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Lower limb perfusion scintigraphy with 99mTc-MIBI scintigraphy and determination of endothelin in diabetic and nondiabetic patients

Perfuziona scintigrafija donjih ekstremiteta sa 99mTc-MIBI i određivanje endotelina kod bolesnika sa dijabetesom melitusom i kod zdravih ispitanika

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Abstract

Background/Aim. Peripheral artery disease (PAD) is a common macrovascular complication in patients with diabetes mellitus (DM) as a result of impairment of homeostatic mechanisms of the endothelium, thus initiating the process of atherosclerosis. The imbalance between endothelium-derived vasodilators and vasoconstrictors plays an important role in the pathogenesis of diabetic microangiopathy, as well as in other vascular complications in diabetes. Perfusion scintigraphy using technetium-99m-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) can be very useful method for evaluation of the lower limbs muscle perfusion. The aim of this study was: to compare the results of dynamic and static studies of lower limbs tissue muscle perfusion scintigraphy with ^{99m}Tc-MIBI (one-day rest-stress protocol) in patients with and without DM and to determine the perfusion reserve for diagnostic evaluation of PAD in patients with DM type 2, as well as to assess the endothelin-1 (ET-1) levels as a vasoconstrictor agent in patients with and without diabetes. **Methods.** Prospective study was performed in 90 patients, divided into two groups according to the presence of DM – patients with DM type 2 (DP), 60/90 (67%), and patients without DM (NDP), 30/90 (33%). Lower limbs tissue muscle perfusion scintigraphy was done with ^{99m}Tc-MIBI including two studies ("rest" and "stress"). **Results.** In the DP group significantly lower pick of radioactivity was detected in comparison with the NDP group, in both phases (rest and stress), for both calves. Lower counts from the static phase were registered in the region of both calves. Lower inter-extremity indexes as well as perfusion reserve were found in the DP group. There was a significant difference in concentrations of ET-1 between groups (higher concentrations were found in tzhe DP group). **Conclusion.** This one-day protocol (rest-stress with ^{99m}Tc-MIBI) of perfusion scintigraphy of lower limbs is considered a useful procedure in PAD assessment in patients with DM type 2, especially the asymptomatic form.

Key words:
diabetes mellitus, type 2; periferal arterial disease; perfusion imaging; lower extremity; endothelin-1.

Apstrakt

Uvod/Cilj. Bolest perifernih arterija predstavlja makrovaskularnu komplikaciju dijabetesa melitusa (DM) koja nastaje kao zbog poremećaja homeostatskih mehanizama endotelijuma i kojom započinje proces arterioskleroze. Poremećaj ravnoteže vazodilatatora endotelnog porekla i vazokonstriktora ima veliku ulogu u patogenezi dijabetičke mikroangiopatije, kao i ostalih vaskularnih komplikacija dijabetesa. Cilj rada je bio da se uporede rezultati dinamičkih i statičkih studija perfizione scintigrafije donjih ekstremiteta sa tehnecijum-99^{m}-metoksiizobutil izonitrilom (99^{m}-Tc–MIBI) (jednodnevni stres/oporavak test) kod osoba sa DM i kod zdravih ispitanika u cilju određivanja perfuzine rezerve u okviru dijagnostičke evaluacije bolesti perifernih arterija kod osoba sa DM tip 2, kao i da se odredi nivo endotelina-1 (ET-1) kao vazokonstriktora kod osoba sa i bez DM. **Metode.** Prospektivnom studijom obuhvaćeno je 90 ispitanika podeljenih u dve grupe prema prisustvu (DP)/odsustvu DM tip 2 (NDP). DP grupu sačinjavalo je 60/90 (67%) bolesnika sa DM tip 2, dok je u grupi NDP bilo 30/90 (33%) ispitanika bez DM tip 2. Perfuziona scintigrafija sa ^{99m}Tc-MIBI mišićnog tkiva donjih ekstremiteta sprovedena je u fazi odmora i fazi stresa. **Rezultati**. U DP grupi ustanovljen je značajno niži pik radioaktivosti u odnosu na NDP grupu ispitanika obostrano i u obe faze sa nižim *inter-extremity* indeksima i sniženom perfuzionom rezervom. Utvrđena je značajna razlika u koncentraciji ET-1 između grupa (veća koncentracija je zabeležena u DP grupi). **Zaključak.** Prikazani jednodnevni protokol perfizione scintigrafije donjih ek-

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stremiteta u fazi odmora i napora je korisna procedura u proceni bolesti perifernih arterija bolesnika sa DM tip 2, naročito u asimptomatskoj formi bolesti.

