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ORIGINAL ARTICLE

Evaluation of Biochemical and Physiological Biomarkers Associated with Non-Alcoholic Fatty Liver Disease (NAFLD) among Pakistani Adults

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ABSTRACT

Background: In Pakistan, non-alcoholic fatty liver disease (NAFLD) is becoming more common, a trend that aligns with rising obesity rates, increased insulin resistance, and broader metabolic issues. This study aimed to investigate the biochemical and physiological indicators associated with NAFLD within a representative group of Pakistani individuals.

Methodology: This cross-sectional study was conducted from December 2022 to June 2023 in tertiary care units of Lahore, Hyderabad and Larkana. Total 120 participants were enrolled, among these, 80 individuals had NAFLD confirmed through radiological methods, while 40 served as healthy controls. Data was collected on standardized anthropometric measures, including body mass index (BMI) and waist circumference, alongside various biochemical parameters such as liver enzymes, lipid profiles, HOMA-IR scores, and levels of C-reactive protein (CRP). Statistical analyses including Student's t-test, Pearson's correlation, and multiple linear regression were used to identify factors that independently predicted alanine aminotransferase (ALT) levels.

Results: The findings revealed that NAFLD patients had significantly elevated values for BMI, waist circumference, ALT, AST, GGT, total cholesterol, triglycerides, HOMA-IR, and CRP compared to the control group ($p < 0.001$ for each parameter). In addition, there were moderate to strong positive correlations between anthropometric measurements and biochemical markers indicating liver damage. Multiple linear regression identified BMI ($\beta = 0.78$, $p < 0.001$), HOMA-IR ($\beta = 2.05$, $p < 0.001$), waist circumference ($\beta = 0.40$, $p = 0.022$), and CRP ($\beta = 0.95$, $p = 0.005$) as independent predictors of ALT levels, collectively accounting for 56% of the variance observed.

Conclusion: The study confirms that NAFLD in the Pakistani population is closely linked to metabolic abnormalities and systemic inflammation. Elevated BMI, insulin resistance, central adiposity, and increased CRP levels emerge as significant indicators of liver injury, underscoring the need for early screening and focused intervention strategies.

Keywords: Non-alcoholic fatty liver disease, NAFLD, biochemical markers, metabolic dysfunction, insulin resistance, Pakistan, inflammation, hepatic injury.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as a critical global health concern and is now recognized as the primary cause of chronic liver disease. This issue is especially alarming in low and middle-income countries, where rapid urban growth, evolving dietary patterns, and increasingly sedentary lifestyles are fueling a rise in metabolic disorders [1]. Over the past decades, these changes have been occurring at a time when rates of obesity, type 2 diabetes, and cardiovascular disease have been increasing rapidly in Pakistan, making NAFLD an important public health problem[2, 3].

Non-alcoholic fatty liver disease (NAFLD) covers a spectrum that begins with simple fat accumulation in the liver (steatosis) and can progress to non-alcoholic steatohepatitis (NASH), a more severe condition that may eventually lead to cirrhosis and hepatocellular carcinoma. The disease's development is complex, driven by multiple factors including insulin resistance, oxidative stress, lipid peroxidation, and chronic inflammation[4]. Early biochemical markers of hepatic injury are liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while assessment of dyslipidemia, systemic inflammation, and insulin resistance are important to understand the underlying metabolic disturbances[5,6].

Assessment of NAFLD is different in the Pakistani population because of the challenges and opportunities presented. Disease progression may be a result of genetic predispositions coupled with regional dietary patterns that favour high carbohydrate and saturated fat consumption. Furthermore, in settings of resource constraint, there is a need for reliable, non-invasive biomarkers, given the limited access to advanced imaging and invasive diagnostic procedures[7]. In recent studies in Pakistan, the utility of composite indices like the fibrosis 4 (FIB4) index and the NAFLD fibrosis score has been stressed for predicting liver fibrosis and guiding clinical management. Novel inflammatory and metabolic markers emerging data further support the development of diagnostic algorithms that would facilitate early intervention strategies[8].

The study aimed to investigate the biochemical and physiological markers of NAFLD in the Pakistani population. The study attempts to elucidate the pathophysiological mechanisms behind NAFLD by synthesizing clinical assessments, laboratory parameters, and anthropometric measurements to inform diagnostic and therapeutic approaches that are effective and region-specific[5,9].

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MATERIALS AND METHODS

Study Design and Setting: This cross-sectional study was conducted from December 2022 to June 2023 in tertiary care units of Lahore, Hyderabad and Larkana. The aim was to evaluate the biochemical and physiological markers linked to non-alcoholic fatty liver disease (NAFLD) within a representative local cohort. The study protocol received Institutional Review Board approval and adhered to the Declaration of Helsinki, with written informed consent obtained from every participant.

Study Population and Sampling: A total of n=120 participants were included, consisting of n=80 patients with radiologically confirmed NAFLD and n=40 healthy controls who were matched for age and sex. Patients were considered eligible if they were between 18 and 65 years of age and had hepatic steatosis as confirmed by abdominal ultrasonography. Individuals with significant alcohol consumption (defined as more than 20 g/day for

women and over 30 g/day for men), viral hepatitis, autoimmune liver disorders, or any other identifiable liver diseases were excluded. The control group was comprised of subjects without any clinical or biochemical indicators of liver disease.

