Research Article

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Evaluation of Hepatitis C Virus Genotype and Viremia Prevalence in a Tertiary Care Hospital in Ankara, Türkiye

Ankara'da Üçüncü Basamak Bir Hastanede Hepatit C Virüs Genotip ve Viremi Prevalansının Değerlendirilmesi

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ABSTRACT

Objectives: According to 2023 the World Health Organization data, around 50 million people globally have chronic hepatitis C virus (HCV) infections, presenting an ongoing public health challenge. This study aimed to evaluate HCV prevalence, viremia rates, and genotype (GT) distribution among HCV-positive cases in Ankara.

Materials and Methods: In this study, anti-HCV results from 308,309 patients were evaluated. Anti-HCV tests were analyzed using the Cobas 8000 system, and quantitative HCV ribonucleic acid polymerase chain reaction (PCR) tests were performed on the Cobas 8800 real-time PCR system. A commercial PCR-based Bosphore HCV genotyping kit v5 was used to determine HCV GTs.

Results: The anti-HCV prevalence was 0.38%, HCV viremia prevalence was 0.04%, and the viremia rate was 11.1% (131/1,179). The viremia rate was 6.4% in 2022, 12% in 2023, and 10.9% in 2024 (p=0.25). The highest HCV viremia prevalence was in those aged 70 and above (0.07%), while the highest HCV viremia rate (16.7%) occurred in the 0-29 age group (both p<0.001). Among foreign patients, the anti-HCV prevalence, HCV viremia prevalence, and viremia rate were 2.3%, 0.4%, and 18.8%, respectively, whereas in Turkish citizens, these rates were 0.3%, 0.03%, and 10%, (p<0.001, p<0.001, p=0.008, respectively). The most common GT was GT1 (55.7%).

Conclusion: This study has demonstrated that HCV prevalence and viremia rates are lower compared to global data. GT1 has been identified as the predominant GT. The higher viremia rates observed in the young population and foreign individuals highlight the importance of early diagnosis and screening programs in these groups.

Keywords: Hepatitis C virus, viremia, genotype, PCR, prevalence

ÖZ

Amaç: 2023 Dünya Sağlık Örgütü verilerine göre dünya genelinde yaklaşık 50 milyon insan kronik hepatit C virüsü (HCV) enfeksiyonu ile mücadele etmekte, bu da süregelen bir halk sağlığı sorunu teşkil etmektedir. Bu çalışma, Ankara'daki HCV-pozitif olgular arasında HCV prevalansı, viremi oranları ve genotip (GT) dağılımını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: Bu çalışmada, 308.309 hastadan alınan anti-HCV sonuçları değerlendirilmiştir. Anti-HCV testleri Cobas 8000 sistemi ile analiz edilmiş ve kantitatif HCV ribonucleic acid polimeraz zincir reaksiyonu (PCR) testleri Cobas 8800 gerçek zamanlı PCR sistemi ile gerçekleştirilmiştir. HCV GT'lerini belirlemek için ticari bir PCR temelli Bosphore HCV genotyping kit v5 kullanılmıştır.

Bulgular: Anti-HCV prevalansı %0,38, HCV viremi prevalansı %0,04 ve viremi oranı %11,1 olarak bulunmuştur. Viremi oranı 2022'de %6,4'ten 2023'te %12'ye ve 2024'te %10,9'a yükselmiştir (p=0,25). En yüksek HCV viremi prevalansı 70 yaş ve üzeri grupta (%0,07) görülürken, en yüksek viremi oranı %16,7 ile 0-29 yaş grubunda tespit edilmiştir (her ikisi de p<0,001). Yabancı hastalarda anti-HCV prevalansı, HCV viremi prevalansı ve viremi oranı sırasıyla %2,3, %0,4 ve %18,8 iken, Türk vatandaşlarında bu oranlar sırasıyla %0,3, %0,03 ve %10 olarak bulunmuştur (sırasıyla p<0,001, p<0,001, p=0,008). En yaygın GT, GT1 (%55,7) olmuştur.

