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Original Article

Correlation of lipid profile with C3d and Carotid Intima-Media thickness in Type 2 Diabetic patients.

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Abstract

Background: Complement activation occurs in the vessel's wall as serum lipids start to get deposited, marking the beginning of atherosclerosis formation. Carotid intima-media thickness (IMT) is a widely used tool for detecting atherosclerosis. This study objective was to correlate lipid profile with C3d levels and Carotid IMT in type 2 Diabetic patients.

Methodology: This case-control study comprised 120 male and female participants between 35 to 65 years of age. Of them, 90 type 2 diabetes patients were stratified according to the diabetes duration into four groups, Group A (n=30) <5 years of diabetic, Group B (n=30) 5-10 years of diabetic, Group C (n=30) >10 years diabetic and non-diabetic subjects healthy controls in Group D (n=30). The Anthropometric measurements (height, weight, and BMI), serum C3d level, carotid intima-media thickness (carotid IMT) &, serum levels of Cholesterol, Triglycerides, Low-density lipoprotein (LDL), and High-density lipoprotein (HDL) were evaluated.

Results: In Type 2 Diabetic patients with moderate to high levels of serum Cholesterol, the C3d levels were found to be significantly raised (10.58 ±3.87 µg/ml and 15.37 ± 2.51 µg/ml, respectively), but Carotid IMT was increased in patients with high Cholesterol (0.98 ± 0.11 mm). Patients with moderate to high levels of Triglycerides had C3d 11.03 ± 3.85 µg/ml and 13.66 ± 4.30 µg/ml, respectively, but Carotid IMT was observed to be raised in patients with higher triglycerides levels (1.00 ± 0.11 mm). Also, in patients with moderate to high serum LDL C3d were 10.26 ± 3.10 µg/ml and 13.14 ± 4.08 µg/ml, respectively, whereas Carotid IMT was increased in patients with high levels of LDL (0.93 ± 0.13 mm). The C3d (12.12 ± 4.42 µg/ml) and Carotid IMT (0.90 ± 0.13 mm) levels were increased as HDL decreased significantly.

Conclusion: Serum C3d can be used as an early marker of atherosclerosis formation, which increases as lipid levels exceed the normal range in diabetic type 2 patients compared to carotid IMT detected in patients with higher lipid parameters.

Keywords

C3d, Carotid Intima-Media Thickness, Type 2 Diabetes, Atherosclerosis.

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Introduction

Diabetes mellitus is a metabolic disorder, the hallmark of which is the presence of high levels of glucose in the blood[1]. Based on etiology, diabetes is broadly grouped as diabetes type 1, and diabetes type 2, the latter type of diabetes occurs due to resistance of tissues to the insulin[1]. On average, 462 million people were diagnosed as diabetics in 2019, according to an international diabetic federation. Out of every 10 individuals, 9 were diagnosed as Type 2 diabetics, with Asians being more preponderant to Type 2 Diabetes than individuals from other ethnic background[2]. Pakistan is ranked the fourth major country in Asia with the highest number of diabetic cases[2].

The number of cases with diabetes-related complications is on the rise with each passing day, which is likely to escalate further, posing a substantial risk to the health care system. Coronary artery atherosclerosis being one of the most dangerous complications occurring in Diabetic Type 2 patients[3]. Coronary atherosclerosis patients without Type 2 Diabetes have significantly reduced rates of cardiovascular events in contrast to the patients with Type 2 Diabetes[4]. The intimal thickness of carotid arteries which signifies the process of atherosclerosis formation, is widely used as an indicator for future stroke and myocardial infarction[5].

