Tbilisi State Medical University

With the right of a manuscript

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"ASSESSMENT OF ENDOTHELIAL FUNCTION MARKERS TO EVALUATE EFFICACY OF QUINAPRIL THERAPY OF MICROANGIOPATHIES IN TYPE 2 DIABETES MELLITUS "

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ABSTRACT

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The work was performed at the Department of Cardiomyopathy and Ischemic Heart Disease of the National Center of Therapy

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The Abstract of the Thesis was delivered on----------2006г.

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Problem Actuality

Despite the progress in medicine, cardio-vascular diseases (CVD), mainly atherosclerotic changes in blood vessels, remain the leading cause of mortality, morbidity and disablement in the world.

According to the data published by the WHO, 1/3 of annual death is caused by cardio-vascular pathologies. Data presented by R.O Banow et al., indicate that 80% of people with diabetes die of cardiovascular diseases, 75% of them having coronary artery disease. Today scientists consider diabetes mellitus (DM) as CVD.

According to M. J. Knol et. al there are currently 200 million people with diabetes worldwide. Type 2 (T2DM) accounts for 90% of all cases. Due to the rise in risk factors that is caused by the lifestyle DM is spreading rapidly. T2DM affects small (microangiopathy) and large (macroangiopathy) vessels.Microangiopathy is characteristic for diabetic retinopathy, neuropathy and nephropathy, while macroangiopathy in DM is manifested by atherosclerosis, that damages such vital organs as heart and brain. In DM, atherosclerosis is a multifactoral process, that involves hyperglycemia, hyperlipidemia, oxsidative stress, hyperinsulinemia and complex inter-relations between coagulation and fibrinolysis systems. Micro- and macroangiopathy are the main causes of loss of working ability and death in T2DM patients.

It is estimated that micro- and macroangiopathy in T2DM results from the dysfunctions of blood vessel endothelium. Improvement of endothelial functions occupies one of the central positions in T2DM management, as it permits to reduce morbidity and mortality rates. It must be mentioned here, that endothelial dysfunctional are observed both in DM and CVD. Disfunction of blood vessel endothelium is one of the early signs of atherosclerosis. Thus, improvement of the endothelial functions is considered to be the urgent problem.

It is well-known that intracellular oxidative stress plays an important role in the pathogenesis of endothelial dysfunctions in DM – hyperglycemia activates oxidation of the free radicals. Superoxide and hydroxyl radicals are formed, that, in turn, causes intracellular (including intimae) oxidation of the LDH cholesterol that aggravates endothelial dysfunctions. It is expressed by the reduction in endothelial vasodilatation (drop in NO) and rise in endothelial vasoconstriction. One of the main pathophysiologic changes in T2DM is resistence of peripheral tissues to insulin, or insulinresistence. Insulinresistence provokes activation of sympatho-adrenal (SAS) and rennin-angoiotensin-aldosterone (RAAS) systems. When RAAS is activated, the balance between vasoconstriction (endothelin-1 and angiotensin - II) and vasodilatation (prostacyclin and NO) factors is disturbed, this resuts in the development of endothelial disfunction. Thus, RAAS activation may comprise one of the pathogenic circles of manifested micro- and macroangiopathy in T2DM. It is known that when renine-angiotensine-aldosterone system is activated, angiotensin-II plays the role of the mediator of the ongoing processes and angiotensine-converting enzyme (ACE) is involved in its production. Thus, lately the study of the effect of ACE-inhibitors became the problem of today. It is clear, that creation of preparations, that could reduce insulinresistence and hyperinsulinemia; restore disturbed functions of the endothelium is very urgent. ACE-ihibitors, that can interfere with bradikinin dissociation, are a potent stimulators of NO. Besides, while blocking superoxide-anion production, they interfere with NO inactivation.

ACE-inhibitors were used to treat arterial hypertension (AH) already 25 years ago, and today they are still successfully used in the therapy of AH, heart failure, myocardial infarction and diabetic/non-diabetic nephropathy.

During past years the possibility to use ACE-inhibitors in patients with increased risk of CVD (including those with DM, peripheral disease and cerebral blood flow disturbances in anamnesis) is widely considered. The group of lipophilic preparations (Perindopril, Ramipril, Quinapril, Trandonapril, etc), that are characterized by tissue specificity and especially, vascular selectivity, are the most prospective in the study of the effect of ACE-inhibitors on the endothelial dysfunction. Various ACE-inhibitors differ according to their ability to bind to tissue ACE. According to their binding ability to tissue ACE, tritium-marked ACE-inhibitor activating line is as follows:

Quinaprilat = Benazeprilat > Ramiprilat > Perindoprilat > Lizinoprilat > Enalaprilat > Fozinopril > Captopril.

Many trials were carried out during past years and TREND was one of them. Normotensive patients with stable angina pectoris and without severe hyperlipidemia and signs of heart failure were enrolled in the trial. Patients were treated with Quinapril, 40mg/daily for 6 months. In parallel, function was assessed with administration of acetylcholine in coronary arteries. As repeated post- treatment examinations showed initial vasoconstriction in reply to the acetylcholine administration in coronary arteries significully dropped and vasodilation was partially normalized after 6 month treatment with Quinapril

Vasoprotective effect of ACE-inhibitors may be connected not only to the revealing of Ag-II synthesis in tissues, but with increase in bradikinin and respectively, influence on NO production.

Thus, the results of the TREND trial revealed, rhat Quinapril significantly improved endothelial function.

Everything stated above stresses the urgency of the problem, especially as detection of drugs, that have positive effect on the endothelial functions, permit to look optimistic on the reduction of morbidity and mortality rates in T2DM.

The Aim of the Study

The aim of the present work was to study the effect of Quinapril treatment on the endothelial dysfunctions in T2DM.

