryati, dr., MS., Sp.PK (K)

**PENELITI LAIN : 1. Dr. Puspa Wardhani, dr., Sp.PK (K)
2. dr Ummi Maimunah, Sp.PD, KGEH, FINASIM
3. dr Rennilia Nita**

UNIT / LEMBAGA / TEMPAT PENELITIAN : RSUD Dr. Soetomo

DINYATAKAN LAIK ETIK

**Bertaku dari : 02/01/2023 s.d 02/01/2024
Surabaya, 2 Januari 2023**

KETUA

**(Prof. Dr. Hedy Hendarto, dr., SpOG (K))
NIP. 19610817 201601 6 101**

**) Sertifikat ini dinyatakan sah apabila telah mendapatkan stempel asli dari Komite Etik
Penelitian Kesehatan*