RESEARCH ARTICLE

Osteoprotegerin and Interleukin-37 are Correlated with Liver Diseases in Chronic Hepatitis B Virus (HBV)-infected Subjects

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Abstract

BACKGROUND: Various biological markers have been proposed to predict subclinical events in subjects with chronic hepatitis B virus (HBV). However, studies regarding chronic HBV infection are still limited. The current study aimed to investigate the relationship between osteoprotegerin (OPG) and interleukin-37 (IL-37) serum levels in chronic HBV subjects.

METHODS: Sixty subjects with chronic HBV infection without previous treatment and 30 healthy subjects were included in this study. Blood samples were withdrawn and examined for biochemical parameters through the use of enzyme-linked immunosorbent assay (ELISA) assessments. Moreover, anthropometric estimations and medical histories were performed on all subjects through a standard self-administered questionnaire.

RESULTS: A highly significant elevation (p<0.01) in serum levels of alanine transaminase (ALT), aspartate transaminase (AST), and IL-37 and a significant increase (p<0.05) in serum level of OPG were observed in chronic HBV subjects compared with the controls. The data demonstrated that OPG had a positive correlation with IL-37 and that both OPG and IL-37 had significantly positive correlations with ALT and AST. Furthermore, the area under the receiver operating characteristics (ROC) curve (AUC) was computed for ALT, AST, OPG, and IL-37 (AUC = 0.831, 0.829, 0.608, and 0.618, respectively) which could be potentially greater predictive biomarkers in chronic HBV subjects.

CONCLUSION: The positive correlation of OPG and IL-37 with ALT and AST and the high positive value of AUC for OPG and IL-37 shows that OPG and IL-37 could to be potential inflammatory biomarkers for the early onset of liver disease and hepatocellular carcinoma in chronic HBV-infected subjects.

KEYWORDS: osteoprotegerin, interleukin-37, hepatitis B virus, inflammation, liver

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Introduction

Hepatitis B virus (HBV) infects the liver and can result in both acute and chronic illness.(1) The average risk of carcinogenesis is now thought to grow with time in chronic HBV subjects with chronic inflammation.(2) Recent research revealed that chronic HBV may not directly cause liver inflammation, hepatocellular damage, or liver cancer but rather these diseases occur as a result of the immunological response to chronic HBV, which is primarily caused by the alteration of cellular immune function.(3)

Osteoprotegerin (OPG) is a tumor necrosis factor (TNF) receptor superfamily excreted glycoprotein.(4) OPG has critical anti-inflammatory and anti-apoptotic properties in addition to its significant function in regulating bone

metabolism.(5) Moreover, OPG has been demonstrated to function as a survival factor in various cell types.(6) This action is caused by the binding of the TNF-related apoptosis-inducing ligand (TRAIL), inhibiting the apoptosis process in sensitive cells.(7) On the contrary, inhibiting the association between RANK:RANKL on dendritic cells (DCs) and activated T-cells is another function of OPG in immune regulation.(8) Compared to mutated DCs, OPG knockout DCs outlive their counterparts, secrete more proinflammatory mediators, and promote T-cell growth. (9) OPG is produced by DCs in reaction to Toll-like receptor (TLR) agonists. Overexpression of this substance throughout chronic infection may add to both DCs' poor ability to stimulate T cells and prevention against metabolic bone disease.(10) On the other hand, interleukin-37 (IL-37), a cytokine of IL-1 family, is secreted by a broad range of cells and tissues.(11) IL-37 receptor signaling results in several intracellular switches being triggered, which in turn causes the downregulation of proinflammatory genes and the suppression of the production of inflammatory cytokines. (12) Inhibiting inflammatory mediators in malignancies, autoimmune illnesses, and infectious diseases enables IL-37 to possess a powerful immunosuppressive effect against innate and acquired immune responses.(13) On the contrary, according to some research, IL-37 may affect the immune pathogenesis of this infection by being linked to enhancing liver disease.(14)

Since there is evidence that OPG and IL-37 have distinct modulatory and immunosuppressive roles in infections and inflammation and that their levels are affected from the early onset of infection and inflammation, we hypothesized that OPG and IL-37 could be serum predictors of liver disease in chronic HBV subjects. A detailed understanding of these biomarkers is crucial for clarifying their biological action in the blood serum of subjects.(15) Hence, the present study was conducted to investigate the relationship between the levels of these potential blood biomarkers in chronic HBV subjects and compare them with healthy controls.