Atherosclerosis is the disorder of enhanced inflammation in the intimal layer of arteries that occurs in the vessel wall over time, persisting for the whole life once it has been formed. The evidence suggests abnormally high complement system activation, the part of innate immunity[6]. The complement protein C3 (C3) is activated by all the pathways involved in the complement proteins activation, namely classic, lectin, and alternate, which leads to the generation of common C3 convertase with C3d signifying the product of terminal degradation of complement protein C3[7]. Since 1950 activation of the complement system has been observed in atherosclerotic lesions because Cholesterol in vascular walls gives rise to the activation of complement proteins[7]. C3d has been found in increasing concentration in patients with type 2 diabetics[8]. The alternate pathway of the complement system activates C3 as the low-density lipoprotein (LDL) starts being deposited in the vessel's intimal layer with the production of terminal C3d[9]. The role of complement activation is to remove the excess LDL deposited in the wall. However, over time, it becomes detrimental to the vessel by recruiting immune cells and activating the adaptive immunity[9]. Many previous studies on both animals and humans have indicated the direct association of complement activation in atherosclerosis[9]. Enhanced levels of C3d have been reported in patients with coronary artery atherosclerosis[7].

The detection of atherosclerosis formation before time is critical in order to save the patient's life from debilitating heart attack and stroke affecting significant vessels of the heart and brain due to which C3d has been extensively studied as it is closely associated with atherosclerosis with raised C3d levels documented in the serum and locally in advanced atherosclerotic lesions[7]. Although Figueredo et al. found the levels of C3d were increased in Type 2 Diabetic individuals, however, C3d association with lipid parameters in blood has not been studied in Diabetic Type 2 patients[8].

This study's goal was to evaluate the degree of complement activation by the serum measurement of C3d and Carotid IMT with the levels of Cholesterol, Triglycerides, LDL, and HDL in Diabetic Type 2 patients.

Methodology

This was the case-control study carried out in the Physiology Department from September 2020 to April 2021. The sample size for the study was calculated to be 79. A total of 120 participants comprising both males and females were included in the study, out of which 90 were Diabetic Type 2 patients between the age group of 35 to 65 years, recruited from the medicine ward 7, who met the inclusion criteria for the study, and 30 participants as healthy controls were recruited from the general population. The approval for ethical consideration of the study was acquired from the institutional review board of Jinnah postgraduate medical

Centre (Ref. No.F.2-81/2019-GENL/17476/JPMC). All study participants were counseled about the research purpose conducted in detail. The consent in written form was acquired from all the patients and controls participating in the study. The relevant facts were then entered into a performed questionnaire.

The study subjects were then divided into groups based on the duration of Type 2 Diabetes; each group comprised 30 subjects. Group A were Type 2 Diabetics patients with a period of diabetes of fewer than 5 years. Group B had Type 2 Diabetics for 5 to 10 years. Group C was Type 2 Diabetics for more than10 years of disease duration, while Group D was age and weight-matched healthy controls. The inclusion criteria for the study were; both males and females, Type 2 Diabetic patients between 35 to 65 years, and healthy individuals as controls. The patients who didn't give consent or had Type 1 Diabetes, with a history of smoking, other addiction, or cardiovascular diseases, were excluded. Furthermore, patients with chronic inflammatory diseases such as Rheumatoid Arthritis, Systemic lupus erythematosus, or those on medicines for lower lipid levels for the last three months or antihypertensive drugs insulin treatment were also excluded from the study.

A short history and basic bio-data about age and gender were taken from all the 120 participants recruited for the study. The patients with already diagnosed Diabetes Type 2 were recruited, and the duration of diabetes since the diagnosis was recorded. The individuals in the study with fasting blood glucose of less than 100 mg/dl (after at least 8 hours of fasting, according to the American Diabetic Association) were recruited as controls. All the study participants were then assessed for anthropometric, biochemical, and radiological parameters.

The anthropometric measurements including weight (kilograms) and height (meter), were recorded and the Body mass index (BMI) was calculated using the Quetelet index formula. According to WHO Asian-BMI classification, individuals with 18.5-22.99 kg/m^2 BMI were taken as normal, individuals with BMI in the range of 23-24.99 kg/m^2 were considered overweight, and BMI of individuals above 25 kg/m^2 were considered obese.

