Research Article

Serum Glycoprotein Chondrex (YKL-40) and High Sensitivity C-Reactive Protein (hscrp) in Type 2 Diabetic Patients in Relation to Cardiovascular Complications

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Abstract

In Type 2 diabetes, C-Reactive Protein (CRP) as an inflammatory marker may be elevated. The glycoprotein Chondrex or YKL-40 is over expressed in many inflammatory conditions. The aim is to study serum hsCRP and YKL-40 in Type 2 diabetic patients in relation to cardiovascular complications.

Methods: Eighty subjects were divided into 3 groups: GROUP 1: 16 apparently healthy controls, GROUP 2: 16 patients suffering from Type 2 DM without cardiovascular complications and GROUP 3: 48 patients suffering from Type 2 DM with cardiovascular complications.

Results: High sensitivity CRP levels were significantly elevated in the diabetic group compared to the diabetic group without cardiovascular complications (p=0.024). YKL-40 was significantly higher in patients with type 2 diabetes mellitus than controls (p=0.017) and cardiovascular complications (p=0.001) contributed to its greater elevation. YKL-40 was positively correlated with triglycerides, systolic and mean blood pressure in the group of diabetic patients without cardiovascular complications and with duration of diabetes and urinary albumin to creatinine ratio in the group with cardiovascular complications. By drawing receiver operating characteristic (ROC) curve between diabetic patients without and with cardiovascular complications the AUC for hsCRP was (0.676, p=0.036) and for YKL-40 was (0.743, p=0.004). By studying the diagnostic performance, YKL-40 had a better specificity and positive predictive value than hsCRP.

Conclusion: YKL-40 has a better specificity and positive predictive value than hsCRP in discriminating between diabetic patients with cardiovascular complications from those without cardiovascular complications.

Introduction

Chondrex or YKL-40 identified in 1989 [1] is related in amino acid sequence to the human glycoprotein chitinase protein family, but YKL-40 has no chitinase activity [2]. The abbreviation YKL-40 is based on the one letter code for the first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight of YKL-40 [3].

The protein YKL-40 contains a single polypeptide chain of 383 amino and has several names: "Human cartilage glycoprotein-39 (HC gp39)", [4] "Breast regressing protein 39 Kd (brp-39)", [5] "38-KDa heparin-binding glycoprotein (Gp38k)", [6] "Chitinase-3-like 1 protein (CHI3L1)", [7] "Chondrex", [8] and "40 KDa mammory gland protein (MGP-40)" [9].

The YKL-40 can play a role as sentinels that trigger responses to parasites, infections and / or antigen challenge. It attracts eosinophils and T cells to sites of parasitic infections, generates or modulates tissue inflammation and / or remodeling [10].

In normal bone marrow the myelometamyelocytes express YKL-40 protein, and it is stored in the specific granules of neutrophil granulocytes and released from fully activated cells [11].

Previous workers found that, the median serum YKL-40 in healthy subjects, was 43 μg/L (range, 20-184 μg/L; 5-95% interval, 20-124 μg/L), values of YKL-40 increases with age with no difference between men and women [12] and no circadian variability in its level [13].

It is suggested that any change in serum YKL-40 levels more than 20% should be considered to be indicative of significant change [12].

Low grade inflammation and activation of the innate immune system play a role in the common pathogenesis of both insulin resistance and endothelial dysfunction and subsequently...
the development of type 2 diabetes and atherosclerosis. CRP still remains the most validated predictor of cardiovascular events is being supplemented by studies on emerging markers [14]. Dysfunction of the vascular endothelium is considered an important factor in the pathogenesis of diabetic micro- and macroangiopathy [15,16].

A previous study showed that YKL-40 plays a role in endothelial dysfunction in relation to cell migration, reorganization, and tissue remodeling during atherogenesis [16]. YKL-40 promotes chemotaxis, cell attachment, spreading, and migration of vascular endothelial cells, suggesting that YKL-40 promotes the process of atherosclerotic plaque formation, in which vascular smooth muscle cells (VSMCs) are induced to migrate through the intima in response to exogenous signals [3]. YKL-40 also modulates vascular endothelial cell morphology by promoting the formation of branching tubes, indicating a role of YKL-40 in angiogenesis by stimulating the migration and reorganization of VSMCs. Furthermore, YKL-40 is produced and secreted by monocytes during differentiation to macrophages and is also secreted by activated macrophages [17]. Substantial evidence indicates that YKL-40 participates in monocyte differentiation and macrophage activation as part of the endothelial dysfunction and the processes during early stages of atherosclerosis and seems to be of pathogenic importance in the low-grade inflammation that precedes the development of cardiovascular disease [13,17].

