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Exploring the Correlation Between Vitamin D Levels and Serological Markers in Liver Diseases: Insights from a Cross-Sectional Study

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Abstract. *Background/Aim: This study investigated the correlation between vitamin D levels and serological markers of liver diseases in two groups of patients: the control group (CG) and the study group (SG). Materials and Methods: The study analyzed data on vitamin D levels categorized as insufficient, sufficient, and optimal, along with serological markers, such as alpha2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin total, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, total cholesterol, and triglycerides. Results: The results indicate significant differences in vitamin D levels between the two groups, particularly in SG, where vitamin D levels varied according to its status and correlated with serological markers. Marker levels, including alpha2-macroglobulin, glucose, and total cholesterol, were notably higher in SG compared to CG, suggesting a potential association with non-alcoholic fatty liver disease (NAFLD). Further analysis using Pearson correlation revealed a strong, inverse relationship between vitamin D levels and FibroTest, NashTest, alpha2-globulin, and glucose. Additionally, increasing FibroTest and NashTest stages, as well as levels of alpha2-macroglobulin and glucose, were associated with lower vitamin D levels in SG. Conclusion: These findings under-score the complex interplay between vitamin D and serological markers in NAFLD, highlighting the potential significance of vitamin D levels in disease progression. Further research is warranted to elucidate the mechanisms underlying this relationship and its clinical implications.*

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Liver problems such as non-alcoholic fatty liver disease (NAFLD) are not showing signs of resolution worldwide; on the contrary, they are increasing and represent a global health problem with a significant impact on the quality of life and life expectancy of individuals (1). In recent decades, research in the field of liver medicine has advanced considerably, bringing into focus new methods and technologies for the diagnosis and management of these conditions. Among them, FibroMax has garnered increasing attention due to its ability to provide accurate and non-invasive information about the stage of liver fibrosis and other concomitant liver damage (2).

The stage of liver fibrosis is crucial in determining the prognosis and treatment options for patients with chronic liver disease (3). FibroMax is a non-invasive assessment method that combines several serological markers and patient demographic characteristics to estimate the degree of liver fibrosis and other associated lesions. In this study, we aimed to explore the effectiveness and relevance of using FibroMax in the diagnosis and monitoring of liver diseases, taking into account the relevant scientific data and studies in the literature (4).

Diagnosing liver disease is a complex process, involving a combination of medical history, physical examination, laboratory tests, and medical imaging. For the evaluation of liver fibrosis, liver biopsy has long been considered the gold standard. However, liver biopsy is associated with certain inconveniences and risks, such as patient discomfort, possible complications, and variability in the interpretation of results (5).

Table I. *Demographic description*.

Variables		N	%	Groups				χ^2	*p*-Value
				CG		SG			
				N	%	N	%		
Age (Mean/SD)		54.31	11.90	49.67	12.66	55.70	11.43	1.862	0.172
Sex	Male	28	43.1	5	33.3	23	46.0	0.743	0.389
	Female	37	56.9	10	66.7	27	54.0		
Environment	Urban	26	40.0	5	33.3	21	42.0	0.356	0.551
	Rural	39	60.0	10	66.7	29	58.0		

CG: Control group; SG: study group; χ^2: Chi square coefficient; *p*: statistical significance; SD: standard deviation.

Cirrhosis is associated with a hypermetabolic state, heightened protein catabolism, reduced glycogen storage and glucose oxidation, and heightened lipid oxidation, all of which lead to a compromised nutritional status. Consequently, there is also a deficiency in fat-soluble vitamins such as vitamin A and vitamin D due to the mentioned mechanisms (6). It is generally accepted that there is a deficiency of vitamin D in viruses, but it has been marked in hepatitis B and C viruses (7). This deficiency was also observed in type 2 diabetes, with liver-related outcomes (8).

Paraclinical tests used in the diagnosis of liver diseases include alpha2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, cholesterol, and triglycerides. Alpha-2-macroglobulin, with a molecular weight of 850 kDa, constitutes one-third of the total α2-globulins and quantitatively dominates the electrophoretic fraction α2 (9). It consists of two polypeptide chains linked by disulfide bridges and is synthesized at both hepatic and extrahepatic levels, including monocytes, lymphocytes, endothelial cells, and fibroblasts. It inhibits a series of enzymes, including elastase, collagenase, and enzymes involved in coagulation. Increased values are associated with acute and chronic hepatitis, acute inflammatory syndromes, nephrotic syndrome, and diabetes mellitus, while low values are associated with acute pancreatitis, septicemia, and intravascular coagulation (10). Haptoglobin and apolipoprotein A1 are synthesized in the liver and intestine and are indicated when assessing the severity and stage of intravascular hemolysis and acute inflammatory processes. About 70% of haptoglobin is associated with an exaggerated intensification of the atherosclerotic process, especially at the coronary level. Total bilirubin, GGT, ALT, AST, glucose, cholesterol, and triglycerides are markers of typical liver function and are commonly used in initial screenings (11).

FibroMax offers a non-invasive alternative to liver biopsy for evaluating liver fibrosis. It utilizes a combination of serologic markers, such as alpha-2-macroglobulin levels, haptoglobin, and hyaluronan levels, along with patient demographics, such as age and sex, to calculate a fibrosis score. FibroTest is a blood test that evaluates the degree of liver fibrosis by measuring specific biomarkers. ActiTest assesses liver inflammation, SteatoTest detects the presence of liver fat, NashTest identifies non-alcoholic steatohepatitis, and AshTest evaluates liver stiffness. These tests provide valuable information about the liver's health and the presence of underlying liver conditions, such as fibrosis, inflammation, fatty liver disease, and NASH. The diagnostic accuracy of FibroMax in detecting liver fibrosis has been supported by several clinical studies (12). Numerous clinical studies have investigated the effectiveness of FibroMax in the diagnosis and monitoring of liver diseases. The results showed that FibroMax had significant sensitivity and specificity in detecting high-grade liver fibrosis, comparable to that of liver biopsy (4).

Vitamin D, a fat-soluble vitamin, plays a crucial role not only in calcium homeostasis, the immune system, and normal development, but also in various physiological processes (13-15). Across numerous epidemiological cohort studies globally, high prevalence rates of vitamin D deficiency and insufficiency have been identified, highlighting them as significant health concerns requiring attention. Additionally, a correlation has been observed between collagen formation and serum levels of vitamin D, indicating an important role in metabolic syndrome and the management of inflammatory processes within the body (16).

The aim of this study was to establish a potential correlation between the blood levels of vitamin D and the paraclinical parameters of NAFLD, with the goal of detecting health risks earlier in patients with varicose vein problems and improving their quality of life.

