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INFLAMATORNI KARDIOVASKULARNI MARKERI RIZIKA I NIJEMA ISHEMIJA MIOKARDA KOD PACIJENATA SA TIPOPOM 2 DIJABETESA

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Abstract

**Background/Aim.** A special feature of Coronary Heart Disease (CHD) in patients with type 2 diabetes (T2D) is that it is often asymptomatic and occurs as a consequence of cardiovascular autonomic neuropathy. Dysregulation of the autonomic nervous system is associated with elevated values of inflammatory markers such as highly sensitive C-reactive protein (hs-CRP) and interleukin 6 (IL-6) which accelerate atherosclerosis and the occurrence of cardiovascular complications in patients with T2D.

The aim of the study was to evaluate the importance of determining inflammatory cardiovascular risk markers IL-6 and hs-CRP in screening for the presence of CHD in asymptomatic patients with T2D.

**Methods:** The study included 169 patients with T2D, without any symptoms and signs of CHD. Ergometric testing proved or ruled out the presence of silent CHD. The levels of hs-CRP and IL-6 were determined by ELISA.

**Results:** IL6 values were significantly higher in patients with positive ergometric test (6.83±1.99 pg/mL) compared to patients with negative ergometric test (3.04±1.39 pg/mL) (p<0.001). We also found that hs-CRP values in patients with positive ergometric test was significantly higher in comparison to patients with negative ergometric test (6.37±2.25 vs 1.67±1.41 mg/L; p <0.001). Combinations of IL-6 and hs-CRP with age, HbA1c values and duration of diabetes, presented through three binary logistic regression models, are significant predictors of silent CHD proven by ergometric testing, i.e. with their increase in the probability of positive ergometric testing increased too (p <0.01).

The sensitivity of the associated finding of elevated IL-6 and hs-CRP values in the detection of silent CHD by ergometric testing was 90% and the specificity was 86%.

**Conclusion:** hs-CRP and IL-6 are significant predictors of silent CHD, and their determination could be recommended in improving cardiovascular risk stratification in asymptomatic patients with T2D.

**Key words:**

*diabetes mellitus, coronary heart disease, high-sensitivity C-reactive protein, interleukin 6.*
Apstrakt

Uvod/Cilj: Posebna karakteristika koronarne bolesti srca (KBS) kod pacijenata sa dijabetesom tipa 2 (T2D) je da je često asimptomatska i javlja se kao posljedica autonomne neuropatije kardiovaskularnog sistema. Disregulacija autonomnog nervnog sistema povezana je s povišenim vrijednostima upalnih markera kao što su visoko senzitivni C-reaktivni protein (hs-CRP) i interleukin 6 (IL-6) koji ubrzavaju aterosklerozu i pojavu kardiovaskularnih komplikacija kod pacijenata sa T2D.

Cilj studije bio je procijeniti značaj određivanja inflamatornih kardiovaskularnih markera rizika IL-6 i hs-CRP u skriningu na prisustvo KBS kod asimptomatskih pacijenata sa T2D.

Metode: Studija je obuhvatila 169 pacijenata sa T2D, bez simptoma i znakova KBS. Ergometrijskim testiranjem dokazano je ili isključeno prisustvo njime KBS. Nivoi hs-CRP i IL-6 određeni su ELISA metodom.

Rezultati: Vrijednosti IL-6 bile su veće kod pacijenata sa pozitivan ergometrijskim testom (6,83 ± 1,99 pg / ml) u odnosu na pacijente sa negativnim ergometrijskim testom (3,04 ± 1,39 pg / ml), što se stistički značajno razlikuje (p <0,001). Takođe smo dokazali da su vrijednosti hs-CRP kod pacijenata sa pozitivan ergometrijskim testom značajno veće u odnosu na pacijente sa negativnim ergometrijskim testom (6,37 ± 2,25 vs 1,67 ± 1,41 pg /l; p <0,001). Kombinacije IL-6 i hs-CRP sa godinama, vrijednostima HbA1c i trajanjem dijabetesa, predstavljene kroz tri binarna logistička regresiona modela su značajni prediktori njime KBS dokazane ergometrijskim testom tj. sa njihovim povećanjem vjerovatnoća pozitivnog ergometrijskog testa takođe se povećava (p <0,01).

