

GENDER-RELATED DIFFERENCES IN INFLAMMATORY AND LIPID PARAMETERS IN PATIENTS WITH DIABETES MELLITUS TYPE 2

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Diabetes mellitus type 2 (DM type 2) is one of the most common health problems worldwide. Diabetics have increased risk for development of wide spectrum of atherosclerotic complications, at the basis of which are inflammation and diabetic dyslipidemia. Increasing of relative risk for coronary artery disease development is higher in females with DM type 2.

The aim of this study was determination of characteristic inflammatory and lipid disorders in type 2 diabetics and their association with patient gender.

The study involved 35 patients with DM type 2 and stabile angina pectoris. Besides anamnesis, all patients underwent clinical examinations (measure of blood pressure, body height and weight and calculation of body mass index). Inflammatory markers (sedimentation in I and II hour-SE I and SE II, C reactive protein-CRP, fibrinogen concentration and leukocyte count) as well as lipid parameters (total cholesterol, LDL and HDL cholesterol concentration and triglycerides) were determined in all the patients.

Total cholesterol and triglycerides concentrations were higher in females with DM type 2 compared to males ($p < 0,05$). There were not significant gender differences in HDL and LDL cholesterol concentration. All inflammatory markers (SE I and SE II, CRP, fibrinogen concentration and leukocyte count) were higher in females with DM type 2 and CAD compared to men ($p < 0,05$). In males, there was a strong positive correlation between SE I and SE II with total LDL ($p < 0,05$) and HDL cholesterol concentrations ($p < 0,01$). Concentration of CRP was only significantly connected with triglycerides concentration ($p < 0,05$). There was a strong association between leukocyte count and increased triglycerides ($p < 0,05$) and low HDL cholesterol concentration ($p < 0,01$). In females, there was only a strong positive correlation between SE II ($p < 0,01$) and CRP concentration ($p < 0,05$) and triglycerides.

Women with DM type 2 and clinically manifest CAD (stable angina pectoris) are at higher risk for development the cardiovascular complications as they have more prominent lipid and inflammatory disorders than men. *Acta medica Medianae 2008;47(3): 39-43.*

Key words: diabetes mellitus, inflammation, lipid disorders, dyslipidemia

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Introduction

Diabetes mellitus type 2 (DM type 2) is one of the most common health problems worldwide. It represents 80 to 90% of all DM types. DM type 2 incidence has risen considerably over the last three decades. The World Health Organization estimates that by the year 2025, the number of patients will have surpassed 300 million. In the USA, there are currently 15 to 16 million people with diabetes, whether diagnosed or not, and additional 25 to 30 million people with pre-diabetes or those with low glucose tolerance. Diabetes incidence in our country is between 2 and 3 %, which sums a figure of 200 to 300 000 patients. The percentage is even higher if people with the low glucose tolerance are taken into consideration.

Patients diagnosed with DM type 2 are more likely to develop a wide range of complications, leading to premature disability and death. The mortality rate among diabetics is 2 to 3 times higher than in the general population. It is considered that the inflammation plays the major role in the development of diabetes and the subsequent complications. Epidemiological, clinical and experimental studies point to the liason between the low degree inflammation and diabetes mellitus type 2. Along with the pro-inflammatory state, DM type 2 is characterized by heavy lipid disorders and diabetes dyslipidemia. In DM type 2 patients, changes in the composition and concentration of all lipoprotein particles occur, which are manifested by the increased overall and LDL cholesterol, the increased triglycerides, the decreased HDL cholesterol, the postprandial lipidemia onset and the changes in apoprotein apo B / apo A ratio.

Atherosclerosis is a chronic inflammatory disease, where the inflammation and lipid disorders play a key role in the initiation, progression and rupture of atherosclerotic plaque (8). This process is based on endothelial dysfunction and activation of T- lymphocytes (9).

