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Studying the Clinical Value of Glial Cell Line-Derived Neurotrophic Factor and Other Biological Markers in Patients with Chronic Liver Diseases

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Abstract

Background: Chronic liver disease (CLD) is a degenerative process characterized by hepatic dysfunction and systemic alterations. Glial cell line-derived neurotrophic factor (GDNF), a member of the TGF-β protein family group, has been implicated in cellular survival and tissue response to injury. The aim of this study is to assess the clinical value of selected biological markers, with a focus on glial cell line–derived neurotrophic factor (GDNF), in the diagnosis of patients with chronic liver diseases. Methods: The study included 82 participants: 63 patients with CLD and 19 healthy controls. Serum GDNF levels were measured, along with liver and kidney function tests and complete blood count. Statistical analyses were conducted to compare results across the two categories. Results: Levels of GDNF were markedly higher in the CLD group (median: 2.51 ng/mL) compared to the control group (median: 1.75 ng/mL, P = 0.001). CLD patients showed significant variations in liver function tests, consisting of elevated bilirubin, AST, GGT, and INR, and decreased albumin. Hematological findings revealed lower hemoglobin, hematocrit, and RBC counts, alongside increased RDW and neutrophil-to-lymphocyte ratio. Platelet indices showed reduced counts and altered morphology. GDNF exhibited promising diagnostic ability in distinguishing patients with CLD from controls with an AUC of 0.743, sensitivity of 82.5% and specificity of 63.2%. Conclusion: The increased GDNF levels in CLD patients suggest a potential role as a non-invasive biomarker. Significant biochemical and hematological alterations further highlight the systemic impact of CLD. These findings support the need for further research into the diagnostic and prognostic utility of GDNF in liver disease.

Keywords: Chronic Liver disease (CLD), Platelets indices, Neutrophile to lymphocyte ratio (NLR), Glial cell line-derived neurotrophic factor (GDNF).

Introduction

Chronic liver disease (CLD) is defined as a lasting damage to liver function. It often develops silently, with few or no symptoms in

the early phases. In the advanced stages, patients usually develop jaundice, fatigue and abdominal discomfort. Fibrosis and cirrhosis are the outcomes of the ongoing inflammation, liver tissue destruction, and regeneration that characterize CLD. Recent literature highlights metabolic dysfunction, viral hepatitis, and alcohol related liver injury as main factors driving to CLD around the world **(Mak et al.,2024)**. An advanced stage of chronic liver disease is cirrhosis, which causes the liver's structure to be disrupted, extensive nodules to develop, vascular reorganization, neo angiogenesis, and extracellular matrix deposition. The mechanism of fibrosis and cirrhosis at a cellular level is the recruitment of stellate cells and fibroblasts, leading to fibrosis. Chronic liver disease is a prevalent clinical condition, with recent studies focusing on its etiological factors, management methods and clinical manifestations **(Sharma and Nagalli, 2023).**

Chronic liver diseases are a key factor of mortality and morbidity around the world with around 800,000 deaths per year resulting from liver cirrhosis (**Gitau and Menge, 2020).** CLD represents a rising global health concern, with an estimated 1.7 billion individuals affected worldwide as of 2021, driven primarily by non-alcoholic fatty liver disease (NAFLD), viral hepatitis, and alcohol-related liver injury **(Marcellin et al., 2023**). Among these, NAFLD—recently redefined as metabolic dysfunction-associated steatotic liver disease (MASLD)—has emerged as the most prevalent cause, affecting about 30.2% of the global adult population, with the highest rates observed in the Middle East and North Africa, reaching up to 42.6% **(Younossi et al., 2024)**. In Egypt and similar regions, the burden of MASLD-related cirrhosis is steadily increasing, particularly among younger populations, reflecting both epidemiological transition and metabolic shifts. This rising prevalence highlights the urgent need for targeted screening strategies and public health interventions to reduce progression to cirrhosis and hepatocellular carcinoma **(Alshahrani et al.,2025).**

As reported by the National Vital Statistics Report 2017 from the Center for Disease Control and Prevention, about 4.5 million individuals had cirrhosis and CLD. Recent estimates report that more than 100 million Americans are affected by some types of liver disease. Furthermore, many individuals remain undiagnosed, especially those with early phase cases like fatty liver disease **(Sharma and Nagalli, 2023).**