Senzitivnost udruženog nalaza povišenih vrednosti IL-6 i hs-CRP u detekciji njime KBS putem ergometrijskog testiranja bila je 90%, a specifičnost 86%.

Zaključak: hs-CRP i IL-6 su značajni prediktori njime KBS i njihovo određivanje bilo bi značajno u poboljšanju stratifikacije kardiovaskularnog rizika kod asimptomatskih pacijenata sa T2D.

Ključne riječi: dijabetes melitus, koronarna bolest srca, visoko senzitivni C-reaktivni protein, interleukin 6.
Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in three out of four type 2 diabetes (T2D) patients. A special feature of CVD in patients with diabetes is that it is often asymptomatic and occurs as a consequence of cardiovascular autonomic neuropathy. The absence of pain during ischemic myocardial episodes, atypical and mild symptoms of acute myocardial infarction delay the start of treatment, causing increased morbidity and mortality in patients. In patients with T2D, autonomic dysfunction is thought to lead to the development of silent episodes of ischemia and silent infarction.

Recent studies have shown that autonomic nervous system dysregulation is associated with elevated inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6) and their determination could reflect the severity of atherosclerosis as well as the risk of developing future cardiovascular events in T2D patients.

In order to identify presence of coronary heart disease (CHD) in asymptomatic patients with diabetes, only an approach based on detailed assessment of traditional cardiovascular risk factors has still been recommended for the time being. Predicting the risk of cardiovascular events occurrence and progression of atherosclerosis and correlation of inflammatory agents in its progression has increasingly been the focus of research.

Traditional risk factors for CVD, such as high LDL cholesterol, low HDL cholesterol, hypertension and smoking explain only a part of cardiovascular risk in T2D patients.

At present, atherosclerosis is considered an inflammatory disease, given the key role of inflammation in all stages of the occurrence and development of atherosclerotic process, and the inflammatory nature of atherosclerosis is manifested by correlation of inflammatory marker levels in blood with its occurrence and progression. Vascular complications arise primarily as a consequence of endothelial dysfunction and inflammatory processes that play a role not only in initiation but also in the progression of atherosclerosis. Therefore, it is crucial to determine other risk factors for CVD occurrence such as progressive inflammatory tissue response to continuous deposition and modification of lipoproteins in the vascular wall.
The aim of the study was to evaluate the significance of determining inflammatory markers IL-6 and hs-CRP as atherosclerosis markers, during screening for presence of CHD in asymptomatic patients with T2D.

**Patients and methods**

Our examination was conducted at the Republic of Srpska University as a cross-sectional study, that included 169 type 2 diabetic patients, men (n=71) and women (n=98). All subjects underwent ergometric testing, and based on the obtained results, they were divided into two groups. The first group consisted of 117 type 2 diabetic patients without the presence of CHD, proven by the absence of symptoms and a negative ergometric test. The second group consisted of 52 type 2 diabetic patients with silent ischemic heart disease, proven by a positive stress test.

All subjects underwent an anamnestic interview after which they all gave their written consent to participate in the study. After that, a physical examination was performed defining the anthropometric measures. Calculation of Body Mass Index (BMI) for the assessment and monitoring of nutritional status was performed according to Quetelet's formula: BMI = body weight in kg/square of body height in meters (kg/m²). Subjects with T2D with CHD, with a history of cerebrovascular, peripheral vascular and malignant diseases were excluded from the study. Also, all the subjects who had an acute or chronic infection or who have been receiving corticosteroids or immunosuppressants within their therapy were excluded from our study.

