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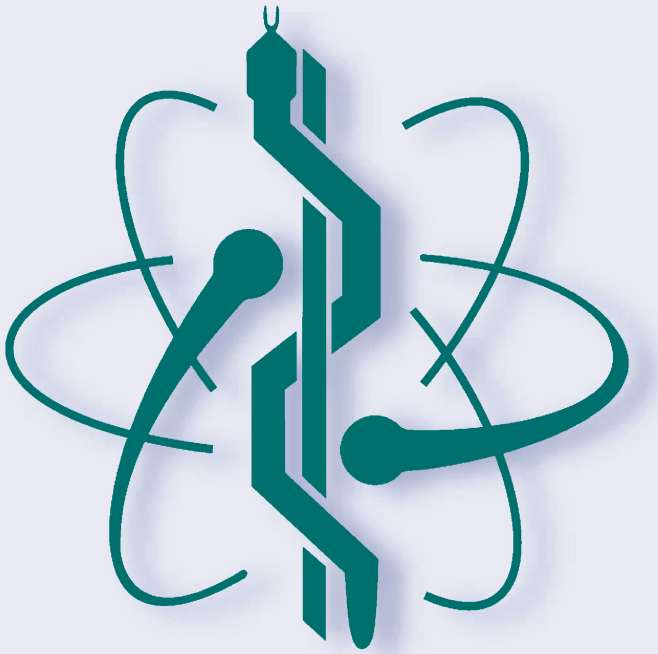

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Simona Vlad • Nicolae Marius Roman
Editors

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Ciprian Roman, "Dr. Constantin Papilian" Emergency Military Hospital of Cluj-Napoca, Romania
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Preface

The 6th "Conference on Advancements of Medicine and Health Care Through Technology"—MediTech 2018 took place in Cluj-Napoca on October 17–20, 2018.

The MediTech Conference has become an international academic forum for clinical engineers and doctors alike for researchers in medicine, medical physics, and biomedical engineering.

The lectures of the conference highlighted many concerns in the field of medical engineering. The authors, Romanians and foreigners, presented the latest research results in universities, clinics, medical devices domain, hospitals, etc.

It was a good opportunity for all participants to exchange their know-how and build up an academic collaboration in one of the most important fields of science and technology—medical engineering.

We were honored for the second consecutive attendance of Prof. Kang-Ping Lin, IFMBE Secretary General, from Chung-Yuan Christian University, at the official opening of the conference. He said:

> I am greatly honored to be invited at this international conference (MediTech-2018). On behalf of IFMBE, I am delighted to show all participants that the mission of IFMBE is to encourage, support and assist BME activities all over the world. At the same time, we promote exchange and cooperation of Science and Technology, integrate industrial technology and clinical application through advancement of research, development, application, management of technology and education training. MediTech Conference becomes a brand well known and respected in Romania and surrounding countries. It is important to point out that this conference was organized by Romanian National Society for Medical Engineering and Biological Technology with Technical University and University of Medicine and Pharmacy from Cluj-Napoca which should be an example for conferences in biomedical engineering and also for research in that area.

All papers submitted for presentation went through a review process and were evaluated by two reviewers. The papers chosen to be presented at the conference were accompanied by manuscripts to be published in these *Proceedings*.

We would like to kindly thank all participants, speakers, academics, researchers, doctors, members of the Technical University, "Iuliu Hatieganu" University of Medicine and Pharmacy, Military Emergency Hospital Cluj-Napoca, the members of the Scientific and Organizing Committees for their hard work and dedication and we hope that they will continue supporting MediTech Conference.

Cluj-Napoca, Romania

Prof. Nicolae Marius Roman
MediTech 2018 Conference Chair

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Part I
Clinical Engineering Assessment

Spectrofluorimetric Characterization of Serum Pentosidine and Retinol Binding Protein in Healthy Rats and Rats with Streptozotocin-Induced Diabetes

D. M. Ciobanu, L. E. Olar, R. Ştefan, G. Roman, and I. Papuc

Abstract

Recently, the fluorescence techniques have become increasingly important in medical diagnostics. Moreover, there is a growing need to introduce cost-effective and no time-consuming techniques for the investigation of various fluorophores in humans and animals with diabetes mellitus. In the studied literature, the newly diagnosis of diabetes mellitus and, subsequently, the risk of developing diabetes complications are reported to be correlated with the production of serum fluorophores pentosidine and retinol binding protein. As far as we are aware, there has been no study on the simultaneous fluorescence evaluation of pentosidine and retinol binding protein in biological fluids obtained from animals. In the present study, the emission intensity and levels of serum pentosidine and retinol binding protein were monitored in both healthy rats and rats with streptozotocin-induced diabetes. The results showed that the height of the peak at ~382 nm attributed to the presence of pentosidine in the serum, and the height of the peak at ~465 nm attributed to retinol binding protein in the serum were significantly higher in rats with streptozotocin-induced diabetes compared to healthy control rats. Also, their contributions to the total fluorescence of serum were significantly higher in rats with streptozotocin-induced diabetes compared to healthy control rats. Thus, fluorescence spectroscopy might be a reliable and useful technique that can be successfully applied in the evaluation and monitoring of serum pentosidine and retinol binding protein in both healthy rats and rats with streptozotocin-induced diabetes.

Keywords

Pentosidine • Retinol binding protein • Diabetes mellitus • Rats • Fluorescence spectroscopy

1 Introduction

Due to the increased number of animals and humans diagnosed with diabetes mellitus worldwide [1, 2], finding new methods and protocols for diabetes' biomarkers evaluation is mandatory. Pentosidine and retinol binding protein represent two of the recent studied compounds in diabetes mellitus [3–6]. Their increased levels in different body fluids and tissues were associated with the newly diagnosis of diabetes mellitus and, subsequently, with a higher risk of developing diabetes complications [3, 5, 7, 8]. However, as far as we are aware, there are no studies that have described the simultaneous fluorescence of both serum pentosidine and serum retinol binding protein in animals with diabetes mellitus compared to healthy controls. Thus, we decided to conduct a study in order to evaluate the levels of both pentosidine and retinol binding protein using spectrofluorimetry in the serum samples obtained from healthy rats and rats with streptozotocin-induced diabetes.

