Research Article

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A Decade of HBV-DNA Testing: Trends in Positivity and Viral Load in a Tertiary Referral Laboratory in Türkiye

Türkiye'de Bir Üçüncü Basamak Referans Laboratuvarında On Yıllık HBV-DNA Testi: Pozitiflik ve Viral Yük Eğilimleri

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ABSTRACT

Objectives: Hepatitis B virus (HBV) infection is a leading cause of liver-related morbidity and mortality worldwide. HBV-DNA tests, used to detect viremic patients and monitor viral load, may also provide insights into transmissibility and public health implications. This study aimed to analyze HBV-DNA tests performed in our hospital over a period of 10.5 years.

Materials and Methods: Results of 45,624 HBV-DNA tests performed in the molecular microbiology laboratory between January 1, 2015, and July 31, 2025 were collected from the electronic records. The HBV-DNA test was performed using real-time polymerase chain reaction. Annual test numbers, positivity rates, and viral load changes were analyzed using linear regression.

Results: On average, 4,310 tests were performed annually, of which 27.4% were positive. Throughout the study period, the median age of the tested patient population increased significantly by 7-8 years; the positivity rate of the tests declined from 37% to 25.6%; and the median viral load of patients testing positive and the quartile extremes decreased significantly by 1-log. The number of tests conducted and the positivity rate decreased significantly during the coronavirus disease 2019 (COVID-19) pandemic compared to previous years, by 35.4% and 37.6%, respectively. Although the COVID-19 pandemic and regional earthquakes temporarily reduced access to testing, these short-term disruptions did not alter the overall downward trends.

Conclusion: The decline in positivity and the increase in patient age suggest that vaccination reduced infections in younger populations, thereby leading to an aging HBV-positive cohort. The reduction in viral load reflects effective treatment and monitoring; however, follow-up may have been disrupted by the pandemic. Strengthening access to antiviral therapy among individuals with undiagnosed chronic HBV could accelerate reduction in HBV prevalence.

Keywords: HBV, HBV-DNA, PCR, viral load, trend analysis

ÖZ

Amaç: Hepatit B virüsü (HBV) enfeksiyonu, dünya genelinde karaciğerle ilişkili morbidite ve mortalitenin önde gelen nedenlerinden biridir. Viremik hastaların tespitinde ve viral yük takibinde kullanılan HBV-DNA testleri, aynı zamanda bulaştırıcılık ve halk sağlığı açısından da önemli bilgiler sağlayabilir. Bu çalışmada hastanemizde 10,5 yıllık bir dönemde yapılan HBV-DNA testlerinin analiz edilmesi amaçlanmıştır.

Gereç ve Yöntemler: 1 Ocak 2015-31 Temmuz 2025 tarihleri arasında moleküler mikrobiyoloji laboratuvarında gerçekleştirilen toplam 45.624 HBV-DNA testinin sonuçları elektronik kayıt sisteminden alınmıştır. HBV-DNA testi gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle yapılmıştır. Yıllık test sayıları, pozitiflik oranları ve viral yük değişimleri doğrusal regresyon analizi ile değerlendirilmiştir.

Bulgular: Yıllık ortalama 4.310 test yapılmış olup, bunların %27,4'ü pozitif bulunmuştur. Çalışma dönemi boyunca, test edilen hasta popülasyonunun ortalama yaşı 7-8 yıl artmış, testlerin pozitiflik oranı %37'den %25,6'ya düşmüş, pozitif hastaların medyan viral yükü ve çeyrek değer aralıkları ise 1-log azalmıştır. 2019 koronavirüs hastalığı (COVID-19) pandemisi döneminde yapılan test sayısı ve pozitiflik oranı, önceki yıllara göre sırasıyla %35,4 ve %37,6 oranında azalmıştır. COVID-19 pandemisi ve bölgesel depremler testlere erişimi geçici olarak azaltmış olsa da, bu kısa süreli aksamalar genel düşüş eğilimini değiştirmemiştir.

Sonuç: Pozitiflik oranındaki düşüş ve hasta yaşındaki artış, aşılama programlarının genç popülasyondaki enfeksiyonları azalttığını ve HBV pozitif hasta grubunun yaşlandığını göstermektedir. Viral yükteki azalma etkin tedavi ve izlem uygulamalarını göstermektedir; ancak pandemi sürecinde hasta takibinde aksamalar yaşanmış olabilir. Tanı almamış kronik HBV hastalarında antiviral tedaviye erişimin kolaylaşması, ülkemizde HBV prevalansını hızla azaltabilir.

