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# Correlation between hemoglobin A1c and lipid profile in Bosnian diabetic patients - gender differences

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**Abstract:** The prevalence of Type 2 *diabetes mellitus* (T2D) as a metabolic disease is rapidly rising worldwide. The purpose of this study was to determine concentrations of hemoglobin A1c (HbA1c), fasting glucose, and lipid profile in a total of 104 adults, 24 patients with newly diagnosed Type 2 diabetes (40-60 years of the age), 40 Type 2 diabetes, and 40 healthy subjects as control group (the same ages). On the basis of these results, we were able to assess the differences due to gender and age in tested population as well as the relationship between glycemic control (HbA1c) and serum lipid profile. Therefore, we properly evaluated the importance of HbA1c as an indicator of dyslipidemia in patients with T2D in selected Bosnian population. Hemoglobin A1c was determined by immunoturbidimetric assay, while fasting glucose and lipid profile were analyzed according to standard clinical methods on BT PLUS 2000-Biotechnic Instruments Bioanalyzer. We found that glycated hemoglobin concentrations in newly diabetic subjects were higher than those in other two groups. Statistically significant differences between study populations were seen at the level of glucose, cholesterol, HDL and LDL cholesterol, HbA1c, triacylglycerol and patient age. Also, our results have shown the significant negative correlation between HbA1c and cholesterol and HDL levels ($p^{***}<0.001$) while positive correlation was observed with glucose and patient age ($p^{***}<0.001$) in all study groups. According to our results, hemoglobin A1c can also be used as a predictor of dyslipidemia and thus early diagnosis of dyslipidemia can be used as a preventive measure for the development of cardiovascular diseases in patients with Type *2 diabetes mellitus.*

## INTRODUCTION

Diabetes mellitus (DM) represent a chronic metabolic disease which was accompanied by hyperglycemia, either because the body does not produce enough insulin, or cells do not respond to the insulin. As a disease, it is characterized with acute and chronic complications, which contributed to diabetes morbidity and mortality rates. Chronically elevated glucose is an important etiologic factor for macro and microvascular complications and the diagnostic feature of DM.

For a long time, HbA1c was used as a reliable marker in glycaemia control which correlated well with the risk of long-term diabetes complications (Kautzky-Willer A., 2015; IDF Recommendations, 2017). In 2010, the American Diabetes Association (ADA) recommended the use of HbA1c as a diagnostic marker for diabetes and categories for increased risk of diabetes (formerly known as prediabetes) (Reynolds et al., 2006; Lyons et al., 2012;

Gupta et al., 2017). Persons with HbA1c of 6.5% and above are to be diagnosed as diabetes and those with HbA1c between 5.7-6.4% are considered to be in categories for increased risk of diabetes (Paterson A. D, 2017; Sherwani et al., 2016; Sequeira and Poppitt, 2017). HbA1c levels differ for different diabetes patients, depending on their history of diabetes and whether they are on tablets or long-term and/or short-term insulin dosage (Cheneke et al., 2016; International Diabetes Federation, 2017; Xu et al., 2014).

Numerous studies have shown that patients with Type 2 diabetes (T2DM) have an increased prevalence of dyslipidemia, which contributes mostly to a high risk of cardiovascular diseases (CVDs) in these patients. In all of these studies, HbA1c proved to be independent risk factor for CV disease. Moreover, for each 1% rise in HbAc levels, there is 18% rise in cardiovascular risk. The same type of risk was reported even when HbA1c concentration were within normal range (Syed and Khan, 2011; VinodMahato et al., 2011). Since, the data related to diagnostic significance of hemoglobin A1c and lipidemia status are often contradictory and lacking for different world populations, in this work we tried to evaluate the importance of hemoglobin A1c as an indicator of dislipidema in selected Bosnian male and female population. The effect of age influence was also evaluated.

