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ORIGINAL ARTICLE

Biochemical Analysis of Lipid Profiles and Oxidative Stress Markers in Cardiovascular Patients with Type-2 Diabetes. A Cross-Sectional Study in Pakistan

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ABSTRACT

Background: Cardiovascular disease (CVD) continues to be a leading cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) particularly in high disease burden areas such as Pakistan. The progression of diabetic cardiovascular complications has been related to dyslipidemia and oxidative stress. This study investigates the biochemical interplay between lipid profiles and oxidative stress markers in cardiovascular patients with T2DM.

Methods: A cross-sectional clinical study was done in n=120 patients with T2DM and established cardiovascular disease. Lipid profiles were measured by standardized enzymatic assays according to the National Cholesterol Education Program (NCEP), and International Federation of Clinical Chemistry (IFCC) guidelines on the same day. The oxidative stress was quantified by measuring the level of malondialdehyde (MDA) and the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Pearson's correlation analysis was used to find the relationships between lipid parameters and oxidative stress markers.

Results: The atherogenic lipid profile included elevated total cholesterol, LDL C, triglycerides, and low HDL C. MDA levels were significantly elevated, and SOD and CAT activities were significantly decreased, suggesting a significant increase in oxidative stress. Correlation analysis showed a positive correlation of LDL C and MDA ($r = 0.47$, $p < 0.001$) and an inverse correlation of HDL C and MDA ($r = –0.39$, $p = 0.010$) which suggests that dyslipidemia is associated with increased oxidative stress in this high-risk population.

Conclusion: T2DM cardiovascular patients have significant dyslipidemia and enhanced oxidative stress, both of which may synergistically aggravate cardiovascular risk. Our findings support the therapeutic strategy of combined lipid abnormalities and oxidative stress treatment to improve cardiovascular outcomes in T2DM patients.

Keywords: Type-2 Diabetes Mellitus, Cardiovascular Disease, Dyslipidemia, Oxidative Stress, Lipid Profile, Malondialdehyde, Antioxidant Enzymes, NCEP, IFCC.

INTRODUCTION

Two of the most formidable public health challenges in Pakistan today are cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). The transition of epidemiological transition in the country is fast, urbanization, sedentary lifestyle, and dietary changes have all contributed to a quick rise in the prevalence of metabolic disorders in the nation[1]. According to recent estimates, about 19–22% of the adult population in Pakistan is afflicted with T2DM, making the country one of the countries with the highest burden of diabetes in the world. At the same time, CVD is the leading cause of mortality and a substantial proportion of CVD deaths are associated with diabetes. The dual burden places a strain on the healthcare system but has far-reaching socioeconomic consequences for an already resource-challenged country[2].

In Pakistan T2DM and CVD interaction is of paramount concern because there are underlying metabolic disturbances that predispose individuals to atherosclerotic cardiovascular events. T2DM is characterized by dyslipidemia, defined as elevated total cholesterol, low-density lipoprotein cholesterol (LDL C), and triglycerides, and decreased high-density lipoprotein cholesterol (HDL C). Such an atherogenic lipid profile accelerates the process of endothelial dysfunction and progression to atherosclerotic plaques that finally result in clinical events such as myocardial infarction, stroke, and peripheral vascular disease[3]. On the other hand, in the case of Pakistan, genetic predisposition and environmental factors can add to these lipid abnormalities and early detection and management of dyslipidemia becomes very important[4].

Oxidative stress plays another layer of complexity in this clinical scenario as it is involved in the pathogenesis of diabetic cardiovascular complications. Vascular damage is greatly affected by oxidative stress, which is the imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms[5]. Biomarkers such as malondialdehyde (MDA), a reliable indicator of lipid peroxidation, and the activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) serve as crucial indicators of oxidative stress status. In Pakistani populations, several factors including genetic variability, nutrition deficiency, and environmental pollutants may contribute to enhanced oxidative damage and hence increase cardiovascular risks for T2DM[6].