The study was conducted in compliance with the Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Ethics Committee of the Republic of Srpska University Clinical Centre in Banja Luka gave their consent for approval of the research protocol.

**CHD diagnosis**

Ergometric testing was performed on a General Electric treadmill type T-2100. Testing was performed according to the standard Bruce protocol. The test was evaluated as positive in subjects with horizontal or descending ST-segment depression equal to or >1mm for 60-80 ms after the J-point, at least in three successive QRS complexes, as well as in patients who experienced ST-segment elevation during the stress test that was characterized as pathological if it occurred with the same characteristics, as well as ST-segment depression
(>1mm, lasts longer than 60-80 ms). The test was defined as positive and negative, and the patients in whom it was described as inconclusive were not considered.

**Laboratory analysis**

Biochemical blood tests for laboratory processing were taken in the morning after a 12-hour overnight fasting. The total cholesterol, HDL cholesterol, LDL cholesterol and serum triglycerides were measured directly, by homogeneous enzymatic procedure on INTEGRA® 400 plus analyser, manufactured by Roche, and HbA1c and urine albumin concentration in a 24-hour urine by a turbidimetric assay method. Determination of the levels of inflammatory markers hs-CRP and IL-6 was performed using ELISA (R&D Systems, Inc., Minneapolis, USA). It is a quantitative sandwich enzyme-linked immunosorbent assay technique. Blood serum was used for this test. The blood was then centrifuged at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at -70°C. A commercial calibrator was used for calibration. Subjects with hs-CRP values above 10 mg/L were excluded from the study because such hs-CRP values indicate the presence of acute inflammatory disease. An hs-CRP value of 1 mg/L indicates a low risk for CVD; from 1-3mg/L=moderate risk; from 3-10 mg/L = high risk 14. The lowest level of IL-6 detectability in the serum was 1.5 pg/ml 15. The coefficients of variation of the test were 5%. Calibrations of the testing instrument were performed as recommended by the manufacturer within the given specifications.

**Statistical analysis**

The data were analysed using a commercially available statistical programme (SPSS 17.0 for Windows; SPSS, Chicago, IL, USA). Continuous variables are summarized as mean ± SD or as a percentage of frequency. Categorical variables are expressed as proportions (percentage), Student’s t-test (for continuous variables) or chi-square proportion test (for categorical variables) were used. Appropriate descriptive and analytical methods (absolute and relative numbers, t test, Wilcoxon test, Mann-Whitney U test) were also used. Multiple logistic regression was applied to predict and evaluate one variable based on the value of the other variable or multiple variables. The significance level was less than 0.05.
Results

Screening test for presence of CHD was performed in 169 asymptomatic patients mean age 58.71 ± 6.76; range from 40 to 70 years with T2D without a history of any CVD. The presence of silent CHD was proven in 52 subjects using ergometric testing, while 117 subjects were without CHD. We examined whether there were differences in cardiovascular risk factors between the study groups.

Table 1 shows a comparison of demographic and risk factors between subjects with positive or negative ergometric test result. Subjects did not differ significantly by gender. The patients with positive ergometric test (silent CHD) were older with a longer duration of diabetes and a higher incidence of smokers compared to the patients with negative ergometric test result (p <0.05). The difference in BMI between the study groups with positive or negative ergometric test was not statistically significant. Prevalence of hypertension as well as HbA1c values were statistically significantly higher in subjects with positive ergometric test compared to the patients with negative ergometric test (p<0.05). Regarding the lipid parameters, total LDL cholesterol as well as triglycerides, were significantly higher in the group of subjects with positive ergometric test (p<0.05), whereas the values of HDL cholesterol did not differ significantly between the study groups. In subjects with with positive ergometric test, microalbuminuria was present in 47 subjects (90.4%). In subjects in whom CHD was not detected (negative ergometric test), microalbuminuria was present in 20 subjects (17.1%), which was statistically significantly different (p <0.001).

