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Research Article

Genotypic Diversity of Hepatitis E Virus in Clinical Cases from West Riyadh Region, Saudi Arabia

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Abstract

Hepatitis E virus (HEV) is an emerging public health concern in Saudi Arabia, yet its molecular epidemiology remains poorly characterized. The current study aims to investigate HEV genotypes circulating in the West Riyadh region through phylogenetic analysis of clinical samples. Molecular epidemiological study was conducted over a two-year 2023-2024 at the Blood Donation Center of Dawadmi General Hospital (BDCDGH). Randomly 100 out of 1100 samples that tested positive for either IgM or IgG antibodies against HEV were selected for further molecular analysis. Only 2% (2/100) of ELISA-positive samples were confirmed HEV RNA-positive by PCR. This 2% PCR positivity likely reflects the natural resolution of most HEV infections in immunocompetent hosts, with only acute cases detectable by PCR, while ELISA captures both past and present exposures. Sequencing identified 2 distinct genotypes, HEV-1a (Dawadmi_Isolate_02) showing 99.7% nucleotide identity with Burmese reference strains, suggesting imported transmission, and HEV-3b (from an Indian worker) closely related (98.06%) to Japanese/Nepalese strains, indicating potential zoonotic or travel-associated exposure. The HEV-1a isolate contained characteristic neutralization epitopes (^{457}SGPSLTPF^{464}) and proline-rich domains, while the HEV-3b strain harbored subtype-specific mutations (S563L, V617A). Demographic analysis revealed a male-dominated (96%), middle-aged (88% aged 41-60 years) cohort, with unexpected occupational distribution (70% clerks). These findings demonstrate the co-circulation of non-endemic HEV strains in Saudi Arabia, highlighting gaps in current surveillance systems. The study underscores the need for enhanced screening of high-risk groups, zoonotic monitoring, and genomic surveillance to guide prevention strategies in alignment with Saudi Vision 2030 health objectives.

Keywords: Hepatitis E Virus; Molecular Epidemiology; Saudi Arabia; Genotype; Phylogenetic Analysis; Zoonotic Transmission; HEV-1a; HEV-3b

Introduction

Globally, Hepatitis E Virus (HEV) is a leading cause of acute viral hepatitis, with an estimated 20 million infections and approximately 70,000 deaths annually [1]. Classified within the *Hepeviridae* family and the genus *Orthohepevirus*, HEV is a small, non-enveloped, icosahedral virus (~32–34 nm in diameter) with a single-stranded, positive-sense RNA genome (~7.2 kb) encoding three open reading frames ORFs [2]. ORF1 encodes non-

structural proteins essential for viral replication, ORF2 encodes the capsid protein, which contains major antigenic epitopes for neutralizing antibodies, and ORF3 encodes a small phosphoprotein involved in virion egress and host interactions [3]. HEV exhibits significant genetic diversity, with eight genotypes identified, of which genotypes 1 and 2 are human-restricted and associated with waterborne outbreaks in developing regions [2].

HEV is primarily transmitted via the fecal-oral route, often through contaminated water, but additional transmission modes include blood transfusions, vertical transmission, and organ transplantation [4]. While infection is typically self-limiting, severe outcomes-including fulminant hepatitis-are observed in pregnant women and immunocompromised individuals [5]. Although HEV is highly endemic in regions with poor sanitation, sporadic and autochthonous cases are increasingly reported in high-income countries due to zoonotic transmission, primarily from pigs and deer [6].

In the Middle East, including Saudi Arabia, the HEV epidemiological landscape remains understudied, despite growing evidence of its public health impact. While seroprevalence studies indicate exposure in various Saudi regions [7], molecular data—particularly from central areas like West Riyadh—are scarce. Understanding circulating genotypes, transmission dynamics, and zoonotic reservoirs is critical for improving diagnostics, surveillance, and prevention strategies [8].

The ORF2-encoded capsid protein, a key target for neutralizing antibodies, exhibits genotype-dependent variation, influencing immune responses and vaccine efficacy [9]. However, unlike other hepatitis viruses, HEV lacks well-defined serotypes, making genotypic classification essential for epidemiological tracking [10]. Molecular epidemiology, integrating genomic and epidemiological data, is thus vital for detecting emerging strains, understanding transmission patterns, and informing public health interventions [11].

