Original Research Article

Deranged Haematological Profile and Dyslipidaemia in Diabetes Induced Nephropathy

Abstract

Background: Diabetic nephropathy (DN) is a serious complication of diabetes mellitus, and its prevalence has been increasing in the developed countries. It may be characterized by dyslipidaemia and deranged haematological indices that play a role in the development of late diabetes complications. Therefore, there is an urgent need to develop strategies to diagnose the patients of diabetic nephropathy during its acute phase.

Objective: The aim of this study was to determine haematological indices and lipid profile in diabetic nephropathy in comparison with diabetic controls

Methodology: The present study was conducted on 150 DN patients with 150 control age group of 30-50 years to determine the haematological profile. For the analysis of lipids, fats and fatty substances lipid panel was performed in both groups.

Results: Current data indicated that the level of Haemoglobin A1c, Prothrombin time, White blood cells, platelet count, Low density lipoproteins and triglyceride is raised while a significant reduction in Red blood cells, and high density lipoproteins levels were noted in patients with diabetic nephropathy as compared to the diabetic individuals.

Conclusion: The study suggests that both the haematological and lipid profile are highly disturbed in DN patients that may serve as a hallmark for early diagnosis of nephropathy in diabetic patients.

Keywords: *Diabetic nephropathy (DN), End-stage renal disease (ESRD), Diabetes mellitus (DM), LDL, HDL.*

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INTRODUCTION

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Diabetes mellitus has increasing prevalence worldwide with certain ethnic and racial groups of Asia and Africa at a greater risk [1]. The number of people suffering from diabetes has been increasing due to aging population, urbanization, and low physical activity. Poorly controlled diabetes leads to various complications such as nephropathy, retinopathy, neuropathy and

oxidative stress causing oxidative damage to tissues and cells [2, 3]. The overall temporal burden of hyperglycaemia is responsible for DM complications and adverse outcomes [4].

Diabetic nephropathy (DN) is considered as one of the main micro vascular complication of diabetes mellitus (DM) and has become the most general single cause of end-stage renal disease (ESRD) worldwide [5], traditionally defined as progressive rise in urine albumin excretion together with increasing blood pressure, leading to declined glomerular filtration, and eventually ESRD. Disease is also characterized by renal morphological and functional alterations such as glomerular hyper filtration, glomerular and renal hypertrophy, increased urinary albumin excretion, increased basement membrane thickness, and meningeal expansion with the accumulation of extracellular matrix proteins such as collagens, fibronectin and laminin [6-8]. Diabetic nephropathy results in profound deregulation of several key enzymes and metabolic pathways; this eventually contributes to disordered high-density lipoprotein (HDL), cholesterol and triglyceride-rich lipoprotein levels.[9] High total cholesterol and non-HDL-cholesterol levels, and low HDL-cholesterol levels are significantly associated with an increased risk of developing renal dysfunction in healthy people. Carbohydrate metabolism directly affects lipid profile in diabetes [10,11] Also insulin deficiency may influence the level of free fatty acid and can cause changes in lipid metabolism. Abnormalities in lipid profile have also been seen to play an important role in the increased vascular risk associated with type-2 DM.[10,11]

The main objective of this study was to evaluate haematological parameters and lipid profile in patients with diabetic nephropathy. It is believed this study will uplift awareness for the need of both haematological and lipid analysis in patients with diabetic nephropathy so the necessary steps can be taken to optimize their management at the earliest to prevent advanced complications.

METHODOLOGY

Study Design, period and area

The study was a case control (retrospective) study which was conducted between January to December 2018. The present research work was conducted at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

Study participants:

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Total numbers of patients were three hundred (300) out of them one hundred and fifty (150) patients of diabetic nephropathy with the age group of 30-50 years and One hundred and fifty age-matched diabetic controls without nephropathy (150) included in the current study. All the patients suffering from diabetic nephropathy with the confirmed clinical reports were recruited. The patients with the history of smoking, metabolic dysfunction, malnutrition, on statins for abnormal lipid treatment, on anticoagulant therapy and who had chronic diseases were excluded from the study.

Sampling Technique:

Convenient non-probability sampling technique was employed to select the study participants.

Ethical consideration:

Ethical clearance was obtained from the "Research and Ethics Committee" at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan. An informed consent from every patient was taken before the start of research work.