Materials and Methods

This cross-sectional study was conducted at the „Venus Vascular Center" in Oradea from 2023 to 2024, prior to invasive surgical interventions such as vein stripping. The study was conducted in accordance with the Declaration of Helsinki and approved by the

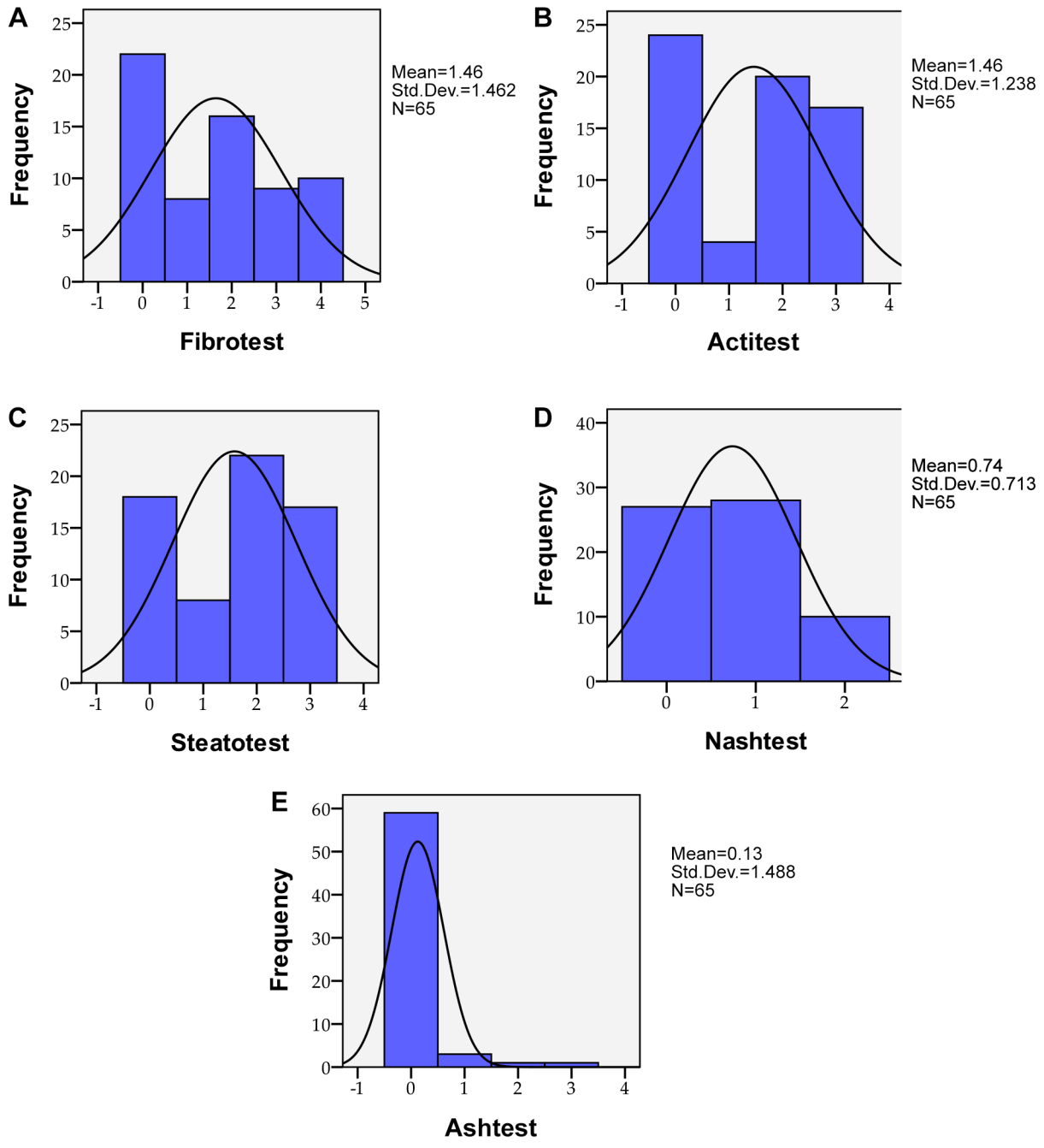

Figure 1. *Graphical representation of FibroMax noninvasive tests: FibroTest (A), ActiTest (B), SteatoTest (C), NashTest (D), and AshTest (E) in this study cohort.*

Institutional Review Board (or Ethics Committee) of the University of Oradea (protocol code CEFMF/1 from 31 January 2023 and date of approval). The cohort of 65 patients was divided into two groups: the control group (CG), consisting of 15 individuals (23.1%), who did not present any form of liver disease, and the research group (SG), comprising 50 patients (76.9%) with NAFLD, which was the primary inclusion criterion. All patients in the SG group were monitored for paraclinical analyses, FibroMax, and vitamin D levels. Informed consent was obtained from all subjects involved in the study.

Exclusion criteria included patients under 18 years old, hospitalized individuals, and those with other serious associated diseases that could potentially modify the results, such as cancer, AIDS, cystic fibrosis, or other autoimmune diseases.

The cohort descriptions for various variables used in the evaluation of liver diseases and associated non-invasive serological tests are presented based on their mean values. These variables included age, sex, and environment (Table I), as well as serological levels of specific markers, such as alpha2-macroglobulin, haptoglobin,