Interestingly, macrophages in atherosclerotic plaques express YKL-40 mRNA, particularly macrophages located deep in the lesion, and the highest YKL-40 expression is found in macrophages in the early lesion of atherosclerosis [18].

There is still limited knowledge about YKL-40 in diabetic patients.

**Aim**

The study of serum C-Reactive Protein (hsCRP) and Chondrex (YKL-40) in Type 2 diabetic patients in relation to cardiovascular complications.

**Methods**

This case-control study was done in accordance to ethical standards of Medical Research Institute based on Helsinki Declaration revised in 2000. Eighty subjects, recruited consecutively from February to July 2014, after taking their consents, from the diabetes clinic of MRI, were divided as: **Group 1**: 16 apparently healthy volunteers, **Group 2**: 16 patients suffering from type 2 DM without clinically evident cardiovascular complications and **Group 3**: 48 patients suffering from type 2 DM with cardiovascular complications. All subjects were of comparable age and sex. Subjects with acute or chronic inflammation, autoimmune disease or malignancy were excluded.

To all subjects the following was done: Complete physical Examination, B mode ultrasonography for CIMT and fundus examination. Complete urine analysis, urinary albumin, creatinine and calculation of urinary albumin to creatinine ratio. Fasting and postprandial glucose, glycated hemoglobin, creatinine and uric acid, Alanine Aminotransferase (ALT) activities, lipid profile, estimated glomerular filtration rate, also hsCRP and YKL-40 [19]. Statistical analysis done using SPSS program version 20 (Statistical Package of social sciences, Chicago, USA) [20].

**For quantitative variables**

Kolmogrov - Smirnov test for normality was used to test for the degree of deviation from normal distribution across all variables. The data of the variables were summarized in the form of: the mean as a measure of central tendency and the Standard Deviation (SD) as a measure of dispersion for normally distributed and as median and range for abnormally distributed. Pearson and Spearman correlation coefficients were used to calculate correlation between variables. *P*<0.05 is considered significant. ROC curve was drawn by sensitivity on y-axis and 1-specificity on the x-axis. The highest Youden’s index calculated gave the best cutoff. The greater the area under the curve was the best curve.

**Results**

Table 1 shows statistical significance of some clinical findings of the studied groups.

Table 2 shows statistical significance of levels of some studied parameters in the studied groups.

The median value of hsCRP was significantly higher in the group of diabetic patients without cardiovascular complications (group 2) when compared to the control group (group 1), and its median value was significantly increased in the group of diabetic patients with cardiovascular complications (group 3) when compared to both the control group (group 1) and the group of diabetic patients without cardiovascular complications (group 2) (Table 2).

Table 3: shows significant correlations of the studied groups.

Table 4: shows clinical (sensitivity, specificity, positive predictive value, negative predictive value and efficiency) for hsCRP and YKL-40 in the studied groups.

Table 5: shows clinical (sensitivity, specificity, positive predictive value, negative predictive value and efficiency) for hsCRP and YKL-40 in diabetic patients.

YKL-40 had a better sensitivity, negative predictive value and efficiency than hsCRP in discriminating between diabetic patients and controls. However, it had a better specificity and positive predictive value than hsCRP in discriminating between diabetic patients without cardiovascular complications from those with cardiovascular complications. (Table 4&5).

**Figure 1**: Bar chart showing YKL-40 levels in both diabetic groups in relation to the control group.

**Figure 2** revealed that level of serum YKL-40 in the group of diabetic patients without cardiovascular complications (group 2) was significantly increased when compared to the control group (group 1). Also the level of serum YKL-40 in the group of diabetic patients with cardiovascular complications (group 3) was significantly higher when compared to both the control group (group 1) and the group of diabetic patients without cardiovascular complications (group 2).

