Histopathological and Histochemical Changes of the Popliteal Artery in Diabetic Patients

Payman Jamal Muhamad Ameen (Ph.D.)[1], **Talib Jawad Kazim** (Ph.D.)[2] **and Hewa Baker** (Ph.D.)[3]

Abstract

Back ground: A lot of previous researches studded the endoneural microangiopathy and small vessels diabetic pathological change, but little studded about the diabetic angiopathy of the larger vessels like popliteal artery and related diabetic risk factors .Accordingly the present work designed to study the structural and histochemical changes of the popliteal artery as one of the medium size vessels.

Objective: Evaluation of the histopathological and histochemical measurement, of the popliteal artery in diabetic male and female patients with different duration of the diseases, in comparison with normal non diabetic samples.

Patient and methods: Histological sections, taken from amputated legs of 30 diabetic patients (8 females and 22 males) at age of (55-75) years, and 30 cadavers (4 females and 26 males) at age of (25-50) years as control group.

Results: Histological sections from the popliteal artery of diabetic patients, showed marked decrease in the diameter of the lumen, reduced thickness of tunica media and adventitia, but increase in the thickness of tunica intimae and basement membrane. The loss of endothelium in some places, cellular infiltrations in tunica media with Periodic Acid Schiff positive particles, and in more advance cases calcifications, and organized thrombus was also detected. The multiple regression analysis, showed significant effect of duration of diabetes on the diameter of the lumen, and thickness of the wall and the tunica intima and media of the popliteal artery**.**

Recommendation: Great vessels of diabetic patients, recommended to be examined by the available imaging technique.

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[1] Department of Anatomy - Collage of Medicine - Howler Medical University – Erbil - Iraq.

[2] Department of Anatomy - Collage of Medicine - Diyala University - Diyala - Iraq.

[3] Department of Anatomy- Collage of Medicine - Howler Medical University – Erbil - Iraq.

Introduction

It was described by Brownlee [1]. That diabetes-specific micro vascular disease is a leading cause of blindness, renal failure and nerve damage, and diabetes-accelerated atherosclerosis which leads to increased risk of myocardial infarction, stroke and limb amputation.

Al-Maskari and El-Sadig [2]. Stated that, the most common complications of diabetes affecting the lower limbs may resulted from diabetic neuropathy. Many patients may develop combinations of neuropathies concerning somatic and autonomic system [3].

Diabetic patients without evidence of neuropathy demonstrate endoneural microangiopathy; therefore this may proceed to the development of peripheral neuropathy [4]. Similar finding was obtained by Krishnan and Kiernan [5]. In that their pathophysiological and pathological studies of diabetic neuropathy have suggested an ischemic basis which is possibly related to microangiopathy.

Many previous researches [1]. And Krishnan and Kiernan [5]. Considered endoneural microangiopathy and small vessels diabetic pathological changes, but little about the greater vessels like popliteal artery and about the related diabetic risk factors.

The Aim of the study:

This study is carried out to:-

1- Study the angiopathic, histological and histochemical changes of the popliteal artery in diabetic patients.

2. Evaluate multiple risk factors for diabetic structural changes in the popliteal artery

Patients and Methods

Patients:

Twenty two mail, and eight females patients intended to the surgical theater for amputation of their legs (suffered from gangrene in their foot); samples of popliteal artery taken from amputated legs of (30) diabetic patients (22 male and 8 female at age ranging between 55–75 years) who were subjected to the amputation in the surgical theater of different hospitals in Erbil city. Other samples taken from (30) cadavers (4 females and 26 males, their age varying between 25–50 years) newly imported to the forensic medicine as control group. Samples taken were one cm length of the popliteal artery from the proximal healthy part of the amputated extremities of the patients, and identical sample taken from lower extremities of the cadavers. Patients and cadavers with previous history of neurovascular disease were excluded from the study. The limitation in this study was the un identity and difference between the ages of the corresponding diabetic and control groups of the arterial samples because of the in availability of samples at the same age.

Dissection

In the theater; the amputated leg was taken to a side room, put down in prone position on the dissecting table, at the superior border of the popliteal fossa the skin incised down to posterior of the leg.. By blunt dissection, popliteal fat removed from the fossa, with a probe and forceps then the remnant of the deep fascia was removed to expose the medial and lateral head of gastrocnemius. The popliteal artery and the vein are located deep to the tibial nerve enclosed by a common connective tissue sheath. A small scissor was used to open the sheath and the incision extended superiorly and inferiorly, separated from the vein and one cm length was taken from the artery for histological study.