Ključne reči:
dijabetes melitus, insulin nezavisni; bolest perifernih arterija; perfuziono snimanje; noga; endotelin-1.

Introduction

Peripheral artery disease (PAD) is a common macrovascular complication in patients with diabetes mellitus (DM) as a result of impairment of homeostatic mechanisms of the endothelium, thus initiating the process of atherosclerosis. The normal, healthy endothelium regulates vascular tone and structure and exerts anticoagulant, antiplatelet, and fibrinolytic properties. The maintenance of vascular tone is accomplished by the release of numerous dilator and constrictor substances. A major vasodilative substance released by the endothelium is nitric oxide (NO), originally identified as endothelium-derived relaxing factor (EDRF). The endothelium also produces vasoconstrictor substances, such as endothelin-1 (ET-1) (the most potent endogenous vasoconstrictor identified to date) and angiotensin II. Angiotensin II not only acts as a vasoconstrictor but also as pro-oxidant, and stimulates production of ET-1. ET-1 and angiotensin II promote proliferation of smooth muscle cells and thereby contribute to the formation of atherosclerotic plaque. Activated macrophages and vascular smooth muscle cells, characteristic cellular components of atherosclerotic plaque, produce large amounts of ET-1 [1].

The imbalance between endothelium-derived vasodilators and vasoconstrictors initiates a number of events/processes that promote or exacerbate atherosclerosis. They include increased endothelial permeability, platelet aggregation, leukocyte adhesion, and generation of cytokines. Decreased production or activity of NO, manifested as impaired vasodilation, and increased production of ET, may be one of the earliest signs of atherosclerosis. All these processes play an important role in the pathogenesis of diabetic microangiopathy, as well as in other vascular complications in diabetes [2, 3]. Development of endothelial dysfunction involves several biological mediators including increased expression of ET-1 and altered expression of ET receptors [4]. Increased endothelial ET-1 expression enhances lipid biosynthesis and accelerates the progression of atherosclerosis.

There are a number of diagnostic procedures that, according to the accepted protocols for this vasculopathy, are successively involved in different levels of diagnosis. Despite good anatomic information for the large arteries provided by computed angiography, it is insufficient for the small vessels perfusion [5]. Perfusion scintigraphy using technetium-^{99m}-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) can be very useful for evaluation of the lower limbs muscle perfusion. After intravenous application, ^{99m}Tc-MIBI is rapidly cleared from the circulation and preferentially is accumulated in muscular tissues (including heart) proportionally to regional blood flow [6, 7]. These characteristics of ^{99m}Tc-MIBI make it very suitable for examining regional blood flow, visualization with gamma camera, as well as getting quantitative parameters for regional blood flow changes, including quantitative assessment of tissue perfusion in basal conditions (rest study) and after workload (stress study).

The aim of this work was to compare the results of dynamic and static studies of lower limbs tissue muscle perfusion scintigraphy (TMPS) with ^{99m}Tc-MIBI (one-day rest-stress protocol) in patients with and without DM type 2 and to determine the perfusion reserve for diagnostic evaluation of PAD in patients with DM type 2. Also, the aim was to assess differences of ET-1 levels between two groups of patients (with and without diabetes).

Methods

TMPS was performed through one-day rest-stress protocol with ^{99m}Tc-MIBI. The study was approved by the Ethics Committee and all subjects signed double informed consent form. This was a prospective study performed in 90 patients, divided into two groups according to the presence of DM type 2 – patients with DM (DP) 60/90 (67%), and patients without DM (NDP) 30/90 (33%). In the NDP group, 10 (33.33%) patients had hypertension (HTA), 8 (26.67%) were obese, 7 (23.33%) had hyperlipidemia (HLP) and 6 (20%) were smokers. Analyzing the symptoms, 18 (60%) had calf pain, 11 (36.67%) complained of numbness, and 7 (23.33%) had cold lower extremities. In the DP group 44 (73.33%) had HTA, 26 (43.33%) HLP, 20 (33.33%) were smokers, 50% were obese, 48 (80%) had calf pain, 34 (56.67%) had numbness and 24 (40%) complained of cold legs.