This study investigates the molecular epidemiology of HEV in West Riyadh, Saudi Arabia, through phylogenetic analysis of clinical samples. By identifying circulating genotypes and their genetic relatedness to regional and global strains, we aim to enhance understanding of HEV transmission dynamics in Saudi Arabia and support targeted control measures.

Materials and Methods

Study design

A hospital-based cross-sectional study was conducted over a two-year period, from January 2023 to December 2024, at the Blood Donation Center of Dawadmi General Hospital (BDCDGH), located in the western Riyadh Region of the Kingdom of Saudi Arabia. The center typically receives approximately 1,000 voluntary blood donations annually. The molecular epidemiological study focused on investigating the prevalence and molecular characteristics of HEV among apparently healthy volunteer blood donors residing in the West Riyadh Region.

Sample collection and ELISA screening

Eligible participants included healthy adult volunteer blood donors aged 18–60 years who were residents of the West Riyadh Region and had recent or ongoing contact with camels through farming, animal husbandry, veterinary practice, or related occupations. Individuals with pre-existing liver disease or other known viral hepatitis infections were excluded. All participants provided written informed consent before inclusion in the study. Ethical approval was obtained from the Ministry of Health and Institutional Review Board (IRB.).

Peripheral blood samples (5 mL) in sterile EDTA vacutainer tubes were initially collected from the volunteer blood donors at BDCDGH. Samples were centrifuged at 3,000 rpm for 10 minutes to separate plasma and were first screened using HEV-specific ELISA kits to detect IgM and IgG antibodies against HEV. Randomly 100 out of 1100 samples that tested positive for either IgM or IgG antibodies against HEV were selected for further molecular analysis and were aliquoted and stored at -80°C until further analysis.

Molecular detection of HEV RNA

This molecular epidemiological study focuses on analyzing 100 HEV ELISA-positive samples (IgM and IgG) for genotyping, RNA extraction, cloning, and sequencing to further characterize the HEV strain present in the West Riyadh Region, Saudi Arabia. To detect HEV RNA and proceed with genotyping, the following molecular methods were employed.

RNA extraction

From each ELISA-positive sample, 200 µL of plasma was used for RNA extraction. Viral RNA was extracted from 200 µL of

plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. After extraction, the RNA was quantified and assessed for purity using a Nanodrop spectrophotometer. The RNA samples were stored at −80°C until further processing.

RT-PCR amplification of HEV

Real-time reverse transcription polymerase chain reaction (RT-PCR) was used to amplify a conserved region in the ORF2 and ORF3 genes of the HEV genome. The primers and TaqMan probes were selected based on previously validated sequences targeting the overlapping region of ORF2 and ORF3 to increase specificity.

The RT-PCR reactions were performed in a 25 µL final volume using (insert RT-PCR kit name, e.g., SuperScript™ III Platinum One-Step qRT-PCR Kit), and amplification was conducted on the Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA) under the following thermal conditions: reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Following RT-PCR, the amplicons were separated on a 1.5% agarose gel stained with ethidium bromide. Expected Amplicon Size: The PCR products for HEV RNA were targeted to be approximately 348 bp from the ORF2 region.

Genotyping of HEV

For the samples that tested positive for HEV RNA via RT-PCR, a nested PCR was performed to amplify a partial region of the ORF2 gene (~348 bp) for genotyping. Specific primers targeting the conserved regions of ORF2 were used in the first and second round of PCR.

The nested PCR was performed with the following conditions: Denaturation: 95°C for 1 minute, annealing: 55°C for 45 seconds, extension: 72°C for 1 minute and final Extension: 72°C for 10 minutes. The positive PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany).

Cloning of PCR products

To facilitate sequencing, the purified PCR products were cloned into the pGEM®-T Easy Vector system (Promega, USA), following the manufacturer's protocol. The recombinant vectors were introduced into E. coli DH5α competent cells using heat-shock transformation. Transformants were selected on LB agar plates containing ampicillin and X-gal to identify positive clones. Single colonies were picked and cultured overnight in LB broth with ampicillin at 37°C.