**Figure 2** shows ROC curve for hsCRP and YKL-40 between controls and diabetics.

**Figure 3** shows that YKL-40 had a bigger area under the Receiver Operating Characteristic (ROC) curve than hsCRP indicating that YKL-40 was better than hsCRP in discriminating between diabetic patients without and with cardiovascular complications.
Table 1: Statistical significance of some clinical findings of the studied groups.

| Item                  | Group 1 (n=16) | Group 2 (n=16) | Group 3 (n=48) |
|-----------------------|---------------|---------------|---------------|
| **Age (years)**       |               |               |               |
| Mean                  | 44.06         | 48.38         | 54.96         |
| SD                    | ±11.82        | ±8.11         | ±9.32         |
| p                     | NS            | NS            | 0.001*        |
| p1                    | NS            | NS            | NS            |
| **Sex**               |               |               |               |
| Males                 | 2 (12.5%)     | 5 (31.3%)     | 11 (22.9%)    |
| Females               | 14 (87.5%)    | 11 (68.8%)    | 37 (77.1%)    |
| FE p                  | NS            | NS            | NS            |
| FE p1                 | NS            | NS            | NS            |
| **Weight (kg)**       |               |               |               |
| Mean                  | 69.94         | 88.56         | 98.04         |
| SD                    | ±11.13        | ±14.00        | ±22.91        |
| p                     | 0.026*        | 0.000**       |               |
| p1                    | NS            | NS            | NS            |
| **Systolic BP (mmHg)**|               |               |               |
| Mean                  | 118.75        | 125           | 135.21        |
| SD                    | ±7.19         | ±11.55        | ±15.29        |
| p                     | NS            | NS            | 0.000**       |
| p1                    | NS            | NS            | 0.035*        |
| **Diastolic BP (mmHg)**|             |               |               |
| Mean                  | 77.81         | 83.44         | 86.91         |
| SD                    | ±5.15         | ±8.7          | ±12.49        |
| p                     | NS            | NS            | 0.008*        |
| p1                    | NS            | NS            | NS            |
| **Mean BP (mmHg)**    |               |               |               |
| Mean                  | 91.44         | 97.28         | 102.98        |
| SD                    | ±5.70         | ±9.38         | ±12.94        |
| p                     | NS            | NS            | 0.003*        |
| p1                    | NS            | NS            | NS            |
| **CIMT (mm)**         |               |               |               |
| Mean                  | 0.55          | 0.67          | 0.99          |
| SD                    | ±0.16         | ±0.13         | ±0.40         |
| p                     | NS            | NS            | 0.000**       |
| p1                    | NS            | NS            | 0.003*        |
| **Duration of diabetes(years)** |       |               |               |
| Median                | -             | 1.0           | 12            |
| Min-max               | -             | 0.10-17.0     | 4.0-40.0      |
| p                     |               |               | 0.000*        |

n: number of subjects.
p: statistical significance from control group.
p1: statistical significance from group of diabetic patients without cardiovascular complications.
*p-value < 0.05 was considered statistically significant.
**p-value < 0.001 was considered highly significant.

**Group 1:** control group.
**Group 2:** group of diabetic patients without cardiovascular complications.
**Group 3:** group of diabetic patients with cardiovascular complications.
SD: standard deviation. Min-Max: minimum-maximum.

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Table 2: Statistical significance of levels of some studied parameters in the studied groups.

