Achievement of LDL-cholesterol target levels and other cardiovascular risk factor targets in patients with type 2 diabetes treated with lipid-lowering medications:
a cross-sectional study.

A thesis submitted in fulfilment of the requirements for the degree of
Master of Science in Medical Research Methodology

By

Anastasia Erythropoulou-Kaltsidou

Thessaloniki, December, 2017
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A MSc thesis submitted in fulfilment of the requirements for the degree of Master of Science in Medical Research Methodology At The Faculty of Health Sciences School of Medicine Aristotle University of Thessaloniki

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Word count
9,847
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Prologue

This thesis with the title “Achievement of LDL-cholesterol target levels and other cardiovascular risk factor targets in patients with type 2 diabetes treated with lipid-lowering medications: a cross-sectional study” was written for the MSc in Medical Research Methodology, during the academic year 2017 – 2018.

Diabetes mellitus (DM) is a common metabolic disease, which is associated with increased risk for developing a cardiovascular disease. The aim of this study is to assess if the patients with type 2 DM (T2DM) who are on lipid-lowering therapy achieve the targets for prevention of a cardiovascular disease, as they are defined by the guidelines for management of dyslipidemias of European Society of Cardiology and European Atherosclerosis Society in 2016.

I would like to thank my supervisor, Dr. Bekiari Eleni, Lecturer of Internal Medicine in Aristotle University of Thessaloniki, for her support. I also want to thank the Associate Professor Dr. Tsapas Apostolos for his assistance. I greatly appreciate also the support of Karagiannis Thomas for giving me a constant feedback during the writing of the thesis. Furthermore, I would like to thank the doctor Boutel Dimitrios, the Director of the diabetes outpatient clinic at the hospital of Giannitsa, who helped me a lot during the conduct of the study. I also appreciate the help of the three nurses working in this diabetes clinic, Gkini Chrisoula, Sakavara Stolina and Thasitou Eustratia. Finally, I want to thank Martasidou Aikaterini, the Director of the laboratory department of Hospital of Giannitsa, for giving me the essential material about the function of the laboratory analyzers.

Thessaloniki, December 2017
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
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<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
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<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<tr>
<td>HbA1c</td>
<td>Hemoglobin A1c</td>
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<td>HDL</td>
<td>High-density lipoprotein</td>
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<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl CoA</td>
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<td>IDL</td>
<td>Intermediate-density lipoprotein</td>
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<td>IQR</td>
<td>Interquartile range</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
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<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
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<tr>
<td>NA</td>
<td>Not applicable</td>
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<tr>
<td>NGSP</td>
<td>National Glycohemoglobin Standardization Program</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Care Excellence</td>
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<tr>
<td>Non-HDL-C</td>
<td>Non-high-density lipoprotein cholesterol</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PCSK9</td>
<td>Proprotein convertase subtilisin/kexin type 9</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>STROBE</td>
<td>STrengthening the Reporting of OBservational Studies in Epidemiology</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
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<tr>
<td>SREBPs</td>
<td>Sterol regulatory element binding proteins</td>
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<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
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<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
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<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
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<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1. Introduction
1.1. Diabetes mellitus: definition, classification and epidemiology

DM is a common metabolic disease, which is characterized by hyperglycemia due to a disorder in insulin secretion, in insulin action or the combination of both (1). DM as chronic hyperglycemic condition can cause serious damage in many organs such as kidneys, eyes, nerves and arteries. According to American Diabetes Association (ADA), DM is classified into four categories (2):

i. Type 1 diabetes (T1DM)
ii. T2DM
iii. Gestational diabetes mellitus (GDM)
iv. Specific types of diabetes by other causes (for example monogenic diabetes syndromes, diabetes induced by drugs, diabetes due to diseases of exocrine pancreas).

T1DM is characterized by the immune-associated destruction of β cells of the pancreatic islets which has as a result the absolute insulin deficiency (1). Autoantibodies to insulin, glutamic acid decarboxylase, insulinoma-associated autoantigen 2 and zinc transporter 8 are present in more than 90% of newly diagnosed patients with T1DM (3). Although T1DM occurs mostly in childhood, it can occur at any age (4).

T2DM is caused by the combination of insulin resistance and inadequate insulin secretion (1). The majority of the diabetic patients, almost 90 – 95% of them, belongs to this category of diabetes (5). During the last years, there is a trend of increasing the incidence of T2DM (6, 7).

GDM is the diabetes which has been first diagnosed during the second or third semester of pregnancy in women that did not have diabetes before the pregnancy (2). Women with GDM have an increased risk for developing later T2DM (8, 9).

The last decades, there is a great increase in the number of patients with DM. According to World Health Organization (WHO), in 1980 the total number of diabetic patients worldwide was 108 million, whereas in 2014 the number was almost quadrupled (422 million) (10). It is also estimated that in 2035 the prevalence of DM will rise up to 592 million. (10, 11). DM was the main cause of death for 1.6 million people in 2015 and it is estimated by WHO that DM will be the seventh most frequent cause of death in 2030 (12).
1.2. Diagnosis of DM

According to ADA the diagnosis of DM is based on the following criteria (2):

- Fasting plasma glucose $\geq 126$ mg/dL.
- The 2-hour plasma glucose $\geq 200$ mg/dL during a 75-g oral glucose tolerance test (OGTT).
- Hemoglobin A1c (HbA1C) $\geq 6.5\%$. The measurement of HbA1C should have been performed in a laboratory which uses a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to Diabetes Control and Complications Trial (DCCT) reference essay.
- Random plasma glucose $\geq 200$ mg/dL in a patient with hyperglycemic symptoms or crisis.

If there is not clear evidence of hyperglycemia, the tests should be repeated to confirm the results and establish the diagnosis of DM.

1.3. Complications of DM

The early diagnosis and treatment of DM is of great importance in order to prevent or delay the complications, which are caused by hyperglycemia (13). The complications of DM are divided into the following two groups (14):

- Macrovascular complications:
  - coronary artery disease
  - cerebrovascular disease
  - peripheral artery disease
- Microvascular complications:
  - diabetic neuropathy
  - diabetic nephropathy
  - diabetic retinopathy

Vascular damage is caused by hyperglycemia through different pathways: production of advanced glycated end products, polyol and hexosamine pathways, activation of protein
kinase C and increased diacylglycerol levels, mitochondrial dysfunction, accumulation of reactive oxygen species, increase in the generation of free radicals and increase of the oxidative stress (15, 16, 17). The above mechanisms have as a result the endothelium dysfunction, which leads to the pathogenesis of macrovascular and microvascular complications.

According to United Kingdom Prospective Diabetes Study (UKPDS), there is a significant association between hyperglycemia and the complications of DM (18). For each 1% reduction of HbA1c, the risk of related-to-diabetes deaths were reduced by 21% (p < 0.0001), the risk for myocardial infraction reduced by 14% (p < 0.0001) and the risk for microvascular complications reduced by 37 % (p < 0.0001) (18). The proper glycemic management of diabetic patients is very important in order to prevent complications and reduce mortality.

1.4. Dyslipidemia: definition and epidemiology

According to the Hellenic Society of Atherosclerosis, dyslipidemias are quantitative or qualitative disorders of metabolism of lipoprotein particles [low-density lipoprotein (LDL), chylomicrons, high-density lipoproteins (HDL), very low-density lipoproteins (VLDL)], which transfer the lipids in the human body (19). Dyslipidemia is characterized as a primary major risk factor for developing atherosclerotic cardiovascular disease (20). According to WHO, in 2008 the worldwide prevalence of increased total cholesterol in the adult population (>= 5 mmol/ or >= 190 mg/dL) was 39 % (21). It is estimated that the elevated cholesterol causes 2.6 million of deaths (21).

1.5. Cholesterol, triglycerides and lipoproteins

Cholesterol and triglycerides consist the lipids existing in the human body (19). The most important role of cholesterol is that it is a structural element of cell membranes (22, 23). It is also a precursor of all steroid hormones and of bile acids (22, 23). Triglycerides are fatty acid esters of glycerol and they are the main fat deposit (23).

Cholesterol can be received either by diet or be synthetized de novo, mainly in the liver (24). The rate of cholesterol synthesis is regulated by changes in the amount and activity of 3-
hydroxy-3-methylglutaryl CoA reductase (HMG-CoA-reductase) (24). Cholesterol is synthetized in three stages: the first stage includes the synthesis of isopentenyl pyrophosphate from acetyl Coenzyme A, in the second stage, six molecules of isopentenyl pyrophosphate are consolidated and the result is the formation of squalene and during the third stage the cyclization of squalene takes place in order to form a tetracyclic product which is converted into cholesterol (25).

Triglycerides are esters in which three molecules of fatty acids are linked with glycerol (23). They are also come from the diet or are synthetized by liver (23).

