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Clinical significance of Hepatitis B virus Genotypes B and C

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Abstract

Aims: Not many researchers report the significance of Genotypes B and C and their implications in sub-Saharan African countries like Nigeria, hence this research aims at finding out this significance in Plateau state.

Methodology and Results: A Real-Time TEL-PCR-HBV reagent system based on SYBR Green PCR technology was used for the qualitative detection or viral load single-plex detection of hepatitis B Virus (HBV) for 24 Hepatitis B Virus Surface Antigen (HBsAg) positive mothers and their infants. The PCR reaction involved denaturation of the sample at 95^{0}C for 3 minutes for one cycle, followed by 40 cycles consisting of 95^{0}C for 0.15seconds, annealing at 60 ^{0}C for 0.15seconds and final extension at 72^{0}C for 0.10 seconds. HBV DNA from 48 subjects (24 mothers paired to 24 children) was amplified. The most prevalent genotype was C (66.7%) followed by genotype B (33.3%). It was also observed that the mix genotypes; 5 (83.3%), samples contained both A, B and D; 2 (100.0%,) samples contained both C and D; and 2 samples contained genotypes A, B, C and D in mothers, while mix genotype was also observed in infants; 1 sample contained both genotypes A and B, and another 1 (16.7%) sample contained genotype A, B and D.

Conclusion, Significance and Impact of Study: This study discovered that just like knowledge of the clinical significance of other genotypes in the transmission of HBV is relevant, Genotypes B and C have high clinical significance as well particular in Nigeria as a developing nation

Keywords: Hepatitis B virus; Genotypes; Chronic hepatitis B; Anti-viral therapy; Viral mutation

1. Introduction

Around 296 million people worldwide are chronically infected with the hepatitis B virus (HBV), which causes roughly one million deaths each year from liver disorders such hepatocellular carcinoma (HCC) and liver cirrhosis (LC) [1]. HBV is a tiny, encased DNA virus that spreads by way of an RNA bridge. The HBV virus is an enclosed pathogen with four widely overlapping open-reading frames, C, X, P, and S, with a genome made of partly double-stranded circular DNA. Hepatitis B e antigen (HBeAg) and hepatitis B core antigen (HBcAg) are encoded by gene C, which contains the sequences of pre-core (preC) and core proteins. The transactivating protein X is encoded by X (HBx). S encodes three surface antigen proteins, while P encodes the DNA polymerase protein with reverse transcriptase (RT) [2, 3, 4]. The 10 established HBV genotypes (A to J) consisting of sequence variants greater than 8% of the whole genome and showing diverse regional distributions were found by a global geographic analysis resulting from HBV treatment [5, 6, 7]. The prognosis and clinical characteristics of different HBV genotypes are linked to each other. According to certain research, for instance, genotype D is closely linked to acute hepatitis [9], genotype A preferentially progresses to chronic hepatitis B [8], genotype B is more resistant than genotype C, and genotype C has a higher aggressive disease risk of HCC than genotype B [10, 11]. A major contributing factor to LC, HCC, and chronic hepatitis B (CHB) is HBV infection. The onset and progression of different liver disorders have been linked to distinct HBV genotypes and genomic alterations.

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However, due to contradicting evidence from various geographic locations, our understanding of HBV genotypes and genetic variants in HBV patients is quite restricted and contentious [12, 13, 14]. Different geographical distributions of genotypes and subgenotypes have been observed in populations worldwide [15, 16].

The most widely distributed genotypes in Europe, Africa, and North America are A and D. While genotype E is primarily found in Africa, genotypes B and C are more common in East and South-East Asia. Native groups from South America, Central America, and Alaska comprise the unique American continent population known as genotype F [17, 18, 19]. Patients with genotypes A or B have been demonstrated to respond to interferon therapy more favorably than those with genotypes C and D. Furthermore, compared to those infected with (sub)genotypes A, B, D, and F4, those infected with (sub)genotypes C and F1b displayed a delayed HbeAg to anti-Hbe seroconversion. Furthermore, mounting data indicates that (sub)genotypes C and F1b are closely linked to the early and quick development of chronic infection and the evolution of HCC [20, 21, 22]. Although the other genotypes are typically located where horizontal transmission is the primary route, genotypes B and C are common in very endemic places where perinatal or vertical transmission plays a significant part in the viral propagation. As a result, HBV genotyping can be used as an epidemiological tool to look into HBV transmission and geographic evolution [23].

