



## Association of serum YKL-40 levels with urinary albumin in Egyptian patients with type 1 diabetes mellitus

Ahmed M.Hussein<sup>\*</sup>, Mahmoud Rizk<sup>\*</sup>, Abdelmonaem Ahmed<sup>\*</sup>,  
Mona Eltokhy<sup>\*\*</sup>, Azza Abo Senna<sup>\*\*</sup>, Magda Ataa<sup>\*\*</sup>

<sup>\*</sup>Internal Medicine Department, Faculty of Medicine, Benha University, Egypt

<sup>\*\*</sup>Clinical Pathology Department, Faculty of Medicine, Benha University, Egypt

Corresponding Author: Ahmed M.Hussein

### Abstract

**Background:** A positive association between elevated circulating YKL-40 levels and increasing levels of albuminuria have been described in patients with type 1 diabetes indicating a role of YKL-40 in the progressing vascular damage resulting in microvascular disease. **Aim of study:** Was to evaluate serum YKL-40 level and its association with albuminuria in Egyptian type 1 diabetic patients. **Subjects and Methods:** Study was carried out on 50 patients from those attending diabetes clinics of Banha University Hospital they were divided into two groups: **Group (a):** Includes 20 healthy individuals their ages ranged from 20-35 years (12 males and 8 females), they were clinically free with normal laboratory findings. **Group (b):** Includes 30 diabetic patients (18 males and 12 females), their ages ranged from 30-48 years with type 1 DM and microalbuminuria. The following laboratory investigations were performed to all patients: Fasting plasma glucose, HbA1c, serum lipid profile, serum creatinine, estimated glomerular filtration rate, urinary albumin / creatinine ratio (ACR), serum YKL-4. **Results:** Our results show: Statistically significant increase in the mean of fasting blood sugar (FPG), hemoglobin A1c (HbA1c), microalbumin in urine, ACR levels in diabetic group compared to control group. Significant increase in the mean of low density lipoprotein cholesterol (LDL-C), triglycerides, serum creatinine, urinary creatinine and YKL-40 levels in diabetic group compared to control group. The mean of high density lipoprotein cholesterol I (HDL-C) and estimated glomerular filtration rate (eGFR) levels show a highly statistically significant decrease in diabetic group compared to control group. A significant positive correlation between ACR and each of age and duration of diabetes. Significant positive correlation between ACR and each of FPG, HbA1c, triglycerides, serum creatinine and microalbumin in urine and negative correlation with eGFR. Significant positive correlation between YKL-40 and each of age and duration of diabetes. Significant positive correlation between YKL-40 and each of FPG, HbA1C, HDL, triglycerides, serum creatinine, microalbumin in urine, urinary creatinine and ACR, and negative correlation with eGFR. No significant difference of sex distribution among the studied groups. Insignificant difference in the mean of (diastolic and systolic blood pressure, total cholesterol level in diabetic group compared to control group). No significant correlation was found between ACR and systolic or diastolic blood pressure. No significant correlation between ACR and each of total cholesterol, LDL-C, HDL-C and urinary creatinine. No significant correlation between YKL-40 and systolic or diastolic blood pressure. No significant correlation between YKL-40 and each of total cholesterol and LDL-C. **Conclusion:** It was concluded that Egyptian type 1 diabetic patients serum YKL-40 levels are significantly elevated and have a linear association with albuminuria. These data suggest that YKL-40 levels could be a tool to assess the risk of diabetic microangiopathy in early stage of type 1 diabetes.

**Keywords:** Type 1 Diabetes, YKL-40, Albuminuria.

## 1. Introduction

Diabetes Mellitus is commonly associated with both microvascular and macrovascular complications and associated with multiple disorders including metabolic, cellular and blood coagulation disturbances leading to many complications affecting various organs such as kidney, retina, peripheral nerves, microvascular and macrovascular compartments<sup>1</sup>.

About 246 million people worldwide had diabetes in 2007. The global figure of people with diabetes is projected to increase to 370 million in 2030. As the prevalence of diabetes has risen to epidemic proportions worldwide, diabetic nephropathy has become one of the most challenging health problems<sup>2</sup>.