CLD early diagnosis is crucial for effective intervention. It progresses silently until advanced phases. Classic diagnostic tools like liver biopsy although considered the gold standard, are invasive and result in problems such as bleeding, pain and sampling error. This has led to growing demand for reliable, noninvasive markers that can accurately detect liver dysfunction and monitor progression of disease. Glial cell line-derived neurotrophic factor (GDNF) is a glycosylated, disulfide-linked homodimer that belongs to a distant subfamily within the transforming growth factor-beta (TGF-β) superfamily. Clinical investigations have demonstrated elevated GDNF levels in both the parietal cortex and plasma of individuals with recurrent major depressive disorder. Moreover, GDNF expression has been shown to increase markedly in response to cytotoxic stimuli, including exposure to ionizing radiation. Furthermore, GDNF levels are elevated in some cancer cell types **(Yang et al.,2022).** The present study aimed to evaluate the diagnostic potential of serum GDNF levels in chronic liver disease by comparing affected patients with healthy controls, while also assessing related clinical laboratory parameters.

Patients and Methods

***Ethical Approval**:*

The Research and Ethical Committees of Al-Azhar Faculty of Medicine, Damietta, Egypt, approved this research. IRB00012367-25-02-006.

Participants:

The study included 82 participants, comprising 63 patients with CLD and 19 healthy controls. CLD diagnosis was based on clinical, biochemical, and imaging findings.

Clinical diagnosis of CLD:

The diagnosis of chronic liver disease (CLD) was based on a combination of clinical evaluation, laboratory testing, and imaging findings, in accordance with established hepatology guidelines **(EASL, 2021; AASLD,**

2023). Clinical criteria included a history of chronic hepatic symptoms or known liver disease lasting more than six months. Laboratory investigations comprised liver function tests, complete blood count, and viral hepatitis serology. Abdominal ultrasonography was performed to assess liver echotexture, surface nodularity, and signs of portal hypertension. Non-invasive fibrosis scores such as the FIB-4 index and APRI score were calculated. In selected cases, the diagnosis was made when clinical and paraclinical findings consistently indicated chronic hepatic injury or fibrosis.

Blood Samples:

The blood samples were collected without an anticoagulant in dry, clean test tubes. Blood was allowed to clot for 30 minutes at room temperature (25°C) for serum separation. The sera were then separated by centrifugation at 3000 rpm for 15 minutes at 25°C. The top yellow serum layer was carefully pipetted without disturbing the white buffy layer and transferred into Eppendorf tubes. Part of the serum samples was used to determine the levels of the other biochemical parameters. The remaining serum samples were aliquoted and stored at –80°C until used for the determination of glial cell line-derived neurotrophic factor (GDNF). Standard hematological techniques were employed for WBCs count, Neutrophils%, Lymphocytes%, RBCs count, HGB level and platelets count using hematology analyzer device Cell Tac MEK - 6510 - 6500. Japan. INR was determined using a thromboplastin-based prothrombin time assay performed on a semi-automated coagulation analyzer (Coatron M1, TECO GmbH, Germany), following the manufacturer's instructions.

Biochemical Analysis:

Serum samples were collected from all participants one day prior to any surgical intervention (if applicable). The following biochemical parameters were measured using standardized assays and methods at the Gastroenterology Surgery Center, Mansoura, Egypt: Creatinine (Cr), Uric Acid (UA), Potassium (K), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, total bilirubin (T.bili), direct bilirubin (D.bili), international normalized ratio (INR). All biochemical analyses were performed in a blinded manner. The individuals conducting the assays were unaware of the participants' clinical diagnosis to minimize potential bias.

Biochemical parameters were quantified using standardized, validated commercial assay kits in accordance with international laboratory protocols. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using commercially available kit (Biomatik, China; AST: Cat. #EKF57543, ALT: Cat. #EKF58248). Serum creatinine levels were measured using a colorimetric enzymatic assay (Cat. #EU3134; FineTest, China). Alkaline phosphatase (ALP) activity was assessed by a kinetic colorimetric method based on the recommendations of the International Federation of Clinical Chemistry (IFCC), using a kit from Spinreact S.A.U. Gamma-glutamyl transferase (GGT) activity was evaluated using a kinetic enzymatic colorimetric kit (Cat. #E-BC-K126-M;Elabscience,China.), while total and direct bilirubin concentrations were determined via the diazo method, also employing kits from Spinreact S.A.U. Serum albumin was measured using the bromocresol green (BCG) dye-binding method with a dedicated assay kit (Cat. #C035-2-1; NJ Bioengineering Institute, China) following the manufacturer instructions.

Estimation of serum GDNF levels:

The level of glial cell line-derived neurotrophic factor (GDNF) in serum samples was measured using a commercially available human GDNF ELISA kit **(**Cat. No. E0122Hu, BT-Laboratory, China), based on the sandwich enzyme-linked immunosorbent assay (ELISA)principle.