Methods

Study Design and Subjects Recruitment

A case-control clinical study involving subjects recruited from Al-Mawany and Al-Basra Teaching Hospitals in the Province of Basra, Iraq, was carried from October 2022 to January 2023.Chronic HBV subjects were diagnosed by clinicians using criteria from the European Association for the Study of the Liver (EASL), the 2017 Clinical Practice Guidelines for the Management of Chronic Hepatitis B, and the 2012 update to the Asian-Pacific Consensus Statement on the Management of Chronic Hepatitis.(16,17) The study's protocol has been approved by The Ethics and Behavioral Research Committee (Scientific Committee) of the Department of Basic Sciences, College of Nursing, University of Basrah, Province of Basra, Iraq (No. 7/54/936 in 02/10/2022), and was carried out in compliance with the Declaration of Helsinki. All subjects received a thorough explanation of the processes and signed the informed consent forms.

The chronic HBV subjects who were 30–60 years old and had positive serum HBV surface antigen (HBsAg) for at least 6 months without antiviral medications were included in the current research. The chronic HBV subjects who suffered from metabolic liver disorder, hypertension, genetic syndromes, cardiomyopathy, autoimmune diseases, neoplastic disease, heart failure, kidney dysfunction, hormonal abnormalities, urinary tract infections, and epilepsy and postmenopausal women (18,19) were excluded. The healthy control group included healthy subjects aged 30–60 years without chronic HBV (even in the family history). The minimum number of subjects required for this study was 57 as per sample size calculations based on confidence level = 95% and confidence interval = 10). (14) All of the subjects had stable clinical circumstances for at least three months. The demographic data were collected through a structured interview conducted when the subjects were being visited. Age, HBV duration, health habits (smoking, drinking alcohol, and exercise), medical history, and current medications were determined through a standard self-administered questionnaire.

Samples Collection

In the morning between 09:00 AM and 10:00 AM, samples were collected from subjects after fasting for 12 hours and 30 minutes of relaxing. Ten mL of fresh venous blood was drawn from the subjects' veins and transferred to a plain tube (without anticoagulant), where it may clot for 20 minutes at room temperature. Next, it was spun at 402 x g for 20 minutes for extracting the serum. The samples were immediately utilized for the measurement of study's parameters, while the remaining samples were kept in deep freezing at -80°C until they were needed.

Measurement of Glucose, Urea, Creatinine, ALT, AST, and Total Bilirubin with UV-Vis Spectrophotometer

Laboratory kits from Linear Chemicals S.L.U., Barcelona, Spain were used to determine the serum levels of glucose

(Cat. No.: 1129015, sensitivity: 3.5 mA/mg/dL), urea (Cat. No.: 1156015, sensitivity: 8.900 mA/min/mg/dL), creatinine (Cat. No.: 1123010, sensitivity: 25 mA/mg/dL), alanine aminotransferase (ALT) (Cat. No.: 1105010, sensitivity: 0.280 mA/min/U/L), aspartate aminotransferase (AST) (Cat. No.: 1109010, sensitivity: 0.3 mA/min/U/L) and total bilirubin (Cat. No.: 1112005, sensitivity: 0.073 A/mg/dL) by UV-Vis Spectrophotometer (UV-EMC-LAB, Duisburg, Germany). Samples were exposed with light that spans the UV to visible spectral spectrum using a source of light (typically 190 to 900 nm), then the amount of light absorbed, transferred, or scattered at each spectrum were determined.

