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Evaluation of Changes in Serum Thyroid Hormone Levels in Patients with Hepatitis B Infection

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Abstract

Purpose: We aimed to determine the differences in the levels of serum thyroid hormone (free T4 [FT4]) and thyroid stimulating hormone [TSH]) as biomarkers for hepatitis B virus (HBV) infection status, with respect to age and sex.
Methods: We retrospectively analyzed serum samples from 200 patients who underwent HBV testing from August 2022 to September 2022. Serum samples were collected from patients suspected of having HBV infection who visited this hospital. Thyroid hormone levels were measured, and patients were grouped according to age and sex.
Results: Differences in TSH and FT4 levels in the serum of patients in the HBV-positive and -negative groups were not significant. Among the HBV-positive patients in the younger age group (<60 years), TSH and FT4 levels were 1.78 ± 0.09 μIU/mL (normal: 0.4–5.0 μIU/mL) and 1.24 ± 0.02 ng/mL (normal: 0.8–1.9 ng/mL), respectively, whereas among the HBV-positive patients in the older age group (≥60 years), TSH and FT4 levels were 2.22 ± 0.17 μIU/mL and 1.24 ± 0.07 ng/mL, respectively.
Conclusions: The presence of HBV did not markedly affect serum thyroid hormone levels. Our findings shed light on the conflicting evidence on the association between thyroid hormone levels and HBV infection.
We, Hyeokjun Yun and Bo Kyeung Jung are co-first authors which made substantial contribution equally to the conception and designed of this work. Jae Kyung Kim, In soo Rheem and Kap No Lee made significant contributions to the acquisition and analysis of the data.

Keywords: Hepatitis B; Serology; Thyroid hormone; CLIA

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1. INTRODUCTION

Hepatitis B is known to cause acute functional deterioration of the liver that is associated with acute-on-chronic liver failure (ACLF) [1–3]. Hepatitis B reportedly causes pathologic and metabolic symptoms, including thyroid dysfunction [4–11,14]. Thyroid dysfunction is reported in many chronic illnesses, such as hyperthyroidism and hypothyroidism, and is also observed in severe liver disease caused by hepatitis B. Thyroid hormones (T3 and T4) and thyroid stimulating hormone (TSH), which exerts its action via the TSH receptor, are the main biological markers that are used for evaluating thyroid function [4].

Thyroid hormones are involved in liver physiology because they regulate metabolism, protein synthesis, detoxification process, and bile production [3]; therefore, alterations in their levels can affect liver function. Previously, serum TSH levels were found to be negatively correlated with the severity of hepatitis B virus (HBV)-related ACLF [5,9]. Additionally, the serum levels of free T4 (FT4) were reported to be negatively correlated with the severity of HBV-related ACLF, and levels of TSH were higher in patients with HBV infection without ACLF than in non-infected patients with HBV [6]. In contrast, other studies did not report any meaningful relation between HBV and thyroid function [4,7-8,13]. Further, studies that have addressed changes in thyroid hormone levels in patients with HBV-related cirrhotic liver diseases have not addressed the relationship between thyroid function and HBV infection [7-8,]. Owing to the conflicting evidence, the importance of the levels of serum thyroid hormones as a prognostic factor to determine disease severity remains to be established in patients with HBV.

Thus, the present study aimed to investigate whether FT4 and TSH levels are specifically altered in patients with HBV and to determine whether FT4 and TSH can be used as prognostic markers for assessing the severity of HBV infection.

2. EXPERIMENTS

2.1 Ethical Approval

This study was approved by the appropriate ethic committee (Certificate No. 2022-10-030). Informed consent was obtained from all study participants.

2.2 Study Design and Participants

This retrospective analysis included 200 patients who underwent testing for HBV infection between August 2022 and September 2022 at the Dankook University Hospital (Cheonan province, South Korea). HBV-positivity was determined using blood samples with reverse-transcription quantitative polymerase chain reaction. Patients were divided into two groups based on the following criteria: age, sex, and HBV infection status (positive or negative).

2.3 Assessment of TSH and FT4 levels

TSH and FT4 levels were measured using an electrochemiluminescence immunoassay (ECLIA) quantitative analyzer (cobas e411, Roche, Mannheim, Germany). Serum TSH and FT4 levels were measured using cobas e601 and cobas e602, respectively. The data were evaluated by the diagnostic test department at the Dankook University according to the protocols of Elecsys TSH and FT4 (cobas e411). Data regarding the thyroid parameters were obtained from 200 serum samples, which revealed positive findings following confirmation of the HBV diagnosis.