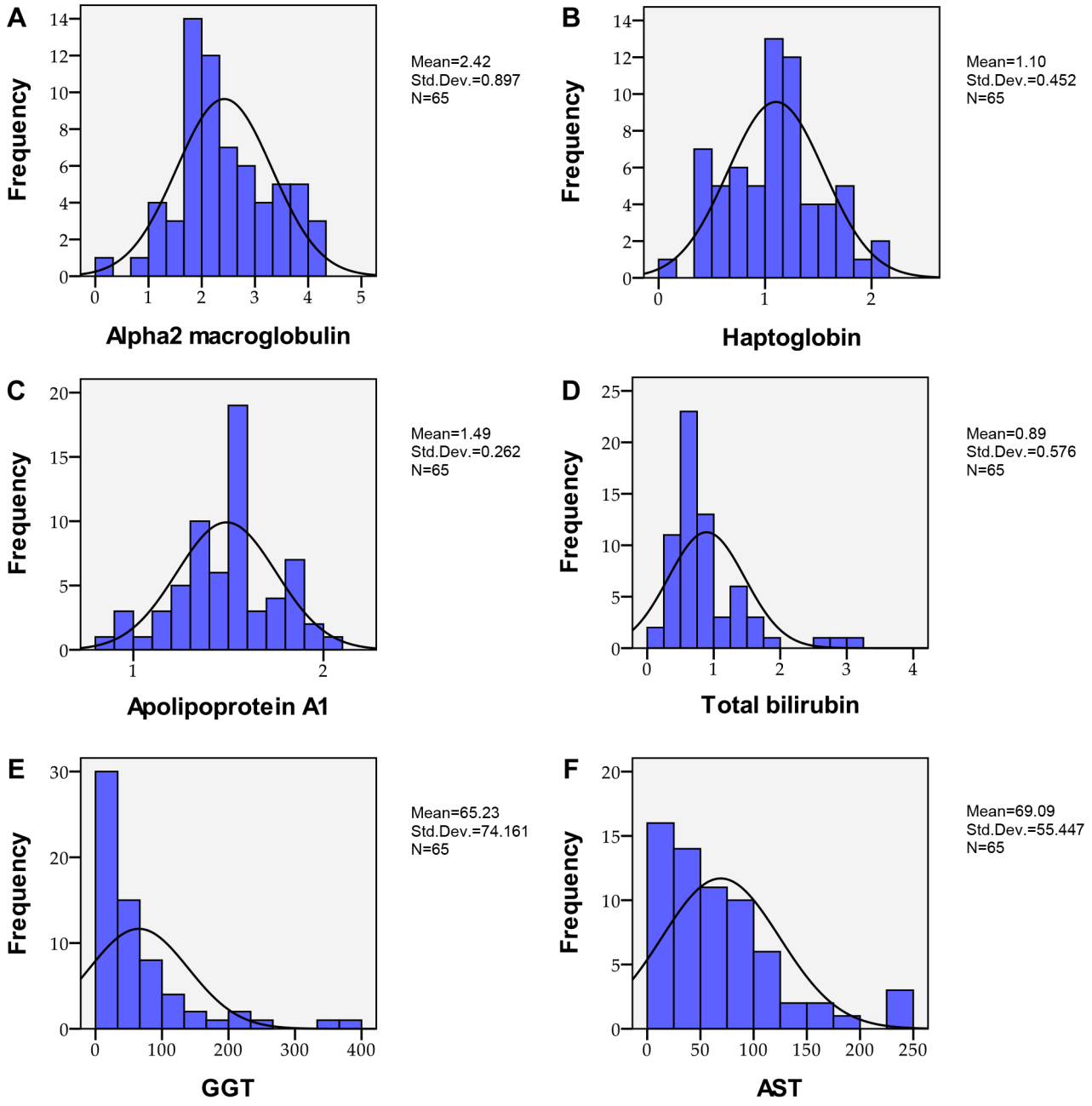

Figure 2. *Continued*

apolipoprotein A1, total bilirubin, GGT, ALT, AST, glucose, total cholesterol, and triglycerides, as well as FibroTest, ActiTest, SteatoTest, NashTest, and AshTest results.

FibroMax investigation. The FibroMax investigation, developed by BioPredictive, comprises five different non-invasive tests: FibroTest, ActiTest, SteatoTest, NashTest, and AshTest. It utilizes an algorithm combining results from serum biochemical markers (alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, gamma-glutamyl transpeptidase – GGT, alanine-aminotransferase ALT, aspartate-aminotransferase AST, basal blood glucose, cholesterol, triglycerides) with patient demographics (age, sex, weight, and height) to assess liver damage.

Paraclinical analysis. Clinical evaluations were conducted at the medical office, while paraclinical parameter evaluations were performed in authorized laboratories. Paraclinical examinations included assessments of Alpha2_macroglobulin, haptoglobin,

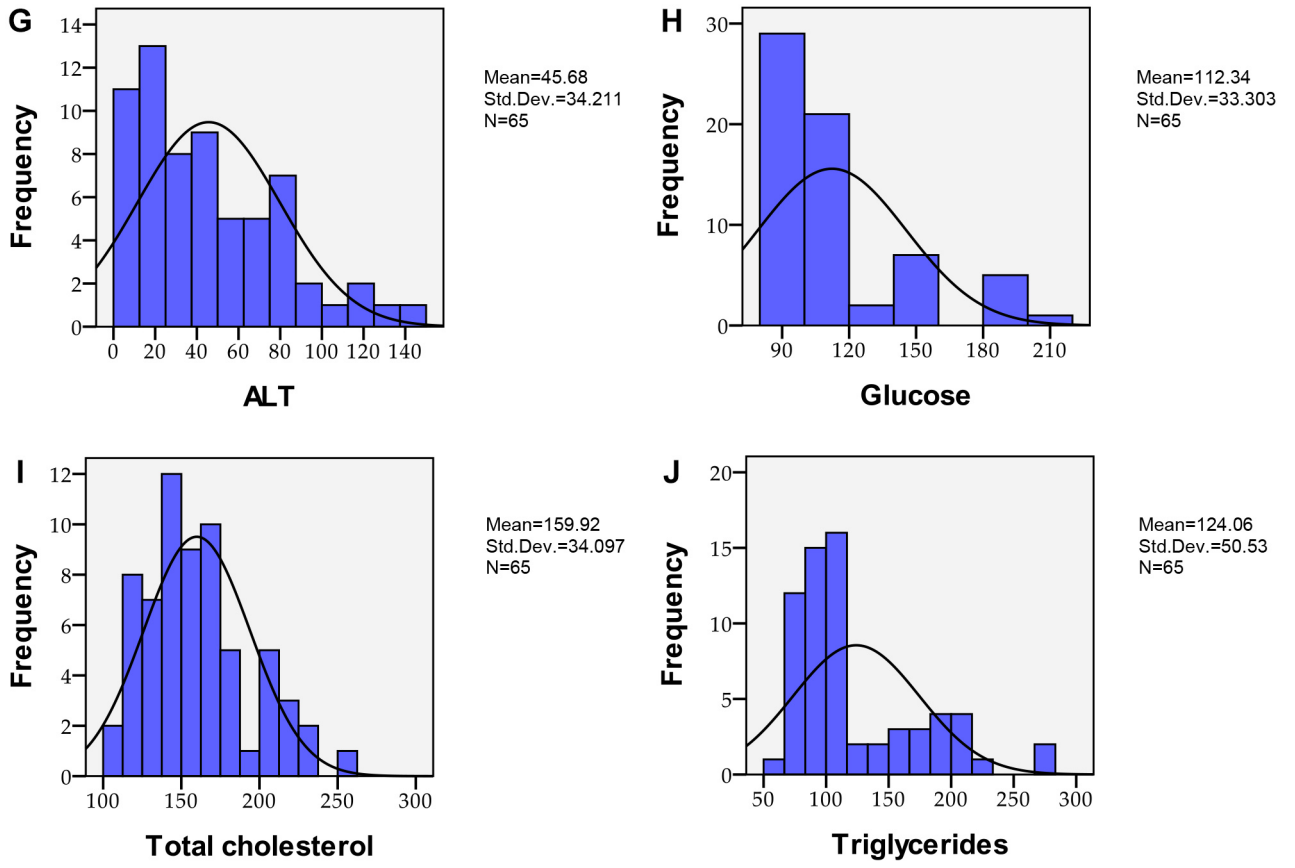

Figure 2. *Graphical representation of paraclinical tests in this study cohort, including alpha2-macroglobulin (A), haptoglobin (B), apolipoprotein A1 (C), total bilirubin (D), gamma-glutamyl transferase (GGT) (E), alanine aminotransferase (ALT) (F), aspartate aminotransferase (AST) (G), glucose (H), cholesterol (I), and triglycerides (J).*

apolipoprotein_A1, Bil_T, GGT, ALT, AST, glucose, Chol_T, and triglycerides. A detailed local clinical examination was also carried out, considering complete medical histories.