Cholesterol and triglycerides are transported in the plasma associated with lipoproteins particles (24). Lipoproteins are classified into the following five categories based on their content of cholesterol, triglycerides and proteins:

- chylomicrons
- VLDL
- intermediate-density lipoprotein (IDL)
- LDL
- HDL

Cholesterol is transferred through LDL to peripheral tissues and returns to the liver by HDL, which prevents the high accumulation of cholesterol in the other tissues of the body (23).

1.6. Dyslipidemia in T2DM

The most common type of dyslipidemia in T2DM is characterized by increased levels of triglycerides and decreased levels of HDL-cholesterol (HDL-C) (26, 27). The levels of LDL cholesterol (LDL-C) are not significantly different in diabetic patients compared with non-diabetic patients. In the Strong Heart Study, it was found that mean LDL-C level in participants with DM was lower than in participants without DM (28). But, in the same study, it was found that in diabetic participants the LDL particle size was smaller than in non-diabetic participants and that LDL-C was a strong independent predictor of coronary heart disease in diabetic patients (28).
The pathogenesis of dyslipidemia in DM (high triglycerides, decreased HDL-C and elevated small dense LDL particles) involves many mechanisms. Insulin resistance and increased flux of the free fatty acids in the liver have as a result the increased production of triglycerides in liver (29). Furthermore, the low levels of HDL-C in diabetic patients may be related with the resistance of insulin which prevents the upregulation of apo A-I production, which is the main protein in HDL particle (29). In DM, the production of inflammatory cytokines is elevated and has as a result the increased insulin resistance. This insulin resistance leads in downregulation of apo A-I and HDL production (29).

In diabetic patients, LDL-C levels are not elevated in comparison with general population, but LDL particles are more atherogenic (30). So, the reduction of LDL-C levels in patients with DM may be more beneficial for reducing the risk of cardiovascular disease than in non-diabetic patients (30). The Collaborative Atorvastatin Diabetes Study supported that treatment with statin reduced the cardiovascular risk in diabetic patients even without having increased LDL-C levels before the treatment (30).

1.7. Management of dyslipidemia

According to the guidelines of European Society of Cardiology and European Atherosclerosis Society in 2016, the management of dyslipidemias is based on lifestyle modifications and lipid-lowering medications (31).

1.7.1. Lifestyle modifications

The lifestyle modifications, in order to improve the lipid profile and prevent cardiovascular disease, are the following (31):

- reduction of dietary trans or saturated fat intake (32)
- increase of the intake of dietary fibers (33)
- increase of physical activity (34)
- smoke cessation (35, 36)
- weight loss (37, 38).
The role of the above interventions is of great importance in order to change the lipid levels and reduce the risk of developing a cardiovascular disease.

1.7.2. Lipid-lowering medications

Drugs used for treating hypercholesterolemia, according to the European Society of Cardiology and European Atherosclerosis Society are the following (31):

- statins
- cholesterol absorption inhibitors (ezetimibe)
- bile acid sequestrants (cholestyramine, colestipol, colesevamel)
- proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors
- nicotinic acid
- drug combinations.

For patient with elevated fasting triglycerides, the available drugs are: statins, fibrates, nicotinic acid and n-3 polyunsaturated fatty acids (PUFAs) (31).

1.7.2.1. Statins

The use of statins is associated with reduction of total cholesterol, LDL-C and triglycerides levels and reduction of not only cardiovascular, but also all-cause mortality (31, 39, 40). Statins are inhibitors of HMG-CoA reductase (41). They reduce the synthesis of endogenous cholesterol, by inhibiting the main enzyme which is involved in the production of cholesterol. The function of HMG-CoA reductase is to convert HMG-CoA into mevalonic acid, which is a precursor of cholesterol (41). The decrease of cholesterol intracellularly induces the activation of a protease, which has a result the release of sterol regulatory element binding proteins (SREBPs) from the endoplasmic reticulum (42). Next, SREBPs are transferred in the nucleus and promote the expression on LDL-receptor gene. The decrease of intracellular cholesterol in the hepatocytes induces an increase of LDL-receptors in the hepatocytes, which leads to the decrease of the LDL-C which circulates in the blood (31, 41). However, except for their effect on LDL-C reductions, statins have more beneficial pleiotropic effects. Specifically, they improve the endothelial dysfunction, they increase the stability of
atherosclerotic plaques, they inhibit the aggregation of the platelets and they have anti-inflammatory properties (43, 44, 45).

The proportion of LDL-C reduction is not the same for all statins (46). From comparisons between the statins, the greatest reduction of LDL-C (more than 40%) was observed with two statins: atorvastatin and rosuvastatin (at a dose of 20 mg or higher daily) (46). Treatment with statin is characterized by a large interindividual variability on reduction on LDL-C levels (47).

All statins are metabolized in the liver by cytochrome P450 (CYP) isoenzyme systems, except from pravastatin which is metabolized by enzymes in liver cytosol (48). Atorvastatin, simvastatin and lovastatin are metabolized by CYP3A4, whereas fluvastatin is mainly metabolized by CYP2C9 (48). Drug interactions with statins are caused by induction or inhibition of CYP450 isoenzymes.

The use of statins is associated with adverse effects. The most important of them are: elevations of transaminases, myopathy and rhabdomyolysis (48, 49, 50, 51). For statin-use, the incidence of rhabdomyolysis was estimated to be 3.4 per 100,000 person-years (52). It is also found that the use of statins is associated with either a slight increase in the new-onset DM or with an adverse glycemic control in patients with DM (53, 54, 55). The highest risk of developing an adverse effect is caused by atorvastatin, whereas fluvastatin has the lowest risk for any adverse effect (56).

1.7.2.2. Cholesterol absorption inhibitors (ezetimibe)

In case of intolerance to statins or need for an adjuvant medication to reduce LDL-C levels, the use of ezetimibe is advisable (57). According to the findings of systematic reviews, the monotherapy with ezetimibe (at a dose of 10 mg per day) reduced significantly the LDL-C levels by 18.58% compared to placebo (58), whereas the addition of ezetimibe to a current statin therapy reduced significantly the LDL-C level by 23.6 % compared to the combination of statin with placebo (59).

Ezetimibe inhibits the absorption of cholesterol by targeting the protein Niemann-Pick C1-Like 1, which is expressed on the surface of the jejunal enterocytes (60). Ezetimibe blocks the absorption of cholesterol and reduces the delivery of the intestinal cholesterol into the liver.
The concentrations of LDL-C in the liver are decreased leading to the increase of the expression of LDL-C receptors in the surface of cells in the liver. This results in the reduction of circulating LDL-C (57).

Ezetimibe is not metabolized by cytochrome P450, so it does not interact with other medications which are metabolized via this pathway, for example statins. Ezetimibe is mainly metabolized by glucoronidation in the liver and in the intestine and formation of ezetimibe-glucuronide (61). Ezetimibe is mainly excreted in the feces, whereas a smaller excretion is performed via urine (57, 61).

Ezetimibe is a well-tolerated lipid lowering medication. According to randomized double-blinded studies, the adverse effects related to liver, muscles or gastrointestinal system did not differ from placebo (62, 63).

1.7.2.3. Bile acid sequestrants (cholestyramine, colestipol, colesevelam)

The three bile acid sequestrants, cholestyramine, colestipol and colesevelam, interrupt the enterohepatic circulation of bile acids (64, 65). Specifically, they bind to the bile acids and prevent them from entering into circulation. The liver needs to synthetize more bile acids from cholesterol. The utilization of hepatic cholesterol for the synthesis of bile acids increases the expression of LDL-C receptors on the surface of hepatic cells. This has as a result the decrease in the circulating LDL-C (64, 65).

Except for LDL-C reduction, it has been shown, that colesevelam improves the glycemic control in diabetic patients, when it is added on the other antidiabetic medications (66, 67).

Bile acid sequestrants may interact with many drugs because they interrupt their absorption (68). Colesevelam presents less drug-interactions than cholestyramine or colestipol (69, 70).

The bile acids sequestrants have not systemic adverse effects because they are not absorbed by the gastrointestinal system (71). The main gastrointestinal side effects are constipation up to 39 %, flatulence, bloating and dyspepsia (71, 72).
1.7.2.4. PCSK9 inhibitors

The PCSK9 inhibitors are monoclonal antibodies which inactivate the PCSK9, which is a hepatic protease that promote the degradation of LDL receptor (73, 74). The prevention of the function of PCSK9 decreases the degradation of LDL receptors and promotes the clearance of circulating LCL-C from plasma. According to the results from systematic review, PCSK9 inhibitors reduces the LDL-C levels by 53.86 % and the risk for cardiovascular events (odds ratio 0.86) compared with placebo (75).

PCSK9 inhibitors are administered by subcutaneous injection every two weeks (31). They do not have interactions with other drugs (31). The most important adverse effects of PCSK9 inhibitors are: nasopharyngitis, reactions at the site of the injection, upper respiratory tract infection, myalgias and nausea (76).