Briefly, the detection buffer contains an antibody combined with a fluorescent dye that specifically binds to thyroid markers, TSH and FT4. When the detection buffer and serum sample are mixed, the antibody in the detection buffer and thyroid-related antigen in the sample form an antigen–antibody complex. The immune response is converted into a fluorescence signal and the concentration is calculated via a dedicated measuring device (cobas e411). The result is presented on the reader as ng/mL for FT4 and µIU/mL for TSH. A fluorescence-labeled control protein of known concentration was included in the reaction, and the intensity of this control was used as a quality control for the assay.

2.4. Analysis of TSH and FT4 levels

The 200 serum samples used for this study were anonymously submitted to the laboratory for routine measurement of thyroid markers with ECLIA. The TSH and FT4 concentrations were within the normal analytical range specified by Roche (Basel, Switzerland), namely 0.1–100.0 μIU/mL and 0.1–8.0 ng/dL, respectively. According to the Korean Society of Laboratory Medicine, the normal ranges for TSH and FT4 are 0.4–5.0 μIU/mL and 0.8–1.9 ng/dL, respectively.

2.5 Statistical Analysis

Normally distributed continuous data are presented as the mean ± standard deviation. Independent t-test was used to analyze significance between the groups. All statistical analyses were performed using GraphPad Prism (Version 7.00.159, GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $p<0.05$.

3. RESULTS

3.1 Demographic and Clinical Characteristics

The patients were divided into two groups on the basis of age: the younger age group included the patients between 0 to 59 years of age (n=140), while the older age group included the patients ≥60 years of age (n=60) (Table 1).

Table 1. Demographic features and clinical findings of the participants with respect to age and sex

Parameter		Younger age group (0–59 years, n=140)		P_1	Older age group (≥60 years, n=60)		P_2
		Hepatitis B Negative (n=61)	Hepatitis B Positive (n=79)		Hepatitis B Negative (n=35)	Hepatitis B Positive (n=25)	
Sex	Male (%)	43 (70%)	44 (56%)	*NS*	18 (51%)	15 (60%)	*NS*
	Female (%)	18 (30%)	35 (44%)	*NS*	17 (49%)	10 (40%)	*NS*
TSH (μIU/mL)		1.45 ± 0.14	2.28 ± 0.19	<0.005	2.18 ± 0.26	2.44 ± 0.44	<0.05
FT4 (ng/dL)		1.35 ± 0.08	1.28 ± 0.03	<0.005	1.21 ± 0.03	1.24 ± 0.07	<0.005

TSH, Thyroid Stimulating Hormone; FT4, Free T4; NS, not significant.

Of the 200 patients included in this study, 104 were HBV positive, of whom 79 (76%) and 25 (24%) belonged to the younger and older age groups, respectively. Of the 96 patients without HBV, 61 (41%) and 35 (59%) belonged to the younger and older age groups, respectively (Table 2).

Table 2. Demographic features and thyroid hormones levels with respect to Hepatitis B infection status.

Parameter	Hepatitis B Negative (n=96)	P_1	Hepatitis B Positive (n=104)	P_2

		Younger age group (n=61)	Older age group (n=35)		Younger age group (n=79)	Older age group (n=25)	
Sex	**Male (%)**	43 (70%)	18 (51%)	*NS*	44 (56%)	15 (60%)	*NS*
	Female (%)	18 (30%)	17 (49%)	*NS*	35 (44%)	10 (40%)	*NS*
TSH (µIU/mL)		1.78 ± 0.19	2.47 ± 0.37	<0.0001	2.22 ± 0.17	2.44 ± 0.44	<0.008
Free T4 (ng/dL)		1.24 ± 0.02	1.21 ± 0.03	<0.03	1.21 ± 0.02	1.24 ± 0.07	<0.0001

TSH, Thyroid Stimulating Hormone; FT4, Free T4; NS, not significant.

3.2 Comparison of Thyroid Hormone Parameters between Patients with and without HBV

In patients with HBV, the TSH and FT4 levels were 2.27 ± 0.16 µIU/mL and 1.22 ± 0.02 ng/dL, respectively (Figure 1(a)). Whereas, in patients without HBV, the TSH and FT4 levels were 2.00 ± 0.12 µIU/mL and 1.23 ± 0.02 ng/dL, respectively (Figure 2(b)). Hence, thyroid hormone levels did not differ significantly between the patients with and without HBV.

