ASPECTOS HEMORREOLÓGICOS NA MICROVASCULATURA DE DIVERSAS PATOLOGIAS / HEMORHEOLOGICAL ASPECTS IN THE MICROVASCULATURE OF SEVERAL PATHOLOGIES

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ABSTRACT

We evaluated morphological changes in several pathologies using computerized videocapillaroscopy, and related hemorheological patterns using the laser assisted optical rotational red cell analyzer (LORCA). In addition, tissue oxygenation was measured using two oximeters with Combi sensors (Periflux 5000, Perimed).

The study included four groups of patients (pts) that were compared with a control group. **Group A** *Controls* (n=25: 15 males [M] and 10 females [F] aged 36±3 years); **Group B** *Diabetic* pts n=32 (IDDM pts n=20: 12 M and 8 F aged 43±4 years; NIDDM pts n=12: 6 M and 6 F aged 45±3 years); **Group C** *Glaucoma* pts n=30 (16 M and 14 F aged 42±5 years); **Group D** *Liver failure* pts n=6 (3 M and 3 F aged 44±5 years); **Group E** *Hypertensive* pts n=50 (smokers n=28: 12 M and 16 F aged 40±4 years, and non-smokers n=22: 12 M and 10 F aged 38±3 years). In all patients hemorheological measurements were made using the LORCA (including red blood cell [RBC] deformability and aggregability), morphology was evaluated using computerized videocapillaroscopy (magnification 200 x), and transcutaneous oxygen partial pressure measurements ($TcpO_2$) were made with the Periflux 5000.

In patients with diabetic microangiopathy: the capillary loops in 50% (16/32) of these pts showed formations such as 'deer horns', 72% (23/32) showed formations such as 'elephant nose', and in 45% (14/32) formations such as a 'cork screw'; in diabetics with POAD an important capillary rarefaction was found in 26% (9/32) of the pts. In glaucoma patients, in 84% (25/30) we observed 'capillary meandering' and images such as 'a comb'. In patients with more complicated pathology capillary rarefaction was found in 70% (21/30) of the patients. An improvement in the perfusion of non-functional loops

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was found in deceased patients who had suffered liver failure one week after liver transplantation in 90% (5/6) of the studied cadavers. In non-smoking hypertensives morphological changes were found in 25% (6/22) of the patients, and in hypertensive smokers in 47% (13/28). RBC deformability was detected using LORCA and expressed as the Elongation Index (EI), and RBC aggregability was detected using LORCA and expressed in t½ (seconds) indicating the RBC aggregability peak. Group A controls: EI 0.59±0.02; t½ 3±1 sec; Group B: IDDM EI 0.55±0.01; t½ : 2±0.5 sec p<0.05; NIDDM EI 0.56±0.01; t½ 2±0.2 sec p<0.04; Group C glaucoma: EI 0.56±0.01; t½ 2±0.3 sec p<0.05; Group D liver failure: EI 0.56±0.02; t½ 2±0.4 sec p<0.03; Group E hypertensives: smokers EI 0.56±0.02; t½ 2±0.6 sec p<0.04; non-smokers EI 0.57±0.02; t½ 2±0.6 sec p<0.04 compared with controls. We also measured the $TcpO_2$ at the dorsum of the right foot as a standard site representing peripheral control of microvasculature perfusion. Group A 96±11 mmHg; Group B IDDM 74±9 mmHg p<0.05; NIDDM 76±8 mmHg p<0.05; Group C glaucoma 75±9 mmHg p<0.05; Group D liver failure 69±6 mmHg p<0.05; Group E hypertensives: smokers 70±5 mmHg p<0.05, non-smokers 77±9 mmHg p<0.05 compared with controls.

This study presents an interesting and complete methodology to evaluate the microcirculation in different pathologies that induce changes in the microvasculature.

Key-words: Hemorheology, microcirculation, videocapillaroscopy, LORCA, tissue oxygenation.

INTRODUCTION

The first to describe the circulatory system in detail was Sir William Harvey (1628; Fig. 1); however, he did not explain the connection between the venous and the arterial system in his *De Motu Cordis*. In Italy the studies of Marcello Malpighi (1661; Fig. 2) have been very important, particularly his *De pulmonibus Observationes Anatomicae*. In his studies Malpighi described preliminary but nevertheless very important observations about the capillary system.