Data collection and Laboratory methods:

Five ml of venous blood was drawn from patients of diabetic nephropathy and control individuals. Then the sample was centrifuged within two hours at 3000 rpm. After the centrifugation, the serum was separated and stored at -70ºC for the examination. The sample was transferred in the laboratory for further processing.

Haematology profile:

Complete blood count comprising red blood cell count, white blood cell count and differentials, and platelets were determined from the remaining whole blood that was placed in EDTA test tubes using ABX Micros 60 Haematology Analyser (Horiba-ABX, Montpellier, France).

Estimation of HBA1C is done by high performance liquid chromatography. HBA1C values are expressed as percentage i.e <7% is considered normal. Prothrombin time was estimated by Quick's method.

The biochemical measurements made were triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels in blood by spectrophotometric method and enzymatic method.

STATISTICAL ANALYSIS:

Data was analysed using Statistical Package for Social Sciences (SPSS, Version 17.0). Normally distributed data was analysed using T-test. A p-value of < 0.05 was considered statistically significant.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY GROUP:

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A total of 300 (150 diabetic control, 150 diabetic nephropathy) participants were included in this study. The mean age of DN patients was 45.61±17.89yrs and mean age of healthy group was 42.85±5.15yrs. The mean weight and BMI of nephropathy patients group were 51.61±11.50kg and 25.4±3.2kg/m^2 respectively, as compared to normal subjects' 41.22±2.25 kg and 23.2±3.2kg/m^2, respectively.

The mean systolic and diastolic blood pressure in patients suffering from diabetic nephropathy was recorded to be 130.33±6.75mmHg and 80.75±2.27mmHg , respectively, while in the control group mean systolic and diastolic BP values of 121.91±2.24mmHg and 78.56±1.19mmHg, respectively, were observed. (Fig 1)

HEAMOTOOGICAL PROFILE:

The mean value of Red Blood Cells (RBCs) in the control group was 6.58±2.88µg/dl and in the DN group was 4.59±1.45 µg/dl. The mean White Blood Cells (WBCs) were 6.59±1.59 (×10^9/L) and 8.59± 2.48 (×10^9/L) in the control group and DN group, respectively. There was a significant raise in WBCs in the study subjects. Platelets were also found to be increased 255.7±82.0 x 10^3/ul in the DN group as compared to the control group 246.3±67.4 x 10^3/ul. A poor glycemic control was observed in the DN group indicated by HbA1C value of 9.55±3.01 as compared to the controls7.59±1.77. The mean prothrombin time assessed in DN patients was increased (16 seconds) as compared to the control group (13 seconds) (Fig 2)

LIPID PROFILE:

The mean values of total cholesterol and triglycerides were significantly increased in the patients with diabetic nephropathy (5.2±1.2mmol/L and 2.58±1.43mmol/L) as compared to healthy individuals (4.92±0.97mmol/L and 2.27±1.0 mmol/L), respectively. An elevated trend of LDL

was found in diseased group (3.2±1.2mmol/L) in comparison with control group (1.1±5.5mmol/L). However, the mean value of HDL was significantly reduced in the patients with diabetic nephropathy (1.8±0.7 mmol/L) as compared to healthy control (3.5±1.2 mmol/L) (Fig 3).

DISCUSSION

Diabetic nephropathy is a condition characterized by the occurrence of uncontrolled secretion of urine albumin, loss of glomerular filtration rate and glomerular lesions. Different epidemiological studies determine that family history ethnicity, abnormal haematological profile, gestational diabetes, elevated blood pressure, dyslipidaemia and obesity are the major risk factors of diabetic nephropathy.[15] Other putative risk factors include external environmental pressure such as smoking and inhalation of other toxins, elevated glycosylated haemoglobin level (HbA1c), proteinuria and elevated systolic pressure.[16] Similar trend could be seen in the present study in which the levels of HbA1c, PLTs and WBCs were increased among the patients. The literature suggests that in case of early detection of Diabetes among the patients it is necessary to estimate the haematological profile and detect the changes in comparison to the controls. In the present study, WBCs, Platelets and HbA1c were significantly higher in the study DN group which is in line with many other reported studies. Literature signifies that the raised levels of the haematological variables is the reason for inflammation, thrombosis, and coagulation among patients. A study by Adane et al., in 2021 indicated that the White blood cells and platelets were higher in patients of Diabetes as compared to the controls.[17] Another similar study conducted in 2015 by Biadgo et al., indicated an increasing trend in both the white blood cells and platelets among the diabetics.[18] Systematic review and meta-analysis of cross-sectional and prospective studies have shown that the number of peripheral WBCs such as basophils, eosinophils and neutrophils increased with no change in the number of monocytes in patients with diabetic nephropathy.[19] Furthermore, a study suggested that high platelet activity enhances vascular complications in DM patients and altered platelet morphology and function can be reflected as a factor for risk of platelet reactivation in patients with diabetes. [20] The results of these studies coincide with the present study results. Another study observed that

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diabetics are prone to anemia due to reduced kidney function and decreased production of erythropoietin hormone, which ultimately leads to decreased red blood cell count.