Diabetic nephropathy is a major microvascular complication & the leading cause of end-stage renal disease worldwide. Under hyperglycemic condition, it has been demonstrated that the renal tubular epithelium highly expresses apoptosis regulatory genes<sup>3</sup>.

YKL-40 is a 40 kDa heparin- and chitin-binding glycoprotein also known as human cartilage glycoprotein 39 (HC-gp39), 38-kDa heparin-binding glycoprotein or chitinase-3-like protein 1 (CHI3L1)<sup>4</sup>.

YKL-40 is an inflammatory glycoprotein involved in endothelial dysfunction by promoting chemotaxis, cell attachment, migration, reorganization and tissue remodelling as a response to endothelial damage. YKL-40 protein expression is seen in macrophages and smooth muscle cells in atherosclerotic plaques with the highest expression seen in macrophages in the early lesion of atherosclerosis. YKL-40 levels are elevated both in patients with type 1 and type 2 diabetes, known to be at high risk for the development of cardiovascular diseases when compared to non-diabetic persons. A positive association between elevated circulating YKL-40 levels and increasing levels of albuminuria have been described in patients with type 1 diabetes indicating a role of YKL-40 in the progressing vascular damage resulting in microvascular disease<sup>5</sup>.

## 2. Subjects and Methods

The present study was conducted at internal medicine and clinical pathology departments of Banha University Hospitals. It was carried out on 50 subjects during the period from April 2014 to April 2015. They were classified into two groups:

**Group (a) control group:** It included 20 apparently healthy individuals (12 males and 8 females), their ages ranged from 20-35 years. They were clinically free with normal laboratory findings.

**Group (b) diabetic group (Cases):** It included 30 patients (18 males and 12 females), their ages ranged from 30-48 years with type 1 DM (T1DM) and microalbuminuria (defined by spot morning albumin/creatinine ratio 30 mg/g).

**Inclusion criteria:** All patients were type 1 DM according to the criteria for diagnosis of diabetes (ADA, 2008): HbA1c 6.5%, or FPG 126 mg/dl (7mmol/l) or 2-h plasma glucose 200 mg/dl (11.1mmol/l). In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose 200 mg/dl (11.1 mmol/l).

**Exclusion criteria:** Neoplasm, Liver dysfunction, Autoimmune disease, Acute infection and Cardiovascular disease.

All individuals included in this study were subjected to the following: Full history taking (age, sex, duration of the disease). Clinical examination with special stress on the following: duration of diabetes, state of diabetic control, mode of treatment, presence of complications e.g. [hypertension, microvascular complications (nephropathy, neuropathy & retinopathy), and macro-vascular complications (cerebral, coronary & peripheral atherosclerosis)].

**Laboratory investigations** were done in the form of: Fasting plasma glucose, HbA1c, serum lipid profile, serum creatinine, estimated glomerular filtration rate: by *Cockcroft – Gault Formula*<sup>6</sup>

$$GFR (ml / min) = \frac{(140 - age(years)) \times (weight(Kg))}{72 \times s.creatinine (mg / dl)} \times 0.85 \text{ if female}$$

Urinary albumin / creatinine ratio, serum YKL-40.

### Sampling:

1) The Blood samples were obtained by peripheral venipuncture from all subjects, after at least 10 hours fast were divided into two parts:

The first part (1 ml) was collected on EDTA tubes for fasting plasma glucose and HbA1c. The second part (4 ml) was collected in sterile tubes then put in water bath at 37 C for 30 minutes then centrifuged for 10 minutes then the resultant serum was divided into

aliquots and stored at -20 C for measurement of serum lipid profile, serum creatinine, serum YKL-40. Frozen samples were allowed to thaw and brought to room temperature only before analysis. Hemolysed samples were discarded, repeated freezing and thawing was avoided.

2) A single early morning urine sample was obtained in a clean sterile cup for estimation of albumin /creatinine ratio.