The blood samples by venipuncture method into the 5 ml syringe were collected to estimate C3d, Cholesterol, Triglycerides, LDL, and HDL. The sample for the Serum C3d estimation was then shifted to the Ethylenediaminetetraacetate (EDTA) containing vacutainers, centrifuged, and the plasma collected was then stored at -800ºC. Serum C3d was then estimated using C3d antibody Enzyme-linked immunosorbent assay (ELISA) Kit Cat no.E5115Hu. The Cholesterol, LDL, Triglycerides, and HDL were analyzed after 8 hours of fasting.

Both right and left common carotid artery were assessed for Carotid IMT measurement, which was done via B–mode ultrasound using a 7.5 MHz probe in a quiet, dark room in the Department of Radiology, JPMC. The Carotid IMT in the range of 0.4-0.7 mm[10] is considered normal, with the cut-off value for atherosclerosis at 0.9 mm[11].

All data were collected and then analyzed via SPSS version 23.0. The frequency with percentage was used to represent the qualitative data, and quantitative data were reported by mean and standard deviation. An Independent t-test was applied to observe the differences in HDL levels with C3d and Carotid intima-media thickness. One-way ANOVA was used to observe the differences in the groups stratified according to the duration of diabetes and lipid parameters in groups stratified according to Cholesterol, Triglycerides, and LDL levels in Diabetic Type 2 patients with the C3d and Carotid IMT levels. A P-value of <0.05 was regarded as a value with a statistically significant difference.

Results

Table 1 represents the age, gender, and BMI of the studied subjects. Out of 120 subjects, 74 (62%) were females. The type 2 Diabetic patients comprising 90 individuals were then stratified according to the duration of disease into group A (<5 years of

diabetes type 2), group B (5-10 years of diabetes type 2), and group C (>10 years of diabetes type 2). At the same time, group D was non-diabetic healthy control comprising 30 subjects. The subjects in Group C had an older population (56.7% of patients older than 55 years) than the patients in other groups, i.e., groups A, B, and D. The number of obese individuals was also higher in Group C, i.e., 20 out of 30 (66.7%) as compared to participants in group B, A and D.

Table 1: Age, Gender, and BMI stratified distribution of participants among groups.

Variable		A (N=30)	B (N=30)	C (N=30)	D (N=30)
Age	35 to 44 years	18(60)	11(36.7)	-	16(53.3)
	45 to 54 years	10(33.3)	19(63.3)	13(43.3)	9(30)
	55 to 64 years	2(6.7)	-	17(56.7)	5(16.7)
Gender	Male	10(33.3)	13(43.3)	11(36.7)	12(40)
	Female	20(66.7)	17(56.7)	19(63.3)	18(60)
BMI (kg/m^2)	Normal	8(26.7)	6(20.0)	5(16.7)	10(33.33)
	Overweight	3(10.0)	7(23.3)	5(16.7)	7(23.33)
	Obese	19(63.3)	17(56.7)	20(66.7)	13(43.33)

Variables are denoted as frequency (percentage).
Group A < 5 years type 2 diabetics; Group B 5-10 years type 2 diabetics; Group C > 5 years type 2 diabetics; Group D age and weight-matched healthy controls.

Table 2 shows Cholesterol levels were higher in group C (265.60 ± 53.36 mg/dl) as compared to groups B, A, and D ($p < 0.001$). The triglycerides, LDL was increased in group C (269.80 ± 98 mg/dl and 161.33 ± 23.31 mg/dl) as compared to group A and B diabetic type 2 patients and also in group D. This signifies that the duration of diabetes type 2 increases the lipid levels become increasingly abnormal with statistical significance between groups ($p<0.001$). However, the HDL levels were noted to be decreased as the duration of diabetes increased, i.e., much lower HDL were detected in Group C (37.00 ± 7.82 mg/dl) patients as compared to groups B, A, and D ($p<0.001$).