Statistical Analysis:

All statistical analyses were performed using of PASW Statistics software, version 26.0 (SPSS Inc., Chicago, IL, USA), for all analyses. Quantitative data were presented as mean ±standard deviation when normally distributed, or as median (interquartile range) for non-normally distributed data. To evaluate significant differences in continuous variables between the control cohort and the chronic liver disease (CLD) group, an independent samples t-test was applied for normally distributed data,

while the Mann-Whitney U test was utilized for data that did not conform to a normal distribution.

Results

Characteristic features of studied groups.

CLD group had a significantly higher median age (57 years) compared to the control group (26 years) (P < 0.001), while the gender distribution was comparable between groups (P = 0.954). Hepatitis B surface antigen (HBsAg) positivity was negligible in both groups, whereas HCV antibody positivity was considerably higher in the CLD group (7.9%) compared to none in the control group (P = 0.001). The CLD patients showed a range of Child-Pugh score, with only 31.9% in class A, while the control group was entirely score A (P = 0.001). There was in the APRI and FIB4 scores, higher proportion of advanced class (≥1 and ≥3, respectively) with (P= 0.001) for both (Table 1).

Table (1) Characteristic features of studied groups.

Variable	Control Group N=19	Chronic Liver Disease Group N=63	P-value
Age (yr)	26 (24-34)	57 (22,5-62)	0.001
Gender			
Male n (%)	14 (73.7)	46 (73)	0.954
Female n (%)	5 (26.3)	17(27)	
HBs Ag n (%)			
+ve	0(0)	1(1.6)	0.377
-ve	19 (100)	57 (90.5)	
HCV Ab n (%)			
+ve	0(0)	5(7.9)	0.001
-ve	19 (100)	27 (42.9)	
Child n (%)			
A	19 (100)	23 (31.9)	0.001
B	0(0)	2 (33.3)	
C	0(0)	7(28.3)	
APRI n (%)			
1	18 (94.7)	27 (42.9)	0.001
2	1 (5.3)	15 (23.8)	
3	0(0)	21 (33.3)	
FIB4 n (%)			
1	19(100)	4(9.3)	0.001
2	0(0)	14(32.6)	
3	0(0)	25(58.1)	

HBsAg - Hepatitis B surface antigen, HCVAb - Hepatitis C virus antibody, Child - Child-Pugh score, APRI - Aspartate Aminotransferase to Platelet Ratio Index, FiB4 - Fibrosis-4 Index.

Serum level of glial cell line derived neurotrophic factor in the studied groups.

The chronic liver disease (CLD) group exhibited a significantly higher median GDNF level (2.51 ng/ml; IQR: 1.77–3.33) compared to the control group (1.75 ng/mL; IQR: 1.20–2.04) with a P-value of 0.001(Table 2).

Table (2) Serum level of Glial Cell Line Derived Neurotrophic Factor in the studied groups.

Variable Median (IQR)	Control Group N=19	Chronic Liver Disease Group N=63	P-value
GDNF (ng/mL)	1.75 (1.20–2.04)	2.51 (1.77 – 3.33)	0.001

GDNF - Glial Cell Line-Derived Neurotrophic Factor

Serum levels of kidney profile in the studied group

Creatinine (Cr) levels were comparable between the groups, with identical medians of 0.80 mg/dl (P = 0.744). Potassium (K) and uric acid (UA) levels also showed no significant differences, with mean ± SD values of 4.30±0.449 mEq/L and 5.02±0.937 mg/dl in the control group versus 4.04±0.58 and 4.81±1.75 in the CLD group (P = 0.136 and 0.644). Sodium (Na) levels were slightly lower in the CLD group, with a median of 137 mEq/L compared to 141 mEq/L in controls, approaching statistical significance (P = 0.051) (Table 3).

Table (3) Serum levels of kidney profile in the studied group.

Variable	Control Group N=19	Chronic Liver Disease Group N=63	P-value
Cr (mg/dl) Median (IQR)	0.80 (0.70 – 0.90)	0.80 (0.70 – 0.90)	0.744
K(mEq/L) Mean ± SD	4.30±0.449	4.04±.58	0.136
UA (mg/dl) Mean ± SD	5.02±0.937	4.81±1.75	0.644
Na(mEq/L) Median (IQR)	141 (137.0-139.0)	137(132.5 – 140)	0.051

Cr - Creatinine, K – Potassium, UA - Uric Acid, Na.- Sodium

Serum levels of different liver profile in studied groups.

