ORIGINAL PAPER

doi: 10.5455/aim.2024.32.112-116
ACTA INFORM MED. 2024, 32(2): 112-116
Received: NOV 26, 2024
Accepted: DEC 28, 2024

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Direct Reciprocal Interaction Between Platelet Count and HBeAg Status in HBsAg-positive Pregnant Women

ABSTRACT

Background: Chronic hepatitis B virus (HBV) infection is a global health issue with a significant impact on pregnant women, mainly due to the interplay between liver function and hematological changes. The liver plays a key role in erythropoiesis and systemic hemostasis. In HBeAg-positive pregnant women, platelet dynamics may be uniquely influenced by the interaction of HBV, immune modulation in pregnancy, and liver function. This area remains underexplored. **Objective:** Our study aimed to analyze the interaction between HbeAg status with others preclinical factors by using the matrix correlation and multidimensional statistics methods. **Methods:** We used SEM (Structural Equation Modeling) to demonstrate and quantify the direct reciprocal interaction between platelet count and HBeAg status in HBsAg-positive pregnant women. **Results:** We found the quantity of platelet, with the optimal threshold is 201x10^3cells/ml, directly relates with HBeAg status (R =0.24) and negatively correlates with ratio of AST on ALT (R=-0.139). In case of HbeAg positive, the risk ratio having a high quantity of platelet (>201x103cells/ml) and high AST/ALT ratio (>1.42) is 2.16[1.23,3.80] (p<0.05). SEM model shows that platelet count has a direct impact on HBeAg (p<0.05, Coefficient =0.24) and indirectly through the AST/ALT ratio. This impact is greater than the direct impact from HBeAg on platelet count (p < 0.05, coefficient = 0.23). **Conclusion:** Research results show a complex relationship between platelet count, AST/ALT ratio and HBeAg in patients with chronic hepatitis B. The direct interaction between platelet count, HBeAg status, and AST/ALT ratio suggests intriguing complex immuno-biochemical responses to chronic hepatitis B virus (HBV) infection.

Keywords: Chronic hepatitis B virus infection; Pregnant women; Platelet; Ratio AST/ALT.

1. BACKGROUND

Chronic hepatitis B virus (HBV) infection is a global health issue with a significant impact on pregnant women, mainly due to the interplay between liver function and hematological changes. The liver plays a key role in erythropoiesis and systemic hemostasis. Liver damage can be indirectly assessed via blood concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamyl transpeptidase (GGT), while prolonged increase total bile acid (TBA), total bilirubin (TBIL) and bilirubin direct (DBIL), decrease albumin, total protein, A/G ratio can reflect hepatic function. Liver damage can result in anemia (low hemoglobin and RBC counts) and thrombocytopenia (low platelets) (1, 2). During pregnancy, these effects may be exacerbated by physiological changes and phases of active HBV infection, such as immune clearance or antiviral treatment. While AST and ALT are established markers of liver inflammation, their relationship with hematological parameters during pregnancy remains underexplored.

2. OBJECTIVE

This study aims to investigate the transient effects of elevated AST, ALT, and their ratio (AST/ALT) on hemoglobin, RBC, and platelet counts in HBV-infected pregnant women, with a focus on understanding these dynamics during antiviral treatment or immune clearance phases and their potential impact on maternal and fetal outcomes (3).

3. MATERIAL AND METHODS

Study Population

From 2020 to 2021, we performed a pilot study on pregnant women who had frequent check-ups and deliveries at Thai Nguyen National Hospital in Vietnam, and 60 HBsAg-positive pregnant women were finally included in the study. The "Study on the risk of mother-to-child transmission of hepatitis B virus with T cell immunity and gene variation in pregnant woman HBsAg(+)" was recognized and accepted by the Institutional Review Board for Ethics in Biomedical Research of Hanoi Medical University's Institutional Ethics Council (Number NCS22/HMU-IRD), and all participants' rights were protected. Written informed consent was obtained from all patients. Pregnant women visiting Thai Nguyen National Hospital in Vietnam are advised about the risk of hepatitis B virus. They are also advised about the necessary treatment. Only pregnant women who are HBsAg-positive and delayed HBV treatment were included in this study.