Anahtar Kelimeler: HBV, HBV-DNA, PCR, viral yük, trend analizi

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Introduction

Human hepatitis B virus (HBV) is a hepatotropic virus belonging to the family *Hepadnaviridae*, with an icosahedral symmetry, an enveloped capsid structure, and a circular, partially double-stranded DNA genome (1). The relatively small genome (3.2 kb) transforms into the covalently closed circular DNA form in the nucleus of the infected cell and encodes the viral polymerase, core, envelope, surface, and X antigens (2). Similar to retroviruses, HBV replicates via an RNA intermediate and causes persistent infection by integrating into the host cell genome. To date, at least 10 genotypes (A-J) of HBV have been identified, each with distinct geographical distribution patterns and varying clinical severity (3).

The HBV forms a stable virion that can remain infectious for months under appropriate conditions outside the human body. It spreads through various transmission routes, primarily parenteral, sexual, and vertical (4). Following infection of a susceptible host, HBV leads to two main outcomes: acute infection or chronic infection. The risk of chronic infection is particularly high when infection occurs in early childhood, whereas infections in young adults are cleared without progressing to chronicity in about 90% of cases (5).

Chronic HBV infection is the most important clinical condition in which the pathogen threatens human health, and is one of the leading causes of chronic liver failure and hepatocellular carcinoma (HCC). A modeling study based on data from 170 countries reported that nearly 270 million people worldwide were living with chronic HBV infection by 2022 (6). The World Health Organization (WHO) announced that 1.2 million people are infected with HBV each year and that 1.1 million deaths occur annually due to chronic HBV-related complications, particularly cirrhosis and HCC (7). Due to this major global health burden, WHO has classified HBV as a major public health threat and set a target of reducing new cases by 90% by 2030 (8).

Türkiye is among the countries with a low-to-moderate prevalence of HBV. According to the 2017 report by the Ministry of Health, there were approximately 3 million chronic HBV patients in the country, and the incidence of the disease significantly decreased following the implementation of vaccination programs (9). Shortly after the licensing of antiviral drugs that inhibit viral replication, they were reimbursed under the national health insurance scheme and administered to patients with chronic HBV who had indications for treatment. In addition, numerous awareness programs have been carried out in the country to increase public knowledge about HBV. Despite all these efforts, there is insufficient scientific data on changes in the prevalence of HBV-DNA-positive patients in our community and on their viral loads.

Monitoring HBV-DNA is critical for establishing treatment indications for an individual patient, as well as for tracking viral concentrations, liver inflammation, and disease progression. Furthermore, analyzing cumulative HBV-DNA test results collected from a population over an extended period can provide valuable information about the effectiveness of preventive measures implemented to date and allow us to predict disease progression within the community prospectively. In this study, we aimed to investigate positivity rates and changes in patients' viral load over the past decade by examining HBV-DNA tests performed in our hospital, which is a tertiary regional referral center for molecular diagnosis.

Materials and Methods

Study Design and Scope

This study was conducted in the molecular microbiology laboratory of a tertiary university hospital with 1,500 beds. HBV-DNA real-time polymerase chain reaction (PCR) test results generated in the laboratory between January 1, 2015, and July 31, 2025, were retrospectively retrieved from the hospital's electronic database. Patient demographic data (age and sex), HBV-DNA results (positive or negative) obtained by PCR, and viral load levels detected in the serum samples of positive patients were evaluated in this study. Regardless of the test request date, HBV-DNA test results completed within the study period were included in the analysis. For repeated tests in the same patient within six months, if positive, the result with the highest viral load was included; if negative, the result of a single test was considered.

HBV-DNA Analysis

Blood samples collected from patients were centrifuged at 3,000 rpm to separate the serum. Viral DNA in serum samples was extracted using the EZ1 Virus Mini Kit (Qiagen, Germany) on the QIAsymphony SP instrument (Qiagen, Germany), according to the manufacturer's instructions. Detection of viral DNA was performed by real-time PCR using artus HBV QS-RGQ kits and the Rotor-Gene Q system (Qiagen, Germany).