Several studies have shown that the increase of a relative risk for the onset of CVD is higher in women than in men with diabetes(10). This is the consequence of women losing "the privilege" they have had over men when it comes to morbidity and mortality of the general population. Higher mortality rate in women with diabetes can partly be explained by the unfavorable levels of HDL and VLDL cholesterol. Values of HDL-C<1.3 mmol/l and VLDL cholesterol >0.5 mmol/l are good indicators of CVD mortality in women with diabetes, and can be helpful in identifying women who would benefit the most from the antilipemic therapy (11). Similar findings are also shown for the system information parameters which are significantly higher in women with DM type 2 (12).

Bearing in mind all of these complex changes that accompany diabetes, the aim of this study was to determine characteristic inflammatory and lipid disorders in type 2 diabetics and their association with the patients'gender.

Patients and methodology

The study was carried out on 35 patients with DM type 2, treated in the lipid department of the Institute of Niska Banja and the Clinic of Endocrinology, Clinical Center in Nis, between January and September 2007. The study involved patients diagnosed with DM type 2 and stable angina pectoris that lasted at least one year. The patients were divided into two gender groups: men (n=23) and women (n=12). All patients underwent a relevant medical nutrition therapy and were treated with oral anti-diabetics accompanied by cardio- therapy. The study excluded DM type 2 patients treated with insulin and hypolipemics, as well as the patients diagnosed with acute coronary syndrome as well as the more severe diseases of respiratory, hepatobiliary and digestive systems.

Medical history was taken, and all the relevant data about the duration of CVD, dyslipidemia and diabetes were included. All patients underwent clinical examinations (measurement of arterial pressure, body height and weight, and the body mass index calculation). Inflammatory and lipid markers were determined in all patients from the blood samples taken early in the morning (after patients had fasted for over 12 hours).

The following inflammatory markers were determined:

1st and 2nd hour sedimentation rate (SE I and SE II) using the Westergreen method.

Highly sensitive CRP values were determined using Dade Behring Company test on the Dimension Expand analyzer.

Fibrinogen values were determined using turbidimetric method. Normal reagents are set between 2.0 - 4.0 g/l

The number of leukocytes was determined using the Haematolog H1 - Technicon blood

count auto analyzer. Normal ranges are set between 6-8 x10⁹/l

The following lipid markers were determined:

Total serum cholesterol and triglycerides, using Bayer Company reagents on Technicon - Axon Bayer analyzer. Normal ranges are set between 3.63 - 5.5 mmol/l; and 0.5 -1.97 mmol/l.

HDL and LDL cholesterol were determined from the serum by a direct enzymatic colorimetric analysis without precipitation, using Dade Behring reagents on the Dimension Expand Dade Behring analyzer. Normal laboratory values were: HDL: (1.0 - 1.55 mmol/l in men and 1.2 - 1.7 mmol/l in women) and LDL: (2.6 - 3.9 mmol/l).

Systolic and diastolic blood pressure values were determined using a mercury manometer on the patients' left upper arm in 3 consecutive measurements, whereby an average value was taken into consideration. All the results were expressed as the average value ± SD. For the statistical significance of the results, Student's t-test was used, as well as Pearson's Correlation Coefficient. Data processing was done using Microsoft Office Excel 2003 program package in Windows XP professional environment.

Results

General characteristics are shown in Table 1

Table 1. General characteristics of patients

	women	men
number (%)	12 (34)	23 (66)
age	63± 1.10	62.75±5.97
Duration of diabetes (years)	16.5±2.64	13.7±9.83
Duration of CVD (years)	2.01±1.2	7.5±2.73*
Duration of dyslipidemia (years)	7.65±2.73	2.1±0.75*
Fasting glycemym (mmol/l)	9.2±2.3	6.9±1.83***
BMI (kg/m ²)	25.37±3.49	28.96±1.32***
Systolic TA (mmHg)	142.27±22.51	132.14±18.17
Diastolic TA (mmHg)	80.71±8.29	85.91±10.68

Data are given as the average value ±SD or as an absolute number (%)

*p<0.05; ***p<0.001

Student's t-test didn't show any significant differences in the age average and the duration of diabetes. However, the duration of coronary diseases was significantly longer (p<0.05) while the duration of dyslipidemia was significantly shorter (p<0.05) in men, than it was in women. The quality of glycoregulation determined by the values of fasting glycemym proved to be significantly lower in men than in women (p<0.001). The degree of obesity expressed through BMI was relevantly higher in men when compared to women (p<0.001).