Diagnostic Criteria

We collected clinical data for pregnant women who were HBsAg-poóitive. Serum/plasma samples were analyzed for viral markers (HBsAg, HBeAg, HBV DNA copies/ml) and other preclinical factors: platelets (x103 cells/ml), time of prothrombin (second), ratio of prothrombin (%), hemoglobin (g/L), red blood cell (RBC) count (x106cells/ml), creatinine (µmol/L), AST (U/I), ALT (U/I).

Statistical Analysis and Structural Equation Modeling

The data collection, storage, and analysis of this study were conducted using R.4.1.0 package tools, following our previous case - control study. (4). Structural Equation Modeling (SEM) is a multivariate technique used in neuroscience to represent causal relations among variables based on a structural model, such as connections between brain regions in fMRI data, through path coefficients indicating the strength of relationships. SEM is a suitable method to quantify the relationships between the factors we are researching. In particular, point out cause-effect relationships, thereby providing further research steps (5).

4. RESULTS

Elevated the interaction Platelet count – HBV _ratio AST/ALT

Results of analysis of the correlation matrix of biochemical indices showed that Platelet had a stronger positive correlation with HBeAg than other factors (R=0.24, p=7.74x10-2). It is necessary to consider in more detail whether this correlation is direct or indirect. (Figure 1, Supplemetary 1).

In the method of calculating the optimal cutpoint value, we see that the value of 201x103 cells/ml of platelets has high specificity of 0.6 and sensitivity of 0.75. Among other factors, only platelet has the clearest cutpoint value. This also raises the idea that, perhaps, HBeAg and Platelet are directly linked.

With the Platelet cutpoint value, we also find that the optimal threshold value of the AST/ALT ratio is 1.42. (Figure 3)

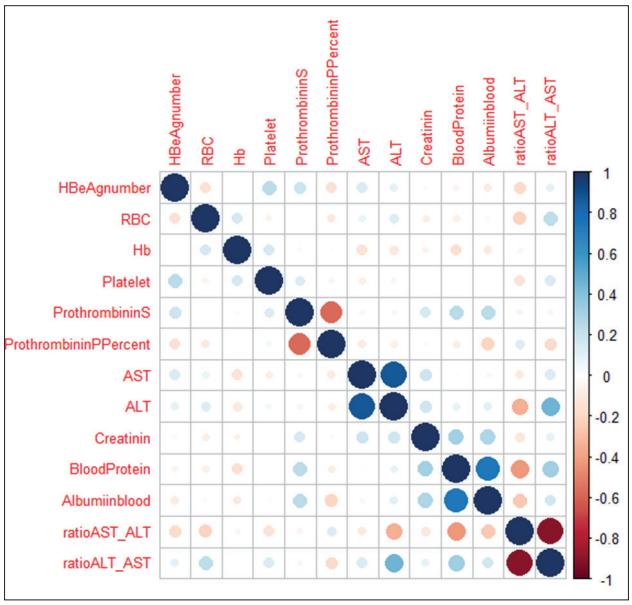

Figure 1. Heatmap of correlation matrix

The Forest plot clearly shows two important points: Heterogeneity in the forest plot is often tested using Cochran's Q test, with the null hypothesis (H_0): there is no significant difference between studies (homogeneous studies). Heterogeneity $p < 0.05$ indicates that our studies included in the meta-analysis were significantly different. The RR re-