Sonuç: Bu çalışma, HCV prevalansı ve viremi oranlarının küresel verilere kıyasla daha düşük olduğunu ortaya koymuştur. Genç nüfus ve yabancılar arasında daha yüksek viremi oranları, bu gruplarda erken tanı ve tarama programlarının önemini vurgulamaktadır. Pangenotipik tedavilerin yaygın kullanımına rağmen, GT1'in baskın olmaya devam etmesi, bu tedavilerin GT dağılımı üzerinde önemli bir etki yaratmadığını göstermektedir.

Anahtar Kelimeler: Hepatit C virüsü, viremi, genotip, PCR, prevalans

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Introduction

Hepatitis C virus (HCV) is a single-stranded, enveloped RNA virus belonging to the *Hepacivirus* genus of the *Flaviviridae* family (1). According to 2023 data from the World Health Organization (WHO), approximately 50 million people worldwide are estimated to be chronically infected with HCV, with around 1.5 million new infections reported annually. Chronic HCV infection remains a significant global public health issue (2). In Türkiye, data from 2018 indicate that approximately 250,000 to 500,000 individuals over the age of 18 are infected with HCV (3).

HCV transmission occurs through multiple potential routes, with blood transfusion and intravenous drug use being the most common. Additionally, factors such as orodental procedures, piercings, tattoos, sharing of shaving equipment, sexual contact, and perinatal transmission: increase the risk of infection (4,5).

In the clinical course of individuals infected with HCV, it is known that 15-45% of cases spontaneously clear the infection within six months due to an effective immune response while 55-85% of cases progress to chronic hepatitis. In untreated cases, approximately 80% of patients with chronic infection are at high risk of developing severe complications such as liver fibrosis, cirrhosis, and hepatocellular carcinoma (6,7).

The HCV genome exhibits high genetic diversity due to the absence of error-correcting mechanisms in RNA-dependent RNA polymerase and the virus's rapid replication capacity. As a result of genetic differences, eight distinct genotypes (GTs) and 93 different subtypes of HCV have been identified (8). While the distribution of GTs varies by geographic region, GT1 and 3 are the most common worldwide. Additionally, GT2 is predominant in West Africa and South America, and GT4 and 6 are prevalent in some regions of North Africa and East/Southeast Asia (9). In Türkiye, GT1 is known to be the dominant GT. However, Türkiye's position as a crossroads between Europe and Asia, coupled with increasing migration in recent years, is thought to contribute to changes in the GT distribution (10,11).

Despite the availability of pangenotypic direct-acting antiviral (DAA) treatments, determining the HCV, GT still plays an important role in optimizing treatment duration and response rates. Therefore, GT determination remains clinically valuable in treatment planning (12).

The diagnosis of HCV infection primarily involves HCV antibody testing, HCV core antigen testing, HCV RNA detection, and genotyping. Initially, an antibody test is used to detect antibodies against HCV. In cases where HCV antibodies are reactive, a quantitative real-time polymerase chain reaction (PCR) test is performed to confirm active infection by detecting HCV-RNA. The HCV core antigen test can also be used to identify active infection. Genotyping of HCV is typically carried out using PCR-based methods, sequencing, and hybridization-based tests (10,13).

In this study, we aimed to determine the prevalence of HCV, HCV viremia, and the GT distribution of HCV-positive cases in a tertiary care hospital in Ankara, the second-largest city in Türkiye by population.

Materials and Methods

Study Design

This study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: 2024-805, dated: 28.08.2024). In this retrospective, single-center, cross-sectional study, patients from all age groups who underwent anti-HCV testing between November 2022 and July 2024 at University of Health Sciences Türkiye, Ankara Etlik City Hospital were evaluated. A total of 308,309 patients were included in the study. Demographic data (age, gender, ethnicity) and laboratory results (anti-HCV, HCV-RNA, HCV GTs) were obtained from the hospital's information management system. Patients without accessible HCV test results (anti-HCV, HCV-RNA, or HCV GT) were excluded from the study. Taking repeated test results into account, only the first sample from patients who were anti-HCV reactive, who underwent multiple tests, was included in the analysis. Patients who were anti-HCV reactive but did not undergo HCV-RNA testing were excluded from the study.

To evaluate changes in HCV prevalence and GT distributions, patients were divided into four age groups: 0-29 years, 30-49 years, 50-69 years, and 70 years and older.