1.7.2.5. Nicotinic acid

Nicotinic acid is characterized as a broad-spectrum lipid drug. It decreases LDL-C and total cholesterol levels, increases HDL-C levels and reduces triglycerides levels (77). However, its use is limited because of its adverse effects, the most common of which is a strong cutaneous vasodilation, that is called flushing (77).

1.7.2.6. Fibrates

Fibrates are first line therapy for treating hypertriglyceridemia (78). Their mechanism of action is performed via activation of peroxisome proliferator-activated receptor alpha. The result is to increase the catabolism of triglyceride-rich particles and decrease VLDL production (78). Fibrates decrease triglycerides levels and increase HDL levels (79).

Fenofibrate does not interact with statins and can be used as combination with statins. Unlike to fenofibrate, gemfibrozil should not be combined with statin therapy (80). The most important side effects are: myopathy, pancreatitis, elevations of liver enzymes, cholelithiasis and venous thrombosis (31, 80).
1.7.2.7. N-3 fatty acids

N-3 fatty acids decrease triglycerides levels and reduce also the risk of cardiovascular events (81, 82). Their exact mechanism of action is not well understood (31).

1.8. Other cardiovascular risk factors

Cardiovascular disease is the leading cause of death globally (83). According to the available data, cardiovascular diseases leads to death more than 4 million of people in Europe every year (83).

It is known, that DM is an important risk factor for cardiovascular disease. Diabetic patients are at higher risk of myocardial infraction and death than non-diabetic patients (84, 85). But except for DM, the other major risk factors according to the American Heart Association are the following (86):

- Smoking
- High blood pressure
- High total cholesterol and LDL-C
- Low HDL-C
- Advancing age
- Obesity
- Lack of physical activity

Other risk factors are: family history, ethnic and psychosocial characteristics, high triglycerides, small LDL particles, high homocysteine in serum, high lipoprotein(a), prothrombotic and inflammatory factors (fibrinogen, C-reactive protein) (86).

1.9. Cardiovascular risk factor targets for prevention of cardiovascular disease

According the European Society of Cardiology, the targets for prevention of development a cardiovascular disease are (31):

- No exposure to tobacco smoke.
- Consumption of vegetables, fish and fruit and avoidance of saturated fats.
- Physical activity: 2.5 to 5 hours moderately vigorous exercise every week or 0.5 to 1 hour the most days.
- Body mass index (BMI) 20 – 25 kg/m², waist circumference less than 94 cm in men and less than 80 cm in women.
- Blood pressure less than 140 / 90 mmHg.
- LDL-C levels less than 70, 100 and 115 mg/dL and non-HDL-C levels less than 100, 130 and 145 mg/dL, in very high-risk, in high-risk and in low- or moderate-risk patients respectively.
- HbA1c less than 7%.

1.10. Aim of the study

Diabetic patients are at increased risk for cardiovascular disease (87). In these patients, the proper management of dyslipidemia, another risk factor for cardiovascular disease, is essential. The primary target for lipid control is LDL-C (31). Until now, there is not any other study in Greece, which assesses if patients with T2DM who are on lipid-lowering therapy, achieve the target for LDL-C and the targets for the other cardiovascular risk factors, in order to prevent the development of cardiovascular disease.

In our study, we will examine if patients with T2DM who are treated with lipid-lowering medications achieve the targets for LDL-C for prevention of a cardiovascular disease, as they are presented by the European Society of Cardiology (31). We will also examine if they meet the targets for non-HDL-C, HbA1C, BMI, waist circumference, physical activity and smoking.
2. Materials and methods
2.1. Study design and objective

Methods and results are reported according to the STROBE statement for observational studies. The STROBE checklist is presented in the supplementary material (paragraph 8.1.2.)

We performed a cross-sectional study to evaluate the achievement of LDL-C target levels for prevention of cardiovascular disease in patients with T2DM who are treated with lipid-lowering medication. Furthermore, we examined if they had achieved the targets for other factors (non-HDL-C, HbA1c, BMI, smoking, physical activity), which are associated with increased risk for developing a cardiovascular disease. The secondary objective of our study is to examine if there is an association between the levels of LDL-C and other variables (gender, years with diabetes, HbA1c, eGFR, BMI, waist circumference, smoking, physical activity). It was also examined the association of non-HDL-C with the same variables.

According to the European Society of Cardiology / European Atherosclerosis Society guidelines for management of dyslipidemias in 2016, the target levels of lipids and other risk factors for cardiovascular disease prevention, which were evaluated in our study, were the following, (31):

- LDL-C < 70 mg/dL for patients at very high risk for cardiovascular disease or LDL-C < 100 mg/dL for patients at high risk for cardiovascular disease. (Diabetic patients with target organ damage or with a major risk factor such as smoking, hypertension or dyslipidemia are at very high risk, whereas the other patients with DM are at high risk).
- Non-HDL-C secondary target for patients at very high risk is < 100 mg/dL and for patients at high risk is < 130 mg/dL.
- HDL-C: there is no target level, but levels > 40 mg/dL in men and > 48 mg/dL in women are associated with lower risk.
- Triglycerides: there is no target level, but levels <150 mg/dL are associated with lower risk.
- HbA1c < 7%.
- BMI 20 – 25 kg/m², waist circumference < 94 cm in men, and < 80 cm in women.
- Smoking: no exposure to tobacco in any form.
- Physical activity: 2.5-5 hours moderately vigorous physical activity per week or 30 – 60 minutes most days.
2.2. Setting

For this observational study, we collected data from patients with T2DM who were on lipid-lowering medication and visited the diabetes outpatient clinic at Hospital of Giannitsa. The patients who visit this diabetes outpatient clinic are residents of Giannitsa town or of a nearby area. Patients visit periodically the clinic for routine examination about every three months. For every patient, there is a file where his/her data are recorded. These data include personal data (name, year of birth, address, telephone number, year when diabetes was diagnosed, medical history), laboratory measurements (HbA1c, complete blood cell count, urea, creatinine, sodium, potassium, fasting glucose, transaminases, lipids, albumin, creatinine phosphokinase, lactate dehydrogenase, thyroid-stimulating hormone, urinalysis), other measurements (weight, blood pressure at the time of their visit), data about complications of DM (for example findings of funduscopic examination) and the list with their medications (for diabetes and for other diseases). The diabetes outpatient clinic accepts patients twice a week. Each time fast thirty patients have an appointment.

2.3. Participants

This cross-sectional study included consecutive patients who visited the diabetes outpatient clinic at Hospital of Giannitsa from 19th of July 2017 until 27th of September 2017 and were eligible for participating in the study. Every patient was included in the study only once. In case of having more than one visits of the same patient, only the first visit in this particular time period was taken into account.

To be eligible a patient to participate in the study should fulfil the following criteria:

A. They should have been diagnosed with T2DM at least 3 months before. The diagnosis of DM was based on the diagnostic criteria of ADA in 2017 (88). There was not any change in the diagnosis of DM based on previous diagnostic criteria by ADA (for example in 2010 or 2013) (89, 90). The criteria for diagnosis of DM are the following:
i. Fasting plasma glucose more than or equals to 126 mg/dl. For this examination is required no food administration for at least eight hours before blood sample collection.

ii. 2-hour plasma glucose more than or equals to 200 mg/dl during the performance of a 75-g OGTT. The OGTT should be performed as described by WHO, using a glucose load which is equal to 75 g anhydrous glucose. The OGTT is performed in the morning after fasting for at least 10 hours. During the last three days, the diet should be rich in carbohydrates (more than 150 grams carbohydrate per day). Smoking and any muscular effort is not permitted during the test. The contraindications for the test are: gastrectomy or enterectomy, any disease of gastrointestinal system which causes impaired resorption, any intercurrent disease or when the diagnosis of DM have already been established (91).

iii. HbA1C test more than or equals to 6.5%. The measurement of HbA1C should have been performed by the use of a method which has the certification of NGSP and has been standardized to the DCCT reference essay.

iv. A random plasma glucose more than or equals to 200 mg/dl in a patient with symptoms of DM or with hyperglycemic crisis.

For the first three criteria, in order to confirm the diagnosis, the test result should be repeated unless the diagnosis is clear on clinical basis (classic symptoms of hyperglycemia such as polyuria, polyphagia and polydipsia or a hyperglycemic crisis). The clinical features will help to distinguish between T1DM and T2DM. Patients will T1DM have usually one of the following: BMI less than 25 kg/m², age of diagnosis below 50 years, ketosis, fast weight loss, history of immune disease. When the classification is uncertain, the measurement of C-peptide or/and diabetes-specific antibodies titres could help to distinguish between the types of diabetes, according to the National Institute for Health and Care Excellence (NICE) guidelines (92, 93).