When we analysed inflammatory markers (IL-6 and hs-CRP) we found that IL-6 values significantly higher in patients with positive ergometric test (p<0.001) (Table1). Similarly, we also found that hs-CRP values in patients with positive ergometric test was significantly higher in comparison to patients with negative ergometric test (Table 1).

The results of combinations of IL-6 and hs-CRP with age show that increasing the value of IL-6 by one unit increases the possibility of a positive ergometric test by 1.439 times, increasing the value of hs-CRP by one unit by 1.830 times while increasing the patient's age by one year increases the possibility of a positive ergometric test by 1.160 times. (Table 2).
Combining IL-6 and hs-CRP with Hba1c, the results of our study showed that an increase in IL-6 by one unit increased the possibility of a positive ergometric test by 1.495 times, hs-CRP values by 1.565 times, while an increase in HbA1c by one unit increased the possibility of a positive test for 1.471 times. (Table 2).

The results of combinations of independent variables IL-6 and hs-CRP with DM duration show that increasing the value of IL-6 by one unit increases the possibility of a positive ergometric test by 1.581 times, increasing hs-CRP by one unit increases the possibility of a positive ergometric test for 1.663 times while increasing the duration of DM by one unit increases the possibility of a positive test 1.293 times (Table 2).

Within all three analyzed models, combinations of IL-6 and hs-CRP with age, HbA1c values and duration of diabetes, show that these values, presented through three binary logistic regression models, are significant predictors of silent CHD proven by ergometric test i.e. with their increase in the probability of positive ergometric test increased too (p <0.01). (Table 2).

ROC analysis was used to test the predictive powers of IL-6 with a positive ergometric test. Using the multivariate logistic registration model used in ROC analysis, the clinical accuracy of the diagnostic procedure was a good; The area under the curve AUC of 0.925 (CI: 0.882 to 0.969). IL-6 has a sensitivity of 90.4% and a specificity of 82.9%. (Figure 1).

ROC analysis was also used in testing the predictive power of hs-CRP with a positive ergometric test. Using the multivariate logistic regression model used in the ROC analysis, the clinical accuracy of the diagnostic procedure was a good. The area under the curve AUC of 0.934 (CI: 0.895 to 0.972). Hs-CRP has a sensitivity of 88.5% and a specificity of 80%. (Figure 2).

We examined the significance of the associated risk of increased hs-CRP and IL-6 values in the detection of silent CHD proven by ergometric testing. In patients with T2D in whom silent CHD was detected, 47 (90%) subjects had a finding of associated risk, while in 5 (10%) there was no associated risk. In patients with T2D without CHD, there was an associated risk in 16 (14%), while in 101 (86%) subjects it did not exist. This difference in frequency distribution is statistically significant (p <0.001). (Figure 3).
Discussion

Our study demonstrated that a large percentage of patients with T2D have silent CHD and that elevated levels of inflammatory markers (IL6 and CRP) represent a strong marker for presence of silent CHD.

Previous research has shown that silent CHD in people with diabetes varied and that there was a need to define the degree of cardiovascular risk in people with silent CHD who could benefit from screening. In the detection of ischemia in asymptomatic diabetics (DIAD) study were randomly assigned to either stress testing and 5-year clinical follow-up or to follow-up only. A total of 22% of patients had silent ischemia. The prevalence of silent myocardial ischaemia in our study was 29%, which is mostly consistent with previously published literature. Due to all the above, coronary artery disease in patients with diabetes is a diagnostic and therapeutic challenge.

Although T2D alone is a large and independent risk factor for occurrence of CVD, coexistence of traditional cardiovascular risk factors significantly increases the risk for occurrence of silent CHD in T2D patients. The Multiple Risk Factor Intervention Trial showed that multiple risk factors in the same patient significantly increased the overall cardiovascular risk. Gaede et al. reported that an intervention directed at multiple risk factors significantly improves cardiovascular prognosis. This is supported by the results of our study which showed that the patients with silent CHD were older, with longer duration of diabetes, higher prevalence of hypertension, poorer glucose regulation, higher values of total cholesterol, LDL cholesterol and triglycerides as well as higher prevalence of albuminuria compared to subjects who did not have CHD, while HDL cholesterol levels and BMI had no statistical significance between the study groups.