The Objectives of the Study

To achieve the stated above aim we assessed endothelial markers prior to and 6 months post treatment initiation with Quinapril. Following objectives were put – to study listed below parameters in T2DM patients before and 6 months after the initiation of the treatment with Quinapril:

1. Markers of the endothelial functions (NO, PAI-I)
2. Heart microcirculation
3. Microalbuminuria
4. Fundus of the eye

Scientific Novelty

For the first time we studied:

1. The functional state of small blood vessels in T2DM using myocardial perfusion scintigraphy .
2. The role of ACE-inhibitor, Quinapril in the treatment of manifested microangiopathy in Georgian population with T2DM.
3. According to factor of endothelium (NO) we assessed the functional state of small blood vessels (myocardial microcirculation and microalbuminuria) in T2DM pacients.

Practical Value of the Study

In the present work single- photon emission computer tomography (SPECT) was used to demonstrate that in patients with T2DM myocardium microcirculation disorders are observed not only during the physical load, but at rest, as well.

Myocardial perfusion scintigraphy can detect coronary artery disease (CAD) at an early stage and reveal T2DM patients with increased risk of CAD. We observed, that Quinapril

positively affects the functional state of small vessels, increases NO concentration in blood plasma and reduces microalbuminuria levels.

Quinapril, today, is intensively used in these patients at the CAD and Cardiomyopathy Dept. of the National Center of Therapy. Besides, perfusive scintigraphy of the myocardium (with SPECT) is widely used in management of patients with T2DM.

Approval of the Work

The work was approved at the meeting of the Scientific Council of the National Canter of Therapy on May 24, 2006 (Record of Proceedings No 8).

Publications: Three works were published on the subject of the present study.

Composition and Structure of the Study

The present work contains Introduction, Literature Review, Clinical Data, Methods of the Study, Results Obtained, Discussion, Conclusions and List of Literature. The work consists of 105 pages, it contains 7 Tables, 12 pictures and 6 Figures.

Materials and Methods

Data obtained for patients with T2DM who were treated in - and out - patiently at the CAD and Cardiomyopathy Dept. of the National Center of Therapy in 2004-2006. Totally 75 patients were enralled in the study (age range 37-79yrs, males – 40, females – 35).

Inclusion criteria: revealing

- Type 2 diabetes mellitus

Exclusion criteria:

- Heart failure
- Arterial hypertension, II-III stage (WHO-ISH, 1999)
- Heart attack
- Kidney failure with creatinine clearance <95ml/min
- 3-4-fold increase in ALT (alanine-amino-transferase), AST (aspartat-amino-transferase) and GGT (gamma-glutamin-transferase) activity in blood plasma
- Total CHOL>250mg/dl, LDL-CHOL>150mg/dl

Sixty two patients met listed above criteria.

Mean diabetes duration was 8.6±6.3 yrs. Oral hypoglycemic agents (glybenclamide, metformin hydrochloride, glyclazide, glymepiride) were used to control T2DM.

Arterial hypertension, I stage (WHO-ISH, 1999) was registered in 32 patients.

The trial was open, non-randomized.

Quinapril was administered at a dose of 40 mg/daily.

Two weeks prior to the study initiation nitrates, diuretics and calcium-antagonists were quitted in all the patients, while administration of statines was stopped 1 month prior to the study onset.

In case of necessity,β-blockers (except Nebilet) were prescribed.

The patients were divided into the following groups:

- according to sex – 30 females (mean age 59.2±7.1 yrs) and 32 males (mean age 59.3±9.4 yrs)
- according to age – 34 patients <60 yrs, and 28 patients ≥60 yrs.

- according to arterial blood pressure levels

Arterial blood pressure (ABP), blood glucose – fasting (FBG) and post-prandial (PPBG), HbA1c, lipid profile, ejection fraction of the left ventricle (LVEF), blood serum NO and PAI-I concentrations, albumin levels in 24-h urine, myocardial perfusion and eye fundus were tested and assessed in all the groups.

Following indices were registered in 62 mentioned above patients before the study was initiated:

- mean SBP – 141.0±14.1 mmHg, mean DBP – 84.0±6.4 mmHg
- FBG – 127.6±19.2 mg/dl, PPBG – 143.3±24.9mg/dl
- T-CHOL – 214.5±22.8 mg/dl, HDL-CHOL – 41.6±9.6 mg/dl, LDL-CHOL – 141.8±23.9 mg/dl, TG – 211.3±54.8 mg/dl
- LVEF – 57.0±3.9%
- BMI – 31.7±4.71 kg/m^2

Arterial hypertension was registered in 32 (51.61%) patients (stage I, WHO-ISH 1999;meanABP – 153.0±5,3/87,0±5.0mm/Hg).

Repeated examinations were performed at month 3 and 6 of the study. All listed above parameters were tested at each visit. Glycemia control was assessed with HbA1c.

Prior to and at month 6 post study initiation NO and PAI-I in blood serum, and albumin (ALB) in 24-h urine were measured. Perfusion scintigraphy of the myocardium was performed in 11 cases and eye fundus examination was performed in all the patients.

ALB was measured with immunoturbidimetric method using the low measurement limit (Biosystems) and following criteria were applied:

- ALB<30 mg/24h – proteinuria not registered
- 30mg/24h<ALB<300 mg/24h – microalbuminuria type proteinuria present.
- ALB>300mg/24h – macroalbuminuria registered

Before the onset of the Study mean AL levels were 66.0±77.8 mg/24h; in 32 cases (51.61%) microalbuminuria was found (mean ALB levels – 114.0±83.6mg/24h).

PAI-I was measured with immunoenzyme method (ELISA, Chromolize PAI-I assay). Mean value of PAI-I before the treatment initiation was 20.5±15.0.