In Holland, Antoni van Leeuwenhoek (1675) devised a prototype instrument to directly study red blood cells (RBC): i.e. a microscope. In a letter to the Royal Scientific Society of London (1686) he reported his observations on RBC in the narrow capillaries described by Malpighi[1]. Subsequently, many other researchers made various scientific contributions. For example, in 1895 the 'Starling Law' described the oncotic and osmotic pressure in the filtration mechanism of vessel-tissue absorption; Starling observed that the contraction energy of the myocardiocytes is directly related to their initial length[2].

T. Lewis (1917), A. Krogh (1922) and Zweifach (1940) marked the birth of scientific knowledge on the microcirculation. In subsequent years studies were published on hemorheology and blood viscosity (L. Dintenfass 1971, T. Di Perri 1979, S. Forconi 1987, H. Meiselman 1989, M.R. Hardeman 1991); as well as on microhemodynamics (R. Del Guercio 1986), and on ischemia/reperfusion, especially during organ transplantation (G. Cicco 2003).

Today molecular and cellular biology are able to explain the relationships between the endothelium and the blood stream, leukocyte rolling and adhesion.

Endothelial functions are not included in the normal balance of endothelial cell stimulation and/or inhibition and are able to favour circulatory diseases. It then became interesting to study the hemorheological and morphological changes in tissue oxygenation in the microcirculation of various patients: including diabetics, hypertensives, patients suffering from lipoidoproteinosis, those with peripheral arterial occlusive disease (PAOD), with systemic pathologies such as systemic lupus erythematosus (LES), progressive systemic sclerosis (PSS= scleroderma), those undergoing organ transplantation (liver, kidney), and those suffering from primary open angle glaucoma (POAG).

AIM

The aim of this study was to evaluate the morphological changes in several pathologies using computerized videocapillaroscopy (Mitsubishi, Videocap, Japan: magnification 200 x), the relative hemorheological patterns using the laser assisted optical rotational red cell analyzer (LORCA), and tissue oxygenation using two oximeters with combi sensors to measure PO_2 and PCO_2 (Periflux 5000, Perimed).

MICROCIRCULATION

According to Chambers and Zweifach (1940) the microcirculation is organized into many units: artery, capillary, arteriolar-venular anastomosis (not always present), veins and perivascular areas. The arteriolar-capillary joints and preference channels are very important. A simple capillary

Fig. 1 – Sir William Harvey

Fig. 2 – Marcello Malpighi

classification was made according to Bunnet (1958) and Rodhin (1973):

A) Related to the basilemma characteristics:

1) type A continuous basilemma
2) type B discontinuous basilemma (sinusoids)

B) Related to the endothelium characteristics:

1) type 1 continuous and thick endothelium (subcutaneous tissues)
2) type 2 continuous and thin endothelium (skeletal and smooth muscles, lungs, fat, central and peripheral nervous system)
3) type 3 fenestrated endothelium (endocrine and exocrine glands)
4) type 4 discontinuous endothelium (liver spleen, bone marrow)

C) Related to the perivascular tissues:

1) type α evident perivascular tissues
2) type β detached perivascular tissues

MATERIAL AND METHODS

In the present study four groups of patients were compared with a control group: Group A Controls, Group B Diabetes, Group C Glaucoma, Group D Liver Failure, and Group E Hypertension. All patients underwent a) hemorheological measurements using the LORCA which measures RBC deformability and RBC aggregability; b) morphological evaluation using computerized videocapillaroscopy (Videocap, Mitsubishi, Japan) i.e. a microscope/video imaging technique with a magnification of 200 x; and c) transcutaneous oxygen partial pressure measurement ($TcpO_2$) (Periflux 5000, Perimed). In addition, in Group C (30 glaucomatous patients) we also measured the RBC surface acetylcholinesterase (ACE) and the cytosolic calcium as hemorheological patterns during treatment of this pathology.

Videocapillaroscopy

Many pathologies are known to have related capillary abnormalities, e.g. endocrine, psychiatric, cardiovascular, and rheumatic diseases (Table 1).

Videocapillaroscopy enables the study of vessels in the microcirculation providing information on their length, number, tortuosity, ectasia, stenosis, etc.[3]. Recently our Center has been using computerized videocapillaroscopy (Videocap, Mitsubishi, Japan; magnification 200 ×) to evaluate the microvasculature throughout the body.

We generally follow the Lee Classification[3]:

Level 0 – no avascular area;
Level 1 – 1 or 2 avascular zones with small dimensions;
Level 2 – more than 2 avascular areas with extended dimensions;
Level 3 – confluent avascular zones (desertification).