Phylogenetic analysis and whole genome sequencing are the gold standards for HBV genotyping [24, 25]. While this method is quite sensitive and may identify novel and recombinant genotypes, it is costly, takes a long time, and identifies the dominant genotype in genotype mixtures primarily [24, 25]. Single gene sequencing is a substitute for whole genome sequencing.

Both sequence length and degree of homology affect the sensitivity of single gene sequencing. A commercial direct sequencing assay kit (TRUGENE HBV Genotyping Kit; Siemens Medical Solutions Diagnostics, NY, USA) is available, which is an essential remark to emphasize [26].

This test is a two-in-one assay that allows for the simultaneous detection of HBV sequence mutations and genotypes in plasma or serum specimens in around 8 hours. Reverse hybridization can identify both single and mixed genotypes; Innogenetics invented the technique, which is marketed under the name INNO-LiPA [24].

In Africa, especially in Plateau State, Nigeria, there is a dearth of information about the biological traits of genotypes. Few in-vivo investigations have directly compared virological parameters across genotypes since there are insufficient replication models, and most studies comparing HBV genotypes have been limited to genotype comparisons between genotypes B and C in Asia and genotypes A and D in Europe [27]. We do not currently have the knowledge necessary to identify the relationship between HBV genotypes and the course of HBV-infected illnesses. To better understand the clinical importance of HBV genotypes B and C in the three senatorial zones of Plateau state, north central Nigeria, is the goal of our research.

2. Materials and Methods

2.1. Genotyping of HBV

Sample size was 24 HBsAg mothers who were tested positive out of 260 pregnant mothers in a research [28]. Real-Time TEL-PCR-HBV is a reagent system based on SYBR Green PCR technology, for the qualitative detection or viral load single-plex detection of hepatitis B Virus (HBV). The set-up is made up of three microliters (3 µl) of the extracted HBV DNA which were amplified in a TEL-PCR. A 25 µl reaction Master-mix was prepared for 50 samples including the positive and negative control to be tested, it was then mixed properly and centrifuged. Aliquot of 22 µl of the mixed master-mix was dispensed into each PCR reaction tube, and 3 µl of the extracted DNA and 3 µl of the positive and negative controls were used. The primer-mix and DNA were mixed thoroughly and also the controls with the Master-Mix by pipetting up and down. The reaction tubes were covered with appropriate optical lids and centrifuged with a microtiter plate rotor for 30 seconds at 1000 x g. The resulting samples were finally transferred to a CFX-96 Bio-Rad machine which was allowed to run for 40 cycles. The PCR reaction was set as follows, denaturation ofA the sample at 95 0 C for 03.00 minutes for one cycle, followed by 40 cycles consisting of 95 0 C for 00.15seconds, annealing at 60 0 C for 00.15seconds and final extension at 72 0 C for 0.10 seconds which was entered into A HP EliteBook 830 G58 generation which was used to monitor the PCR assay real-time graph as shown in figures 1.