^{99m}Tc-MIBI scintigraphy

Lower limbs TMPS with ^{99m}Tc-MIBI is a non-invasive, functional method that evaluates tissue perfusion in resting condition (rest study) and after workload (stress study), as visually as well as through several quantitative parameters.

Tissue muscle perfusion studies were done with planar technique, with two-headed gama camera (DHV MEDISO Nucline SPIRIT), low energy high resolution collimator (LEHR). Before the initiation of the rest study the patient was positioned in resting mode for 20–30 minutes (separate isolated room was used to avoid external influence and the patients were instructed to remain in a horizontal position during this period of resting mode). The rest study was started with a dynamic phase of tissue-muscle vascularization of both calves after iv. application of 300 MBq of ^{99m}Tc-MIBI, (the rest study time interval was 7 minutes, consisted of 28 frames, with time interval 15s per frame) (Figure 1), followed with a whole body scan (WBS) for tissue perfusion of the whole body in posterior-anterior (PA) position, matrix size 512 × 1024 × 16, speed 15 cm/min.

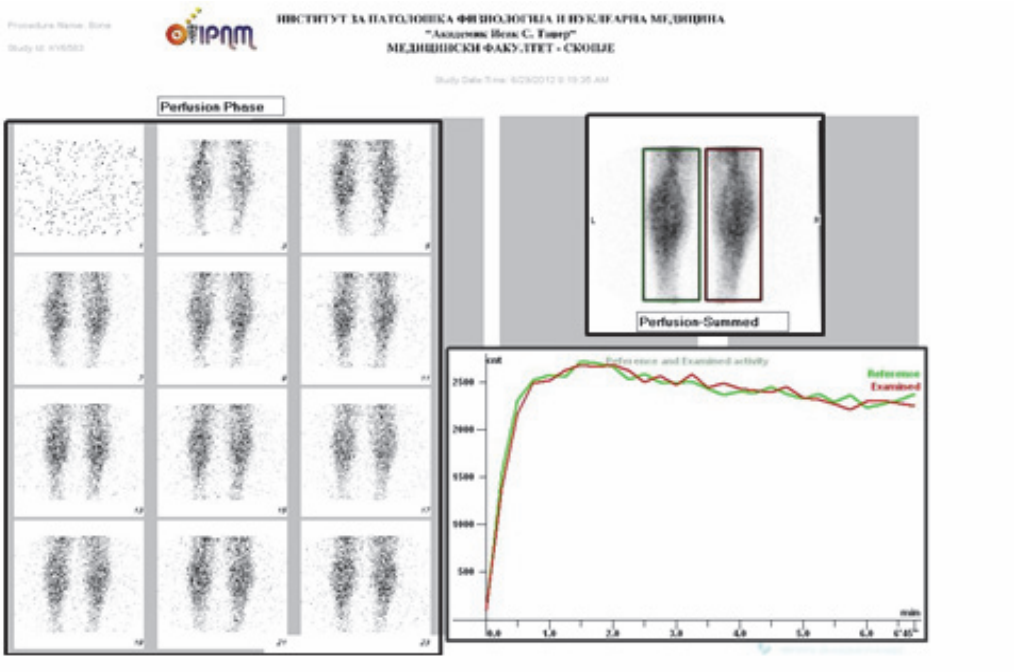

Fig. 1 – Dynamic phase of both calves in the rest study.