Albumin levels were significantly lower in the CLD group (median: 3.6 g/dL) compared to the control group (median: 4.5 g/dL, P < 0.001). Total bilirubin (T. bili) and direct bilirubin (D. bili) levels were markedly elevated in the CLD group, with medians of 1.35 mg/dL and 0.70 mg/dL, respectively, compared to 0.60 mg/dL and 0.15 mg/dL in

controls (P < 0.001). The CLD group also exhibited significantly higher levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and international normalized ratio (INR), with P < 0.001 for all. Alanine aminotransferase (ALT) levels were slightly elevated in the CLD group, but this difference was not statistically significant (P = 0.147) (Table 4).

Table (4) Serum levels of different liver profile in studied groups.

Variable Median (IQR)	Control Group N=19	Chronic Liver Disease Group N=63	P-value
Alb (g/dL)	4.50 (4.40-4.80)	3.6(3.0 – 3.9)	<0.001
T. bili(mg/dL)	0.60(.50 -.70)	1.35 (0.80 – 3.12)	<0.001
D.bili(mg/dl)	0.15(.10-.25)	0.70 (.300 – 1.50)	<0.001
ALP(IU/L)	5(5 -5)	6 (5.0 – 8.0)	<0.001
AST(U/L)	21(20- 21)	41.5 (30.0 – 69.0)	<0.001
ALT(U/L)	23(21 – 27)	28.5 (21.0 – 48.0)	0.147
GGT(U/L)	17(12 – 27)	46 (30.50 – 89.50)	<0.001
INR `	1(1 – 1.10)	1.2 (1.0 – 1.5)	<0.001

Alb - Albumin, T.bili - Total Bilirubin, D.bili - Direct Bilirubin, ALP - Alkaline Phosphatase, AST - Aspartate Aminotransferase, ALT - Alanine Aminotransferase, GGT - Gamma-Glutamyl Transferase, INR - International Normalized Ratio.

Red Blood Cells and Their indices in the studied groups.

The CLD group exhibited significantly lower RBC counts (median: 4.2 $\times 10^6/\mu L$) compared to the control group (median: 5.20 $\times 10^6/\mu L$, P < 0.001). Hemoglobin (HGB) and hematocrit (HCT) levels were also markedly reduced in the CLD group, with mean ± SD values of 11.31±2.34 g/dL and 34.06±6.96%, respectively, compared to 14.26±1.51g/dL and 41.79±5.38% in controls (P < 0.001 for both). RDW level was significantly e higher in the CLD group (median: 14.5%) versus the control group (median: 12.30%, P < 0.001), improved interpretation of RDW. MCV, mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) showed no significant differences between the groups (P = 0.195, 0.814, and 0.358, respectively) (Table 5).

Table (5) Red Blood Cells and Their indices in the studied groups

Variable	Control Group N=19	Chronic Liver Disease Group N=63	P - Value
RBCs$\times 10^6$ (cell/µL) Median (IQR)	5.20(4.60 – 5.40)	4.2(3.42 – 4.60)	<0.001
HG (g/dl) Mean ± SD	14.26 ± 1.51	11.31±2.34	<0.001
HCT (%) Mean ± SD	41.79±5.38	34.06±6.96	<0.001
MCV (fL) Median (IQR)	82.46(80.16 – 86.20)	85(80.63 – 90.0)	0.195
MCH(Pg) Median (IQR)	28.5(27.8 – 29.25)	28.7(26.80 – 30.60)	0.814
MCHC(g/dL) Mean ± SD	34.30±1.72	33.28±2.57	0.358
RDW (%) Median (IQR)	12.30(11.50 – 13.05)	14.5(12.95- 16.90)	<0.001

RBC - Red Blood Cell Count, HG – Hemoglobin, HCT – Hematocrit, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration, RDW - Red Cell Distribution Width.

White Blood Cells and Their indices in the studied groups.

The CLD group exhibited a significantly lower median WBC count (4.3 $\times 10^3/\mu L$) compared to the control group (5.7 $\times 10^3/\mu L$, P = 0.039). Lymphocyte count (Lymph No) was notably lower in the CLD group (median: 1.02 $\times 10^3/\mu L$) compared to the control group (median: 2.50 $\times 10^3/\mu L$, P < 0.001), and lymphocyte percentage (Lymph %) was decreased in the CLD group (mean ± SD: 27.28 ± 12.06%) compared to the controls (mean ± SD: 43.7 ± 7.5%, P < 0.001). On the other hand, neutrophil count (Neut No) and percentage (Neut %) were higher in the CLD group, but the difference in neutrophil count was not significant (P = 0.650), while the neutrophil percentage was significantly increased (mean ± SD: 58.17 ± 14.07% in CLD vs. 43.7 ± 8.4% in controls, P < 0.001). The neutrophil-to-lymphocyte ratio (NLR) was significantly higher in the CLD group (median: 2.34) in contrast to the control group (median: 0.96, P < 0.001) (Table 6).