Statistical Analysis

Patients' demographic data and their HBV-DNA-positive or -negative status were expressed as numbers (n) and percentages (%). Viral loads detected in positive patients were expressed in copy/mL, and annual median viral load values were calculated. For each year, viral loads of positive patients were ranked from highest to lowest, and median viral load values for the quartiles (the upper 25%, upper-middle 25%, lower-middle 25%, and the lowest 25%) were calculated. Over the study period, which exceeded 10 years, annual changes in patients' median age, positivity rates, median viral load among positive patients, and median viral load across quartiles were evaluated using linear regression analysis. Categorical variables were compared using Pearson's chi-square test. A p-value <0.05 was considered statistically significant.

Ethical Approval

This study was approved by the İnönü University Health Sciences Scientific Research Ethics Committee (approval no: 2025/8344, date: 16.09.2025). Consent was not obtained because the study was retrospective.

Results

A total of 45,624 test results were included in the study. The average numbers of tests per month and per year during the study period were 359 and 4,310, respectively. Among the patients, 37.5% were female and 62.5% were male. During the study, 12,520 (27.44%) samples were HBV-DNA positive, with a female-to-male distribution of 39.7% and 60.3%, respectively. Notably, the number of tests conducted in 2020 decreased significantly by 35.4% compared with the average of the previous five years (3058 vs. 4731, p=0.008), and the positivity rate in 2021 was significantly lower than that of the previous six years (19.1% vs. 30.6%, p=0.033). The gender characteristics, number of tests performed, and results by year are shown in Table 1.

The median age of patients who underwent HBV-DNA testing increased from 46 years in 2015 to 54 years in 2025. The median age of positive patients rose from 42 years in 2015 to 49 years by the end of the study, while that of negative patients increased from 49 to 56 years over the same period. Linear regression analysis confirmed a significant annual increase in median age [estimate=0.836, standard error (SE)=0.045, p<0.001, 95% confidence interval (CI): 0.736-0.937]. Changes in patients' ages across the study years are shown in Figure 1.

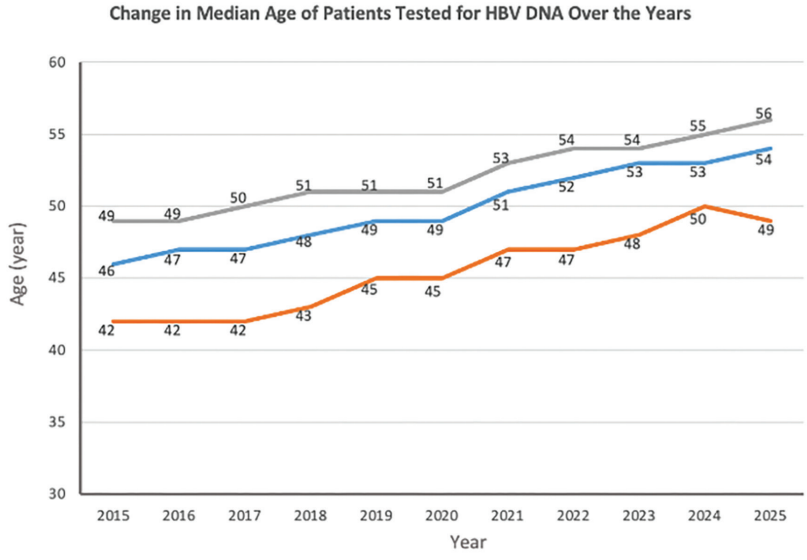

Figure 1. Median age of patients tested for HBV-DNA by year (2015-2025). The blue line represents all patients, the orange line indicates HBV-DNA positive patients, and the grey line indicates HBV-DNA negative patients

HBV: Hepatitis B virus

Table 1. HBV-DNA test data by year and demographic characteristics of tested patients

Years	HBV-DNA				Sex (n)		Age (median; year)
	No of tests	Positive (n, %)		Negative (n)	Female	Male	
2015	4526	1705	37.7	2821	1673	2853	46
2016	4739	1424	30.04	3315	1724	3015	47
2017	5029	1578	31.4	3451	1899	3130	47
2018	4593	1405	30.6	3188	1773	2820	48
2019	4771	1320	27.7	3451	1796	2975	49
2020	**3058***	743	24.3	2315	1122	1936	49
2021	3904	746	**19.1****	3158	1453	2451	51
2022	4791	1099	22.9	3692	1818	2973	52
2023	3378	728	21.6	2650	1262	2116	53
2024	4171	1091	26.2	3080	1612	2559	53
2025	2664	681	25.6	1983	956	1708	54

