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ORIGINAL ARTICLE

A Clinical Evaluation of Lipid Profiles and Oxidative Stress Biomarkers in Hypertensive Individuals with Type-2 Diabetes

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ABSTRACT

Background: Cardiovascular disease risk is increased in the coexistence of hypertension and type-2 diabetes, at least in part, by dyslipidemia and oxidative stress. This study investigates the interrelationship between lipid profiles and oxidative stress biomarkers in hypertensive individuals with type-2 diabetes in Pakistan.

Methods: The study involved n=120 participants, 80 of whom were hypertensive diabetic patients, and 40 matched age and sex, which were performed as a cross-sectional study. Lipid profiles (total cholesterol, LDL, HDL, and triglycerides) were measured in fasting blood samples by enzymatic colorimetric assay. Plasma malondialdehyde (MDA) levels were quantified and the activities of antioxidant enzymes, superoxide dismutase (SOD), and catalase were determined via spectrophotometric methods to assess oxidative stress. The groups were compared using statistical analysis to find correlations between lipid parameters and oxidative stress markers.

Results: The BMI and blood pressure of controls were significantly higher than hypertensive diabetic individuals. Biochemical analyses showed that the study group had markedly increased total cholesterol, LDL cholesterol, triglycerides, and low HDL cholesterol ($p < 0.001$). In addition, the study group had increased MDA levels and decreased SOD and catalase activities ($p < 0.001$), indicating increased oxidative stress and impaired antioxidant defense.

Conclusion: The results support a key role of dyslipidemia and oxidative stress in hypertensive type 2 diabetic subjects and contribute to their apparent increased cardiovascular risk. These results underscore the importance of integrated management strategies aimed at lipid abnormalities and oxidative stress to improve cardiovascular outcomes in a high-risk population.

Keywords: Hypertension, Type-2 Diabetes, Dyslipidemia, Oxidative Stress, Malondialdehyde, Antioxidant Enzymes, Cardiovascular Risk, Pakistan

INTRODUCTION

Pakistan is presently undergoing a vast epidemiological transition where the prevalence of noncommunicable diseases like T2DM and hypertension is increasing. Once the diseases of affluence, these conditions are now common in all strata of society, mainly because of rapid urbanization, sedentary lifestyles, and changing dietary habits[1]. In Pakistan, the coexistence of hypertension with T2DM is now emerging as a major public health challenge as it is responsible for a large proportion of the burden of cardiovascular disease (the leading cause of mortality in the country)[2].

There is a unique genetic and environmental predisposition of the Pakistani population for the manifestation and progression of metabolic disorders. Individuals with T2DM and hypertension also have a high risk of dyslipidemia and oxidative stress as a result of factors such as high carbohydrate intake, ubiquitous use of trans fat, and limited access to preventive health care services[3]. A common metabolic abnormality seen in diabetic patients is dyslipidemia (elevated total cholesterol, low-density lipoprotein (LDL), and triglycerides with low high-density lipoprotein (HDL)). These lipid abnormalities are coupled with hypertension and increase the risk of atherosclerotic cardiovascular complications even more[4].

Vascular damage and atherogenesis are mediated by an imbalance of reactive oxygen species (ROS) production and the antioxidant defense of the body, termed oxidative stress. Dyslipidemia and oxidative stress are a topic of greater clinical significance in the context of Pakistan, where lifestyle modifications and early disease detection are suboptimal[5]. Oxidative stress markers, like malondialdehyde (MDA), and decreased activities of endogenous antioxidant enzymes have been shown to mediate the progression of endothelial dysfunction and subsequent cardiovascular events[6].

Despite the recognized effect of these metabolic derangements, there is a dearth of systematic local studies exploring the interrelationship between lipid profiles and oxidative stress biomarkers among hypertensive individuals with T2DM in Pakistan. It is essential to study region-specific risk factors and potential therapeutic targets given the heterogeneity of the Pakistani population with its different dietary practices, environmental exposures, and genetic backgrounds[7]. It is important to understand these associations for the refinement of diagnostic strategies as well as for the formulation of targeted interventions to mitigate the cardiovascular risks associated with both a 'dual burden of disease'[8].

Thus, this study aimed to assess the lipid profiles and oxidative stress biomarkers in hypertensive subjects with T2DM in the Pakistani context. Doing so allows it to offer a critical look into the metabolic perturbations that underlie cardiovascular disease in this high-risk group[9]. The findings are expected to contribute to a better understanding of the pathophysiological mechanisms at play and to inform the development of comprehensive management protocols tailored to the specific needs of the Pakistani population[10].

MATERIALS AND METHODS

Study Design and Setting: It was a cross-sectional study conducted from May 2021 to May 2022 in a tertiary care hospital in Pakistan. The study was conducted by the ethical standards of the Declaration of Helsinki according to the provisions of the Institutional Ethical Review Committee. Adherence to ethical standards throughout the study was ensured by the fact that all participants gave written informed consent before enrolment.