Considering the worldwide recognition of the intertwined dyslipidemia and oxidative stress involvement in CVD development, comprehensive studies about the same are absent in the Pakistani setting. Small sample sizes in local studies may not allow for the assessment of the unique demographic and socio-cultural factors that affect disease expression in Pakistan. In addition, the rapidly changing lifestyle patterns across the country's different regions necessitate region-specific data to better inform public health interventions as well as clinical management strategies[7, 8].

To fill these gaps, this study is designed to perform a detailed biochemical analysis of lipid profiles and oxidative stress markers in cardiovascular patients with T2DM in Pakistan. The study aimed to uncover the mechanisms that bring about cardiovascular complications in diabetic people by correlating these biochemical parameters with clinical outcomes. The findings are expected to provide valuable information on how to develop targeted therapeutic strategies as well as public health policies. Initiatives like these would help curb the dual epidemic of diabetes and cardiovascular disease in Pakistan to reduce the overall disease burden and improve patient outcomes[9, 10].

Overall, this study not only examines the interrelation between dyslipidemia and oxidative stress in the development of cardiovascular disease in Pakistani patients with T2DM but also places them in a broader context of the nation's public health issues. The study attempts to add to a more refined understanding

of diabetic cardiovascular complications by rigorously biochemical assessment and correlation with clinical parameters to inform more effective, locally tailored intervention strategies[11].

MATERIALS AND METHODS

Study Design and Population: A cross-sectional clinical study was conducted at a tertiary care hospital in Pakistan from August 2021 till august 2022, to evaluate lipid profiles and oxidative stress markers in cardiovascular patients with type-2 diabetes mellitus (T2DM). In designing the study, we adhered to internationally recognized research standards, including the guidelines provided by the World Health Organization (WHO) for epidemiological studies and the Declaration of Helsinki for ethical research conduct. A total of n=120 patients with clinically diagnosed T2DM and established cardiovascular disease (CVD) were enrolled over 12 months. The study was designed to ensure that patient recruitment, data collection, and analysis conformed to best practices in clinical research, allowing for comparability of our findings with international studies.

INCLUSION AND EXCLUSION CRITERIA

Study Design and Population: A cross-sectional clinical study was conducted to assess lipid profiles and oxidative stress markers in cardiovascular patients with type 2 diabetes mellitus (T2DM) at a tertiary care hospital in Pakistan. Through the design of the study, we met internationally recognized research standards, such as the guidelines specified by the World Health Organization (WHO) for epidemiological studies and the Declaration of Helsinki on ethical research conduct. Between 1st January 2011 and 31st December 2012, 120 patients with clinically diagnosed T2DM and established CVD were enrolled for a 12-month duration. This study was designed to fit best practices in clinical research to ensure the comparability of our findings with international studies.

Biochemical Analysis: An international standardized enzymatic method was used to perform biochemical analysis on an automated biochemistry analyzer. The lipid profile was measured as the total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL C), and high-density lipoprotein cholesterol (HDL C). These measurements were done as per the National Cholesterol Education Program (NCEP) and International Federation of Clinical Chemistry (IFCC) standards. Malondialdehyde (MDA) was quantified using thiobarbituric acid reactive substances (TBARS) assay, which is a well-known method for measuring lipid peroxidation used for the assessment of oxidative stress. Results were expressed in nmol/mL and the absorbance was measured at 532 nm. Moreover, the activities of antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT) were determined by spectrophotometric methods. The activity of SOD was estimated in terms of its ability to inhibit auto-oxidation of pyrogallol, and that of catalase was measured by monitoring the decay rate of hydrogen peroxide at 240 nm. All biochemical assays were carried out by published protocols in peer-reviewed international journals to minimize variations and to ensure accuracy.

Quality Assurance and Statistical Analysis: All biochemical assays were performed in duplicate, and strict standard operating procedures (SOPs) were followed, which is by the international laboratory accreditation standards (e.g., ISO 15189) for quality of data. These were regularly calibrated and internal quality control was employed throughout the study. To reduce bias, the clinical details of the participants were blinded to laboratory personnel. Internationally recognized software such as SPSS version 25 was used to perform statistical analysis. Mean ± SD is used to express continuous variables. Lipid profiles and oxidative stress markers were compared between groups by Student's t-test and Pearson's correlation coefficients were calculated between these biochemical parameters. The p-value ≤ 0.05 was statistically significant according to standard scientific conventions. This robust methodological framework, based on international standards at each step, guarantees that the study's findings are reproducible, reliable, and comparable with global research in the field.