B. They should be treated with one or more lipid-lowering drug(s) for at least 3 months. A patient is eligible if he/she receives a lipid-lowering drug which has an effect on
decreasing the levels of LDL-C. These LDL-C-lowering drugs include: statin, ezetimibe, colesvelam, cholestyramine, PCSK9 inhibitors and nicotinic acid (niacin) (94). Colestipol, which is a bile acid sequestrant, is not available in Greece. A patient is also eligible when he/she is treated with a drug combination, for example statin with ezetimibe, if at least one of the drugs has an effect on lowering the LDL-C levels.

Patients who receive only fibrates or N-3 fatty acids are not eligible for the study.

C. They should have visited the diabetes outpatient clinic at hospital of Giannitsa between 19th of July until 27th of September and have recent results of lipid levels (LDL-C, total cholesterol, HDL-C, triglycerides) and HbA1c. The measurements of lipids levels and HbA1c should have been performed at the laboratory of hospital of Giannitsa. To be eligible, the measurements should have been performed the day of the visit or within the last two months or within the next 30 days after their visit. There is no age limitation for participating in the study.

The exclusion criteria for our cross-sectional study are the following:

i. Patients with T1DM or GDM.

ii. Pregnant women.

iii. Patients with missing data (without recent results of LDL-C, total cholesterol, triglycerides, HDL-C and HbA1c).

iv. Patients with triglycerides levels > 400 mg/dL, because the Friedewald formula, which is used for calculation of LDL-C levels, cannot be used (31).

The main eligibility criteria are summarized in the table 1.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with T2DM, treated with lipid-lowering medication for at least three months.</td>
<td>Patients with T1DM or GDM.</td>
</tr>
<tr>
<td>No age limitation.</td>
<td>Pregnant women.</td>
</tr>
<tr>
<td></td>
<td>Patients with inadequate data (missing data for lipids, HbA1c).</td>
</tr>
<tr>
<td></td>
<td>Triglycerides levels &gt; 400 mg/dL.</td>
</tr>
</tbody>
</table>

Table 1. Inclusion and exclusion criteria for selection participants to the study.
The patients were divided into two groups based on their total cardiovascular risk, as it is suggested by the European Society of Cardiology and European Atherosclerosis society (31):

- very high risk
- high risk

As patients of very high risk of cardiovascular disease were characterized those patients with target organ damage or a major risk factor (smoking, hypertension, dyslipidemia). As target organ damage caused by diabetes is characterized one of the following:

- diabetic retinopathy, estimated by the findings of funduscopic examination
- diabetic neuropathy, estimated by neurological examination
- established cardiovascular disease (history of stroke, myocardial infraction, angina, peripheral vascular disease)
- diabetic nephropathy, estimated by diminished estimated glomerular filtration rate (eGFR < 60 ml/min/1.73m² which is calculated by the Modification of Diet in Renal Disease (MDRD) equation) and microalbuminuria.

The diabetic nephropathy is estimated by decreased eGFR (less than 60 ml/min/1.73m²) and microalbuminuria (by the use of urinary albumin-to-creatinine ratio (mg/g) or the use of urinary albumin concentration (mg/l)) (95, 96). The eGFR was calculated by using a prediction equation. We used the MDRD formula because it is more accurate than Cockcroft Gault formula (97). So, in our study, a participant was characterized as having diabetic nephropathy, if he or she had eGFR < 60 ml/min/1.73m² and in presence of microalbuminuria (urinary albumin-to-creatinine ratio >= 30 mg/g Cr) (98). The microalbuminuria was measured by Alere Afinion AS100 Analyzer Menarini. We should have checked for the presence of microalbuminuria in case of having a patient without any other target organ damage or a major risk factor for cardiovascular disease, in order to decide in which risk group should be classified.

Hypertension is defined as systolic blood pressure equals or more than 140 mmHg or diastolic blood pressure equals or more than 90 mmHg after repeated examination (99). In our study, a patient was considered as hypertensive if he or she was on anti-hypertensive treatment or
if he/she had three different measurements of blood pressure in his/her house or in the outpatient clinic over the 140 mmHg or over 90 mmHg for the systolic and diastolic pressure retrospectively.

As dyslipidemia is defined in our study as having a previous level of non-HDL-C more than 160 mg/dl, LDL-C more than 130 mg/dl, triglycerides more than 150 mg/dl or HDL-C less than 40 mg/dl for men or less than 50 mg/dl for women, according to the National Lipid Association Recommendations for Patient-centered management of Dyslipidemia (100). Patients who were on lipid-lowering therapy because of having a history of dyslipidemia, not for prophylactic reasons, were considered as having dyslipidemia.

2.4. Variables

For collecting the data, there was a questionnaire for each patient, which was filled in by the primary investigator. The data that was collected with the questionnaire were the following:

- date of visit at the diabetes outpatient clinic
- serial number of the patient (1, 2, 3...)
- age (in years)
- gender (male / female)
- educational level (primary / secondary / tertiary)
- place of residence (Giannitsa / other)
- years of diabetes
- antilipidemic medication(s)
- antidiabetic medication(s)
- medical history, co-morbidities
- values of LDL-C (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), HDL-C (mg/dL), non-HDL-C(mg/dL), HbA1c (%), creatinine (mg/dL)
- eGFR (mL/min/1.73m²)
- weight (kg)
- height (m)
- BMI (kg/m²)
• waist circumference (cm)
• smoking (yes / no)
• physical activity (at least 2.5 hours every week or at least 30 minutes most days).

The lipid-lowering medications were classified into four categories according to the intensity of LDL-C reduction (101). These categories are:

a. Low-intensity cholesterol-lowering therapy (decrease LDL-C < 30%).
b. Mild-intensity cholesterol-lowering therapy (decrease LDL-C 30-49%).
c. High-intensity cholesterol-lowering therapy (decrease LDL-C 50-60%).
d. Very-high-intensity cholesterol-lowering therapy (decrease LDL-C > 60%).

The table below shows the classification of the lipid-lowering medication (101).

<table>
<thead>
<tr>
<th>Low-intensity cholesterol lowering therapy (reduction of LDL-C &lt; 30 %)</th>
<th>Mild-intensity cholesterol lowering therapy (reduction of LDL-C 30-49 %)</th>
<th>High-intensity cholesterol lowering therapy (reduction of LDL-C 50 – 60 %)</th>
<th>Very-high intensity cholesterol lowering therapy (reduction of LDL-C &gt; 60 %).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin 10 mg</td>
<td>Atorvastatin 10 - 20 mg</td>
<td>Atorvastatin 40 – 80 mg</td>
<td>Atorvastatin 40 – 80 mg + Ezetimibe 10 mg</td>
</tr>
<tr>
<td>Pravastatin 10 - 20 mg</td>
<td>Rosuvastatin 5 – 10 mg</td>
<td>Rosuvastatin 20 – 40 mg</td>
<td>Rosuvastatin 20 – 40 mg + Ezetimibe 10 mg</td>
</tr>
<tr>
<td>Lovastatin 10 – 20 mg</td>
<td>Simvastatin 20 – 40 mg</td>
<td>Simvastatin 20 – 40 mg + Ezetimibe 10mg</td>
<td></td>
</tr>
<tr>
<td>Fluvastatin 40 mg</td>
<td>Pravastatin 40 mg</td>
<td>Pravastatin 40 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td>Pitavastatin 1 mg</td>
<td>Lovastatin 40 mg</td>
<td>Lovastatin 40 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td>Ezetimibe 10 mg</td>
<td>Pitavastatin 2 – 4 mg</td>
<td>Fluvastatin 80 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluvastatin XL 80 mg</td>
<td>Pitavastatin 2 – 4 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pitavastatin 1 mg + Ezetimibe 10 mg</td>
<td>Atorvastatin 10 – 20 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Simvastatin 10 mg + Ezetimibe 10 mg</td>
<td>Rosuvastatin 5 – 10 mg + Ezetimibe 10 mg</td>
<td></td>
</tr>
<tr>
<td>Pravastatin 20 mg + Ezetimibe 10 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluvastatin 40 mg + Ezetimibe 10 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovastatin 20 + Ezetimibe 10 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Categories of lipid-lowering therapy based on reduction of LDL-C levels.

This classification refers only to patients receiving statin, ezetimibe or the combination of both. Patients, if there were any, who received another LDL-lowering medication (for example colesevelam, cholestyramine, PCSK9 inhibitors or nicotinic acid) would be also categorized according to the percentage of LDL-C reduction, based on the findings of systematic reviews or randomized controlled trials. Specifically, it has been found, that PCSK9 inhibitors reduces LDL-C by 53.86% (95% confidence interval 49.08, 58.64) in comparison with placebo (102). So, PCSK9 inhibitors are categorized as high intensity cholesterol lowering therapy. Colesevelam was found according to the results of three double-blind randomized controlled studies (103, 104, 105) to reduce LDL-C levels by 12.8% to 16.7% (in all of the three studies, p < 0.001). So, treatment with colesevelam will be characterized as low intensity cholesterol lowering therapy. It was also found that cholestyramine reduced the LDL-C level 12.6% in comparison with placebo (106). So, cholestyramine is categorized as low intensity cholesterol therapy. Niacin is also categorized as low intensity cholesterol therapy, because it was found to decrease LDL-C less than 30% (about 16.7%) (107).

