The Risk of Acute or Reactivated Hepatitis E Virus Infection at a Liver Transplant Center in Brazil: Observational Cohort Study

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Article

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Abstract: Hepatitis E virus is a major etiological agent of chronic hepatitis in immunosuppressed individuals. Studies on HEV in the context of liver transplantation have produced mixed results, most of the data coming from studies conducted in high-income countries. This was a prospective cohort study of liver transplant recipients in southeastern Brazil. Recipients were systematically followed for one year, with the objective of determining the prevalence, incidence, genotype, and natural history of HEV infection in this population. We included 107 liver transplant recipients and 83 deceased donors. Positivity for anti-HEV IgG was detected in 11 (10.2%) of the recipients and in eight (9.7%) of the donors. None of the patients tested positive for HEV RNA at baseline or during follow-up. There were no episodes of reactivation or seroconversion, even in cases of serological donor-recipient mismatch or in recipients with acute hepatitis. Acute and chronic HEV infections seem to be rare events in the region studied. That could be attributable to social, economic, and environmental factors. Our data indicate that, among liver transplant recipients, hepatitis E should be investigated only when there are elevated levels of transaminases with no defined cause, as part of the differential diagnosis of seronegative hepatitis after transplantation.

Keywords: hepatitis E; liver transplantation; prevalence; incidence; natural history; Brazil

1. Introduction

As has been demonstrated previously, hepatitis E virus (HEV), which is a non-enveloped RNA virus of the family *Hepeviridae*, is a major etiological agent of acute hepatitis and liver failure in the general population. It has recently been shown to be a cause of chronic hepatitis and cirrhosis in immunosuppressed individuals, especially in solid organ transplant (SOT) recipients [1–5].

Cases of human HEV infection are caused by the species *Orthohepevirus A*, which comprises eight genotypes [1]. Genotype 3 (HEV-3), which is transmitted mainly through the consumption of pork, is the main genotype responsible for chronic hepatitis in immunosuppressed individuals, whereas genotypes 1, 4, and 7 have been implicated only in rare cases [6].

Studies of hepatitis E in liver transplant recipients (LTRs) have produced quite variable results, most of the data coming from prevalence studies conducted in high-income countries, mainly in Europe [5]. Data regarding the clinical evolution of hepatitis E in SOT recipients are also discordant, reports ranging from benign evolution to a high frequency of chronic disease [7,8]. Given that

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geographic, socioeconomic, and cultural differences seem to lead to variability in the presentation of HEV infection, the aim of this study was to determine the prevalence, incidence, genotype, and natural history of HEV infection among LTRs in southeastern Brazil.

2. Materials and Methods

2.1. Study Design and Patient Selection

This was a prospective study of LTRs submitted to periodic collection of blood samples to identify markers of HEV infection. We included patients ≥ 18 years of age who underwent liver transplantation between June 2019 and December 2020 at the organ transplant center of the Hospital Municipal Vila Santa Catarina, in the city of São Paulo, Brazil. The hospital is operated by the Hospital Israelita Albert Einstein, also based in the city of São Paulo.

Recipients were followed for one year, during which time blood samples were collected periodically for serology (for anti-HEV IgG and IgM antibodies) and for quantitative reverse transcription PCR (qRT-PCR): in the pretransplant period; monthly for the first 6 months after transplantation; and at 9 and 12 months after transplantation. That periodicity was necessary to avoid missing transient viremia and to determine the behavior of the viral load over time. When a qRT-PCR was positive for HEV, genotyping was performed. When available, blood samples collected from subsequently deceased donors were also submitted to serology and qRT-PCR. When an LTR developed acute hepatitis during the follow-up period, we performed additional serology for anti-HEV IgM and IgG antibodies, as well qRT-PCR for HEV, to determine the incidence of acute hepatitis E in the posttransplant period. In recipients found to be infected with HEV, qRT-PCR was also performed during clinical events that required hospitalization, such as any other severe infection or acute rejection. Epidemiological, clinical, and biochemical data were collected from all of the recipients evaluated.