## Methods:

### *Estimation of urinary microalbumin:*

#### **Principle of the method:**<sup>7,8,9,10</sup>

Albumin in the urine sample causes agglutination of the latex particles with anti –human albumin. The agglutination of the particles is proportional to the albumin concentration and can be measured by turbidimetry.

**Reference values:**<sup>11</sup> Urine, adults: Up to 15 mg/L.

All previous tests were done on Automated chemistry analyzer Biosystem A15.

Calculation of the albumin/creatinine ratio in mg/g.

If the urine microalbumin is 10 mg/L and the urine creatinine is 100 mg/dL, then the albumin/creatinine ratio is 10 mg/g. In this example, you first need to multiply the urine creatinine value by 10 in order to convert mg/L (i.e., 100 mg/dL  $\times$  10 dL/ L= 1000 mg/L). Then simply divide the urine albumin value (10 mg/L ) by the urine creatinine value (1000 mg/L) to arrive at the ratio (10 mg/L /1000 mg/L=0.01), then multiply by 1000 to express the value as (mg albumin /g creatinine). If the two values are already in the same units, simply divide the albumin value by the creatinine value and then multiply by 1000<sup>12</sup>. Normal value of ACR is < 30 mg/g, microalbuminuria ACR is 30-300 mg/g, and macroalbuminuria ACR is >300 mg/g<sup>13</sup>.

### *Assay of human (YKL-40 / CHI3L1)*

#### **Test principle :**<sup>11</sup>

The kit uses a double –antibody sandwich enzyme – linked immunosorbent assay (EIISA) to assay the level of Chitinase-3-like protein 1 (YKL-40/CHI3L1) in samples and it is supplied by SunRed company. Chitinase-3-like protein 1 (YKL-40/CHI3L1) was added to monoclonal antibody enzyme well which is

pre-coated with Human Chitinase-ion3-like protein 1 (YKL-40/CHI3L1) monoclonal antibody, incubation then, (YKL-40/CHI3L1) antibodies labeled with biotin were added, and combined with streptavidin –HRP to form immune-complex; then incubation and washing were carried out again to remove the uncombined enzyme. Then chromogen solution A,B were added the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance Chitinase-3-like protein 1 (YKL-40/CHI3L1) of sample were positively correlated.

### **Statistical analysis**

The results were analyzed using SPSS (version 16) statistical package for Microsoft windows. Data were presented as a number and percentage for qualitative variables and as a mean and standard deviation for quantitative continuous variables. The significance of difference between mean values of paired observation was performed using paired t-test. Pearson correlation coefficient (r) was applied to test association of two quantitative variables in the same patients.

**Significance of results:** The corresponding P value for each test was directly computed by the microprosessor. Non–significant difference when P>0.05. Significant difference when P<0.05. Highly significant difference when P<0.01.<sup>14</sup>

## **3. Results**

The present study comprised of 30 type 1 diabetic patients (18 males &12 females) and 20 apparently healthy subjects serving as a control group (12 males and 8 females). The results were presented in 6 tables.

**Table (1):** Shows no significant difference of sex distribution among the studied groups. **Table (2):** Shows a statistically significant increase in the mean of FPG, HbA1c, microalbumin in urine, ACR levels in diabetic group compared to control group (**P < 0.01**). There was a statistically significant increase in the mean of LDL-C, triglycerides, S.creatinine, urinary creatinine and YKL-40 (**P < 0.05**) levels in diabetic group compared to control group. While the mean of HDL-C and eGFR levels shows a highly statistically significant decrease in diabetic group compared to control group (**P < 0.01**). No statistically significant difference was detected in the mean of total cholesterol (**P 0.5**) levels in diabetic group compared to control group. **Table (3):** Shows a significant positive correlation between ACR and each of age