HCV Antibody Analysis

Anti-HCV testing was performed using the Roche Elecsys anti-HCV test kit (Roche Diagnostics GmbH, Mannheim, Germany). This test kit is based on the electrochemiluminescence immunoassay method for detecting antibodies against HCV. A reference value of anti-HCV signal/cut-off ratio ≥1.0 was considered indicative of a reactive test result.

Quantitative HCV-RNA Analysis

HCV-RNA analysis in plasma samples was performed using the fully automated extraction and PCR amplification processes of the Cobas® 8800 system (Roche Diagnostics GmbH, Mannheim, Germany), which integrates all analysis steps into a single device. The limit of detection for the test was set at 15 international units (IU)/mL, and the lower limit of quantification was also 15 IU/mL.

HCV Genotyping

HCV genotyping in the study population was performed using the commercial PCR-based Bosphore HCV genotyping kit v5 (Anatolia Geneworks, Türkiye). This kit targets the NS5B region to detect the six major HCV GTs and their most common subtypes (GTs1, 2, 3, 4, 5, 6 and subtypes 1a, 1b). The results were evaluated according to the manufacturer's instructions.

Statistical Analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences version 27.0 software (International Business Machines Corporation). The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables that did not follow a normal distribution were expressed as the median (interquartile range) and minimum-maximum values; comparisons between two groups were made using the Mann-Whitney U test while the Kruskal-Wallis test was

used for comparisons between more than two groups. Categorical data were presented as frequencies and percentages, and the chi-square test or Fisher's exact test, where appropriate, was used to assess the relationship between groups. A p-value of <0.05 was considered statistically significant.

Results

Anti-HCV Antibody Positivity and HCV Viremia Rates

Anti-HCV antibody positivity was detected in 1,179 of 308,309 patients, with a prevalence of 0.38% [95% confidence interval (CI): 0.36-0.40]. The prevalence of viremic HCV infection in the entire tested patient population was calculated as 0.04% (131 out of 308,309 patients) (95% CI: 0.04-0.05). The HCV viremia rate was 11.1% (131/1,179) (95% CI: 9.37-13.05).

Significant differences in anti-HCV and HCV viremia prevalence were observed across different age groups. Anti-HCV prevalence increased with age; the lowest prevalence was observed in the 0-29 age group at 0.2% (192/97,155), while the highest was found in the 70 and older group at 0.8% (339/39,840) (p<0.001). Similarly, the prevalence of HCV viremia was lowest, at 0.03%, in the 0-29 age group (32/97,155) and the 50-69 age group (28/86,645) while it was highest in the 70 and older group (32/39,840) (p<0.001). The highest viremia rate was detected in the 0-29 age group at 16.7% (32/192), while the lowest was in the 50-69 age group at 7.1% (28/392) (p<0.001).

An analysis by year revealed that anti-HCV prevalence was highest in 2022 (0.5%) and showed a gradual decline in subsequent years, reaching its lowest level in 2024 (0.3%) (p<0.001). The prevalence of HCV viremia remained stable, in the years 2022 (0.03%), 2023 (0.04%), and 2024 (0.03%) (p=0.12). The HCV viremia rate was lowest in 2022 (6.4%) and peaked in 2023 (12%) before decreasing in 2024 (10.9%), although this change was not statistically significant (p=0.25) (Table 1).

In terms of ethnicity, anti-HCV prevalence, HCV viremia prevalence, and the viremia rate were found to be 2.3% (139/6,159), 0.4% (26/6,159), and 18.8% (26/139) in foreign nationals, while these rates were 0.3% (1,040/302,150), 0.03% (105/302,150), and 10% (105/1,040), respectively, in Turkish citizens. These differences were statistically significant (p<0.001, p<0.001, p=0.008, respectively).

HCV Genotype Prevalence

Among 70 HCV patients in the study, the most frequently detected GT was GT1, observed in 55.7% of cases. Among GT1 subtypes, GT1b was the most common at 30%. GT3 was identified in 28.6% of cases, while both GT2 and GT4 were found in 7.1% of cases. GT5 was rarely isolated in one patient (1.4%).