2.2. Definitions

To characterize new or reactivated HEV infection, the following definitions were used:

- HEV infection: anti-HEV IgM positivity, anti-HEV IgG positivity, or positivity for HEV on qRT-PCR.
- Primary HEV infection: anti-HEV IgM positivity, anti-HEV IgG positivity, or positivity for HEV on qRT-PCR in a patient previously testing negative for anti-HEV IgG or IgM antibodies.
- Reactivated HEV infection: positivity for HEV on qRT-PCR, at any time after transplantation in a recipient who, at the time of transplantation, had tested positive for anti-HEV IgM or IgG antibodies but negative for HEV on qRT-PCR.
- Chronic HEV infection: positivity for HEV on qRT-PCR for a period of three months or more.
- Acute hepatitis: increase to at least double the normal value in the concentrations of the hepatic transaminases alanine aminotransferase (ALT) and aspartate aminotransferase or of the canalicular enzymes gamma-glutamyl transferase and alkaline phosphatase.

2.3. Serology

In serum samples, we quantified anti-HEV IgM and IgG antibody titers, using commercial in vitro kits (recomWell HEV IgM/IgG; Mikrogen GmbH, Neuried, Germany), which are based on the principle of an indirect sandwich ELISA with recombinant antigens from the open reading frame 2 and open reading frame 3 regions of the HEV genome, expressed in *Escherichia coli*.

2.4. HEV RNA Detection

All samples were submitted to molecular analysis. For the extraction of viral RNA, we used the QIAamp MinElute Virus Spin extraction kit (QIAGEN, Hilden, Germany), and we used the RealStar HEV RT-PCR kit 2.0 (Altona Diagnostics, Hamburg, Germany) for the detection of HEV. To rule out the possibility of false-negative results, we also subjected every sample to an in-house PCR-based assay that is capable of differentiating the various strains of HEV [9].

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2.5. Statistical Analysis

Means and standard deviations were reported for continuous variables. For categorical variables, frequencies and percentages were reported. Statistical analyses were performed with JASP software, version 0.141.0 (https://jasp-stats.org/).

2.6. Ethical Aspects

All procedures were performed in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the 2013 Declaration of Helsinki and with the guidelines established in the 2018 Declaration of Istanbul. The study was approved by the Research Ethics Committees of the Hospital Israelita Albert Einstein and the Federal University of São Paulo. All subjects gave their informed consent for inclusion before they participated in the study.

3. Results

3.1. HEV Infection in the Pretransplant Period

Between June 2019 and December 2020, we recruited 107 LTRs and 83 donors. We collected a total of 765 blood samples.

In the pretransplant period, the anti-HEV IgG test result was positive in 11 (10.2%) of the recipients, positive in eight (9.7%) of the donors, and inconclusive in three (1.6%) of the recipients. The anti-HEV IgM test result was positive in one (0.9%) of the recipients and inconclusive in one (1.2%) of the donors.

The demographic, clinical, and epidemiological characteristics of the recipients are described in Table 1. Among the 107 recipients, the mean age was 53.5 years and most (72.9%) were men. Almost all (96.3%) of the recipients lived in urban areas, and the majority (83.2%) reported eating pork.

Table 1. Baseline characteristics of liver transplant recipients evaluated between 2019 and 2020.

Characteristic	(N=107)
Age (years), mean ± SD	53.5 ± 13.13
Male, n (%)	78 (72.9)
More than one underlying disease, n (%)	44 (41.1)
Underlying disease, n (%)	
Alcoholic liver cirrhosis	34 (31.8)
Chronic hepatitis C	24 (22.4)
Cryptogenic cirrhosis	15 (14.0)
Autoimmune hepatitis	9 (8.4)
Non-alcoholic steatohepatitis	7 (6.5)
Other	18 (16.8)
Region of birth, n (%)	
Southeastern Brazil	77 (72.0)
Northeastern Brazil	23 (21.5)
Central-west Brazil	3 (2.8)
Southern Brazil	2 (1.9)
Northern Brazil	1 (0.9)
Region of residence, n (%)	
Southeastern Brazil	101 (94.4)
Central-west Brazil	4 (3.7)
Northeastern Brazil	2 (1.9)