Table (6) White Blood Cells and

Their indices in the studied groups.

Variable	Control Group N=19	Chronic Liver Disease Group N=63	P - Value
WBC ×10³ (cell/µL) Median (IQR)	5.7(4.2 – 6.9)	4.3(3.22- 6.03)	0.039
Lymph No×10³ (cell/µL) Median (IQR)	2.50(1.90-3.05)	1.02(0.73 – 1.6)	<0.001
Neut No×10³ (cell/µL) Median (IQR)	2.16(1.70 – 3.10)	2.68(1.55 – 3.50)	0.650
Lymph % Mean ± SD	43.7±7.5	27.28±12.06	<0.001
Neut % Mean ± SD	43.7±8.4	58.17±14.07	<0.001
NLR Median (IQR)	0.96(0.80-1.22)	2.34(1.44 – 3.43)	<0.001

WBC - White Blood Cell Count, Lymph no - Lymphocyte Number, Neut no - Neutrophil Number, Lymph % - Lymphocyte Percentage, Neut % - Neutrophil Percentage, NLR - Neutrophil-to-Lymphocyte Ratio.

Platelets and Their indices in the studied groups

Platelet count (PLT) was significantly lower in the CLD group (median: 105 ×10³/µL) compared to the control group (median: 209 ×10³/µL, P < 0.001), reflecting thrombocytopenia commonly associated with liver disease. PDW was considerably higher in the CLD group (median: 18.60%) than in controls (median: 13%, P = 0.043), suggesting increased variability in platelet size. Pct was markedly decreased in the CLD patients (median: 0.089%) compared to controls (median: 0.149%, P < 0.001). Conversely, platelet large cell ratio (PLCR) was higher in the CLD group (median: 33.9%) than in controls (median: 28.40%, P = 0.002). MPV and PLR showed no significant differences between the groups (P = 0.116 and 0.739, respectively) (Table 7).

Table (7) Platelets and Their indices in the studied groups

Variable Median (IQR)	Control Group N=19	Chronic Liver Disease Group N=63	P - Value
PLT×10³ (cell/µl)	209(191.9 – 252.9)	105(60.25-166.77)	<0.001
PDW (%)	13(11.60 – 10.20)	18.60(13.35-20.25)	0.043
MPV (fL)	9.30(6.50 – 10.50)	9.45(7.37 – 11.30)	0.116
Pct (%)	0.149(0.126-0.206)	0.089(0.056-0.144)	<0.001
PLCR (%)	28.40(21.60 – 30.60)	33.9(30.7- 41.1)	0.002
PLR	83.6(71.22 – 112.22)	90.9(60.28 – 134.4)	0.739

PLT - Platelet Count, PDW - Platelet Distribution Width, MPV - Mean Platelet Volume, Pct - Plateletcrit (percentage of blood volume occupied by platelets), PLCR - Platelet Large Cell Ratio, PLR - Platelet-to-Lymphocyte Ratio.

Area under curve (AUC), sensitivity, and specificity of serum glial cell line-derived neurotrophic factor for diagnosis of patients with Chronic Liver disease

The area under the curve (AUC), sensitivity (Sen%), specificity (Spe%), and 95% confidence intervals (CI) for GDNF in diagnosing chronic liver disease (CLD) patients was shown in (**Table.8**). Glial cell line-derived neurotrophic factor (GDNF) demonstrated a strong AUC of 0.743, with an 82.5% sensitivity and 63.2% specificity at cutoff of 1.62, and *P* value of (*P* < 0.001) (Table 8) (Fig 1).