Rapid vitamin D test. For assessing vitamin D levels, a cassette-type rapid test was utilized to semi-quantitatively detect 25-hydroxyvitamin D in whole blood samples obtained *via* fingerstick. The JusChek (Bucharest, Romania) rapid test was employed to determine vitamin D levels (25 (OH) D3). Results were divided into three categories: Insufficient (0-10 μg/ml), Adequate (10-29 μg/ml), Optimal (30-100 μg/ml).

Statistical evaluation. Data analysis was performed using Statistical Product and Service Solutions (version 20; IBM, Armonk, NY, USA). Demographic variables, procedure frequency, and cost data were assessed for trends across the two study groups and over the surveyed time points. Calculations included means, frequency ranges, standard deviations, and statistical significance tests using Student's *t*-test and the chi-square test. The Bravais–Pearson correlation coefficient measured the relationship between variables. A significance level of $p<0.05$ indicated statistical significance, while $p<0.01$ suggested a high level of statistical significance. Post hoc analysis (Bonferroni) was conducted for additional subgroup analysis.

Results

The average age of the subjects was 54.31±11.90 years, ranging from 28 to 78 years with a higher proportion from rural areas (60%). The average values for the non-invasive FibroMax tests were as follows: FibroTest 1.65±1.46, ActiTest 1.46±1.23, SteatoTest 1.58±1.15, NashTest 0.74±0.71, and AshTest 0.13±0.48. The graphical representation of the data at the cohort level can be seen in Figure 1.

Regarding specific serological markers, the mean for alpha2-macroglobulin was 2.42±0.89, for haptoglobin it was 1.10±0.45, for apolipoprotein A1 1.49±0.26, for total bilirubin 0.89±0.57, for GGT 65.23±74.16, for ALT 69.09±55.44, for AST 45.68±34.21, for glucose 112.34±33.30, for total cholesterol 159.92±34.09, and for triglycerides 124.06±50.52 (Figure 2).

Statistical analysis of the differences between the two research groups. Demographic data and characteristics of the two groups of patients, namely CG (control group) and SG (study group), along with the results of analysis of variance

Table II. *Paraclinical parameters in the two groups.*

Parameters	Groups				χ^2	*p*-Value
	CG		SG			
	Mean	SD	Mean	SD		
Alpha2 macroglobulin	2.03	0.12	2.54	0.99	2.676	0.102
Haptoglobin	1.19	0.10	1.08	0.51	0.440	0.507
Apolipoprotein A1	1.65	0.16	1.44	0.27	6.815	0.009**
Total bilirubin	0.66	0.17	0.96	0.63	1.573	0.210
GGT	17.33	3.52	79.60	79.16	24.879	0.000**
ALT	22.67	8.34	83.02	56.02	21.868	0.000**
AST	12.00	1.69	55.78	32.81	31.040	0.000**
Glucose (Mean/SD)	88.20	8.00	119.58	34.62	22.155	0.000**
Total cholesterol (Mean/SD)	149.40	14.85	163.08	37.57	0.670	0.413
Triglycerides (Mean/SD)	87.07	10.17	135.16	52.54	16.527	0.001**

CG: Control group; SG: study group; χ^2: Chi square coefficient; *p*: statistical significance; SD: standard deviation. *Significant at $p<0.05$ (2-tailed). **Significant at $p<0.01$ (2-tailed).

(ANOVA) tests to assess differences between these groups, are presented in Table II.

Age (Mean/SD): CG: The mean age was approximately 49.67 years with a standard deviation of 12.66; SG: The mean age was approximately 55.70 with a standard deviation of 11.43. The result of the ANOVA test indicated a lack of statistical significance ($p=0.949$), suggesting no significant differences between the mean ages of the patients in the two groups.

Sex: CG: 33.3% male and 66.7% female; SG: 46.0% male and 54.0% female. The ANOVA test result showed a significant difference ($p=0.039$*), indicating a different sex distribution between the two groups.

Environment: CG: 33.3% of patients lived in urban environments, and 66.7% lived in rural environments; SG: 42.0% of patients lived in urban areas, and 58.0% lived in rural areas. The ANOVA test showed a lack of statistical significance ($p=0.168$), indicating that the distribution of patients according to the environment does not differ significantly between the two groups. Table I presents the results of the comparison of different characteristics and serological tests between the two groups of patients: CG (control group) and SG (study group).

In summary, regarding demographic characteristics, there were no significant differences between the groups in terms of age and living environment. However, there was a significant difference in sex distribution between the two groups, with a higher percentage of men in SG compared to CG.

Regarding the results of the serological tests, significant differences existed between the groups in most of the analyzed parameters. Mean levels of serological markers, such as alpha2-macroglobulin, haptoglobin, total bilirubin, GGT, ALT, AST, glucose, total cholesterol, and triglycerides were significantly higher in SG compared to CG.

Significant differences also existed in the results of FibroTest, ActiTest, SteatoTest, NashTest, and AshTest serological tests between the two groups, with a significantly higher prevalence of abnormal results in SG compared to CG.

Patients in the SG exhibited higher levels of serological markers associated with liver disease and a higher prevalence of abnormal non-invasive serological test results compared to those in the CG. These findings may have clinical relevance in the identification and management of liver diseases in medical practice.

The FibroMax parameters were measured using FibroTest, ActiTest, SteatoTest, NashTest, and AshTest (Figure 3).

For FibroTest, the CG exhibited the following distribution of fibrosis stages: F0 (n=15, 100.0%), F1 (n=0, 0.0%), F2 (n=0, 0.0%), F3 (n=0, 0.0%), and F4 (n=0, 0.0%). In contrast, the SG demonstrated varying percentages across the fibrosis stages: F0 (n=7, 14.0%), F1 (n=8, 16.0%), F2 (n=16, 32.0%), F3 (n=9, 18.0%), and F4 (n=10, 20.0%). Statistical analysis using the Chi-square coefficient (X2) revealed a significant difference between the two groups ($p=0.001$**).