In capillaroscopy the morphologic or static parameters (Table 2) and the functional or dynamic (Table 3) parameters are very interesting.

Capillaroscopy can be used in clinical practice (Table 4) as well as in various fields of research (Table 5)[4].

Table 1 – Pathologies often with related capillary abnormalities

1. Endocrine diseases
• Diabetes
• Hypothyroidism
• Addison's disease
2. Psychiatry
• Schizophrenia
• Epilepsy
• Oligophrenia
3. Cardiovascular diseases
• Arterial hypertension
• Right heart failure
4. Rheumatic diseases
• Progressive systemic sclerosis (PSS)
• Dermatomyositis
• Eosinophile fascitis
• Mixed connective phlogosis
• Raynaud's syndrome and disease
• Rheumatic fever
• Systemic lupus erythematosus (LES)
• Psoriatic arthritis
• Cryoglobulinemia

Table 2 – Morphological or static parameters

• Visibility
• Loop morphology
• Loop orientation
• Capillary density
• Loop length
• Loop diameter
• Loop-dermal papilla distance
• Loop tortuosity
• Ectasia and microaneurism
• Megacapillaries
• Avascular zones
• Neoangiogenesis
• Microbleedings

Table 3 – Functional or dynamic parameters

Capillary flow:
• Continuous or ribbon-like
• Granular or with microaggregates
• Intermittent or with plasma gaps
• With or without stops

Table 4 – Capillaroscopy in clinical practice

• Angiology (POAD)
• Phlebology
• Rheumatology (PSS, LES)
• Cardiology (hypertension)
• Vascular surgery
• Dermatology
• Surgery
• Oncology
• Endocrinology (diabetes, hypothyroidism)
• Psychiatry (schizophrenia, epilepsy, oligophrenia)

Many capillary morphological changes have been found during routine use of videocapillaroscopy:

1. normal loops, such as 'hairpins'
2. tortuous loops, such as 'corkcrews, candelabra, glomerulus';
3. branched loops, such as 'deer horns, shamrock' (Fig. 3);
4. arborescent loops, such as 'thicket, claw';
5. loops such as 'cactus';
6. loops such as 'shoal of fishes' or 'elephant nose' (Fig. 4) in diabetes;
7. apical or lateral small ectasia;
8. capillary loop enlargement (in the efferent loop or in the apical zone);
9. megacapillary in 'gigantic loop';
10. loops such as 'pearl necklace collar.

In loop delivery we have observed:

1. ordered, regular, such as 'comb' loops
2. meandering delivery

Table 5 – Capillaroscopy in scientific research

- Mapping of the regional architecture of the microvessels.
- Response of the regional microvessels to physico-mechanical, hemodynamic, neuroendocrine, postural and other stimulations.
- Response of the microvessels to pharmacological stimuli as well as to endogenous and exogenous stimulations.
- Monitoring of the efficacy of drugs.
- Study of microcirculation-tissue relationships in patients of different ages
- Regional dynamic hemorheology.

3. focal absence
4. a gigantic loop with a few normal loops around it.

LORCA

The LORCA system is an interesting method for the study of RBC deformability and aggegability. Laser beams allow the detection of changes in RBC deformability and their aggregability[5-7].

Oximeters

Oxygen partial pressure is measured using the Periflux 5000 (Perimed) with combi sensors.

RESULTS AND DISCUSSION

In patients with diabetic microangiopathy (Group B): in 50% (16/32) the capillary loop showed formations such as 'deer horns', in 72% (23/32) a formation such as 'elephant nose', and in 45% (14/32) a formation such as a 'cork screw'. In diabetics with POAD an important capillary rarefaction was found in 26% (9/32) of the patients. In glaucoma (Group C): in 84% (25/30) of the patients we observed 'capillary meandering' and images such as 'a comb'. In patients with more complicated pathology capillary rarefaction was found in 70% (21/30) of the group.

We also found an improvement in the perfusion of non-functional loops in patients who had died due to liver failure one week after liver transplantation (Group D) in 90% (5/6) of the studied cadavers. In Group E: in non-smoking hypertensives we found morphological changes such as 'tortuous loops' in 25% (6/22) of the patients, and in 47% (13/28) of the smoking hypertensive patients.