Since the inflammatory process is an integral part of the evolution of atherosclerosis, the use of CRP as a biomarker becomes very useful in combination with the control of classic risk factors such as lipid levels, changes in eating habits, weight loss, regular physical activity, glycemic control and smoking cessation. The interrelation of these risk factors for CVD are strategies to reduce cardiovascular events in primary and secondary prevention.

Due to the relationship between high CRP plasma levels and cardiovascular mortality and morbidity risk it is important to establish a primary care line to decrease CVDs. For this, it is essential the evaluation of cardiovascular risk factors to stop their progression. Several
prospective studies having CRP as a central target have shown the benefits of primary prevention. In 1999, the MONICA-Augsburg study performed in a sample of 936 asymptomatic men, concluded that the increase in hs-CRP leads to a 19% increased risk of fatal and non-fatal coronary events\textsuperscript{21}. In the same way, the PREVEND study in 8139 asymptomatic men and women observed a relationship between hs-CRP and angiographic characteristics and consequently clinical instability of the atherosclerotic plaque\textsuperscript{22}. A six-year follow-up study of healthy middle-aged men showed that baseline IL-6 levels greater than 2.28 pg / ml were associated with a 2.3-fold higher risk of future myocardial infarction, which is why IL-6 was also identified as a significant predictor risk of cardiovascular events\textsuperscript{23}. Recent research has reported the importance of inflammation in the development and progression of atherosclerosis as well as the possibility of using inflammatory markers to assess cardiovascular risk. Among the several biomarkers proposed in cardiovascular risk stratification is CRP, which would be used in the identification of individuals at risk for developing CVD, but this is not yet recommended in the guidelines\textsuperscript{24, 25, 26}. Prospective clinical case-control studies Physician’s Health Study and Women Health Study, have identified CRP as a strong, independent risk factor for CHD\textsuperscript{27, 28}. Previous research has shown that hs-CRP was a predictor of CVD, even after adjusting to traditional risk factors indicating that hs-CRP may provide additional significant prognostic information in cardiovascular risk assessment\textsuperscript{29}. Also, elevated levels of IL-6 in serum is correlated with the development of CAD, which is why IL-6 has become an important cytokine in assessment of atherosclerosis in people with T2D, as evidenced by two large genetic studies reporting correlation between IL-6 receptor signalling and CVD\textsuperscript{30, 31, 32}. It has been also suggested that elevated IL-6 values were correlated with an increased risk of future myocardial infarction even after adjustment in initial differences in total cholesterol, HDL-cholesterol, BMI, blood pressure, diabetes mellitus, family medical history, alcohol consumption and doing physical activity\textsuperscript{33, 34}. Also, elevated levels of IL-6 can play a predictive role in occurrence of CVD, thus providing a potential prognostic means in detection of CVD\textsuperscript{35}. The results of our research also support these studies. We concluded that the combined finding of increased values of IL-6 and hs-CRP posed a high risk of the presence of silent CHD in T2D patients.
In this study, we also analysed the significance of inflammatory cardiovascular risk markers (CRP, IL-6) for the appearance of silent CHD in patients with T2D. In subjects with silent CHD, there was a direct correlation with IL-6 and hs-CRP values that were significantly higher compared to the subjects without CHD. The results of our study showed that IL-6 and hs-CRP are significant predictors of silent CHD and showed that the association of IL-6 and hs-CRP with age, HbA1c values and duration of diabetes are significant predictors of silent CHD proven by ergometric testing.