Among 90 patients of Type 2 Diabetes, 45 (50%) had Cholesterol levels of less than 200 mg/dl, 11(12%) patients had Cholesterol levels between 200 to 239 mg/dl and 34 (36.9%) patients had Cholesterol above 240 mg/dl. The HDL levels were above 40 mg/dl in 34 (37.7%) patients while 56 (62.2%) had HDL below 40 mg/dl. The LDL levels were below 100 mg/dl in 25 (27.7%) patients, 26 (28.8%) patients had LDL between 100 to 129 and 39 (43.3%) had LDL above 130 mg/dl. Out of 90 patients 19 (20.65%) had Triglyceride below 150 mg/dl, 23 (25.5%) had Triglyceride 150-200 mg/dl and 48 (53.3%) had Triglyceride above 200 mg/dl.

Table 2: Comparison of Lipid profiles among groups.

Variable	A (N=30)	B (N=30)	C (N=30)	D (N=30)	p-value
Cholesterol (mg/dl)	183.27±31.92	223.57±67.36	265.60±53.36	175.23±27.59	<0.001***
Triglycerides (mg/dl)	147.47±26.98	200.97±94.81	269.80±98	105.23±29.08	<0.001***
HDL (mg/dl)	44.77±7.60	40.10±7.16	37.00±7.82	50.17±9.75	<0.001***
LDL (mg/dl)	102.93±23.82	129.17±32.57	161.33±23.31	96.27±21.28	<0.001***

Results are denoted as Mean ±SD; p-value<0.05*, <0.01**, <0.001***
Group A < 5 years type 2 diabetics; Group B 5-10 years type 2 diabetics; Group C > 5 years type 2 diabetics; Group D age and weight matched healthy controls

Table 3 compares C3d and Carotid IMT in Type 2 Diabetes patients grouped according to normal, moderate, and higher Cholesterol, Triglycerides, LDL, and HDL levels. The C3d levels were increased in the patients with moderate to higher levels of Cholesterol. Still, carotid IMT was observed to be raised in patients with higher levels of Cholesterol. The patients with moderate to a higher increase in Triglycerides and LDL levels had significantly increased C3d levels in contrast to Carotid IMT, which was increased in patients with high levels of triglycerides and LDL ($p < 0.001$). Both C3d and Carotid IMT were increased as HDL levels fell below 40mg/dl in diabetic type 2 patients.

Table 3: Comparison of C3d and Carotid IMT with lipid profile in Type 2 Diabetic patients.

Lipid Parameter	Levels (mg/dl)	C3d (µg/ml) Mean ±SD	p-value	Carotid IMT (mm) Mean ±SD	p-value
Cholesterol	<200	6.37±3.65	<0.001*	0.66±0.11	<0.001*
	200-239	10.58±3.87	<0.001*	0.85±0.05	<0.001*
	>240	15.37±2.51	<0.001*	0.98±0.11	0.001*
Triglyceride	<150	9.08±3.30	<0.001*	0.63±0.12	<0.001*
	150-200	11.03±3.85	<0.001*	0.79±0.11	<0.001*
	>200	13.66±4.30	<0.001*	1.00±0.11	<0.001*
LDL	<100	7.84±3.17	<0.001*	0.58±0.08	<0.001*
	100-129	10.26±3.10	<0.001*	0.74±0.10	<0.001*
	>129	13.14±4.08	<0.001*	0.93±0.13	<0.001*
HDL	<40	12.12±4.42	0.036*	0.90±0.13	<0.001*
	>40	10.28±3.77		0.68±0.12	

*P-value<0.05 is considered statistically significant.