( $r=0.6$ ,  $p<0.01$ ) and duration of diabetes ( $r=0.9$ ,  $p<0.01$ ). No significant correlation was found between ACR and systolic blood pressure, and diastolic blood pressure ( $p 0.05$ ). **Table (4):** Shows a significant positive correlation between ACR and each of FPG ( $r = 0.5$ ,  $p<0.01$ ), HbA1c ( $r = 0.9$ ,  $p<0.01$ ), triglycerides ( $r =0.3$ ,  $p<0.05$ ), S.creatinine ( $r =0.4$ ,  $p < 0.01$ ) and microalbumin in urine ( $r =0.96$ ,  $p < 0.01$ ) and negative correlation with eGFR ( $r =-0.7$ ,  $p < 0.01$ ). There is no significant correlation between ACR and each of total cholesterol, LDL-C, HDL-C and urinary creatinine ( $p 0.05$ ). **Table (5):** Show a significant positive correlation between YKL-40 and each of age

( $r=0.57$ ,  $p<0.01$ ) and duration of diabetes ( $r=0.8$ ,  $p<0.01$ ). No significant correlation between YKL-40 and systolic blood pressure or diastolic blood pressure ( $p 0.05$ ). **Table (6):** Show a significant positive correlation between YKL-40 and each of FPG ( $r =0.6$ ,  $p <0.01$ ), HbA1C ( $r =0.8$ ,  $p <0.01$ ), HDL ( $r =0.3$ ,  $p <0.05$ ), triglycerides ( $r =0.5$ ,  $p <0.01$ ), S.creatinine ( $r =0.4$ ,  $p < 0.01$ ), microalbumin in urine ( $r =0.9$ ,  $p < 0.01$ ), urinary creatinine ( $r =0.6$ ,  $p <0.01$ ) and ACR ( $r =0.8$ ,  $p <0.01$ ) and negative correlation with eGFR ( $r =-0.5$ ,  $p < 0.01$ ). While there is no significant correlation between YKL-40 and each of total cholesterol and LDL-C ( $P 0.05$ ).

**Table (1):** Significant difference of sex distribution among the studied groups

Sex Studied Groups	Female		Male		Total		P value
	No.	%	No.	%	No.	%	
Control group: (n=20)	8	40	12	60	20	100	> 0.05
Diabetic patients: (n=30)	12	40	18	60	30	100	
Total	20	40	30	80	50	100	

**Table (2)** Statistically significant increase in the mean of FPG, HbA1c, microalbumin in urine, ACR levels in diabetic group compared to control group

Group Variable	Control	Diabetics	t	P
	Mean±SD	Mean±SD		
FBG (mg/dl)	84.1±7.2	213.2±121.9	4.7	<0.01
Hb A1c(%)	4.8±0.5	9.5±0.9	21.9	<0.01
Total cholesterol (mg/dl)	168.9±8.6	172.9±24.4	0.7	0.5
LDL(mg/dl)	95.1±13.7	103.9±10	2.5	<0.05
HDL(mg/dl)	55.5±13.5	45.2±6.2	3.2	<0.01
Triglycerides (mg/dl)	96±13.1	109.5±26.7	2.1	<0.05
Serum creatinine (mg/dl)	0.8±0.1	0.9±0.2	2.1	<0.05
eGFR(ml/min)	110.2±4.4	99.3±5.6	7.3	<0.01
Microalbumin in urine (mg/l)	7.5±2	49.2±9.7	18.9	<0.01
Urinary creatinine (mg/dl)	34.6±4.5	38.6±5.7	2.7	<0.05
ACR(mg/g)	22±8	128.3±27.5	16.8	<0.01
Ykl-40 (ng/ml)	111.9±37.6	202.4±32.6	9	<0.05

**Table (3)** Shows a significant positive correlation between ACR and each of age and duration of diabetes

<i>Parameters</i>	<i>ACR</i>	
	<i>r</i>	<i>P</i>
<i>Age (years)</i>	0.6	<0.01
<i>Duration of DM (years)</i>	0.9	<0.01
<i>SBP (mmHg)</i>	-0.01	0.9
<i>DBP (mmHg)</i>	0.02	0.87

**Table (4)** Shows a significant positive correlation between ACR and each of FPG, HbA1c, triglycerides , S.creatinine and microalbumin in urine