## EXPERIMENTAL

### *Subjects*

A total of 104 participants (40 controls, 24 newly diagnosed T2D patients and 40 diabetic) have been screened for plasma lipid profile, fasting plasma glucose (FPG), and hemoglobin A1c (HbA1c) after obtaining informed consent. Participants involved in this study were free of evidence of hepatitis B or C viral infection or active liver and kidney damage, and were selected on the basis of presence of history of diabetes mellitus type 2 (T2D) for more than five years. Initial diagnosis of T2D was established by a specialist of internal medicine who used World Health Organization (WHO) criteria for diagnosis of the disease accompanied by classical symptoms such as polyuria, polydipsia, polyphagia as well as a weight loss of over 10 kg or glycemia levels more than 11.1 mmol/L obtained after performing the oral glucose tolerance (GT) test. Newly diagnosed T2D patients were between 40-80 years old and with a recent diagnosis of T2D (less than 12 weeks or 6 months) and were not taking any medication i.e. were without therapy. Nondiabetic (controls, C) were of approximately same age (40-80 years old), with normal GT (fasting plasma glucose less than 6.2 mmol/L and two hours postprandial glycemia less than 7.8 mmol/L). All research involving human subjects and material derived from it in this study was done in accordance with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000) and write consistent of participants.

### *Sample Analysis*

Blood samples were withdrawn by using sterile syringe from 12 to 14 hours of overnight fasting diabetic patients and control subjects in the morning. All samples, after collection in sterile tubes were centrifuged at 3000 rpm for 10 minutes and serum was stored at 4°C. Fasting blood glucose concentration was measured by an enzymatic glucose hexokinase method, lipid profile analyzed by standard methods, while immunoturbidimetric assay was used for measurement of HbA1c. Glucose, lipids and HbA1c were measured on BT PLUS 2000-Biotechnic Instruments Bioanalyzer (Rome, Italy).

### *Principle of immunoturbidimetric assay for HbA1c*

There is a number of techniques that can be used to measure hemoglobin A1c because different and various factors may affect the accuracy of HbA1c measurements i.e. contribute older age, female gender, duration diabetes and others.

Briefly, immunoturbidimetric method used in this protocol, utilizes the interaction of antigen and antibody to directly determine HbA1c in whole blood. Mouse antihuman A1c monoclonal antibodies are added to whole blood, latex A1c antihuman A1c antibody complex is formed. Agglutination occurs when goat anti-mouse immunoglobulin G (IgG) polyclonal antibody interacts with the monoclonal antibody. After that, the amount of agglutination is measured as absorbance is proportional to the amount of A1c absorbed onto the surface of latex particles. One dried blood spot corresponding to 8 µl of blood was punched out and dispensed in 400 µl of hemolysis reagent, mixed well, and kept at room temperature for 30 min for complete lysis. In addition, the hemolysate was incubated with latex reagent followed by the addition of antibody reagent containing mouse antihuman A1c antibody and goat anti-mouse IgG polyclonal antibody in glycine buffer and further incubation followed by measurement of absorbance at 600 nm. For this analysis, hemolysate was prepared by incubating 10 µl of blood with 500 µl of hemolysis reagent for 10 min, and the measurement carried out on a BT PLUS Autoanalyzer.

### *Statistical analysis*

All statistical analyses were done by SPSS (version 17.0 for Windows, SPSS Inc; Chicago, IL, USA). *P* values smaller than 0.05 were accepted as significant. The compare means of the differential values between each of biochemical parameters and HbA1c was done by ANOVA test. Within the program, nonparametric Mann-Whitney U-test was used in order to estimate differences in glucose, hemoglobin A1c, and lipid concentration between groups. Spearman's correlation coefficient was calculated in order to analyze the relationships between the study variables.

## RESULTS AND DISCUSSION

The study was conducted on 104 participants of both genders, ranging from 35 to 65 years. In our study, HbA1c concentrations, in newly diagnosed T2D patients of both sexes were slightly higher when compared to T2D subjects (7.2% and 6.8, respectively) which is represented in Figure 1a and 1b. This finding is expected because

these patients were not taking any medications (without therapy) and correlates with the results of Lin L-K. et al., 2017; Paterson A. D., 2017; Sherwani et al., 2016. The concentrations of FPG and HbA1c, as expected, were significantly higher in newly diagnosed diabetics and T2D group of patients of both sexes, compared to Controls (Figures: 1a, 1b, 2a, 2b, 3a and 3b). Results for tested clinical parameters for all study participants are presented in Table I.