Table (8). Area under curve (AUC), sensitivity, and specificity of serum glial cell line-derived neurotrophic factor for diagnosis of patients with Chronic Liver disease

Variables	AUC	Cutoff	Sen%	Spe%	95% CI	P value
GDNF (ng/ml)	0.743	1.62	82.5%	63.2%	0.618-0.868	<0.001

GDNF - Glial Cell Line-Derived Neurotrophic Factor, AUC- Area Under the curve, CI- Confidential Interval

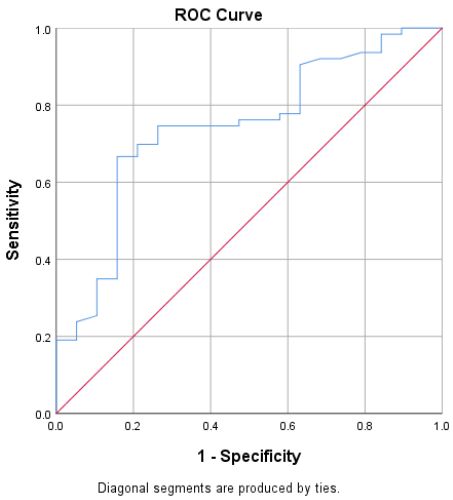

Fig 1. ROC curve for GDNF for diagnosis of patients with chronic liver disease

Discussion

Chronic liver diseases (CLD) have a wide range of causes, including, long -term alcohol misuse, infections, autoimmune disease, and metabolic diseases. The last stage of chronic liver disease, cirrhosis, is recognized by vascular reorganization, neo-angiogenesis, the deposition of an extracellular matrix, disturbance of liver architecture and the development of extensive nodules. Liver cirrhosis may be classified into two phases, the compensated cirrhosis and the decompensated cirrhosis. There are no symptoms during the compensated cirrhosis, while in the decompensated one, portal pressure escalates above the normal range(>5mmHg). Worldwide epidemiological analysis underscores a growing burden of CLD driven primarily by metabolic dysfunction associated steatotic liver disease and alcohol related liver disease (**Yu et al.,2024).**

Glial cell line-derived neurotrophic factor (GDNF) is a robust protein that belongs to the transforming growth factor-beta (TGF-β) superfamily. It was basically introduced as a survival factor, but other research has revealed that GDNF has much broader biological functions. While historically studied in Parkinson disease context, current research suggests its dual upregulation in neural cell types during neurodegredation, highlighting significant value in inflammation and brain repair. It has fundamental role not only in the development and maintenance of the central and peripheral nervous systems but also in organogenesis, spermatogenesis, and cellular responses in various tissues. GDNF is encoded by the GDNF gene, which is situated on chromosome 5p13.2 in humans **(Sidorova and Saarma,2020).**

The main signaling pathways activated by the GDNF-RET complex include the PI3K/AKT pathway, which promotes cell survival and has anti-apoptotic effects, and the MAPK/ERK pathway, which is associated with differentiation and cell proliferation. Src family kinases may also be involved in mediating downstream effects. These signaling mechanisms are critical not only in normal physiology but also in pathological conditions, such as cancer (**Guo et al., 2024).**

Beyond its neural roles, GDNF has been found to participate in tissue repair, neuroinflammation, and certain cancer processes. Elevated levels of GDNF have been implicated in tumor progression and resistance to therapy in some cancers, whereas in other contexts, GDNF may have protective effects. For instance, its neuroprotective properties have prompted investigation into its therapeutic potential in neurodegenerative diseases like Parkinson's disease. In liver disease, increasing evidence reported that GDNF may have diagnostic and prognostic ability. Furthermore, previous studies reported that GDNF levels are elevated in hepatic stellate cells and liver tissues of patients with advanced fibrosis(F4) **(Zhuang et al.,2023).**

In our study, gender distribution was similar between the categories (P=0.954), indicating that female and male participants were equally represented in both study individuals. The percentage of HBV positivity was similar between the groups, while HCV was considerably higher in CLD group compared to none in the controls(P=0.001).

Level of GDNF was notably higher in the CLD group versus control group (P=0.001). This is in line with studies suggesting that GDNF reduces hepatocytes apoptosis and oxidative stress while concurrently being involved in carcinogenesis. GDNF activates survival pathways, such as PI3K/Akt and ERK signaling, which counteract apoptosis signals. A study by Zhang et al. investigated serum GDNF levels in individuals with liver fibrosis. This study, which employed ELISA to quantify GDNF concentrations, demonstrated significantly increased serum GDNF in patients

with liver fibrosis versus controls. The study focused on circulating levels of GDNF and did not involve histological analysis of liver tissue **(Zhang et al.,2019).**

The serum kidney profile analysis revealed comparable creatinine, potassium, and uric acid levels between the control and chronic liver disease groups, suggesting that overall kidney function, as indicated by these markers, was not significantly compromised in the CLD cohort in this study. This finding aligns with some studies indicating that renal impairment is not always present in the early stages of CLD. Monitoring NA levels is essential for prognostication and CLD management However, the slightly lower sodium levels in the CLD group, approaching statistical significance, warrant further consideration **(Kiani & Zori,2023).** Hyponatremia is a recognized complication of advanced liver disease, often associated with fluid retention and altered hormonal regulation **(Ginès et al., 2018).** While not statistically significant in this instance, this trend suggests the need for careful monitoring of electrolyte balance in CLD patients.