Measurement of Insulin, OPG and IL-37 with Enzyme Linked Immunoabsorbance Assay (ELISA)

Sandwich human ELISA laboratory kits from BT-Laboratory, Shanghai, China were utilized to measure the levels of serum insulin (Cat. No.: E0010Hu, sensitivity: 0.11 mIU/L), OPG (Cat. No.: E1558Hu, sensitivity: 0.023ng/ml) and IL-37 (Cat. No.: E1947Hu, sensitivity: 4.56 ng/L) by enzyme-linked immunosorbent assay (ELISA) (Humareader/Human/Germany). All procedures were performed according to the kit protocol. The plate was read using a Varioscan® ELISA reader (Thermo Fisher Scientific) with an absorbance of 450 nm. Insulin, OPG, and IL-37 levels were measured based on a standard curve plotted.(20)

Measurements of BMI and HOMA-IR

Body mass index (BMI) was calculated by the equation: BMI (kg/m^2) = weight (kg)/(height)^2 (m). While insulin resistance (IR) was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation (21): HOMA-IR = Fasting insulin (μIU/mL) × Fasting glucose (mg/dL)/405.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corporation, Armonk, NY, USA) was utilized for the statistical analysis. The data was ordinarily dispensed, and the analysis of variances was utilized for comparing the groups before Dunnett's t-test was applied to determine the statistical significance. The correlation was obtained using a scatter plot. Binary logistic regression analysis was utilized. The sensitivities and specificities, as well as the 95% confidence interval, were calculated by the receiver operating characteristics (ROC) curve, created by plotting sensitivity (y-axis) against 1-specificity (x-axis) and computing the area under the ROC curve (AUC). The Pearson correlation method was utilized for establishing the correlations. The values of one group were often bigger (or lower) than the values of the comparison. A value of $p<0.05$ was deemed statistically significant, a value of $p<0.01$ was deemed highly significant, and an AUC value close to 0 (or 1) implied a strong diagnostic value.

Results

Subjects Characteristics

A total of 140 subjects were selected to participate in the present study. Fifty subjects were excluded because they did not match the inclusion and exclusion criteria. Only 60 chronic HBV subjects were enrolled in this study. These subjects were matched with 30 healthy controls. There were no significant differences in age (42.27±10.12 *vs.* 43.86±11.45 years) and gender ratio (men/women) (17/43 *vs.* 6/24) of chronic HBV subjects and healthy controls. In addition, the results demonstrated that chronic HBV subjects had a non-significant change ($p>0.05$) in BMI, glucose, insulin, HOMA-IR, urea, creatinine, and total bilirubin levels in comparison to healthy controls as displayed in Table 1. On the other hand, the obtained data in Table 1 indicated that chronic HBV subjects had a highly significant ($p<0.01$) increase in levels of serum ALT and AST. Furthermore, the obtained data from binary logistic regression analysis illustrated that ALT and AST had a highly significant increase ($p=0.000$, and $p=0.007$, respectively) compared to the control group, which might be utilized as potential markers in chronic HBV subjects. Moreover, the acquired AUC data revealed that ALT and AST might be more accurate predictive biomarkers in chronic HBV subjects (AUC = 0.831, and 0.829, respectively), whereas BMI, glucose, insulin, HOMA-IR, urea, creatinine, and total bilirubin could not be predictive biomarkers in chronic HBV subjects as demonstrated in Figure 1 and Figure 2.

OPG Level and Correlation with Other Parameters

The acquired data in Table 1 revealed that chronic HBV subjects had a significant ($p<0.05$) increase in OPG level in comparison to the healthy group. In addition, the obtained data from binary logistic regression analysis illustrated that OPG level had a significant ($p=0.033$) increase compared to the control group, suggesting that it may be used as a marker in chronic HBV subjects. Furthermore, the acquired AUC data revealed that OPG might be a more potent predictive biomarker in chronic HBV subjects (AUC = 0.608) as demonstrated in Figure 2. Moreover, Figure 3 demonstrated

Table 1. Serum markers levels of healthy controls and chronic HBV subjects.

Parameters	Mean±SD		*p*-value
	Chronic HBV-infected Subjects	Healthy Subjects	
BMI (kg/m^2)	29.72±1.62	29.80±1.00	0.809
Glucose (mg/dL)	80.40±3.61	80.78±2.24	0.598
Insulin (µU/mL)	11.73±2.82	11.87±1.58	0.805
HOMA-IR	2.33±0.60	2.36±0.31	0.809
Urea (mg/dL)	33.87±7.40	33.32±6.13	0.727
Creatinine (mg/dL)	1.06±0.31	1.07±0.21	0.888
Total Bilirubin (mg/dL)	0.86±0.29	0.87±0.17	0.870
ALT (U/L)	67.13±35.25	28.07±7.72	0.000*
AST (U/L)	33.87±19.27	19.61±5.89	0.000*
OPG (ng/mL)	2.03±1.22	1.45±0.43	0.013*
IL-37 (pg/mL)	106.48±66.87	59.17±8.73	0.000*