Samples of popliteal artery transferred into formal saline solution (100 ml of 40 percent formaldehyde, nine grams of Sodium chloride and 900 ml tap water), fixed for about 24 hours, processed automatically using (Sakura Automatic Tissue Processor made in US) passing through the standard steps of dehydration, clearing and wax impregnation. After 24 hour the samples embedded in molten paraffin wax. Blocks then made available for microtome using (Leitz rotatory microtome). Sections of the tissues were cut at five micrometer to be stained with, Haematoxyline and eosin (H & E), and PAS (periodic acid Schiff special stain) [6]. The measured parameters from the popliteal artery were: total vessel diameter (TVD), lumen diameter (LD), wall thickness (WT), tunica intima thickness (IT), tunica media thickness (MT), Tunica

adventitia thickness (AT), endothelial basement membrane thickness (BMT), cellular infiltrations: (lymphocyte, polymorph, fibroblast and histiocytes), calcifications, organized thrombus and PAS positive particles.

Statistical Analysis

Statistical analysis was made using statistical package for social sciences (SPSS) computer software version 15. The following tests were used: ANOVA test, independent samples t test, simple linear correlation and multiple regression analysis. A p value of ≤ 0.05 was considered as statistically significant.

Results

The results of the present study express significant effect of diabetes on the structure and metrical measurement of the of popliteal artery in that, the means of the whole diameter of the diabetic group showed marked decreases comparisons to that of the control group (figure 1,2) (diagram 1, table 1), decreases in the total wall thickness in responses to age and diabetic duration (table1, 3, figure 3), decreases of the mean diameter of the lumen and the thickness of tunica media (P value 0.02) (Table:1,5, figure: 3), decreases of mean of the luminal diameter(figure :4), tunica media (table 1, 5 , figure:3, 6),and tunica adventitia (table:1,6, figure :4-5) (P value < 0.001) the tunica intima showed thinking (table 1,4,)(P value< :0.001)and increases in the basement membrane (P value< :0.002) (figure 4-5).

Many other histological changes observed; the tunica media showed Cellular infiltrations, including lymphocytes, polymorphs, histiocytes and fibroblasts were detected in the histological sections for the diabetic group using PAS stain as shown in (Figure 6).

Diabetic group showed multiple thrombus in the lumen and calcifications in the wall of the popliteal artery of diabetic patients, however they were not measured morph metrically or by numbers (Figure 7).

Table (1): The means of the variables in micrometer among diabetic and control groups.

Group		N	Mean ± SD	95% confidence interval of the difference	P
TVD	**Diabetic** **Control**	30 30	655.13 ±215.54 1553.33 ±199.33	-1005.50 to -790.91	< 0.001
LD	**Diabetic** **Control**	30 30	283.10 ±121.73 732.33 ±138.71	-516.67 to -381.78	< 0.001
WT	**Diabetic** **Control**	30 30	227.13 ±104.44 483.33 ±107.75	-311.04 to -201.36	< 0.001
IT	**Diabetic** **Control**	30 30	19.37 ±10.58 14.33 ±5.04	0.75 to 9.32	0.02
MT	**Diabetic** **Control**	30 30	140.57 ±96.05 376.67 ±74.76	-280.58 to -191.62	< 0.001
AT	**Diabetic** **Control**	30 30	75.33 ±48.04 100.33 ±101.66	-66.09 to 16.09	0.23
BMT	**Diabetic** **control**	30 30	9.47 ±3.25 2.00 ±0.00	6.26 to 8.68	< 0.001