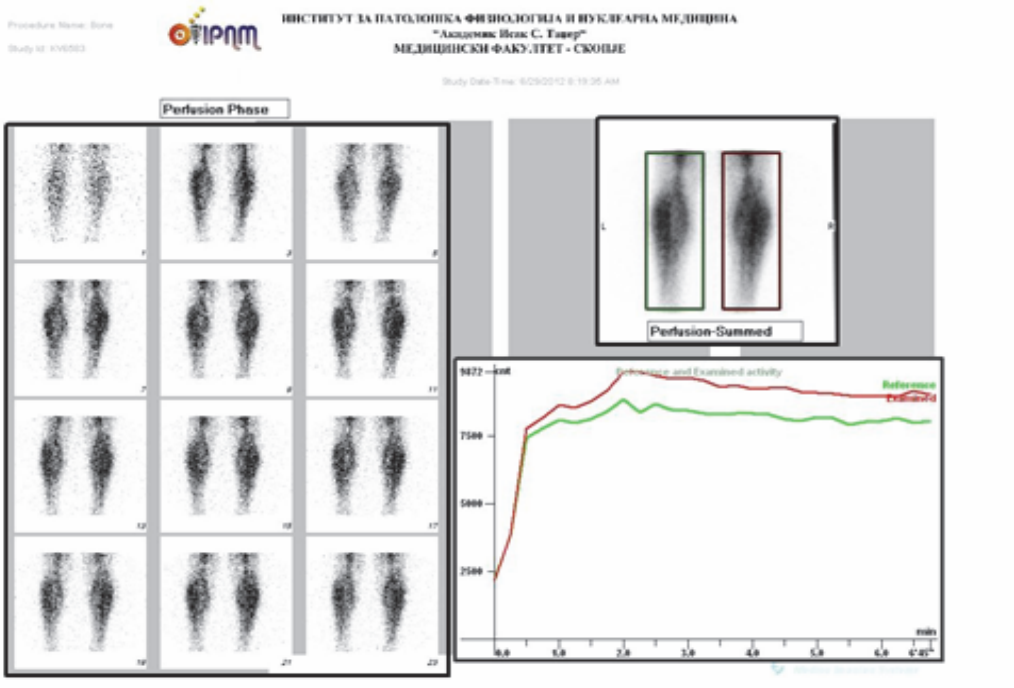

Fig. 2 – Dynamic phase of both calves in the stress study.

The stress study was carried out afterwards and the patient was instructed to perform 30 flexion/extensions of both feet, followed by iv. application of 600 MBq ^{99m}Tc-MIBI, when the dynamic phase was started with the same acquisition protocol as in the rest study (Figure 2). After application of the radiopharmaceutical, the patient performed another 30 flexion/extension of the feet. WBS was performed afterwards (with the same aquistion as in the rest study) (Figure 3).

With quantitative analyses of the dynamic phase, radioactive curves were constructed in a time manner (time activity curve – TAC) above the region of interest (Figure 2), positioned above both calves and these parameters were investigated: T maximum (Tmax) – time of maximal uptake of the tracer in each calf and impulses collected in Tmax; radioactivity in 1st minute in calves – (radioactivity above calf in 1st minute) × 100 / maximal radioactivity above calf.

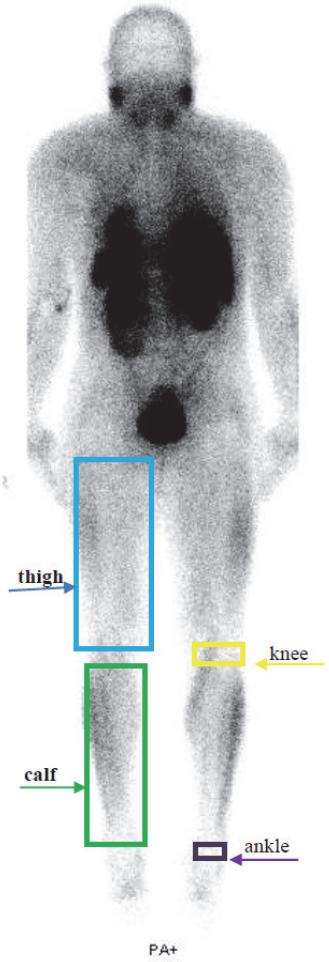

Fig. 3 – Whole body scan in the stress study.

With quantitative analyses of WBS, with registered impulses in the ROI, positioned over calves and the whole body these indices were evaluated: radioactivity in calves – accumulated impulses in both calves in both studies, after drawing symmetrical ROI (Figure 3); intra-extremity index – (for both studies) left calf/left ankle (LC/LA) and right calf/right ankle (RC/RA); index calf/whole body (for both studies) – left calf/whole body and right calf/whole body; perfusion reserve (PR %) for both calves – as a percent of grow of the tissue blood flow in stress study, in comparison with the rest study, was calculated with the formula:

$$PR\ (\%) = \frac{\text{(radioactivity in calf in stress} - \text{radioactivity in calf in rest)}}{\text{radioactivity in calf in rest}} \times 100\%$$

Endothelin-1 measurements

For the determination of ET-1 in our study we used a commercial RIA by the manufacturer Phoenix Pharmaceuticals, Inc. After blood withdrawal, the samples were centrifuged and the serum was stored in a refrigerator at -20°C until analyses performed simultaneously for all samples. ET-1 measurements were taken by a competitive radioimmunoassay. The method is based on a competitive reaction of the analyte (ET-1 in the test sample) and the radiolabelled endothelin (^{125}I-endothelin) in the kit, for the limited amount of antibody-specific antibodies in each of the test tubes. According to the competitive conditions, there is an inverse correlation of the bound radioactivity in the formed immune complex and the concentration of the analyte ET-1. The procedure for the determination of ET-1 was carried out in accordance with the conditions and protocol prescribed by manufacturer.

