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Investigation Of The Relationship Between Dietary Intake Patterns And Levels Of Oxidized Low-Density Lipoprotein (Ox-LDL) In Individuals With Type 2 Diabetes Mellitus

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Abstract

Diabetes mellitus is considered as a metabolic disorder characterized by high level of glucose in blood stream. Diabetes is associated with impaired carbohydrate, fat and protein metabolism resulting from either the deficiency of insulin secretion or decreased sensitivity of cells to recognize insulin action that leads to insulin resistance. In the diabetic environment low density lipoprotein (LDL) are typically exposed to high level of circulating glucose and in result glycated LDL are formed. Similarly, some LDL molecules are modified by reactive oxygen species (ROS) and become oxidized low-density lipoprotein (Ox-LDL) particles. This study was done to determine the association between dietary intake and Ox-LDL. Physical, clinical and biomedical assessment was done by using food frequency questionnaire and taking blood samples. The concentration of Ox-LDL was determined by using human Ox-LDL Elisa kit. Pearson correlation and regression analysis were used to determine the correlation between Ox-LDL concentration and body mass index (BMI), calories intake, fruit intake, blood glucose, carbohydrate (CHO) intake, processed foods, fat intake and no. of servings. Study has found that Ox-LDL levels increased in diabetic patients. Ox-LDL is positively correlate with BMI, calories, blood glucose, and no. of servings, fat intake, processed foods and CHO intake. Thus, Ox-LDL can be reduced by low CHO, good fat and fruit intake.

Keywords: *Oxidized LDL, Diabetes Mellitus, Dietary Intake, Processed foods, BMI, Calories*

INTRODUCTION

Diabetes mellitus is considered as metabolic disease characterized by a high level of glucose in the bloodstream. Diabetes is associated with impaired carbohydrate, fat, and protein metabolism resulting from the deficiency of insulin secretion or insulin insensitivity of cells to recognize insulin action which leads to insulin resistance. The poorly controlled Blood sugar level can lead to serious health complications that damage various organs and cause diabetes nephropathy, neuropathy, retinopathy and cardiovascular diseases (Sami *et al.,* 2017). Insulin is the hormone that is secreted by the pancreas, acts as a carrier that regulate blood glucose in the body. The consumed food is broken down the in bloodstream. This sugar goes into the bloodstream and signals the pancreas to secrete insulin. It takes blood glucose enter into the body cells so that it can be used for energy purposes. Insulin also signals the liver to store extra glucose in the form of glycogen for later use (Nakrani *et al.*, 2020). Blood sugar enters into the cells and signals the pancreas to decrease the secretion of insulin. Lower insulin level signals the liver to release stored glycogen so energy is always available even in fasting state. This is the normal functioning but this can disturb in response to chronic over nutrition or when there is increased consumption of refined and processed carbohydrates. This inadequate intake of food causes a lot of glucose to enter the bloodstream. The pancreas releases more insulin to get blood sugar into the cell. Over time cells stop responding to all that insulin. The pancreas keeps making more insulin in response of excessive glucose to make cell

respond. But the pancreas can't keep up and the blood glucose in bloodstream keeps on rising and there's a lot of insulin too (Thorens, 2024). This signals the liver and muscle to store extra glucose in the form of glycogen. But the capacity of liver and muscle to store glucose is limited. Liver sends excess glucose to fat cells to be stored as fat in the body. This cause increased concentration of cholesterol, LDL, triglycerides. That eventually cause obesity insulin resistant. The stage is set for Diabetes mellitus. It also involved in carbohydrate metabolism (Kumar *et al.*, 2019; Faisal *et al.*, 2024).