Values of lipid markers are shown in Table 2.

Table 2. Values of lipid markers

lipides (mmol/l)	women	men	total
Total cholesterol	7.57±0.73*	6.27±1.24	6.7±1.24
Tryglicerides	3.89±0.25**	2.43±0.89	2.91±1.02
HDL cholesterol	1.15±0.1	1.14±0.34	1.15±0.28
LDL cholesterol	4.87±0.86	4.13±1.35	4.38±1.16

Data are shown as average values ±SD

*p<0.05; **p<0.01

Statistical data analysis showed that the total cholesterol values (p<0.05) and values of triglycerides (p<0.01) are significantly higher in women with DM type 2 when compared to men (p<0.05). At the same time, there is no substantial gender difference in the values of HDL and LDL cholesterol (Table 2)

Characteristics of the inflammatory markers in patients with DM type 2 and stabile angina pectoris are shown in Table 3

Table 3. Inflammatory risk factors for the onset of CVD

	women	men	total
Number of leucocytes (G/l)	9.63±1.02***	5.38±0.73	6.23±1.87
Fibrinogen (g/l)	2.89±0.6*	2.11±0.54	2.26±0.58
C-reactive protein (mg/l)	10.5±2.6***	3.73±1.67	5.1±3.2
SE I (mm/h)	18.36±9.39***	6.23±4.95	
SE II (mm/h)	39.45±18.54***	14.92±11.56	

* p<0.05; **p<0.01; ***p<0.001

All examined inflammatory markers were significantly higher in women with diabetes mellitus and CVD when compared to men (Table 3).

Correlation between lipid and inflammatory markers in men and women tested with Pearson's Coefficient of Correlation is shown in Tables 4 and 5.

Table 4. The correlation between lipid and inflammatory markers in men

	SE I	SE II	CRP	fibrinogen	leukocytes
Total cholesterol.	0.6*	0.6*	-0.4	0.3	0.1
LDL cholesterol.	0.7*	0.6*	-0.5	0.2	0.06
Tryglicerides	-0.3	-0.3	0.5*	0.2	0.6*
HDL cholesterol.	0.8**	0.8**	-0.2	0.4	-0.6**

* p<0.05; **p<0.01; ***p<0.001

In men with DM type 2 and stable angina pectoris, there was a strong positive correlation between values of SE I and SE II and the value of total LDL (p<0.05) and HDL cholesterol (p<0.01). Only CRP values were significantly related to the concentration of triglycerides (p<0.05) and the decrease of HDL cholesterol (p<0.01) (Table 4).

Table 5. The correlation of lipid and inflammatory markers in women

	SE I	SE II	CRP	fibrinogen	leukocytes
Total cholesterol	-0.06	0.1	0.1	0.09	-0.3
LDL cholesterol.	-0.3	-0.3	-0.1	-0.05	-0.03
triglycerides	0.3	0.9**	0.8*	0.3	-0.08
HDL cholesterol	-0.2	-0.7	-0.3	-0.2	0.1

* p<0.05; **p<0.01

In women with DM type 2 and stabile angina pectoris there was a strong positive correlation between SE II (p<0.01) and CRP (p<0.05) and the values of serum of triglycerides (Table 5).

Discussion

The female patients had higher values of total cholesterol and triglycerides when compared to the male patients (Table 2). This is in accordance with the meta-analysis which proved the presence of unfavorable lipoprotein profiles in the female diabetics which was not the case with the male diabetics (13). This indicated a higher risk of the possible cardio vascular complications in female diabetics, because the decreased HDL-C and the increased LDL- C were proven to have a stronger prognostic importance than the values of hyperglycemia in UKPDS study (14).