**Table I.** Clinical and biochemical characteristics in study patients

| Parameter | Newly diagnosed Type 2 diabetics | Type 2 diabetics | Controls |
|---|---|---|---|
| Number | 24 | 40 | 40 |
| Gender (M/F) | 14/10 | 19/21 | 9/31 |
| Glucose, mmol/L | 8.20±0.29 | 9.91±0.43 | 5.28±0.10 |
| TAG, mmol/L | 2.08±0.15 | 2.61±0.22 | 2.29±0.18 |
| Total cholesterol, mmol/L | 4.53±0.20 | 5.32±0.13 | 5.82±0.17 |
| HDL-C, mmol/L | 1.17±0.13 | 1.09±0.07 | 1.72±0.05 |
| LDL-C, mmol/L | 2.47±0.19 | 2.97±0.13 | 3.07±0.17 |
| HbA1c, % | 7.2±0.1 | 6.8±0.2 | 4.5±0.1 |

*data presented as mean±SEM; Triacylglycerols, TAG; High-density lipoproteins, HDL-C; Low-density lipoproteins, LDL-C

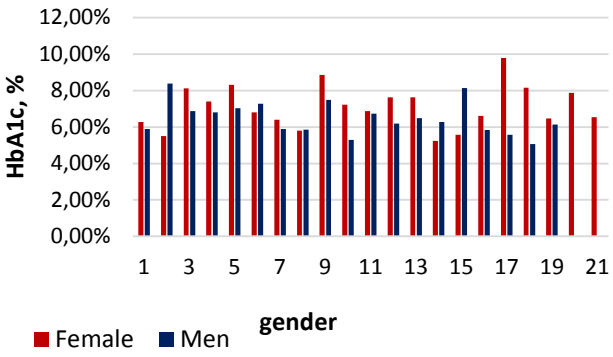


**Figure 1a.** Concentration of HbA1c in T2D groups by gender

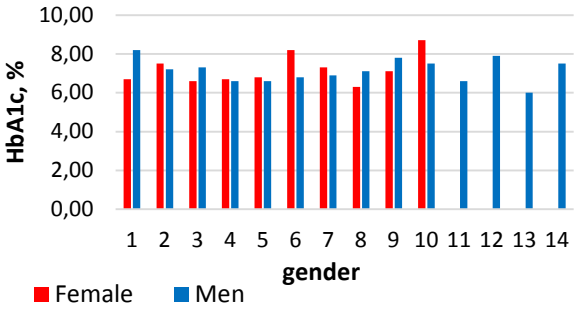


**Figure 1b.** Concentration of HbA1c in ND-T2D groups by gender

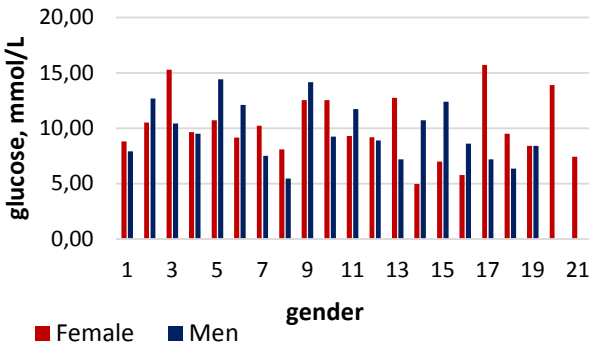


**Figure 2a.** Concentration of glucose in T2D groups by gender

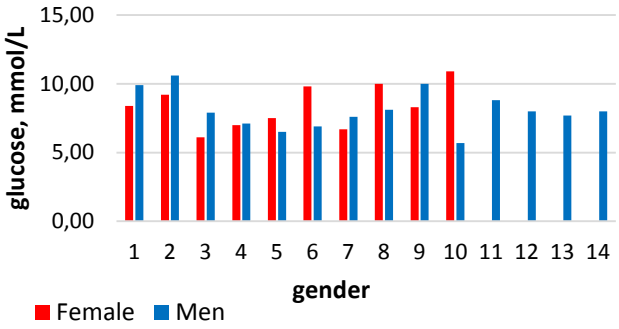


**Figure 2b.** Concentration of glucose in ND-T2D groups by gender

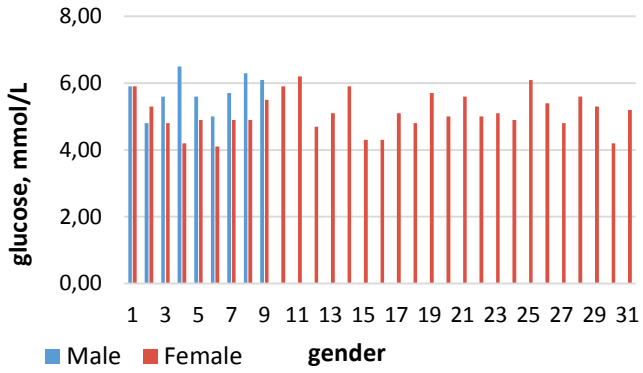


**Figure 3a.** Concentration of glucose in Control groups by gender

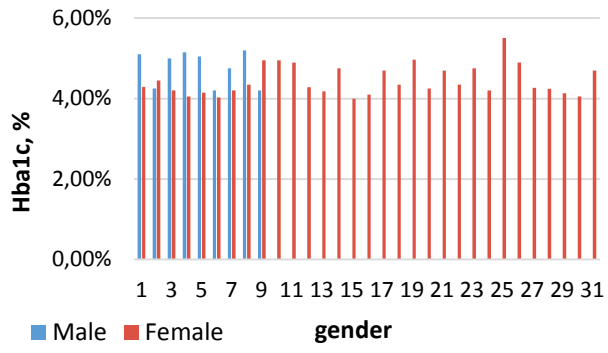


**Figure 3b.** Concentration of HbA1c in Control groups by gender

Numerous studies showed that the levels of HbA1c are strongly correlated with FPG and lipid concentrations (Barua et al., 2014; Cheneke et al., 2016; Hussain et al., 2017), as observed in our results (Figures 4, 5, and 6). This means that apart from a reliable glycemic control, HbA1c can also be used as a predictor of dyslipidemia. Early diagnosis of dyslipidemia can be used as a preventive measure for the development of vascular