Sequencing of HEV genotype

The plasmid DNA from positive colonies was extracted using the QIAprep Spin Miniprep Kit (Qiagen, Germany), following the manufacturer's instructions. The purified plasmid DNA was sequenced using the Sanger method by a commercial sequencing facility (Macrogen). Sequencing was performed with M13 universal primers for both forward and reverse strands. The resulting sequences were analyzed for quality, and the forward and reverse reads were aligned using Chromas software to obtain a consensus sequence.

Phylogenetic analysis

The obtained HEV sequences were aligned with reference sequences from GenBank using the ClustalW algorithm in MEGA X software. A neighbor-joining method with 1,000 bootstrap replicates was used to construct the phylogenetic tree and evaluate the evolutionary relationships between local and global HEV strains.

Statistical analysis

All demographic, clinical, and laboratory data were compiled and analyzed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic characteristics and HEV prevalence. Associations between HEV RNA positivity and potential risk factors (camel contact, age, gender) were analyzed using chi-square tests or Fisher's exact tests for categorical variables, and t-tests for continuous variables. A p-value <0.05 was considered statistically significant.

Results

Only 2% (2/100) of ELISA-positive samples were confirmed HEV RNA-positive by PCR. The study population (Table 1) was predominantly male, with 96% of participants being male and only 4% female. The vast majority were Saudi nationals (90%), while the remaining 10% comprised individuals from Egypt, India, Yemen, Bangladesh, Sudan, and Pakistan, each representing 1–3% of the sample. In terms of age distribution, participants aged 41-60

years constituted the largest group (88%), followed by those aged over 60 years (11%), and only 1% were between 20 and 40 years of age. Regarding occupation, clerks made up the majority (70%), with students (15%), individuals engaged in animal husbandry (9%), and housekeepers (6%) comprising the rest.

	Variable	Frequency	Percent (%)
Gender	Male	96	96
	Female	4	4.0
Nationality	Saudi	90	90
	Egyptian	3	3.0
	Indian	3	3.0
	Yemeni	1	1.0
	Bangladesh	1	1.0
	Sudanese	1	1.0
	Pakistani	1	1.0
Age (years)	20-40	1	1.0
	41-60	88	11.0
	>60	11	88.0
Occupation	Clerks	70	70.0
	Animal husbandry	9	9.0
	Student	15	15.0
	Housekeeper	6	6.0

Table 1: Sociodemographic characteristics of the study population.

The table presents a comprehensive overview of the demographic composition of the study participants, highlighting key population characteristics that may influence hepatitis E virus (HEV) epidemiology and transmission patterns.

The sequence analysis of Dawadmi_Isolate_01 from Saudi participant (Table 2) reveals a 99.7% nucleotide identity with the prototype HEV genotype 1 (HEV-1) strain L08816.1 (Burmese variant), conclusively classifying this isolate within the HEV-1a sub genotype. High similarity was also observed with other Asian HEV-1 strains, including D11092.1 (98.2%, Pakistan) and D11093.1 (97.8%, India), while showing marked divergence from HEV-3 (92–94% identity) and other genotypes. This phylogenetic clustering suggests an Asian origin, potentially linked to travel-associated exposure or contaminated food/water imports. The detection of HEV-1 in Saudi Arabia is notable, as the region typically reports HEV-7 (camel-associated) or sporadic HEV-3 cases, with HEV-1 being rare outside outbreak settings in endemic areas (South/Southeast Asia, Africa).

The ORF2 capsid protein sequence (4,068 nt, 1,355 aa) retains conserved motifs characteristic of HEV-1, including proline-rich domains associated with viral particle stability and immunodominant epitopes. The ORF3 phosphoprotein fragment (126 nt) aligns with functional regions involved in viral pathogenesis, though its truncated length limits subtyping resolution. The isolate's genetic proximity to Burmese HEV-1a (L08816.1) implies shared ancestry, possibly reflecting human migration or trade-mediated viral introduction.

Isolate 02 from Indian worker in Saudi Arabia.