<i>Parameters</i>	<i>ACR</i>	
	<i>R</i>	<i>P</i>
<i>FBG (mg/dl)</i>	0.5	<0.01
<i>Hb A1C (%)</i>	0.9	<0.01
<i>Total cholesterol (mg/dl)</i>	-0.07	0.6
<i>LDL-C (mg/dl)</i>	-0.1	0.4
<i>HDL-C (mg/dl)</i>	0.2	0.2
<i>Triglycerides (mg/dl)</i>	0.3	<0.05
<i>Serum creatinine (mg/dl)</i>	0.4	<0.01
<i>eGFR (ml/min)</i>	-0.7	<0.01
<i>Microalbumin in urine (mg/l)</i>	0.96	<0.01
<i>Urinary creatinine (mg/dl)</i>	0.2	0.2

**Table (5)** Show a significant positive correlation between YKL-40 and each of age and duration of diabetes

<i>Parameters</i>	<i>YKL-40</i>	
	<i>R</i>	<i>P</i>
<i>Age (years)</i>	0.57	<0.01
<i>Duration of DM (years)</i>	0.8	<0.01
<i>SBP (mmHg)</i>	0.04	0.8
<i>DBP (mmHg)</i>	0.06	0.7

**Table (6)** Show a significant positive correlation between YKL-40 and each of FPG, HbA1C, HDL, triglycerides, S.creatinine, microalbumin in urine

<i>Parameters</i>	<i>YKL-40</i>	
	<i>R</i>	<i>P</i>
<i>FBG (mg/dl)</i>	0.6	<0.01
<i>Hb A1C (%)</i>	0.8	<0.01
<i>Total cholesterol (mg/dl)</i>	-0.02	0.9
<i>LDL-C (mg/dl)</i>	-0.1	0.4
<i>HDL-C (mg/dl)</i>	0.3	<0.05
<i>Triglycerides (mg/dl)</i>	0.5	<0.01
<i>Serum creatinine (mg/dl)</i>	0.4	<0.01
<i>eGFR (ml/min)</i>	-0.5	<0.01
<i>Microalbumin in urine (mg/l)</i>	0.9	<0.01
<i>Urinary creatinine (mg/dl)</i>	0.6	<0.01
<i>ACR (mg/g)</i>	0.8	<0.01

## Discussion

Patients with insulin dependent diabetes mellitus (IDDM) are well known to be at a high risk of vascular diseases and dysfunction of vascular endothelium which is considered as an early step in the development of diabetic complications<sup>15</sup>.

The long-term prognosis of type 1 diabetes is associated with the development of organ complications. With respect to life expectancy, diabetic nephropathy plays a pivotal role. In the U.S. as well as in industrialized European countries, diabetes is an important leading cause of renal failure in adults<sup>16</sup>. Type 1 diabetes mellitus has longer life expectancy with higher impact, earlier end-stage renal disease (ESRD), difficulties in control during adolescence in addition to long term complications often overlooked. Moreover, patients with type 1 diabetes face a 20-50% probability of developing ESRD requiring dialysis or renal transplantation<sup>17</sup>. A number of risk factors may influence the onset and/or the progression of diabetic nephropathy (DN); including duration of diabetes, metabolic control, puberty, hypertension, hyperlipidemia, genetic influence, and smoking<sup>18</sup>. Early detection of diabetic nephropathy and timely control of blood pressure have a pivotal role in the prevention of end stage renal disease in diabetic patients. The first clinical sign is

microalbuminuria. Even slightly abnormal urinary albumin excretion (UAE) is associated with early renal and vascular damage<sup>19</sup>. YKL-40 is a 40 KDa heparin and chitin binding glycoprotein, which is expressed and secreted by a variety of cells including neutrophils, monocytes, macrophages, chondrocytes, synovial cells, smooth muscle cells, endothelial cells and tumour cells and is readily detected in the blood of normal individuals. Elevated circulating levels of YKL-40 have been observed in patients with asthma, metastatic breast cancer, cardiovascular disease, diabetes and hepatic fibrosis. In many of these disorders, YKL-40 correlates with disease activity and its expression is believed to reflect distinct pathways in disease pathogenesis<sup>20</sup>. The aim of the present study is to evaluate serum YKL-40 levels and its association with albuminuria in Egyptian type 1 diabetic patients.