| Item            | Group 1 (n=16) | Group 2 (n=16) | Group 3 (n=48) |
|-----------------|---------------|---------------|---------------|
| **FSG (mg/dl)** |               |               |               |
| Mean            | 92            | 167.56        | 209.83        |
| SD ±9.18        | ±79.7         | ±88.35        |               |
| P               | 0.022*        | 0.000**       | NS            |
| **PPG (mg/dl)** |               |               |               |
| Mean            | 105.38        | 244.31        | 291.67        |
| SD ±11.63       | ±95.25        | ±109.95       |               |
| P               | 0.000**       | 0.000**       | NS            |
| **HbA1C (%)**   |               |               |               |
| Mean            | 5.31          | 7.72          | 9.25          |
| SD ±0.37        | ±1.26         | ±2.09         |               |
| P               | 0.001*        | 0.000**       | 0.010*        |
| **Creatinine (mg/dl)** |       |               |               |
| Median          | 0.9           | 1.0           | 1.15          |
| Min-Max         | 0.7-1.1       | 0.7-1.2       | 0.7-5.3       |
| P               | NS            | NS            | 0.000**       |
| **Uric acid (mg/dl)** |       |               |               |
| Mean            | 3.89          | 4.81          | 6.21          |
| SD ±0.82        | ±1.12         | ±2.53         |               |
| P               | NS            | NS            | 0.001*        |
| **ALT (U/L)**   |               |               |               |
| Mean            | 13.5          | 20.56         | 20.08         |
| SD ±4.07        | ±8.47         | ±8.38         |               |
| P               | 0.035*        | 0.013*        | NS            |
| **TC (mg/dl)**  |               |               |               |
| Mean            | 182.12        | 211.44        | 203.04        |
| SD ±19.6        | ±37.27        | ±64.8         |               |
| P               | NS            | NS            | NS            |
| **HDL-C (mg/dl)** |            |               |               |
| Mean            | 61.06         | 43.14         | 43.65         |
| SD ±12.33       | ±7.32         | ±11.56        |               |
| P               | 0.000**       | 0.000**       | NS            |
| **LDL-C (mg/dl)** |            |               |               |
| Mean            | 104.7         | 140.16        | 123.79        |
| SD ±19.27       | ±33.14        | ±55.99        |               |
| P               | 0.047*        | NS            | NS            |
|                      | Group1 | Group2 | Group3 |
|----------------------|--------|--------|--------|
| **TG (mg/dl)**       | Median | 79     | 182    | 158.5  |
|                      | Min-Max| 38-143 | 71-332 | 46-1031|
|                      | P      | 0.000**| 0.000**|
|                      | p1     | NS     | NS     |        |
| **Urine albumin (mg/l)** | Median | 11.2   | 10.55  | 71.6   |
|                      | Min-Max| 1.4-28.2| 3.3-32.4| 2.8-3014.8|
|                      | P      | NS     | 0.000**|
|                      | p1     | NS     | 0.001**|
| **Urine creatinine (mg/dl)** | Median | 128    | 123.7  | 77.9   |
|                      | Min-Max| 40-212 | 40.5-315.5| 17.8-201.5|
|                      | P      | NS     | 0.003* |
|                      | p1     | NS     | NS     |        |
| **Alb/cr ratio (mg/g)** | Median | 10.54  | 12.73  | 93.8   |
|                      | Min-Max| 1.6-28.5| 2.28-29.6| 3.3-8025.7|
|                      | P      | NS     | 0.000**|
|                      | p1     | NS     | 0.000**|
| **eGFR (ml/min/1.73m2)** | Mean   | 91.13  | 108.26 | 84.61  |
|                      | SD     | ±10.85 | ±32.60 | ±47.10 |
|                      | p      | NS     | NS     |        |
|                      | p1     | NS     | NS     |        |
| **hsCRP (mg/L)**     | Median | 0.875  | 3.58   | 9.79   |
|                      | Min-Max| 0.16-2.73| 0.38-17.2| 0.3-92.9|
|                      | p      | 0.000**| 0.000**|
|                      | p1     | 0.024* |
| **YKL-40 (ng/mL)**   | Mean   | 56.64  | 165.34 | 266.62 |
|                      | SD     | ±48.21 | ±122.74| ±116.62|
|                      | p      | 0.017* | 0.000**|
|                      | p1     | 0.005* |

n: number of subjects.

p: statistical significance from control group.

p1: statistical significance from group of diabetic patients without cardiovascular complications.

*p-value < 0.05 was considered statistically significant.

**p-value < 0.001 was considered highly significant.

Group1: control group.

Group2: group of diabetic patients without cardiovascular complications.

Group3: group of diabetic patients with cardiovascular complications.

SD: standard deviation. Min-Max: minimum-maximum.

FSG=fasting serum glucose, PPG=post prandial glucose, HbA1C=glycated hemoglobin A1C

ALT=alanine aminotransferase, TC=total cholesterol, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, TG=triglycerides, Alb/C=albumin/creatinine ratio, eGFR=estimated glomerular filtration rate, hsCRP=high sensitivity C-reactive protein, YKL-40=Chondrex.
Table 3: Significant correlations of the studied groups.