According to the data from International Diabetes Federation stated that about 420 million people with diabetes live in the world, the prevalence rate is 8.8%.out of this 75% people live in developing countries. It is predicted that by 2040, approximately 642 million people will be effected form type 2 diabetes (Oguntibeju, 2019). In diabetesvery low-density (VLDL) lipoprotein are secreted from the liver into the blood streamthen transported to the capillaries and exposed to lipoprotein lipase. The role of this enzyme is to breakdown VLDL particle thereby converting them into intermediate density lipoprotein (IDL), which results in the formation of free fatty acid (FFA) that can released into body tissue. IDL circulates back to the liver from the peripheral tissue once in the liver the particles are acted upon by the enzyme hepatic lipase. the enzyme that specifically breakdown IDL. This result in the production of fatty acid(FA) and low-density lipoprotein (LDL). Triglycerides rich lipoprotein particles are able to enter liver hepatocyte because of large open pores LDL retain Apo-B 100 protein and return to the circulation. Some of these particles return to the general circulation other LDL particles bind to LDL receptors found on the surface of hepatocyte and thereby get clear from the circulation (Trpkovic *et al.,* 2015)

In the diabetic environment LDL are typically exposed to high level of circulating glucose from this exposure this LDL become glycated LDL. Similarly, some LDL molecules are modified by reactive oxygenated speciesROS and becomes oxidized LDL particles. These modified LDL particles have low affinity to LDL receptors on the surface of hepatocyte for normal clearance. From liver these modified LDL particles move into blood circulation and travels to the coronary arteries. They gain access to endothelial barrier permeability and penetrate to sub endothelial surface once in the sub endothelial matrix. There is increased retention of these small density lipoprotein. The remaining particles goes in sub endothelial matrix for specific interval of time and undergoes further modification. These retained LDL particles are susceptible to oxidation and become oxidized small density lipoprotein particle (Parthasarathy *et al.,* 2010). Dietary habits and lifestyle has the crucial role in the management of this pathological mechanism. Therefore, aim of this study is to investigate the relationship between dietary habits and ox-LDL in diabetic patients.

MATERIAL AND METHODS

The following study was directed in department of nutritional sciences, in Government College University, Faisalabad. Methods which were followed and materials which were utilized are described as under

Design and Participants

This experimental study was conducted on 50 patients. Samples were collected from District Head quarter(DHQ)hospital Faisalabad and the study duration was three months.

Data Collection

Data was collected from the 35–70year patients with type II diabetes

After selecting the particpants physical examination, recall of dietary and medical history, processed foods and alcohol intake were assessed by using HbA1c, fasting glucose, food frequency questionnaire, respectively

Anthropometric characteristics including BMI with their caloric intake were measured using standard formula (weight/height^2). Diabetes related risks was evaluated. Baseline venous 50 samples of blood were collected overnight fasting of approximately 12 hours for determination the concentration of blood sugar levels and oxidized low density lipoprotein particles.

In first interactionpatients received the training how to go through 7-day dietary intake history including their cooking methods and ingredients that were used in cooking. The final session clarified any objectives or queries.

Assessment of dietary intake

Dietary assessment questionnaire, food frequency questionnaire and Pakistan food composition tables were used. Food frequency questionnaire was developed under the supervision of senior dietitians. Pakistan food composition table 2001 was used for the estimation of calories and serving size of different food groups. finalize our classification for dietary glycated products intake on previous clinical researches that determine low and high dAGE's intake in humans. Therefore, for this research BMI, higher than 25kg/m^2 and caloric intake more than 1600 kcal were considered high.

The oxidized LDL particles have short period of life in the blood and it is expected that it can easily cleared from the body by reticulo endothelial system. Moreover, small dense but significant amounts of oxidized LDL are detectable in the blood sample. Sandwich base enzyme-linked immune sorbent assays technique was used to determine the concentration of oxidized LDL. (Trpkovic *et al.,* 2015)

Biochemical Analysis

Sample collection and storage:

Bloodsamples were collected and left for 35 minutes, after that centrifuged the sample for 15 minutes at 3000 rpm to obtain serum from the blood samples. Then the test tubes were properly labeled with the name and number of patients assign to them and sent directly to the biochemistry laboratory. For long term storage of serum, the samples were stored at -80 ° C until the date of analysis.