*: The annual number of HBV-DNA test was significantly lower than the annual average of the test numbers conducted in the previous five years (3058 vs. 4731, p=0.008).
**: The positivity rate detected in 2021 was found to be significantly lower than the annual average of previous six years positivity rate (19.1% vs. 30.6%, p=0.033). Pearson chi-square test
HBV: Hepatitis B virus

The positivity rate of HBV-DNA tests decreased from 37.7% in 2015 to 25.6% in 2025. Linear regression analysis confirmed a significant decreasing trend in HBV-DNA positivity (estimate=-1.175, SE=0.352, p=0.009, 95% CI: -1.972 to -0.378).

The median viral load of positive patients decreased from 16,625 copies/mL in 2015 to 6,730 copies/mL in 2025. Trend analysis revealed a statistically significant 1-log reduction in the median viral load over the study period (β=1070.8, SE=187.4, p<0.001, R^2=0.784).

The change in the median viral load of positive patients is shown in Figure 2. Considering the quartiles based on viral load values, patients in the highest (p=0.016, R^2=0.491) and lowest quartiles (p<0.001, R^2=0.491) demonstrated a significant 1-log reduction in median viral load over the years. However, the decreases observed in the upper-middle and lower-middle quartiles were not statistically significant. The changes in median viral load levels across quartiles over the study period are presented in Figure 3.

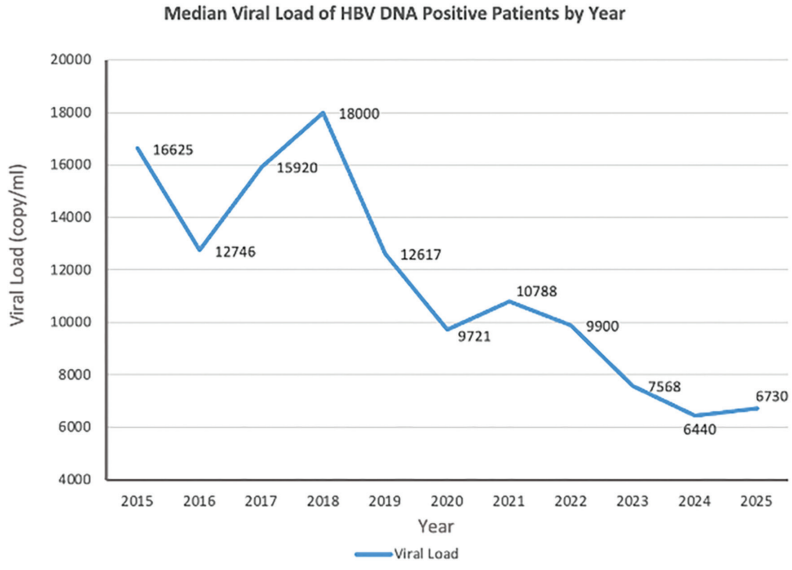

Figure 2. Median viral load of HBV-DNA positive patients by year (2015-2025)
HBV: Hepatitis B virus

