Use of Endothelin-1 (ET-1) and von Willenbrand Factor (vWF) as biological markers in patients with normo and microalbuminuria

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Abstract: - Microalbuminuria is a well-known marker of endothelial dysfunction in general and is even more specific in the renal endothelium dysfunction. Early diagnosis and even prevention of microalbuminuria depends on finding effective and feasible methods to help the diabetic patient in preventing in time one of the most important diabetic complications, the nephropathy. One of these methods is finding new novel biomarkers that can define and measure the endothelial dysfunction in diabetic patients.

Keywords: - Biological markers, endothelin 1, von Willenbrand factor, albuminuria, endothelial dysfunction

I. INTRODUCTION

Diabetes today is one of the five mayor causes of death, mostly because of its complications in the cardiovascular and renal systems. In 2010 there were 285 million people suffering from diabetes and projections for 2030 are almost a double of that number (1). 90% of diabetic patients have type II diabetes, not depended in insulin. Diabetic nephropathy accounts for 35-40 % of the diabetic complications and ends up in damaging the function of the kidney in 10 - 15 % of the cases (2).

Microalbuminuria is considered one of the best markers for diabetic nephropathy (3, 4). It shows the damage of the glomerular endothelium and its progression towards renal failure (4). Finding the microalbuminuria in the diabetic patient has been the first sign of the renal damage in diabetic patients (5). We have tried to find more sensitive and early markers in diagnosing the endothelial dysfunction in these patients. Two of those novel markers are chosen for our study: ET-1 and von Willebrand factor.

Endothelin was discovered in 1988 as a small peptide composed of 21 amino acids and was found to have a potent vasoconstrictor effect. It is mostly produced by endothelial cells, but it is also produced in small quantities in the cortex of the adrenal gland, myocardial cells, smooth muscle cells surrounding the blood vessels, tubular renal cells, glomerular mesangial cells, glial cells, macrophages, mast cells and pituitary cells. There are three different forms of Endothelin named ET-1, ET-2 and ET-3. The most important isomer is Endotheline 1 (ET-1) which is produced in the endothelial cells and is the most potent vasoconstrictor (6, 7).

In normal conditions there is a perfect balance between the production of vasodilatatory substances from the endothelium (NO, prostacyclin -1 and the hyperpolarizing factor EHP) and vasoconstrictor substances (mainly Endothelin-1 and Angiotensin II) (8, 9). During prolonged periods of stress caused by hyperglycemia and resistance to insulin, as it happens during diabetes type II, there is a shift in the balance with more vasoconstrictors being produced compared to vasodilating agents. This leads to endothelial dysfunction, defined by an increase of vasoconstriction, proinflammatory and procoagulatory status of the vessels. In the glomerular endothelium this changes affect the permeability of the filtration barrier leading to microalbuminuria (10).
MA is measurable and we can use it as a marker for evaluating dysfunction in renal glomerular endothelium. We have taken into our study patients with microalbuminuria and without microalbuminuria.

VWF is another glycoprotein released by the endothelium. It plays an important role in platelet adhesion to extracellular matrix during endothelial damage and thrombogenesis. It also binds to factor VII in plasma stabilizing its structure (6). It is normally stored in the Weibel Palade bodies in the endothelial cells and is secreted during damage of endothelium. VWF is considered as a golden standart for measuring the endothelial dysfunction. Its plasma level increases during vascular injury and vasculopathy related to diabetes (11, 12). Being one of the most useful and specific markers in the vascular damage and thromb formation during endothelial damage (13) we have measured it in the plasma of plasma of our patients in the study.

II. MATERIAL AND METHODS

100 patients were included in the study where 80 patients were diagnosed with type 2 diabetes in the last 2-5 years. They were all presented at the Tirana Polyclinic of Specialities during September 2010 – December 2013 and were under treatment for diabetes with oral therapy (they were diagnosed according to the guidelines from the Expert Comity on Diabetes Diagnosis). 20 individuals, part of the control group, were selected at the same age with the diabetic patients, but were in perfect health.