Regarding ActiTest, the CG displayed the following distribution of activity stages: A0 (n=15, 100.0%), A1 (n=0, 0.0%), A2 (n=0, 0.0%), and A3 (n=0, 0.0%). Meanwhile, the SG exhibited varying percentages across the activity stages: A0 (n=9, 18.0%), A1 (n=4, 8.0%), A2 (n=20, 40.0%), and A3 (n=17, 34.0%). The statistical analysis indicated a significant difference between the groups ($p=0.001$**).

For SteatoTest, the CG demonstrated the following distribution of steatosis stages: S0 (n=15, 100.0%), S1 (n=0, 0.0%), S2 (n=0, 0.0%), and S3 (n=0, 0.0%). Conversely, the

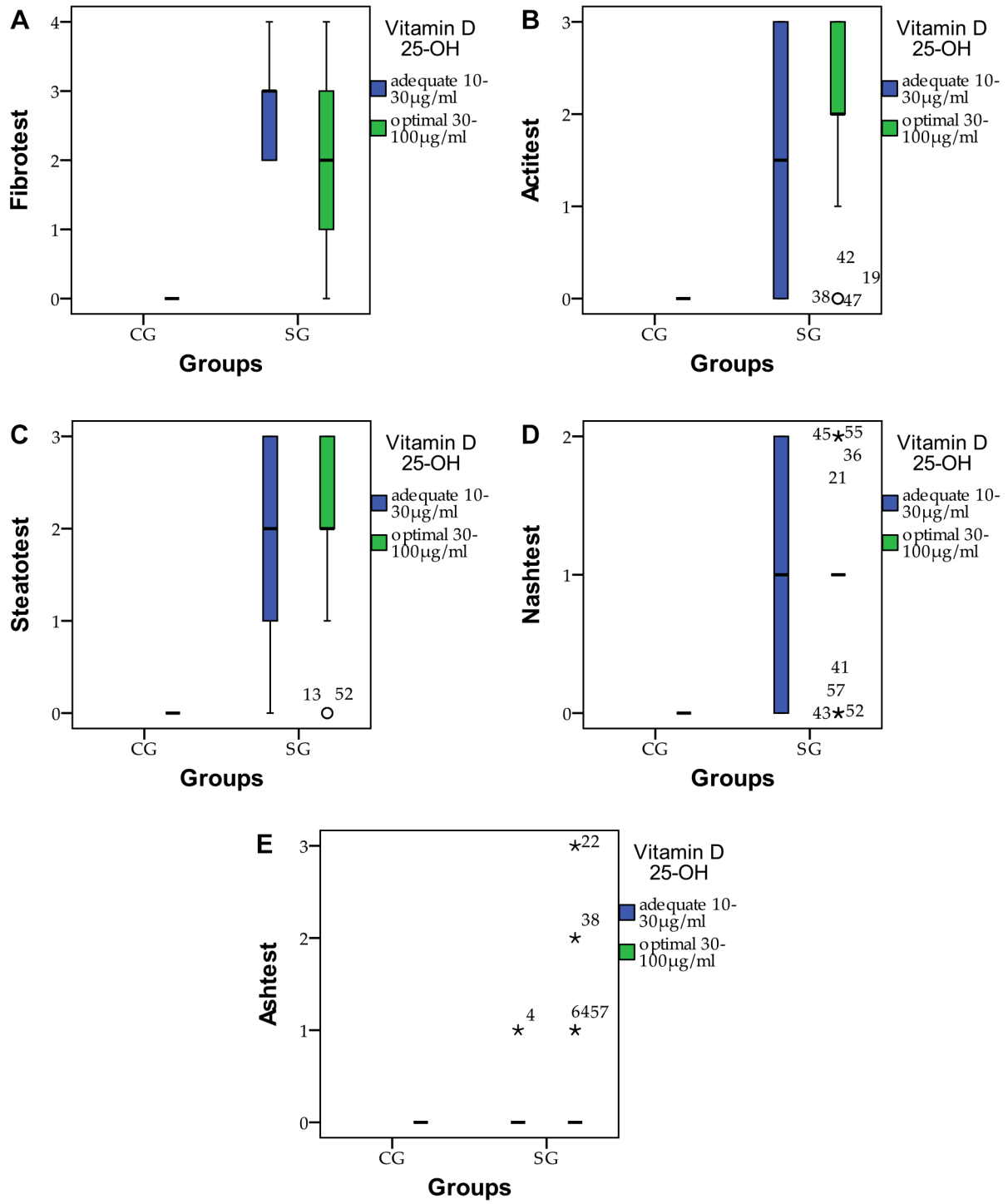

Figure 3. *Graphical representation of Fibromax noninvasive tests: FibroTest (A), ActiTest (B), SteatoTest (C), NashTest (D), and AshTest (E) in the two research groups, depending on the vitamin D levels.*

SG showed varying percentages across the steatosis stages: S0 (n=3, 6.0%), S1 (n=8, 16.0%), S2 (n=22, 44.0%), and S3 (n=17, 34.0%). A statistically significant difference was observed between the two groups (p=0.001**).

Regarding NashTest, the CG had the following distribution of the necrosis stages: N0 (n=15, 100.0%), N1 (n=0, 0.0%), and N2 (n=0, 0.0%). Conversely, the SG exhibited varying percentages across the necrosis stages: N0