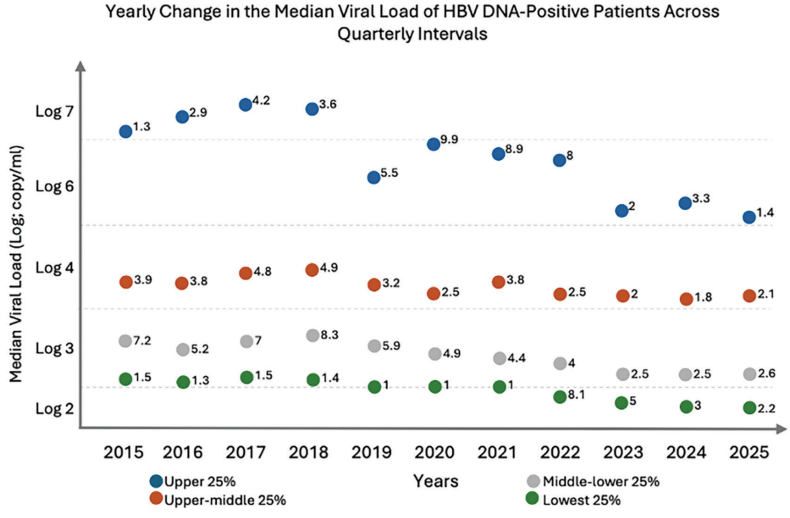

Figure 3. Changes over the years in the median viral load measured in quartile groups formed by ranking viremic patients from highest to lowest viral load. A significant 1-log reduction was observed in patients in the upper and the lowest quartiles during the study period; however, although a decrease in median viral load was also noted in the two middle quartiles, these were not statistically significant
HBV: Hepatitis B virus

Discussion

Liver diseases caused by HBV are an important public health problem both in our country and worldwide. Published data have reported that one in three individuals in Türkiye has been exposed to HBV, and that 40-50% of liver transplantations are HBV-related (9). In a study included 334 liver transplant patients in Türkiye, liver failure due to HBV was found in 115 patients (34.4%) (10). Therefore, important strategies have been adopted in Türkiye to combat HBV, and the country has begun aligning with the WHO's 2030 HBV elimination targets.

Nationwide vaccination campaigns play a major role in prevention strategies. First introduced into the immunization schedule in 1998 under the hepatitis B control program, the HBV vaccine has already been administered to every child within the expanded vaccination program from birth as part of the routine schedule (11). According to Ministry of Health data, supplementary vaccination campaigns for primary and secondary school students, especially between 2005-2009, played a major role in reducing the incidence of acute HBV from 12.0 per 100,000 in 2005 to 1.9 in 2017 (9). Another strategy implemented to curb transmission has been the strict legal regulation and control of other high-risk routes of HBV spread, such as blood transfusion, dental procedures, cosmetic practices, and sex work. In addition, since spousal transmission has been identified as an important route in Türkiye, (4,12) individuals applying for marriage have been included in HBV screening, and when one partner is found positive, awareness programs are conducted to prevent viral transmission among them. Another preventive measure has included community awareness activities, such as public information campaigns on World Hepatitis Day (July 28).

In Türkiye, treatment services have also been implemented to reduce HBV-related health problems. For this purpose, antivirals and immunomodulatory drugs, once licensed for clinical use, began to be reimbursed by the national health insurance scheme for eligible patients. According to the joint guidelines prepared by the Turkish Association for the Study of the Liver and the Viral Hepatitis Society, all first-degree relatives and sexual partners of HBV-positive individuals are recommended to undergo testing for HBV serological markers. If negative, vaccination is advised, and patients with chronic HBV infection should be closely monitored both for antiviral treatment indications and potential complications (13).

The initial diagnosis of HBV patients is most often made serologically by detecting viral antigens and antibodies. In particular, HBV-DNA tests are used in chronic infections both for monitoring viral load and for evaluating treatment response. In this study, HBV-DNA test results from a regional molecular microbiology laboratory, spanning more than 10 years, were analyzed to assess the possible impact of HBV control efforts in Türkiye over the past 20 years on patients and test outcomes. Patient age was analyzed according to three parameters: the ages of all patients tested, positive patients, and negative patients. Over the 10.5-year study period, all three parameters showed a significant and progressive increase of 7-8 years (Figure 1). This finding is one of the most important results of our study, indicating that the HBV-tested population in our region is aging steadily, without a substantial influx of younger patients. If this trend continues, chronic HBV infection will become a disease that characteristically affects the geriatric population, while the HBV-positive burden will significantly decrease over the coming decades. In its 2017 report, the Ministry of Health noted an increase in the ages of chronic HBV patients (9). However, detailed information on the extent and trend of this increase is lacking, and developments after 2017 have not been investigated. A multicenter community survey published in 2015 found that the median age of hepatitis B surface antigen (HBsAg)-positive individuals in Türkiye was between 40-49 years, (12) coherent with the median age of 46 years for our HBV-positive patients in that year. Importantly, our study shows that HBV-positive individuals have continued to age steadily since 2015. This change is likely driven by the widespread vaccination campaigns implemented over the last 30 years, which have rendered children and young people immune to HBV and protected them against infection.