NO was measured in venous blood; samples were placed in polyethylene tubes (40 mm in length), frozen in liquid nitrogen at -196^{0}C and placed in the spectrometer resonator using quartz duar. With computer and recorder we registered EPR signals on EPR-Spectrometer (РЭ – 1307). Mean NO value before the Study was 20.5±4.3mm/mg

Blood circulation in the myocardium was assessed with photon-emission computer tomography (SPECT) prior to and at month - 6 post treatment initiation. Radiopharm-preparation (RPP) – Tc 99 m Sestambi was administered. A one-day study protocol was used. SPECT at rest and at the pick of the physical load after the administration of the RPP were performed during one day.

SPECT analyses were performed based on a segmental scheme.

A 20-segment model was used (Picture 1). In each segment RPP inclusion was assessed with 5-score system (0 = normal, 1 = supposed, 2 = moderate reduction, 3 = severe reduction, 4 = absence of inclusion).

Three global perfusion indices were used:

1. SSS (summarized stress score), that is obtained by summarizing all segment scores during the stress;
2. SRS (summarized rest score), that is obtained by summarizing all segment scores at rest.
3. SDS (summary difference score) or reversibility score, that is obtained by summarizing all segment score differences between stress and rest.

SPECT scintigraphy detected RPP distribution disturbances, if segment scores in two or more segments were ≥2. When SSS was <4, there were no disorders in the myocardial perfusion; with SSS of 4-8, we had mild myocardial perfusion disorders, while SSS >8 indicated to severe ones.

SDS or reversibility score is the index of myocardial ischemia; when

0< SDS<1 – no myocardial ischemia is present

2< SDS<4 – mild myocaroial ischemia is present

SDS > 4 - moderate- to- sever myocardial ischemia is present

Myocardial perfusion disorders were registered in all the patients before the study was initiated; mean SRS was 10.45, and mean SSS was 9.73.

Fundus examination was performed with ophthalmoscope. In 16 (25.8%) patients diabetic rtinopathy (DR) was revealed – 10 of them (62.5%) had stage 1DR and 6 (37.5%) had stage 2 DR ; in 44 (70.9%) cases hypertonic angiopthy was revealed/ 30(68.2%) – stage 1, and 14(31.8%) – stage 2 hypertonic retinopathy.

The patients' data were entered into the Microsoft office Excel Data Base. The data was processed using MINITAB 11.12 for Windows Statistical program. The data obtained were processed with descriptive and analytical statistical methods; SD was calculated; difference in numeric indices was estimated by P-value calculation. Basic Statistics Methods, such as Descriptive Statistics. One Sample t-test (Confidence intervals for the Mean), Pearson Product Moment Correlation Coefficient, ANOVA-method, One-way Analyses of Variance and Mann-Whitney Method for Small and Non-Homogenous Groups were used.

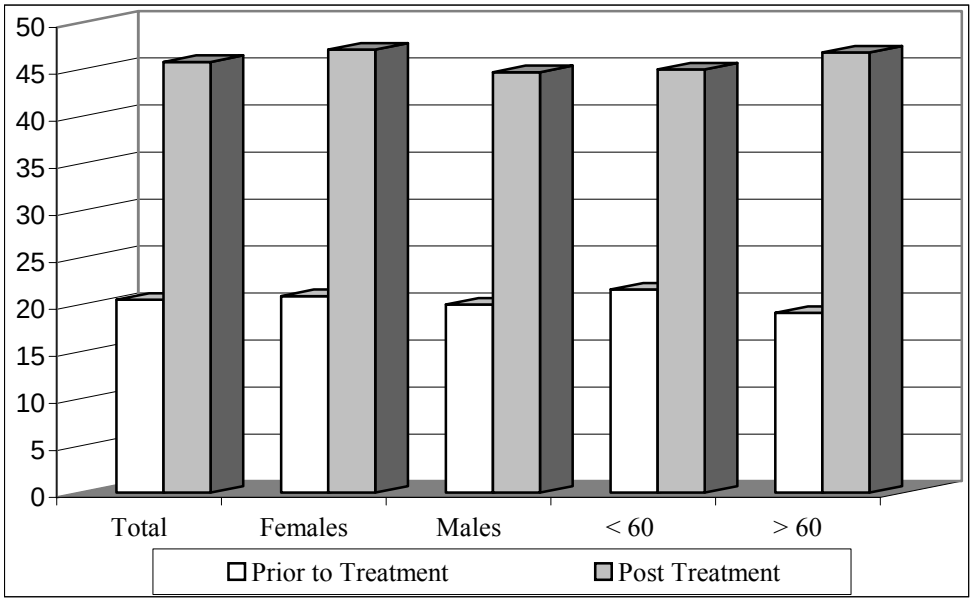
Results of the Study and Discussion

Various indices were studied prior to and at month 6 post Quinapril treatment initiation.

At month 6 post treatment initiation mean concentration of NO in the study group increased by 25 (20.5±4.3mm/mg vs. 45.8±14.9mm/mg); it increased by 26 in females (20.9±4.1mm/mg vs. 47.1±15.3mm/mg), and by 25 in males (20.0±4.6mm/mg vs. 44.7±14.6mm/mg); in < 60 yrs age group it raised by 23 (22.0±4.2mm/mg vs. 45.0±13.1mm/mg) and in ≥ 60 yrs age group by - 28 (19.0±4.2mm/mg vs. 47.0±17.2mm/mg); concentration of NO increased 2–fold in all the groups (Fig. 1).

Prior to and 6 month post Quinapril therapy was initiation we studied PAI-1 and NO indices both, in hypertensive and normotensive patients. Mean NO concentrations in hypertensive patients increased by 26, while in normotensive ones – by 25; concentration of NO increased 2-fold in both groups (Fig. 1).