Along with the inflammation, dyslipidemia is another deep-seated characteristic of diabetes. Wexler and associates confirm that values of LDL and HDL cholesterol are significantly higher in women with DM type 2, whether or not they suffer from a coronary disease. This leads to the conclusion that diabetes neutralizes protective impact that female sex hormones have on the cardiovascular system adding up a certain cardiovascular risk (15), which is in compliance with the results of this study.

All examined inflammatory markers were remarkably higher in women with DM and CVD, than in men (sedimentation rate in I and II hour, number of leukocytes, fibrinogen concentration and CRP) (Table 3). Inflammation is the initial change in the progression of atherosclerosis, alongside other cardiovascular complications. Many prospective studies have identified pro-inflammatory cytokines, acute phase proteins and several indirect inflammatory markers as the predictors of DM type 2.

Natali A. and associates have proven that the values of sedimentation are remarkably higher in patients with diabetes than in healthy people whereas they are significantly higher in women diabetics than in men diabetics (16) which has also been confirmed in our study. Certain data indicate that sedimentation is higher in women than in men, and increases over age, irrelevant of gender (17). It is higher in postmenopausal women than in premenopausal women of the same age; but significantly lower in postmenopausal women on substitution estrogen therapy (18).

The results of MONICA study investigating the significance of CRP values in prediction of atherosclerotic diseases, point to the fact that the increased hsCRP values are related to diabetes, and have a great predictive role for the onset of atherosclerotic diseases, CVD, as well as PVD. It has also shown that the number of leukocytes is in a similar correlation with a metabolic syndrome and later cardiovascular complications (20). Long time ago, prospective studies discovered the connection between the number of leukocytes and the development of CVD. Framingham study showed that in patients without CVD, there was a significant correlation between the number of leukocytes and the first onset of CVD (21). In compliance with the results of the conducted study, it was proven that women diabetics when compared to men have a more distinct pro-inflammatory state and are in a higher risk of the development of a coronary event in time (Table 3).

It is well known that cholesterol has an enormous impact on erythrocyte sedimentation rate, which can be explained by electrostatic changes on the erythrocyte membrane (22). On the contrary, however, there is evidence that the sedimentation rate increases significantly in healthy people with hypercholesterolemia (23,24). Levels of total, LDL and HDL cholesterol shows strong correlation with the sedimentation rate in men but not in women included in this study; which leads to the conclusion that hypercholesterolemia accelerates erythrocyte sedimentation rate in men, the way it does in healthy people; whereas in women, this mechanism is extinguished (Tables 4 and 5). The results of our study confirm that the erythrocyte sedimentation rate is significantly higher in women, and that it is strongly related to the levels of triglycerides, which are known as surrogate markers of bad glucoregulation and hyperglycemia in the condition of insulin resistance (25). In the light of this discovery, the fact remains

that the increased CRP is in significant correlation with the values of triglycerides in both men and women subjected to this study. Bearing in mind that CRP is the strongest biomarker for the onset and progression of CVD, we found that its prognostic value surpasses LDL-C and Framingham risk score (26, 27). Alongside being the powerful risk marker CRP shows pro-inflammatory and pro-atherogene characteristics. It represents the parameter of the body's total inflammatory response and a relevant indicator of the anti-inflammatory therapy efficiency.

One detailed analysis showed that the increased value of the number of leukocytes was significantly correlated with the existence of metabolic syndrome and dyslipidemia (20); which was compatible with the results of this study, as far as male patients were concerned but not the female (Table 4 and 5). At the same time, the study showed that the number of leukocytes was connected to hypertension and hyperglycemia; while values of fibrinogen were connected to low HDL -C (20).

Correlation of inflammatory and lipid disorders in women is found mainly by triglyceride concentration, as an indirect marker of bad glucoregulation and insulin resistance; while this correlation in men is found through all lipid functions. This confirms that men benefit more from the correction of lipid disorders, while in women these disorders are relatively independent pathogenetic mechanisms in atherogenesis.