Blood Glucose test

Baseline venous samples were collected after 12 hours of fasting for the determination of blood glucose. Then these samples were centrifuged for 15 min at 3000 rpm separate the serum from blood. These samples have been stored in freezing state until essential for analysis (Rao *et al.,* 2004).

Oxidized LDL test:

Human oxidized LDL Elisa kit by Elabsciences were used for the identification and measurement of oxidized LDL with a sensitivity of 37.5pg/mL, this kit can be used to quantitatively determine the amount of human oxidized LDL in serum, plasma, and other biological fluids. The range of its detection is 62.5-4000pg/ml. The Sandwich-base method was employed by this ELISA kit. This kit includes a tiny ELISA plate that was previously coated with an antibody that is specific to human Ox-LDL structure. Samples (or standards) combined with a particular antibody and applied to ELISA substrate sources. Then, an Avidin-Horseradish Peroxidase (HRP) combination and a particular biotinylated detection antibody for Human Ox-LDL were added to the appropriate micro plate well and incubated. The cleaned free pieces. To each source, a substrate solution is applied. They are the only sources that have human Ox-LDL, the modified detected antibody will show in blue color.

Statistical Analysis

Versions 9.4 and 22.0 of the Statistical Software for Social Sciences (SPSS) were used for the statistical analyses. The level of significance was set at 0.01 for determining the sample size. To ascertain the relationship between Ox-LDL concentration and various study parameters, Pearson correlation and regression techniques were applied. Statistics were considered significant at a P value of 0.05. According to various metabolic indicators, the rate of LDL oxidation alone was examined (Montgomery, 2017).

RESULTS AND DISCUSSION

Association of Ox-LDL with BMI

The relationship between Oxidized LDL concentration and BMI in the whole sample size of the study conclude that elevated concentration of Ox-LDL is associated with higher BMI. Average BMI is (26kgms^{-2})

The type 2 DM group's BMI was markedly elevated (P 0.001).

According to studies, obesity poses a significant risk for type 2 diabetes, its cardiovascular problems, as well as a standalone risk for cardiovascular disease. BMI and Ox-LDL concentration significantly positively correlated in the current study, according to the findings (p 0.001). This could account for the higher prevalence of type 2 diabetes and cardiovascular issues seen in obese people in numerous earlier investigations. It has long been known that Ox-LDL plays a crucial part in the production of foam cells and has a high correlation with the risk of CVD. Our findings indicate that obese patients had greater Ox-LDL levels.(Njajou *et al.,* 2009)

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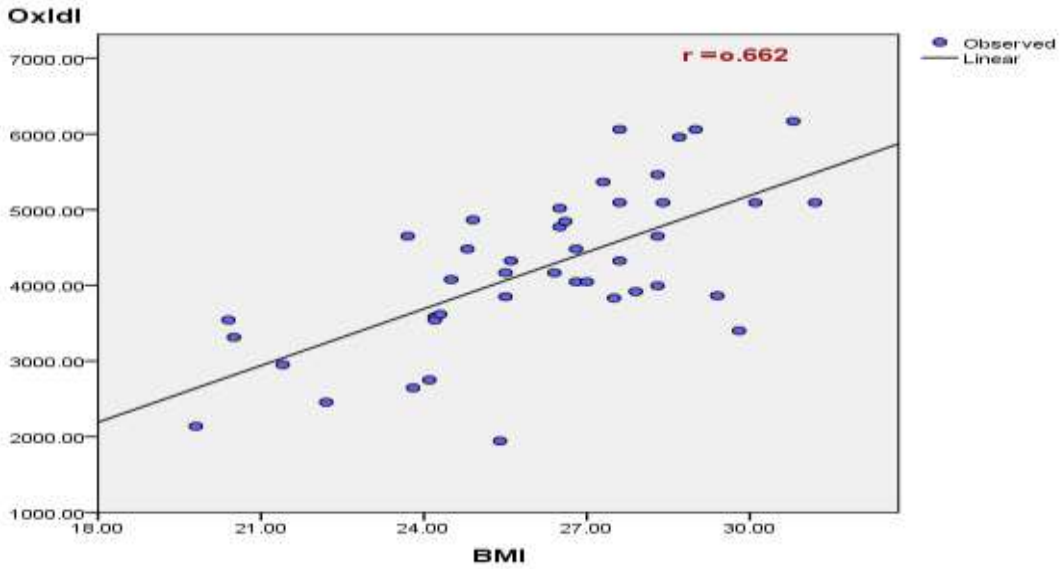