The antidiabetic therapy was divided in the following three categories: non-insulin medication (which includes oral drugs or non-insulin injectable drugs), insulin and combination of both treatments.
2.5. Data sources / measurements

2.5.1. Clinical measurements

For estimating weight, every patient was weighed without shoes and wearing light clothes. For the measurement, it was used the same weighing scale. The brand of the used weighing scale was ADE.

The height was measured by a stable stadiometer (height rod). The brand of the stadiometer that we used to measure height of each patient was Seca. Each patient was barefoot during the measurement.

BMI was calculated based on the following formula:

\[ \text{BMI (kg/m}^2\text{)} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \]

In other words, the result comes from dividing weight in kilograms by the square of height in meters. According to WHO, for adults over 20 years old, there are four categories of nutritional status based on BMI (108):

i. Underweight (BMI under 18.5 kg/m²)
ii. Normal weight (BMI 18.5 – 24.9 kg/m²)
iii. Pre-obesity (BMI 25 – 29.9 kg/m²)
iv. Obesity (BMI >= 30 kg/m²). For this category, there are the following three subcategories:
   a. Obesity class I (BMI 30 – 34.9 kg/m²)
   b. Obesity class II (BMI 35 – 39.9 kg/m²)
   c. Obesity class III (BMI >= 40 kg/m²).

The waist circumstance was measured with a measuring tape. For the measurement, the patient removed the upper clothing until his/her waist and was in upright position. The measurement was performed in horizontal plane, in the middle between the lower ribs and the iliac crest (109).

2.5.2. Laboratory measurements

Lipid levels (total cholesterol, triglycerides, HDL-C, LDL-C) were measured in the laboratory at hospital of Giannitsa. The measurements performed by the AU680 Beckman Coulter analyzer.
An enzymatic assay is used for the quantitative determination of total cholesterol levels in human serum. More specifically, the enzymes cholesterol esterase, cholesterol oxidase and peroxidase take part in chemical reactions, which have as final result the creation of a chromophore from the cholesterol esters. The levels of cholesterol are identified by spectrophotometric measurement. The specimens of patients’ blood are serum and plasma with ethylenediaminetetraacetic acid (EDTA) or plasma that is heparined and not hemolyzed.

An enzymatic essay is also used for the quantitative determination of triglycerides levels in the sample. Enzymes like lipases, glycerol kinase, glycerol phosphate oxidase, peroxidase participate in chemical reactions and the result is the formation of a chromophore, which is measured at 660/800 nm. The raise in absorption is proportional to the concentration of triglycerides of the sample. The types of the specimens are serum or plasma with EDTA or heparinized plasma.

For the qualitative determination of HDL-C levels, an enzymatic assay was also used. Cholesterol esterase, cholesterol oxidase and a chromogen system react with HDL cholesterol and produce a blue complex, which is measured at 600/700 nm. The increase in absorption is proportional to the concentration of HDL. The specimen that is used is serum or heparinized plasma (fasting or not).

Non-HDL-C is calculated from the total cholesterol minus HDL-C.

LDL-C is calculated using the Friedewald formula (1): $\text{LDL-C (mg/dL)} = \text{total cholesterol (mg/dL)} - \text{HDL} - (\text{triglycerides (mg/dL)})/5$. In case of high levels of triglycerides (more than 400 mg/dL), the above formula cannot be used for calculating LDL-C levels. It is also unreliable in case of non-fasting blood sample.

$\text{HbA}_{1c}$ was measured by Alere Afinion AS100 Analyzer Menarini by the brand Menarini.

The eGFR was calculated based on MDRD formula, based on age, serum creatinine, race and gender.
2.6. Bias

This type of observational study is prone to selection bias, because it included only the diabetic patients who visited the outpatient diabetes clinic of hospital of Giannitsa at a particular time period. To reduce the selection bias, we included in the study every consecutive patient who visited the clinic this period and fulfilled our eligibility criteria.

In this study, we tried to diminish the measurements errors by using the same measurement instrument for all participants and following the same way of measurement (for example measurement of weight with light clothes and without shoes).

The levels of HbA1c can be influenced by some factors (110). Iron or vitamin B12 deficiency, reduced erythropoiesis, increased erythrocyte life span, alcoholism can lead to increased HbA1c, whereas administration of vitamin B12, iron, erythropoietin or hemoglobinopathies can have as a result a reduced HbA1c. These factors affect the HbA1c levels and have as a result, false high or false low levels of HbA1c.

2.7. Study size

Before starting designing the study, we calculated the study size that should be collected using the OpenEpi Collection of Epidemiologic Calculators. The calculation of the required sample size was based on the population size and the anticipated frequency. According to the Greek electronic prescription in year 2014-2015, in Greece, the prevalence of DM, based on the prescription of medication, was 7% (720,764 individuals) (111). Prevalence of T1DM was only 0.24%. For Greek population, there are not adequate data on prevalence of dyslipidemia in diabetic patients. Based on a multicenter study in China, the prevalence of dyslipidemia in T2DM is 67.1% and the proportion of diabetic patient with dyslipidemia who receive treatment is 55.9%. Among the diabetic patients who are on lipid-lowering therapy, 39.4% achieve the LDL-C target less than 100 mg/dL and, for patients with previous cardiovascular disease, the percentage of patients who achieve LDL-C less than 70 mg/dL is 15.3% (112). Based on these data, the calculated sample size of our study is 199 (with confidence level 95%) and 86 (with confidence level 80%).
2.8. Ethics

For performing our study, we obtained the approval of scientific committee of the hospital of Giannitsa (protocol number: Giannitsa, 22-09-2017, Γ5β/οικ.8636). The questionnaires were filled in anonymously and after having the oral consent of each participant.

2.9. Statistical methods

The statistical analysis was performed by RStudio. The percentage of patients who achieved the targets of lipids and other risk factor targets for prevention of cardiovascular disease were calculated. For each qualitative variable, we calculated the number of cases and the relative frequency. For each continuous variable, we also calculated the mean and the standard deviation (SD) for the normally distributed variables and the median and the interquartile range (IQR) for the variables that were not normally distributed. We checked the normality by using the Kolmogorov Smirnov test. Chi-square tests of independence or Fisher exact tests were used to investigate if there is an association in achieving the targets of LDL-C or non-HDL-C between the participants with HbA1c < 7% and those with HbA1c >= 7%. Linear regression model was used to assess if there is any relationship between LDL-C and the other explanatory variables (age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking, physical activity). The same model will be used to assess the relationship between non-HDL-C and the same explanatory variables. The level of significance was set at 0.05.
3. Results
3.1. Patients’ characteristics

Ninety-six individuals were enrolled in this study. Males were 49% (n=47) and females were 51% (n=49). The mean age of the participants was 68.2 (SD = 9.8). The median of duration of DM was 10 years (IQR = 11). We divided the participants into three categories, based on the duration of DM. In the first group, the percentage of participants with duration of DM less than five years was 17.7% (n = 17), in the second group, the percentage of participants with duration between five and ten years was 40.6% (n = 39) and the last group, the percentage of participants with duration of DM more than ten years was 41.7% (n = 40). In figure 1, a bar chart is presented, based on the duration of DM.

![Bar chart of the duration of DM](image)

**Figure 1. Classification of the participants based on their duration of DM.**

Among participants, 29 (30%) were residents of Giannitsa, whereas 67 (70%) were residents of another town or of a village. The majority of the participants (83%, n = 80) completed primary education, whereas 14% of the participants (n = 13) and 3% of them (n = 3) completed secondary and tertiary education, respectively. The nutritional status of the participants according to the classification of WHO based on BMI (108) is presented in the figure 2. None of the participant were underweight. Among participants, eight were normal, 29 were pre-obese and 58 were obese.
Figure 2. Classification of the participants based on their BMI (normal, pre-obese and obese).

The number of participants who were treated only with a statin was 78 (81%), while 18 participants (19%) were treated with a combination of two LDL-lowering drugs. Four participants (4%) received also a fibrate, in addition to the LDL-lowering drug.

Based on the classification of LDL-lowering therapy according to the reduction of LDL levels, which is presented in the table 2, the participants were divided in the following three categories depending on the intensity of their LDL-lowering medication(s), as it is depicted in the figure 3. None of them received low-intensity LDL-lowering therapy. Seventy-five participants (78%) received mild-intensity LDL-lowering therapy, 20 participants (21%) were treated with high-intensity LDL-lowering therapy and one participant (1%) received very-high-intensity LDL-lowering therapy.
All of the participants were patients with T2DM who were treated with antidiabetic medications. Based on their antidiabetic therapy, they were categorized into the following three groups: patients who were treated with non-insulin medications (oral or injectable), patients who were treated with insulin and patients who were treated with the combination of insulin and non-insulin medications. The percentages of non-insulin-treated, insulin-treated and combination-treated patients were 58% (n = 56), 9% (n = 9) and 32% (n = 31), respectively. The classification is presented in the figure 4.
3.2. Targets for prevention of cardiovascular disease

All of the participants were at very-high total cardiovascular risk level.