Study Population and Recruitment: Two groups of n=120 participants were enrolled. Eighty hypertensive individuals with type-2 diabetes mellitus (T2DM) diagnosed according to the American Diabetes Association (ADA) criteria were the study group (n = 80). The control group (n = 40) consisted of age and sex-matched healthy volunteers with no history of hypertension, diabetes, or cardiovascular disease. The participants were rigorously screened and excluded if they had acute infections, chronic inflammatory conditions, renal or hepatic dysfunction, malignancies, or had used antioxidant supplements in the three months prior.

Clinical Evaluation and Data Collection: A detailed medical history collection and a thorough physical examination were done on all enrolled participants. Height and weight, as well as body mass index (BMI), were obtained by a stadiometer and a calibrated

electronic scale respectively, and the BMI was calculated. To ensure that blood pressure readings were standardized and reproducible, blood pressure was measured using a calibrated, standardized sphygmomanometer under controlled conditions.

Blood Sample Collection and Processing: Each participant fasted venous blood samples (approximately 10 mL) were collected after an overnight fast of at least 10 hours. Immediately, the blood samples were processed, and serum was separated by centrifugation at 3000 rpm for 10 minutes, aliquoted, and then stored at -80°C until biochemical analyses were performed. In this manner, sensitive biomarkers were degraded as minimally as possible.

BIOCHEMICAL ASSAYS

Lipid Profile Analysis: Standard enzymatic colorimetric assays were used to determine serum lipid profiles. Using validated commercial kits, total cholesterol and high-density lipoprotein (HDL) cholesterol levels were measured and the triglyceride levels were measured using a colorimetric method. If applicable, the Friedewald equation was used to calculate low-density lipoprotein (LDL) cholesterol. Strict quality control protocols were followed and all measurements were done on an automated biochemical analyzer.

Assessment of Oxidative Stress Biomarkers: Plasma levels of malondialdehyde (MDA), a key marker of lipid peroxidation, were evaluated by measuring the levels of thiobarbituric acid reactive substances (TBARS). In this method, plasma samples were reacted with thiobarbituric acid under acidic conditions at 95°C, and the formed chromogen was quantified spectrophotometrically at 532 nm. Additionally, superoxide dismutase (SOD) and catalase activity was measured using spectrophotometric methods. The pyrogallol autoxidation assay was based on the inhibition of this reaction, and the catalase activity was measured by the decomposition rate of hydrogen peroxide at 240 nm.

Quality Control Procedures: All the biochemical assays were performed in duplicate to ensure the reliability and reproducibility of measurements. Concurrently with patient samples, calibration curves, and quality control samples were run with all laboratory equipment being calibrated regularly according to the manufacturer's specifications. To minimize variability, as well as to improve the accuracy of the data, these measures were put in place.

Statistical Analysis: The data were analyzed using SPSS software (version XX; IBM Corp, Armonk, NY, USA). Mean ± standard deviation (SD) was used to express continuous variables, and frequencies and percentages to present the categorical variables. The Shapiro-Wilk test was used to test if the data was normal. The independent samples t-test was used for normally distributed variables, whereas Mann Whitney U test was used for non normally distributed data. The relationships between lipid parameters and oxidative stress biomarkers were evaluated by calculating Pearson's correlation coefficient. Moreover, multivariate regression analysis was performed to account for confounding variables such as age, sex, and BMI. Statistically significant was considered a two-sided p-value less than 0.05.

Ethical Considerations: The study was performed ethically. This protocol was approved by the Institutional Ethical Review Committee and all participants gave written informed consent. Anonymization of participant data and storage of all records in a secure manner ensured confidentiality. All procedures conformed to the ethical standards of the institution and the general principles of the Declaration of Helsinki.

RESULTS

Demographic and Clinical Characteristics: The study group consisted of 80 hypertensive individuals with type 2 diabetes and the control group of 40 healthy controls; a total of 120 participants were enrolled in the study. Age and sex distribution of both groups were similar, denoting proper matching. But, as predicted, the study group had much higher body mass index (BMI) and blood pressure readings (systolic and diastolic) than the control group. These differences exemplify the current clinical profile of hypertensive diabetic patients and underscore the greater cardiovascular risk associated with them. The detailed demographic and clinical data are summarized in Table 1 below.

Table 1: Demographic and clinical characteristics of the study and control groups.