Fig 3.1 Association of Ox-LDL with BMI

In study we found that Oxidized LDL was increased in patients with higher BMI, by applying statistical correlation found the value (r = o.662) in all subjects the correlation was highly significant (p<0.001). BMI had a strong positive correlation with concentration of ox-LDL in all participants of studied groups.

Association of Ox LDL with Caloric intake

The relationship between Ox-LDL concentration and excessive calories intake in the whole population of the study confirmed that increased caloric intake cause increased in concentration of oxidized LDL. Our study found that there is a significant association between high caloric intake and increased Ox-LDL concentration. Average caloric intake is (1678.57kcal). Excessive caloric intake cause obesity which compromises the carbohydrate and fatty acid metabolism. Obesity is implicated as cause of oxidative stress which includes higher formation of mitochondrial reactive oxygen species and cause increased in inflammation. Higher caloric intake and insulin resistance are associated with elevated oxidative damage.

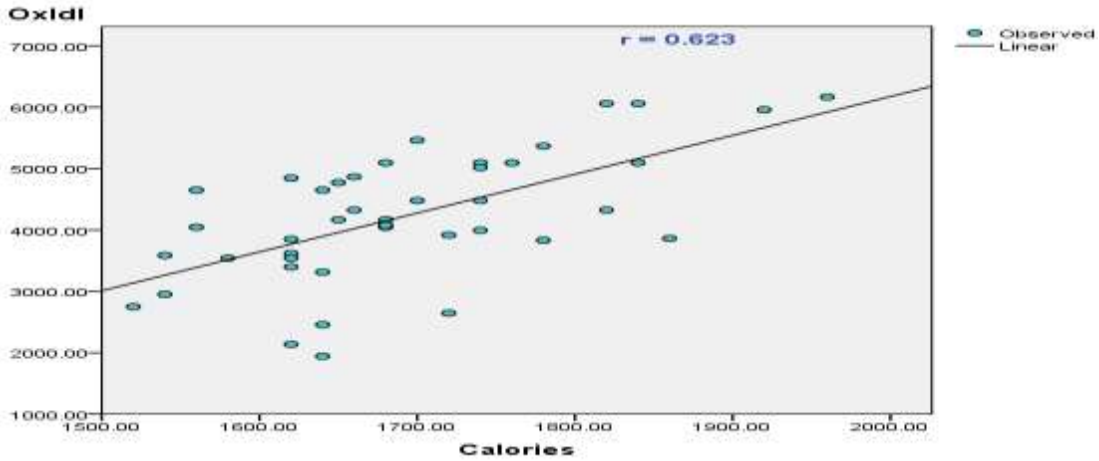

Fig 4.2 Association of Oxidized LDL with Caloric intake

Oxidized LDL was found to be increased with excessive calories intake, by applying Pearson correlation coefficient (r = o.623) in all patients the association was highly significant (p<0.001). Excessive calories intake had a strong positive correlation with increased concentration of ox-LDL in all participants.

Association of Oxidized LDL with fasting blood glucose

The relationship between Ox-LDL concentration and higher blood sugar levels in all the participants of research confirmed that Positive and significant associations with ox-LDL were observed for hyperglycemia.