The 80 diabetic patients were divided in two groups based on the level of albuminuria measured in the urine during 24 hours.

1. The first group was composed of 40 patients with normoalbuminuria (excreted albumin 0 – 30 mg/24 h)

2. The second group of 40 patients with microalbuminuria (excreted albumin 30 – 300 mg/24 h)

In the plasma of all the patients and the control group we measured the level of ET-1 and vWF. 100 cc of blood was taken from the patients and it was separated in two vacuum tubes with 5cc K3EDTA in each of them.

In measuring ET-1 blood was processed for 10 minutes at 2500 rotations per minute and plasma was separated and conserved at – 70 °C. Before use, they were put in laboratory water bath at 37 °C. We used an ELISA kit from the DRG with calibration curbes and three phases of incubation. The readings were done by ELISA HUMA HS of Human Germany with a reading filter 450 nm, correcting filter 630 nm, measuring unit ng/ml and 5 numbers of standart. Standart values were: a. S1 0.01ng/ml, b. S2 0.1ng/ml, c. S3 1.0ng/ml, d. S4 10ng/ml e. S5 100ng/ml. Each test was done in two parallels and the results were taken referring to the calibration curve.

In measuring vWF the blood was processed for 15 minutes at 2000 – 2500 rotations per minute. Plasma was processed within 24 hours kept at 2-8 °C. It can also be kept for one month at – 20 °C and before use be put in laboratory water bath at 37 °C. The reagent we used was STA-LIATEST VWF: AG. Measurements were done using STAGO –COMPACT a closed automated system. Reading was done by dosing the antigene by immunoturbimetric method using a thin filter (540 nm). Reading was based on the increase of turbidity at the microparticle of latex suspension measured by photometry. The Calibrator was prepared by vW calibrator. The calibrator curbe has a linear regression and the value of the calibrator is 102%.

50 l serum was taken with 0.7 l buffered reagent vWF + 0.7 l latex reagent vWF. When the latex microparticles with the bound specific antibodies are in contact with the plasma vWF, causes a reaction which leads to the agluttination of the microparticles. This phenomena causes an increase in the turbidity of the mixture resulting in an increase of the environmental absorbance. The amplitude of this is related to the level of vWF in examined plasma. Normal levels for adults are between 50–160 %. Control values are between 76–96 %.

III. STATISTICS ANALYSIS

All data was presented in average value and standart deviation. Discrete data was presented in absolute value and percentage. Data was processed and presented in tables and graphics (Bar diagram, linear graphic, space filling graphic, box-plot and scatter diagram). Differences between variables were analysed by the “t” student test (in comparing the two groups) and by the ANOVA analysis (when comparing more than two groups).

Differences between groups for discrete variables were done using the Hi-square test. The relationship between the variables was analyzed through Pearson and Kendal’stau correlation coefficient. Values of p<0.05 were considered significant. SPSS 19.0 was used for analyzing the data.
IV. RESULTS

100 persons were included in the study with an average age of 55.78±7.72 years old, from whom 39% were male and 61% were female.

Tab 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55.78±7.72</td>
</tr>
<tr>
<td>Years with diabetes</td>
<td>2.27±2.03</td>
</tr>
<tr>
<td>ET-1</td>
<td>1.18±0.52</td>
</tr>
<tr>
<td>VWF</td>
<td>1.523±0.46</td>
</tr>
</tbody>
</table>

100 patients were included in the study where 80 patients were diagnosed with type 2 diabetes in the last 2-5 years and 20 patients were part of the control group. The 80 diabetic patients were divided in two groups. The first group was composed of 40 patients with normoalbuminuria (excreted albumin 0 – 30 mg/24 h) and the second group of 40 patients with microalbuminuria (excreted albumin 30 – 300 mg/24 h). ET-1 and vWF were measured in their blood.