Fig. 1 NO Indices Prior to and 6 - Month Post Quinapril Initiation



NO elevation in all the study groups was statistically evident (Table 1 and 2). If literary data are taken into consideration, this rise indicates to the improvements in the endothelial functions.

PAI-I indices prior to and post treatment initiation did not differ statistically in either group (Table 1 and 2). Though in one of the previous studies it was shown that Quinapril improved PAI-I, and this change was statistically evident.

Table 1. NO and PAI Indices Prior and Post Treatment Initiation

Group	N	NO mm/mg			PAI-I		
		Prior to	Post	P	Prior to	Post	P
Total	62	20.5±4.3	45.8±14.9	0.000	20.5±15.0	19.4±13.5	0.68
Females	30	20.9±4.1	47.1±15.3	0.000	20.7±13.5	21.6±13.8	0,80
Males	32	20.0±4.6	44.7±14.6	0.000	20.3±16.6	17.4±13.0	0,44
< 60	34	21.6±4.2	45.0±13.1	0,000	25.5±15.7	23.5±14.0	0,56
≥ 60	28	19.1±4.2	46.8±17.0	0,000	14.4±11.8	14.5±11.1	0,96

The data obtained in our Study may be explained by the fact that various factors influenced the hemostasis system. Besides, we did not mean to study other components of hemostasis, that, in turn, influence PAI-I.

Table 2. NO and PAI-1 Indices Prior to and Post Treatment Initiation

Group	No	NO mm/mg			PAI-1		
		Prior to treatment	Post treatment	P	Prior to treatment	Post treatment	P
Normotensive Patients	30	20.20±4.32	45.4±16.6	0.000	22.0±14.3	16.8±11.0	0.12
Hypertensive Patients	32	20.7±4.4	46.2±13.3	0.000	19.1±15,.	21.9±15.2	0.48

Microcirculation of the myocardium was studied to assess the effect of Quinapril on the state of small blood vessel functions.

Microcirculation in the myocardium was studied in 11 men (age range 36-71yrs, mean age 55.27±9.28), all were normotensives; in 3 T2DM patients, beside a heart attack in anamnesis, diagnosis of CAD was based on the results of coronorography.

Before therapy with Quinapril was initiated, specificity of myocardial perfusion scintigraphy was studied in middle-aged patients with T2DM.

At rest decrease in the myocardial perfusion was observed in all the cases. SPECT showed that RPP distribution during physical load improved in 6 out of 11 patients (54,5%) who were diagnosed with T2DM (Picture 2). Diabetes duration in this group was 5- 6 yrs, DM was well-controlled and lipid profile indices were not elevated significantly.

In one case RPP inclusion in every segment during stress did not change, e.i SRS and SSS equelled to 9 (Picture 3); this indicates to moderate disorders of the perfusion. Based on results obtained, we may presume, that the patient had the increased risk of CAD development. DM duration in this patient was 5yrs, FBG was 110mg/dl, PPBG was 130mg/dl, TG levels were elevated – 275mg/dl.

SPECT showed that RPP distribution during physical load deteriorated in 4 patients. In three out of the four patients SPECT results indicated to the presence of moderate- to – severe ischemia (SDS>4), in all the 3 cases CAD had been diagnosed. In the fourth patient, results of the SPECT (mainly SSS, that was 14, and SDS of 5) indicated to severe perfusion disturbances and moderate myocardial ischemia, respectively (Picture 4) . It means that T2DM, in this case, was associated with CAD. Though, CAD was not diagnosed in this patient before SPECT was performed, and there were no changes on the ECG during stress – test. Diabetes duration here

was 11 yrs, HbA1c was 6.01%, T-CHOL - 210mg/dl, HDL-CHOL – 34.4mg/dl, LDL-CHOL - 153.96mg/dl, TG – 118.2mg/dl, I.A – 5.1.

Thus, using myocardial perfusion scintigraphy, we diagnosed CAD in 1 (12.5%) patient with T2DM in anamnesis, and revealed 1 patient with high risk of CAD development; this patient also had T2DM. It is worth mentioning here that patients with T2DM often do not have anginal symptoms of myocardial ischemia, all symptoms are blurred, thus the condition may become critically severe, with the silent course of myocardial ischemia. At the same time, levels of glycemia control do not correlate tightly to the degree of the coronary obstruction and CAD development frequency; though, particularly high correlation is observed with morbidity and mortality indices. Thus, it is of utmost importance to timely reveal myocardial ischemia and high risk patients when DM is present and select methods of their adequate management. Appropriate treatment improves quality of life of these patients, that, in turn, permits to increase their life-expectancy. Thus, SPECT makes it possible to reveal CAD at an early stage, to detect high risk patients and to select appropriate therapeutic approach.

Examinations at month 6 post study initiation showed improvement in the myocardial perfusion. At rest, improvement was registered in 9 cases (81.8%); mean – by 25-30% (Fig. 2). We found statistically evident positive changes in indices of perfusion scintigraphy of the myocardium, (Table 3). In 7 patients (77.8%) myocardial perfusion became normal (Picture. 5), and in the

Table 3. Perfusion Indices Prior to and Post Treatment Initiation

Myocardium Perfusion Indices	Type 2 Diabetes Mellitus N = 11		P**P**
	Prior to Treatment	Post Treatment	
SRS	11.27±3.10	6.36±4.15	0.0057
SSS	10.18±4.81	6.18±3.66	0.042

remaining 2 cases (22.2%) perfusion improvement was reflected by the reduction in the number of the segments, where RPP inclusion was reduced before treatment (Picture. 6).