These findings strengthened the association between hyperglycemia and increased level of oxidized LDL. Because high concentration of glucose has been describing to stimulate vascular formation of free radicals.

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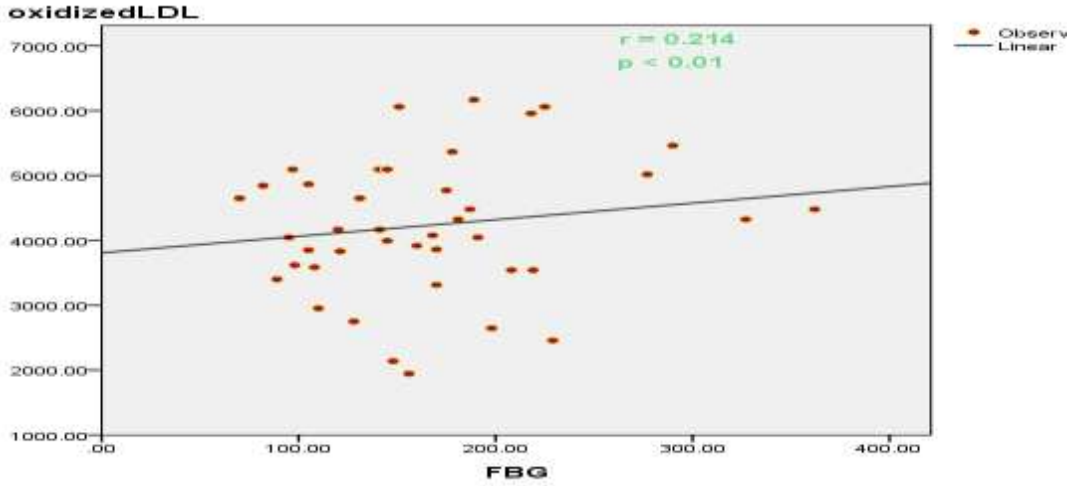

Fig 3.3 Association of Oxidized LDL with fasting blood glucose

Oxidized LDL was found to be increased with increased in fasting blood glucose level, by applying Pearson correlation coefficient (r = o.214) in all patients the correlation was highly significant (p<0.001). Higher fasting blood glucose level had a strong positive correlation with ox-LDL concentration in all subjects of studied groups.