Hernandes et al., pointed out in 2013 that an increase in HbA1C of one percent was associated with a 30% to 40% increase in chronic kidney disease which supports the present study's raised percentage of HbA1C.[21] A study in 2015 in CKD cohort indicated that HbA1C prediabetes values are associated with higher mortality rates among the patients.[22] Another study by Lee et al., in 2020 suggested that variation in HbA1C levels is considered as an independent risk factor for a decline in renal function in patients with type 2 diabetes.[23] These studies, results are in accordance with the present study.

It is known that many factors affect lipid levels in diabetes because carbohydrate metabolism directly affect lipid metabolism.[24,25] There have been a number of observational studies showing that lipid abnormalities are associated with a reduction in kidney function in the general population. It is uncertain if it is the lipid abnormalities that cause the reduction in kidney function, or if impaired renal function or proteinuria itself cause both the lipid abnormalities and reduction in renal function. The current study indicated that the level of Low density lipoproteins (LDL) and triglycerides (TG) were raised and the levels of High density lipoproteins (HDL) were decreased in patients with diabetic nephropathy. In a study by Srinidhi Rai et al., it was pointed out that the values of TG and LDL were significantly higher in type 2 diabetes with nephropathy as compared to the controls.[26] A study measured plasma TG and HDL levels as predictors of development of diabetic kidney disease in 2016. It was seen that diabetic Dyslipidemia with high TG and low HDL levels is an important risk factor for the development of diabetic kidney disease over a period of 4 years.[27] Another study by Kachawa et al., emphasised that Dyslipidemia is a leading cause of end stage renal disease in DN and lipids represent a useful clinical tool in assessing the advancement of renal disease in DN.[28] All these studies strengthen the results of the present study in terms of lipid derangements seen in DN patients as compared to the controls.

CONCLUSION

Current study observed severe derangements in haematological parameters and lipid metabolism in patients with diabetic nephropathy. Our study showed that the levels of White blood cells, Platelets and HbA1C were significantly raised in Diabetic nephropathy as compared to the healthy controls. This is an important risk factor for various cardio and cerebrovascular disease which can be detected with less expensive and simple blood screening in diabetic patients. Routine screening and treatment of these abnormal indices is recommended to minimize DM-related complications. Also our study indicated severe dyslipidemia associated with DN patients. Increased LDL, triglyceride and reduced HDL levels also indicate increased risk for atherosclerotic diseases in diabetics. So, Lipid profile may represent a useful clinical tool in not only identifying patients at a higher risk of kidney disease but also an assessment of development and progression of renal disease. The limitation of this study is it does not evaluate a cause-effect relationship between variables and diabetic nephropathy patients.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES:

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1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;94(3):311–21.

2. Comazzi S, Spagnolo V, Bonfanti U. Erythrocyte changes in canine diabetes mellitus: in vitro effects of hyperglycaemia and ketoacidosis. J Comp Clin Path. 2004;12(4):199–205.

3. Elalamy I, Chakroun T, Gerotziafas GT, Petropoulou A, Robert F, Karroum A, et al. Circulating platelet-leukocyte aggregates: a marker of microvascular injury in diabetic patients. Thromb Res. 2008;121(6):843–8.

4. Roberto T, Dodesini AR, Lepore G. Lipid and renal disease. J Am Soc Nephrol. 2006;17:S145–7.

5.Moradi M, Rahimi Z, Amiri S. AT1R A1166C variants in patients with type 2 diabetes mellitus and diabetic nephropathy. J Nephropathol 2015;4:69-76.

6.Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: worldwide difference of prevalence and risk factors. J Nephropharmacol 2016;5:49-56.

7.Akbari F, Shahinfard N, Mirhoseini M, Shirzad H, Heidarian E, Hajian S, Rafieian-Kopaei M. Impacts of Hibiscus esculentus extract on glucose and lipid profile of diabetic rats. J Nephropharmacol 2016;5:80-85.