The target level of LDL-C (< 70 mg/dL) for cardiovascular disease prevention was achieved by 27% of the participants (n = 26), whereas 73% of the participants (n = 70) did not meet the target. The above results are presented in the figure 5. The mean of LDL-C was 86.9 mg/dL (SD = 29.2). For the participants who achieved the LDL-C target, the mean of LDL-C was 53.2 mg/dL (SD = 10.5), while for those who did not achieve the target, the mean of LDL-C was 99.4 mg/dL (SD = 23.4).
Figure 5. Pie chart of achievement of the LDL-C target.

The secondary target for non-HDL-C (< 100 mg/dL) for cardiovascular disease prevention was achieved by 34% of the participants (n = 33), whereas the percentage of those who did not meet the target was 66% (n = 63). These results are presented in the figure 6. The median of non-HDL-C was 118 mg/dL (IQR = 38.3). For the participant who achieved the non-HDL-C target, the median was 83 (IQR = 21), while for those who did not achieve the target, the median of non-HDL-C was 125 (IQR = 27.5).
Figure 6. Pie chart of achievement of the non-HDL-C target.

The percentage of males who had HDL-C > 40 mg/dL was 43% (n = 20), while the percentage of females who had HDL-C > 48 mg/dL was 47% (n= 23), as they are presented in the figures 7 and 8. These levels of HDL-C (> 40 mg/dL in males and > 48 mg/dL in females) indicate lower risk for cardiovascular disease. The median HDL-C was 43 mg/dL (IQR = 15.5). For males, the median of HDL-C was 39 mg/dL (IQR = 11) and for females it was 48 mg/dL (IQR = 17). For males achieving HDL-C > 40 mg/dL, the median of HDL-C was 48.5 mg/dL (IQR = 13.3), whereas for females achieving HDL-C > 48 mg/dL, the median was 59 m/dL (IQR = 12.5).

Figure 7. Pie chart of achievement HDL-C > 40 mg/dL in males.
Figure 8. Pie chart of achievement HDL-C > 48 mg/dL in females.

Among participants, 56% (n = 54) had triglycerides levels less than 150 mg/dL, while 44% (n = 42) had triglycerides levels equal or more than 150 mg/dL, as it is depicted in the figure 9. The median for triglycerides was 141 mg/dL (IQR = 56.8). The median of triglycerides for participants with triglycerides < 150 mg/dL was 115.5 mg/dL (IQR = 38.5) and for those with triglycerides equal or more than 150 mg/dL, the median was 172.5 (IQR = 38.5).
The target for HbA1c (less than 7%) was achieved by 51% of the participants (n = 49), as it is shown in figure 10. The median of HbA1c was 6.9% (IQR = 0.93). For the participants who achieved the HbA1c target, the median was 6.6% (IQR = 0.5) and for the participants who did not achieve the target for HbA1c, the median was 7.6% (IQR = 1.55).
The percentage of non-smokers was 88% (n = 84), as it is presented in the figure 11.

![Pie chart of achievement of the target for smoking](image)

*Figure 11. Pie chart of achievement the target for smoking.*

The percentage of the participants who had moderately vigorous physical activity for 2.5 to 5 hours or at least 30 to 60 minutes at most days was 30% (n = 29).

![Pie chart of achievement of the physical activity target](image)

*Figure 12. Pie chart of achievement the physical activity target.*
The target level of BMI, that means BMI between 20 and 25 kg/m², was achieved by 8% (n = 8), as it is shown in figure 13. The percentage of participants with BMI more than 25 kg/m² was 92% (n = 88). None of the participants had BMI less than 20 kg/m². The mean BMI was 31.3 kg/m² (SD = 5.2). For participants achieving the target for BMI, the mean of BMI was 24 kg/m² (SD = 0.9), whereas for those not achieving the target for BMI, the mean of BMI was 32 kg/m² (SD = 4.9).

![Pie chart of achievement of the BMI target](image)

### Pie chart of achievement of the BMI target

<table>
<thead>
<tr>
<th>Achievement of the BMI target</th>
<th>Not achievement of the BMI target</th>
</tr>
</thead>
<tbody>
<tr>
<td>8%</td>
<td>92%</td>
</tr>
</tbody>
</table>

**Figure 13. Pie chart of achievement of BMI target.**

Waist circumference less than 94 cm had two males (4%). None of the females had waist circumference less than 80 cm. The mean waist circumference was 112.6 cm (SD = 12.3). Based on the gender, the mean waist circumference was 114.6 cm (SD = 14) for males and 110.7 cm (SD = 10.3) for females. For males achieving the target for waist circumference, the mean was 91.5 cm (SD = 0.7) and for males not achieving the target, the mean was 115.6 cm (SD = 13.4).
Figure 14. Pie chart of achievement of waist circumference target in males.

All the results for achieving the target levels for cardiovascular risk factors are summarized in the table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Target</th>
<th>Number of patients (Relative frequency (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>&lt; 70 mg/dL</td>
<td>26 (27)</td>
</tr>
<tr>
<td>Non-HDL-C</td>
<td>&lt; 100 mg/dL</td>
<td>33 (34)</td>
</tr>
<tr>
<td>HDL-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&gt; 40 mg/dL</td>
<td>20 (43)</td>
</tr>
<tr>
<td>Females</td>
<td>&gt; 48 mg/dL</td>
<td>23 (47)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt; 150 mg/dL</td>
<td>54 (56)</td>
</tr>
<tr>
<td>HbA1c</td>
<td>&lt; 7%</td>
<td>49 (51)</td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
<td>84 (88)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Yes</td>
<td>29 (30)</td>
</tr>
<tr>
<td>BMI</td>
<td>20 – 25 kg/m²</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Waist circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&lt; 94 cm</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Females</td>
<td>&lt; 80 cm</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 3. Summary of the number of participants who achieve the targets for prevention of a cardiovascular disease.
3.3. Assessing the association between achieving LDL-C or non-HDL-C target and HbA1c target.

To assess if there is an association between those who achieved the target of LDL-C and those who achieved the target of HbA1c, we performed the chi-square test of independence.

For the two categorical variables, we obtained the following frequency table (table 4):

<table>
<thead>
<tr>
<th></th>
<th>HbA1c target</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>8</td>
<td>26</td>
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<tr>
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<td>31</td>
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<tr>
<td>summary</td>
<td>49</td>
<td>47</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 4. The frequency table for the two categorical variables (LDL-C target, HbA1c target)

After performing the chi-square test of independence, we found out that there is an association between achieving LDL-C target and achieving HbA1c target (p = 0.0298 < 0.05).

To assess if there is an association between those who achieved the target of non-HDL-C and those who achieved the target of HbA1c, we performed again the chi-square test of independence.

The frequency table is presented below (table 5).

<table>
<thead>
<tr>
<th></th>
<th>HbA1c target</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>yes</td>
<td>no</td>
<td>summary</td>
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<tr>
<td>Non-HDL-C target</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>19</td>
<td>14</td>
<td>33</td>
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<tr>
<td>no</td>
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<tr>
<td>summary</td>
<td>49</td>
<td>47</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 5. The frequency table for the two categorical variables (non-HDL target, HbA1c target).

After performing the chi-square test of independence, we found out that there is not an association between achieving non-HDL-C target and achieving HbA1c target (p = 0.35 > 0.05).
3.4. Assessment of the association between LDL-C and other variables

We applied the linear regression analysis to assess the association between the quantitative variable LDL-C and the following nine variables: age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking and physical activity.