In conclusion, our study showed that a large percentage of T2D patients had silent CHD. Elevated levels of inflammatory cardiovascular risk markers hs-CRP and IL-6 value are strong markers of the presence of silent CHD in asymptomatic T2D patients. Given that traditional risk factors for CVD explain only a part of cardiovascular risk in T2D patients, and current screening recommendations are based on their use, it would be important to include the determination of inflammatory cardiovascular risk markers in order to improve cardiovascular risk stratification in asymptomatic patients with T2D. By doing so, we would be able to reduce the incidence of cardiovascular complications occurrence and apply appropriate treatment modalities in a timely manner, whether it was a conservative or invasive treatment.

Ultimately, there are some limitations of our study. First, the study was single centre trial assay with relatively small number of subjects. Second, this study was cross sectional, without appropriate follow up, so our study could not demonstrate, in the long term, the incidence of silent CHD or the influence of investigated markers to the future appearance of CHD. This could be main reason to extended the investigation to a larger number of subjects and a longer follow-up in the future in order to get stronger results.
Table 1.
Demographic and anthropometric characteristics of the T2D patients with positive or negative ergometric test

| Characteristics | Patients with positive ergometric test | Patients with negative ergometric test | p value |
|----------------|---------------------------------------|---------------------------------------|---------|
| n=52           | n=117                                 |                                       |         |
| Gender (male/female) | 24/28                               | 47/70                                 | ns      |
| Smoking, n (%) | 32 (38.5)                             | 16 (13.7)                             | <0.05   |
| Age (years) | 58.71 ± 6.76                          | 54.98 ± 6.69                          | <0.05   |
| Duration of DM (years) | 10.52 ± 4.60                      | 7.08± 3.19                            | <0.05   |
| BMI, (kg/m2) | 27.6 ±1.58                            | 27.2±1.46                             | ns      |
| Systolic BP (mm Hg) | 139.90 ± 11.7                        | 128.16 ± 10.72                        | <0.05   |
| Diastolic BP (mm Hg) | 88.56 ± 9.3                          | 81.03± 7.27                           | <0.05   |
| HbA1c, (%) | 9.16 ±1.91                            | 7.43± 1.08                            | <0.05   |
| Total cholesterol, (mmol/l) | 6.07± 1.33                          | 5.37± 1.11                            | <0.05   |
| LDL cholesterol, (mmol/l) | 3.97±1.11                           | 3.33 ±0.86                            | <0.05   |
| HDL cholesterol, (mmol/l) | 1.17 ±0.2                            | 1.14 ±0.37                            | ns      |
| Triglyceride, (mmol/l) | 2.35±1.1                             | 2.06 ± 1.37                           | <0.05   |
| Microalbuminuria, n (%) | 47 (90.4)                           | 20 (17.1)                             | <0.001  |
| IL-6,(pg/mL) | 6.83±1.99                             | 3.04±1.39                             | <0.001  |
| Hs-CRP, (mg/L) | 6.37±2.25                            | 1. 67±1.41                            | <0.001  |

DM: diabetes mellitus; BMI: Body Mass Index; BP: blood pressure; HbA1c: Glycosylated haemoglobin; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; IL-6: interleukin 6; hs-CRP: highly sensitive C-reactive protein
Table 2. Binominal logistic regression analysis of risk factors for prediction of ergometric test amongst the study population