complications in both, patients with T2D and newly diagnosed patients (Lyons et al., 2012; Martins et al., 2012). In this work, positive correlation between hemoglobin A1c and total cholesterol (p**<0.01) and negative correlation between HDL-C levels and HbA1c (p***<0.001) was observed. As expected, HbA1c and glucose levels showed strong positive correlation (p***<0.001). The same type of correlation was obtained for patient age and concentrations of HbA1c (Figure 7), result reported in numerous other publications (Syed and Khan, 2011; Martins et al., 2012; Hussain et al., 2017; Vijayakumar et al., 2017).

With aging, the function of pancreatic islets gradually declines, tissue sensitivity to insulin and insulin receptor activity slowly decreases, while muscle tissue gradually reduces, and the consumption of glucose generally decreases. Under the combined effect of all of these factors, blood glucose increases with age incrementally, so that the HbA1c levels are also elevated, especially with increase age. These observations were confirmed in our study (p***<0.001). When diabetic patients were further classified on the basis of gender, statistically significant differences between groups were seen at the level of glucose, cholesterol, HDL and LDL cholesterol, triacylglycerols, HbA1c, and patient age (Table II). Similar results were reported by Innoke et al., 2012; Kautzky-Willer et al., 2015; Ma Q et al., 2016. Interestingly, we showed statistically significant positive correlation between hemoglobin A1c levels with glucose and patient age in both genders (Figures: 8, 9, 10, and 11).

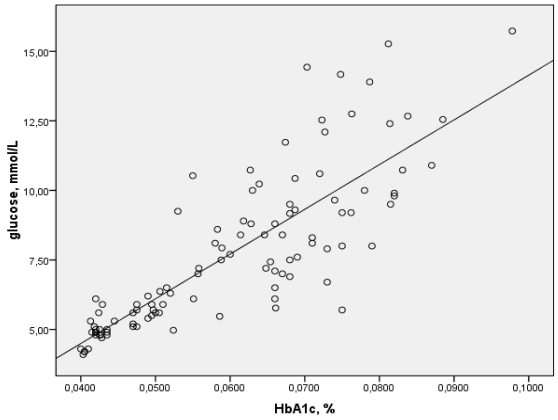


**Figure 4.** Spearman´s correlation coefficient between glucose and HbA1c levels in studied patients (*r=0.859, p*****<0.001*)