The study was conducted at internal medicine and clinical pathology departments of **Banha University Hospitals**. It was carried out on 50 subjects (30 type 1 diabetic patients and 20 apparently healthy individuals working as control group) during the period from April 2014 to April 2015. All the procedures used in this study were approved by the medical ethics committee of the faculty of medicine, Banha University, Egypt. An informed consent was obtained from all subjects in this study.

All individuals included in this study were subjected to full history taking, thorough clinical examination, with special stress on the following: (state of diabetic control, mode of treatment, presence of complications e.g. hypertension, microvascular complications (nephropathy, neuropathy & retinopathy). Laboratory investigations were done in the form of: Fasting plasma glucose, HbA1c, serum lipid profile, serum creatinine, urinary albumin/creatinine ratio, estimated GFR by *Cockcroft - Gault* equation and serum YKL-40.

The results of the present work showed no significant difference of sex distribution among the studied groups.

This is in concordance with *Demirbilek et al. (2013)* who reported that female/male ratio in T1DM patients was generally equal in their Turkish study.

This is against *Schonle et al. (2001)*, *Ackerman (2006)*, *Gonzalez et al.(2010)* and *Ostrauskas et al. (2011)* who reported significant male predominance in type 1 diabetic patients. This predominance can be explained by differences in environmental exposures between females and males and sex hormones which affect the function of the immune system.

The results of the present work showed insignificant difference in the mean of diastolic blood pressure and systolic blood pressure levels in diabetic group compared to control group. These results were in concordance with *Graziella et al.*<sup>21</sup> and *Sakamoto et al.*<sup>22</sup> which reported that there was no significant difference between the diabetic and control groups in systolic and diastolic blood pressure while *Rathcke et al.*<sup>5</sup> reported that there was no significant difference in systolic blood pressure between the diabetic group and control groups but there is a significant difference in diastolic blood pressure between diabetic group and control group. The results of the present study showed a statistically significant increase in the mean of FPG and HbA1c in diabetic group compared to control group. The increase in FPG and HbA1c in diabetic patients than control group were in concordance with many studies<sup>23,24,25,26</sup>. In diabetic patients glucose reacts with amino groups on proteins to form covalently bonded glycated products. An example of such product is glycated hemoglobin used to monitor the long term control of glucose in diabetic patients. *The Microalbuminuria Collaborative Study Group*<sup>27</sup> and *Thomas and Hostetter*<sup>28</sup> made observational studies correlating HbA1c concentration with the

development and progression of microalbuminuria and overt nephropathy. These results confirm that, uncontrolled state of diabetes reflected by elevated fasting plasma glucose and HbA1c with long duration of diabetes, facilitate the progression of diabetic nephropathy through glucose-induced tissue injury<sup>29</sup>. The results of the present work showed a statistically significant increase in the mean of triglycerides, LDL-C and decrease in HDL-C and no statistically significant difference in the mean of total cholesterol levels in patient group compared to control group. These results were in concordance with *Stenier*<sup>30</sup>, *Seligman*<sup>31</sup> *Bokemark*<sup>32</sup> and *Udawat*<sup>33</sup>.

Diabetic hypertriglyceridemia is based on enhanced hepatic VLDL secretion and diminished VLDL and chylomicron clearance<sup>34,35</sup>. Under conditions of poor glycemic control, LDL-C of diabetic patients contain an elevated proportion of triglycerides, mostly at the expense of cholesterol. Alteration in the lipid composition or size of LDL particles have consequences for their binding to lipoprotein receptors: Triglyceride-rich and small dense LDL show reduced cellular uptake via the LDL receptor leading to accumulation of LDL-C in the vascular system<sup>36</sup>. A decrease in HDL-C in diabetic patients has often been reported in combination with altered composition of HDL-C<sup>32</sup>. Similar to VLDL and LDL, HDL particles contain an increased proportion of triglycerides. Triglycerides-rich HDL shows a faster catabolic rate than normal HDL, followed by a decreased number of circulating HDL particles<sup>37</sup>.