Distribution of HCV Genetype by Gender

GT1 was the most common GT in both male (55.8%) and female (55.6%) patients. GT1a was more prevalent in males (39.5%), while GT1b was more frequent in females (51.9%) (p<0.001) (Table 2).

Distribution of HCV Genetype by Ethnicity

Among patients with GT analysis, 78.6% (55/70) were of Turkish nationality. GT1 was the most frequently detected GT in both Turkish (58.2%) and foreign patients (46.7%) (p=0.186). After GT1, the most common GTs in Turkish patients were GT3 (27.3%) and GT2 (9.1%), while in foreign patients, GT3 (33.3%) and GT4 (13.3%) were the most common. Additionally, GT1b was more frequently detected in the Turkish population, whereas

Table 1. Prevalence of anti-HCV and HCV viremia, and viremia rates from 2022 to 2024

	Anti-HCV prevalence				
Years	**Total, n**	**Anti-HCV reactive, n**	**%**	**95% CI**	**p-value**
2022	26,382	125	0.47	0.39-0.56	0.002
2023	170,701	679	0.39	0.37-0.43	
2024	110,015	375	0.34	0.31-0.38	
Total	308,309	1,179	0.38	0.36-0.40	
	HCV viremia prevalence				
	Total, n	**HCV-RNA positive, n**	**%**	**95% CI**	
2022	26,382	8	0.03	0.01-0.06	0.240
2023	170,701	82	0.04	0.04-0.06	
2024	110,015	41	0.03	0.03-0.05	
Total	308,309	131	0.04	0.04-0.05	
	HCV viremia rate				
	Total*, n	**HCV-RNA positive, n**	**%**	**95% CI**	
2022	125	8	6.4	2.80-12.22	0.177
2023	679	82	12	9.72-14.77	
2024	375	41	10.9	7.96-14.54	
Total	1,179	131	11.1	9.37-13.05	

*Patients who underwent HCV-RNA testing. HCV: Hepatitis C virus; RNA: Ribonucleic acid, CI: Confidence interval

Table 2. Demographic and laboratory characteristics of patients based on HCV GT

	HCV GT							
	GT1	GT1a	GT1b	GT2	GT3	GT4	GT5	p-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
n=70	39 (55.7)	18 (25.7)	21 (30)	5 (7.1)	20 (28.6)	5 (7.1)	1 (1.4)	
Gender								
Male	24 (55.8)	17 (39.5)	7 (16.3)	4 (9.3)	12 (27.9)	2 (4.7)	1 (2.3)	<0.001
Female	15 (55.6)	1 (3.7)	14 (51.9)	1 (3.7)	8 (29.6)	3 (11.1)	0 (0)	
Etnicity								
Turkish	32 (58.2)	14 (25.5)	18(32.7)	5 (9.1)	15 (27.3)	3 (5.5)	0 (0)	0.273
Foreign nationals	7 (46.7)	4 (26.7)	3 (20)	0 (0)	5 (33.3)	2 (13.3)	1 (6.7)	
Age*	42	28	58	28	31	31	-	<0.001
IQR	28-61	23-32	42-66	24-58	26-44	24-51	-	
Age range	11-84	16-67	11-84	24-86	19-64	22-54	-	
HCV-RNA†	5.7	5.5	6.0	7.1	5.8	6.3	-	0.063
IQR (25-75)	4.7-6.7	4.4-6.4	5.0-6.7	6.8-7.4	4.6-6.8	5.0-7.0	-	
Range	0.7-7.6	0.7-7.0	2.7-7.5	6.7-7.4	0.7-7.1	5.0-7.0	-	

HCV: Hepatitis C virus, GT: Genotype, IQR: Interquartile range, *: Median, †: $\log_{10}$ IU/mL, IU: International unit

GT1a was more common among foreign patients (p=0.280). No mixed HCV GT infections were detected in the study population (Table 2).

Distribution of HCV Genetype by Age Groups

The median age of patients infected with GT1b was 58 years, while patients infected with GT1a were younger, with a median age of 28 years. The median age for those infected with GT3 was determined to be 31 years. The age differences between GTs were statistically significant (p<0.001). GT1a was the most common GT in the 0-29 age group (44%). GT3 was most prevalent in the 30-49 age group (40%), while GT1b was the most frequently detected GT in the 50-69 age group (66.7%) and the 70 years and older group (80%). GT5 was only identified in the 30-49 age group (4%) (p=0.001) (Figure 1).