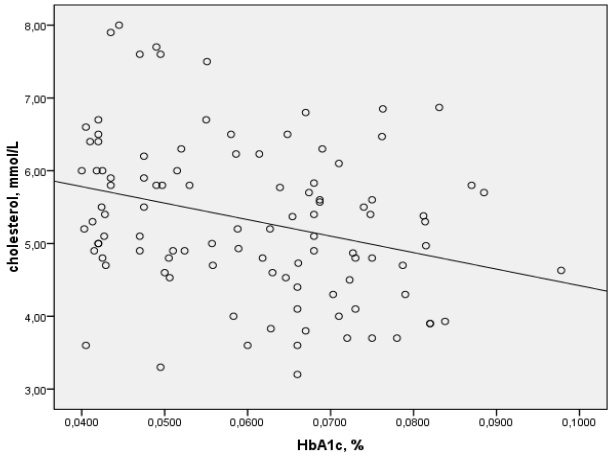


**Figure 5.** Spearman´s correlation coefficient between HbA1c and cholesterol levels in studied patients (*r=-0.295, p****<0.01*)

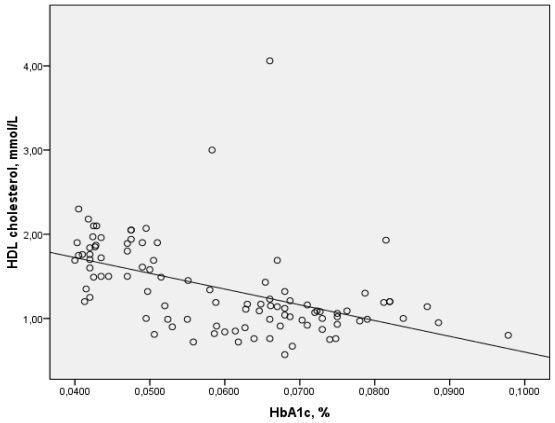


**Figure 6.** Spearman´s correlation coefficient between hemoglobin A1c and HDL cholesterol levels in studied patients (*r=-0.606, p*****<0.001*)

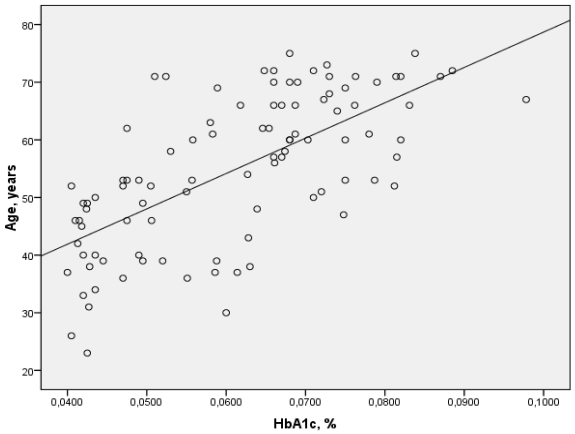


**Figure 7.** Spearman´s correlation coefficient between hemoglobin A1c and patient age in studied patients (*r=0.673, p*****<0.001*)

**Table II.** Serum lipid profile, glucose, glycated hemoglobin and patient age for male and female in all diabetic participants.

| Participants | Gender | Glucose (mmol/L) | Cholesterol (mmol/L) | HDL (mmol/L) | LDL (mmol/L) | TAGs (mmol/L) | HbA1c (%) | Age (years) |
|---|---|---|---|---|---|---|---|---|
| N=42 | **Male** | 8.32±0.38 | 4.91±0.14 | 1.20±0.10 | 2.54±0.13 | 2.68±0.19 | 6.34±0.17 | 57.70±2.24 |
| N=62 | **Female** | 7.32±0.37 | 5.61±0.14 | 1.47±0.05 | 3.13±0.13 | 2.16±0.14 | 5.79±0.20 | 52.88±1.58 |
| **Total N=104** | ***p*-value** | **0.050** | **0.001** | **0.013** | **0.003** | **0.030** | **0.040** | **0.050** |