Tab 2.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normoalbuminuria</th>
<th>Microalbuminuria</th>
<th>Control group</th>
<th>Total</th>
<th>F value</th>
<th>p* value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55.18±9.325</td>
<td>59.70±7.928</td>
<td>51.78±3.422</td>
<td>55.78±7.722</td>
<td>6.094</td>
<td>0.004</td>
</tr>
<tr>
<td>Years with diabetes</td>
<td>3.00±1.342</td>
<td>3.90±1.210</td>
<td>.00±0.000</td>
<td>2.27±2.029</td>
<td>76.207</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Von Willebrand F</td>
<td>1.523±0.5006</td>
<td>1.809±0.1410</td>
<td>1.206±0.4799</td>
<td>1.523±0.4619</td>
<td>11.650</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ET_1</td>
<td>1.20±0.485</td>
<td>1.23±0.504</td>
<td>0.87±0.572</td>
<td>1.18±0.519</td>
<td>7.315</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Analysing average values for the variables above through one way ANOVA (Bonferroni procedure), was noticed an important difference between groups. The group with microalbuminuria has the highest average age, more years with diabetes, higher levels of ET-1 and vWF.

Relationship between ET-1, von Willenbrant factor with microalbuminuria

<table>
<thead>
<tr>
<th>Variables</th>
<th>r*</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbuminuria</td>
<td>.303</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>.495</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

* Pearson correlation coefficient Tab 3

Through the Pearson correlation coefficient was noticed an important connection between ET-1 levels and microalbuminuria (p<0.05) as well as between vWF and microalbuminurise (p<0.001).
V. DISCUSSION ENDOTELIN ET-1 AND ALBUMINURIA

Affected by stress acting for a long time, like in the case of type 2 diabetes (hyperglycemia and insulin resistance), elevated levels of vasoconstrictors are produced by the endothelial cells. These substances tip the balance between vasoconstriction and vasodilation in favor of vasoconstriction leading to endothelial dysfunction (10, 14). This is noticed very early at the glomerular endothelium where the filtrations barrier is disrupted. It starts with the glycocalix, but it continues until microalbuminuria is made present (15, 16). The level of microalbuminuria is proportional with the level of the damage (16). Measuring microalbuminuria gives us the opportunity to use it as a marker for measuring endothelial dysfunction in renal glomerular endothelium (17, 18, 19, 20). That’s why we have in our study patients with microalbuminuria and normoalbuminuria. In our study, in the first group with normal albuminuria ET-1 levels were 1.20±0.485 (0.87±0.572 at the control group), while at the second group with microalbuminuria ET-1 levels were 1.23±0.504. Based on this findings we can say that ET-1 starts to increase during the first year of diabetes where it seems also that the endothelial dysfunction is present. This proves that hyperglycemia starts to affect the vascular endothelium by changing its homeostasis. ET-1 values with microalbuminuria, a well-known marker of endothelial dysfunction in renal endothelium, were statistically important (p<0.05). We can come to the conclusion that ET-1, as a biomarker, is also very useful in the evaluation of the endothelial dysfunction in renal glomerular cells. Reason for this change it is believed to be the disruption of the glycocalix layer, glomerular filtration and the endothelial cells itself, by hyperglycemia and the ROS produced by damaged cells. Hyperglycemia causes metabolic cell changes which tip the balance in the production of the oxidative substances and free radicals, increase in oxidative stress, activation of the C protein kinase affecting the lowering of the activity of eNOS leading to smaller NO levels, a vasodilatory and antiatherogenic substance (17, 18, 19, 20). Lowering the NO level, hyperglycemia increases the expression of ET-1 wich is a potent vasoconstrictor and an antagonist of NO (24, 25). The lowering of the biodisponibility of NO causes an increase in the expression of pro inflammatory cytokines leading to adhesion and chemotaxis for monocytes and neutrophils at the site of the damage (26, 27). The disruption of the endothelial glycocalix (cell coat) from the proinflammatory cytokines and TNF-α, will lead to failure of the endothelium to maintain it’s negatively charged plasma membrane leading to microalbuminuria (28, 15). Receptors to Endothelin are two types, A and B (29). Through A receptors ET-1 acts in the kidney and partakes in the salt related hypertension (30). This may include the use of iNOS (inflammatory NOS), and the B receptors (31). One of the mechanisms endotelin acts at the glomerular endothelium is its interaction with nephrin, a protein affecting directly on the glomerular filtration barrier (32, 33). So we have the damage of the filtration barrier starting with the glycocalix and latter affecting the glomerular endothelium, which manifests the endothelial dysfunction. This is the cause of microalbuminuria.