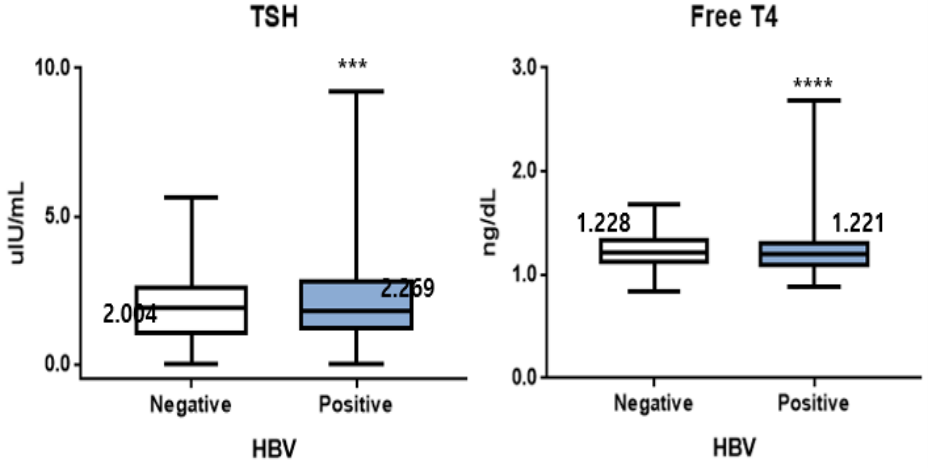

Figure 1 (a) . Comparison of TSH level following hepatitis B virus infection status. (p-value < 0.0005)
Figure 1 (b) . Comparison of FT4 level following hepatitis B virus infection status. (p-value < 0.0005))

3.3 Thyroid Hormone Levels in Patients with HBV with respect to Age

Regarding patients with HBV, for those in the younger age group, TSH and FT4 levels were 2.22 ± 0.17 µIU/mL and 1.21 ± 0.02 ng/dL, respectively; whereas for those in the older age group, TSH and FT4 levels were 2.44 ± 0.44 µIU/mL and 1.24 ± 0.07 ng/dL, respectively. Hence, HBV-related thyroid hormone changes were not significant with respect to age (Figure 2)

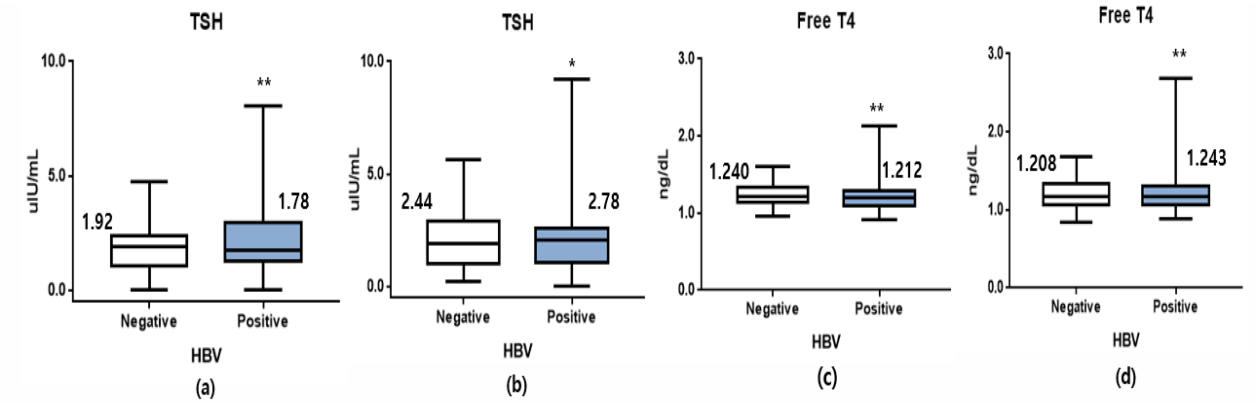

Figure 2. Comparison of thyroid hormone levels (TSH and FT4) with respect to age. TSH levels in the younger (0–59 years) (a) and older age groups (≥60 years) (b) and FT4 levels in the younger (0–59 years) (c) and older age groups (≥60 years) (d). FT4, free T4; HBV, hepatitis B virus; TSH, thyroid stimulating hormone.

3.4 Thyroid Hormone Levels in Patients with HBV with respect to Sex

Figure 3(a) and 3(b) shows, in male patients with HBV, TSH and FT4 levels were 2.07 ± 0.20 µIU/mL and 1.52 ± 0.04 ng/dL, respectively, whereas in male patients without HBV, the respective values were 1.91 ± 0.14 µIU/mL and 1.60 ± 0.04 ng/dL . In female patients with HBV, TSH and FT4 levels were 2.53 ± 0.26 µIU/mL and 1.49 ± 0.06 ng/dL, respectively, whereas in female patients without HBV, the respective values were 2.47 ± 0.35 µIU/mL and 1.41 ± 0.06 ng/dL (Figure 3(c) and 3(d)). The results indicate that TSH levels were slightly higher in both sexes in patients with HBV, whereas FT4 levels were higher in female patients with HBV and lower in male patients with HBV, compared to their levels in corresponding sexes without HBV. However, none of these trends were statistically significant (Figure 3).