*data presented as mean±SEM

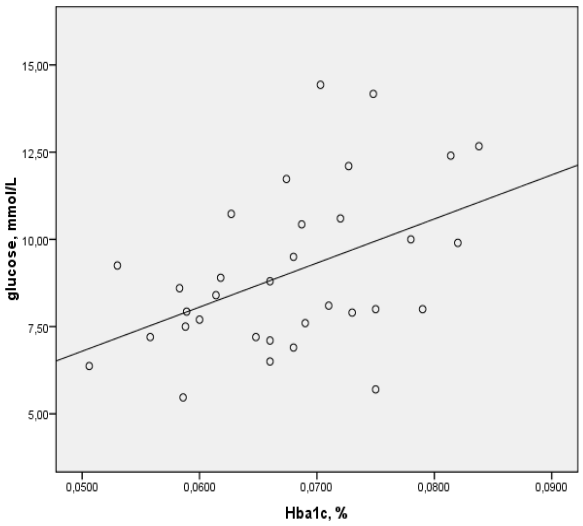


**Figure 8.** Spearman´s correlation coefficient between hemoglobin A1c and glucose in studied male diabetic patients (*r=0.444, p*$^{**}$*<0.01*)

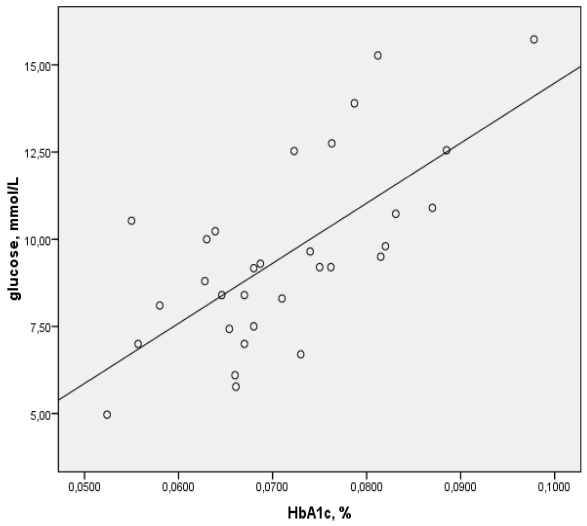


**Figure 10.** Spearman´s correlation coefficient between hemoglobin A1c and glucose in studied female diabetic patients (*r=0.623, p*$^{***}$*<0.001*)

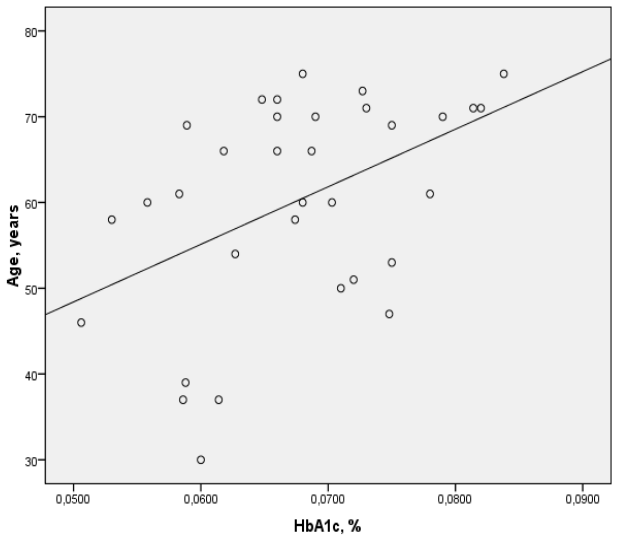


**Figure 9.** Spearman´s correlation coefficient between hemoglobin A1c and patient age in studied male diabetic patients (*r=0.450, p*$^{**}$*<0.01*)