First, we ran a univariate analysis. Only the variables which had $p < 0.2$ in the univariate analysis were included in the multivariate analysis. These variables with $p < 0.2$ were: age, gender and HbA1c. The results of univariate and multivariate linear regression analysis of LDL-C on age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking and physical activity are summarized in the table 6.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted β</th>
<th>95% CI</th>
<th>p-value</th>
<th>Adjusted β</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>-0.407</td>
<td>(-1.016, 0.201)</td>
<td>0.187</td>
<td>-0.316</td>
<td>(-0.933, 0.3)</td>
<td>0.311</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Ref.</td>
<td>Ref.</td>
<td>NA</td>
<td>Ref.</td>
<td>Ref.</td>
<td>NA</td>
</tr>
<tr>
<td>Male</td>
<td>9.979</td>
<td>(-1.761, 21.719)</td>
<td>0.095</td>
<td>9.357</td>
<td>(-2.322, 21.036)</td>
<td>0.115</td>
</tr>
<tr>
<td><strong>Duration of diabetes (years)</strong></td>
<td>-0.431</td>
<td>(-1.152, 0.29)</td>
<td>0.238</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>4.706</td>
<td>(0.25, 9.161)</td>
<td>0.039</td>
<td>3.783</td>
<td>(-0.808, 8.373)</td>
<td>0.105</td>
</tr>
<tr>
<td><strong>eGFR (mL/min/1.73m²)</strong></td>
<td>-0.003</td>
<td>(-0.216, 0.21)</td>
<td>0.977</td>
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<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>-0.06</td>
<td>(-1.21, 1.089)</td>
<td>0.917</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>-0.036</td>
<td>(-0.521, 0.448)</td>
<td>0.882</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Ref.</td>
<td>Ref.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Physical activity</td>
<td>β</td>
<td>CI</td>
<td>P</td>
<td>Ref.</td>
<td>Ref.</td>
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</tr>
<tr>
<td>-------------------</td>
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<td>--------</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>----</td>
</tr>
<tr>
<td>Yes</td>
<td>2.321</td>
<td>(-15.685, 20.328)</td>
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<tr>
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<td>Ref.</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Yes</td>
<td>2.042</td>
<td>(-10.925, 15.009)</td>
<td>0.755</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

| β | coefficient of explanatory variable, CI: Confidence Interval, NA: not applicable |

Table 6. Results of univariate and multivariate linear regression analysis of LDL-C on age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking and physical activity.

Based on the results of the multivariate linear regression analysis, none of the variables is significantly associated with the variable LDL-C (all p-values > 0.05), adjusted for the other variables. In the univariate analysis, the variable HbA1c was positively associated with the variable LDL-C and this association is statistically significant (p = 0.039, CI: 0.25, 9.161). The interpretation of this result is that for every 1% increase in HbA1c, LDL-C is increased by about 4.7 mg/dL (p = 0.039 < 0.05), but this change is not significant when adjusting for the variables age and gender (p = 0.105 > 0.05).

3.5. Assessment of the association between non-HDL-C and other variables

In order to assess the association between the non-HDL-C and other variables (age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking and physical activity), we applied again the linear regression analysis. The variables gender, duration of diabetes and HbA1c that had p < 0.2 in the univariate analysis were included in the multivariate analysis. The results of this univariate and multivariate linear regression analysis are presented in the table 7.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Multivariate</th>
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<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>-0.373</td>
<td>(-1.072, 0.326)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Male</td>
<td>10.516</td>
<td>(-2.955, 23.987)</td>
</tr>
<tr>
<td><strong>Duration of diabetes (years)</strong></td>
<td>-0.587</td>
<td>(-1.41, 0.236)</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>-4.179</td>
<td>(-0.969, 9.327)</td>
</tr>
<tr>
<td><strong>eGFR (mL/min/1.73m²)</strong></td>
<td>-0.109</td>
<td>(-0.351, 0.134)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>0.306</td>
<td>(-1.008, 1.621)</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>0.055</td>
<td>(-0.499, 0.61)</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Yes</td>
<td>1.881</td>
<td>(-18.736, 22.498)</td>
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<tr>
<td><strong>Physical activity</strong></td>
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<td></td>
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<tr>
<td>No</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Yes</td>
<td>2.28</td>
<td>(-12.565, 17.125)</td>
</tr>
</tbody>
</table>

β: coefficient of explanatory variable, CI: Confidence Interval, NA: not applicable

Table 7. Results of univariate and multivariate linear regression analysis of non-HDL-C on age, gender, duration of diabetes, HbA1c, eGFR, BMI, waist circumference, smoking and physical activity.
Based on the results of the linear regression analysis, the variables duration of diabetes and HbA1c are significantly associated with the variable non-HDL-C ($p < 0.05$), adjusting for the three variables of the multivariate analysis. More specific, it was found that for every one year increase in the duration of diabetes, there is an expected significant decrease in non-HDL-C about 0.88 mg/dL ($p = 0.042$, CI: -1.72, -0.03), adjusting for gender and HbA1c. Furthermore, for every 1% increase in HbA1c, there is an expected significant increase in non-HDL-C of about 5.52 mg/dL, adjusting for gender and duration of diabetes ($p = 0.042$, CI: 0.196, 10.849).
4. Discussion
4.1. Principal findings

Cardiovascular disease is an important cause of mortality for diabetic patients (113). Except for diabetes itself, dyslipidemia is a major risk factor for cardiovascular disease and often coexists with DM (113). Our cross-sectional study assessed the management of dyslipidemia in patients with T2DM. Furthermore, it was examined if the diabetic patients achieved the targets of other risk factors for prevention of a cardiovascular disease, based on the guidelines of the European Society of Cardiology and European Atherosclerosis Society for management of dyslipidemias in 2016 (31).

Our findings indicate that the percentage of patients with T2DM who were on lipid-lowering therapy and met the target for LDL-C less than 70 mg/dL is low (27%). It means that fast three out of every four patients did not achieve the target for LDL-C in order to reduce the risk for a cardiovascular event in the future, although they received antilipidemic therapy. Given that the LDL-C level is a strong predictor of cardiovascular disease in diabetic patients (28), even in presence of the characteristic diabetic dyslipidemia (consisting of high triglycerides and low HDL-C), we can understand the importance of maintaining the levels of LDL-C less than 70 mg/dL.

The secondary target for non-HDL-C less than 100 mg/dL was achieved by only 34% of the participants. The majority of the patients with T2DM (about two-thirds of them) had levels of non-HDL-C higher than the target. Although LDL-C levels is the primary target for managing dyslipidemia, non-HDL-C is also an important target because it represents the content in cholesterol of all atherogenic lipoproteins (LDL, IDL and VLDL) (114).

Less than the half of the participants achieved the desired level of HDL-C (more than 40 mg/dL for males and 48 mg/dL for females), which indicates lower possibility for developing a cardiovascular disease. The percentage of females who had HDL-C above the limit was higher than males (47% and 43%, respectively). The desired level of triglycerides (less than 150 mg/dL) was achieved by more than the half of the patients (56%). The management of triglycerides and HDL-C is also important, because the elevated triglycerides to HDL-C ratio is associated with higher risk for developing a cardiovascular disease (115, 116).

About half of the diabetic patients (51 %) achieved the target for HbA1c (less than 7 %). The proper glycemic control is of great importance for the diabetic patients, because high HbA1c
levels and the chronic hyperglycemia are associated with increased risk for cardiovascular diseases and mortality (117, 118).

The majority of the participants (88 %) were non-smokers. The exposure in cigarette smoke is associated with increased risk for coronary artery disease and myocardial infraction (119). Cigarette smoking via oxidative stress leads to atherothrombotic disease and cardiovascular dysfunction (119). Cessation of smoking is very important for the prevention of a cardiovascular disease.

According to our study, only 30 % of the participants achieved the goal for the physical activity (at least 2.5 hours every week or half an hour for the most days of the week). The percentage of participants with BMI 20 – 25 kg/m² was very disappointing (only 8%). The rest of the participants had BMI above the target level. The percentage of participants with waist circumference within the target level, was extremely low. Only 4% of diabetic men had waist circumference less than 94 cm and none of the diabetic females had waist circumference less than 80 cm. Lack of physical activity and obesity are associated with increased cardiovascular mortality (120). In patients with T2DM, physical activity is not only associated with reduced risk of cardiovascular disease and mortality (121), but also with reduction in HbA1c (122). The reduction in HbA1c is greater with structured physical activity of more than 150 minutes per week than with physical activity of less duration (122). Obesity is associated with increased risk of cardiovascular disease and may have an effect on atherosclerosis (123). Abdominal obesity, which is assessed by waist circumference, is also strongly associated with cardiovascular disease, even in patients with not elevated BMI (124). Therefore, encouraging patients with T2DM for participating in physical activity and for losing weight is of great importance.

Based on the results from our statistical analysis, there is an association of achieving the LDL-C target and HbA1c target. In our univariate analysis, LDL-C levels are significantly positively associated with HbA1c levels (p = 0.039), but there is not a statistically significant association in the multivariate analysis (p = 0.105). In the multivariate analysis, it is also found, that non-HDL levels are significantly positively associated with HbA1c levels (p = 0.042) and negatively associated with the duration of DM (p = 0.042). In conclusion, the proper glycemic management is important, because HbA1c is one of the targets for prevention of a cardiovascular disease and may be also associated with the lipid management.
4.2. Limitations of the study

Our study has some limitations. The calculated sample size that was needed for our study was 199 (with confidence level 95 %) and 86 (with confidence level 80 %). In our study, which took place from 19th of July 2017 until 27th of September 2017, the eligible participants were 96. Our sample size was less than the calculated one (in order to have confidence level 95 %), but it is satisfying for having confidence level 80 %. In our study, we recruited only patients who visited the diabetes outpatient clinic of hospital of Giannitsa at specific dates (from 19th of July until 27th of September 2017). Maybe the results would be different if we had expanded our study, including patients that visited other outpatient diabetes clinics or private doctors. In our study we did not assess if the patients with T2DM achieved the target of blood pressure for cardiovascular disease prevention. The reason for not including blood pressure to the examined risk factors was that the measurement of blood pressure should be performed at least in two separate visits in the outpatient clinic, in order to make the conclusion that a patient have elevated blood pressure, above the target (125). But, in our study we took in account only one visit (the first one) of every patient. Furthermore, due to the white coat effect, the measured blood pressure in the outpatient diabetes clinic could be false higher (126). Due to these bias in blood pressure measurement, we decided that we would not include blood pressure into the examined risk factors.