Data Collection and Measurements: Data were obtained through structured questionnaires, clinical interviews, and standardized measurement methods. Anthropometric data—including height, weight, and waist circumference—were collected using calibrated equipment. The body mass index (BMI) was calculated by dividing the weight (in kilograms) by the square of the height (in meters). Blood samples, taken after a fasting period of 10–12 hours, were analyzed in accredited hospital laboratories. Biochemical assessments included liver function tests (ALT, AST, GGT, and alkaline phosphatase), a lipid profile (total cholesterol, LDL, HDL, and triglycerides), and indicators of glucose metabolism (fasting blood glucose and insulin). Insulin resistance was evaluated using the homeostatic model assessment (HOMA-IR) index, and serum C-reactive protein (CRP) levels were measured to gauge systemic inflammation. The degree of hepatic steatosis was assessed through abdominal ultrasonography performed by experienced radiologists following standardized protocols.

Statistical Analysis: Data were analyzed using SPSS version 26.0 Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were reported as frequencies and percentages. Comparisons between NAFLD patients and controls were made using Student's t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed variables. The chi-square test was applied to analyze categorical data. Pearson's correlation coefficients were computed to explore the relationship between anthropometric measurements and biochemical markers within the NAFLD group. A multiple linear regression model was then employed to identify independent predictors of ALT levels, with BMI, HOMA-IR, waist circumference, and CRP included as independent variables. A two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

The study enrolled a total of n=120 participants (80 patients with non-alcoholic fatty liver disease [NAFLD] and 40 healthy controls). NAFLD patients were 46.0 ± 11.0 years of age, while controls were 43.0 ± 10.5 years of age (p = 0.12). Gender distribution of groups did not significantly differ (NAFLD 62.5% males vs controls 60.0%, p = 0.78). Nevertheless, anthropometric measurements were significantly different. The controls had a body mass index (BMI) of 24.0 ± 2.8 kg/m² and a waist circumference of 85.0 ± 7.8 cm and the NAFLD patients had a BMI of 28.5 ± 3.8 kg/m² (p < 0.001) and a waist circumference of 97.0 ± 9.5 cm (p < 0.001) as shown in table 1.

Liver enzyme levels were notably higher in NAFLD patients compared to controls. Specifically, the mean alanine aminotransferase (ALT) level in the NAFLD group was 50.0 ± 18.0 IU/L, whereas in controls it was 22.0 ± 7.0 IU/L (p < 0.001). Similarly, aspartate aminotransferase (AST) levels averaged 45.0 ± 15.0 IU/L in NAFLD patients versus 20.0 ± 5.5 IU/L in controls (p < 0.001). Gamma glutamyl transferase (GGT) was also elevated, with NAFLD patients recording 68.0 ± 28.0 IU/L compared to 30.0 ± 10.0 IU/L in the control group (p < 0.001). Additionally, the NAFLD group showed higher lipid levels, with total cholesterol at 212.0 ± 32.0 mg/dL versus 182.0 ± 27.0 mg/dL, and triglycerides at 195.0 ± 50.0 mg/dL compared to 125.0 ± 35.0 mg/dL (p < 0.001 for both). The insulin resistance measure, HOMA-IR, was significantly greater in NAFLD patients (3.4 ± 1.0) relative to controls (1.9 ± 0.5, p < 0.001), and serum C-reactive protein (CRP) levels were substantially increased in the NAFLD group (5.8 ± 2.0 mg/L versus 2.8 ± 1.0 mg/L, p < 0.001) as shown in table 2.

Within the NAFLD group, the relationships between anthropometric measures and biochemical markers were assessed using Pearson's correlation coefficients. A moderate positive relationship was identified between body mass index (BMI) and alanine aminotransferase (ALT) (r = 0.43, p < 0.001), as well as between waist circumference and ALT (r = 0.46, p < 0.001). Similarly, both BMI (r = 0.50, p < 0.001) and waist circumference (r = 0.53, p < 0.001) showed significant correlations with the HOMA-IR index. Additionally, positive associations were observed between C-reactive protein (CRP) levels and both BMI (r = 0.44, p < 0.001) and waist circumference (r = 0.47, p < 0.001), suggesting that greater adiposity is linked to heightened systemic inflammation as shown in table 3.

In NAFLD patients, alternative predictors of ALT levels were determined by a multiple linear regression analysis. Independent variables of the regression model included BMI, HOMA-IR, waist circumference, and CRP. Overall, the model was statistically significant (F[4,75] = 19.5, p < 0.001), and the variance in ALT levels accounted for 56% (adjusted R^2 = 0.56). These regression coefficients were BMI (β = 0.78, p < 0.001), HOMA-IR (β = 2.05, p < 0.001), CRP (β = 0.95, p = 0.005), and waist circumference (β = 0.4, p = 0.022). These results indicate that metabolic parameters and inflammatory status are important independent predictors of hepatic enzyme elevations in NAFLD patients as shown in table 4.