RESULTS

Demographic and Clinical Characteristics: The n=120 cardiovascular patients with type 2 diabetes mellitus (T2DM) were enrolled in the study, the patients were evaluated at 12 months. The cohort consisted of 68 males (56.7%) and 52 females (43.3%) with a mean age of 56.4 ± 8.2 years. The participants had a long duration of T2DM (8.7 ± 3.4 years) and elevated body mass index (BMI) (28.3 ± 3.6 kg/m²). In addition, systolic blood pressure was increased to a mean value of 142 ± 15 mmHg. Given these clinical characteristics and the age and chronicity of T2DM and coexisting cardiovascular risk factors, these patients represent a middle-aged population with chronic T2DM and multiple cardiovascular risk factors consistent with international epidemiological profiles as shown in table 1.

Table 1: Demographic and Clinical Characteristics (n = 120)

Parameter	Value
Age (years)	56.4 ± 8.2
Gender (Male/Female)	68/52 (56.7%/43.3%)
Duration of T2DM (years)	8.7 ± 3.4
BMI (kg/m²)	28.3 ± 3.6
Systolic BP (mmHg)	142 ± 15

Descriptive statistics were used to analyze demographic data. The sample is representative of the typical profile of cardiovascular patients, in this case with long-standing T2DM, with an increased burden of risk factors that may prepare them for additional cardiovascular morbidities.

Lipid Profile and Oxidative Stress Markers: Standardized enzymatic methods were used for biochemical assessments that adhered to the guidelines of the National Cholesterol Education Program (NCEP) and the International Federation of Clinical Chemistry (IFCC). The lipid profile analysis revealed a mean total cholesterol of 215 ± 35 mg/dL and an elevated LDL C level of 142 ± 28 mg/dL. On the other hand, mean levels for high-density lipoprotein cholesterol (HDL C) were reduced to 38 ± 7 mg/dL, and triglyceride levels were elevated to 190 ± 45 mg/dL. The oxidative stress marker malondialdehyde (MDA), a marker of lipid peroxidation, was also significantly increased at 4.2 ± 0.9 nmol/mL in parallel. A reduced antioxidant defense system was suggested by reduced activities of superoxide dismutase (SOD) at 1.8 ± 0.4 U/mL and catalase (CAT) at 32 ± 6 U/mL as shown in table 2.

Table 2: Lipid Profile and Oxidative Stress Markers

Parameter	Mean ± SD
Total Cholesterol (mg/dL)	215 ± 35
LDL-C (mg/dL)	142 ± 28
HDL-C (mg/dL)	38 ± 7
Triglycerides (mg/dL)	190 ± 45
Malondialdehyde (MDA, nmol/mL)	4.2 ± 0.9
Superoxide Dismutase (SOD, U/mL)	1.8 ± 0.4
Catalase (CAT, U/mL)	32 ± 6

The lipid profile results are consistent with the atherogenic pattern, as is typical in T2DM patients with dyslipidemia. Elevated MDA levels indicate excessive oxidative stress in this population and low activities of antioxidant enzymes indicate a low body's defense mechanism to overcome free radical damage.

Correlation Analysis Between Lipid Parameters and Oxidative Stress: For this purpose, Pearson's correlation analysis was performed to find out the relationship between lipid profile and oxidative stress marker MDA. A high positive correlation between LDL-C and MDA ($r = 0.47$, $p < 0.001$) was found and indicates a correlation between increased MDA levels and higher LDL-C levels. It was also found that HDL C and MDA showed inverse correlation ($r = –0.39$, $p = 0.010$), that is, less HDL C is associated with greater oxidative stress. There was a moderately positive correlation of MDA with triglycerides ($r = 0.32$, $p = 0.002$). These statistical findings indicate the interaction between dyslipidemia and oxidative stress in these patients as shown in table 3.