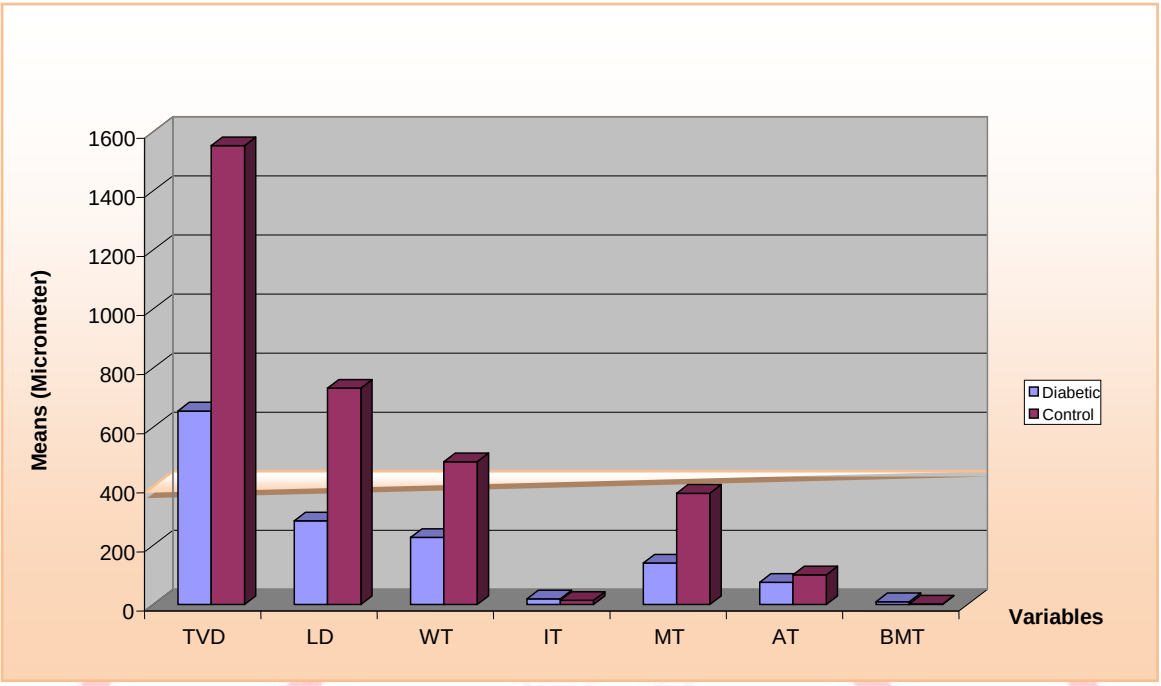

Diagram (1): Means of variables among the study groups

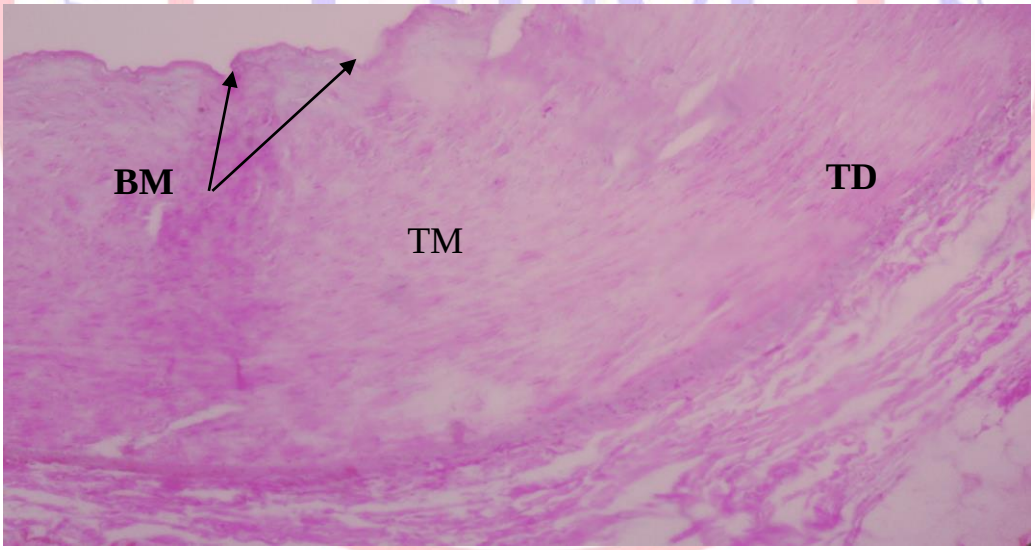

Figure (1): Section through the wall of popliteal artery (PAS staining) showing normal thickness of the wall (BM=basement membrane, TM=Tunica media, TD=Tunica advantetia). X 100**.**