Association of Oxidized LDL with Oil and Ghee

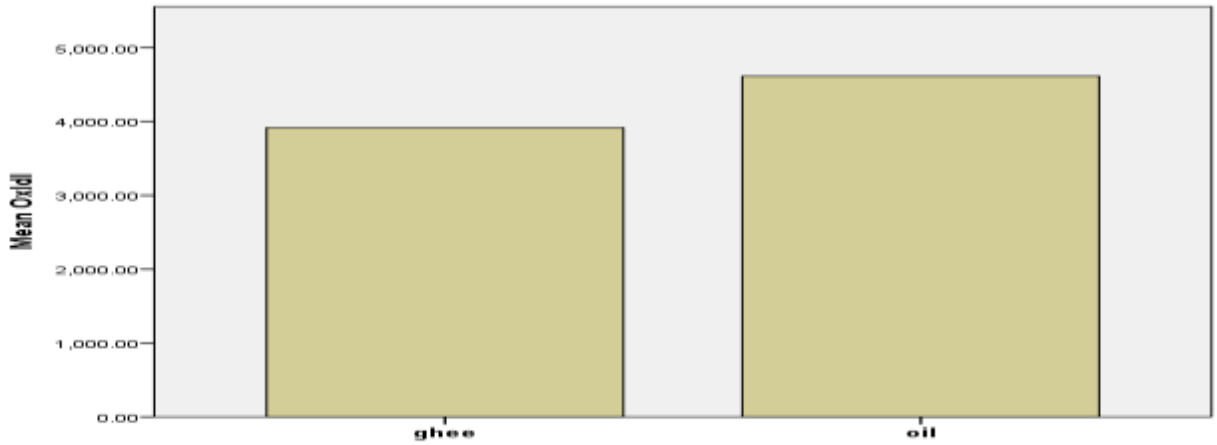

Fig 3.4 Association of Ox-LDL with Oil & Ghee

Oxidized LDL was found to be increased with intake of hydrogenated oil as compared to ghee, by applying Pearson correlation coefficient (r = o.577) in all patients the correlation was significant (p<0.001). High intake of processed oil had a strong positive correlation with increased ox-LDL concentration in all subjects of studied groups. We hypothesized that there was negative correlation between animal fat/ghee and concentration of oxidized LDL.

Hydrogenated oils are a major source of trans-fat in Pakistan, where they contribute to inflammation and the generation of reactive oxygen species (Hosseinabadi and Nasrollahzadeh, 2022).

Association of Oxidized LDL with processed foods

In this category, dry heat-processed foods including snacks, chips, and cookies had the maximum dAGE per gram of food. This is probably because adding items like butter, oil, cheddar, eggs, and nuts dramatically accelerates the production of dAGE during dry thermal processing. Long-term high-temperature frying of fat and meat may increase the amount of advanced glycated end products. Except when prepared with added fats, grains, beans, breads, vegetables, fruits, and milk were among the foodstuffs with the lowest dAGE values. For instance, the amount of dAGEs in cookies was more than ten times greater than that in low-fat bread, buns, or bagels. Increased cross-linking of collagen caused by the production of Advanced Glycation End Products is a significant change (AGEs).

These changes of proteins are lipid- and carbohydrate-dependent and result from both oxidative and non-oxidative processes. Carbonyl stress is the accumulation of AGEs by glycosylation, glycoxidation, lipoxidation, or the creation of carbonyl compounds. This condition has been linked to atherogenesis and pulmonary hypertension as well as diabetes and uremia. (Sharma *et al.,* 2015; Maldonado-Pereira *et al.*, 2023)

Fig 3.5 Association of OxLDL with Processed foods

Oxidized LDL was found to be increased with the intake of processed foods/bakery items, by applying Pearson correlation coefficient (r = o.701) and regression model in all patients the correlation was highly significant (p<0.001). Intake of processed foods had a strong positive correlation with increased ox-LDL concentration in all subjects of studied groups. We hypothesized that there was positive correlation between intake of processed foods and concentration of oxidized LDL.

Association of Oxidized LDL with Fruit intake

Oxidized LDL was found to be increased with 4-5 servings of fruits per day, by applying Pearson correlation coefficient (r = o.687) in all participants the relationship was significant (p<0.001). 4-5 servings of fruits per day had a strong positive correlation with increased levels of ox-LDL in all patients of studied groups. We hypothesized that there were negative correlation between 2-3 servings of fruits a day and concentration of oxidized LDL.

Fructose is naturally present in fruit, present in comparatively small doses compared to processed foods, and fruit consumption has been epidemiologically linked to a reduction in obesity. Minute dose of fructose is excreted from the GI track to create sugar and therefore it cannot enter in the portal circulation. Moreover, in people with diabetes Fructose, but not glucose, boosts the hepatic SREBP1c and lipids synthesis genes when substantial quantities of high-fructose fruits are consumed together with an HFD. This enhances lipid synthesis and decreases hepatic insulin sensitivity (Bergheim *et al.,* 2008; Bacchetti *et al.*, 2019)