Results

The DP group had significantly lower pick of radioactivity detected in the dynamic phase in comparison with the control group, in both studies (rest and stress) for both calves (Table 1). The number of impulses in the 1st minute for both calves was also significantly lower in the DP group in both studies, as well (Table 2).

Table 1
Number of impulses accumulated at the peak of radioactivity for both calves

Peak of radioactivity	Group	Rest	Stress
		mean ± SD	mean ± SD
Tmax RC	DP	2,158.75 ± 410.6	7,223.62 ± 1,383.4
	NDP	2,427.40 ± 278.8	8,019.47 ± 946.3
p-value		0.0018**	0.0057**
Tmax LC	DP	2,234.75 ± 423.7	7,240.07 ± 1,673.8
	NDP	2,445.43 ± 384.1	7,995.53 ± 1,098.3
p-value		0.024*	0.028*

RC – right calf; LC – left calf; DP – diabetic patients; NDP – nondiabetic patients; SD – standard deviation. *$p < 0.05$, **$p < 0.01$ (Student's *t*-test for independent samples).

Table 2
Number of counts accumulated in the 1st minute of dynamic phase

T1min	Group	Rest	Stress
		mean ± SD	mean ± SD
RC	DP	1,949.32 ± 404.9	6,752.88 ± 1,248.6
	NDP	2,230.87 ± 284.4	7,671.73 ± 978.1
	p-value	0.001**	0.00068**
LC	DP	2,048.45 ± 435.1	6,924.87 ± 1,314.9
	NDP	2,248.6 ± 442.1	7,646.87 ± 1,080.5
	p-value	0.044*	0.011*

RC – right calf; LC – left calf; DP – diabetic patients; NDP – nondiabetic patients; SD – standard deviation. *$p < 0.05$, **$p < 0.01$ (Student's *t*-test for independent samples).

The accumulated counts in the region of both calves was insignificantly lower in the DP group compared to the NDP group in the rest study and significantly lower in the stress study ($p = 0.018$). The counts accumulated in the rest study were for LC 16,967.78 ± 3,520.9 in the DP group vs. 17,726.83 ± 3,285.3 in the NDP group, while for RC they were 17,228.07 ± 4,287.5 in diabetic patients vs. 17,772.87 ± 3,242.2 in nondiabetic ones.

Table 3
Intra-extremity index for both calves in both studies (rest and stress)

Variable	Group	Rest		Stress	
		mean ± SD	median	mean ± SD	median
LC/LA	DP	82.17 ± 23.72	74.47	368.16 ± 110.6	356.5
	NDP	82.79 ± 23.31	88.09	389.06 ± 110.1	399.8
	p-value	0.7 (ns)		0.2 (ns)	
RC/RA	DP	84.48 ± 29.09	79.86	368.91 ± 111.9	346.6
	NDP	81.65 ± 19.08	83.23	385.46 ± 104.8	374.6
	p-value	0.86 (ns)		0.43 (ns)	

LC/LA – left calf/left ankle; RC/RA – right calf/right ankle; DP – diabetic patients; NDP – nondiabetic patients.
ns – non-significant (Mann-Whitney test).

In the stress study total counts for the LC these values were 75,546.95 ± 15,864.5 in the DP group, 84,098.9 ± 19,954.7 in the NDP group and for the RC values were 75,059.9 ± 14,851.9 in the DP group and 83,972.8 ± 19,489.8 in the NDP group.

Median for intra-extremity index of the left and right calf was lower in diabetic patients vs. nondiabetic ones, without significance (Table 3). Non-significant differences in indices of calf/whole body were registered in both studies for both calves (Table 4).

Table 4
Index calf/whole body

Variable	Group	Rest	Stress
		mean ± SD	mean ± SD
LC/WB	DM	1.98 ± 0.4	3 ± 0.5
	NDP	1.78 ± 0.3	3.05 ± 0.6
	p-value	0.23 (ns)	0.69 (ns)
RC/WB	DM	2 ± 0.4	2.98 ± 0.5
	NDP	1.8 ± 0.3	3.05 ± 0.6
	p-value	0.24 (ns)	0.55 (ns)

LC/WB – left calf/whole body; RC/WB – right calf/whole body; DP – diabetic patients; NDP – nondiabetic patients; SD – standard deviation.
ns – non-significant (Mann-Whitney test).