4.3. Comparison of the main result with the existing literature.

In our study, LDL-C target level (less than 70 mg/ dL) were achieved by 27% of the participants that were on lipid-lowering medication, whereas in a similar cross-sectional study in Chinese patients with T2DM performed by Yan et al. (112), the LDL-C target (less than 70 mg/dL) of those who received lipid-lowering therapy, was achieved by only 15.3 %. This percentage is smaller than in our study, perhaps due to the different ethnicity or the much larger sample size of the study in China. Kennedy et al. also demonstrated in their study (127), that only 15.7 % of very high risk diabetic patients had LDL-C less than 70 mg/dL. This percentage is also quite lower in comparison with our study’s result. Mithal et al., in a cross-sectional study in Indian diabetic patients (128), found out that the percentage of the diabetic patients with overt cardiovascular risk, who had LDL-C less than 70 mg/dL, was 22.87 %.
4.4. Implications for practice and research

Effective management of dyslipidemia, a modifiable risk factor, in patients with T2DM is very important for preventing cardiovascular disease. Based on the results of our study, the percentages of patients with T2DM, who achieved the targets for LDL-C and non-HDL-C, are low (27% and 34%, respectively), although they received lipid-lowering medications. Therefore, the lipid-lowering therapy should be more intensive, in order to decrease the levels of LDL-C and non-HDL-C and diminish the risk for cardiovascular disease. Furthermore, the patients should be encouraged to make lifestyle modifications (like restriction of saturated fat intake) in order to improve their lipid profile.

Proper glycemic control is also of great importance for diabetic patients. In our study, fast the half of the patients (51%) had HbA1c less than 7%. Therefore, the antidiabetic therapy should be intensified to achieve the glycemic target, but taking into account the comorbidities, the life expectancy and the risk of hypoglycemia for each patient (129). Modifications in diet are also necessary for better glycemic control.

According to the results of our study, the percentage of the diabetic patients who have the ideal BMI and waist circumference for reducing the risk for developing a cardiovascular disease is extremely low. This means, that it is urgent to encourage patients to lose weight. Except for interventions in diet, there are some antidiabetic drugs (such as metformin, glucagon like peptide 1 receptor agonist or sodium-glucose cotransporter 2 inhibitors) that promote weight reduction (130, 131, 132). These medications could have a favorable effect on obese patients. Moreover, we should also focus on physical activity, because only 30% of the participants in our study reported that participate in moderately vigorous physical activity.

This cross-sectional study included only a small number of patients with T2DM that received antilipidemic medications. In the future, a multi-center study in Greece with greater sample size could be performed, in order to examine if the patients with T2DM achieve the lipid targets and the other risk factor targets for prevention of a cardiovascular disease. And if the percentages of the diabetic patients who meet the targets are low, then a more intensive approach is needed, in order to reduce the risk of developing a cardiovascular disease in patients with T2DM.
4.5. Funding

This study received no funding to be carried out.
5. Conclusions
5.1. Main conclusions

- Cardiovascular disease is an important cause of mortality for diabetic patients.
- Dyslipidemia is a major risk factor for developing atherosclerotic cardiovascular disease.
- Twenty seven percent of patients with T2DM who were on lipid-lowering therapy achieved the target for LDL-C (less than 70 mg/dL).
- The percentage of the participants who met the target for non-HDL-C (less than 100 mg/dL) was 34%.
- HDL-C more than 40 mg/dL had 43% of the men and more than 48 mg/dL had 47% of the women.
- Triglycerides less than 150 mg/dL had 56% of the participants.
- HbA1c less than 7% was achieved by 51% of the participants.
- Non-smokers were 88% of the patients.
- Thirty percent of the patients with T2DM were participating in physical activity.
- BMI between 20 and 25 kg/m² was achieved by 8% of the participants, whereas the rest of them were above of it.
- Waist circumference less than 94 cm had 4% of men. No woman had waist circumference less than 80 cm.
- There was found that there is an association between achieving LDL-C target and HbA1c target (p = 0.03).
- In the univariate analysis, HbA1c levels was significantly positively associated with LDL-C levels (p = 0.039), but this association was not significant in the multivariate analysis, adjusted for age and gender (p = 0.105).
- In the multivariate analysis, non-HDL levels are significantly positively associated with HbA1c levels (p = 0.042) and negatively associated with the duration of DM (p = 0.042).
- The lipid and glycemic management of patients with T2DM should be more intensive.
- The patients with T2DM should be more encouraged to lifestyle modifications (such as losing weight, physical activity).
6. Abstract

Introduction

Patients with DM are at increased risk for developing a cardiovascular disease. In these patients, the proper lipid and glycemic management is essential to reduce the risk.

Aim

To examine if patients with T2DM who are treated with lipid-lowering medications achieve the LDL-cholesterol (LDL-C) target level and other cardiovascular risk factor targets for prevention of a cardiovascular disease.

Material and methods

A cross-sectional study was carried out in the diabetes outpatient clinic at Hospital of Giannitsa from 19th of July until 27th of September 2017. A total of 96 patients with T2DM who were on lipid-lowering therapy were included in the study. The main cardiovascular risk factors that were examined were: LDL-C, non-HDL-C, HbA1c, BMI, waist circumference, physical activity and smoking. Laboratory measurements were used to assess the lipids and HbA1c levels. Weight, height and waist circumference of each participant were measured.

Results

Out of 96 patients, males were 49%. The percentage of the participants who achieved LDL-C less than 70 mg/dL and non-HDL-C less than 100 mg/dL was 27% and 34%, respectively. HbA1c less than 7% was achieved by 51%. Non-smokers were 88%. The target of physical activity was achieved by 30%. The target for BMI (between 20 and 25 kg/m²) was achieved by 8%, whereas the target for waist circumference less than 94 cm was met by 4% of men and less than 80 cm by none of the women.

Conclusions

This study has revealed that the percentage of patients with T2DM that achieved the target for lipids is low, although they received lipid-lowering medications. More intensive antilipidemic therapy and lifestyle modifications for patients with T2DM are needed, to reduce the risk for developing a cardiovascular disease.
7. References


42. Statins: are they wonder drugs? Br J Cardiol. 2014; Available from: http://bjcardio.co.uk/2014/03/statins-are-they-wonder-drugs/


92. NICE guideline. Type 1 diabetes in adults: diagnosis and management | Guidance and guidelines | NICE. 2015; Available from: https://www.nice.org.uk/guidance/ng17/chapter/1-Recommendations#diagnosis

93. NICE Guideline N 18. NCC for W and CH (UK). Diabetes (Type 1 and Type 2) in Children and Young People: Diagnosis and Management. 4 Diagnosis of diabetes. National Institute for Health and Care Excellence (UK); 2015.


Blackburn H KASE et al. The Lipid Research Clinics Coronary Primary Prevention Trial Results. JAMA [Internet]. 1984 Jan 20;251(3):351.


Body mass index - BMI. 2017 Sep 10; Available from: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi


Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus; Available from: http://www.who.int/cardiovascular_diseases/report-hba1c_2011Edited.pdf


## 8. Appendix

### 8.1. Supplementary material

#### 8.1.1. Questionnaire

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<tr>
<td>Duration of DM (years)</td>
<td></td>
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<tr>
<td>Residency (Giannitsa/other)</td>
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</tr>
<tr>
<td>Educational level (primary/secondary/tertiary)</td>
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<table>
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<td>Waist circumference (cm)</td>
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<td>Smoking (yes/no)</td>
<td></td>
</tr>
<tr>
<td>Physical activity (yes/no)</td>
<td></td>
</tr>
</tbody>
</table>

| LDL-C (mg/gL)                |       |
| Total cholesterol (mg/gL)    |       |
| HDL-C (mg/gL)                |       |
| Triglycerides (mg/gL)        |       |
| Non-HDL-C (mg/gL)            |       |
| Creatinine (mg/dL)           |       |
| eGFR (mL/min/1.73m²)         |       |
| HbA1c (%)                    |       |
8.1.2. STROBE checklist for methods and results

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<td><strong>Methods</strong></td>
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</tbody>
</table>