Another key finding of our study was a downward trend in both the number and proportion of HBV-DNA-positive patients. The average positivity rate was above 32% during the first four years (2015-2018) and dropped to around 23% during 2022-2025. Additionally, the median viral concentration in positive patients has shown a significant decrease of 1-log. When viral loads among viremic patients were analyzed by quartile, the median viral load in both the highest and lowest quartiles declined significantly over time. The concurrent decrease in both the proportion of viremic patients and the viral loads of these patients suggests that antiviral treatment has been effective in Türkiye. Antiviral agents suppress viral replication, markedly reducing the viral load, even below the limit of detection. A meta-analysis of 328 published studies found that antiviral treatment, without causing major adverse effects, was an independent factor associated with HBsAg loss, HBV-DNA clearance, and reduced cirrhosis and HCC in chronic HBV patients (14). In a Turkish study involved 17 pregnant women with chronic HBV infection and high viral loads, antiviral therapy reduced viral load to below 10,000 IU/mL without significant adverse effects in mothers or fetuses, achieved an average 2-log reduction in two-thirds of patients, and reduced liver enzyme levels in about 90% of patients (15). These results highlight the potential of antiviral therapy to significantly reduce vertical transmission, a critical route of HBV spread in the community.

By suppressing HBV replication to such an extent, antiviral therapy reduces hepatic inflammation and hepatocyte damage, halts progression to cirrhosis and HCC, and lowers the likelihood of transmission to others. Nevertheless, a multicentre Turkish study in 2015 involving approximately 5,500 participants found that only a small proportion of HBsAg-positive individuals were aware of their condition (12). Moreover, given that treatment non-adherence in Türkiye ranges from 18-30%, with even higher rates in some particular groups (16,17), ensuring that as many HBV patients as possible receive therapy, and improving adherence and continuity among those under treatment, appears as major goals of the HBV control program today.

In our study, the number of tests was significantly lower in 2020, and the positivity rate was lowest in 2021, coinciding with the COVID-19 pandemic. This may have been due to hospitals being overwhelmed with pandemic cases, curfews, and public announcements advising older adults to stay at home. Similarly, another decline was observed in 2023 after the major earthquakes in our region on February 6, although it was not significant. Following this disaster, many people experienced housing problems; about one-quarter of the city's population migrated to other regions. Indeed, notable declines have been reported in the detection of many infectious diseases after both the pandemic and the earthquakes (18-20), and our study confirmed that HBV diagnosis and follow-up processes were significantly affected by these unexpected events. Taking appropriate precautions and ensuring preparedness can help minimize the impact of such disasters on HBV control programs.

On the other hand, in light of our results and the available literature, we believe that the emergence of antiviral resistance or the spread of non-vaccine HBV serotypes could threaten the success of control programs. To sustain the decreasing trend in HBV infection in the community, it is essential not only to provide broader vaccine coverage but also to monitor rare vaccine-breakthrough cases and treatment-resistant strains.

Study Limitations

This study has some limitations. First, it is a retrospective study conducted at a single-center and based on laboratory records. Second, detailed clinical information such as patients' treatment status, disease stage, and vaccination history was not available in the laboratory database.

Conclusion

In this study, more than ten years of data from a laboratory serving as the sole molecular microbiology facility for a population representing approximately 1% of Türkiye were analyzed. The findings indicate that the HBV-infected population has been gradually ageing as a result of vaccination and other preventive measures. Transmission among the young population has effectively decreased, and both the proportion of viremic patients and their viral loads have fallen, indicating reduced transmissibility. Although unforeseen events such as the pandemic and earthquakes have disrupted diagnostic and follow-up processes, they have not yet had a significant negative impact in the short term. For Türkiye to achieve full success in HBV control, identifying and enrolling undiagnosed and untreated HBV patients in care and treatment programs will be crucial

Ethics

Ethics Committee Approval: This study was approved by the İnönü University Health Sciences Scientific Research Ethics Committee (approval no: 2025/8344, date: 16.09.2025).

Informed Consent: Consent was not obtained because the study was retrospective.

Footnotes

Authorship Contributions

Concept: E.S.T, Y.Y., B.O., Design: E.S.T, Y.Y., B.O., Data Collection or Processing: E.S.T., M.G., Analysis or Interpretation: Y.Y., M.G., Literature Search: E.S.T., M.G., B.O., Writing: E.S.T., Y.Y., B.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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