Perfusion reserve (PR) of calves (LC, RC) was calculated with the formula: (ROI stress-ROI rest) × 100% / ROI rest. The results showed insignificantly lower PR of LC in diabetic patients compared to nondiabetic ones (40.25 ± 14.7 vs. 44.77 ± 10.3, respectively; $p = 0.32$). Significant difference in PR of RC was registered in diabetic patients in relation to nondibaetic ones (40.02 ± 11.2 vs. 44.53 ± 10.5 in nondiabetic ones, respectively; $p = 0.045$).

There was a significant difference in concentrations of ET-1 between groups (higher concentrations were found in diabetic patients) (Table 5).

Diabetes mellitus is a chronic disease caused by impaired insulin secretion or insulin resistance. Peripheral arterial disease in diabetes is a consequence of an atherogenic process in the lower limb arteries accelerated by multifactorial pathophysiologic mechanisms underlying DM. This process is accompanied also with atherotrombosis in vasculature of other organs including coronary and cerebrovascular system. Having in mind all complications arising from this pathological condition it is of great clinical significance to recognize the early abnormalities in the peripheral circulation. The precise assessment of the prevalence of PAD in diabetic patients is aggravated by the high prevalence of asymptomatic forms, peripheral neuropathy, and the absence/impared function of pain perception, as well as the present limitation of screening methods for its diagnosis. Therefore, in the resolution of asymptomatic and subclinical forms of PAD in these patients, both preventive and diagnostic and curative medical procedures should always be included.

Table 5
Endotelin-1 concentration (pg/mL)

DP	NDP	*p*
mean ± SD	mean ± SD	
105.22 ± 8.8	98.58 ± 8.6	0.042*

DP – diabetic patients; NDP – nondiabetic patient; SD – standard deviation.
***$p < 0.05$ (Student's *t*-test for independent samples).**

Discussion

For this purpose in nuclear medicine ^{99m}Tc-labelled perfusion tracers are used to provide better image quality as well as quantitative processing of the scans. Radiopharmaceutical that was used in our study, ^{99m}Tc-MIBI, is a lipophilic cationic component that injected into animals is distributed into the tissues proportionally to blood flow and is retained in the mitochondria. Given the negative plasma membrane potential and even more negative mitochondrial membrane potential, both potentials contribute to a strong driving force for ^{99m}Tc-MIBI accumulation and sequestration in the mitochondrial matrix. Studies showing that cultured myocardial cells accumulate ^{99m}Tc-MIBI 1,000 times more in mitochondria than in the cytosol, have contributed to its wide application in the field of nuclear cardiology [8]. Biodistribution and kinetics of the ^{99m}Tc-detected components allowed combining myocardial perfusion with perfusion of the lower limbs.

The results from our study clearly pointed to abnormal microvascular perfusion in the affected regions of lower limbs, while the quantification of the tested parameters indicated the extent of perfusion insufficiency. Lower number of

accumulated counts was detected in both calves for both phases in the diabetic patients. In the rest phase of the left calf, total count number was 75,546.95 ± 15,864.5 in the DP group, and 84,098.9 ± 19,954.7 in the NDP group. For the right calf the total count number was 75,059.9 ± 14,851.9, and 83,972.8 ± 19,489.8, respectively for both groups. Still significant decrease of the counts was registered in the stress phase only, due to reactive hyperemia. This is a state when under resting conditions, the limb uses all possible resources for blood supply and self-protection from ischemic consequences, such as collateral circulation and vasodilator response under the action of stimuli that are excreted in response to hypoxia or steel phenomenon. However, under loading conditions it is unable to raise the blood flow to a higher level in order to provide an appropriate metabolic response to the effort.

Perfusion of the lower extremities was also performed in the study of Taillefer [9] in 35 patients using method of post-occlusive reactive hyperemia and resting state. Regions of interest over both thighs and calves were drawn in PA position of imaging, and afterwards inter- and intra-extremity index were calculated. Paradoxically, larger uptake showed muscle blood supply from significantly stenosed blood vessels, which resulted in false positive and false negative results.