Parameter	Control Group (n = 40)	Study Group (n = 80)	p-value
Age (years)	50.3 ± 5.1	52.1 ± 6.2	0.08
Sex (M/F)	22/18	44/36	0.95
Body Mass Index (kg/m²)	24.5 ± 2.1	27.1 ± 3.0	< 0.001
Systolic Blood Pressure (mmHg)	120.5 ± 7.8	140.3 ± 9.5	< 0.001
Diastolic Blood Pressure (mmHg)	80.2 ± 5.3	90.4 ± 8.1	< 0.001

Biochemical Parameters: The biochemical evaluation showed a significant difference in lipid profile and oxidative stress biomarkers between the study and control groups. The dyslipidemia profile of the hypertensive diabetic group was markedly high in total cholesterol, LDL cholesterol, and triglycerides and low in HDL cholesterol. Furthermore, the study group showed significant changes in lipid abnormalities as well as in markers of oxidative stress. Plasma levels of malondialdehyde (MDA) were increased, a marker of lipid peroxidation, and SOD and catalase activities were markedly decreased. These biochemical findings help understand why hypertensive type-2 diabetics may be at increased risk for cardiovascular disease. Table 2 below presents the detailed biochemical parameters.

In summary, the data reflect that hypertensive subjects with type 2 diabetes have marked changes in clinical and biochemical parameters. Well-documented risk factors for cardiovascular disease, the study group also had higher BMI and blood pressure readings. Biochemically, this group was dyslipidemia, i.e., they had increased total cholesterol, LDL cholesterol, and triglycerides and decreased HDL cholesterol. At the same time, the levels of MDA and the activities of SOD and catalase were found to be elevated, indicating that these individuals are subjected to higher oxidative stress. The results here give a picture of the metabolic disturbances that probably contribute to the elevated cardiovascular risk of hypertensive diabetic patients.

Table 2: Comparison of lipid profile and oxidative stress biomarkers between control and study groups.

Biochemical Parameter	Control Group	Study Group	p-value
Lipid Profile			
Total Cholesterol (mg/dL)	180.4 ± 19.2	220.7 ± 28.5	< 0.001
LDL Cholesterol (mg/dL)	110.5 ± 14.6	150.2 ± 24.8	< 0.001
HDL Cholesterol (mg/dL)	50.2 ± 8.0	40.3 ± 6.5	< 0.001
Triglycerides (mg/dL)	150.3 ± 20.7	200.5 ± 35.6	< 0.001
Oxidative Stress Markers			
Malondialdehyde (MDA, nmol/mL)	2.5 ± 0.5	4.0 ± 0.7	< 0.001
Superoxide Dismutase (SOD, U/mL)	1200 ± 100	900 ± 90	< 0.001
Catalase (U/mL)	3000 ± 250	2500 ± 300	< 0.001

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DISCUSSION

This study has found that hypertensive individuals with type 2 diabetes have a significant interplay of dyslipidemia and oxidative stress and the findings are particularly pertinent in the Pakistani context[11]. The study group exhibits elevated total cholesterol, LDL cholesterol, and triglycerides with reduced HDL cholesterol levels, which are in keeping with the dyslipidemia profile that is frequently reported in diabetic patients with hypertension. It is known that these lipid abnormalities are associated with the development of atherosclerotic plaques and increased risk of cardiovascular events[12].

Additionally, the increased lipid peroxidation evident in the study group is reflected by the significantly higher malondialdehyde (MDA) levels in the study group. Oxidative stress is a pivotal player in the pathogenesis of vascular damage by promoting endothelial dysfunction and inflammation which are precursors to develop cardiovascular complications[13]. In addition, the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase are also reduced which further compounds the oxidative stress burden and further decreases the body's capacity to neutralize reactive oxygen species (ROS) [14].

Given this, these findings have great clinical significance in the Pakistani setting where urbanization and lifestyle changes have increased the prevalence of noncommunicable diseases. Local dietary patterns, genetic predispositions, and a lack of preventive healthcare services may all play some role in the observed biochemical alterations, and together the burden of cardiovascular disease is increasing[15]. Our study emphasizes the requirement of integrated management that extends beyond blood pressure and glycemic control to include lipid management and oxidative stress reduction to lower cardiovascular risk[16].

While the cross-sectional design does provide a snapshot of the current metabolic state, the causal inference cannot be inferred. Furthermore, the sample size is large enough for preliminary analysis, but the results need to be confirmed by larger studies to confirm these findings and study underlying mechanisms in more detail[17]. Future research should also include longitudinal studies to evaluate the effect of interventions, including lifestyle modifications and antioxidant therapies, on lipid profiles, oxidative stress biomarkers, and overall cardiovascular outcomes[18, 19].

CONCLUSION

Our study shows that hypertensive persons with type 2 diabetes have significant dyslipidemia and increased oxidative stress that contribute to cardiovascular risk. The lipoprotein changes, elevated MDA, and reduced antioxidant enzyme activities indicate a complex metabolically disturbed state in this population at high risk. These findings suggest a need for a holistic and multi-target approach for managing hypertensive diabetic patients in settings such as Pakistan where the disease burden of noncommunicable diseases is increasing. Further interventional studies are needed to determine if such metabolic derangements can be reversed and determine if such metabolic derangements are related to reduced cardiovascular morbidity and mortality in this highly vulnerable population.

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