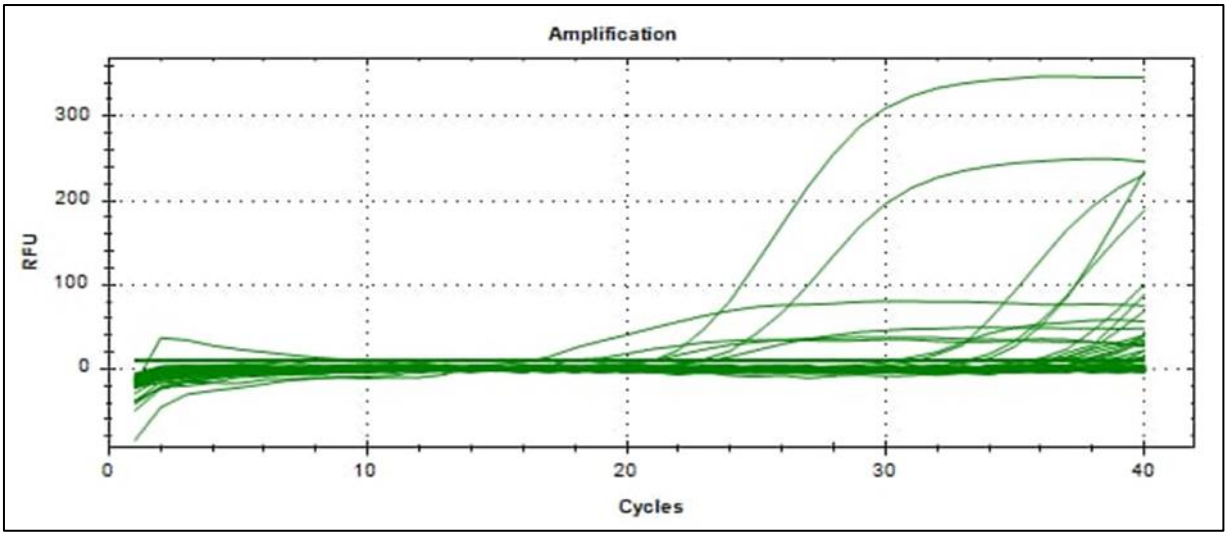

Figure 1 Amplification graph for HBV Genotyping using CFX 96 Biorad PCR machine

2.2. Quality Assurance

A qualified supervisor reviewed the data every day to ensure it was accurate and comprehensive. With consistent results, the fast HBsAg test kit's performance was assessed with established controls that came from blood donors who had undergone ELISA testing. Three participants with sera of positive HBeAg underwent the same procedure and had the same result. A colored band to the control (C) line also represents control procedures that increases the validity of the outcome.

2.3. Data Analysis

A Statistical Package for Social Science (SPSS); IBM Corp., Armonk, NY, USA version 26 was used to code, enter, and analyze the research data. We used the mean plus standard deviation (SD) or proportion to characterize the data. For categorical predictors, the X^2 test (or Fisher's exact test, if appropriate) was used to evaluate the relationship between participant characteristics and outcome variables (HBsAg positive). To find variables that have been connected independently, the multivariate logistic regression model included all explanatory variables in the bivariate analysis with a p-value ≤ 0.05. The investigation of the impact of different factors on the incidence of HBV infection was done using odds ratios (OR) and their 95% confidence intervals (CI). A statistically significant p-value was defined as less than 0.05.

3. Results and Discussion

Hepatitis B Virus Genotypes B and C in Mothers and Their Infants

HBV genotyping was conducted for the 24 HBsAg positive mothers discovered in this research. 8 mothers were positive for genotype B out of the 24 HBsAg positive mothers, with only 1 of their babies being tested positive; 4 babies were detected positive for genotype B while their mothers were negative. A total of 5 mothers were tested positive for genotype C out of which only 1 baby was also positive for the genotype, 3 babies were detected positive for genotype C while their mothers were negative. A further distribution analysis compared to genotypes A, D and E revealed that a total of 17 mothers were tested positive for genotypes A to D while a total of 16 babies were tested positive for the same range of genotypes (table 1). No mother (0.00%) and 1(100.0%) baby was respectively positive for genotype A only; The same result was also found for genotype B only; 2 mothers (50.0%) and 2 babies (50.0%) were respectively positive for both genotypes C and E only, 1(50.0%) mother and 1(50.0%) baby were respectively positive for genotypes A and E only. In addition, 1(100.0%) baby was positive for both genotypes A and B only, while 3(75.0%) babies and 1(25.0%) mother was positive for genotypes D and E only. Similarly, 2(100.0%) mothers were positive for genotypes A, B and D with no baby being tested positive for all the three genotypes; 4(66.7%) mothers were tested positive for genotypes A, B, D and E while 2(33.3%) babies were positive for all the four genotypes; 1(100.0%) mother was positive for genotypes A, B, C and E with no baby being tested positive for the four types, while 1(100.0%) mother was tested positive for genotypes A, C, D and E with no baby being tested positive for the four types. Finally, 1(100.0%) baby was confirmed positive for genotypes A, B, C, D and E.