that the correlations between OPG level and ALT, AST, and IL-37 were positive and highly significant (p<0.01) (r=0.818, r=0.969, and r=0.907, respectively). Meanwhile, OPG showed a non-significant negative correlation (p>0.05) with glucose, insulin, HOMA-IR, and total bilirubin and non-significant positive correlations (p>0.05) with BMI, urea, and creatinine.

IL-37 Level and Correlation with Other Parameters

The obtained data in Table 1 indicated that chronic HBV subjects had a highly significant (p<0.01) increase in levels of serum IL-37 compared to the healthy group. Furthermore, the obtained data from binary logistic regression analysis illustrated that IL-37 level had a significant (p=0.013) increase compared to the control group, which might be utilized as potential marker in chronic HBV subjects. Furthermore, the acquired AUC data revealed that IL-37 could be potentially greater predictive biomarker in chronic HBV subjects (AUC = 0.618) as demonstrated in Figure 2. Additionally, IL-37 showed a highly significant positive correlation (p<0.01) with ALT, AST, and OPG (r=0.925, r=0.845, and r=0.907, respectively). Moreover, IL-37 had non-significant negative correlations (p>0.05) with glucose, insulin, and total bilirubin and non-significant positive correlations (p>0.05) with BMI, HOMA-IR, urea, and creatinine as illustrated in Figure 4.

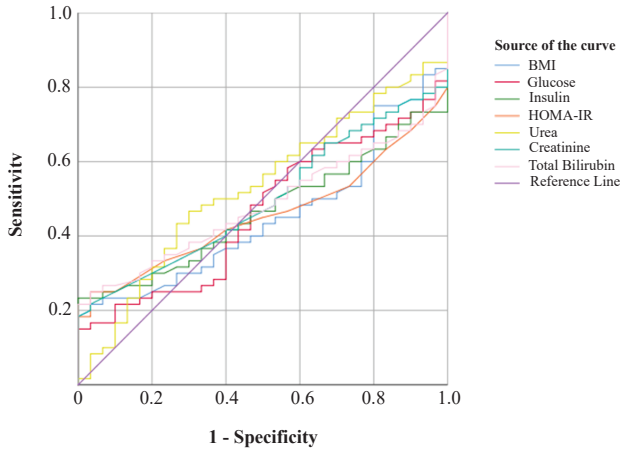

Figure 1. The ROC curve for BMI, serum glucose, insulin, HOMA-IR, urea, creatinine and total bilirubin in chronic HBV and healthy control subjects.

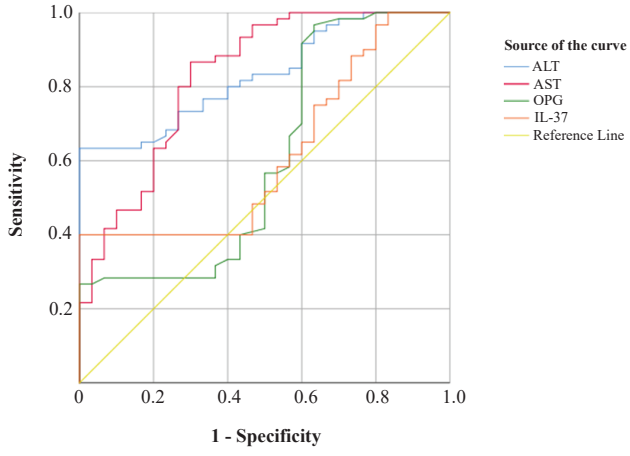

Figure 2. The ROC curve for serum ALT, AST, OPG and IL-37 in chronic HBV and healthy control subjects.