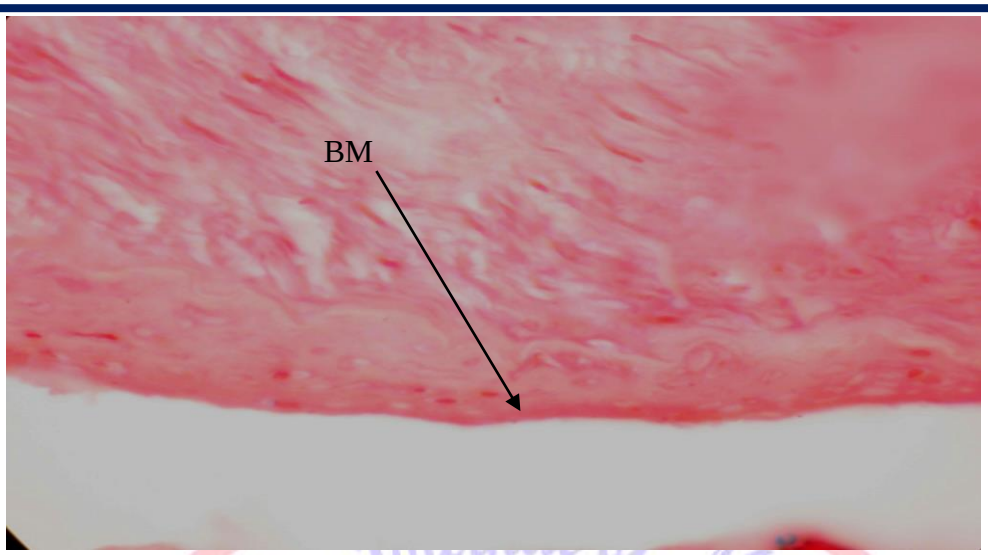

Figure (2): Section through the wall of popliteal artery from control specimen showing normal architecture. PAS X 1000.

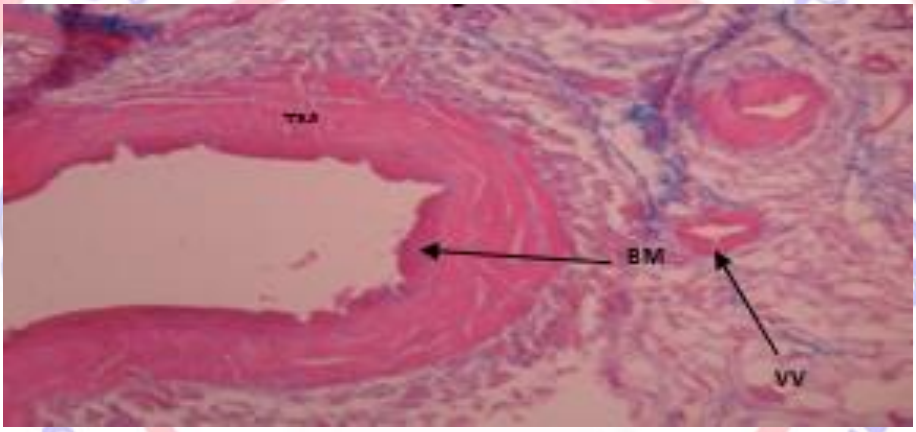

Figure (3): Section through the wall of popliteal artery from diabetic patient ((reduced thickness of T media ™ and thickened endothelial basement membrane (BM) (VV=Vasa vasorun). PASX

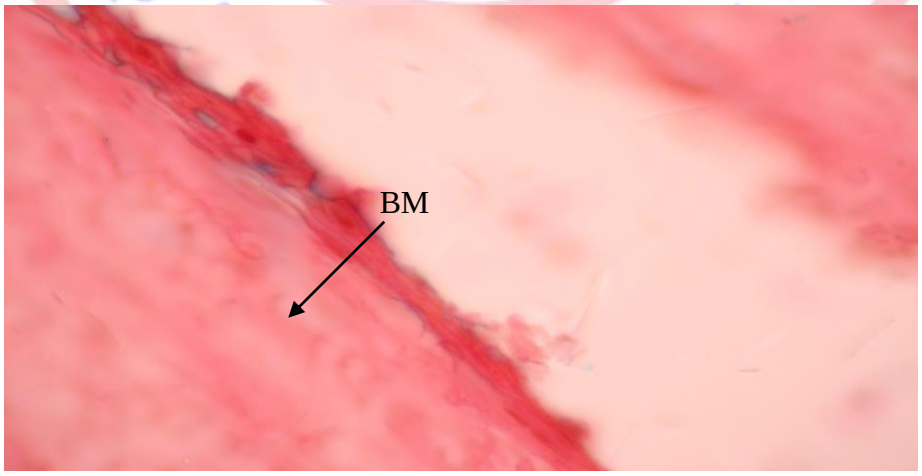

Figure (4): Section through the wall of popliteal artery from diabetic specimen showing