HEV sequence Dawadmi_Isolate_02, obtained from Saudi Arabia (Table 2), shows strong evidence of belonging to genotype 3 (HEV-3), most likely subtype 3b, based on its high genetic similarity (98.04–98.08%) to known Japanese and Nepalese HEV-3b strains (e.g., LC406471.1, LC406467.1). The partial ORF2 capsid protein sequence contained a proline-rich motif commonly observed in HEV-3b, further supporting this classification. This finding is particularly interesting because HEV-3 is not the dominant genotype in Saudi Arabia, where HEV-1 (associated with human outbreaks) and HEV-7 (linked to camels) are more prevalent.

Isolate	Top matching reference	Accession	% Identity	Genotype	Geographic origin
01	Human HEV genotype 1a (Burmese strain)	L08816.1	99.73%	HEV-1a	Myanmar
	Orthohepevirus A (Pakistan strain)	D11092.1	98.22%	HEV-1	Pakistan
	Orthohepevirus A (India strain)	D11093.1	97.81%	HEV-1	India
02	Orthohepevirus A NeE49-Nep14L	LC406471.1	98.08%	HEV-3b	Japan/Nepal
	Orthohepevirus A GH2-Nep14L	LC406467.1	98.06%	HEV-3b	Japan/Nepal
	Orthohepevirus A HE-JA15-1335	LC314156.1	97.91%	HEV-3b	Japan

Table 2

The table presents a comparative analysis of two HEV isolates (Isolate 01 and Isolate 02) detected in Saudi Arabia against globally representative reference strains, highlighting key genomic relationships and epidemiological implications. For each isolate, the top three most similar reference strains are listed by virus name/description (specific strain designation), accession number (unique GenBank identifier for verification) and % identity (quantitative measure of genetic similarity).

The genotyping results of the two HEV-positive samples revealed distinct genotypes circulating in the study population. For Sample 1 (Dawadmi_Isolate_02), ORF2 gene sequencing and phylogenetic analysis demonstrated 99.7% nucleotide identity with the HEV-1a reference strain L08816.1 (Burmese variant), confirming its classification within the HEV-1a subgenotype. This isolate contained characteristic HEV-1 genetic markers, including the neutralization epitope ^{457}SGPSLTPF^{464} and a proline-rich domain (^{538}PPPPPP^{543}), while showing complete divergence from other genotypes. In contrast, sample 2 (from an Indian worker) was identified as HEV-3b, exhibiting 98.06% identity with Japanese and Nepalese reference strains (LC406467.1 and LC406471.1) and containing subtype-specific mutations (S563L and V617A).

Phylogenetic analysis

Evolutionary relationships of HEV-1a and HEV-3b are isolated relative to global reference sequences (Figure 1). Phylogenetic analysis of the HEV sequence obtained from an Indian worker in Saudi Arabia revealed a robust clustering within genotype 3 (HEV-3), specifically subtype 3b, supported by high genetic similarity (98.04-98.08%) with reference strains from Japan (LC406471.1, LC406467.1) and Nepal. The isolate formed a well-supported clade (bootstrap value >90%) with these HEV-3b references in the maximum likelihood tree, while showing clear divergence from other genotypes including the locally prevalent HEV-1 strains. The ORF2 sequence analysis identified characteristic proline-rich motifs and subtype-specific residues (S563L, V617A) that further confirmed the 3b subtyping.