In 2 cases we observed perfusion deterioration at rest SRS1mean – 9.5, SRS2mean – 12.0. If before treatment RPP distribution was disturbed in 2 – 2 segments, 6 month post treatment initiation it was lowered in 3 -3 segments (Picture. 7). In both cases repeated HbA1c tests revealed glicemia control deterioration (HbA1c at entry – 7.5%, at month-3 – 8.2%, at month-6 – 8.8%

Myocardial perfusion was also studied during physical load. Stress SPECT revealed statistically evident improvement in perfusion 10.18 ± 4.81 vs 6.18 ± 3.66, P = 0.042. Though when compared to at rest values this improvement comprised only 20% (Fig. 2).

Perfusion amelioration was registered in 9 cases SSS1mean – 9.18 and SSS2mean – 4.72

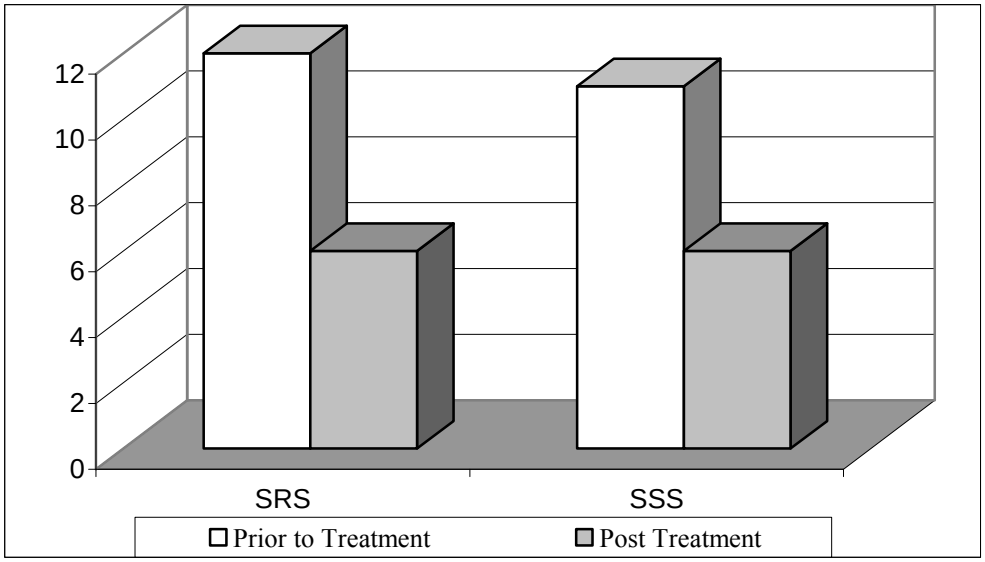
In 3 cases (27.2%) perfusion disorders were no more observed SSS< 4 (Picture. 8);

In other 3 cases (27.2%) severe perfusion disorder was replaced by a mild one (Picture 9);

And in the remaining 3 patients (27.2%) we observed relative improvement of the myocardial perfusion(Picture 10).

RPP distribution deteriorated in 2 cases (18.1%) – SSS1mean – 5.5 and SSS2mean – 8.0 (Picture 11). We observed aggrevation of both, stress and at rest perfusion disordersiIn the same pacients.

Fig. 2 Indices of the Myocardial Perfusion in Patients with Type 2 Diabetes Mellitus



We may presume that such amelioration of perfusion is related to the improvements in endothelial functions caused by the treatment with Quinapril. Correlation between the changes in the perfusion scintigraphy indices and increase in NO in these patients support the presumption (r = 0.457). Besides, all factors that, independent of Quinapril, can positively effect myocardial perfusion were excluded. Mainly, BMI indices did not change significantly after 6 month treatment period –31.7±4.71 kg/m^2 vs 30.2 ± 5.7 kg/m^2. Lipid profile indices did not change significantly in the main group after 6 month of treatment with Quinapril (Table 4).

In the group of patients, where SPECT was used to assess myocardium circulation, we did not observe statistically evident changes in lipid profile indices 6 month after the initiation of Quinapril therapy: T-CHOL – 224.0 ± 14.1mg/dl vs 225.3 ± 17.5mg/dl; HDL – CHOL – 45.3 ± 15.2mg/dl vs 44.2 ± 18.2mg/dl ; LDL – CHOL – 135.2 ± 19.6 mg/dl vs 136.5 ± 17.3mg/dl; TG – 224.5 ± 87.2mg/dl vs 225.8 ± 93.1mg/dl;

Statistically not evident changes in the lipid profile indices can be explained by the fact that antiatherosclerotic therapy was not initiated in our patients. One month prior to the Quinapril treatment onset statin administration was quitted to exclude their role in endothelial function amelioration.

Based on the results of our Study, we may suggest that improvements in endothelial functions after Quinapril treatment cause rise in functional blood vessel number that provides amelioration of the myocardial perfusion.

Table 4. Lipid Profile Indices Prior to and Post Quinapril Initiation

	Type 2 Diabetes Mellitus			
	N = 62			
	Indices of Lipid Profile			
	T –CHOL mg/dl	**HDL-CHOL mg/dl**	**LDL- CHOL mg/dl**	**TG mg/dl**
Prior to Treatment	214.5±22.8	41.6±9.6	141.8±23.9	211.3±54.8
Post Treatment	217.1±24.3	39.6±13.3	143.0±25.2	212.5±57.5
P	0.53	0.34	0.78	0.91

It is worth mentioning here, that good glycemia control is an indispensable condition; otherwise, NO inactivation will take place, due to the effect of superoxide radicals. This will interfere with amelioration of the endothelial function and Thus, Quinapril will be less effective,

when such conditions are present. Elevated glycemia levels could explain deterioration of myocardial perfusion in 2 of our patients treated with Quinapril.