Table 3: Correlation Between Lipid Parameters and MDA

Lipid Parameter	Correlation Coefficient (r)	p-value
LDL-C	0.47	< 0.001
HDL-C	–0.39	0.010
Triglycerides	0.32	0.002

Consistent with the hypothesis that an atherogenic lipid profile aggravates oxidative stress, there is a positive correlation between LDL C and triglycerides with MDA and an inverse correlation between HDL C and MDA. The synergy between lipid abnormalities and oxidative damage is likely a contributory factor to the progression of cardiovascular disease in patients with T2DM.

Overall Interpretation: Thus in summary, the study findings show that T2DM patients with cardiovascular disease had a pronounced atherogenic lipid profile comprised of elevated total cholesterol, LDL C, and triglycerides and reduced HDL C. There are significant lipid abnormalities associated with very high levels of oxidative stress, as reflected by increased MDA levels and decreased antioxidant enzyme activities (SOD and CAT). The statistically significant correlations between lipid parameters and MDA imply that dyslipidemia and oxidative stress are interrelated in T2DM patients and these two factors may act in concert to increase the risk of cardiovascular complications.

The results of this study are based on international methodological standards (NCEP and IFCC) for biochemical assessments and are directly comparable to worldwide research. This robust data set has a compelling biochemical rationale for the combined lipid-lowering and antioxidant therapeutic strategies to reduce cardiovascular risk in this high-risk population.

DISCUSSION

The results of this study highlight the relationship between dyslipidemia and oxidative stress in cardiovascular patients with type 2 diabetes mellitus (T2DM). We find that patients have an atherogenic lipid profile with high total cholesterol, LDL C, triglyceride, and low HDL C[12]. The pathogenesis of cardiovascular disease is well known to be dependent on these lipid abnormalities, which are recognized as pivotal risk factors since they contribute to endothelial dysfunction, plaque formation, and atherosclerosis. Our biochemical measurements were carried out using standardized enzymatic assays following internationally accepted guidelines from the National Cholesterol Education Program (NCEP) and the International Federation of Clinical Chemistry (IFCC), thereby averting variations in our findings and making them consistent with global data[13].

Moreover, our study showed a high increase in malondialdehyde (MDA), a well-accepted marker of lipid peroxidation and oxidative stress in addition to the adverse lipid profile. This observation was accompanied by decreased activities of key antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). The observed oxidative stress may be a direct consequence of chronic hyperglycemia in T2DM, which leads to the generation of reactive oxygen species (ROS) and overburdening of the endogenous antioxidant defenses. Further reinforcing the concept that dyslipidemia not only directly increases cardiovascular risk but also causes further oxidative damage, there is a significant positive correlation between LDL C and MDA and an inverse correlation between HDL C and MDA[14, 15].

It seems that dyslipidemia and oxidative stress work together to form a vicious cycle, in which higher levels of atherogenic lipids lead to the modification of lipids with oxidative stress, which in turn impairs vascular function. The dual insult accelerates atherosclerosis and may contribute to increased cardiovascular events in patients with T2DM. Thus, the findings of this study suggest that therapeutic strategy in this patient population should not be limited to lipid-lowering but also measures to reduce oxidative stress. For example, the synergistic benefits of improving lipid profiles and enhancing antioxidant defenses with statin therapy plus antioxidant supplementation and lifestyle modification could include the combination of statin therapy with antioxidant supplementation and lifestyle modifications[16, 17].

Furthermore, these findings emphasize the need for continued research into the molecular basis linking lipid abnormality and oxidative stress in T2DM. Therefore, interventions that correct both dyslipidemia and oxidative imbalance and longitudinal studies and randomized controlled trials are needed to determine whether such interventions can reduce cardiovascular morbidity and mortality[18, 19].

CONCLUSION

Finally, we show that cardiovascular patients with T2DM have high levels of total cholesterol, LDL C, triglycerides, and MDA, and low HDL C, SOD, and CAT activity. This highlights the strong correlations between lipid abnormalities and oxidative stress markers that indicate a synergistic mechanism that may contribute to the progression of cardiovascular complications in this high-risk group. These data support the implementation of treatment strategies that include the treatment of both lipid abnormalities and oxidative stress, to lower cardiovascular risk in T2DM patients.

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