| Item                              | Correlation coefficient (r) | Significance (p-value) |
|-----------------------------------|----------------------------|------------------------|
| Significant correlations of the control group (Group 1): |
| YKL-40 and age                    | 0.526                      | 0.037                  |
| YKL-40 and TG*                    | 0.559                      | 0.025                  |
| YKL-40 and micro-albumin*         | -0.500                     | 0.048                  |
| hsCRP and uric acid*              | 0.572                      | 0.021                  |
| hsCRP and SBP*                    | 0.504                      | 0.047                  |
| hsCRP and MBP*                    | 0.527                      | 0.036                  |
| Significant correlations of the group of diabetic patients without cardiovascular complications (Group 2): |
| CIMT and HbA1C                    | 0.551                      | 0.027                  |
| CIMT and DBP                      | 0.573                      | 0.020                  |
| CIMT and MBP                      | 0.547                      | 0.028                  |
| YKL-40 and TG*                    | 0.603                      | 0.013                  |
| YKL-40 and SBP*                   | 0.668                      | 0.005                  |
| YKL-40 and MBP*                   | 0.521                      | 0.038                  |
| hsCRP and age*                    | -0.555                     | 0.026                  |
| hsCRP and FSG*                    | 0.703                      | 0.002                  |
| Significant correlations of the group of diabetic patients with cardiovascular complications (Group 3): |
| CIMT and age                       | 0.291                      | 0.045                  |
| CIMT and weight                    | -0.286                     | 0.049                  |
| CIMT and PPG                       | 0.307                      | 0.034                  |
| CIMT and uric acid                | 0.322                      | 0.026                  |
| CIMT and eGFR                      | -0.325                     | 0.024                  |
| CIMT and DBP                       | 0.295                      | 0.042                  |
| CIMT and MBP                       | 0.286                      | 0.049                  |
| YKL-40 and age                     | 0.362                      | 0.011                  |
| YKL-40 and creatinine*             | 0.395                      | 0.005                  |
| YKL-40 and microalbumin*           | 0.302                      | 0.037                  |
| YKL-40 and alb/ cr ratio*          | 0.386                      | 0.007                  |
| YKL-40 and eGFR                    | -0.366                     | 0.011                  |
| YKL-40 and duration of DM          | 0.371                      | 0.009                  |
| hsCRP and LDL*                    | 0.287                      | 0.048                  |

*Spearman correlation.

Table 4: Clinical (sensitivity, specificity, positive predictive value, negative predictive value and efficiency) for hsCRP and YKL-40 in the studied groups.

| hsCRP  | Controls | Diabetics | Sensitivity % | Specificity % | PPV % | % ANN | Efficiency % |
|--------|----------|-----------|---------------|---------------|-------|-------|--------------|
| ≤2.73  | 16       | 14        | 78.13         | 100.0         | 100.0 | 53.33 | 82.50        |
| >2.73  | 0        | 50        |               |               |       |       |              |
| YKL-40 | ≤91.78   | 14        | 82.81         | 87.50         | 96.36 | 56.0  | 83.75        |
| >91.78 | 2        | 53        |               |               |       |       |              |
Table 5: Clinical (sensitivity, specificity, positive predictive value, negative predictive value and efficiency) for hsCRP and YKL-40 in diabetic patients.

|                  | Diabetic without cardiovascular complications | Diabetic with cardiovascular complications | Sensitivity % | Specificity % | PPV % | NPV % | Efficiency % |
|------------------|----------------------------------------------|------------------------------------------|---------------|---------------|-------|--------|--------------|
| **HsCRP**        | ≤3.93                                        | 10                                       | 11            | 77.08         | 62.50 | 86.05  | 47.62        | 73.44        |
|                  | >3.93                                        | 6                                        | 37            |               |       |        |              |              |
| **YKL-40**       | ≤211.42                                      | 12                                       | 15            | 68.75         | 75.0  | 89.19  | 44.44        | 70.31        |
|                  | >211.42                                      | 4                                        | 33            |               |       |        |              |              |

Discussion

Worldwide, it is estimated that 366 million people have diabetes and half of them are not aware that they have the disease [21].

Subclinical systemic inflammation and abnormalities of a wide variety of systemic inflammatory markers, including CRP, have been reported in type 2 DM [22,23]. Furthermore it induces endothelial dysfunction which appears to be the earliest event in atherogenesis, and plays a pivotal role in all phases of atherosclerosis from the initiation of the fatty streak to plaque rupture with culmination in acute coronary syndrome [24].
YKL-40 is expressed and secreted by several cell types of the innate immune system and by differentiated vascular endothelial and smooth muscle cells. A substantial body of evidence indicates that YKL-40 acts not only as an inflammatory marker in relation to both acute and chronic inflammation but also as a growth factor with involvement in extracellular matrix remodeling and angiogenesis. In this regard, YKL-40 was found to be associated with various inflammatory conditions and in early stages of atherosclerosis [25].

Cardiovascular Diseases (CVD) are a major concern considering that the risk of cardiovascular death in patients with T2DM is double the risk of individuals without diabetes [26].

The aim of this work is to study serum human Chondrex (YKL-40) in type 2 diabetic patients in relation to cardiovascular complications.

The present study included eighty subjects divided into three groups. The control group (group 1) included sixteen apparently healthy volunteers (2 males and 14 females); their mean age was 44.06±11.82 years. The group of diabetic patients without cardiovascular complications (group 2) included sixteen patients suffering from type 2 diabetes mellitus without cardiovascular complications (5 males and 11 females); their mean age was 48.38±8.11 years. The group of diabetic patients with cardiovascular complications (group 3) included forty eight patients (11 males and 37 females); suffering from type 2 diabetes mellitus with cardiovascular complications in the form of coronary artery disease (100%), stroke (8.3%) and peripheral artery disease (4.16%) their mean age was 54.96±9.32 years. (Table 1).