8. Adeshara KA, Diwan AG, Tupe RS. Diabetes and Complications: Cellular Signaling Pathways, Current Understanding and Targeted Therapies. Curr Drug Targets 2016;17:1309

9. Vaziri ND, Norris K. Lipid disorders and their relevance to outcomes in chronic kidney disease. Blood Purif 2011;31:189-96.

10. Schaeffner ES, Kurth T, Curhan GC, Glynn RJ, Rexrode KM, Baigent C, et al. Cholesterol and the risk of renal dysfunction in apparently healthy men. J Am Soc Nephrol 2003;14:2084-91.

11. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599-622.

12.Thomas MC. Epigenetic Mechanisms in Diabetic Kidney Disease. Curr Diab Rep 2016;16:31.

13.Sutaria A, Liu L, Ahmed Z.Multiple medication and chronic kidney disease in patients aged 60 and older: a pharmacoepidemiologic perspective. Ther Adv Cardiovasc Dis. 2016.

14.Arévalo-Lorido JC, Carretero-Gómez J, García-Sánchez F. Secondary hyperparathyroidism prevalence and profile, between diabetic and non-diabetic patients with stage 3 to 4 chronic kidney disease attended in internal medicine wards. MiPTH study. Diabetes Metab Syndr 2016.

15.Nazar CMJ, Bojerenu MM. Diabetes education. J Renal Endocrinol 2016;2:02.

16.Sun L, Dutta RK, Xie P, et al. Myoinositol Oxygenase Over-Expression Accentuates Generation of Reactive Oxygen Species and Exacerbates Cellular Injury Following High Glucose Ambience: A New Mechanism Relevant to the Pathogenesis of Diabetic Nephropathy. J Biol Chem 2016.

17. Comazzi S, Spagnolo V, Bonfanti U. Erythrocyte changes in canine diabetes mellitus: in vitro effects of hyperglycaemia and ketoacidosis. J Comp Clin Path 2004;12(4):199-205.

18. Mullugeta Y, Chawla R, Kebede T, Worku Y. Dyslipidemia associated with poor glycemic control in type 2 diabetes mellitus and the protective effect of metformin supplementation. Ind J ClinBiochem 2012;27(4):363-9.

19. Gundogan K, Bayram F, Capak M, Tanriverdi F, Karaman A, Ozturk A. Prevalence of metabolic syndrome in the Mediterranean region of Turkey: evaluation of hypertension, diabetes mellitus, obesity, and dyslipidemia. Metab Syndr Relat Disord 2009;7:427-34.

20. Barbieri M, Ragno E, Benvenuti E, Zito GA, Corsi A, Ferrucci L, et al. New aspects of the insulin resistance syndrome: impact in haematological parameters. Diabetologia 2001;44:1232-7.

21. Hernandes et al Lipid profile. 2013. http://www.diabetes management.com. Accessed 23 Feb

22.Anand, G., Vasanthakumar, R., Mohan, V., Babu, S., & Aravindhan, V. (2014). Increased IL-12 and decreased IL-33 serum levels are associated with increased Th1 and suppressed Th2 cytokine profile in patients with diabetic nephropathy (CURES-134). *International journal of clinical and experimental pathology*, *7*(11), 8008.

23. Lee et al 2020 Novel Model Predicts Diabetic Nephropathy in Type 2 Diabetes Am J Nephrol 2020;51:130–138 https://doi.org/10.1159/000505145

24.Taal MW, GM Chertow, PA Marsden, AS Yu and BM Brenner (2012). Risk factors and chronic kidney disease. Brenner & Rector's The Kidney. 9th ed. Philadelphia: Elsevier Inc758-781.

25.Demirtas L, H Degirmenci, EM Akbas, A Ozcicek, A Timuroglu, A Gurel and F Ozcicek (2015). Association of hematological indicies with diabetes, impaired glucose regulation and microvascular complications of diabetes. International journal of clinical and experimental medicine 8(7):11420.

26. Srinidhi Rai et al Abnormal lipid profile in International Journal of Clinical Biochemistry and Research, October-December 2017;4(4):379-382 381

27. LaPierre D. Dyslipidaemia. 2011. Htttp: //www. Sharinginhealth.Ca/ conditions_and_diseases/dyslipidemia.Html Accessed 23 Apr 2017.

