Introduction

Diabetes mellitus (DM) is a disease as old as the human race. It is a disease with frequency of 0.5% - 3.5% and complications which can cause serious morbidity and mortality [1]. Its etiopathogenetic division is type 1, type 2, and specific forms of gestational diabetes [2].

Oxidative stress is a biochemical mechanism that induces micro and macro angiopathy, hyperpermeability and angio-occlusion with retinopathy [3]. The tissues that take glucose in the state of hyperglycemia independently of insulin are the retina and the lens of the eye, whereas the nerves and endothelium suffer the greatest damage [4, 5].

Non-proliferative retinopathy is indicative of ischemia of the retina, and it is diagnosed by de-
tecting soft exudates, intra retinal micro vascular abnormalities, changes in the veins and arteries - micro aneurysms, hemorrhages, "cotton-wool" exudates and as focal infarction caused by occlusion of pre-capillary arterioles. Changes in the veins include dilatation, twisting the loop (looping), thickening of the beads (leading), and sausage-like segmentations [6].

Visual impairment, particularly in type 2 diabetes mellitus, is caused by maculopathy due to the involvement of fovea by edema, soft and hard exudates and ischemia [7].

Clinical characteristics of proliferative retinopathy are neovascularization of the papillae (NVD - new vessels of disc) and proliferation along the vessels (NVE - new vessels elsewhere) or both at the same time. The newly formed vessels grow from the veins as endophytic proliferations between the retina and vitreous body by using a medium as a substrate for the growth [6, 7]. Severity of the proliferative retinopathy is determined as mild, moderate or severe according to the ratio of the surface covered with vasoproliferative courts with a surface of papillae [8].

Material and Methods

The research, which included 135 subjects, was conducted at the Institute of Occupational Health, Department of Ophthalmology of the Clinical Centre in Kragujevac from November 15th, 2007 to November 15th, 2009. The inclusion criterion was retinopathy, which was diagnosed in 90 patients and they formed the experimental group; whereas the control group consisted of 45 subjects without complications.

The fundus was examined by the methods of ophthalmoscope, with Goldman’s prism and panfundoscopy on the biomicroscope. The duration of disease, age, and body mass index (BMI) were obtained from the research protocols.

Biochemical analyses included fasting and postprandial glycemia. Glycosylated hemoglobin A1C (HbA1C) represented the percentage of hemoglobin that succumbed to non-enzymatic glycosylation of proteins, and was determined by chromatographic separation, photometric measurements at 415 nm (endpoint method). Total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL) - cholesterol, and triglycerides were determined early in the morning before any meal. Total cholesterol was determined by Tinder’s endpoint method. HDL - cholesterol was determined by the precipitation method with fosfoliphosphoric-acid and magnesium chloride, which quantitatively precipitated very low-density lipoprotein (VLDL) and LDL.

Cholesterol fractions of high-density lipoproteins, which remain in the supernatant after centrifugation, were measured as total cholesterol. LDL - cholesterol was calculated indirectly. Triglycerides were determined by the enzymatic - colorimetric method, the so-called endpoint method.

The BMI values were obtained from the standard protocols, i.e. the measured body weight and body height of the subject, which were then inserted into the BMI formula (BMI=body weight (kg)/body height (m²) and calculated.

The degree of risk for diabetic retinopathy as part of obesity was calculated using the standard BMI calculations and BMI balance sheet (BMI ≥ 30 kg/m² - obesity), the BMI – categories being: underweight < 18.5, normal = 18.5-24.9, overweight = 25-29.9, obesity = ≥ 30, obesity grade 1 = 30 to 34.9, obesity grade 2 = 35-39.9, grade 3 - morbid obesity ≥ 40 [9].

Having obtained the written consent from the subjects, the local ethics committee approved the study.

The following statistical analyses were used: methods of descriptive statistics, measures of central tendency (arithmetic mean), variability (SD), T-test, χ² test, Fisher’s test, the method of binary logistic regression and correlation analysis [10].

Results

In the control group, the average duration of disease was 11.71 ± 5.85, and in the experimental group it was 14.40 ± 7.68 years. T-test showed that the difference in disease duration between the experimental and control group was statistically significant, being (p = 0.026). The disease lasted longer in the experimental group, which affected retinopathy.

Since the mechanisms of glucoregulation and the processes related to lipid metabolism were disrupted, it was necessary to examine their correlation with retinopathy. The priority goal of treatment was to establish adequate glucoregulation. The following parameters were used to determine the degree of risk for diabetic retinopathy and obesity as part of it.
the quality of glucoregulation: fasting glycemia, postprandial glycemia and HbA1C values. The average value of fasting glycemia was 7.02 ± 2.20 mmol/L in the control group and it was 8.34 ± 3.18 mmol/L in the experimental group. The analysis of results showed that the difference in fasting glucose between the experimental and control group was statistically significant (p = 0.006). The experimental group had a higher level of fasting glycemia before the first meal, which can be associated with retinopathy (Graph 1).

The obtained value of postprandial glycemia in the control group was 10.44 ± 3.98 and in the experimental group it was 11.76 ± 4.47 mmol/L. The obtained values showed no statistically significant difference (p = 0.096) between the control and experimental group.