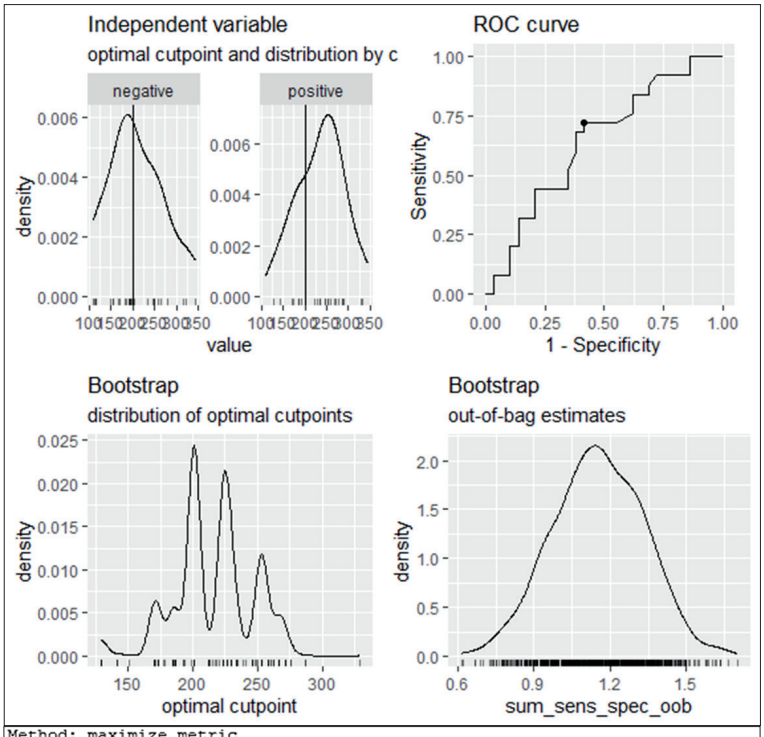

Figure 2. Optimal cut point of platelet count under impact of HBeAg status (negative / positive)

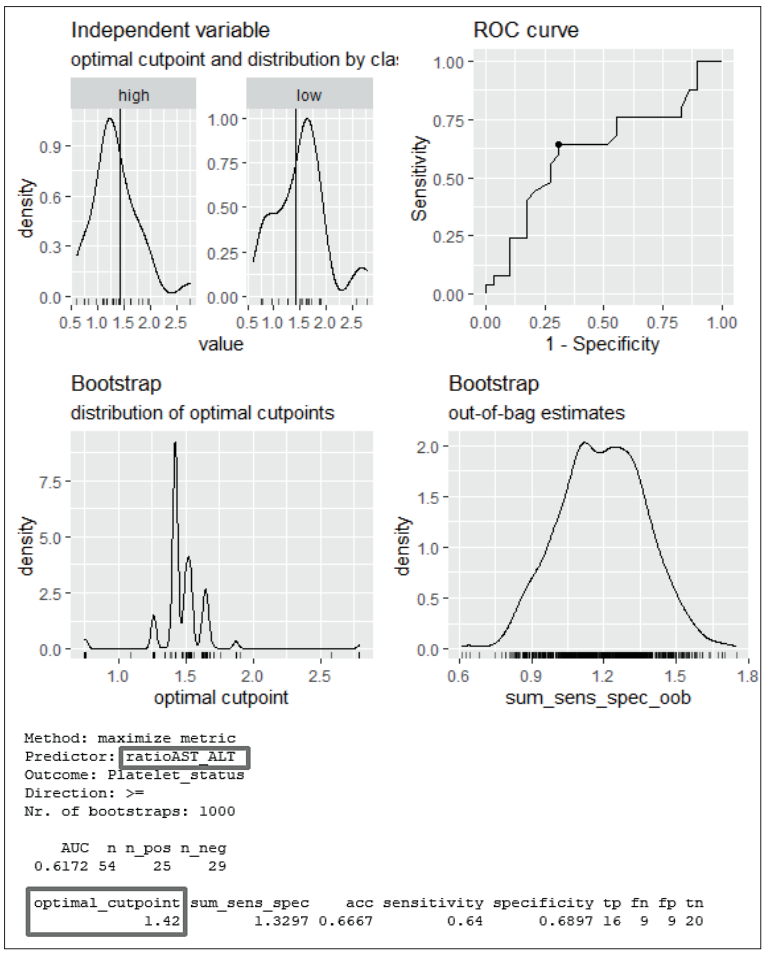

Figure 3. Optimal cut point of AST/ALT ratio under impact of Platelet status (≥201x103cells/ml or <201x103cells/ml)

sults showed that the significant risk ratio and Platelet count for HBeAg and vice versa, with RRs of 2.22[1.43,3.42] and 2.16[1.23;3.80], respectively.

Direct reciprocal interaction

The path coefficient between HBeAg and Platelet count is +0.23, showing that HBeA g has a positive effect on platelet count, meaning that when HBeAg increases, Platelet count also increases, although the level of effect is only moderate. Meanwhile, the ratio of AST/ALT has a negative impact on Platelet with a coefficient of -0.26, indicating that when the ratio of AST/ALT increases, Platelet tends to decrease. This effect is stronger than that of HBeAg in absolute magnitude. In addition, the correlation between HBeAg and AST/ALT ratio was -0.16, showing a weak but negative association, meaning that when HBeAg increased, AST/ALT ratio decreased and vice versa. (Figure 5-A).