VI. FACTOR VONWILEBRAND AND ALBUMINURIA

In our study the vWF as a biomarker of endothelial dysfunction was measured in the plasma of our patients in 100 individuals. 80 of them were diabetic type 2 patients having diabetes for a period of 2-5 years and 20 of them without any known pathology. Average age of the diabetic group with normoalbuminuria was 55.18±9.325 and of the group with microalbuminuria was 59.70±7.928. Average age of the control group was 51.78±3.422.

In the control group there was no albumin in the urine collected in 24 hours (negative) and the vWF level was within normal range 0.50-1.60 (50-160%). This is the result of a normal function of endothelium in general and renal glomerular endothelium more specifically. Endothelium is intact and the balance of functions is preserved.
vWF levels start to go up in the first years of diabetes (2-5 years) and its increase is proportional with the level of microalbuminuria p<0.001. The group of diabetic patients without albuminuria had vWF levels within range: 1.523±0.506 (36 patients out of 40). Only 4 patients had a higher level of vWF (1.602±0.232). These 4 cases had hypertension, suggesting that hypertension is another factor influencing the endothelial dysfunction in diabetic patients. The relationship of vWF and microalbuminuria was very strong and statistically important. All the 40 patients who had microalbuminuria had also higher vWF levels (1.809 ±01410). Age was not related with the vWF level.

All the cases with microalbuminuria in our study (40 patients), has higher levels of vWF. The conection of microalbuminuria with vWF as a biomarker of endothelial dysfunction was very strong (p< 0.001). We sugest that vWF is an important biological marker of renal endothelial dysfunction, which is expressed through microalbuminuria. Microalbuminuria is a well known marker today of endothelial dysfunction in general and of the renal endothelium more specifically (34).

Several studies have shown the conection of microalbuminuria with endothelium dysfunction. One of the mayor ones is the “Steno hypothesis” (4) where microalbuminuria is connected to the endothelial dysfunction not only in diabetic patients, but also in elderly patients without diabetes. Theoretically, an endothelial dysfunction in the renal glomerulus, can cause microalbuminuria through an increase of the glomerular pressure, changes in the permeability of the basement membrane, changes in the podocyte function, inflammatory changes (paracrine model) as we have presented with the chart at the beginning of the discussion (35). Other studies like Stehouwa et al (36, 37), showed that an increase of vWF precedes the appearance of microalbuminuria in the three first years. There are also other studies showing the relation between vWF and endothelial dysfunction preceding microalbuminuria (37).

VII. Conclusion

Based on the data from our study, we can say that both biomarkers ET-1 and vWF are very significant in the early stages of the installation of endothelial dysfunction in the renal glomerulus. Their values measured in the plasma of our patients can give to the clinicist the right to intervene in time for preventing diabetic nephropathy or slow the process of endothelial dysfunction towards diabetic nephropathy and renal damage.

REFERENCES

[8] “Nitric oxide signaling: systems integration of oxygen balance in defense of cell integrity” Gong, Li; Pitari, Giovanni M.; Schulz, Stephanie; Waldman, Scott A.