Pentosidine is formed through the reaction of glucose with lysine, arginine and ribose in the serum [9]. Increased pentosidine formation was described in various diseases associated with oxidative stress, including diabetes mellitus [10]. The evaluation of pentosidine in patients with diabetes may give us a valuable marker of long-term glycemic control which could seriously impact glycated hemoglobin levels [11]. Pentosidine is a fluorescent product which has been quantified and evaluated through various techniques such as: spectrofluorimetry, enzyme-linked immunosorbent assay, high-performance liquid chromatography and mass spectrometry [12]. However, there are limited data regarding the serum measurement of pentosidine in rats with streptozotocin-induced diabetes compared to controls using

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a simple and rapid method for quantitative determination such as the fluorescence technique followed by the Gaussian deconvolution of the obtained spectra.

Retinol is one on the most used parameters for the evaluation of serum vitamin A concentration in living organisms [13]. In the systemic circulation, there are three classes of specific transport proteins for vitamin A and its metabolites, called retinol binding proteins [14, 15]. The cellular retinol binding protein is the most abundant retinol binding protein in most tissues, such as: the liver, kidney and lung [15]. In fact, in order to prevent the disappearance of this retinol binding protein from blood by filtrating in the renal glomeruli, in the systemic circulation appears another protein called transthyretin [16, 17]. It was suggested that the complex formed between retinol and retinol binding protein is more fluorescent than free retinol [16, 18, 19]. The results of several investigations suggest an association of retinol binding protein with the newly diagnosis and presence of insulin resistance, diabetes mellitus and metabolic syndrome [5, 7, 20, 21].

In this paper, we wanted to simultaneously investigate the serum levels of pentosidine and retinol binding protein using fluorescence spectroscopy followed by the deconvolution method of the obtained spectra in an animal model of diabetes mellitus disease.

Therefore, we assessed the heights of the peaks attributed to these serum constituents and their contributions to the total serum fluorescence in rats with streptozotocin-induced diabetes compared to healthy control rats. Thus, eventual changes in the condition of these fluorophores could help the monitoring of animals' physiological response to diabetes mellitus diagnosis and to specific diabetes therapies.

2 Materials and Methods

2.1 Animals

The experiment was conducted in accordance with Romanian laws regarding correct manipulation of laboratory animals and was approved by the local Ethical Committee of University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania.

A number of 10 Wistar rats with the age of 6–7 weeks old, weighing between 250 and 300 g were used in this experiment. They were housed in two stainless steel cages with free access to food and water and under standard environmental conditions: temperature 24 ± 5 °C, relative humidity 60 ± 4%, light/dark cycle (12 h/12 h). The same rats were used in the first and second part of the study (after the administration of streptozotocin and, consequently, the induction of experimental diabetes mellitus).

2.2 Induction of Diabetes

Diabetes mellitus was induced with a single intraperitoneal dose of 60 mg/kg streptozotocin (Sigma, Aldrich). The experimental diabetes was induced in a number of 1–4 days after streptozotocin administration. Rats with blood glucose levels >300 mg/dl were considered as having diabetes mellitus [22].

2.3 Blood Sampling

The venous blood (0.2 ml) was collected from the orbital sinus of healthy rats and rats with streptozotocin-induced diabetes in 0.5 ml Eppendorf tubes. Further, the blood was centrifuged at 1000 g for 10 min. The levels of glycemia were measured using commercially available methods (Hitachi, Roche Diagnostics).

For the spectrofluorimetric analyses, further dilutions of serum (4 µl) were performed (1:500) using physiological serum (NaCl 0.9%).

2.4 Spectrofluorimetric Determination of Pentosidine and Retinol Binding Protein

The spectrofluorimetric analysis was made using a FP-8200 spectrofluorometer (Jasco, Japan) (Fig. 1). The measurements were performed at room temperature using a 1 cm quartz cell. The serum spectra were recorded in a region from 360 to 600 nm using an excitation of 335 nm [23, 24] at a medium sensitivity.

The fluorescence intensity was recorded at the emission maximum of ~380 nm for pentosidine [24] and at ~465 nm [19] for the retinol binding protein, with a wavelength accuracy of ±2 nm.

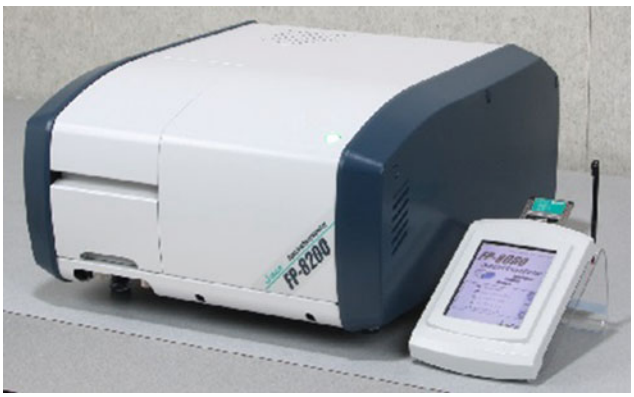

Fig. 1 Spectrofluorometer FP-8200 (Jasco, Japan)

The interpretation of obtained data was done using Origin Pro 8.1 software. Further, we performed the deconvolution of the obtained spectra using the peak analyzer option of Origin Pro 8.1 software in order to have a better quantification of the serum levels of pentosidine and retinol binding protein in rats with and without diabetes [24, 25]. Therefore, utilizing a combination of six Gaussian bands, we established the peaks position and full width at half maximum while the intensity was allowed to vary in order to match the line shape of the experimental spectra (Fig. 2).