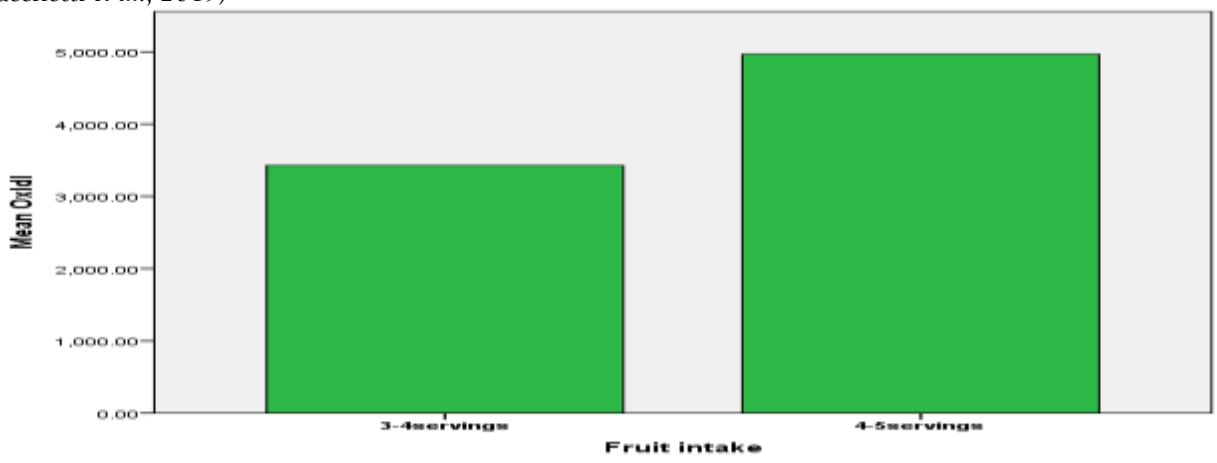

Fig 4.8 Association of OxLDL with Fruit Intake

Three servings of fruit per day showed negative correlation with Oxidized LD (r= -0.701). Patients who consume three servings of fruits per day have less oxidized LDL level as compared to the patients take 4-5 servings of fruits per day.

Table: Association between parameter and Ox-LdL

Parameters	Ox-LDL Chi sq.
BMI	0.00*
Fasting blood glucose	0.174
Oil and Ghee	0.03*
Fruit Intake	0.00*
Processed food	0.01*
Food servings	0.01*
Cabohyderate intake	0.00*
Caloric intake	0.00*

Comparison of clinical variables in normal and diabetic group

The present study investigated the differences in lipid profiles and oxidized LDL levels between individuals with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls. Our findings demonstrate significant alterations in key metabolic markers, particularly lipid parameters and oxidative stress indicators, among diabetic patients compared to their non-diabetic counterparts. Age was not significantly different between the two groups ($p = 0.15$), suggesting that observed differences are less likely due to age disparities and more likely associated with diabetic status. As expected, the body mass index (BMI) was significantly higher in diabetic individuals (29.6 ± 3.3 kg/m^2) compared to controls (24.8 ± 2.3 kg/m^2), with a highly significant p-value ($p < 0.001$), reflecting the well-established link between obesity and T2DM. Glycemic control indicators, namely **fasting blood sugar (FBS)** and **HbA1C**, were markedly elevated in diabetic patients (FBS: 166.4 ± 23.9 mg/dL; HbA1C: 8.3 ± 1.3%) compared to controls (FBS: 88.3 ± 4.9 mg/dL; HbA1C: 5.2 ± 0.4%), both with significant p-values ($p = 0.01$). These values confirm poor glycemic control and reinforce the metabolic disturbances associated with diabetes. In terms of lipid profile, diabetic patients exhibited **higher total cholesterol, triglycerides (TG), and LDL-C levels**, along with **significantly reduced HDL-C levels** ($p \leq 0.01$ for all parameters). These dyslipidemic patterns are consistent with the atherogenic lipid profile commonly observed in T2DM, which contributes to increased cardiovascular risk. Of particular interest is the **dramatic elevation in oxidized LDL (ox-LDL) levels** in diabetic patients (87.7 ± 15.9 mU/L) compared to non-diabetics (41.6 ± 9.8 mU/L), with a highly significant difference ($p = 0.001$). ox-LDL is a key marker of oxidative stress and is known to play a pivotal role in atherosclerosis development. Moreover, the **ratios of ox-LDL to LDL-C and HDL-C** were significantly higher in the diabetic group ($p < 0.001$), suggesting a greater burden of oxidized lipoproteins relative to the total LDL and protective HDL, further indicating heightened oxidative stress and lipid imbalance. These findings align with previous literature suggesting that **hyperglycemia, insulin resistance, and inflammation** in T2DM promote the oxidation of LDL particles, thereby exacerbating endothelial dysfunction and increasing cardiovascular risk.