Chondrex or YKL-40 is a glycoprotein involved in inflammation and endothelial dysfunction. It is a growth factor for various cell types and has an important role in extracellular matrix remodeling and angiogenesis [27].

In this study the mean of serum YKL-40 in the control group, the group of diabetic patients without cardiovascular complications and the group of diabetic patients with cardiovascular complications was 56.64±48.21, 165.34±122.74 and 266.62±116.62 ng/mL respectively. The YKL-40 level was significantly higher in both the diabetic groups without and with cardiovascular complications when compared to the control group (p=0.017, 0.000) respectively. Also the level of YKL-40 was significantly higher in the group of diabetic patients with cardiovascular complications when compared to the group of diabetic patients without cardiovascular complications (p=0.005). (Table 2)(Figure 1).

These results were not in agreement with the work of Kido and colleagues in (2015) [28] but was in accordance with previous results [27,29-31] which stated that YKL-40 level was significantly higher in type 2 diabetes mellitus patients when compared to healthy controls. Also this was consistent with Kim et al, (2012) [31] who stated that the level of serum YKL-40 was markedly increased in type 2 diabetic patients with suspected CAD, compared to those with no evidence of CAD.

Also other studies have demonstrated that serum YKL-40 is increased in patients with acute Myocardial Infarction (MI), [32-34] and in patients with stable chronic CAD [33,35-37].

In the initiation and progression of atherosclerosis, activated macrophages take up lipids and then these lipid-rich macrophages secrete inflammatory mediators that stimulate VSMC migration and proliferation, resulting in atherosclerosis. It is known that YKL-40 is secreted by activated macrophages and neutrophils in different tissues with inflammation, and vascular smooth muscle cells (VSMC). In this regard, YKL-40 may be involved in the early stage of atherosclerosis and CAD [31].

When diabetes is accompanied by other major cardiovascular risk factors such as hypertension, dyslipidemia, and smoking the incidence of atherosclerosis is markedly increased. The atherosclerosis process in diabetes is indistinguishable from that of the non diabetic population, but it begins earlier and is often more extensive and more severe [38].

Dyslipidemia, an established risk factor for Cardiovascular Disease (CVD), is strikingly common in patients with type 2 diabetes, affecting almost 50% of this population [39].

In the current study, the mean HDL-Cholesterol level in both diabetic groups (group 2 and 3) was significantly lower than the control group (p=0.000 for both). Also the median of triglycerides level was significantly higher in both diabetic groups (group 2 and 3) when compared to the control group (p=0.000 for both). The mean LDL-Cholesterol level was significantly higher in the group of diabetic patients without cardiovascular complications (group2) when compared to the control group (p=0.047) (Table 2).

YKL-40 was positively correlated with triglycerides in the group of diabetic patients without cardiovascular complications (r=0.603, p=0.013), (Table 3) similar to the findings of Røndbjerg et al, (2011) [27] Rathicke et al, (2012) [40] and Kim et al (2012) [31].

Carotid-wall intima–media thickness is a surrogate measure of atherosclerosis associated with cardiovascular risk factors and with cardiovascular outcomes [41].

In this study, the mean of the Carotid Intima Media Thickness (CIMT) in the control group, the group of diabetic patients without cardiovascular complications and the group of diabetic patients with cardiovascular complications was 0.55±0.16, 0.67±0.13 and 0.99±0.40 mm respectively. The CIMT was significantly higher in the group of diabetic patients with cardiovascular complications when compared to both the control group and the group of diabetic patients without cardiovascular complications (p<0.000, 0.003) respectively (Table 1).

In vitro studies showed that YKL-40 promotes chemotaxis, cell attachment, spreading and migration of vascular endothelial cells which suggest a role of YKL-40 in the atherosclerotic plaque formation. These in vitro studies are supported by immunohistochemical analysis which has shown in vivo protein expression of YKL-40 in human smooth muscle cells in atherosclerotic plaques [6].

In agreement with a previous study done by Ito et al, (2010) [42] who stated that eGFR is negatively associated with CIMT in type 2 diabetic patients we found that the CIMT was negatively correlated with eGFR in the group of diabetic patients with cardiovascular complications (group 3) (r=-0.325, p=0.024) (Table 3). Also this comes in agreement with another study done by Cho et al, (2011) [43] who stated that CIMT may be a predictor of diabetic nephropathy progression in patients with type 2 diabetes.