Glycosylated hemoglobin values represented a significant, reliable parameter for assessing the quality of metabolic control. The values in the experimental group were 8.22 ± 2.05, while in the control group they were 7.16 ± 1.37 mmol/L. The values of T-tests showed that there was no statistically significant difference in HbA1C between the control and experimental group (p = 0.001). The experimental group had higher HbA1C level, which proves that HbA1C values affect retinopathy (Graph 2).

The average value of triglycerides was 1.92 ± 0.72 in the control group and in the experimental group it was 2.63 ± 1.60 mmol/L. The values of T-test suggest that there is a statistically significant difference in triglycerides between the groups (p = 0.001) and triglyceride levels may be the cause of retinopathy (Graph 3).

The value of cholesterol was 6.25 ± 1.47 and 5.97 ± 1.01 mmol/L in the experimental and control group, respectively. Considering the values of T-test, the null hypothesis can be accepted, according to which no statistically significant difference (p = 0.198) regarding total cholesterol level was found between the control and experimental group. No statistically significant difference (p = 0.088) was found in the HDL value in the blood of subjects from the control and experimental group. The average value of LDL was 3.76 ± 0.66 and 3.95 ± 0.73 mmol/L in the control and experimental group, respectively. The value of T-test shows that the difference in LDL between the experimental and control group was not statistically significant (p = 0.149).

The BMI index was used to assess obesity. The average BMI was 27.66 ± 5.13 and 23.94 ± 2.65 SI – units in the experimental and the control group, respectively. The value of T-test makes it possible to accept the alternative hypothesis that there is a statistically significant difference between the control and the experimental group, which proves the positive affect of obesity on retinopathy (Graph 4).

A statistically significant correlation (r = 0.753, p = 0.000) has been observed between fasting and postprandial glycemia. According to the analysis of the obtained coefficients of correlation, there is a positive direction of the relationship – the higher the fasting glycemia values, the higher the postprandial glycemia values get. The absolute value of the resulting linear correlation coefficient indicates a correlation between the two parameters. Fasting glycemia values were significantly correlated with HbA1c (r = 0.541, p = 0.000). The positive sign indicated the direction of the obtained relationship, an increase in blood glucose, followed by a rise in glycosylated hemoglobin. A statistically significant linear correlation (r = 0.532, p = 0.000) was noticed between postprandial glycemia and hemoglobin. The resulting correlation coefficient indicated a significant correlation between the two parameters. The increase in postprandial glycemia level was accompanied by an increase in glycosylated hemoglobin.

By applying Receiver-Operating Characteristics (ROC) curve it was found that HbA1C might be a marker of retinopathy (r = 0.660, p = 0.002).
The cut-off point was 7.55, the sensitivity was 0.567, and the specificity was 0.711 (Graph 5).

By applying ROC curve, it was found that triglycerides might be markers of retinopathy ($r = 0.640, p = 0.008$). The cut off point was 2.25, the sensitivity was 0.522, and the specificity was 0.711.

By applying ROC curve, it was found that cholesterol cannot be a marker for retinopathy, HDL and LDL cannot be markers for retinopathy. By applying ROC –curve it was revealed that the duration of disease can be a marker for retinopathy ($p = 0.607, p = 0.044$). The cut off point was 14.5, the sensitivity was 0.511, and the specificity was 0.733.

Binary logistic regression showed that the occurrence of retinopathy was affected by HbA1C, triglycerides, cholesterol, LDL. However, the application of the backward method step by step on the previous regression and the elimination of the variables one by one, starting from the one which affects retinopathy least, result in the status showing that retinopathy depends on HbA1C ($p = 0.010$), triglycerides ($p = 0.009$), the duration of disease ($p = 0.032$) and obesity ($p = 0.039$).

The odds ratio for HbA1C was 1.389, which meant that the chance of retinopathy was increased 1.389 times, i.e. 38.9 % if HbA1C increased for the unit, provided that the other variables did not change. The odds ration for triglycerides was 1.872, which meant that the chance of retinopathy was increased 1.872 times, i.e. 87.2%, if the values of triglycerides increased by the unit, provided that the other variables did not change. The odds ratio for disease duration was 1.068, which meant that the chance of retinopathy was increased 1.068 times, i.e. 6.8%, when the duration of diabetes increased by one year, provided that the other variables did not change.

**Discussion**

The most common cause of blindness in working population is diabetes mellitus. The complications of proliferative retinopathy in patients having type 1 diabetes are visual impairments. In patients with type 2 diabetes, the cause of poor vision is maculopathy [11, 12]. Retinopathy is found in about 40% of patients having type 2 diabetes at the moment when the disease is being diagnosed, whereas impaired vision is found in 4.8% of these patients [13]. A great number of risk factors for developing retinopathy has been identified, such as duration of disease, poor glycemic controls, elevated triglycerides, etc; however, there is an opinion that the synergistic effect of the dominant factors is more dangerous, which was confirmed in the study - The Aus Diab Study and the Blue Mountains Eye Study [14, 15].