The current study demonstrated marked alterations in RBC indices among patients with CLD versus the controls, suggesting a high prevalence of anemia in CLD patients. key erythrocyte parameters including RBC count, HGB, and HCT levels were considerably reduced in the CLD group ($P < 0.001$ for all), consistent with prior findings in the literature **(Hu et al., 2023; Mishra et al., 2021)**. These reductions are reflective of the multifactorial etiology of anemia in CLD, which may involve hypersplenism, gastrointestinal blood loss due to portal hypertension, nutritional deficiencies, impaired erythropoietin production, and direct bone marrow suppression.

The anemia observed in CLD is often of the normocytic normochromic type, suggesting preserved red cell morphology despite declined production or high destruction. This was reinforced by the findings in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), which did not differ significantly between CLD patients and controls ($P = 0.195$ and $P = 0.814$, respectively). Similarly, mean corpuscular hemoglobin concentration (MCHC) remained statistically unchanged ($P = 0.358$). These results indicate that while anemia is prevalent, its features are typical of chronic inflammatory conditions, which often present with normal RBC indices **(Scheiner et al.,2020).**

In contrast, RDW was considerably increased in CLD cases ($P < 0.001$), indicating increased anisocytosis and suggesting a heterogeneous population of red cells. This finding may reflect ineffective erythropoiesis or a mixed anemia etiology, potentially involving both iron deficiency and vitamin B12 or folate deficiency. Elevated RDW has been associated with advanced fibrosis poor clinical outcome and portal hypertension **(Haung et al.,2025).**

Anemia in CLD tends to worsen with disease severity, as hemoglobin and hematocrit levels have been shown to correlate negatively with liver function scores such as the Child-Pugh score and MELD system. Both HGB and HCT tend to decrease as liver disease progresses, and their reduction has been significantly correlated with higher Child-Pugh classes **(Abdel-Moneim & Mahmoud, 2023).** Lower levels of hemoglobin have prognostic implications, being correlated with increased mortality and poorer clinical outcomes. These hematological parameters, therefore, work not only as evidence of anemia but also as important markers of disease progression and prognosis **(Schettler et al., 2020).**

Alterations in WBC indices further reflect the underlying pathophysiology of CLD. Leukopenia is frequently observed widely due to hypersplenism associated with portal hypertension, resulting in elevated sequestration of leukocytes in the enlarged spleen. Furthermore, different studies suggested that higher baseline WBC count independently predict the incidence of NAFLD Additionally, bone marrow suppression from chronic inflammation, viral infections, and hepatotoxic insults can contribute to diminished leukocyte production. In contrast, leukocytosis may be observed in specific contexts such as alcoholic liver disease or systemic infections, reflecting acute inflammatory responses **(Hu et al.,2024).**

Elevated NLR has been linked to systemic inflammation, disease severity, complications, and poor survival in cirrhosis. Increased PLR has been associated with the degree of liver fibrosis, specifically in NALFD patients and chronic hepatitis C, suggesting its value as a non-invasive biomarker of liver dysfunction. These indices provide valuable insight into the immune-inflammatory dynamics in CLD and may enhance clinical assessment when integrated into routine

assessment **(D'Amico et al.,2025).**

The serum liver profile exhibited significant alterations in the CLD group. The observed lower albumin values are a well-established evidence of impaired hepatic synthesis capacity in CLD **(Friedman et al., 2019).** Similarly, the significantly elevated total and direct bilirubin levels reflect compromised bilirubin metabolism and excretion, characteristic of liver dysfunction **(Sherlock & Dooley, 2018).** Furthermore, the higher levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and international normalized ratio (INR) further corroborate the presence of liver damage and impaired synthetic function in the CLD group. These enzymes are commonly released into the bloodstream upon hepatocyte injury, and an elevated INR signifies impaired coagulation factor synthesis by the diseased liver. Other studies suggested that despite its prognostic value and inclusion in scoring systems such as MELD, INR does not reliably predict bleeding complications **(Wang et al.,2023).** While ALT levels were slightly elevated in the CLD cases, the lack of statistical significance might suggest that other enzymes, particularly AST, GGT, and ALP, were more sensitive indicators of the predominant type of liver injury in this specific CLD cohort. Overall, the distinct differences in the serum liver profile between the control and CLD groups strongly support the presence of significant hepatic dysfunction in the studied CLD population, consistent with established clinical and biochemical features of chronic liver disease.