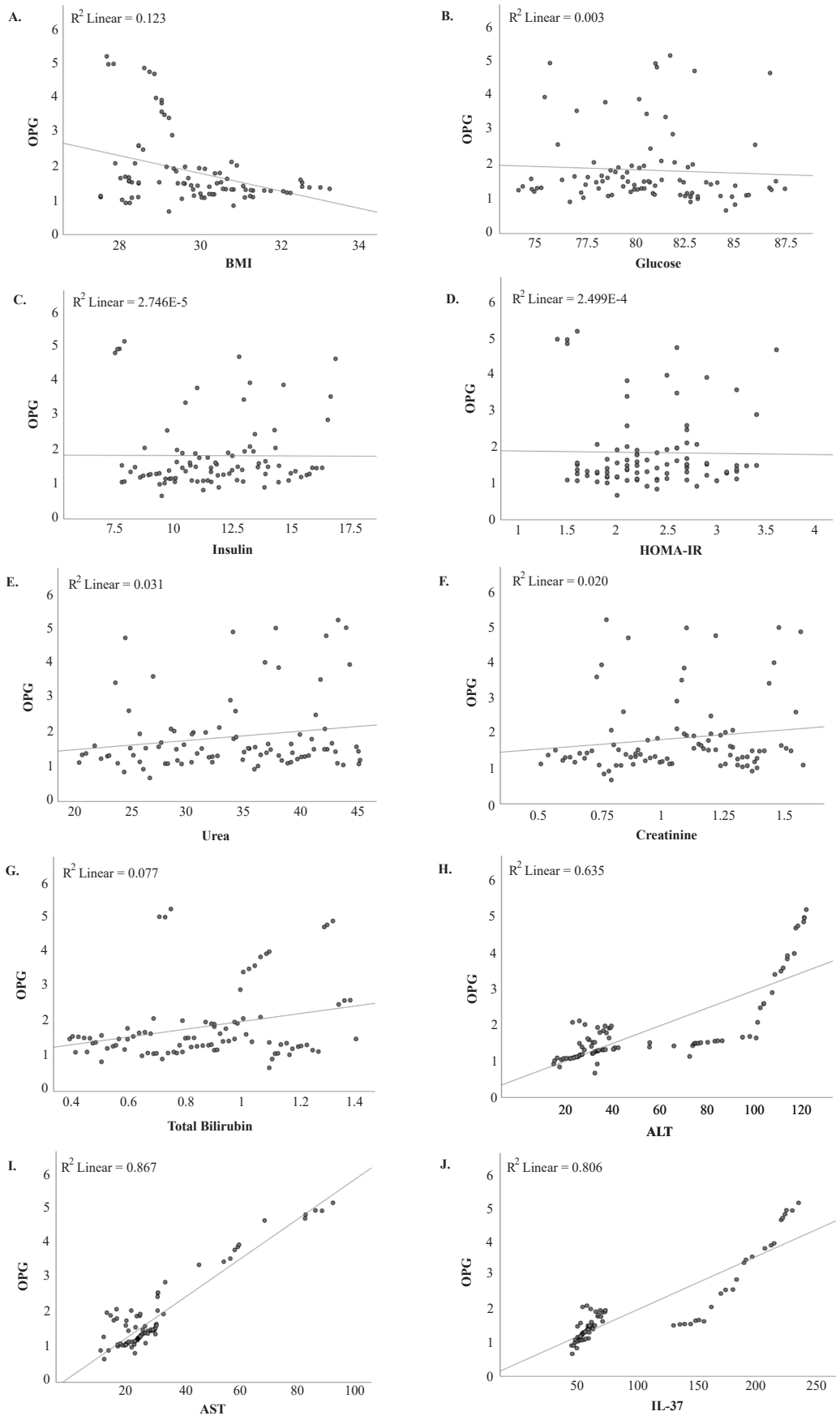

Figure 3. The scatter plot for OPG against (A) BMI, (B) glucose, (C) insulin, (D) HOMA-IR, (E) urea, (F) creatinine, (G) total bilirubin, (H) ALT, (I) AST and (J) IL-37 in chronic HBV subjects.