A statistically significant difference (T-test, $p = 0.026$) was found regarding the duration of disease between the experimental and control group, the average duration of disease being $14.4 ± 7.68$ and $11.71 ± 5.85$ years in the experimental and control group, respectively. A statistically significant correlation ($r = 0.66, p = 0.02$) was found between disease duration and retinopathy. Data obtained by ROC curve define the duration of disease as a marker for retinopathy. Binary logistic regression shows that the incidence of retinopathy depends on the duration of disease ($p = 0.032$), which coincides with the suggestions and recommendations given by the American Diabetes Association (ADA) [1, 16].

Glucoregulation quality is assessed on the basis of fasting glycemia, postprandial glycemia and glycosylated hemoglobin [16]. If glycosylated hemoglobin decreases by 1.5%, or if it is possible to reduce its value to 7 mmol/L, retinopathy is reduced as well. Studies have shown that when HbA1C is decreased by 1%, the incidence of retinopathy is reduced by 37% [17]. There was a statistically significant difference in mean fasting glucose level between the experimental and control group (T-test, $p = 0.006$). According to the analysis of the obtained differences, higher values of fasting glycemia were observed in the group of patients with retinopathy, being $8.34 ± 3.18$, while they were $7.02 ± 2.20$ in the controls.

The comparison of the values of postprandial glycemia in the experimental and control group did not show a statistically significant difference (T-test, $p = 0.096$). There was a statistically significant difference in the average HbA1C values between the experimental and control group ($p = 0.001$). Higher values of this parameter were statistically significant in the patients with retinopathy, being $8.22 ± 2.056$, whereas they were $7.16 ± 1.37$ in the control group. The correlation between the values of fasting glycemia and postprandial glycemia was statistically significant ($r = 0.754, p = 0.000$).
The result of correlation analysis was \( r = 0.754 \), which indicated a significant correlation of two parameters, meaning that the increasing values of glucose led to an increase in postprandial glucose values. The values of fasting glycemia were significantly correlated with HbA1c (\( r = 0.541, p = 0.000 \)). The correlation analysis showed that the correlation coefficient \( r = 0.541 \), which indicated a significant correlation between the two parameters, meaning that the rise in glucose levels led to an increase in HbA1c. A statistically significant correlation (\( r = 0.66, p = 0.02 \)) was found between retinopathy and HbA1c. The values obtained by ROC-curve make it possible to define HbA1c as the most important marker for retinopathy, which is included in the statement - The International Expert Committee [17, 18]. Binary logistic regression shows that the development of retinopathy depends on HbA1c (\( p = 0.10 \)). The assessment of liporegulation quality is based on the measurements of total cholesterol, HDL - cholesterol, LDL - cholesterol and triglyceride levels [18]. No statistically significant difference in the total cholesterol values (T-test, \( p = 0.193 \)) was found between the experimental and control group.

The average value of total cholesterol was 5.97 ± 1.01 and 6.25 ± 1.47 in the experimental and in the control group, respectively. Total cholesterol did not show a statistically significant correlation with retinopathy (\( r = 0.539, p = 0.461 \)). No statistically significant difference (T-test, \( p = 0.088 \)) was found by comparing the values of HDL - cholesterol in patients having retinopathy and those without it, the mean value of this parameter being 1.23 ± 0.24 and 1.23 ± 0.24 in the former and latter, respectively. No statistically significant correlation (\( r = 0.397, p = 0.052 \)) was found between HDL - cholesterol and retinopathy, therefore the effect of HDL - cholesterol to retinopathy has not been proved. The average value of LDL - cholesterol in the patients with retinopathy was 3.95 ± 0.73, whereas it was 3.76 ± 0.66 in the control group. The difference in LDL - cholesterol (T-test, \( p = 0.149 \)) was not statistically significant between the experimental and control group. There was no statistically significant correlation (\( r = 0.578, p = 0.143 \)) between LDL - cholesterol and retinopathy.

A statistically significant difference (T-test, \( p = 0.001 \)) was found by comparing the levels of triglycerides in the experimental and control groups. The experimental group had higher levels of triglycerides. There was no statistically significant correlation (\( r = 0.640, p = 0.008 \)) between the values of triglycerides and retinopathy, which was confirmed in the study - The Multi - Ethnic Study of Atherosclerosis (MESA) [19]. There was a statistically significant difference in BMI - obesity between the experimental and control group (T-test, \( p = 0.000 \)). The average BMI in the group with retinopathy was 27.66 ± 5.13, whereas it was 23.94 ± 2.65 in the control group. The analysis of the obtained correlations shows that the effect of obesity on retinopathy increases as BMI gets higher [19].

**Conclusion**

The duration of illness is associated with retinopathy. There is a positive correlation between the values of the parameters of glucosemetabolism and retinopathy, because the increase in the values of fasting glucose, postprandial glycemnia and glycosylated hemoglobin makes it more likely for retinopathy to develop.

A correlation between triglycerides and retinopathy has also been found. Higher values of triglycerides lead to the development of retinopathy.

Retinopathy seems to occur more frequently in diabetic patients who are prone to obesity.

**References**