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Resident of an urban area, n (%)	103 (96.3)
Level of education, n (%)	
None	1 (1.0)
< 9 years of schooling	18 (17.1)
9 years of schooling	11 (10.4)
High school, incomplete	5 (4.8)
High school, complete	36 (34.3)
College, incomplete	12 (11.4)
College, complete	21 (20.0)
Postgraduate work	1 (1.0)
Previous blood transfusion, n (%)	56 (52.3)
Consumption of pork, n (%)	89 (83.2)
Consumption of game meat, n (%)	40 (37.4)
Consumption of seafood, n (%)	79 (73.8)
Home with sewage system, n (%)	105 (98.1)
Home with treated water, n (%)	105 (98.1)
Contact with domestic animals, n (%)	56 (52.3)
Contact with natural waters, n (%)	55 (51.4)
Previous contact with an HEV-infected individual, n (%)	2 (1.9)
Travel in the last year, n (%)	50 (46.7)
Anti-HEV IgG-positive, n (%)	11 (10.3)
Anti-HEV IgM-positive, n (%)	1 (0.9)

3.2. Positive Anti-HEV IgG Receptors in Pretransplant

Among the 11 recipients who were anti-HEV IgG-positive in the pretransplant period, there were no cases of reactivation, as would have been identified through the detection of HEV RNA in the follow-up examinations, even among the four recipients who were diagnosed with acute hepatitis. Among the four recipients with acute hepatitis, two had an episode of acute cellular rejection, one developed stenosis of the biliary anastomosis, and one had metabolic acidosis with hyperkalemia, with no apparent hepatic cause.

One male recipient who, in the pretransplant period, tested positive for anti-HEV IgG (50 U/mL) and negative for anti-HEV IgM subsequently (two months after transplantation) tested positive for anti-HEV IgM (49 U/mL), with an elevated IgG titer (> 125 U/mL). Four days before the serology, that recipient developed acute hepatitis (ALT, 157 U/L). However, because he also tested positive for cytomegalovirus, with a high viral load (5.01 log copies/mL), on qRT-PCR, it was not possible to confirm a new or reactivated HEV infection. In the other recipients, there was no significant increase in anti-HEV IgG titers in the follow-up examinations. Four of the 11 recipients who were anti-HEV IgG-positive in the pretransplant period had an anti-HEV IgG titer < 20 U/mL in at least one follow-up examination. The seven remaining recipients maintained an anti-HEV IgG titer > 25 U/mL throughout the one-year follow-up period.

3.3. Anti-HEV IgG-Positive Donors

Eight recipients, six of whom were anti-HEV IgG-negative in the pretransplant period, received organs from anti-HEV IgG-positive donors. Among those eight recipients, no seroconversions were identified; nor was any HEV RNA detected in the follow-up examinations.

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4. Discussion

The prevalence of anti-HEV IgG antibodies in the context of liver transplantation is highly variable between countries and even between regions within the same country. A recent meta-analysis that included 18 studies from the United States, Thailand, China, and various European countries, found the pooled estimated prevalence of anti-HEV IgG positivity among LTRs to be 27.2% [10]. The differences in HEV prevalence may be a reflection of geographic and socioeconomic diversity, as well as differences in lifestyle habits and in the performance of the serology assay employed. In France, HEV-3 infection is quite common, especially in the southwestern region of the country, where the seroprevalence of HEV-3 among SOT recipients is nearly 40% [11]. The high prevalence in that region is probably related to the higher consumption of raw or undercooked pork. The Corsican sausages known as figatelli, made of pork liver, constitute one possible source of infection [12]. In other countries, the prevalence rates for HEV-3 infection are lower, such as the 2.9% and 7.1% reported for Japan and Argentina, respectively [13,14]. It has been extensively reported that the Wantai HEV IgG ELISA has the highest sensitivity for HEV detection, with excellent specificity as well. In a meta-analysis of studies conducted in Europe, various commercial kits were tested and the following anti-HEV IgG seroprevalences were reported: 17% for the Wantai kit; 10% for the Mikrogen kit; 7% for an MP Diagnostics kit; 4% for a DiaPro kit; and 2% for an Abbott kit. The Mikrogen kit, which was employed in the present study, has been reported to have a sensitivity of 96.6% and a specificity of 97.1% [15–22].