Viral Load Relationship with HCV Genetype

The median HCV-RNA viral load levels ($\log_{10}$, IU/mL) were highest in patients infected with GT2 at 7.1, although no statistically significant difference in HCV-RNA levels was found between GTs (p=0.063). The demographic and clinical characteristics of patients by HCV GTs are presented in Table 2.

Discussion

HCV remains one of the leading causes of chronic liver disease worldwide, responsible for approximately 400,000 deaths annually. The WHOs Global Health Sector Strategy, launched in 2016, aims to reduce HCV transmission by 90% and HCV-related deaths by 65%, with the ultimate goal of eliminating the virus by 2030. Achieving these targets depends on the widespread use of DAA therapies and increased HCV awareness (14,15,16). Understanding the prevalence of HCV viremia and rates of infection is crucial for assessing disease burden and guiding treatment strategies. Consequently, studies estimating disease burden are necessary

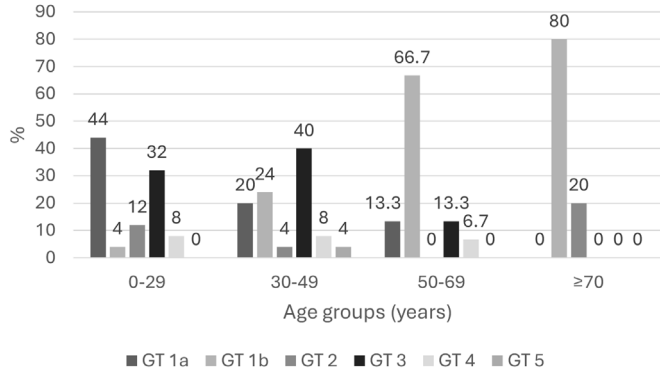

Figure 1. Distribution of HCV genotypes by age group
HCV: Hepatitis C virus

for developing national strategies. In this study, we examined HCV viremia prevalence, viremia rates, and HCV GT distribution at a tertiary care hospital, comparing the findings with data from the literature.

It is important to note that anti-HCV positive individuals do not always have an active infection; thus, the prevalence of HCV viremia is a more specific marker for active infection. Globally, the distribution of HCV infection varies by geographic region. Countries with the highest anti-HCV antibody prevalence include Gabon (4.3%) and Pakistan (4.1%), while nations such as Saudi Arabia, Spain, and the Netherlands report prevalence rates below 0.1% (17). In rural areas, these rates can vary significantly; for instance, in rural Taiwan, prevalence rates as high as 16.7% have been reported (18). A recent study identified the highest HCV prevalence in Egypt (6.3%), followed by the Democratic Republic of Congo (4.3%). Among developed countries, notable findings include a prevalence exceeding 2% in Estonia and Italy (19). In Türkiye, studies on anti-HCV prevalence have reported rates ranging from 0.27% to 2.76% (4,17,20,21). In our study, the anti-HCV prevalence was found to be

0.38%, which falls at the lower end of this range when compared to other studies in Türkiye and globally. This discrepancy may be attributed to the demographic characteristics of the region and population studied.

The prevalence of viremic HCV infection in this study was 0.04%. Globally, a significant decrease in HCV viremia prevalence has been observed over the years. A meta-analysis covering the period from 2015 to 2020 showed substantial changes in global HCV infection prevalence and viremia rates due to treatment efforts. In 2015, the global prevalence of viremia was 0.9%, which decreased to 0.7% in 2020, with the number of viremic infections dropping from 63.6 million to 56.8 million. In 2020, HCV prevalence was highest in Eastern Europe (2.9%) and Central Asia (2.6%). The highest number of viremic infections occurred in South Asia (14.5 million) and East Asia (10 million). In Türkiye, no significant change in viremic HCV prevalence was noted between 2015 and 2020, with the rate remaining at 0.3%. In regions such as Western Europe and North America, HCV prevalence has fallen below 0.1% (16). Another study reported a global viremic HCV prevalence of approximately 1.1%. Regionally, the highest viremia rate was found in sub-Saharan Africa (4.1%), while the lowest was in Western Europe (0.6%) (22). In Türkiye, viremic HCV prevalence has been reported to range from 0.3% to 2.05% (4,20). In this study, the prevalence of viremic HCV infection was 0.04%, significantly lower than the global and national averages. Differences in viremic HCV prevalence in Türkiye may be attributed to regional and demographic variations, as well as the characteristics of the study population, diagnostic methods, and access to healthcare.