Effect of Quinapril on myocardial perfusion was studied in normotensive patients to exclude myocardium perfusion amelioration caused by the drop in ABP due to Quinapril. It is well –known that ACE – inhibitors, including Quinapril, have potent hypotensive properties. In hypertensive patients, enrolled in our study ,administration of Quinapril resulted in significant drop in ABP levels. At entry, 32 (51.6%) patients had AH (females -19; males – 13; 20 patients> 60yrs and 12 patients < 60yrs). Throughout the 6 – month period their ABP was gradually declining (Fig. 3) and at the end of the study it was statistically lower; and this drop was statistically evident in the main group and in the groups of patients of both sexes, and over and below 60yrs (Table 5).

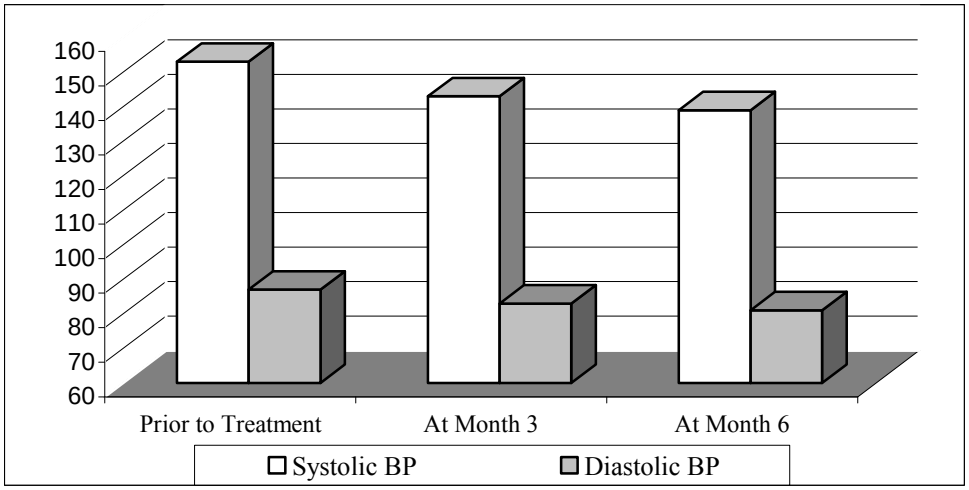
Table 5. ABP Indices Prior to and 6 – Month Post Quinapril Administration in Hypertensive Patients

Group	no	SBP mm/Hg			DBP mm/Hg		
		Prior to treatment	Post treatment	P	Prior to treatment	Post treatment	P
Total	32	152.6 ± 4.9	138.2 ± 5.3	0.0000	86.8 ±5.0	81.1 ± 5.5	0.0000
Females	19	153.16±4.68	139.16± 5.16	0.0000	86.68±5.43	80.95 ±5.73	0.0032
Male	13	151.69±5.27	136.85± 5.49	0.0000	87.08± 4.63	81.38 ± 5.35	0.0080
< 60	12	152.00±5.83	138.33 ± 6.27	0.0000	87.92± 5.3	82.08 ± 5.09	0.012
≥ 60	20	152.90±4.38	138.15 ± 4.86	0.0000	86.20±4.91	80.55 ± 5.77	0.0019

In Quinapril treated hypertensive patients statistically evident drop in ABP levels depends on the blocking of the rennin–angiotensin–aldosterone system; as a result we observe decline in Ag –II plasma levels and catecholamine release, inhibition of vasomotor reflexes, increase in K levels in blood within normal range.

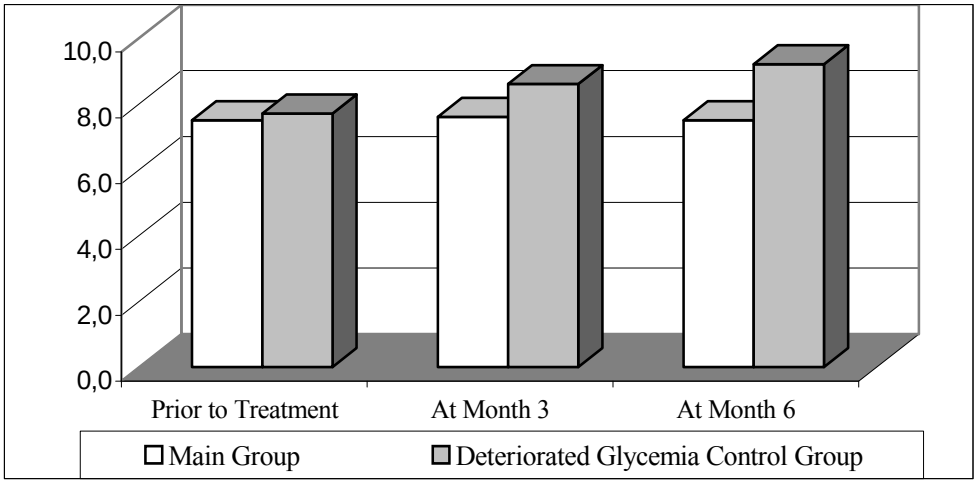
All descried above mechanisms create conditions favourable for ABP decrease. Thus, effect of Quinapril on various markers of ABP is diverse. Positive correlation between SBP/DBP and NO indices was revealed ($r = 0.215$ and $r = 0.242$, respectively).

Fig 3. Variations of ABP in Hypertensive Patient Group



There was no statistically evident difference in HbA1c levels (main group, 62 patients) at entry, at month – 3 and month – 6: entry levels – 7.5 ± 0.6%, 3 – month levels – 7.6 ± 0.8%, 6 – month levels – 7.5 ± 0.4%. Throughout the 6 – month period glycemia levels had increased in 15 patients; among of them females were 9, males – 6.10 patients > 60yrs, 5 patients < 60yrs. Mean HbA1c levels inthis group were as follows: at entry – .,7 ± 0.7%; at month – 3 – 8.6 ± 0.4%; at month – 6 – 9.2 ± 0,3% (Fig. 4).