Zhao Y, Zhang J, Zhang J, Wu J. Diabetes mellitus is associated with shortened activated partial thromboplastin time and increased fibrinogen values. PLoS One 2011; 6(1):16470.

28. Kachhawa K, Varma M, Kachhawa P, Agrawal D, Shaikh M, Kumar S. Study of dyslipidemia and antioxidant status in chronic kidney disease patients at a hospital in South East Asia. J Health Res Rev 2016;3:28-30.

Figure legend

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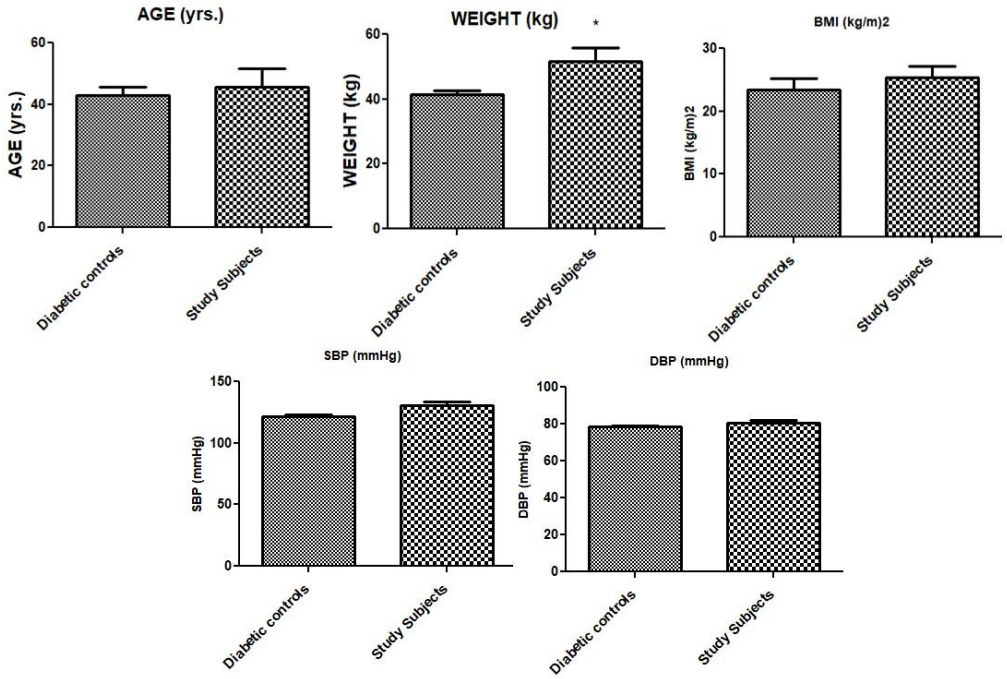