Table: Comparison of clinical variables in normal and diabetic group

Variabale	Normal (n=50	Diabetic patients (n=50	P-value
Age	45.6±4.2	47.1±6.5	0.15
BMI (kg/m2)	24.8±2.3	29.6±3.3	0.00*
FBS (mg/dL	88.3±4.9	166.4±23.9	0.01
HbA1C (%)	5.2±0.4	8.3±1.3	0.01
Total cholesterol (mg/dL)	178.3±21.3	220.8±29.9	0.01
TG (mg/dl)	124.7±17.7	190.2±33.7	0.00*
HDL-C (mg/dl)	51.3±6.5	39.2±5.5	0.001

LDL-C (mg/dL)	10.7.4±19.01	136.5±27.00	0.003
ox-LDL (mU/L)	41.6±9.8	87.7±15.9	0.001
ox-LDL/LDL-C	0.37±0.08	0.71±0.09	0.00*
ox-LDL/HDL-C	0.78±0.11	2.41±0.38	0.001

CONCLUSION

In conclusion, our study has found that oxidized LDL levels increase in diabetic patients as compared to normal. Oxidation of LDL is positively correlate with BMI, Calories, hyperglycaemia, 3 servings of food per day, Oil, processed foods and increased carbohydrate intake. However, concentration of oxidized LDL is negatively correlate with balanced fruit intake. A direct association has been observed between obesity and inflammatory markers, oxidative stress, and susceptibility to oxidative damage or low-density lipoprotein modification.

Excessive caloric intake cause obesity which compromises the carbohydrate and fatty acid metabolism. Our findings strengthened the association between hyperglycemia and increased level of oxidized LDL. Because high concentration of glucose has been describing to stimulate vascular formation of free radicals. We hypothesized that there was negative correlation between less than 50% of carbohydrate intake and concentration of oxidized LDL. Early studies have indicated that high-carb diets stimulate the development of tiny, thick LDL particles that are more vulnerable to oxidation and glycation.

Hydrogenated oils are a major source of trans-fat in Pakistan, where they contribute to inflammation and the generation of reactive oxygen species. Trans-fat increased plasma levels of low-density lipoprotein (LDL) and lowered plasma levels of high-density lipoprotein (HDL). Long-term high-temperature frying of fat and meat may increase the amount of advanced glycated end products. Except when prepared with added fats, grains, beans, breads, vegetables, fruits, and milk were among the foodstuffs with the lowest dAGE values. The habit of refrying meals has harmful impacts on one's health. These breakdown products have been connected to pathophysiological outcomes of oxidative stress when consumed. Vegetable oils that have been heated repeatedly can have negative effects on bone structure and bone cells as well as an increased probability of hypertension, vascular dysfunction, vascular issues, lipoprotein oxidation, and eventually an increased risk of atherosclerosis In people with diabetes Fructose, but not glucose, boosts the hepatic SREBP1c and lipids synthesis genes when substantial quantities of high-fructose fruits are consumed. More studies are recommended to investigate whether increasing the antioxidant capacity either by dietary or medical intervention routes will prevent or reduce oxidation of LDL.

Study shows a positive correlation between obesity, increased caloric intake and hyperglycemia with elevated level of oxidized LDL in diabetic patient. Weight control, management of blood glucose level and balanced intake of fruits are recommended to avoid increase in atherogenic oxidized LDL particles.

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