Fig. 4 Variation of HbA1c Indices in Different Groups



Before the Study on-set microalbuminuria was registered in 32 patients (females -12, males – 20, 13 patients > 60yrs, 19 pacients < 60yrs). Drop in ALB levels in 24 –h urine was statistically evident in all the study groups (Table 6).

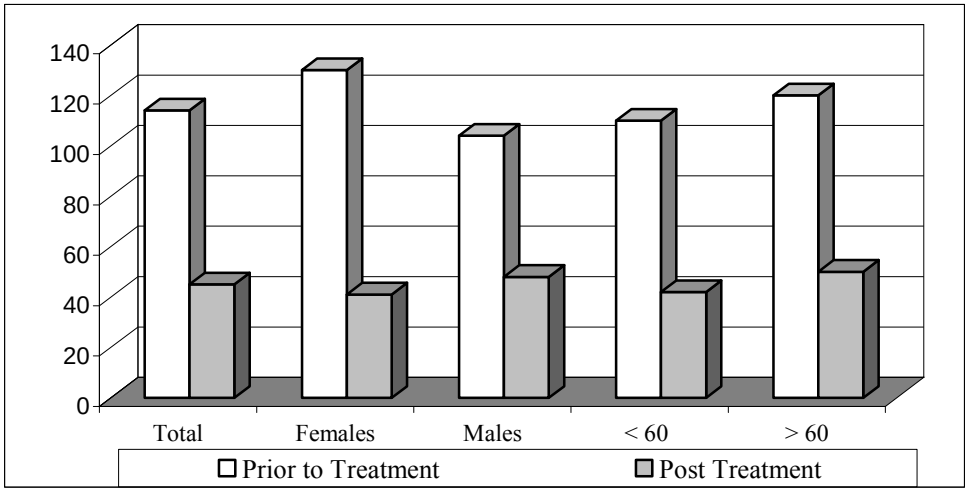
Table 6. ALB in 24 – h Urine in Patients with Microalbuminuria

Group	no	ALB mg/24h	

		Prior to treatment	Post treatment	P
Total	32	114.0 ± 83.6	45.0 ± 39.1	0.0001
females	12	130.4 ± 69.1	40.8 ± 19.6	0.001
males	20	104.1 ± 91.5	48.3 ± 47.4	0.022
< 60	19	110.2 ± 74.7	42.4 ± 39.9	0.0017
≥ 60	13	119.5 ± 98.1	50.1 ± 39.0	0.032

After 6 month of treatment with Quinapril mean ALB concentrations in 24 – h urine dropped by 60.5% for the main group (114.0 ± 83.61 vs 45.0 ± 39.1; this drop was by 52.8% for males (104.1 ± 91.5 vs 48.3 ± 47.4) and by 68.5% for females (130.4 ± 69.1 vs 40.8 ± 19.6); ALB decreased by 61.8% for patients < 60yrs (110.2 ± 74.7 vs 42.4 ± 39.9) and by 57.8% for those > 60yrs (119.5 ± 98.1 vs 50.1 ± 39.0); (Fig. 5).

Fig. 5. MicroALB Indices Prior to and 6 Month Post Quinapril Initiation



At entry microalbuminuria was found in 32 patients. Fifteen of them were hypertensive and 17 normotensive. After 6 month of treatment with Quinapril microalbuminuria levels were statistically lower in all groups (Fig. 6). Mean ALB concentrations dropped by 61.3% in hypertesive patients (163.0 ± 94.7 vs 63.0 ± 46.9) while in normotensive ones they dropped only by 57.1% (70,4 ± 37,3 vs 30.2 ± 22.6); (Table 7).

Decrease in microALB levels both in hyper – and normotensive patients may indicate that drop in ALB concentrations in 24 – h urine is not determined only by a decline in ABP levels. Statistically evident rise in NO concentration after 6 month administration of Quinapril in those patients (as well as in other ones) indicates to the fact that drop in micro ALB observed in normotensive patients is related to the amelioration of endothelial function. correlation between

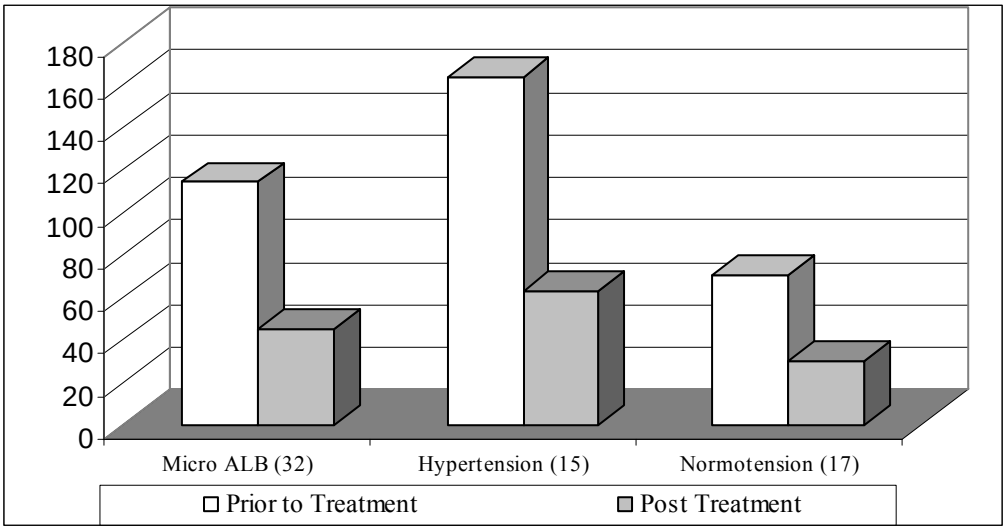
Table 7. MIcroALB Indices Prior to and 6 – month Post Quinapril Initiation in Hypertensive and Normotensive Patients