The anti-HEV IgG prevalence of 10.2% found in the present study is comparable to those reported in two studies employing the Mikrogen kit in the evaluation of LTRs in southeastern Brazil, albeit lower than the 18.7% reported in a study employing the Wantai kit in the evaluation of LTRs in the southern region of the country [16,23,24]. Brazil is the fourth largest pork producer in the world, and the southern region of the country is responsible for 66.12% of the national pork production [21]. It is the region with the highest number of pig farms, as well as the highest consumption of pork and pork products, and the prevalence of anti-HEV IgG positivity has been reported to be higher there than in any other region of the country [17,21,24]. Brazil is also the fifth largest country in the world and has extremely diverse socioeconomic and sanitation conditions, which influence the dynamics of infectious diseases. The fact that a large majority of the recipients in our study were residents of the southeastern region could explain the fact that the prevalence of anti-HEV antibody positivity in our sample was lower than that reported in studies carried out in the southern region.

In our study, the prevalence of anti-HEV IgG positivity among the recipients was similar to that observed among the donors, suggesting that chronic liver disease is not a risk factor for HEV infection. Similarly, the prevalence of anti-HEV IgG positivity was found to be comparable between blood donors and LTRs in the southern region of Brazil [24]. However, in a cohort of immunosuppressed patients and healthy controls in Spain, liver cirrhosis, liver transplantation, and HIV infection were found to be factors independently associated with anti-HEV antibody detection [25]. When compared with the rates reported for kidney transplant recipients, the results are also conflicting. In a study conducted in Italy, the prevalence of anti-HEV IgG positivity was found to be a similar between blood donors and kidney transplant recipients (9.1% and 10.2%, respectively) [26]. However, in another study conducted in Italy, the prevalence of anti-HEV IgG positivity was found to be much higher among individuals with chronic kidney disease than among blood donors and individuals with chronic liver disease (30.7% vs. 7.0% and 9.2%, respectively) [27].

In the present study, no HEV RNA was detected during the follow-up of the 11 recipients who were anti-HEV IgG-positive before transplantation. Therefore, qRT-PCR revealed no reactivation, even among the recipients with acute hepatitis. Certain factors must be considered when analyzing reactivation in anti-HEV IgG-positive recipients: the detection rate of HEV RNA, within and outside the context of SOT; the sensitivity of the commercial kit used; and aspects of the immune response of the recipient.

The HEV RNA detection rate reported in cross-sectional and prospective studies of LTRs is low, ranging from 0% to 4% in most published articles (Table 2) [3,8,11,13,14,25,28–45]. However, in a prospective study conducted in Thailand, involving 711 samples collected from LTRs who were

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followed for 12 months, HEV RNA was detected in 7.7% of the 91 recipients, among whom the authors found the prevalence of anti-HEV IgG positivity to be 52.7% [41]. In a study conducted in France, the authors also identified no reactivation in the first year after SOT, even among the 99 SOT recipients with elevated transaminases who had tested positive for anti-HEV IgG antibodies in the pretransplant period [32]. In Brazil, within and outside the context of SOT, there is a low detection rate for HEV RNA, which has been reported to be undetectable in some studies. In a study of 294 LTRs in southeastern Brazil, HEV RNA was detected in only 5.8%. In a study of 80 LTRs in the southern region of the country, no HEV RNA was detected in any of the recipients [23,24]. Outside the context of SOT, two studies, both conducted in southeastern Brazil, are notable. In a study of 354 HIV-infected patients, among whom the prevalence of anti-HEV IgG positivity was 10.7%, no HEV RNA was detected. In another, more recent study, which included 400 patients with elevated transaminases, an HEV RNA detection rate of 4% was reported [18,46].

Table 2. Prevalence of markers of HEV infection among liver transplant recipients in various countries, 2008–2022.