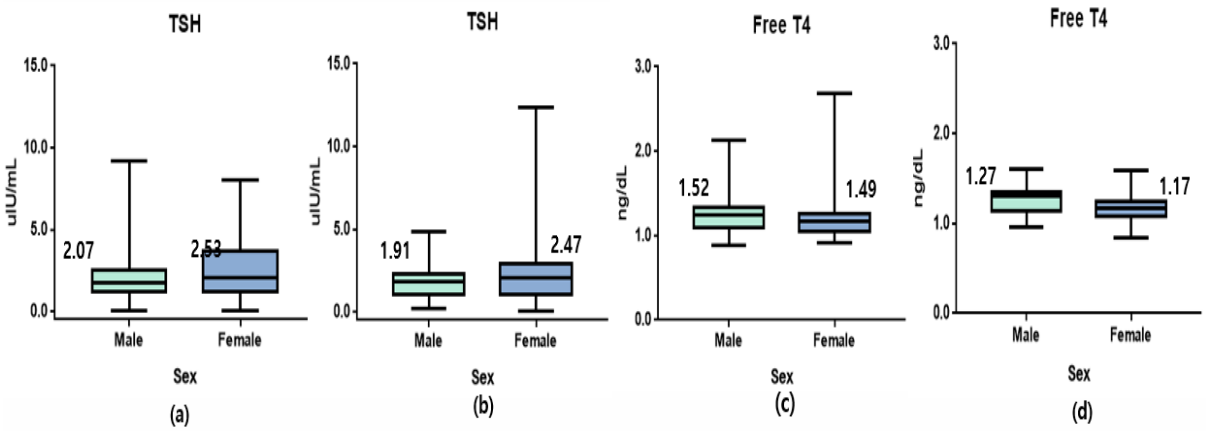

Figure 3. Comparison of thyroid hormone levels (TSH and FT4) with respect to sex. TSH levels in the hepatitis B virus (HBV)-positive (a) and HBV-negative groups (b) and FT4 levels in the HBV-positive (c) and HBV-negative groups (d). FT4, free T4; HBV, hepatitis B virus; TSH, thyroid stimulating hormone.

3.5 Hepatitis B DNA Quantification Levels in relation to Sex and Age in patients with HBV

Figure 4 shows the quantitative units for hepatitis B (HBV unit, IU/mL) in patients with HBV included in this study. Interestingly, male patients with HBV had 140256 ± 105119 HBV units, whereas female patients with HBV had only 55.93 ± 30.58 HBV units (Figure 4 (a)). This difference was statistically significant (p

value < 0.005). Furthermore, a considerable difference in number of HBV units was observed between patients with HBV in the younger (104697 ± 78641 units) and older (262.3 ± 217.2 units) age groups (Figure 4(b)). The number of HBV units was not found to correlate significantly with serum TSH and FT4 levels following HBV infection. Herein, TSH and FT4 values varied with respect to the sex and age of the patients with HBV. These findings suggest that thyroid hormone levels did not correlate with HBV viral load.