In the present study, a significant positive correlation was found between YKL-40 and systolic blood pressure in the group of diabetic
Regarding the duration of diabetes, it was 0.1-17 years, median 1 year for group 2 patients and 4-40 years, median 12 years in group 3 patients. There was a significant increase in the duration of diabetes in the diabetic patients with cardiovascular complications when compared to those without (p=0.000) (Table 1). This goes with the longer duration of diabetes, the more the predisposition to cardiovascular complications being exposed to more risk factors and the pathology of diabetes on the blood vessels.

In the present study, there was a significant positive correlation between YKL-40 and duration of DM in the group of diabetic patients with cardiovascular complications. (r=0.371, p=0.009) (Table 3). This was in agreement with Røndbjerg et al, (2011) [27].

Glycemic control reduces the incidence of cardiovascular events in T2DM [44].

A prospective epidemiological analysis based on the Heart Outcome Prevention Evaluation (HOPE) study also identified a significant relationship between glycemic level and incident cardiovascular events [45]. HbA1c is also the accepted measure of long-term glycaemia in the long-term follow-up study to the Diabetes Control and Complications Trial (DCCT), [46] which have found a lower risk for macrovascular complications with improved glycemia [47]. It was suggested that poor glycemic control is associated with structural changes in the carotid artery that are consistent with early atherosclerosis [48].

In the present work, there was a significant positive correlation between HbA1c and CIMT (r = 0.551, p = 0.027) in the group of diabetic patients without cardiovascular complications (Table 3).

Uric acid, the final oxidation product of purine catabolism, can act as a prooxidant, [49,50] but it may also have a therapeutic role as an antioxidant [51]. Hyperuricemia has been found to be associated with obesity and insulin resistance, and consequently with type 2 diabetes [52,53]. It relates to endothelial dysfunction by inducing antiproliferative effects on endothelium and impairing nitric oxide production and inflammation, e.g., through increased C-reactive protein expression [53,54]. Finally, uric acid may play a role in immune activation with subsequent increased chemokine and cytokine expression [55,56].

In the present study, uric acid level was significantly higher in diabetic patients without cardiovascular complications when compared to the control group (p=0.001) (Table 2), which was the case with Ito et al (2011) [57] who stated that hyperuricemia was associated with diabetic micro- and macroangiopathies. Furthermore, the incidence of coronary heart disease was significantly higher in the patients with hyperuricemia than in those without. Also Rathmann et al (1993) [58] reported that hyperuricemia was associated with coronary heart disease in 4,047 patients with type 2 diabetes mellitus according to a cross-sectional study.

In the present work, serum uric acid level was positively correlated with CIMT in the group of diabetic patients with cardiovascular complications (group 3) (r=0.322, p=0.026) (Table 3). Which was in agreement with a previous study [58].

Increased serum level of uric acid is an independent risk factor for cardiovascular disease. Accumulation of free radicals in the presence of hyperuricemia stimulates lipid peroxidation, which may be responsible for increased CIMT. Moreover it increases platelet adhesiveness and vascular smooth muscle proliferation [59].

Clinical hallmarks of diabetic nephropathy include a progressive increase in urinary albumin excretion and late decline of glomerular filtration rate, which occurs in association with an increase in blood pressure, ultimately leading to end stage renal failure [60].

In the present study, Alb/cr ratio was significantly higher in the group of diabetic patients with cardiovascular complications (group 3) when compared to both the control group (group 1) and the group of diabetic patients without cardiovascular complications (group 2) (p=0.000 for both) (Table 2).

There was a significant positive correlation between YKL-40 and albumin to creatinine ratio in the group of diabetic patients with cardiovascular complications (r=0.366, p=0.011) (Table 3). This was similar to Catalan et al, (2011) [59]. In contrast, other results [27,35] stated that increasing YKL-40 levels were not predicted by a decline in eGFR. Also there was a significant positive correlation between YKL-40 and serum creatinine in the group of diabetic patients with cardiovascular complications (r=0.395, p=0.005) (Table 3). This result was in accordance with Rathcke et al, (2009) [35], Catalan et al, (2011) [25].

In the present study, the median level of YKL-40 in diabetic patients without retinopathy (n=15) was 237.1 (85.15-340.85 ng/ml) and in complicated diabetic patients with retinopathy (n=33) was 287.49 (60.12-511.54 ng/ml) showing a significantly higher levels than in uncomplicated diabetic patients (p=0.048, 0.003) respectively (Table not shown).