				Positivity rate		
				IgG	IgM	HEV RNA
Reference	**Year**	**Location**	**N**	**(%)**	**(%)**	**(%)**
Kamar et al. [3]	2008	France	86	10.4	ND	ND
Haagsma et al. [28]	2009	Netherlands	285	3.9	0.35	0.35
Buti et al. [29]	2010	Spain	82	3.7	0	0
Pischke et al. [30]	2010	Germany	226	4.4	0.88	0.88
Legrand-Abravanel et al. [32]	2011	France	171	12.9	ND	0
Pas et al. [31]	2012	Netherlands	300	ND	ND	1
Buffaz et al. [33]	2014	France (Alps)	206	28.0	1.5	1.5
Riveiro-Baciera et al. [25]	2014	Spain	338	9.5	ND	ND
Abravanel et al. [11]	2014	France	211	37.4	0.5	0
Sherman et al. [34]	2015	United States	113	19.5	0.9	0
Koning et al. [35]	2015	United States	145	42.0	4.1	0
Galante et al. [36]	2015	Germany	287	ND	ND	1.4
Inagaki et al. [13]	2015	Japan	1.893	2.9	0.05	0.12
Nicola et al. [37]	2015	Italy	79	33.0	0	0
Pisano et al. [14]	2017	Argentina	14	7.1	0	0
Agarwala et al. [8]	2018	India	30	46.6	6.6	0
Sinakos et al. [38]	2018	Greece	76	ND	ND	1.3
Reekie et al. [39]	2018	England	226	ND	ND	1.14
Mrzljak et al. [40]	2019	Croatia	242	24.4	ND	0
Komolmit et al. [41]	2020	Thailand	91	52.7	ND	5.5
Darstein et al. [42]	2020	Germany	73	24.7	ND	4.1
Chorami et al. [43]	2021	Iran	122	15.6	ND	ND
Ogut et al. [44]	2022	Turkey	185	31.9	1.6	0
Samala et al. [45]	2022	United States	203	23.2	0	0.5
This study	2021	Brazil	107	10.2	1.9	0

ND, no data (test not performed).

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According to the manufacturer, the kit employed for qRT-PCR in the present study has an analytical sensitivity of 0.20 IU/μL (95% CI: 0.12–0.45). The kit has been used in studies of SOT in Brazil and Europe [24,33,47]. To avoid false-negative results, the samples evaluated in the present study were submitted to a pangenotypic PCR-based assay, as previously described [9]. In one study, that pangenotypic PCR-based assay was shown to have greater sensitivity than the Altona and Mikrogen kits for different dilutions of the World Health Organization standard strain, as well as performing better than either of those kits in samples collected from patients with acute hepatitis [9]. The authors of that study also detected no HEV RNA in any of the patients.

One group of authors suggested that HEV RNA would be detected almost exclusively in the setting of chronic infection, which could be related to the fact that HEV infection typically evolves to chronicity in immunosuppressed patients or to the short window for HEV RNA detection in the course of self-limited acute infection [33]. The effect of the latter factor can be mitigated by collecting blood samples more frequently.

A humoral immune response and a T-cell response are both necessary for viral clearance. The anti-HEV IgG-positive recipients in our sample were infected before transplantation and perhaps managed to clear the virus before undergoing the posttransplant immunosuppression, which would explain why we identified no reactivation among those individuals. Similarly, in a sample of 28 SOT recipients who tested positive for anti-HEV IgG antibodies in the pretransplant period and developed acute hepatitis within the first four months after transplantation, no HEV RNA was detected [11]. In a study conducted recently in the United States, HEV RNA was detected in only one of 203 LTRs, suggesting that HEV chronicity is rare within the liver transplant population in that country [45]. It is possible that HEV chronicity is also rare in Brazil and that larger studies conducted in different regions of the country would lead to the identification of acute infection after transplantation, with occasional conversion to chronic infection and consequent detection of HEV RNA. The low seroprevalence rate among the recipients evaluated in the present study might have also contributed to the failure to identify HEV RNA in the samples analyzed.