Table 1 Distribution of genotype of HBV among HBsAg positive mothers and their infants

Genotype	Patients		Total (n=39)
	Baby (n=19)	Mother (n=20)	
A	1(5.3)	0(0.0)	1(2.6)
B	1(5.3)	0(0.0)	1(2.6)
D	0(0.0)	1(5.0)	1(2.6)
E	6(31.6)	7(35.0)	13(33.3)
A, B	1(5.3)	1(5.0)	2(5.1)
A, E	1(5.3)	1(5.0)	2(5.1)
C, E	3(15.8)	1(5.0)	4(10.3)
D, E	2(10.5)	2(10.0)	4(10.3)
A, B, D	0(0.0)	1(5.0)	1(2.6)
A, B, C, E	0(0.0)	1(5.0)	1(2.6)
A, B, D, E	3(15.8)	4(20.0)	7(17.9)
A, C, D, E	0(0.0)	1(5.0)	1(2.6)
A, B, C, D, E	1(5.3)	0((0.0)	1(2.6)

Patients with single genotype were 16 accounting for 41.0% which was higher in infants (42.1%) than in mothers (40.0%) (table 2). 12 (30.8%) of the patients had a combination of two genotypes with higher proportion (36.8%) in the infants than mothers (25.0%). Patients with multiple (>two) genotypes constitute 11(28.2%); this was higher in mother (35.0%) than in the infants (21.1%). A comparison of patients in relation to number of genotypes shows no association

$$(\chi^2 = 1.127, p=0.569).$$

Table 2 Association between genotype of HBV among HBsAg positive mothers and their infants

Genotype	Patients			χ^2	p-value
	Baby	Mother	Total		
Single	8(42.1)	8(40.0)	16(41.0)	1.127	0.569
Two	7(36.8)	5(25.0)	12(30.8)		
Multiple (>two)	4(21.1)	7(35.0)	11(28.2)		

A characterization analysis shows that 15 (32.3%) of the patients tested positive for genotype A and was higher in the mothers (37.5%) than in the infants (table 3). Patients who were positive for genotype B were 12 representing 25.0% of the studied sample, the proportion been higher in mothers (29.2%). Nine (18.8%) of the patients were positive for genotype C, this was equally higher in mothers (20.8%). Also fifteen (31.3%) of the studied sample were found to be positive for genotype D with lower proportion of 29.2% among the infants than in their mothers. Genotype E had the highest positivity of 68.8%. The proportion was higher (70.8%) in mothers than the infants who had had 66.7%.

However, it was found that percentage of positivity with respect to all the genotypes were higher in the mothers than in the infants with no significant difference ($p>0.05$). This indicates that in there is no association in the characterization of HBV genotypes circulating among HBsAg positive mothers and their infants across the various genotypes.

Table 3 To characterize the HBV genotypes circulating among HBsAg positive mothers and their infants

Genotypes	Patients			χ^2	p-value
	Baby	Mother	Total		
Genotype A					
Negative	18(75.0)	15(62.5)	33(68.8)	0.873	0.350
Positive	6(25.0)	9(37.5)	15(31.3)		
Genotype B					
Negative	19(79.2)	17(70.8)	36(75.0)	0.444	0.505
Positive	5(20.8)	7(29.2)	12(25.0)		
Genotype C					
Negative	20(83.3)	19(79.2)	39(81.3)	0.137	0.712
Positive	4(16.7)	5(20.8)	9(18.8)		
Genotype D					
Negative	17(70.8)	16(66.7)	33(68.8)	0.097	0.755
Positive	7(29.2)	8(33.3)	15(31.3)		
Genotype E					
Negative	8(33.3)	7(29.2)	15(31.3)	0.097	0.755
Positive	16(66.7)	17(70.8)	33(68.8)		

Distribution of genotypes in relation to patients shows that single genotype (A, B, C and D) were found to be more in babies than in their mothers. Multiple genotypes were more in mothers than in babies except A, B, C and D. In both cases there were no significant difference in the proportions ($p>0.05$) as observed in table 4.