However, in 120 patients with NAFLD, liver enzymes were significantly elevated, and dyslipidemia, insulin resistance, and systemic inflammation were present, compared to controls. Both BMI and waist circumference were strongly associated with biochemical markers of liver injury and metabolic dysfunction. Multiple regression analysis further demonstrated that predictors of elevated ALT levels were independent of BMI, HOMA-IR, waist circumference, and CRP, indicating that metabolic and inflammatory factors together contribute to hepatic health in NAFLD.

Table 1. Demographic and Anthropometric Characteristics

Parameter	NAFLD Patients (n = 80)	Controls (n = 40)	p-value
Age (years)	46.0 ± 11.0	43.0 ± 10.5	0.12
Male (%)	62.5%	60.0%	0.78
BMI (kg/m²)	28.5 ± 3.8	24.0 ± 2.8	<0.001
Waist Circumference (cm)	97.0 ± 9.5	85.0 ± 7.8	<0.001

*Data are expressed as mean ± standard deviation (SD) or percentages. Comparisons were performed using the student's t-test for continuous variables and the chi-square test for categorical variables.

Table 2. Biochemical Parameters

Parameter	NAFLD Patients (n = 80)	Controls (n = 40)	p-value
ALT (IU/L)	50.0 ± 18.0	22.0 ± 7.0	<0.001
AST (IU/L)	45.0 ± 15.0	20.0 ± 5.5	<0.001
GGT (IU/L)	68.0 ± 28.0	30.0 ± 10.0	<0.001
Total Cholesterol (mg/dL)	212.0 ± 32.0	182.0 ± 27.0	<0.001
Triglycerides (mg/dL)	195.0 ± 50.0	125.0 ± 35.0	<0.001
HOMA-IR	3.4 ± 1.0	1.9 ± 0.5	<0.001
CRP (mg/L)	5.8 ± 2.0	2.8 ± 1.0	<0.001

*Values are reported as mean ± SD. Statistical significance was assessed using the student's t-test.

Table 3. Correlations in NAFLD Patients

Variable	ALT (IU/L)	HOMA-IR	CRP (mg/L)
BMI	r = 0.43	r = 0.50	r = 0.44
Waist Circumference	r = 0.46	r = 0.53	r = 0.47

*All correlations were statistically significant (p < 0.001).

Table 4. Multiple Linear Regression Analysis Predicting ALT Levels in NAFLD Patients

Variable	β Coefficient	Standard Error	p-value
BMI	0.78	0.15	<0.001
HOMA-IR	2.05	0.48	<0.001
Waist Circumference	0.40	0.18	0.022
CRP	0.95	0.32	0.005

*The regression model was developed using a stepwise approach, with statistical significance set at p < 0.05.

DISCUSSION

This study finds that metabolic dysfunction is associated with hepatic injury in the Pakistani population with non-alcoholic fatty liver disease (NAFLD) to a very high degree. NAFLD patients had significantly higher BMI, waist circumference, and adverse biochemistry when compared with controls, including elevated liver enzymes, dyslipidemia, increased insulin resistance, and heightened systemic inflammation[10]. These observations highlight the multifactorial pathophysiology of NAFLD in which both adiposity and metabolic disturbances are essential for liver injury. Previous research confirms the strong correlations between anthropometric measures (BMI and WC) and hepatic enzymes (ALT) and that central obesity is an important factor in the progression of NAFLD[11, 12].

Additionally, the independent predictors for ALT levels were found by the multiple linear regression analysis to be HOMA-IR, BMI, waist circumference, and CRP. Based on this model, both metabolic and inflammatory parameters are main contributors to hepatic damage in NAFLD patients[13]. These findings are in line with the broader literature, which has time and again demonstrated the key role of insulin resistance and chronic inflammation in the development and progression of NAFLD. The study's results also help support the increasing demand for non-invasive biomarkers to aid in early identification and risk stratification of NAFLD, particularly in resource-constrained settings like Pakistan[14, 15].

Nevertheless, some limitations should be acknowledged. Given the cross-sectional design of the study, it is not possible to make causal inferences regarding metabolic dysfunction and liver injury. Moreover, the relatively small sample size (n = 120) may provide constraints on the generalizability of the findings[16]. These associations should be validated in future research with larger, longitudinal cohorts and a potential role of early intervention strategies in altering disease progression explored[17, 18].

CONCLUSION

The results of this study show that the presence of NAFLD in the Pakistani population is highly correlated with increased adiposity, dyslipidemia, insulin resistance, and systemic inflammation that lead to hepatic injury as reflected by elevated ALT levels. These results indicate that BMI, HOMA-IR, waist circumference, and CRP have independent predictive value for elevations in liver enzymes, indicating the critical interplay between metabolic and inflammatory pathways in the pathogenesis of NAFLD. In addition to adding to the understanding of the regional aspects of the disease, these findings reinforce the importance of routine screening and early intervention to prevent the progression of NAFLD. These biomarkers should be validated in larger populations, and the efficacy of targeted therapeutic strategies for patients with NAFLD should be evaluated in future studies.

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