Discussion

This case-control study was done to determine the association of Lipid profile with a fragment of complement C3 degradation product C3d and Carotid IMT in Diabetic Type 2 patients. The current study's findings indicate that serum C3d levels increase in Type 2 Diabetic patients along with an increase in Cholesterol, Triglycerides, LDL levels above the normal range. It also signified the massive activation of the complement system as soon as lipid levels begin to rise above their respective standard value compared to the Carotid IMT found to be raised in patients with higher levels of Cholesterol, Triglycerides, and LDL. Both C3d and Carotid IMT were negatively correlated with HDL levels, i.e., as HDL decreases, both C3d and Carotid IMT increase. The Cholesterol in the intimal vessel layer causes the activation of complement protein C3 with ultimate degradation of C3 leading to the progression of atherosclerotic lesions[12]. The observations of the current study are in concordance with the study by Praliya et al., where the postprandial lipid profile was compared with the Carotid IMT in which the cholesterol, triglycerides, and LDL levels were positively correlated with the Carotid IMT and negative correlation of HDL with Carotid IMT were found[1]. Also, the study by Sharma and Shah[13] on diabetic type 2 patients with retinal changes found the association of cholesterol, triglycerides, and LDL with the Carotid IMT. However, the association of Carotid IMT was found to be negative with the HDL levels. Kumar et al.[14] compared Carotid IMT in people with type 2 diabetes with triglycerides and LDL; a correlation existed between triglycerides and Carotid IMT, but no association was found between LDL and Carotid IMT. Thus it is suggested that the raised levels of cholesterol, triglycerides, and LDL results in the enhancement of atherosclerotic lesions in diabetic patients[15].

The current study results show that cholesterol, triglycerides, and LDL increase as the duration of

diabetes, but HDL levels decrease as diabetes's duration increases. This finding is similar to the study by Biadgo et al., where the cholesterol, triglycerides, and LDL levels were raised in diabetic type 2 in contrast to the healthy controls. However, HDL levels were observed to be reduced in diabetic type 2 compared to the controls[16]. This finding is also similar to a study by Parmar et al. where the levels of cholesterol, triglycerides, and LDL raised as the duration of diabetes type 2 increases[17]. However, the study by Yakubu et al. found the association of cholesterol with a period of diabetes and an increase in cholesterol levels in diabetes type 2 with greater than 5 years of diabetes duration compared to people with type 2 diabetes of lower than 5 years of period[18]. However, contrary to the present study, no significant relation with type 2 diabetes duration was found in terms of Triglycerides, LDL, and HDL. Also, the current study's findings are contrary to Oatamer et al., where no association between abnormal lipids and duration of Diabetes Type 2 was found[19]. The significant deficiency of insulin is the primary culprit in causing dyslipidemia in patients with diabetes type 2[18], where an abnormal glucose level causes glycation of LDL, which is one of the reasons for the accelerated progression of atherosclerosis[20].

The study was carried out with a sample size of 90 type 2 diabetic patients, which is the major limitation. Also, Carotid IMT was estimated via a manual technique that is less accurate in contrast to an automated approach that uses radiofrequency signals.

The serum measurement of C3d can provide a non-invasive tool for aggravated atherosclerosis formation in type 2 diabetic patients with abnormal lipid profiles. The stringent control of lipid levels in type 2 diabetic patients can slow down the earlier activation of complement. Further research in this area is required on a large scale, as also the prognostic role of C3d in patients receiving the lipid-lowering treatment.

Conclusion

The increase in serum Cholesterol, Triglycerides, and LDL levels above the normal range in Type 2 Diabetic patients leads to enhanced complement activation as signified by the elevated presence of C3d was observed. Also, the serum C3d indicating the massive formation of atherosclerosis can be used as a marker of early atherosclerosis detection compared to carotid IMT, which was found in an abnormal range when lipid levels become exceedingly high.

Conflicts of Interest

The authors have declared that no competing interests exist.

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References

1. Praliya RS, Gupta S, Adya CM, Thakur N, Saini S. A Study of Correlation of Postprandial Lipid Profile with Carotid Intima-Media Thickness in Type-II Diabetes Mellitus. 2020;7(8): 330-334.
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2019(157):1-10.
3. Li S, Tang X, Luo Y, Wu B, Huang Z, Li Z, Peng L, Ling Y, Zhu J, Zhong J, Liu J. Impact of long-term glucose variability on coronary atherosclerosis progression in patients with type 2 diabetes: a 2.3 year follow-up study. Cardiovasc Diabetol. 2020;19(1):1-3.
4. Cosentino F, Cannon CP, Cherney DZ, Masiukiewicz U, Pratley R, Dagogo-Jack S, Frederich R, Charbonnel B, Mancuso J, Shih WJ, Terra SG. Efficacy of ertugliflozin on heart failure-related events in patients with type 2 diabetes mellitus and established atherosclerotic cardiovascular disease: results of the VERTIS CV Trial. Circulation. 2020;142(23):2205-2215.