In the SEM model, the relationships are only partially explained. In our result, 87% of the variation in Platelet count is not explained by HBeAg and the AST/ALT ratio, expressed as the residual of Platelet count. The green arrow from Platelet count to HBeAg with a coefficient of 0.24 shows that Platelet count has a direct, positive impact on HBA. This means that when Platelet count increases by 1 unit, HBA increases on average by 0.24 units if other conditions remain unchanged. However, the indirect effect from Platelet count through the AST/ALT ratio (-0.09) on HBeAg is weaker (Figure 5-B), emphasizing the important role of Platelet count in the direct relationship with HBA. (Figure 5-CD).

5. DISCUSSION

Clinical experience suggests that platelet parameters may help assess liver inflammation and fibrosis, and platelet parameters are commonly evaluated during routine blood tests. Regression analysis with the stage of liver fibrosis as the dependent variable and platelet count (PLT), platelet distribution width (PDW), and mean platelet volume (MPV). Age as an independent variable showed that age was significantly positively related to the fibrosis stage, and PLT and PDW were significantly negatively associated with the fibrosis stage ($p<0.05$) (6).

A liver biopsy is the "gold standard" for determining if the liver has inflammation and fibrosis. However, it is invasive. Accurate assessment of the degree of liver fibrosis in patients with a normal ALT level in a non-invasive manner is crucial to ascertain if patients are immune-tolerant (7). Feng M. et al. (2022) observed that, in HBV-infected patients with a normal ALT level, the progression of inflammation and fibrosis could not be determined by hepatic biochemical indices (ALT, AST, albumin, globulin, A/G ratio), imaging (PVW, spleen thickness), or other characteristics (e.g., age) alone. Research Feng M has noted a significant difference in the platelet count between patients with fibrosis stage <2 and patients with fibrosis stage ≥2. Thus, serum ALT

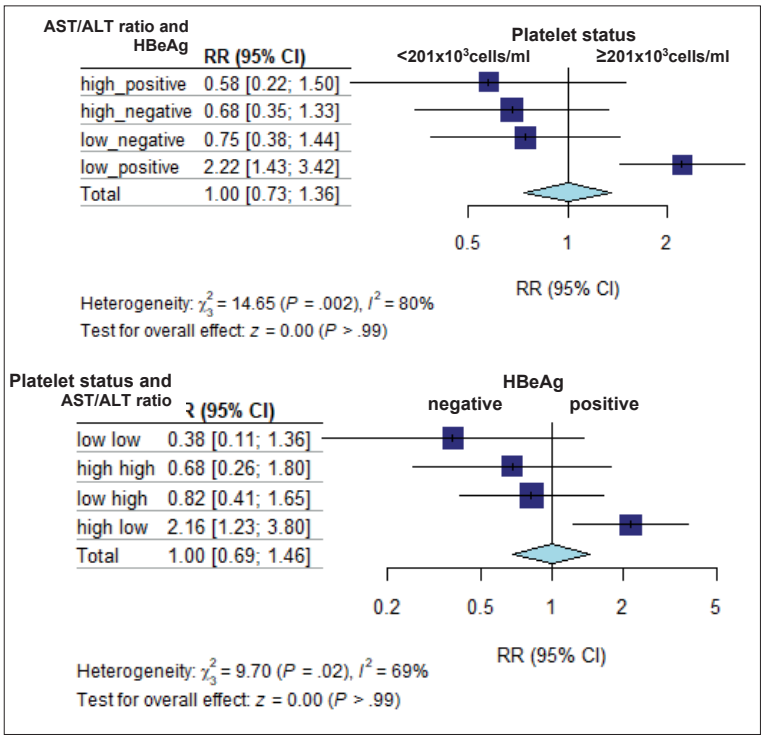

Figure 4. Forest plot. Platelet count high means ≥201x103cells/ml, low means <201x103cells/ml. AST/ALT ratio high means ≥1.42, low means <1.42.