As previously mentioned, one of our LTRs who tested positive for anti-HEV IgG antibodies in the pretransplant period subsequently (after transplantation) developed acute hepatitis, with IgM positivity and an increased IgG titer. The antibody concentration required to protect against HEV infection has not been well established. In a rhesus monkey model of HEV infection, previous HEV infection was shown to be capable of conferring cross-protection against different genotypes, despite the fact that HEV RNA was detected in animals with reinfection [45]. In a study of 91 SOT recipients the reinfection rate was 3.3%. The three cases of reinfection were in patients with quite low pretransplant anti-HEV IgG concentrations, suggesting that antibody levels lower than 7 U/mL are not protective [11].

During the follow-up examinations of the LTRs in our sample, no cases of primary infection were observed among those who were anti-HEV IgG-negative in the pretransplant period, even in cases of serological donor-recipient mismatch or in recipients with acute hepatitis. Transmission of HEV via the graft is a rare event in the SOT setting. There has been at least one reported case of a LTR who was infected via the donated organ, subsequently progressing to chronicity and liver cirrhosis [48]. There has also been a report of two kidney transplant recipients who developed chronic HEV infection after receiving organs from the same donor [49]. That donor had experienced an episode of acute hepatitis, with an ALT level of 110 U/L, an alkaline phosphatase level of 400 U/L, and an HEV load of 2,870,000 IU/mL (6.46 log IU/mL) on RT-PCR, as well as testing positive for anti-HEV IgG and IgM antibodies [49]. The authors of one study evaluated the risk of HEV transmission via the transplanted organ by analyzing viral markers in 15 donors [32]. All 15 donors tested negative for HEV RNA, and only one tested positive for anti-HEV IgM antibodies, the same donor testing negative for anti-HEV IgG antibodies [32]. The fact that HEV transmission via a graft is rare is likely explained by the relatively rapid viral clearance in immunocompetent patients and the rarity of latent virus in donated organs. Primary HEV infection from environmental exposure after transplantation has been reported more frequently, the highest rates having been reported for France and India, whereas rates below 2% have been reported for other countries. In a study of 30 LTRs who were

followed for six months in India, five were found to be infected with HEV after transplantation [8]. In a study of 123 LTRs followed for one year in France, 12 primary HEV infections were identified, translating to an incidence of 4.8 cases/100 person-years [32]. In a subsequent study, also conducted in France, there were three cases of non-graft-related exogenous reinfection during the first year after transplantation among the SOT recipients who tested positive for anti-HEV IgG antibodies in the pretransplant period, which does not seem to have happened among the LTRs evaluated in the present study [11]. That discrepancy could be attributed to the way in which we preselected individuals for transplantation (taking into account social and environmental conditions), less exposure to risk factors associated with HEV infection (due to the posttransplant care recommended), the probable predominance of HEV-3 in Brazil, and the higher proportion of patients who were residents of the southeastern region, where basic sanitation is better and the rates of pork consumption are lower than in other regions of the country.

The originality of our study lies in the longitudinal follow-up of a large number of patients, with the systematic collection of blood samples to assess HEV infection status, rather than the point prevalence of infection and disease caused by the virus. Our study has some limitations. The fact that it was conducted at a single center could have created a selection bias. In addition, the follow-up period was relatively short (only one year). Furthermore, the sample was fairly homogeneous in terms of regional representation, given that most of the patients evaluated resided in southeastern Brazil.

We can conclude that, in the scenario of liver transplantation in Brazil, hepatitis E should be investigated only in recipients with elevated levels of transaminases or canalicular enzymes with no defined cause, as part of the differential diagnosis of seronegative hepatitis after transplantation. Therefore, we believe that pretransplant screening and systematic testing for HEV infection are unnecessary, as is the case for infection with cytomegalovirus and certain other pathogens. Given the size and heterogeneity of the population of Brazil, in terms of habits and socioeconomic conditions, we believe that there is a need for additional studies to evaluate LTRs in other regions of the country. With more extensive data on the prevalence and incidence of HEV infection in the country, it would be possible to determine the cost-effectiveness of systematic screening for such infection in the setting of liver transplantation in Brazil.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Universidade Federal de São Paulo (CAAE 03829318.5.3002.5505) and the Hospital Israelita Albert Einstein (CAAE 03829318.5.0000.0071).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data analyzed during the current study is not publicly available, but it is available upon request from the corresponding author.

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