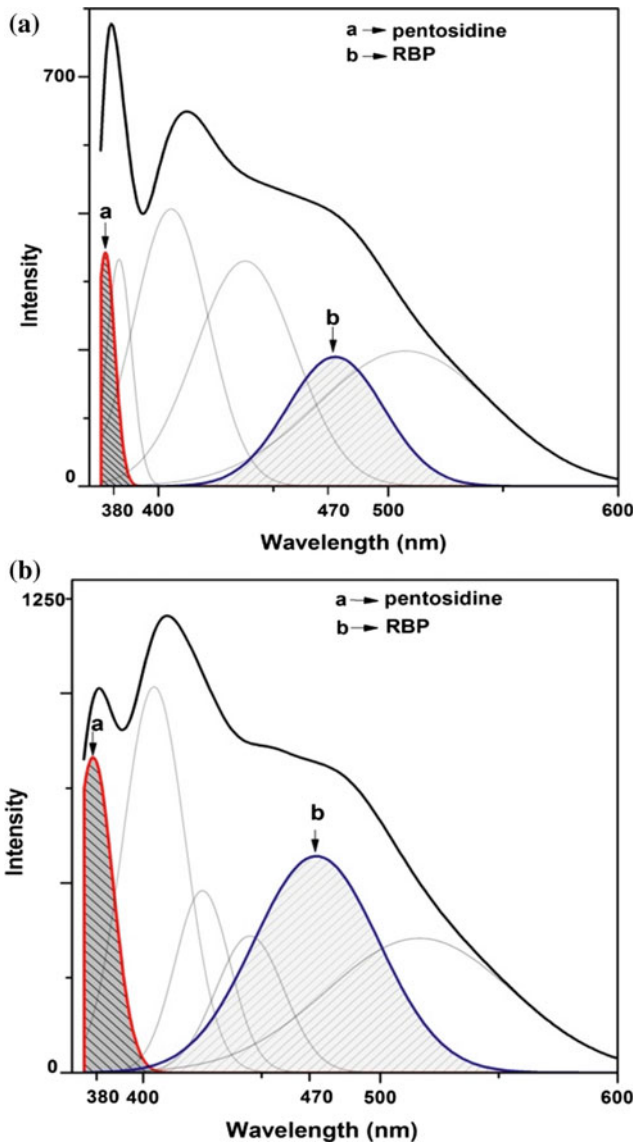

Fig. 2 The deconvolution of fluorescence spectrum of serum collected from rats before (**a**) and after the administration of streptozotocin (**b**) (excitation wavelength of 335 nm)

2.5 Statistical Analysis

Data analyses were performed using R commander version 3.0.1 for Windows. Peak heights and areas under the peak were normally distributed when evaluated using Kolmogorov–Smirnov test, and were expressed as means ±standard deviation. Group comparisons of the mentioned variables were performed using t-test. A p value <0.05 was considered statistically significant.

3 Results

Figure 3 shows the emission intensities of pentosidine and retinol binding protein in the serum of healthy rats and rats with streptozotocin-induced diabetes.

The height of the peak from ~380 nm attributed to the presence of pentosidine in the serum was significantly higher in rats with streptozotocin-induced diabetes compared to healthy rats ($p < 0.001$). Also, significant differences between the rats with streptozotocin-induced diabetes and healthy controls were noticed for the height of the peak near 470 nm attributed to the presence of retinol binding protein in the serum ($p < 0.001$). Moreover, when comparing the average contribution (area under the peak) of pentosidine and retinol binding protein to the total fluorescence of serum in rats with streptozotocin induced diabetes and healthy rats, the results showed that a statistically significant difference exists between the two study groups ($p < 0.001$) (Table 1).

4 Discussions

We evaluated the heights of the fluorescence signals attributed to pentosidine and retinol binding protein and their contributions to the total fluorescence of serum in order to study these biochemical compounds in the serum of healthy rats and how they change with the induction of diabetes mellitus. Concerning the existing connection between an increased pentosidine formation and the presence of diabetes mellitus, it was declared that hyperglycemia promotes the accumulation of pentosidine [10]. In our study, the fluorescence evaluation of pentosidine in rats with streptozotocin-induced diabetes revealed significantly higher peaks and contributions to the total fluorescence of serum compared to healthy control rats.

Therefore, we can declare that the levels of pentosidine in rats with diabetes mellitus were statistically significant higher compared to healthy controls. Similarly, previous studies reported significant difference in pentosidine formation between the patients with diabetes and controls [26]. In the same study, it was mentioned that the presence of complications of diabetes such as retinopathy and chronic