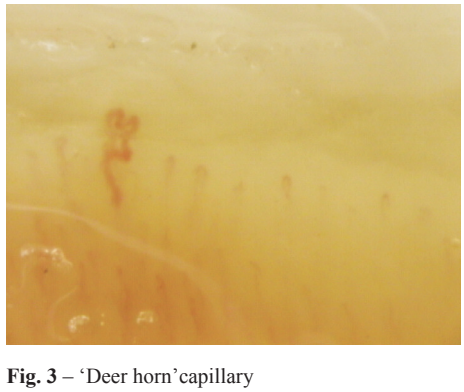

Fig. 3 – 'Deer horn' capillary

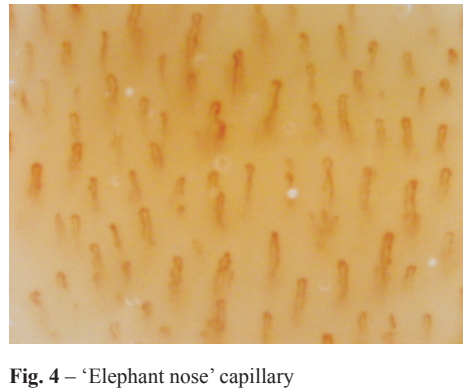

Fig. 4 – 'Elephant nose' capillary

Fig. 5 – LORCA

RBC deformability expressed as the elongation index (EI), and RBC aggregability expressed as t½ (seconds) indicating the time to obtain peak RBC aggregability, were detected using LORCA.

We found a significant decrease in the EI of all groups compared with controls: 0.56±0.01 vs 0.59±0.01 ($p<0.04$) and also a significant decrease in t½ in all groups compared with controls: 2±0.2 ($p<0.04$). The $TcpO_2$ was detected at the dorsum of the right foot as a standard site representing peripheral control of microvasculature perfusion. The $TcpO_2$ appeared significantly decreased ($p<0.05$) compared with controls (Table 6).

Normal RBC deformabiliy is important in the perfusion and oxygenation of peripheral capillaries, especially in ocular tissues (retina, optic nerve, etc.). It is also possible to interfere with RBC deformability using drugs that influence the amount of intracytosolic calcium. The influence of an increase in surface RBC acetylcholinesterase (ACE) may be very important[8]. ACE is an enzyme located on the external surface of the RBC membrane. An increase in ACE induces an increase in cytosolic calcium in RBC and a subsequent decrease of RBC deformability with impairment in microcirculatory blood flow and tissue oxygenation[9-13]. We compared healthy control subjects with glaucoma patients (Group C), thereby exploring other aspects in the same glaucoma group[14-15] (Table 7).

Table 6 – Data on TcpO2 values in the five study groups

Type		TcpO2 (mmHg)	p-value
Group A	Controls	96 ± 11	*
Group B	IDDM	74 ± 9	* <0.05
	NIDDM	76 ± 8	* <0.05
Group C	Glaucoma	75 ± 9	* <0.04
Group D	Liver failure	69 ± 6	* <0.04
Group E	Smokers	70 ± 5	* <0.05
	Non-smokers	77 ± 9	* <0.03

Table 7 – Data on glaucoma patients

Type		Number of patients	Males (M)	Females (F)	Age (years)
Group A	Controls	25	15	10	36 ± 3
Group C1	Glaucoma: topical β-blockers	10	5	5	44 ± 3
Group C2	Glaucoma: carbonic anhydrase inhibitor	10	6	4	45 ± 6
Group C3	Glaucoma: prostaglandins	10	5	5	50 ± 3

CONCLUSIONS

This study using computerized videocapillaroscopy to investigate capillary morphology, LORCA to investigate RBC plasticity, and transcutaneous oximeter to study tissue oxygenation presents an interesting and complete methodology to explore and evaluate the microcirculation in different pathologies that induce changes in the microvasculature. These approaches allow the physician to modulate the therapy during follow-up in patients with these types of pathologies.

In addition, our data show that in glaucomatous patients topical prostaglandins and carbonic anhydrase inhibitors do not significantly (p=n.s.) alter the surface RBC ACE activity in these patients compared with controls. Topical β-blocker drugs in glaucomatous patients are able to induce a significant ($p<0.05$) increase in ACE and thus also an increase in intracytosolic calcium, finally decreasing RBC deformability and therefore tissue oxygen supply. Therefore, we conclude that carbonic anhydrase inhibitors and prostaglandin drugs do not interfere significantly with the intra-ocular microcirculation and hemorheology (particularly the optic nerve blood flow), whereas β-blockers do.

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