The results of the present work showed that eGFR shows a high statistically significant decrease in diabetic group compared to control group. The decrease in eGFR in diabetic patients than control group is in concordance with *Schram*<sup>38</sup>, *Astrup*<sup>39</sup>.

In diabetic patients, nephropathy and hyperlipidemia has been identified as risk factors for more rapid rate of decline in eGFR and mortality. An increased plasma concentration of triglycerides rich apoB containing lipoproteins has been found to be linked to a more rapid decrease in renal function<sup>40</sup>.

However *Alvin and Powers*<sup>41</sup> stated that glomerular hyperperfusion and renal hypertrophy occur in the first years after the onset of DM and are reflected by an increased glomerular filtration rate. Before the onset of overt proteinuria, there are various renal functional changes including renal hyperfiltration, hyperperfusion, and increasing capillary permeability to macro-molecules<sup>42</sup>.

The results of the present work showed a statistically significant increase S.creatinine, urinary creatinine, microalbumin in urine, ACR levels in diabetic group compared to control group.

The significant increase noted in creatinine level in diabetic patients than control group was in concordance with *UK Prospective Diabetes Study Group*<sup>43</sup>, *Chaturvedi and his colleagues*<sup>44</sup> and *Gross et al.*<sup>45</sup> who found significant increase of creatinine level in all diabetic patients, as the progression of diabetic nephropathy starts in early course of diabetes.

*De-Zeeuw et al.*<sup>46</sup> suggested that ACR should be considered a risk marker for progressive loss of renal function. Also they reported that, albuminuria is the predominant renal risk marker in diabetic patients with nephropathy; the higher the albuminuria, the greater the renal risk.

Proteinuria directly contributes to its pathogenesis by including tubulointerstitial pathology. Itself a major correlate of declining in renal function. Other mechanisms such as the enhanced ultrafiltration of growth factors may also contribute<sup>47</sup>.

The progression from microalbuminuria to macroalbuminuria (overt nephropathy) is associated with several risk factors including: elevation of urinary albumin excretion, poor glycemic control, genetic factors, long duration of diabetes, abnormalities in lipid spectrum and haemostatic parameters<sup>48</sup>.

Like other microvascular complications, the pathogenesis of diabetic nephropathy is related to chronic hyperglycemia. There are three major theories, which explain how hyperglycemia might lead to diabetic nephropathy. One hypothesis is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of cellular proteins. A second hypothesis is that hyperglycemia increases glucose metabolism via the sorbitol pathway and this leads to cellular dysfunction. A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to activation of certain isoforms of protein kinase C (PKC), which, in turn, affect a variety of cellular events that lead to DM-related complications. Also growth factors appear to play an important role in DM-related complications<sup>49</sup>.

The results of the present work showed a significant positive correlation between ACR and each of age and duration of diabetes and no significant correlation

between ACR and systolic blood pressure and diastolic blood pressure.

*(Brenner et al.*<sup>50</sup>, *Alder et al.*<sup>51</sup> and *Molitch et al.*<sup>52</sup>) suggested that there is a constant deterioration of renal function of the diabetic patients over time or progression of nephropathy over time.

*Khan et al.*<sup>53</sup> suggest a link between development and progression of diabetic nephropathy and duration of diabetes.

The results of the present work showed a significant positive correlation between ACR and each of FPG, HbA1c, triglycerides, S.creatinine and microalbumin in urine.

Diabetic dyslipidemia plays a major role in the pathophysiology and progression of vascular disease and probably diabetic nephropathy as well<sup>50</sup>. The increased plasma concentration of triglycerides rich Apo-B containing lipoproteins with low levels of HDL-C have been found to be linked to a more rapid decrease in renal function and development of diabetic nephropathy<sup>54,55</sup>.