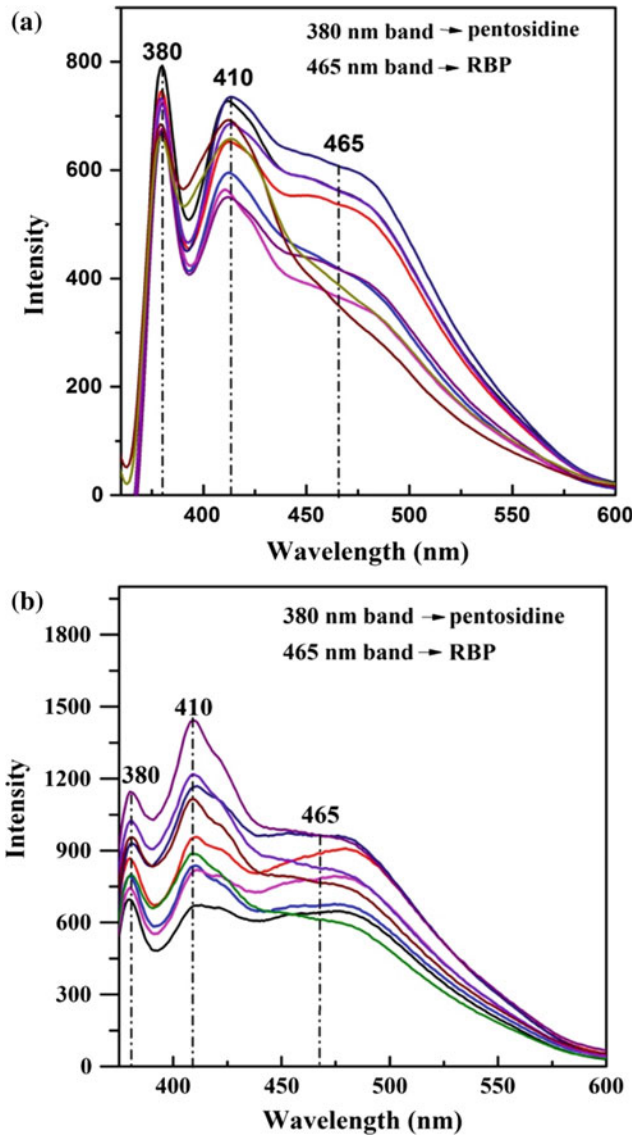

Fig. 3 The emission spectrum of serum pentosidine and retinol binding protein (RBP) in healthy controls (**a**) and rats with streptozotocin-induced diabetes (**b**)

Table 1 The characterization parameters of pentosidine and retinol binding protein that significantly ($p < 0.001$) differentiate between healthy rats and rats with streptozotocin-induced diabetes

	Characterization parameters		
	Peak center (nm)	Peak height (a.u)	Average of the area under the peak (cm^{-1})
Control rats	~380	708.6 ± 44.5	2.3 ± 0.4
	~470	465 ± 95.3	13.3 ± 0.9
Rats with streptozotocin induced diabetes	~380	877.1 ± 135.7	8.0 ± 1.2
	~470	789.4 ± 126.3	24.2 ± 2.7

nm, nanometers; a.u., arbitrary units

kidney disease leaded to significantly higher pentosidine levels [26]. It has been reported that an increased concentration of pentosidine measured by reverse-phase high performance liquid chromatography might be an independent predictor of all-cause and cardiovascular mortality and that it may represent a useful biomarker for monitoring clinical outcome in patients with end-stage renal disease [27]. In our article, we used a cost-efficient and no time-consuming fluorescence spectroscopic method with a high sensitivity and selectivity of detection for the investigation of pentosidine in the serum of rats with streptozotocin-induced diabetes.

We compared the contributions and the heights of the serum peak near 465 nm, attributed to retinol binding protein in healthy control rats and rats with streptozotocin-induced diabetes. Our results showed that both the heights of the peak attributed to retinol binding protein and its contribution to the total fluorescence of the serum were significantly higher in rats with streptozotocin-induced diabetes compared to controls, suggesting higher levels of this product in the presence of diabetes. Our results are somewhat in concordance with a study that reported higher serum and urine concentrations of retinol binding protein in type 2 diabetes mellitus patients compared to healthy controls [28]. Our study showing increased serum levels of retinol binding protein in rats with streptozotocin-induced diabetes highlights the similarities between these two species concerning the retinol metabolism in diabetes mellitus, and therefore, the possibility of using these animals as models in studying the pathogenesis of human diabetes mellitus. According to other studies, the levels of retinol in insulin treated diabetes mellitus patients and in streptozotocin induced diabetes mellitus rats were significantly lower than the levels of control subjects [29]. This finding was supported by numerous observations, such as: lower transthyretin levels or the presence of some problems in the synthesis of retinol binding protein due to altered renal function [30]. As can be observed, at this time, in the literature isn't established whether or not the levels of retinol binding protein are actually increased in patients with diabetes mellitus. Our study brings an improvement to the existing literature and claims an increased level of retinol binding protein in rats with streptozotocin induced diabetes mellitus when compared to healthy control rats. Also, we describe an increased serum retinol level in rats with streptozotocin induced diabetes.

5 Conclusions

In conclusion, the contributions of both pentosidine and retinol binding protein to the total serum fluorescence and also the heights of these peaks attributed to the presence of these biochemical compounds in serum are significantly

higher in rats with streptozotocin-induced diabetes mellitus compared to healthy control rats. On the basis of these data, we could claim the fact that the levels of pentosidine and retinol binding protein are significantly higher in the serum of rats with streptozotocin-induced diabetes compared to controls.

In addition, fluorescence spectroscopy represents a reliable and no time-consuming technique that can be applied successfully in the evaluation and monitoring of both serum pentosidine and retinol binding protein in animals diagnosed with diabetes mellitus.

Future studies should better investigate the correlation of pentosidine and retinol binding protein as determined by fluorescence spectroscopy with others biochemical compounds such as hyperglycemia and glycated hemoglobin and with the presence of diabetic complications in order to better understand the pathophysiology of these compounds in diabetes mellitus in both humans and animals.

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Conflict of Interest The authors declare that they have no potential conflict of interest relevant to this article.