kategory	No	ALB Mg/24h	P

		Prior to treatment	Post treatment	
MicroALB	32	114,0 ± 83,6	45,5 ± 39,1	0,0001
Hypertension	15	163,0 ± 94,7	63,0 ± 46,9	0,0015
Normotension	17	70,4 ± 37,3	30,2 ± 22,6	0,0008

variations in microALB and NO indices (r = 0.698) also supports this observation. Correlation between variations in microALB and NO indices (r = 0.440) was also found in the group of hypertensive patients. There was also revealed correlation between changes in microALB and BP levels (r = 0.382). Data obtained show that microALB decline in hypertensive patients depends on both, decrease in ABP levels and amelioration of endothelium function. At the same time, it must be stressed here, that nephroprotective effect of ACE – inhibitors and Quinapril, mainly, depends on the selective reduction of intraglomerular ABP, as the glomerular efferent blood vessels are more sensitive to Ag – II, and delation of the mentioned blood vessels, caused by Quinapril effect, does not result in significant drop in intraglomerular pressure and, thus, reduction in glomerular filtration rate.

Fig. 6 MIcroALB Indices Prior to and 6 Month Post Quinapril Initiation in Hypertensive and Normotensive Patients



Fundus examinations with ophthalmoscope after 6 months of Quinapril treatment revealed neither progression, nor regression of the condition (Picture 12). It must be mentioned here that a short observation period (6 months) and low specificity and sensitivity of the method do not permit to make any conclusions based on the results of the Study.

Thus, the results of our study showed that treatment with Quinapril improved functional state of the small blood vessels in patients with T2DM. Positive effect of Quinapril on the functional state of small blood vessels depends on the sharp rise in the concentration of NO – a vasodilating marker of the endothelium. Though, it must be mentioned here, that positive effect of Quinapril on the functional state of small blood vessels was observed only when T2DM was well controlled. The fact that together with the increase in HbA1c levels deterioration of myocardium perfusion is observed exemplifies this fact.

Analysis of the data obtained show that Quinapril significantly improves endothelial functions, thus, it is reasonable to add the drug to the standard treatment of type 2 diabetes mellitus.

It is well – known that postnatal angiogenesis depends on the functional state of the endothelium. Angiogenesis is determined by increase in production and release of growth factors (for example, a vascular endothelium growth factor VEGF), from one side, and by reduction in growth factor inhibitors (for example, angiostatin) from the other. Results of various studies reveal that drop in NO production and its inactivation are associated with elevated angiostatin expression; as a result, development of collaterals is weakened. In chronic hyperglycemia activation of NO is actually diminished under the influence of superoxid radicals. Thus, we may consider, that Quinapril causes elevation of NO and, respectively, significant improvement in endothelial function. It may be a factor favourable for therapeutic angiogenesis; and data, obtained in given below experimental studies support this idea: H. Murohara et al. found that increase in NO intensifies angiogenesis in animal models. J. E. Fabre , A. Rivard et al. studied the effect of Quinapril in animal models and observed that the drug promotes therapeutic angiogenesis in ischemic tissues. It is obvious that the use of such pharmaceutical preparatios in patients with T2DM may reduce the rates of mortality and disablement, caused by microangiopathis. It stands to reason, that significant improvement in endothelial function, due to Quinapril effect, will also positively affect the functional state of large vessels.

Based on the data obtained, following conclusions were drawn and practical recommendations were elaborated.

Conclusion

1. Effect of Quinapril causes significant increase in NO concentrations
2. In type 2 diabetes mellitus administration of Quinapril positively affects microalbuminuria levels both in hypertensive and normotensive patients.
3. Myocardial perfusion scintigraphy – single- photon emission computer tomography (SPECT) is an important assessment method, that permits to diagnose CAD at an early stage and to reveal patients with elevated risk of CAD, when type 2 diabetes mellitus is present.
4. Myocardial perfusion in patients with typa 2 diabetes mellitus is disturbed not only during the stress tests but at rest as well.
5. In type 2 diabetes mellitus Quinapril significantly improves the myocardial perfusion
6. Addition of Quinapril to the standard DM therapy is effective in treatment of micro vascular complications of T2DM, both in males and females, despite their age.

Practical Recommendations

1. It is important to measure microalbuminuria levels (the index of haemodynamics and glomerular filtration disorders) to assess presence of microangiopathy in patients with T2DM
2. It is important to use SPECT to reveal disorders of the myocardial perfusion at an early stage when T2DM is present.
3. It is recommend to include Quinapril in the standard treatment of diabetes mellitus both in males and females, independent of their age. Due to significant amelioration of endothelial function when Quinapril is used, it becomes possible to reduce morbidity and mortality rates in this population.

Publications

1. **Effect of Quinpril on Indices of Myocardial Perfusion Scintigraphy in Type 2 Diabetes Mellitus.** " ANNALS OF BIOMEDICAL RESEARCH AND EDUCATION" № 6 (1) Jauary/March 2006 86- 89 (Co-authors: N. Kipshidze, T. Todua, T. Vakhtangadze).
2. **Assessment of the Improvement of Myocardial Perfusion According to Some Markers of Endothelial Function in Patients with Type 2 Diabetes Mellitus. "** Cardiologe and Internal Medicine XXI ". № 2 (XIV) 2006 page 30 -34.
3. **Myocardial Perfusion Scintigraphy Pattern in Patients with Type 2 Diabetes Mellitus.**
   "Georgian Journal of Radiology". № 2-3 (25) 2006 page 33-36 (Co-authors: N. Kipshidze, F.todua, T. Vakhtangadze, M. Razmadze.).(in Georg).