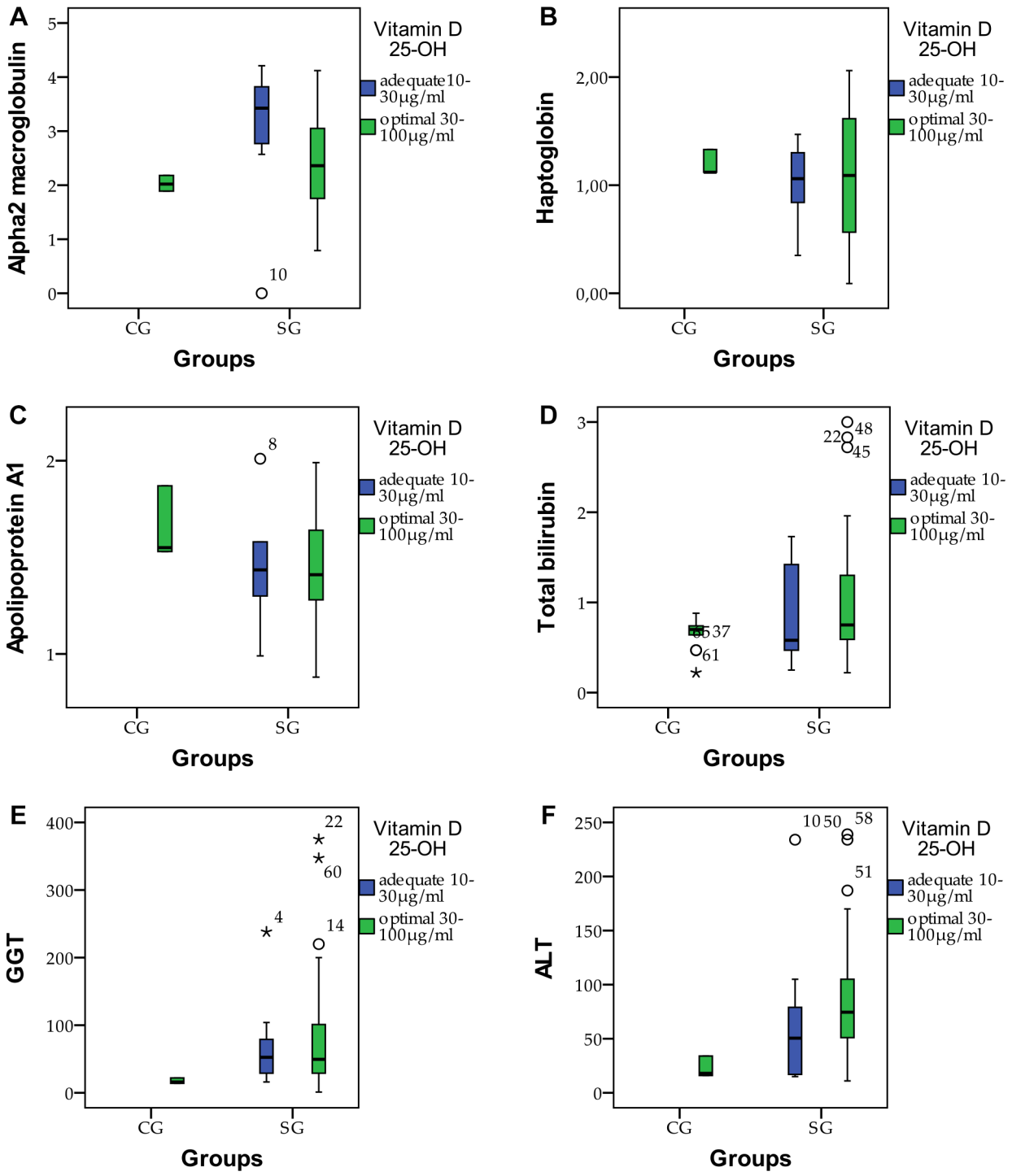

Figure 4. *Continued*

(n=12, 24.0%), N1 (n=28, 56.0%), and N2 (n=10, 20.0%). Statistical analysis indicated a significant difference between the two groups (p=0.001**).

Lastly, for Ashtest, the CG displayed the following distribution of activity stages: H0 (n=15, 100.0%), H1 (n=0, 0.0%), H2 (n=0, 0.0%), and H3 (n=0, 0.0%). Conversely, the SG exhibited varying percentages across the activity stages: H0 (n=44, 89.8%), H1 (n=3, 6.1%), H2 (n=1, 2.0%), and H3 (n=1, 2.0%). Statistical analysis did not reveal a significant difference between the two groups (p=0.206).

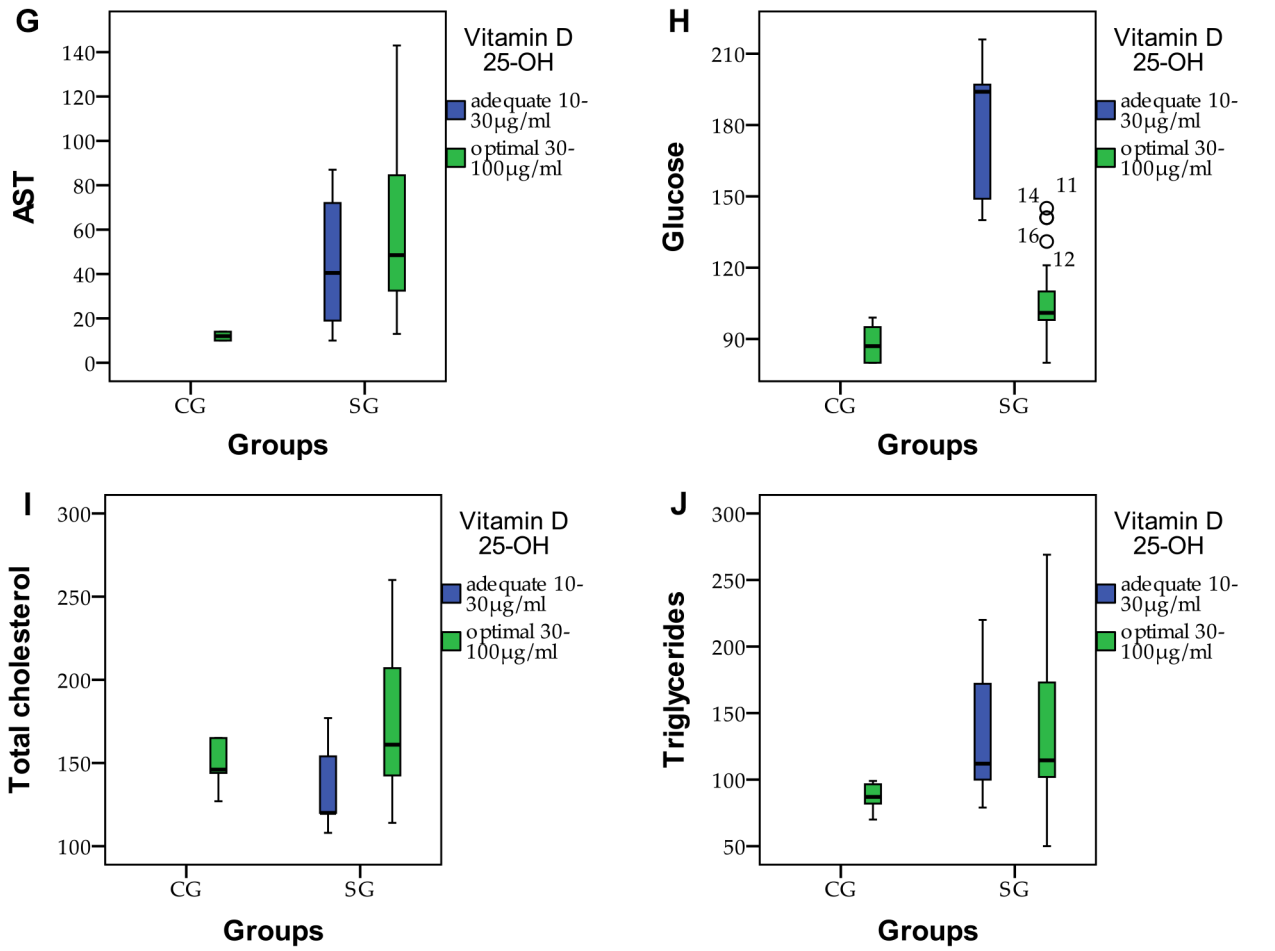

Figure 4. *Graphical representation of paraclinical tests, including alpha2-macroglobulin (A), haptoglobin (B), apolipoprotein A1 (C), total bilirubin (D), gamma-glutamyl transferase (GGT) (E), alanine aminotransferase (ALT) (F), aspartate aminotransferase (AST) (G), glucose (H), cholesterol (I), and triglycerides (J), in the two research groups categorized according to the levels of vitamin D.*