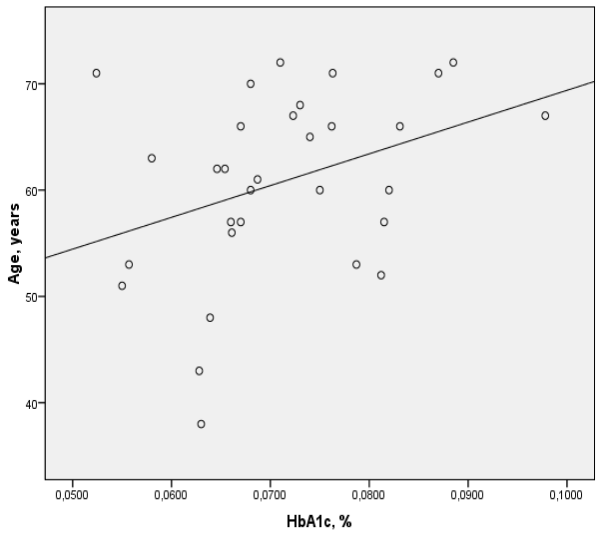


**Figure 11.** Spearman´s correlation coefficient between hemoglobin A1c and patient age in studied female diabetic patients (*r=0.405, p<0.05*)

## CONCLUSION

Type 2 *diabetes mellitus*, as metabolic disorder, results in numerous pathophysiological changes due to hyperglycemia in various systems in the body. Because the complications of disease are related to glycemic control, normoglycemia is an appropriate goal for most of the patients. Analysis of hemoglobin A1c is a gold standard to check long-term glycemia in patients with *diabetes mellitus.* In our study a significant correlation between HbA1c and various circulating lipid parameters was observed. This may indicate that HbA1c can be used as a potential biomarker for predicting dyslipidemia in newly diagnosed diabetics and patients with T2DM. In our study, 59.62% of diabetic female patients and 40.38% of male diabetics were found to have dyslipidemia and positive significant correlation between HbA1c with glucose levels and patient age was demonstrated. Hyperlipidemia in women may be attributed to the effects of sex hormones on body fat distribution. These findings are consistent with some previous studies. The results of our study suggest the importance of glycemic control in managing dyslipidemia and further reducing the risk for CVD in patients with T2DM, shown at the level of significant association of HbA1c with various lipid parameters.

To our knowledge, this is one of the first studies addressing diagnostic value of HbA1c in relation to status of lipidemia in context of gender and age influences performed on Bosnian diabetic population. However, further studies with a higher number of patients involved and better controlled protocols are needed in order to make more definite conclusions.

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## Summary/Sažetak

U svijetu, učestalost Tip 2 diabetes mellitusa (T2D) kao metaboličkog oboljenja, rapidno raste. Cilj ove studije bio je odrediti koncentracije hemoglobina A1c (HbA1c), natašte glukoze i lipidni profil kod ukupno 104 odrasle osobe, 24 bolesnika sa novodijagnosticiranim Tip 2 dijabetesom (40-60 godina starosti), 40 bolesnika sa T2D i 40 referentnih ispitanika kao kontrolne grupe (iste starosne dobi). Na osnovu tih rezultata, mogli smo procijeniti razlike prema spolu i starosnoj dobi u ispitivanoj populaciji, kao i odnos između kontrole glikemije (HbA1c) i lipidnog profila u serumu. Stoga smo pravilno analizirali značaj HbA1c kao indikatora dislipidemije kod bolesnika sa T2D u odabranoj bosansko-hercegovačkoj populaciji. Hemoglobin A1c određen je imunoturbidimetrijskom metodom, dok su natašte glukoza i lipidni profil analizirani prema standardnim kliničkim metodama na BT PLUS 2000-Biotechnic Instruments bioanalizatoru. Nađeno je da je koncentracija glikiranog hemoglobina kod novo dijagnosticiranih dijabetičara veća od izmjerene koncentracije u druge dvije analizirane grupe. Pokazana je statistički značajna razlika na nivou ispitivane populacije (kada se porede biohemijski parametri i nivo HbA1c) u vrijednostima glukoze, holesterola, HDL i LDL holesterola, HbA1c i starosne dobi bolesnika. Također, naši rezultati su pokazali i statistički značajnu negativnu korelaciju između vrijednosti HbA1c i holesterola i HDL holesterola ($p^{***}<0,001$) i pozitivnu korelaciju s koncentracijom glukoze i starosnom dobi bolesnika ($p^{***}<0,001$), u svim ispitivanim grupama. Prema našim rezultatima, hemoglobin A1c, također, može biti korišten kao prediktor dislipidemije i time rana dijagnoza dislipidemije može biti upotrebljena kao preventivna mjera za razvoj kardiovaskularnih oboljenja kod pacijenata sa Tip 2 diabetes mellitusom.