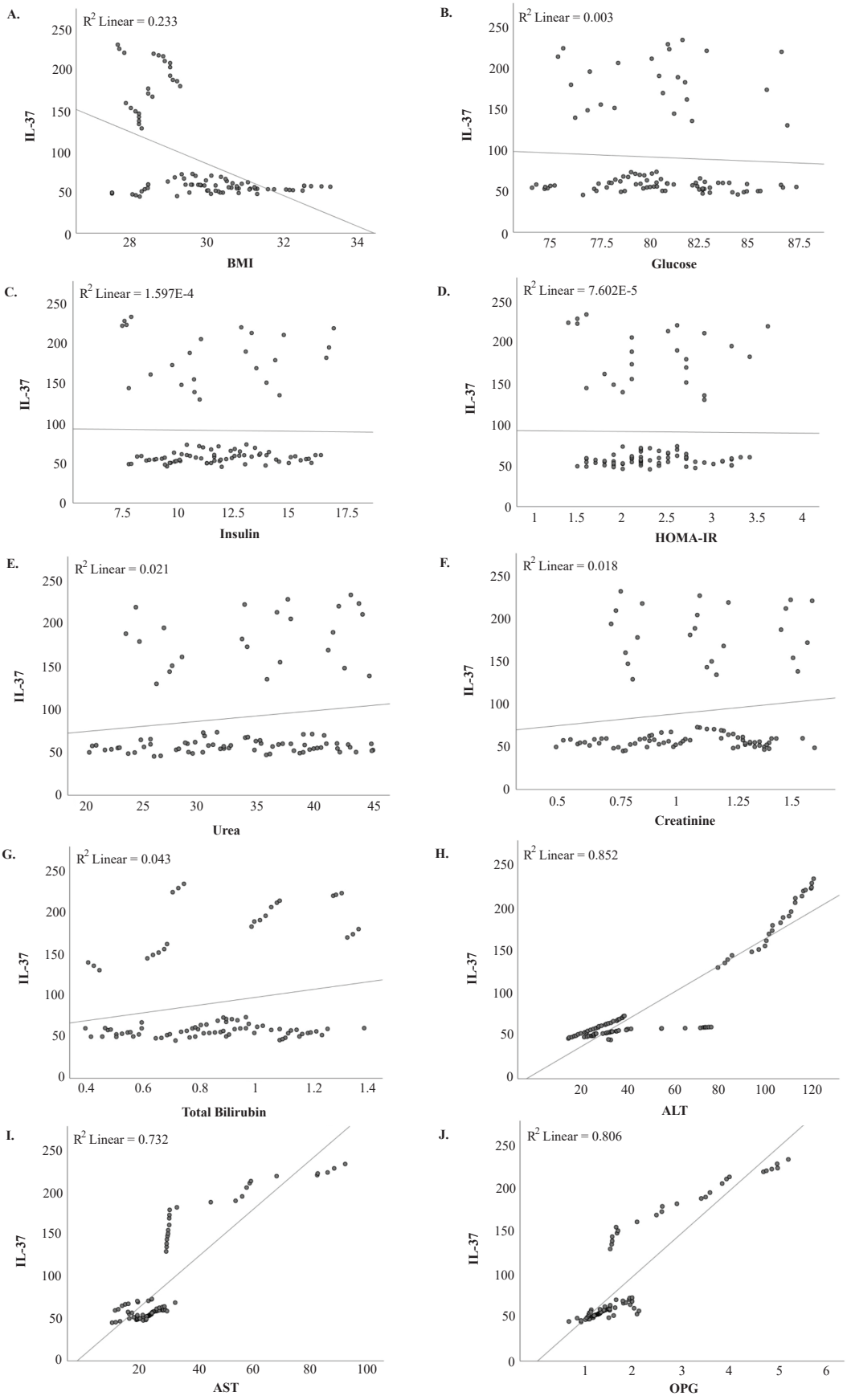

Figure 4. The scatter plot for IL-37 against (A) BMI, (B) glucose, (C) insulin, (D) HOMA-IR, (E) urea, (F) creatinine, (G) total bilirubin, (H) ALT, (I) AST and (J) OPG in chronic HBV subjects.