In summary, the distribution of Fibromax parameters differed significantly between the control and study groups, as evidenced by statistical analysis using the Chi-square coefficient (χ^2) with a significance level of $p<0.05$.

Evolution of parameters in relation to vitamin D levels. The evolution of parameters in relation to vitamin D levels was examined based on data from the serological tests FibroTest, ActiTest, SteatoTest, NashTest, and Ashtest in the two groups of patients (CG and SG).

In the CG, the mean vitamin D level was consistent across all serological test results, maintaining around 66.00 with a standard deviation of 10.73. In the SG, the average vitamin D level varied depending on serological test results. For positive test outcomes, such as FibroTest, ActiTest, SteatoTest, and NashTest, the average vitamin D level was lower in the SG compared to CG, ranging from 38.11 to 58.86, with standard deviations ranging from 10.92 to 23.01. Conversely, for positive AshTest results, the mean vitamin D level was higher, ranging from 48.00 to 62.00, with standard deviations ranging from 18.28 to 32.81.

These findings indicate a potential correlation between liver disease-specific serological test outcomes and vitamin D levels in SG. Lower vitamin D levels were generally observed with positive serological test results, whereas higher vitamin D levels were associated with positive Ashtest results. This correlation may hold significance in the evaluation and management of liver diseases.

The level of vitamin D according to its level (insufficient, sufficient, and optimal) and the serological levels of specific markers for liver diseases in the two groups of patients (CG and SG) is presented in Figure 4. Regarding vitamin D levels, in CG, no statistically significant difference was observed between the three serum vitamin D levels, all patients being in the optimal range.

In SG, the level of vitamin D varied within the three domains according to the levels of specific liver markers.

This variation can provide clues about the correlation with the serological levels of specific markers for liver diseases. Statistically significant differences were recorded in the case of the markers alpha2-macroglobulin, glucose, and Chol T ($p<0.05$). In addition, serological levels of these markers appeared to be significantly higher in SG compared to CG, suggesting a possible association with liver disease in this patient group.

These findings suggest that vitamin D levels might be associated with specific serological markers for liver disease, and more detailed investigation is needed to better understand this relationship and its clinical implications.

Correlations. Upon processing the data using Pearson correlation, a strong but inversely proportional relationship was observed between the levels of vitamin D with FibroTest, NashTest, alpha2-globulin, and glucose. As the stage according to FibroTest and NashTest, as well as alpha2-macroglobulin and blood glucose, increases, it is associated with a lower level of vitamin D (Table III and Figure 5).

Table III. *Pearson correlation of research parameters and vitamin D.*

Pearson correlation		Vitamin D 25-OH
Fibrotest	r	–0.451**
	p-Value	0.001
Nashtest	r	–0.297*
	p- Value	0.016
Alpha2 macroglobulin	r	–0.331**
	p- Value	0.007
Glucose	r	–0.831**
	p- Value	0.001
	N	65

*Significant at $p<0.05$ (2-tailed). **significant at $p<0.01$ (2-tailed).

Discussion

Liver diseases represent a major public health challenge with increasing incidence and prevalence globally. The diagnosis and management of these conditions are crucial to reducing associated mortality and morbidity (17). In recent decades, non-invasive serological tests, such as FibroTest, ActiTest, SteatoTest, NashTest, and AshTest have been developed and validated to assist in the diagnosis and monitoring of liver diseases (18). These tests utilize a combination of serological markers and other clinical parameters to evaluate the degree of liver fibrosis, inflammation, steatosis, and necroinflammatory activity in the liver. In this study, we explored the relevance and effectiveness of these tests in clinical practice, considering existing scientific data (19, 20). In the current study, we concurrently tracked both FibroMax-related tests and specific paraclinical parameters.

Non-invasive serological tests such as FibroTest have gained popularity due to their ability to provide valuable information about liver status without necessitating a liver biopsy (21). FibroTest utilizes a combination of serological markers, such as alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, and bilirubin to assess the degree of liver fibrosis. Several studies have demonstrated that FibroTest correlates well with liver biopsy results across different stages of chronic liver diseases, including chronic hepatitis and cirrhosis (12, 22, 23).

ActiTest is another serological test employed to evaluate liver inflammation and necroinflammatory activity. It measures specific serological markers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other parameters such as the patient's age and sex to estimate the degree of inflammation and necroinflammatory activity in the liver. Research indicates that ActiTest can aid in identifying chronic hepatitis patients with high necroinflammatory activity, thereby assisting in therapeutic decision-making (24-26).

SteatoTest is a serological test utilized to assess the degree of hepatic steatosis or fatty liver. It utilizes a combination of serological markers and clinical parameters to estimate the amount of fat accumulated in the liver. SteatoTest may prove useful in the diagnosis and monitoring of patients with non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AHFD), both of which are increasing globally (27).

NashTest and AshTest are serological tests focused on the diagnosis and evaluation of necroinflammatory activity and fibrosis in non-alcoholic steatohepatitis (NASH) and alcoholic hepatitis (AH), respectively. These tests employ specific serological markers and other clinical parameters to estimate the degree of inflammation, necrosis, and fibrosis in the liver. Identifying and monitoring these pathological features are critical for managing patients with NASH and AH, providing essential information for therapeutic decision-making (28).

Vitamin D deficiency is commonly observed in various chronic liver conditions and has been linked to the onset and progression of NAFLD (29). Advanced liver disease leads to reduced vitamin D hydroxylation, as well as decreased production of albumin and DBP, all contributing to lower levels of 25(OH)D. However, the deficiency of vitamin D in chronic liver disease is not solely attributed to liver synthesis dysfunction, as it is also widespread in non-cirrhotic patients (30). In our study, a marked vitamin D deficiency was outlined in those with NADLD.