The age-related HCV antibody prevalence was highest among those aged 70 and over (0.8%) and lowest among individuals under 29 (0.2%). Studies conducted in Türkiye have confirmed that HCV antibody prevalence increases with age. This rise is likely associated with older individuals who became infected during periods when blood transfusions and medical procedures posed a higher risk of transmission, particularly in the 1970s and 1980s. Additionally, advancements in medical practices in recent years may explain this trend (4). On the other hand, Chlibek et al. (23) found the highest HCV antibody prevalence in the 30-44 age group in the general population, while Suntur et al. (21) reported elevated anti-HCV prevalence in patients aged 18-29. Both studies suggested that the higher prevalence in these age groups may be linked to intravenous drug use (21,23). These data indicate that intravenous drug use plays a significant role in HCV transmission, particularly among younger populations, and that efforts to reduce this behavior are critical for controlling the spread of the infection.

Globally, HCV viremia rates show significant regional variations; for instance, viremia rates as low as 43% have been observed in Central Asia, while rates in South Asia have risen as high as 81% (22,24). Two studies conducted in Türkiye reported viremia rates of 21.3% and 33.17% (10,20). In this study, the HCV viremia rate was significantly lower at 11.1% compared to rates reported in both studies. Several factors may explain the lower viremia rates observed in our study. First, limited HCV-RNA testing among anti-HCV positive individuals may result in underreporting of true viremia rates. Additionally, widespread screening and treatment programs in Türkiye, as well as the increasing use of DAA therapies, may contribute to the decline in viremia rates.

Although HCV GT testing is no longer as crucial in determining treatment as it once was, it remains important in some cases. The first-generation DAA therapies had varying efficacy depending on the HCV GT, making GT determination critical in treatment selection. However, with the development of pan-genotypic DAAs that are effective against nearly all HCV GTs, routine GT testing is no longer necessary. Nevertheless, in certain cases, genotyping is still relevant (25,26). Studies investigating global HCV GT distribution have shown that GT1 is the most common GT at 49.1%, followed by GT3 at 17.9% and GT4 at 16.8% (24). Research conducted in the Middle East and North Africa has reported that GT1 is prevalent in the region. Countries such as Morocco, Algeria, Bahrain, and Libya show higher frequencies of GT2, while GT3 is more common in Afghanistan and Pakistan, and GT4 is dominant in Egypt, Iraq, Qatar, Palestine, and Syria (9). Studies on HCV GT distribution in Türkiye have also shown that GT1 is the most common GT, with GT1b being the most prevalent subtype (10,15,20). In this study, GT1 was identified as the most prevalent GT. While GT1a was dominant in the 0-29 age group, GT1b was the predominant subtype in other age groups. These findings suggest that despite the widespread use of pan-genotypic DAAs, there has been no significant change in HCV GT distribution. However, as pan-genotypic DAAs have been reported to achieve high success rates across all GTs, it is still unclear whether GT distribution will shift over time.

Study Limitations

The fact that the data belong to a single center is one of the limitations of this study.

Conclusion

This study has demonstrated that HCV prevalence and viremia rates are lower compared to those found in global data and that GT1 is identified as the predominant GT. The detection of higher viremia rates among the young population and foreign individuals highlights the necessity of early diagnosis and screening programs in these groups.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: 2024-805, dated: 28.08.2024).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.F.K., A.B., Concept: M.F.K., A.B., Design: M.F.K., G.K., A.B., Data Collection or Processing: M.F.K., G.K., A.B., Analysis or Interpretation: M.F.K., A.B., Literature Search: M.F.K., A.B., Writing: M.F.K., A.B.

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

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