Discussion

Based on extensive research into virus-host interactions, a variety of novel mediators designed to enhance innate and adaptive immune responses are being developed for the treatment of chronic HBV.(22)

Our results indicated a highly significant positive correlation between OPG level and ALT, AST, and IL-37 in chronic HBV subjects in comparison to controls. Our data were consistent with other research that discovered the correlation of OPG with ALT, AST, and IL-37 in chronic HBV subjects.(23,24) This may be due to the response of inflammation in viral hepatitis.(25) OPG is primarily produced by osteoblasts. However, increased OPG production from cells other than osteoblasts in inflammation such as T-cells, B-cells, colonic cells, fibroblasts, and defective osteoblasts may contribute to the elevated levels of OPG in the blood.(26) In addition, liver inflammation in chronic HBV subjects might cause bowel inflammation (Crohn's disease) as peripheral $CD4^+$ Th1 cells exposed to intestinal antigens respond with interferon-gamma (IFN-γ) secretion leading to an increase in the OPG level.(27) Moreover, the proliferation of T cells was insufficiently induced by DCs which are highly effective in stimulating T cell proliferation and releasing large amounts of pro-inflammatory cytokines. Therefore, elevated amounts of circulating OPG in chronic HBV subjects could perhaps be caused by inadequate DC function.(28) Furthermore, DCs can release OPG in response to activation by TLRs. OPG released by the cells of the innate immune system may be induced by the virus, as indicated by the possibility that higher levels of OPG exist.(29) Besides, a subset of autoreactive cells known as invariant NK T cells (natural killer T cells) is presented by the human cluster of differentiation-d (CD1d).(30) By the rapid production of cytokines, these cells may dominate the host's response and realize endogen lipid ligands to cell stress and tissue deterioration.(31) In the liver of chronic HBV subjects, invariant NKT cells significantly increase, raising the serum OPG level. Conversely, increased levels in chronic HBV subjects may aid in preventing osteoporosis, a side consequence of liver illness.(32)

Our data revealed that IL-37 is positively correlated with high significance with ALT, AST, and OPG in chronic HBV subjects in comparison with controls. This is in line with many studies demonstrating that IL-37 is correlated with ALT, AST, and OPG in chronic HBV subjects.(33,34) This could be attributed to inflammation caused by the host immune system's defenses against viral infections.(35) TLR ligands like TLR2 and TLR4 can stimulate the production of IL-37 during an infection. Additionally, lipopolysaccharides (LPS) cause TLR4 to be expressed on cell surfaces, interacting with intracellular adaptors to activate nuclear factor kappa B (NF-κB) and promote the transcription of genes for proinflammatory cytokines.(36) Consequently, the expression of proinflammatory cytokines is increased as well as IL-37, resulting in the inhibition of innate immunity and inflammation.(37) On the contrary, inflammation may cause ulcerative colitis while IL-37 expression may prevent it by lowering the enlistment of the leukocyte population and the secretion of the proinflammatory cytokines to the lamina propria of the colon.(38) Consequently, IL-37 has a feedback mechanism that dampens an aggravated immune response, and its levels are higher in chronic HBV subjects than in controls.(39) Moreover, the elevated levels of IL-37 found in our study's chronic HBV subjects may have resulted from an increase in IL-37 concentration caused by chronic HBV infection that is dependent on virus load.(40) The present study was cross-sectional and retrospective rather than emphasizing the causative results. In addition, the data for both chronic HBV subjects and the general population in the Province of Basra, Iraq, may not accurately represent the actual situation due to the study's small sample size. However, the findings of this research may be applied to the development of therapeutic strategies for the earlier detection, mitigation, or management of chronic HBV subjects in the Province of Basra, Iraq. Future research with a more diverse and larger population might be necessary for validating the role of OPG and IL-37 in chronic HBV, including those with liver illness and liver cancer.

Conclusion

OPG and IL-37 were positively correlated with ALT and AST, which could suggest that these inflammatory biomarkers are playing key roles in the immune response and inflammatory processes of chronic HBV. Moreover, their biochemical estimation may help understand the pathogenic mechanisms, probably causing hepatic dysfunction and liver cancer in chronic HBV subjects.

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Authors Contribution

SAA and AJMA were involved in the conception and planning of the research. SAA, IMAAM, and AJMA performed the data acquisition/collection. SAA, IMAAM, and AJMA calculated the experimental data and performed the analysis. SAA and AJMA drafted the manuscript and designed the figures. SAA, IMAAM, and AJMA aided in interpreting the results. All authors took part in the critical revision of the manuscript.

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