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Eye Examination for Early Detection of Diabetic Neuropathy-Role of Corneal Confocal Microscopy

Georgeta Victoria Inceu, C. L. Vonica, and G. Roman

Abstract

Diabetes mellitus registers an alarming increase globally, taking epidemic proportions. Diabetic neuropathies are considered the most prevalent chronic complications of diabetes, affecting up to 50% of subjects with diabetes during lifetime. Distal symmetric polyneuropathy (DSPN), a length-dependent injury of peripheral nerves is the most frequent type among diabetic patients. Small nerve fibers are the first affected in the natural progression of the disease and their early damage can be assessed using corneal confocal microscopy (CCM). We applied CCM to the study group of 90 patients with type 2 diabetes, to identify early signs of diabetic neuropathy, to stratify patients into classes of severity of diabetic neuropathy and to find potential correlation between clinical, metabolic parameters and diabetic neuropathy severity. In our study, 88.89% of the patients were diagnosed as having DSPN, 37.77% of them with mild form, 38.88% with moderate neuropathy and 12.22% with severe neuropathy. Patients with diabetic neuropathy had a significantly higher BMI ($p = 0.04$) and abdominal circumference, higher HbA1c ($p = 0.36$) and a higher total cholesterol ($p = 0.43$) compared with patients without DSPN. Also, the Toronto Clinical Neuropathy Score was significantly higher in patients with pathological changes of the sub-basal corneal plexus ($p = 0.04$). Patients with DSPN had a significantly longer duration of diabetes ($p = 0.04$) and a worse glycemic control, compared with patients without DSPN. The result of our study proved that CCM can be used as a reliable diagnosis tool for early detection of small nerve fiber damage, considering that corneal sub-basal plexus changes precede clinically detected peripheral nerve changes.

Keywords

Distal symmetric polyneuropathy • Corneal confocal microscopy • Small nerve fibers

1 Introduction

Worldwide, we are witnessing to an impressive dynamic of the increase in the prevalence of diabetes, a chronic, progressive disease that requires continued medical care based on multifactorial risk reduction strategies [1]. Diabetes induced complications represent a major health burden, with an important impact on the quality of life of diabetic patients [2]. Type 2 diabetes is a significant cause of blindness, foot ulcerations and amputation and end-stage renal failure [3]. The economic cost of diabetes is also very high and it's continually rising [4, 5].

Diabetic neuropathies are considered the most prevalent chronic complications of diabetes, affecting up to 50% of subjects with diabetes during lifetime [6]. Diabetes can affect different components of the nervous system, and diabetic neuropathies can manifest in various forms with different clinical manifestations, but the most frequent type of diabetic neuropathy is distal symmetric polyneuropathy (DSPN), a diffuse and length-dependent damage of peripheral nerves [7]. Diabetic peripheral neuropathy is the most important risk factor for foot ulcerations [8–10], which may lead to lower extremity amputation among people with diabetes [11, 12] and drive significant economic cost of diabetic neuropathy. An early diagnosis and proper staging of diabetic neuropathy is essential for therapeutic decisions and early interventions.

In the natural history of diabetic neuropathy, the involvement of different nerves fibers is selective, small nerve fiber being affected earliest in the disease progression, prior to the development of the large fiber injury [13, 14].

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Small Aδ and C nerve fibers of the sensory and autonomic nervous system represents 70–90% of the peripheral nerve fibers. Small nerve fiber loss usually presents with pain, dysesthesia and autonomic dysfunction [13]. The involvement of large fiber may cause loss of protective sensations, numbness, impaired balance and walking. All this increase the risk of falls, foot trauma, ulceration and amputation [15].

The screening and diagnosis of diabetic neuropathy is mainly based on symptoms questionnaires, clinical examination, reflex tests and quantitative sensory tests [16]. Nerve conductions study are used to confirm the diagnosis, even though these electrophysiologic studies can assess only the large-diameter nerve fiber status [17]. To assess small nerve fiber loss, the golden diagnosis test is considered the quantification of intra-epidermal nerve fiber density (IENFD) in skin biopsies [18, 19]. Lately, there is a growing body of evidence that corneal confocal microscopy (CCM) can be used as a reliable screening tool and diagnosis method in diabetic sensorimotor polyneuropathy [20–22]. More than that, CCM can also be used in patients with other types of neuropathy such as Human Immunodeficiency Virus-associated sensory neuropathy [23], chemotherapy-induced neuropathy [24], as well as chronic inflammatory demyelinating polyneuropathy [25].

In people with diabetes have been described several abnormalities in corneal morphology including reduction in corneal nerve bundles, reduced thickness, thinner epithelium [26, 27], and a strong correlation have been demonstrated between injuries of peripheral nerves due to diabetes and damages in corneal sub-basal nerve plexus detected with CCM [28]. So, in the light of these strong evidence, CCM can be considered a surrogate marker of small fiber neuropathy [29].

2 Objectives

A. *To quantify small unmyelinated nerve fiber loss in patients with type 2 diabetes using CCM*
B. *To stratify patients into classes of severity of diabetic neuropathy*
C. *To assess potential correlation between clinical, metabolic parameters and DSPN severity*

3 Materials and Methods

90 patients with type 2 diabetes from Cluj-Napoca Diabetes Clinical Center were included in the study, between September 2012-November 2012. Inform consent was obtained from all the participants All the patients were assessed for anthropometric parameters: weight, height, waist circumference and body mass index (BMI), and laboratory measurements. The presence of DSPN was assessed using Semmes-Weinstein Monofilament Testing (SWMT), vibration perception with the 128-Hz tuning fork, Rapid-Current Perception Threshold (R-CPT) measurements using the Neurometer®, and CCM. The Neurological Symptom Score (NSS) [30] and the Toronto Clinical Neuropathy Score (TCNS) [31] which includes an assessment of symptoms (present or absent), sensory loss (normal or abnormal), and reflexes (normal, reduced, or absent), were used. For the TCNS, based on the outcome, a score of 6–8 denotes mild neuropathy, 9–11 moderate, and ≥ 12 severe neuropathy.

CCM was performed using a Heidelberg Retina Tomograph. This is a confocal retinal microscope with a Rostock module attached that allows it to shrink focal length and so, can make cornea and no retinal section. The images were analyzed manually by an ophthalmologist and four parameters were quantified: the total number of nerves per image (TNN); the number of main branches (NMN), the number of branches connections (NBC), and the branches/connections ratios (BCR) [21]. According to these parameters, DSNP was stratified into three severity classes as presented in Table 1.