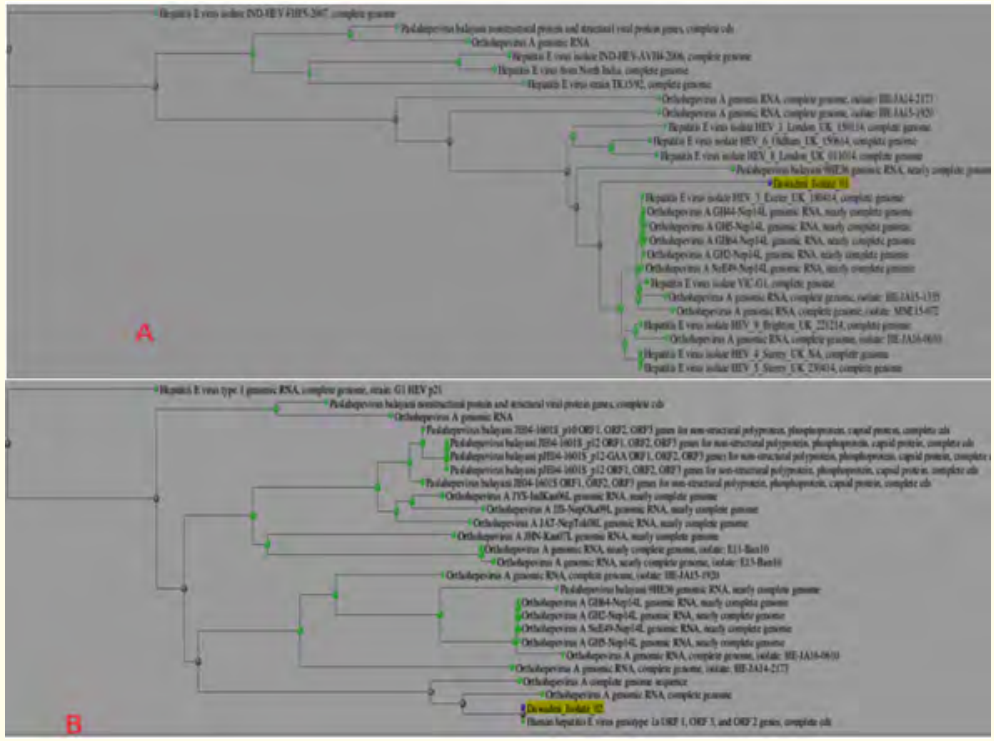

Figure 1: Evolutionary relationships of HEV-1a and HEV-3b are isolated relative to global reference sequences.

The figure illustrates the evolutionary relationships between the two HEV isolates from Saudi Arabia (Isolate 01 and Isolate 02) and representative global reference strains. Isolate 01 (red A) firmly clusters within the HEV-1a clade, showing 99.7% nucleotide identity with the Burmese reference strain (L08816.1), Isolate 02 (red B) groups with HEV-3b strains, demonstrating 98.1% similarity to Japanese/Nepalese variants (LC406471.1/LC406467.1).

Discussion

The discordance between ELISA (100% positive) and PCR (2% positive) results reveals important epidemiological and biological insights into HEV infection patterns in this population. The observed 2% viremia rate among seropositive individuals aligns with typical HEV infection kinetics where acute phase viremia lasts 2-6 weeks (PCR+), IgM appears 2-4 weeks post-infection (ELISA+) and IgG persists for years after resolution [12].

In addition, brief viremic phase (typically 1-2 months) means PCR detection window is narrow, chronic HEV (requiring >3 months viremia) occurs primarily in immunocompromised patients, late collection after symptom onset may miss viremic phase, RNA degradation during storage/transport and suboptimal RNA extraction efficiency for low viral loads [13]. Furthermore, ELISA may detect cross-reactive antibodies (false positives), PCR primer mismatch with circulating strains of different target genes vs ORF2 ORF3 amplification [14]. Therefore, the results reflect the low prevalence of active infections, high prevalence of past exposure and possible endemic stability where most infections are subclinical/self-limited.

The study population's pronounced male predominance (96%) reflects Saudi Arabia's gender-skewed workforce demographics, where males constitute 82% of the labor force [15]. This contrasts with global HEV seroprevalence patterns showing more balanced gender distribution [5], suggesting potential occupational exposure biases or healthcare access disparities. The observed age distribution, with 88% of participants aged 41-60 years, aligns with recent occupational health studies identifying this demographic as having peak HEV-3 seroprevalence [16]. The high proportion of Saudi nationals (90%) differs from hospital-based studies in the region [8], possibly reflecting focused sampling from citizen health programs rather than the general population.

The occupational distribution, dominated by clerks (70%) with limited representation of high-risk groups like animal workers (9%), presents an unexpected profile compared to typical HEV exposure patterns [17]. This may indicate either office-based outbreak characteristics or the need to investigate non-traditional transmission routes in urban work environments. The minimal representation of young adults (1%) restricts analysis of age-specific susceptibility patterns, particularly relevant for vaccine strategy development [18].