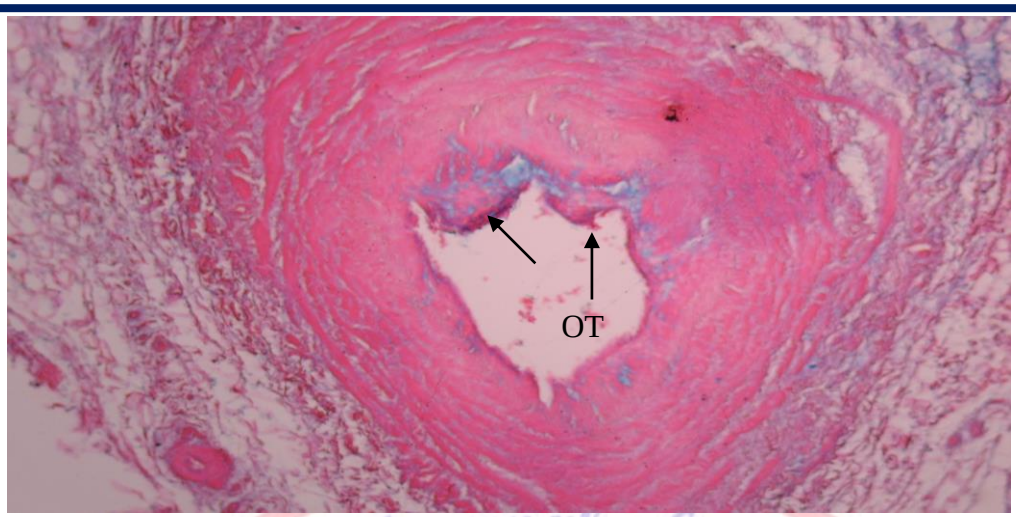

Figure (5): Section from popliteal artery of a diabetic specimen showing organized thrombus (OT) and decreases in the luminal diameter. Stained with PAS X 100.

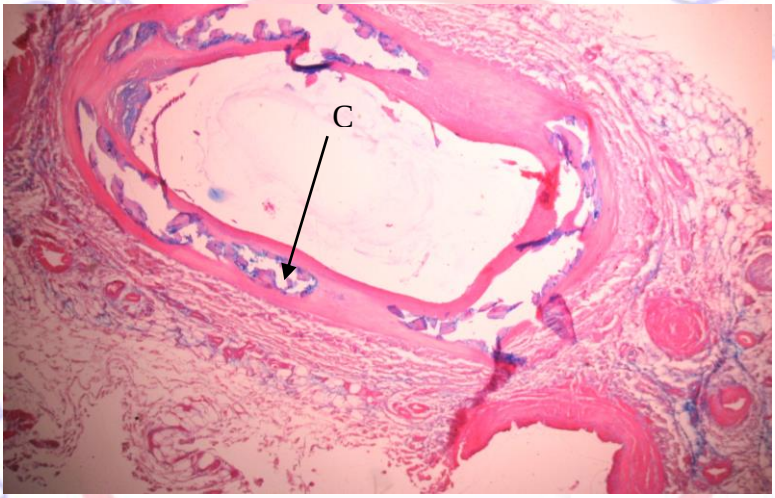

Figure (6): Cross section through popliteal artery of a diabetic specimen showing thickened marked calcification (C) area in T. media of the vessel. Stained with PAS X 40.

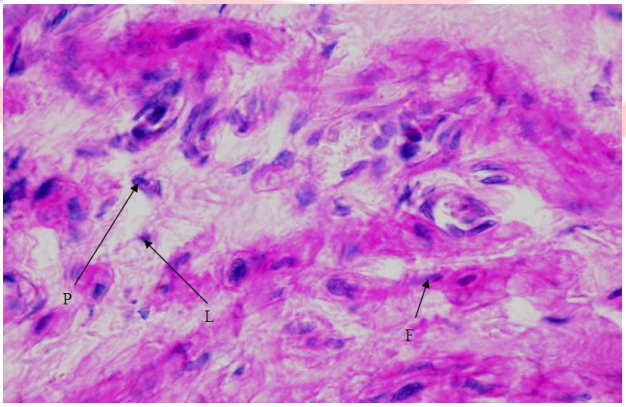

Figure (7): Cross section through the wall of the popliteal artery of a diabetic sample showing cellular infiltration in (arrow) T.media, stained with PAS X 1000. (P=polymorph, L=lymphocyte, F=fibroblast).