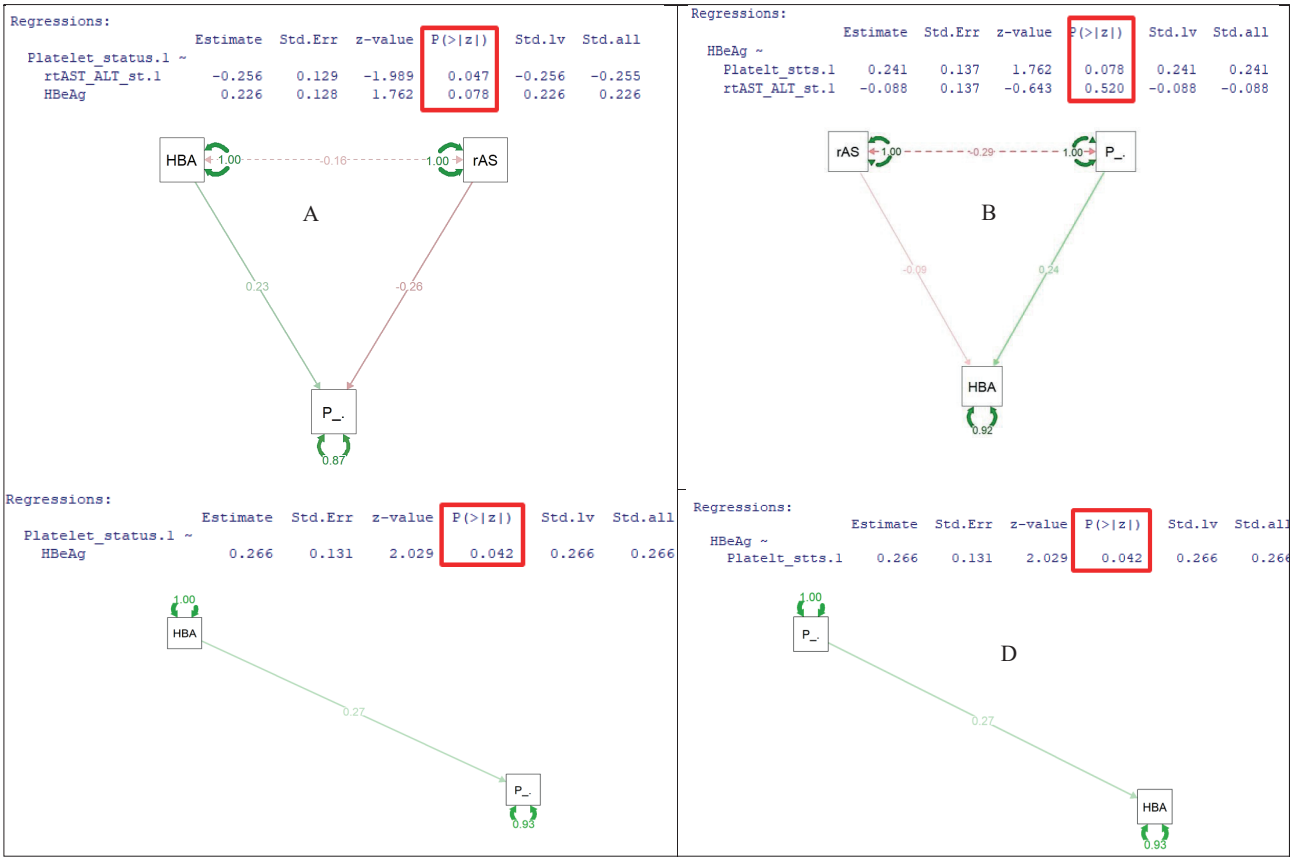

Figure 5. Diagram of SEM. P_: Platelet count, HBA: HBeAg, rAS: ratio AST/ALT

in HBV-infected patients may be expected, but inflammation and progressive fibrosis may still be present in the liver tissue (8). That also means patients with progressive liver fibrosis require antiviral therapy regardless of ALT status (9-11)..

The aim of the present study was to determine the relationships between platelet count (P_), AST/ALT ratio (rAS), and HBeAg antigen (HBA) in chronic hepatitis B patients and whether these could provide a new method for assessing liver damage in HBV-infected pregnant women.

Analyzes of the optimal threshold values for all the biochemical factors we are interested in show that HBeAg status only groups the Platelet count most clearly, into two symmetrical Histograms. The case is similar when using to calculate the threshold value of the AST/ALT ratio under the influence of Platelet count (Suplementary 2, 3, 4).