| Variables | B   | S.E. | p      | OR   | 95% CI. for OR |
|-----------|-----|------|--------|------|---------------|
|           |     |      |        |      | Lower      | Upper |
| Model 1: IL-6 + hs-CRP + Age |     |      |        |      |             |      |
| IL-6      | .364 | .219 | .097   | 1.439 | .937    |       |
| 2.210     |     |      |        |      |           |      |
| hs-CRP    | .604 | .194 | .002   | 1.830 | 1.250   |       |
| 2.678     |     |      |        |      |           |      |
| Age       | .148 | .056 | .008   | 1.160 | 1.039   |       |
| 1.294     |     |      |        |      |           |      |
| Constant  | -7.454 | 1.956 | .000  | .001  |           |      |
| Model 2: IL-6 + hs-CRP + HbA1c |     |      |        |      |           |      |
| IL-6      | .402 | .209 | .054   | 1.495 | .993    |       |
| 2.249     |     |      |        |      |           |      |
| hs-CRP    | .448 | .174 | .010   | 1.565 | 1.112   |       |
| 2.202     |     |      |        |      |           |      |
| HbA1c%    | .386 | .238 | .106   | 1.471 | .922    |       |
| 2.348     |     |      |        |      |           |      |
| Constant  | -7.454 | 1.956 | .000  | .001  |           |      |
| Model 3: IL-6 + hs-CRP + Diabetes duration |     |      |        |      |           |      |
| IL-6      | .458 | .219 | .037   | 1.581 | 1.029   |       |
| 2.429     |     |      |        |      |           |      |
| hs-CRP    | .509 | .184 | .006   | 1.663 | 1.160   |       |
| 2.384     |     |      |        |      |           |      |
| Diabetes duration | .257 | .081 | .001   | 1.293 | 1.105   |       |
| 1.514     |     |      |        |      |           |      |
| Constant  | -7.092 | 1.202 | .000  | .001  |           |      |

**IL-6: interleukin 6; hs-CRP: high-sensitivity C-reactive protein; HbA1c: Glycosylated haemoglobin; OR: odds ratio; CI: confidence interval.**
Figure 1.
ROC curve (receiver operating characteristic) predictive power of IL-6 with positive ergometric test

Area = .825
p < .001
CI .802 - .960
Figure 2.

ROC curve (receiver operating characteristic) predictive power of hs-CRP with positive ergometric test
Figure 3.
Significance of the associated risk of increased hs-CRP and IL-6 values in the detection of silent CHD proven by ergometric testing.

References

1. Mei-Fang Li, Cui-Chun Zhao, Ting-Ting Li et al. The coexistence of carotid and lower extremity atherosclerosis further increases cardio-cerebrovascular risk in type 2 diabetes; Cardiovasc Diabetol. 2016;15:43.
2. Bulugahapitiya U, Siyambalapitiya S, Sithole J, Idris I. Is diabetes a coronary risk equivalent? Systematic review and meta-analysis. Diabet Med. 2009;26:142–148.
3. Dongfang Su, Zhongxia Li, Xinrui Li et al. Association between Serum Interleukin-6 Concentration and Mortality in Patients with Coronary Artery Disease. Research Article 2013;47:209-214
4. Libby P., Ridker PM., Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105: 1135-43.
5. Weiner DA, Ryan TJ, McCabe CH, Luk S, Chaitman BR, Sheffield T, Tristani F, Fisher L. Significance of silent myocardial ischemia during exercise testing in patients with coronary artery disease. Am J Cardiol. 1987; 59:725-729.
6. Laakso M, Lehto S. Epidemiology of risk factors for cardiovascular disease in diabetes and impaired glucose tolerance. Atherosclerosis 1998; 137 (Suppl.): S65–S73

7. Lalić K., M. Medić-Zamaklar. Značaj lipidskih poremećaja za aterogenezu. Priručnik za dijagnostiku i lečenje lipidskih poremećaja, 2004; 57-64. (Serbian)

8. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nature Med. 2002;8:1211–7.

9. Dandona P, Aljada A. A rationale approach to pathogenesis and treatment of type 2 diabetes mellitus, insulin resistance, inflammation and atherosclerosis. Am J Cardiol 2002; 90:27-33.

10. Dongfang Su, Zhongxia Li, Xinrui Li et al. Association between Serum Interleukin-6 Concentration and Mortality in Patients with Coronary Artery Disease. Research Article 2013;47:1209-214

11. Calabro P, Willerson JT, Yeh et al. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation.2003;108:1930-1932.

12. Irace C, De Luca S, Shehaj E et al. Exenatide improves endothelial function assessed by flow mediated dilation technique in subjects with type 2 diabetes: results from an observational research. Diab Vasc Dis Res.2013;10:72-77.