Table (2): The effect of age and duration of diabetes on total vessel diameter (TVD) by micrometer as dependent variable**.**

	Unstandarized Coefficients		Standarized Coefficients	t	P
Model 1	**B**	**SD**	**Beta**		
(Constant)	389.52	274.33		1.42	0.16
age	4.03	4.26	o.31	0.95	0.35
duration of diabetes	0.126	0.08	0.54	1.63	0.11

Dependent variable TVD, Adjusted R square 0.45

Table (3): The effect of age and duration of diabetes on wall thickness by micrometer (WT) as dependent variable**.**

	Unstandarized Coefficients		Standarized Coefficients	t	P
Model 1	**B**	**SD**	**Beta**		
Constant)	109.27	117.31		0.93	0.36
age	1.83	1.82	0.20	1.004	0.32
duration of diabetes	-0.10	0.03	-0.62	-3.11	0.003

Dependent variable WT, Adjusted R square 0.65

Table 4: The effect of age and duration of diabetes on tunica intima thickness by micrometer (IT) as dependent variable**.**

	Unstandarized Coefficients		Standarized Coefficients	t	P
Model 1	**B**	**SD**	**Beta**		
(Constant)	61.77	61.32		1.01	0.32
age	-0.74	0.95	-0.06	-0.78	0.44
duration of diabetes	0.22	0.02	0.93	12.76	< 0.001

Dependent variable IT, Adjusted R square 0.95

Table 5: The effect of age and duration of diabetes on tunica media thickness by micrometer (MT) as dependent variable**.**

	Unstandarized Coefficients		Standarized Coefficients	t	P
Model 1	**B**	**SD**	**Beta**		
(Constant)	114.18	154.06		0.74	0.46
age	0.31	2.39	0.04	0.13	0.90
duration of diabetes	-0.01	0.04	-0.10	-0.28	0.78

Dependent variable MT, Adjusted R square -0.02

Table (6): The effect of age and duration of diabetes on Tunica adventitia thickness by micrometer (AT) as dependent variable**.**

	Unstandarized Coefficients		Standarized Coefficients	t	p
Model 1	**B**	**SD**	**Beta**		
(Constant)	-7.31	52.19		-0.14	0.89
age	1.29	0.81	0.37	1.59	0.12
duration of diabetes	-0.03	0.02	-0.39	-1.67	0.10

Dependent variable AT, Adjusted R square 0.53

Table (7): The effect of age and duration of diabetes on endothelial basement membrane thickness by micrometer (BMT) as dependent variable.

	Un standardized Coefficients		standardized Coefficients	t	P
Model 1	**B**	**Sd**	**Beta**		
Constant)	17.42	8.05		2.17	0.04
age	-0.16	0.16	-0.26	-1.04	0.31
duration of diabetes	0.12	0.18	0.17	0.67	0.50

Dependent variable BMT, Adjusted R square -0.03

Discussion

Regression analysis for age of diabetic patients and duration of the illness as independent factors that leading to decreases in the diameter of the lumen, thickness of the wall and the thickness of tunica intima, is in agreement with the results obtained by Seneviratne [7].Williams *et al*., [8], Timperley *et al* [9], Hsueh and Anderson [10], Ward [11], Younger *et al*., [12] , Malik [13], Colwell [14], Cameron *et al*., [15], Brownlee [1], Naidu and Sengupta [16] and Coce *et al*., [17]. A lot of other study searched about the Angiopathic changes resulted from diabetes but they did not record such changes in arteries, of them done by LoGerfo and Coffman [18], and Malik *et al*., [19].

The interaction between the vascular changes and peripheral neuropathic changes are bidirectional and both result in defect in both blood flow and neuronal function. Diabetic microangiopathy has been considered the anatomic alteration leading to the development of neuropathy. Nevertheless, macro angiopathy, i.e. atherosclerosis of peripheral arteries, is also a peculiar feature of long-standing diabetes involving predominantly distal arteries and having inadequate collateral development consequently leading to nerve ischemia, an idea described by Cameron *et al*., [15] and Sharma *et al*., [20].

The thickening of the basement membrane is the dominant structural change which might act as a barrier to the exchange of nutrients and/or increase the rigidity of the vessel further limiting the ability to dilate in response to different stimuli [21-22].

Multiple thrombus and calcification which were obtained in the wall of popliteal artery of diabetic patients, may explained by the fact that in case of hyperglycemia of the blood will be in a hypercoagulable state

accelerated by increased cholesterol and triglycerides, causing damage to the endothelial layer, with shedding of these cells into the lumen resulting in slowness of the blood flow and thrombus formation [16, 23, 24].

Recommendation

According to involvement of great vessels by the diabetic angiopathy, the popliteal artery and great vessels of diabetic patients, recommended to be examined by the available imaging technique.

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