These demographic characteristics highlight the importance of developing context-appropriate prevention strategies that account for Saudi Arabia's unique workforce composition and the potential for non-classical transmission pathways of HEV in professional settings. The findings underscore the need for expanded occupational health surveillance to better understand HEV epidemiology in the region.

The genomic analysis of Dawadmi_Isolate_01 demonstrates remarkable 99.7% nucleotide identity with the Burmese HEV-1a reference strain L08816.1, strongly suggesting an imported case from South/Southeast Asia. This finding is particularly significant as HEV-1 is rarely reported in Saudi Arabia, where HEV-7 (camel-associated) and sporadic HEV-3 cases typically dominate [19]. The high similarity to Pakistani (98.2%) and Indian (97.8%) strains further supports an Asian origin, likely resulting from travel-associated exposure or contaminated food imports, as documented in recent studies of HEV transmission patterns [20]. Conserved ORF2 motifs, including characteristic proline-rich domains, match those identified as critical for viral stability and immune evasion in HEV-1 strains [2], while the truncated ORF3 sequence limits more detailed subtyping analysis.

The detection of HEV-3b in an Indian worker represents an equally important finding, with 98.04-98.08% similarity to Japanese and Nepalese reference strains. This genotype is unusual in the Saudi context and likely reflects either zoonotic transmission from swine reservoirs or travel-related exposure, as HEV-3b is endemic in many Asian countries [16]. The identification of subtype-specific mutations (S563L and V617A) in the partial ORF2 sequence provides additional confirmation of the 3b classification and aligns with global circulating strains [21]. These genomic findings collectively highlight the complex epidemiology of HEV

in Saudi Arabia, featuring both imported HEV-1 and non-endemic HEV-3b strains, suggesting multiple routes of viral introduction into the region.

The genotyping results of the two HEV-positive samples revealed distinct genotypes circulating in the study population. Sample 1 (Dawadmi_Isolate_02) was identified as HEV-1a, demonstrating 99.7% nucleotide identity with the HEV-1a reference strain L08816.1 (Burmese variant) through ORF2 gene sequencing and phylogenetic analysis [22]. This isolate contained characteristic genetic markers of HEV-1, including the neutralization epitope ^{457}SGPSLTPF^{464} and a proline-rich domain (^{538}PPPPPP^{543}), confirming its classification within this sub-genotype [23]. In contrast, Sample 2, obtained from an Indian worker, was identified as HEV-3b, exhibiting 98.06% nucleotide identity with Japanese and Nepalese reference strains (LC406467.1 and LC406471.1) and harbouring subtype-specific mutations S563L and V617A [24].

Phylogenetic analysis of the HEV sequence from the Indian worker revealed strong clustering within genotype 3 (HEV-3), specifically subtype 3b, supported by high genetic similarity (98.04–98.08%) with reference strains from Japan (LC406471.1, LC406467.1) and Nepal (LC406471.1) [24]. The isolate formed a well-supported clade (bootstrap value >90%) with HEV-3b references in the maximum likelihood tree, while clearly diverging from other genotypes, including the locally prevalent HEV-1 and camel-associated HEV-7 strains [25]. Further analysis of the ORF2 sequence identified characteristic proline-rich motifs and subtype-specific residues (S563L, V617A), reinforcing the 3b subtyping classification [26]. These findings highlight the co-circulation of divergent HEV genotypes in the study population, suggesting multiple sources of infection, including potential zoonotic transmission for HEV-3b [27].

Conclusion

This study highlights the co-circulation of two distinct HEV genotypes (HEV-1a and HEV-3b) in Saudi Arabia, suggesting multiple introduction pathways, including travel-related infections and potential zoonotic spillover. The detection of HEV-1a (99.7% identity with Burmese strains) indicates likely importation from South/Southeast Asia, while HEV-3b (linked to Japanese/Nepalese strains) raises concerns about non-endemic zoonotic or travel-associated transmission.

These results underscore the necessity for targeted public health interventions, including improved diagnostic screening, zoonotic surveillance, and vaccination strategies for at-risk populations. Future research should focus on expanding genomic surveillance to better understand HEV transmission dynamics in Saudi Arabia.

Author Contributions

Conceptualization, methodology, data analysis, writing original draft and review are contributed by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest.

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