Statistical analysis was performed using SPSS 15.0 (SPSS Inc, Chicago, USA). The normality of variables distribution was evaluated using Kolmogorov–Smirnov test. Continuous data were expressed as means ±standard deviation when normally distributed or as median for non-parametric data. Qualitative data were expressed as numbers and percentages. Group comparisons of all variables were performed using t-test for groups with normally distributed data, and Mann–Whitney test for groups with not normally distributed data. A ***p*** values less than 0.05 was considered statistically significant.

Table 1 DSPN severity classes according to the CCM parameters

Parameters	Without DSPN	Mild	Moderate	Severe
TNN	>25	20	15	10
NMB	>8	5–7	2–4	≤ 1
NBC	>20	<15	<10	<5
BCR	<1.25	1.3	1.5	>2

DSPN-distal symmetric polyneuropathy, TNN-the total number of nerves per image, NMB-the number of main branches, NBC-the number of branches connections, BCR-the branches/connections ratios

4 Results

The study group included 90 patients with type 2 diabetes who met the inclusion criteria. Of these, 56.7% (51) were female, and 4.3% (39) were males. The characteristics of the study group are presented in Table 2.

According to the protocol described in Materials and methods, after performing CCM, we assigned the patients according to the presence of diabetic neuropathy and further on the severity classes of diabetic neuropathy: 11.11% (10) without DSPN, 37.7% (34) mild DSPN, 38.8% (35) moderate DSPN, 12.2% (11) severe DSPN. Figure 1 shows the images of patients with and without DSPN.

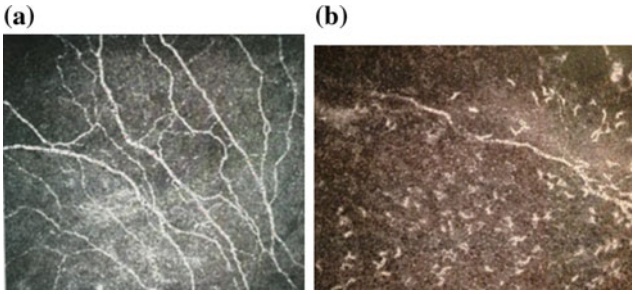

Fig. 1 Sub-basal nerve plexus of a subject without DPN (**a**) and with severe DPN (B)

Table 2 Baseline characteristics of the study group

Parameter	Mean ±SD
No. of patients	90
Mean age (years)	61.58 ± 10.092
Sex (male)	43.3% (n = 39)
Mean diabetes duration (years)	8.31 ± 6.61
Smoking	16.7% (n = 15)
Alcohol	28.9% (n = 26)
Hypertension	82.2% (n = 74)
Total cholesterol (mg/dl)	179.3 ± 40.6
HDL-cholesterol (mg/dl)	45.3 ± 13.3
LDL-cholesterol (mg/dl)	101.3 ± 35.7
Triglycerides (mg/dl)	163.4 ± 69.1
Mean HbA1c (%)	7.5 ± 1.1
BMI (kg/m^2)	31.6 ± 5.14
Retinopathy	23.3% (n = 21)

BMI-body mass index, HDL-high density lipoprotein, LDL-low density lipoprotein, HbA1c-glycated hemoglobin. Data are presented as means ±SD (standard deviation)

Were further analyzed the differences between patients without DSPN and those with DSPN (regardless of severity) (Table 3). Patients with diabetic neuropathy had a significantly higher BMI (p = 0.04) and abdominal circumference, higher HbA1c (p = 0.36) and a higher total cholesterol (p = 0.43) compared with patients without DSPN. Also, the Toronto Clinical Neuropathy Score was significantly higher in patients with pathological changes of the sub-basal corneal plexus (p = 0.04).

Patients with morphological changes of the sub-basal corneal plexus had a significantly longer duration of diabetes (p = 0.04) compared to patients without diabetic neuropathy and additionally, the duration of diabetes correlated positively with the severity of diabetic neuropathy (5.6 ± 3.44 years in patients without DSPN, 8.01 ± 5.86 years in patients with mild DSPN, 9.4 ± 6.53 years in patients with severe DSPN) (Fig. 2).

Table 3 Clinical and biochemical parameters in patients with and without DSPN, assessed by CCM

Parameter	No DSPN	Established DSPN	p-value
Age (years)	60.9 ± 12.67	61.66 ± 9.82	0.87
Diabetes duration (years)	5.6 ± 3.44	8.65 ± 6.85	0.04
Weight (kg)	85.2 ± 15.88	87.96 ± 15.39	0.52
BMI (kg/m^2)	29.57 ± 0.43	31.9 ± 5.28	0.04
WC (cm)	102.7 ± 9.7	110.69 ± 12.37	0.04
HbA1c (%)	6.8(6.7–8.1)	7.59 ± 1.17	0.36
Total cholesterol (mg/dl)	168.7 ± 32.32	180.72 ± 41.52	0.43
HDL cholesterol (mg/dl)	41 ± 14.54	45.9 ± 1.22	0.24
Triglycerides (mg/dl)	169.7 ± 57.56	162.64 ± 70.79	0.41
LDL cholesterol (mg/dl)	93.76 ± 15.46	102.29 ± 37.5	0.39
NSS	7.1 ± 5.92	7.63 ± 4.97	0.7
TCNS	2.1 ± 2.23	3.49 ± 2.71	0.04

BMI-body mass index, WC-waist circumference, HbA1c-glycated hemoglobin, HDL-high density lipoprotein, LDL-low density lipoprotein, NSS- The Neurological Symptom Score, TCNS-The Toronto Clinical Neuropathy Score. Data are presented as mean or median ± SD