Conclusion

Women with DM type 2 who clinically manifest CAD (stable angina pectoris) are at a higher risk for the development of a coronary event in due time as well as cardiovascular complications as they have more prominent lipid and inflammatory disorders than men.

References

- Burke PJ, Williams K, Gaskill PS, Hazuda PH. Rapid Rise in the Incidence of Type 2 Diabetes From 1987 to 1996. Results From the San Antonio Heart Study. *Archives of Internal Medicine* 1999;159:1450-6.
- American Diabetes Association: Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications (Position Statement). *Diabetes Care* 2003; 26(Suppl.1):S51-S61.
- Henry RR. Type 2 Diabetes Care: The Role of Insulin Sensitizing Agents and Practical Implications for Cardiovascular Disease Prevention. *The American Journal of Medicine* 2002;105(1A):20S-26S.
- Ilić S, Deljanin Ilić M, Nikolić A. Akutni koronarni sindromi prvi deo: klasifikacija, patofiziologija i dijagnostika. *Acta Medica Medianae* 2005; 44(1):31-7.
- Zozulinska D, Wierusz-Wysocka B. Type 2 diabetes mellitus as inflammatory disease. *Diabetes Research and Clinical Practice*, 74:S12-S16.
- Haffner S.M. Lipoprotein disorders associated with type 2 diabetes mellitus and insulin resistance. *Am J Cardiol* 2002;90:55i-61i.
- Tan KC, Chow WS, Tam SC, Ai VH, Lam CH, Lam KS. Atorvastatin lowers C-reactive protein and improves endothelium dependent vasodilation in type 2 diabetes mellitus. *J. Clin Endocrinol Metab* 2002;87:563-8.
- Patel S, Celermajer DS, Bao S. Atherosclerosis-Underlying inflammatory mechanisms and clinical implications. *Int J Biochem Cell Biol* 2008;40:576-80.
- Zhou X, Nicoletti A, Elhage R, Hansson G.K. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation* 2000; 102(24):2919-22.
- US Centers for Disease Control and Prevention (CDC). CDC national diabetes fact sheet. Available at: www.cdc.gov/diabetes/pubs/estimates.htm#deaths Accessed July 7, 2005
- William BK, Emerson HT. Epidemiology and Pathology of Sudden Coronary Death. The Framingham Study. *Annals of the New York Academy of Sciences* 1982;382(1):3-21.

12. Jenkins A, Rothena M, Kleina R, Mollera K, Eldridge L, Zheng D, et al. the DCCT/EDIC Research Group. Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDIC cohort. *Journal of Diabetes and Its Complications* 2008; 22:153-63.
13. Bittner V. Perspectives on Dyslipidemia and Coronary Heart Disease in Women. *J Am Coll Cardiol* 2005;46:1628-35.
14. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;352:837-53.
15. Wexler DJ, Grant RW, Meigs JB, Nathan DM, Cagliero E. Sex Disparities in Treatment of Cardiac Risk Factors in Patients With Type 2. *Diabetes Care* 2005; 28: 514-20.
16. Natali A, L'Abbate A, Ferrannini E. Erythrocyte sedimentation rate, coronary atherosclerosis and cardiac mortality. *Eur Heart J* 2003; 24: 639-48.
17. Bain BJ. Some influences on the ESR and the fibrinogen level in healthy subjects. *Clin Lab Haematol.* 1983; 5: 45-54.
18. Rafnsson V, Bengtsson C. Female sex hormones and the erythrocyte sedimentation rate. Results from a population study of women in Goteborg, Sweden. *Scandinavian Journal of Clinical and Laboratory Investigation.* 1981;41:729-33.
19. Koenig W, Sund M, Frohlich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONITORING trends and determinants in Cardiovascular disease (MONICA) Augsburg cohort study, 1984 to 1992. *Circulation* 1999;99:237-42.
20. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. The metabolic syndrome and insulin resistance: relationship to haemostatic and inflammatory markers in older non-diabetic men. *Atherosclerosis* 2005;181:101-8.
21. Kannel WB, Anderson K, Wilson PWF. White blood cell count and cardiovascular disease. *J Am Med Assoc* 1992;267:1253-6.
22. Bottiger L.E. Erythrocyte sedimentation rate and plasma lipids. *Acta Med Scand* 1973;193:53-7.
23. Bottiger LE, Carlson LA, Ekelund LG, Olsson AG. Raised erythrocyte sedimentation rate in asymptomatic hyperlipidemia. *Br Med J* 1973; 2: 681-4.
24. Jong W.C, Soo H.P. Influences of hypercholesterolemia on red cell indices and erythrocyte sedimentation rate in elderly persons. *Clin Chim Acta* 2004; 341:117-21.
25. Đinđić B, Janković R, Savić T, Bojanić V. Antilipemična terapija i problem niskog holesterola. *Acta Medica Medianae* 2004; 43(1):43-7.
26. Tiong A, Brieger D. Inflammation and coronary artery disease. *Am Heart J* 2005;150:11-8.
27. Ridker P, Brown N, Vaughan D, et al. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. *Circulation* 2004;109(Suppl):6-19.