5. Effoe VS, McClendon EE, Rodriguez CJ, Wagenknecht LE, Evans GW, Chang PP, Bertoni AG. Diabetes status modifies the association between carotid intima-media thickness and incident heart failure: The Atherosclerosis Risk in Communities study. Diabetes Res Clin Pract. 2017;128 :58-66.
6. Ge X, Xu C, Liu Y, Zhu K, Zeng H, Su J, Huang J, Ji Y, Tan Y, Hou Y. Complement activation in the arteries of patients with severe atherosclerosis. Int J Clin Exp. 2018;11(1):1-9.
7. Bavia L, Lidani KC, Andrade FA, Sobrinho MI, Nisihara RM, de Messias-Reason IJ. Complement activation in acute myocardial infarction: An early marker of inflammation and tissue injury?. Immunol Lett. 2018;200:18-25.
8. Figueredo A, Ibarra JL, Bagazgoitia J, Rodriguez A, Molino AM, Fernandez-Cruz A, Patino R. Plasma C3d levels and ischemic heart disease in type II diabetes. Diabetes Care. 1993;16(2):445-449.
9. Martin-Ventura JL, Martinez-Lopez D, Roldan-Montero R, Gomez-Guerrero C, Blanco-Colio LM. Role of the complement system in pathological remodeling of the vascular wall. Mol Immunol. 2019;114:207-215.
10. Mahmoud MZ. Sonography of common carotid arteries' intima: media thickness in the normal adult population in Sudan. N Am J Med. 2013;5(2):88-94.
11. Chen XM, Zhang Y, Shen XP, Huang Q, Ma H, Huang YL, Zhang WQ, Wu HJ. Correlation between glucose fluctuations and carotid intima-media thickness in type 2 diabetes. Diabetes Res Clin Pract. 2010;90(1):95-99.
12. Pilely K, Fumagalli S, Rosbjerg A, Genster N, Skjoedt MO, Perego C, Ferrante AM, De Simoni MG, Garred P. C-reactive protein binds to cholesterol crystals and co-localizes with the terminal complement complex in human atherosclerotic plaques. Front Immunol. 2017;8:1-14.
13. Sharma S, Shah P. Correlation of retinal changes and carotid intimal thickness in type 2 Diabetes Mellitus. Endocr Pract. 2019;25:111.
14. Kumar A, Rathore A, Singh AK. Study of Carotid Intima-Media Thickness (CIMT) In Type 2 Diabetes Mellitus and Its Correlation with Glycaemic Control and Inflammatory Marker HsCRP. Int J Med Res Prof. 2020; 6(1); 81-86.
15. Bosevski M, Stojanovska L. Progression of carotid artery disease in type 2 diabetic patients: a prospective cohort study. Vasc Health Risk Manag. 2015;11:549-553.
16. Biadgo B, Abebe SM, Baynes HW, Yesuf M, Alemu A, Abebe M. Correlation between serum lipid profile with anthropometric and clinical variables in patients with type 2 diabetes mellitus. Ethiop J Health Sci. 2017;27(3):215-226.
17. Parmar D, Vidja K, Ghugare B. Impact of the duration of diabetes and age: on lipid profile and glycaemic control in type 2 diabetic patients. J Int Med Res. 2013;2(1):69-72.
18. Yakubu A, Dahiru S, Mainasara AS, Anaja PO, Musa B, Hassan HA. Abnormalities of Serum Lipid Profile in Type 2 Diabetes Mellitus in North-Western Nigeria. 2020;11(4):17-21.
19. Otamere HO, Aloamaka CP, Okokhere PO, Adisa WA. Lipid profile in diabetes mellitus; what impact has age and duration. Br J Pharmacol. 2011; 2(3):135-137.
20. Klisic A, Kavaric N, Jovanovic M, Zvrko E, Skerovic V, Scepanovic A, Medin D, Ninic A. Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci. 2017; 22(122):1-7.