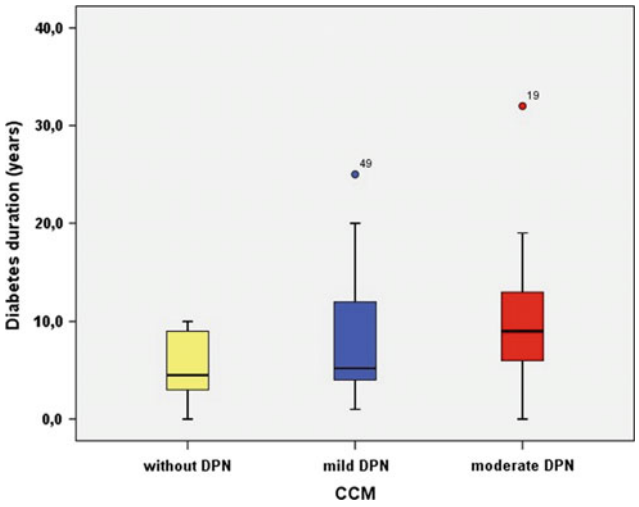

Fig. 2 Significantly higher diabetes duration in patients with higher classes of diabetic neuropathy. CCM-Corneal Confocal Microscopy, DPN-Diabetic peripheral neuropathy

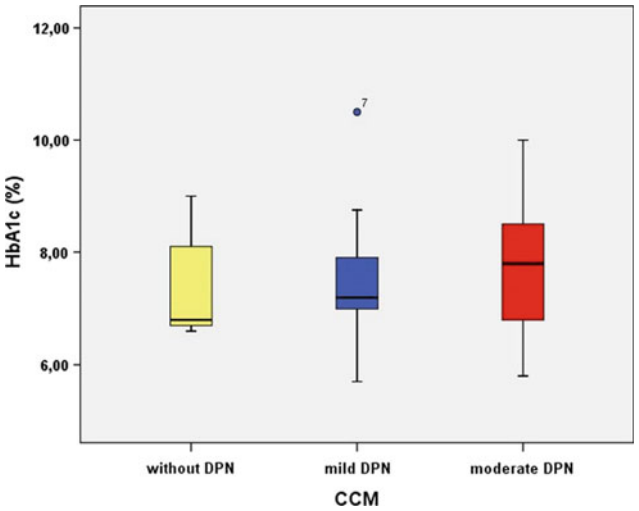

Fig. 3 Correlation between HbA1c and diabetic neuropathy severity

The patients were divided according to HbA1c value in two categories: with good glycemic control (HbA1c < 7%) and suboptimal glycemic control (HbA1c $\geq$ 7%), and the results showed that patients with good glycemic control had a shorter duration of diabetes, better anthropometric indices and better lipid profile compared to patients with unsatisfactory glycemic control (Table 4).

We noticed an increasing trend in glycated hemoglobin value with the increase in the severity of diabetic neuropathy, by analyzing this parameter in the study patients (without DSPN, with mild, moderate and severe DSPN) (Fig. 3).

Patients in the study group were assessed for protective sensation (using the Semmes-Weinstein Monofilament Testing), and the results were compared with those obtained from the sub-basal corneal plexus imaging through CCM. Although a significant percentage of patients with preserved protective sensation were diagnosed with diabetic neuropathy (64 patients), statistical significance was not reached in this case (p = 0.29) (Table 5).

Following the calculation of the Kendall correlation coefficient, there was a tendency for patients with abnormal protective sensitivity to associate with lower severity classes of neuropathy (according to CCM parameters) (Table 6).

Table 4 Characteristics of patients in the studied group according to the HbA1c value

Parameter	HbA1c < 7	HbA1c $\geq$ 7	p-value
Age (years)	63.1 ± 8.47	60.78 ± 10.83	0.17
Diabetes duration (years)	8.26 ± 7.38	8.34 ± 6.25	0.01
BMI (kg/m^2)	30.51 ± 4.65	32.23 ± 5.33	0.27
WC (cm)	108.16 ± 11.22	110.66 ± 12.87	0.18
Total cholesterol (mg/dl)	177.23 ± 36.92	180.52 ± 42.7	0.48
HDL cholesterol (mg/dl)	46.55 ± 14.77	44.72 ± 12.68	0.51
Triglycerides (mg/dl)	158.32 ± 63.12	166.11 ± 72.56	0.04
LDL cholesterol (mg/dl)	99.01 ± 34.27	102.57 ± 36.77	0.24
NSS	7.81 ± 4.78	7.44 ± 5.22	0.04
TCNS	3.1 ± 2.29	3.46 ± 2.88	0.17

BMI-body mass index, WC-waist circumference, HbA1c-glycated hemoglobin, HDL-high density lipoprotein, LDL-low density lipoprotein, NSS-The neurological symptom score, TCNS-The Toronto clinical neuropathy score. Data are presented as mean or median ± SD

Table 5 DSPN diagnosed by using protective sensation testing and CCM

	CCM				
Protective sensation	No DSPN	Mild DSPN	Moderate DSPN	Severe DSPN	Total
Lost	0	5	8	3	16
Normal	10	29	27	8	74
Total	10	34	35	11	90

Data are presented as number of patients. CCM-corneal confocal microscopy, DSPN-distal symmetric polyneuropathy

Table 6 Correlation between CCM and protective sensation

	Protective sensation
Kendall correlation coefficient	−0,24
	0,04

CCM-corneal confocal microscopy

5 Discussions

Corneal confocal microscopy is a method with over 20 years of experience in the evaluation of diabetic neuropathy [32, 33]. This study included 90 patients with type 2 diabetes who were assessed for the presence of diabetic neuropathy using CCM and were then classified on severity classes of diabetic neuropathy according to an original protocol [21]. 88.89% of the patients were diagnosed as having DSPN, 37.77% of them with mild form, 38.88% with moderate neuropathy and 12.22% with severe neuropathy. In the literature, several studies have confirmed and validated the use of CCM in both diagnosis and risk stratification of the disease [28, 34, 35]. Tavakoli et al. [36] assessed 101 diabetic patients (type 1 and 2) for the presence of diabetic neuropathy using CCM. In their study, 66.33% of patients is classified as having diabetic neuropathy (36.63% mild neuropathy, 12.84% moderate and 13.86% severe diabetic neuropathy).