POVEZANOST INFLAMATORNIH I LIPIDNIH POKAZATELJA U ODNOSU NA POL BOLESNIKA OBOLELIH OD DIJABETES MELITUSA TIP 2

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Dijabetes melitus tip 2 (DM tip 2) predstavlja jedan od najčešćih zdravstvenih problema širom sveta. Bolesnici oboleli od DM tip 2 imaju povišen rizik za nastanak i razvoj širokog spektra aterosklerotskih komplikacija, u čijoj osnovi stoje inflamacija i lipidni poremećaji. Povećanje relativnog rizika za nastanak koronarne bolesti veće je kod žena nego muškaraca obolelih od dijabetesa.

Cilj ove studije bio je da se utvrdi povezanost i karakteristike inflamatornih i lipidnih poremećaja u odnosu na pol bolesnika obolelih od DM tip 2.

Studijom je obuhvaćeno 35 bolesnika obolelih od DM tip 2 sa stabilnom anginom pectoris. Pored uzimanja anamnestičkih podataka i kliničkog pregleda (merenje arterijske tenzije, telesne težine i visine, preračunavanje indeksa mase tela), kod svih bolesnika određivani su inflamatorni (sedimentacija u I i II satu, C reaktivni protein-CRP, koncentracija fibrinogena i broj leukocita) i lipidni pokazatelji (koncentracija ukupnog, LDH i HDL holesterola i triglicerida).

Vrednosti ukupnog holesterola i triglicerida su veće kod žena obolelih od dijabetes melitusa tip 2 u odnosu na muškarce ($p < 0,05$), bez razlike u vrednostima HDL i LDL holesterola između polova. Svi ispitivani inflamatorni pokazatelji (SE I, SE II, CRP, fibrinogen, leukociti) bili su značajno veći kod žena sa dijabetes melitusom i KB u odnosu na muškarce ($p < 0,05$). Kod muškaraca postoji jaka pozitivna povezanost između vrednosti SE I i SE II i ukupnog, LDL ($p < 0,05$) i HDL holesterola ($p < 0,01$). Vrednosti CRP jedino su značajno povezane sa koncentracijom triglicerida ($p < 0,05$). Broj leukocita je povezan sa rastom triglicerida ($p < 0,05$) i padom HDL holesterola ($p < 0,01$). Kod žena postoji samo jaka pozitivna povezanost SE II ($p < 0,01$) i CRP ($p < 0,05$) sa vrednostima serumske koncentracije triglicerida.

Kod žena obolelih od dijabetes melitusa tip 2 sa klinički manifestom koronarnom aterosklerozom po tipu stabilne angine pectoris postoji veći rizik za razvoj naknadnih koronarnih događaja i kardiovaskularnih komplikacija uslovljen težim lipidnim i inflamatornim poremećajima. *Acta Medica Medianae* 2008;47(3):39-43.

Ključne reči: diabetes melitus, inflamacija, lipidni poremećaji, dislipidemija