Table 4 Distribution of genotypes in relation to patients

Genotypes	Patients			χ^2	p-value
	Babies	Mothers	Total		
A	2(66.7)	1(33.3)	3	0.333	0.564
B	1(100.0)	0(0.0)	1	-	-
C	2(66.7)	1(33.3)	3	0.333	0.564
D	3(60.0)	2(40.0)	5	0.200	0.655
A, B	0(0.0)	1(100.0)	1	-	-
A, C	0(0.0)	1(100.0)	1	-	-
A, B, D	3(37.5)	5(62.5)	8	0.500	0.480
A, B, C	0(0.0)	1(100.0)	1	-	-
A, B, C,D	1(100.0)	0(0.0)	1	-	-

The percentage of mothers who transferred genotype to their babies is presented in figure 2. Mothers with genotype A transferred more to their babies, followed by mothers with genotype D. The least was genotype B while mothers with genotype C did not transfer any genotype to their infants.

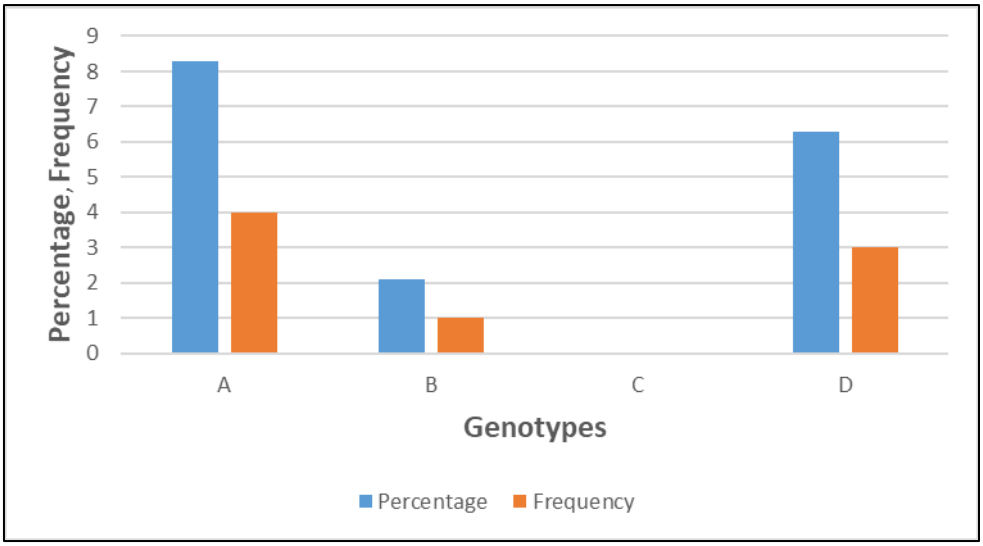

Figure 2 Percentage of mothers who transfer genotype to their babies

HBV genotyping may be a helpful tool for the epidemiologic study of intrafamilial HBV transmission, according to the division of HBV into distinct genotypes and the development of quick and easy genotyping techniques. In order to ascertain potential channels of intrafamilial transmission for each family, we employed a PCR restriction fragment-length polymorphism assay to ascertain the HBV genotypes of HBsAg-positive family members. Our findings added to the body of evidence supporting intrafamilial paternal transmission and highlight the critical role that health authorities play in promoting hepatitis B vaccination campaigns in underdeveloped nations such as Nigeria. Given the role that paternal transmission plays in the intrafamilial spread of HBV, it is possible that the prenatal maternal screening program for HBsAg will not reliably identify all pregnant mothers who require immunization at delivery. In contrast, regardless of the means of intrafamilial transmission, immunization against HBV has been demonstrated to be successful in preventing infection. In contrast to Taiwan, where genotypes B and C account for the majority of strains, more HBV strains were identified in our study with genotype B [29, 30]. Out of the 9 infants that were positive for the genotypes B and C, only 1 of them was found to be positive for both genotypes B and C, while the other 8 were positive for either genotypes B or C. Notable among the findings in this research is that the level of viral loads (cq-values) does not determine the capability of a mother transferring HBV to her infant since some mothers have high or low viral loads and are either positive or negative for genotypes B and C.