Figure 1: DEMOGRAPHIC AND CLINICAL VARIABLES IN DIABETIC NEPHROPATHY

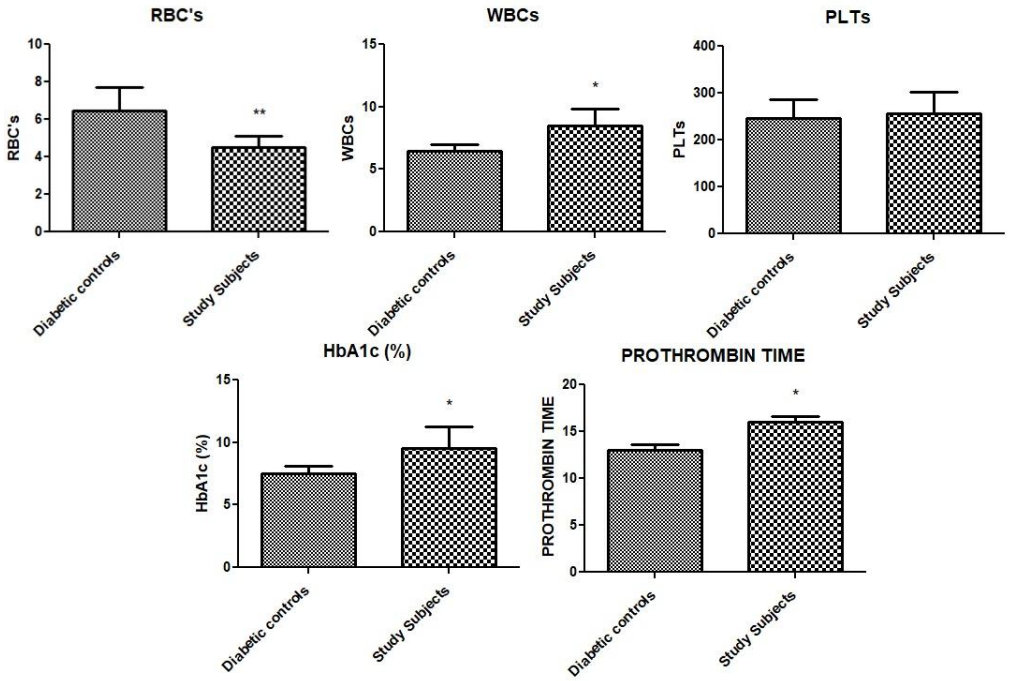

Figure 2: COMPARISON OF HEAMATOLOGICAL INDICIES OF THE STUDY PARTICIPANTS

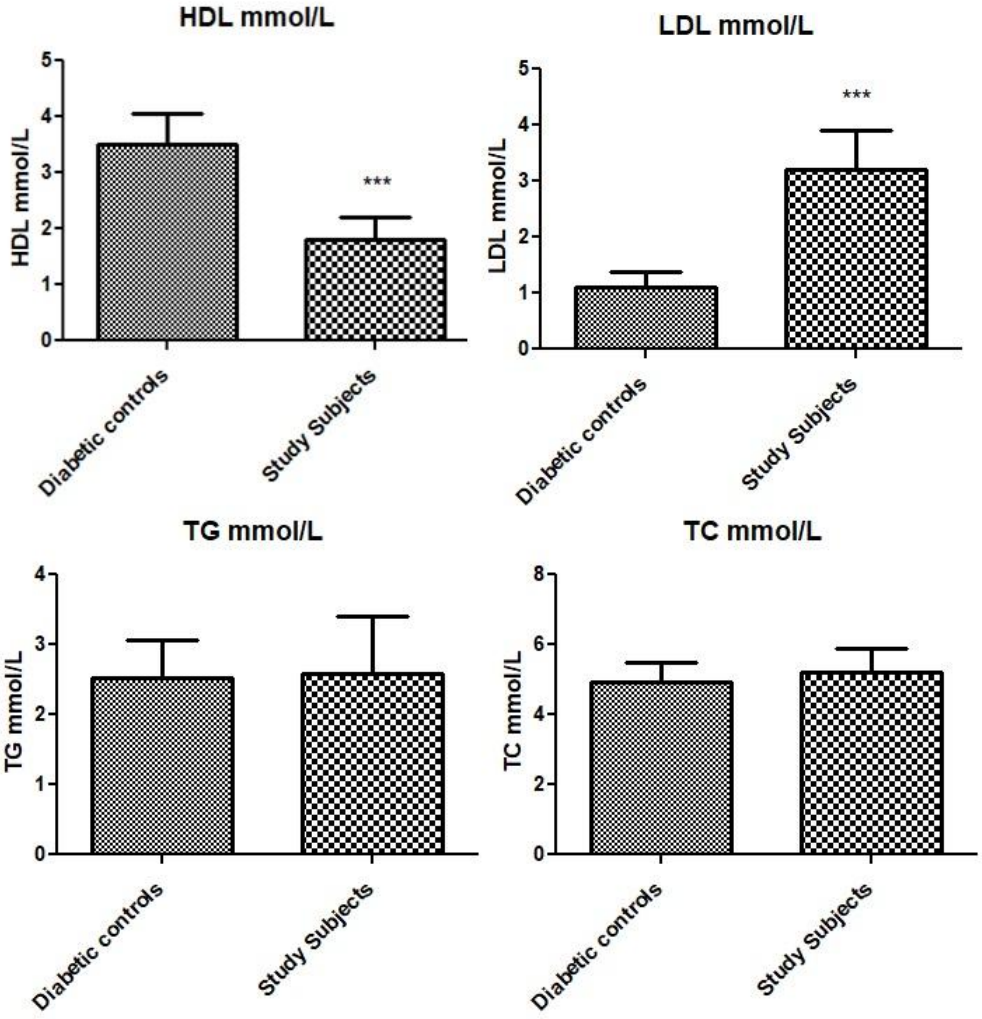

Figure 3: LIPID PROFILE OF DIABETIC CONTROL AND PATIENTS OF DIABETIC NEPHROPATHY