The median level of YKL-40 in complicated diabetic patients without nephropathy (n=16) was 234.76 (60.12-479.33 ng/ml). In complicated diabetic patients with nephropathy (n=32) it was 287.16 (60.79-511.54 ng/ml) showing a significantly higher levels than in uncomplicated diabetic patients (p=0.003) (Table not shown).

The inflammatory processes play a pivotal role in all stages of the development of both acute and chronic atherosclerosis [61].

C-reactive protein, an acute phase reactant synthesized in the liver in response to the cytokine interleukin-6, is a factor in the development of atherosclerotic plaque. Although CRP was initially believed to be only a marker of vascular inflammation, further researches indicates that it also plays an active role in atherogenesis [62].
In addition to YKL-40 the underlying inflammatory process was also demonstrated by the significantly higher hsCRP values in all patient groups compared to the control group (p=0.000). In the present study hsCRP values ranged from 0.16 to 2.73 mg/L with a median of 0.875 mg/L in the control group, from 0.38 to 17.2 mg/L with a median of 3.58 mg/L in the group of diabetic patients without cardiovascular complications, while in the group of diabetic patients with cardiovascular complications it ranged from 0.3 to 92.9 mg/L with a median of 9.79 mg/L (Table 2). Also hsCRP was significantly higher in the group of diabetic patients with cardiovascular complications when compared to the group of diabetic patients without cardiovascular complications (p=0.024), (Table 2).

In the present study there was no correlation between hsCRP and YKL-40 which came in agreement with Thomsen et al, (2010) [30] Catalan et al, (2011) [25] Kim et al, (2012) [31] implying that YKL-40 and hsCRP are produced and secreted independently of each other.

Although hsCRP was used as a predictor of cardiovascular risk [62], Recently it has been evaluated as a prognostic biomarker in patients with IHD. As hsCRP is mainly produced in the liver, it is likely that biomarkers as YKL-40 secreted from inflammatory cells within the atherosclerotic plaque could be superior prognostic biomarker [61].

Using the upper limit of control hsCRP value as a cutoff 2.73 mg/L, the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of hsCRP were 78.3%, 100%, 100%, 53.33% and 82.50% in diabetic patients while using the best cutoff of 91.78 mg/ml YKL-40 sensitivity, specificity, positive predictive value, negative predictive value and efficiency were 82.81%, 87.50%, 96.36%, 56.0% and 83.75%. YKL-40 had a better sensitivity, negative predictive value and efficiency than hsCRP in discriminating between controls and diabetics (Table 4).

Using the best cutoff of 3.93 mg/L the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of hsCRP were 77.08%, 62.50%, 86.05%, 47.62% and 73.44% while using the best cutoff of 211.42 mg/ml YKL-40 sensitivity, specificity, positive predictive value, negative predictive value and efficiency were 91.78%, 56.0% and 83.75%. From the previous results it can be noticed that YKL-40 had a better sensitivity and positive predictive value than hsCRP in discriminating between diabetic patients without and with cardiovascular complications (Table 5).

In the present work, by drawing receiver operating characteristic (ROC) curve between controls and diabetic, the Area Under the Curve (AUC) for hsCRP was 0.897, p<0.001 and for YKL-40 was 0.927, p<0.001 (Figure 2). Whereas in the curve between diabetic patients without and with cardiovascular complications the AUC for hsCRP was 0.676, p=0.036 and for YKL-40 was 0.743, p=0.004. (Figure 3) This indicates that YKL-40 was better than hsCRP in discriminating total diabetic patients from controls and diabetic patients with cardiovascular complications from those without.

Conclusion

The inflammatory YKL-40 was significantly higher in patients with type 2 diabetes mellitus than controls and cardiovascular complications contributed to its greater elevation. YKL-40 was positively correlated with several cardiovascular risk factors such as triglycerides, systolic blood pressure and mean blood pressure in the group of diabetic patients without cardiovascular complications and in the group of diabetic patients with cardiovascular complications, with duration of diabetes mellitus and urinary albumin to creatinine ratio denoting that longer duration of inflammation leads to increased YKL-40 levels with subsequent generalized vascular damage reflected by albuminuria.

There was no correlation between both inflammatory markers YKL-40 and hsCRP implying that they are produced and secreted independently of each other. YKL-40 had a better specificity and positive predictive value than hsCRP in discriminating between diabetic patients without cardiovascular complications from those with cardiovascular complications.

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