The same HBV genotype infection in parents and children is not uncommon. Consequently, genotyping data alone was insufficient to determine the children's viral origins. Nevertheless, it is not impossible for extrafamilial horizontal transmission by homotypic but distinct strains to occur, even though the mother and carrier children's concordance in HBV genotype strongly suggests that parents are potential sources of intrafamilial HBV transmission. In the present study, 4 infants and 3 infants (babies) were found positive for genotype B and C respectively, however their mothers were tested negative. This leaves us with this assumption that it could possibly be because of contaminations from other samples during the testing process or it is of less significance for mothers to be tested positive before their infants can also be found positive for the same type of genotype. It could be a possibility that their positivity was due to paternal transmission though very slim [31]

The most widely distributed genotypes in Europe, Africa, and North America are A and D. While genotype E is primarily found in Africa, genotypes B and C are more common in East and South-East Asia. Native groups from South America, Central America, and Alaska comprise the unique American continent population known as genotype F [17, 18, 19]. This outcome is inconsistent with our research because genotypes B (prevalence of 27.1%) and C (18.8%) were identified in Plateau state, North Central Nigeria. Research has demonstrated that individuals with genotypes A or B infections typically react to interferon therapy more favorably than those with genotypes C and D infections. Furthermore, compared to those infected with (sub)genotypes A, B, D, and F4, those infected with (sub)genotypes C and F1b displayed a delayed HbeAg to anti-Hbe seroconversion. Furthermore, mounting data indicates that (sub)genotypes C and F1b are closely linked to the early and quick development of chronic infection and the eventual evolution of hepatocellular carcinoma [32, 21, 33]. Since genotypes B and C are common in more endemic locations where vertical transmission plays a significant part in the viral propagation, HBV genotyping can be used as an epidemiological method to study HBV geographic development and transmission [34, 35].

Numerous pregnant women and their unborn children were discovered to have coinfections of various HBV genotypes in the current investigation, including genotypes B and C (Table 3). This discovery aligns with other research indicating a high frequency of many dominant genotypes in specific areas. For instance, a high frequency of genotypes B and C is frequently observed in the Asia-Pacific area, but genotypes A and D are frequently found in Western nations [7]. There have been numerous reports of inter-genotypic recombination, or the recombination of various hepatitis B virus (HBV) strains [36]. This phenomenon happens when multiple HBV genotypes co-infect humans, resulting in genetic material exchange across the strains. The clinical management of HBV infection is significantly impacted by the presence of inter-genotypic recombination.

Estimating the course of the disease and formulating the best antiviral treatment plan require knowledge of the HBV genotype [36]. Variations in liver cirrhosis and hepatocellular carcinoma risk, as well as differences in the severity of the disease and response to antiviral medication, have been linked to distinct HBV genotypes [37]. Thus, in order to inform treatment choices, knowledge of the distribution of HBV genotypes and the possibility of inter-genotypic recombination is crucial.

Previous research on HBV recombination indicates that most recombinants that have been found are hybrids of genotypes A/C or B/D, with genotypes A and C showing a greater propensity for recombination than other genotypes [38, 39]. New genotypes that originate from this inter-genotypic recombination of HBV strains have the potential to change the clinical presentation and severity of the disease [38]. Inter-genotypic recombination is exemplified by the identification of HBV genotype I, which was discovered in Vietnam and Laos and is a distinct recombinant between genotypes A, C, and G [38]. This discovery emphasizes how dynamically HBV evolves and how inter-genotypic recombination may affect the genetic diversity and clinical manifestations of the virus.

4. Conclusion

In Conclusion, we found that, although understanding the clinical importance of other genotypes in the transmission of HBV is important, genotypes B and C also have a great deal of clinical value, especially in underdeveloped countries like Nigeria. According to the findings of our study, screening for HBV infection in all family members—including fathers—and HBV vaccination for all children should be promoted in order to lessen horizontal transmission.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Statement of ethical approval

The research was conducted adhering to the Nigerian National Code for Health Research Ethics (NCHRE). The ethical committees of Plateau State Hospital Management Board (Ref: HMB/ADM/423/111/794), Jos University Teaching Hospital (JUTH, Ref: JUTH/DCS/IREC/127/XXX2041), and Bingham University Teaching Hospital (Ref: NHREC/21/05/2005/00700) reviewed and approved this research project to cover the three senatorial zones of the state. Prior to enrollment, the expectant mothers provided written informed permission. The primary investigator (PI) maintained all research subjects' identities and results of their individual laboratory tests private. This study also noted the use of code word safety and other relevant database security procedures.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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