13. Myers J, Arena R, Franklin B, Pina I, Kraus WE, McInnis K, Balady GJ; on behalf of the American Heart Association Committee on Exercise, Cardiac Rehabilitation, and Prevention of the Council on Clinical Cardiology, the Council on Nutrition, Physical Activity, and Metabolism, and the Council on Cardiovascular Nursing. Recommendations for clinical exercise laboratories: a scientific statement from the American Heart Association. Circulation. 2009;119:3144–3161.

14. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of Clinical Investigation, 2003; 111 (12): 1805–12.

15. R&D Systems, human VEGF; Catalogue DVE00, human FGF basic; catalogue DFB50,human IL-1β; catalogue DLB50, human IL-6; catalogue D6050, human TNF-α; catalogue DTA00C. Available at: www.Rndsystems.com/product-results
16. Milan study on atherosclerosis and diabetes (MiSAD) group. Prevalence of unrecognized silent myocardial ischemia and its association with atherosclerotic risk factors in non-insulin dependent diabetes mellitus. Am J Cardiol. 1997;79:134-9.

17. Wackers FJT, Young LH, Inzucchi SE, et al. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care. 2004;27:1954-1961

18. Ales Kotalik, Anne Eaton, Qinshu Lian, Carlos Serrano, John Connett, James D Neaton. A win ratio approach to the re-analysis of Multiple Risk Factor Intervention Trial. Sage Journals 2019; 31389723

19. Gaede P, Vedel P, Larsen N, Jensen G, V. Parving H, H. Pedersen. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med., 2003 Jan 30;348(5):383-93.

20. Denardi CAS, Filho AC, Chagas ACP (2008) C-reactive protein today. Rev SOCERJ, 2008; 2:329-334.

21. Koenig W, Sund M, Fröhlich M, Fischer HG, Löwel H, 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 MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort study, 1984 to 1992. Circulation, 1999; 99: 237-242.

22. Geluk CA, Post WJ, Hillege HL, Tio RA, Tijssen JG, et al. C-reactive protein and angiographic characteristics of stable and unstable coronary artery disease: Data from the prospective PREVEND cohort. Atherosclerosis, 2008; 196: 372-382.

23. Lee KW, Lip GY, Tayebjee M, Foster W and Blann AD. Circulating endothelial cells, von Willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. Blood 2005; 105: 526-32.

24. Ridker P.M, Hennekens C.H, Buring J.E, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. The New England Journal of Medicine, 2000; 342: 836-843.

25. P.M. Ridker, M. Cushman, M.J. Stampfer, R.P. Tracy, C.H. Hennekens. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med, (1997), pp. 973-979
26. Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, et al. Myocardial infarction accelerates atherosclerosis. Nature. 2012;487(7407):325-9.

27. P.M. Ridker, J.E. Buring, J. Shih, M. Matias, C.H. HennekensProspective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation, 98 (1998), pp. 731-733

28. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: areport of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 Suppl 2):S49-73.

29. Shikawa T, Hatakeyama K, Imamura T, Date H, Shibata Y, et al. Involvement of C-reactive protein obtained by directional coronary atherectomy in plaque instability and developing restenosis in patients with stable or unstable angina pectoris. Am J Cardiol. 2003; 91: 287-292.

30. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012; 308(8):788–95.

31. Blake GJ and Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002; 252: 283-294.

32. Ingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisationanalysis.Lancet 2012; 379(9822):1214–1224.

33. Arwar N, Butterworth AS, Freitag DF, GregsonJ,WilleitP,GormanDN,GaoP,Saleheen D, Rendon A, Nelson CP, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies.Lancet 2012;379(9822):1214–1224.

34. Ridker PM, Rifai N, Stampfer MJ and Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767-1772.

35. Lee KW, Lip GY, Tayebjee M, Foster W and Blann AD. Circulating endothelial cells, von Willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. Blood 2005;105:526-32.