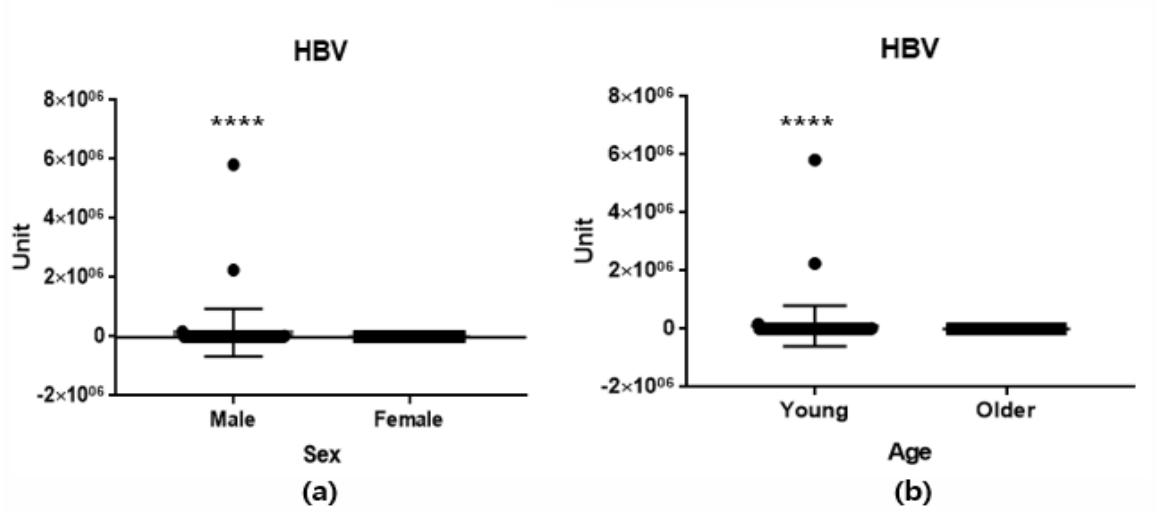

Figure 4. Comparison of the hepatitis B DNA quantitative unit (HBV unit) with respect to sex and age. The HBV unit in the HBV-positive group with respect to sex (a) and age (b). HBV, hepatitis B virus.

4. DISCUSSION

This study focused on exploring the analytical and diagnostic performance of FT4 and TSH levels on ECLIA platforms to assess their validity for clinical use in evaluating the severity of HBV infection. According to the results of ECLIA, no significant correlation was observed between thyroid hormone levels and HBV-positivity.

Several studies have reported that the relationship between the thyroid hormone levels and HBV-related liver functions is weak. In these studies, although FT4 levels were found to be increased, TSH levels were within the normal range [1,8,10]. However, as observed in the present study, no significant differences were observed in the thyroid hormone levels with respect to HBV infection status, age, and sex. Furthermore, the thyroid hormone levels were not significantly correlated with the quantitative trend of the expression of the HBV surface antigen. Thus, a significant relationship between HBV infection and thyroid hormone levels could not be ascertained.

This study has several limitations. Owing to the retrospective nature of the study, other thyroid biomarkers, such as total T3 (a known initial indicator of thyroid metabolic activity) [7,11-12] and free T3 (which is crucial in cellular thyroid metabolism [2,13] and affects the liver and neurons [6,8]), were excluded. However, because TSH and FT4 levels were measured after the confirmation of HBV infection and hospitalization, these two biomarkers are deemed useful for monitoring and predicting the progress of thyroid activity. Furthermore, owing to the administrative approach and temporary operations of the hospital, only patients quantitatively confirmed as positive or negative for HBV infection were included. Therefore, the presence or absence of other hepatic illnesses in inpatients and the criteria for severe or non-severe hepatitis B could not be determined by the symptoms alone. Future research should explore other potential biomarkers, including cardiac and inflammatory makers, for predicting the association of thyroid hormone levels with HBV infection.

This study did not specifically analyze the thyroid values based on the quantitative results of HBV DNA quantification (HBV qPCR). Instead, it compared the thyroid values between patients infected with HBV and those not infected with HBV using the HBV DNA quantification method. While HBV DNA quantification testing can be used to monitor the effectiveness of antiviral therapy, this study did not consider the analysis of virus treatment. The absence of information regarding the treatment status of the virus or the condition of the treatment drugs is a limitation of this study. A more in-depth discussion on the thyroid test results based on the quantitative values of HBV DNA will be addressed in ongoing additional research.

5. CONCLUSION

In this study, the performance of two thyroid hormone assays using CLIA platforms was evaluated. The aim was to assess any changes in serological thyroid hormone levels before and after HBV infection. However, the results indicated no significant alterations in these hormone levels following HBV infection. The relationship between HBV infection and thyroid hormone levels still remains uncertain due to conflicting findings in previous research. The exact mechanism underlying this association remains incompletely understood, emphasizing the need for further investigation.

It is important to note that this study had certain limitations. For instance, it excluded certain thyroid biomarkers and did not consider the presence of other respiratory illnesses or the severity of hepatitis B. Nonetheless, the CLIA method utilized in this study proved to be valuable in providing biomarker data with improved sensitivity and specificity. As a result, these assays can be valuable in the diagnosis and monitoring of various diseases. Moving forward, future studies employing this assay should explore additional metabolic hormones in patients with hepatitis B. Moreover, it would be worthwhile to investigate the correlation between thyroid hormone levels and both hepatitis A and B. These endeavors will contribute to a better understanding of the relationship between thyroid hormones and viral hepatitis infections.

We, Hyeokjun Yun and Bo Kyeung Jung are co-first authors which made substantial contribution equally to the conception and designed of this work. Jae Kyung Kim, In soo Rheem and Kap No Lee made significant contributions to the acquisition and analysis of the data.

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