Glial cell line-derived neurotrophic factor (GDNF) exhibited good diagnostic potential, with an AUC of 0.743, sensitivity of 82.5%, and specificity of 63.2% ($P < 0.001$). GDNF, a neurotrophic factor, has been implicated in liver regeneration and fibrosis modulation, its moderate specificity suggests that while GDNF is sensitive to chronic liver disease, it may also be influenced by extrahepatic conditions, necessitating further validation in diverse cohorts **(Yang et al.,2022).**

One limitation of this study is the noticeable age difference between the control and CLD groups. The median age of the control group was 26 years, compared to 57 years in the CLD group. Since certain biological markers, including GDNF, may be influenced by age-related physiological changes, this discrepancy could act as a confounding factor. Although the observed differences in GDNF levels were statistically significant, future studies should consider age-matched cohorts to more accurately isolate the effect of liver disease.

Additionally, no statistically significant correlation was observed between GDNF levels and Child–Pugh classification, suggesting that while GDNF may have diagnostic value, it may not reflect disease severity. This could be due to the limited sample size, heterogeneity in disease etiology, or the possibility that GDNF expression is altered early in liver disease but not progressively across severity stages. Future studies with larger, age-matched cohorts and stratification by liver disease stage are warranted.

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الملخص العربي

عنوان البحث: دراسة القيمة السريرية لعامل التغذية العصبية المشتق من الخلايا الدبقية ومؤشرات حيوية اخري في المرضي المصابين بأمراض الكبد المزمنة

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الخلفية: أمراض الكبد المزمنة (CLD) هي حالة تقدمية تتميز بخلل في وظائف الكبد وتغيرات جهازية. تم ربط عامل التغذية العصبي المشتق من الخلايا الدبقية (GDNF)، وهو أحد أعضاء عائلة TGF-β الفائقة، ببقاء الخلايا والاستجابة لإصابة الأنسجة. هدفت هذه الدراسة إلى تقييم مستويات GDNF في مصل الدم لدى مرضى مصابون بأمراض كبد مزمنة مقارنةً بالأشخاص الأصحاء، بالإضافة إلى تقييم المعلمات البيوكيميائية والدموية ذات الصلة. الطرق: شملت الدراسة ٨٢ مشاركًا: ٦٣ مريضًا بـأمراض كبد مزمنة و١٩ شخصًا سليمًا. تم قياس مستويات GDNF في المصل، إلى جانب اختبارات وظائف الكبد والكلى وعدد الدم الكامل. أُجريت التحليلات الإحصائية لمقارنة النتائج بين المجموعتين. النتائج: كانت مستويات GDNF أعلى بشكل ملحوظ في مجموعة CLD (الوسيط: ٢,٥١ نانو غرام/مل) مقارنةً بمجموعة الشواهد (الوسيط: ١,٧٥ نانو غرام/مل؛ القيمة الاحتمالية P = 0.001). أظهر المرضي الذين يعانون من امراض كبد مزمنة تغييرات ملحوظة في اختبارات وظائف الكبد، بما في ذلك ارتفاع البيليروبين، وإنزيمات AST، وGGT، وINR، وانخفاض الألبومين. وكشفت النتائج الدموية عن انخفاض في مستويات الهيموغلوبين، والهيماتوكريت، وعدد كريات الدم الحمراء، إلى جانب زيادة في RDW ونسبة العدلات إلى الخلايا اللمفاوية. كما أظهرت مؤشرات الصفائح الدموية انخفاضًا في العدد وتغيرًا في الشكل. أظهر GDNF قدرة تشخيصية واعدة في التمييز بين الأشخاص المصابينن بأمراض كبد مزمنة والأشخاص الاصحاء، حيث بلغت المساحة تحت المنحني ٠,٧٤٣، مع حساسية بلغت ٨٢,٥ % ونوعية بلغت ٦٣,٢%. الاستنتاج: تشير المستويات المرتفعة من GDNF في مرضى CLD إلى دوره المحتمل كواسم حيوي غير جراحي. وتسلط التغيرات البيوكيميائية والدموية الهامة الضوء على التأثير الجهازي لأمراض الكبد المزمنة. وتدعم هذه النتائج الحاجة إلى إجراء المزيد من البحوث حول الفائدة التشخيصية والتنبؤية لـ GDNF في أمراض الكبد.