In several studies, a link between vitamin D deficiency and liver diseases has been observed, along with various mechanisms (31-35). In the 2022 study, not necessarily different serum levels between men and women were observed, but possible differences in the responses of women and men to vitamin D use, as well as inter-individual

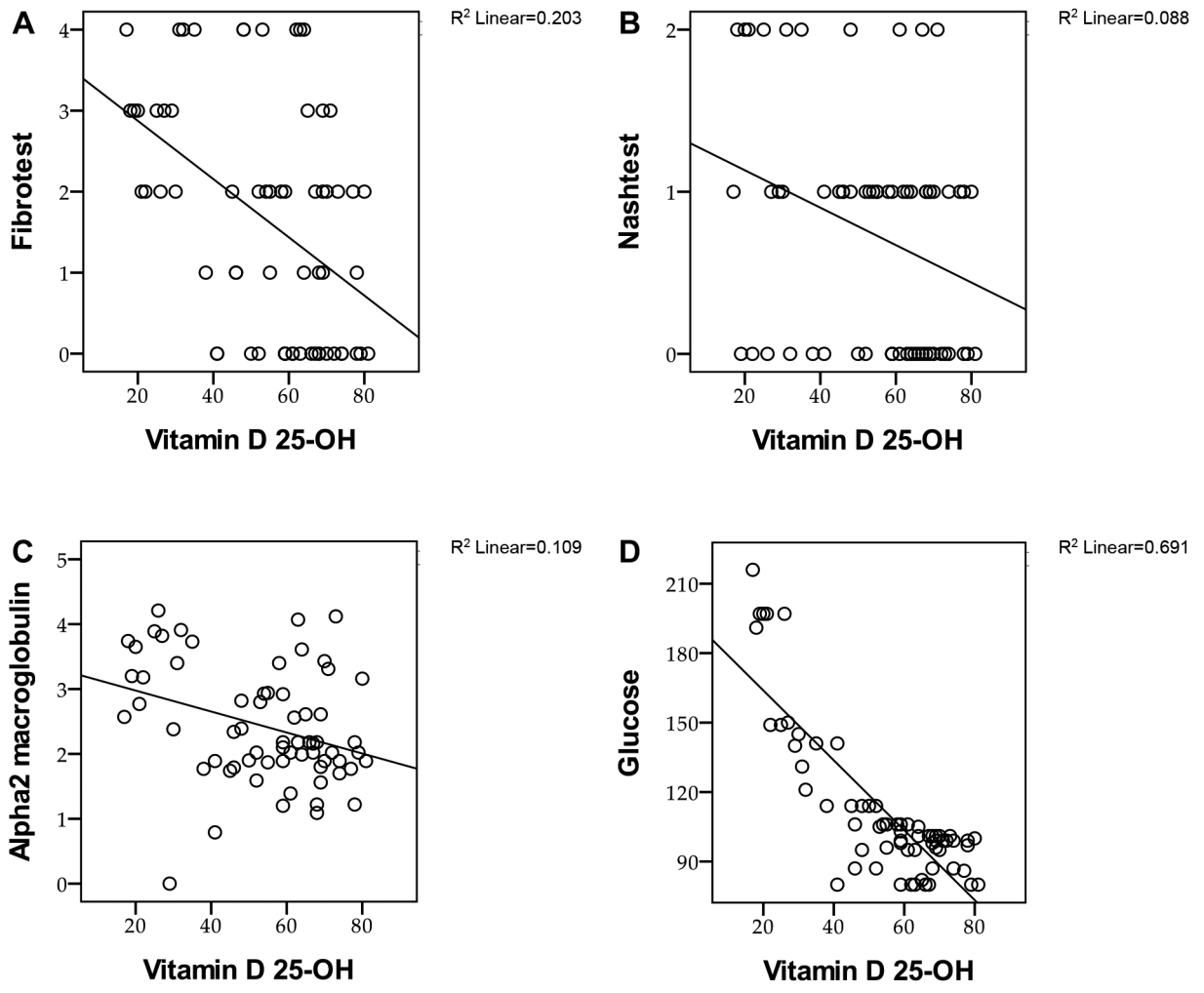

Figure 5. *Graphic representation of the Pearson correlation between vitamin D and FibroTest (A), NashTest (B), Alpha2 macroglobulin (C), and Glucose (D).*

differences in vitamin D metabolism, were noted (36). It has also been observed that prenatal vitamin D deficiency increases the prevalence of histopathological changes in the liver and alters its gene expression profile (37). In 2023, a probiotic and hepatoprotective approach to liver diseases was outlined, in addition to vitamin D, and the effectiveness of the combined treatment was observed (38, 39).

In the context of tangential studies on liver diseases, when these are explored as pre-existing risks in main conditions, such as cardiometabolic diseases, diabetes mellitus, or varicose veins, the number of patients may be smaller due to the high specificity. This was also evident in the current study, where out of 325 individuals, only 65 met the study's inclusion criteria. This limited number of patients can be regarded as a constraint of the study, potentially leading to altered results in a larger cohort.

Conclusion

Vitamin D levels vary according to their status and may provide clues about the correlation with serological levels of specific markers for liver disease in the study group. The serological markers alpha2-macroglobulin, glucose, and total cholesterol showed significant differences between the control and study groups, indicating a possible association with liver diseases.

Serological levels of specific markers for liver disease were significantly higher in the study group compared to the control group, suggesting an increased presence of liver disease in the study group. Pearson correlation analysis revealed a strong but inversely proportional relationship between vitamin D levels and the stages of FibroTest, NashTest, alpha2-globulin, and glucose markers, indicating a possible influence of vitamin D level on liver disease progression.

Increasing FibroTest and NashTest stages, as well as alpha2-macroglobulin and blood glucose levels, were associated with lower vitamin D levels in the study group, suggesting a complex interaction between vitamin D and specific markers for liver disease.

In conclusion, non-invasive serological tests like FibroTest, ActiTest, SteatoTest, NashTest, and AshTest hold significant potential in the diagnosis and management of liver diseases. These tests offer a valuable and less invasive alternative to liver biopsy and can aid in assessing fibrosis, inflammation, steatosis, and necroinflammatory activity in the liver. However, it is essential to recognize that these tests must be utilized within a clinical context and interpreted in conjunction with other pertinent information for each individual patient.

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Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization, A.I.M. and T.C.G.; methodology, S.C.H.; software, T.C.G.; validation, S.D.M., A.I.M. and L.L.; formal analysis, R.D.B.; investigation, D.F.T.; resources, A.I.M.; data curation, A.I.M.; writing—original draft preparation, T.C.G.; writing—review and editing, T.C.G.; visualization, T.C.G.; supervision, T.C.G.; project administration, A.I.M.; funding acquisition, A.I.M. All Authors have read and agreed to the published version of the manuscript.

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