Analyzing patients with and without DSPN, we noticed significant differences between BMI (31.9 ± 5.28 vs. 29.57 ± 3.43 kg/m^2, $p = 0.04$) and abdominal circumference (110.69 ± 12.37 vs. 102.7 ± 9.7 cm, $p = 0.04$) between the two groups. Edwards and colleagues [37] also found statistically significant differences in BMI and abdominal circumference between patients with and without diabetic neuropathy ($p < 0.05$).

We found a statistically significant difference ($p = 0.04$) between duration of diabetes in patients without DSPN (5.6 ± 3.44 years) compared to patients with DSPN (8.65 ± 6.85 years). Additionally, the duration of diabetes had an ascending trend within the severity classes of diabetic neuropathy, more obvious for patients with moderate neuropathy (22.5% of the subjects had diabetes duration less than 5 years, 44.1% between 5 and 10 years, and 52% had a history of diabetes over 10 years). Our results are consistent with those reported in other studies using CCM to assess diabetic neuropathy [36, 38]. In a study involving 231 patients with diabetes [37], the length of small unmyelinated nerve fibers of the sub-basal corneal plexus correlated inversely and statistically significantly with the duration of diabetes mellitus ($r = -0.20$).

HbA1c showed an increase trend with the severity classes of diabetic neuropathy, finding consistent with those reported by Petropoulos and co [39] in their study on 111 diabetic patients and 47 healthy volunteers, assessed by CCM. HbA1c was statistically significantly higher in patients with diabetes and highest values of HbA1c were recorded in patients with severe diabetic neuropathy.

In our studied group, an important number of patients with preserved protective ($n = 64$) and vibratory ($n = 66$) sensation, were actually identified as having pathological changes of the sub-basal corneal plexus, an essential finding that supports the hypothesis that small unmyelinated nerve fiber loss precede the large myelinated nerve fibers damage, which highlights one of the limitations of the conventional methods of diabetic neuropathy assessment, applied in everyday practice, the ability to quantify only the large myelinated nerve fibers. An important percentage of patients with diabetes who had no clinical signs of neuropathy was shown to have abnormal corneal sub-basal nerve plexus changes, demonstrating that corneal changes precede clinically detected peripheral nerve changes.

6 Conclusions

Screening and diagnosis of peripheral diabetic neuropathy remain a challenge for the clinician, as there is no consensus on how to do it. On the other hand, the importance of early detection and adequate management of diabetic neuropathy results from the fact that up to 50% of patients is asymptomatic, with a high risk of painless injuries. Neuropathic symptoms and deficits affect the quality of life and life expectancy of patients with diabetes, inducing a significant rate of morbidity and mortality.

Corneal confocal microscopy is a noninvasive technique to analyze corneal structure and anatomy allowing the study

of the different layers of cornea, the most innervated organ of human body [6].

The results of this study demonstrated the accuracy of confocal corneal microscopy in the diagnosis of peripheral diabetic neuropathy compared to classical advocated methods used to assess diabetic neuropathy. CCM can be considered an important diagnosis instrument for the detection of small fiber neuropathy, the earliest manifestation of DSPN, having the potential to identify patients with minimal signs of neuropathy.

Conflict of Interest The authors declare that they have no conflict of interest.

Statement of Human and Animal Rights

The procedures involving human subjects conducted in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

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Doppler Ultrasonography, a Rapid Evaluation Method of the Major Risk in the Vascular Pathology

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Abstract

The benefit ultrasonography brings in medical practice when dubbed by a competent user is unquestionable. The following considerations support the assertion that the technique is non-invasive for the patient and the user, inexpensive, offering real-time relations as compared to other imaging and high performance techniques such as CT and MRI. In our clinical study, the four patients selected were representative for the above mentioned conclusions, being hospitalized and diagnosed sonographically with: tissue hematoma, pseudoaneurysm, arteriovenous fistula and venous thrombosis. After discharge, patients were clinically and sonographically monitored after 1, 3 and 6 months. The technique has also imposed a choice of treatment appropriate to each individual case, namely a conservative treatment for the patients with hematoma, pseudoaneurysm and arteriovenous fistula. For the patient with venous thrombosis, the prescribed treatment was medical. A surgical treatment (excision and ligation) for the pseudoaneurysm was also discussed. After a 6-month surveillance and delay, thrombosis was performed. We mention that the prognosis is also favorable after a surgical treatment when the evaluation and timing are correctly chosen.

Keywords

Pseudoaneurysm • Arteriovenous fistula • Venous thrombosis • Hematoma

1 Introduction

The Doppler ultrasound is a way of exploring the cardiovascular apparatus based on the Doppler Effect (described in 1842 by Hristian Doppler), which consists in modifying the frequency of the received signal when the emission source and/or the receiver are moving away from one another. At the level of the cardiovascular device, the color Doppler codifies the blood flow through the color providing exceptional user relationships.

Increasing the number of invasive (catheterization) or non-invasive procedures in medical practice as well as a traumatology that is more and more common in daily work has led to an increase in the number of vascular complications, including the following: hematoma with extensive skin blood swellings, pseudoaneurysms, arteriovenous fistulas and venous thrombosis. Post-catheterization, the frequency of complications depends on the nature of the intervention (performed for diagnostic or therapeutic purposes).

In the medical literature, the frequency of reported complications is considered to be below 1% for diagnostic catheterization, up to 9% for coronary angioplasty and over 16% for intracoronary stenting [1–3].

2 Material and Method

This study included a limited number of adult patients aged 42–76 years, namely 2 men and 2 women with severe vascular disease (pseudoaneurysm, arteriovenous fistula and venous thrombosis) admitted to the Recovery Hospital over

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