In 2001, Cosson et al. [10] investigated by thallium-201 scanning circulation in the muscles of the lower limb in diabetic patients without clinical peripheral vascular disease but with a high cardiovascular risk profile and suggested that scanning of the lower limbs coupled with myocardial scintigraphy is a convenient method of investigating peripheral muscle circulation. They found muscle perfusion defects in 42% of the patients, mainly in the calves.

Significantly lower PR of diabetic patients (without peripheral artery disease) versus the control group (without DM), 70.2 ± 10.7% and 98.6 ± 9.4%, respectively were registered in 2004 by Lin et al. [11]. They used method of 60 plantar and dorsal flexions of the right foot and calculated the perfusion reserve by the formula PR = (ROI right foot – left foot)/ROI (right foot) ×100%.

Lower extremity ischemic disease assessed by thallium-201 was also used by Cizmic et al. [12] in evaluation of diabetic angiopathy. Their results of lower extremities perfusion scintigraphy showed reliable indices of muscle microcirculatory perfusion, with statistically significant correlation between the Doppler hemodynamic indices and thallium-201 perfusion scintigraphy.

Younes et al. [13], in 2017, performed 30–40 dorsoplantar flexions and extensions of the right foot in sitting position and afterwards ROI were drawn over both calves. Using the formula: PR = Stress (right foot) – Rest (left foot)/ROI (left foot) × 100%, significantly lower PR was detected in patients with PAD vs. the control group (28.4 ± 20.3% vs. 65.0 ± 11.4%, respectively; $p < 0.001$).

Perfusion muscle scintigraphy of lower limbs can help in the algorithm for starting using more invasive diagnostic methods such as angiography. In 2007, Soyer and Uslu [14] published a case of a patient with intermittent claudication in one leg, a preserved circulation evaluated by the Doppler technique, a striking reduction in perfusion in the stress phase recorded with ^{99m}Tc-MIBI muscular scintigraphy and the detection of multiple stenosis with peripheral arterial angiography. Additionally, through the visual analysis of the scans it is possible to locate regions with impaired microvascular circulation, which would contributed to the appropriate therapeutic modalities.

In our case report of diabetic patient in 2016 we performed TMPS and confirmed diabetic angiopathy in both calves, with a borderline value for perfusion reserve of the left calf – 57%, and a lower perfusion reserve of the right calf – 42% (reference values 50–80%) [15].

Tan et al. [16] used two-day protocol of ^{99m}Tc-MIBI TMPS in patients with Behcet disease, using pharmacologic stress plus adding 30 plantar flexions and extensions of the feet. PR was calculated with the formula: PR % = (ROI stress-ROI rest)/ ROI rest) × 100%. They got significantly lower PR in the control group -3.34 ± 8.7%, vs. 8.6 ± 8.5%.

The detection of PR with the method of TMPS was used in patients with rheumatoid arthritis, as a screening tool in the evaluation of the atherosclerotic process by Amin et al. [17] in 2012. Higher PR were noticed in the control group vs. patients with RA (48.3 ± 27.2% vs. 30.7 ± 22.6%, respectively; $p = 0.015$).

The concentrations of ET-1 showed significant higher mean values in the group of diabetic patients vs. the control group, which is consistent with pathogenetic mechanisms of the ET-1 involvement in the onset of microangiopathy. In that context, in several studies it was found that vascular endothelial dysfunction may precede DM type 2, implying that elevated levels of ET-1 can partly be included in development of the metabolic syndrome, mainly through reduction of insulin sensitivity. Considering conducted studies, it was found that ET-1 increases the production of reactive oxygen species (mainly superoxide anions) and thus contributes to the endothelial activation and consecutive endothelial dysfunction in vascular endothelial cells as the main place of ET-1 production. Also, increased circulating levels of ET-1 may promote the initiation and progression of atherosclerosis by inhibiting endogenous NO production in vascular smooth muscle cells (VSMCs), through its inhibitory effect on endothelial nitric oxide synthase (eNOS), and additionally contribute to the development of microcirculatory disorders [18–20].

Conclusion

This one-day protocol (rest-stress with ^{99m}Tc-MIBI) of perfusion scintigraphy of lower limbs is considered a useful procedure in PAD assessment, especially the asymptomatic form, in patients with DM type 2. The investigation of the functional haemodynamic parameters are important for relevant guidance, treatment and risk stratification of these patients with PAD.

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