The results of the present work showed a statistically significant increase in YKL-40 level in diabetic group compared to control group. Also, there was a significant positive correlation between YKL-40 and each of age and duration of diabetes and no significant correlation between YKL-40 and systolic blood pressure and diastolic blood pressure.

These findings are in concordance with three previous studies performed in type 1 DM. *Rathcke et al.*<sup>5</sup> reported elevated YKL-40 levels in a cohort of Danish, middle aged (50 years) T1D patients, with a long diabetes duration (30 years) and with high prevalence of microvascular complications (more than 50%) and *Sakamoto et al.*<sup>22</sup> showed also elevated levels of YKL-40 in a cohort of Japanese T1D patients younger than the cohort of Danish patients (mean age of 25 years), with less mean duration of diabetes (13 years) and with a lower prevalence of microvascular complications (9% of microalbuminuria, 33% of retinopathy) and *Aguilera et al.*<sup>56</sup> who reported a mean age and duration of diabetes intermediate between the above two studies, but with a much lower prevalence of microangiopathy than the Danish cohort and similar to the Japanese patients.



*Astrup et al.*<sup>39</sup> and *Schram et al.*<sup>38</sup> explained the increase of YKL-40 (chitinase 3-like protein 1) in diabetic patients by being a marker of inflammation and endothelial dysfunction, both of which play important roles in the progression of diabetic complications. It has a role in inflammation and remodeling of extracellular matrix. Clinical studies showed that biomarkers of inflammation and endothelial dysfunction were higher in type 1 diabetic patients with diabetic complications.

The results of the present work showed a significant positive correlation between YKL-40 and each of FPG, HbA1C, HDL-C, triglycerides, serum creatinine, microalbumin in urine, urinary creatinine and ACR and negative correlation with eGFR. On the other hand there is no significant correlation between YKL-40 and each of total cholesterol and LDL.

These results were in concordance with *Rathcke et al.*<sup>5</sup> and *Sakamoto et al.*<sup>22</sup> who described elevated concentrations of YKL-40 in T1D patients with microalbuminuria but against is *Aguilera et al.*<sup>56</sup> reporting that YKL-40 is already increased even at a stage when subclinical microvascular and macrovascular disease is not detected with current clinical procedures or if it is modestly present. The low proportion of T1D patients presenting nephropathy (9%) but showing high YKL-40 compared to controls clearly indicates that at this stage of T1D evolution, inflammatory status is already activated. Moreover, they did not find higher levels of YKL-40 in nephropathy patients at the stage of positive microalbuminuria, and although they did not find differences according to the presence or absence of retinopathy, probably due to the low number of subjects included in their cohort, all the data taken together suggest that more prospective studies are probably needed in order to clarify if relevant changes in the magnitude of YKL-40 concentration are detected over time and in relation to the appearance of microvascular disease.

*Schram et al.*<sup>38</sup>, *Lin et al.*<sup>57</sup> and *Klein et al.*<sup>58</sup> showed that the chronic low grade inflammation and endothelial dysfunction were associated with the occurrence and progression of diabetic microangiopathy including nephropathy and retinopathy. Thus, it is possible that YKL-40 plays an important role in the pathogenesis of diabetic microangiopathy via intermediating low grade inflammation and endothelial dysfunction.

*Gerstein et al.*<sup>59</sup>, *Rossing et al.*<sup>60</sup>, *Johansen*<sup>61</sup> and *Molitch et al.*<sup>62</sup> showed that the presence of albuminuria and decline of estimated GFR are not only a predictor of progression of nephropathy in type 1 diabetic patients, but also a risk factor for cardiovascular disease with or without diabetes mellitus. Inflammation and endothelial dysfunction are thought to be key processes in the progression of atherosclerosis. YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubule, indicating that YKL-40 may function in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells.

## 5. Conclusion

In conclusion, serum YKL-40 levels were elevated in type 1 diabetic patients and associated with increasing level of albuminuria. YKL-40 could be a predictor to assess the risk of diabetic microangiopathy in the early stage in type 1 diabetic patients.

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