In studying the relationship between platelet count (P_), AST/ALT ratio (rAS) and HBeAg antigen (HBA) in chronic hepatitis B patients, the SEM model showed that Platelet count has a direct effect positively affects HBA with a coefficient of 0.24, meaning that as platelet count increases, HBeAg levels also increase. This may explain that in the stage of the disease that has not yet progressed to cirrhosis, the platelet count is normal or slightly increased due to the active virus, leading to HBeAg positivity. At the same time, platelet has an indirect effect on HBeAg through AST/ALT ratio. Specifically, it negatively affects AST/ALT ratio with a coefficient of -0.29, showing that as platelet count increases, rAS decreases. Because rAS is a marker that is often increased in liver damage or fibrosis, a decrease in ratio AST/ALT suggests better liver function. Furthermore, AST/ALT ratio negatively affects HBeAg with a coefficient of -0.09, meaning that a high AST/ALT ratio may be associated with lower HBeAg levels, often seen in cirrhosis when viral activity is reduced. Platelet counts may increase during active HBV infection, which may enhance understanding of the observed relationships. During active HBV infection, the immune system responds to viral replication, leading to inflammation. This inflammation can stimulate megakaryocytes - bone marrow cells responsible for platelet production - to increase platelet production (12). An increase in platelet count during active HBV infection may correlate with decreased AST/ALT ratio, reflecting improved hepatic function or reduced liver inflammation and fibrosis. Elevated platelet counts can indicate better liver function, as thrombocytopenia is often associated with advanced liver disease and fibrosis. Higher platelet levels show a healthier liver environment with less fibrosis and inflammation. Consequently, improved liver function leads to the normalization of liver enzymes, resulting in a lower AST/ALT ratio. Therefore, the inverse relationship between platelet count and AST/ALT ratio may indicate liver health in chronic hepatitis B patients. From there, it shows that the AST/ALT ratio is an interfering factor in the association of Platelet count and HBeAg (13).

Overall, the SEM model emphasizes the important role of platelet count and AST/ALT ratio in assessing viral activity and degree of liver damage. Simultaneous monitoring of these indicators not only provides information about the progression of hepatitis B but also supports effective disease management and treatment.

6. CONCLUSION

Elevated AST, ALT, and their ratio are associated with transient reductions in Hb, RBC, and platelet levels in HBV-infected pregnant women, particularly during immune clearance or treatment phases. Monitoring these parameters and

tailoring management strategies are crucial for optimizing maternal and fetal outcomes.

- **Funding:** The study belongs to the collaborative research between Germany and Vietnam. It was funded by the BMBF (German Bundesministerium für Bildung un Forschung) under reference no. 01DP19001 for its participation in the project "Identification of viral and host factors in vertical mother-to-child transmission of hepatitis B virus" and by the MOST of Vietnam (Ministry of Science and Technology of Vietnam) under reference no. 232/QD-BKHCN for its participation in the project "Apply DNA Sequencing and ELISPOT technologies in evaluation of HBV transmission risk from mother to children.
- **Institutional Review Board Statement:** The institutional ethics committee legally approved the study of Hanoi Medical University (Number NCS22/HMU-IRD). The Institutional Review Board for Ethics in Biomedical Research – Hanoi Medical University agreed to approve the study: "Study on the risk of mother-to-child transmission of hepatitis B virus with T cell immunity and gene variation in pregnant woman HBsAg (+)" and the rights of all participants were protected. Written informed consent was obtained from all patients.
- **ICMJE Statement and Conflict of interest:** The authors declare that there is no conflict of interest.
- **Funding Statement:** The authors received no specific funding for this work.
- **Author's contributions:** NTD and HCT designed the present study. BTTH received the grant for the study. HTNTr and BTTH performed the data collection, the experiments. CTH performed the data mining and SEM study. HTNTr and HCT wrote the main manuscript. NTD revised the manuscript and supervise. All authors read and approved the final manuscript.
- **Acknowledgments:** We thank the BMBF of Germany and MOST of Vietnam for supporting the bilateral scientific cooperation between Germany and Vietnam. We acknowledge the participation of patients, healthcare workers, midwives, and technicians who participated and assisted us in completing this research.

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