© Med Sci Monit, 2012; 18(2): CR58-64
PMID: 22293878

WWW.MEDSCIMONIT.COM
Clinical Research

Received: 2011.06.29
Accepted: 2011.08.01
Published: 2012.02.01

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Periodontal microcirculation in diabetics: An *in vivo* non-invasive analysis by means of videocapillaroscopy

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Source of support: Departmental sources

Summary

Background: Diabetes mellitus is today considered a society-wide disease of a chronic/degenerative nature. Among the secondary effects of diabetes, the one that interests the dental surgeon most is diabetic parodontopathy. The aim of this study was to underline and objectify microcirculatory variations at a periodontal mucous level in type 2 diabetics.

Material/Methods: The study enrolled 80 subjects: 40 subjects with a diagnosis of diabetes mellitus type II (18 males and 22 females, between 44 and 85 years of age); and 40 healthy subjects (17 males and 23 females, between 44 and 78 years of age). All the subjects, both diabetic and healthy, were submitted to a videocapillaroscopic examination of the mucosa of the oral cavity.

Results: The measurements concerning the density (expressed in the number of loops/mm^2) of the capillary loops presented differences between the healthy subjects and the diabetic subjects. The average periodontal capillary density (DC-P) was clearly superior in diabetic subjects (35.62±10.40 n°loop/mm^2) compared to healthy subjects (17.55±3.88 n°loop/mm^2). The statistical analysis was performed by means of the Mann Whitney test. The value of P (p=0.000000986), well below the level of significance, demonstrates the high significance of the results obtained.

Conclusions: The increase in capillary density could suggest the presence of active inflammatory phenomena or, more probably, a tendency to a greater susceptibility to inflammatory phenomena. Ultimately, this study shows that there is some peripheral damage to microcirculation at the masticatory mucous level in diabetic subjects and that such alterations can be instrumentally objectified and quantified through the videocapillaroscopic method.

key words: **periodontal disease • mouth mucosa • microscopic angioscopy • microcirculation**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=882456

Word count: 2780
Tables: 2
Figures: 9
References: 23

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Background

Diabetes mellitus, which is today considered a society-wide disease of a chronic/degenerative nature, includes a group of metabolic disorders characterized by a hyperglycemic state [1] that may derive from the altered secretion or the altered uptake and peripheral action of insulin. Individuals with by diabetes face microvascular and macrovascular complications.

Among the secondary effects of diabetes, the one that interests the dental surgeon most is diabetic parodontopathy. Several studies have attested to a close link between diabetes and periodontal disease, due to alterations in microcirculation in the periodontium and in oral mucosa [2–4].

In the presence of hyperglycemia, the metabolic process of the endothelial cells encounter an excessive oxidative stress that in turn increases non-enzymatic glycation and the oxidation of proteins. Therefore the excess of glucose in the blood favors the glycation of low density lipoproteins, the basic process behind atherosclerosis (macroangiopathy) and peripheral microangiopathic phenomena [5].

At an oral mucous and periodontal level, the microcirculatory damage is consequential to the glycation of proteins. It is clinically expressed by an increased tendency for tissue atrophy (above all on the back of the tongue) and by an increased tendency for the development of periodontal disease, which is also caused by the increase of glucose in sulcular fluid [6].

Videocapillaroscopy is an interesting way of studying microcirculation because of the possibility of examining small vessels *in vivo* by means of a microscope. From the study of periodontal microcirculation, the presence of characteristic capillaries can be observed – the arterial and venous ends with their different diameters; the presence and number of capillary crossing; and the capillary density. Videocapillaroscopy can have a predictive role in evaluating the presence of inflammatory phenomena [7].

Until now, it appears that no *in vivo* studies on oral and periodontal microcirculation have been performed that show a correspondence between variations in the periodontal capillary bed and the diabetic pathology.

Thanks to its particular anatomical and histological characteristics and easy accessibility, oral mucosa is indicated as being the preferential centre for capillaroscopic examination.

The aim of this study was to underline and objectify microcirculatory variations at a periodontal mucous level in subjects diagnosed with type 2 diabetes.

Material and Methods

Eighty subjects were enrolled and divided into 2 groups: 40 subjects with a diagnosis of diabetes mellitus type II (18 males and 22 females, between 44 and 85 years of age); and 40 healthy subjects (17 males and 23 females, between 44 and 78 years of age) (Table 1).

All the subjects gave their consent for the capillaroscopic examination to be performed and for the processing and use of their personal medical data in scientific papers, in accordance with Italian laws on privacy and the use of personal data (Law 675/1996, art. 1–29).

Table 1. Data of age and sex of enrolled patients.

	Group A (Type II Diabetics)	Group B (Healthy Subjects)
Age (mean±SD)	63.82±11.13	64.51±11.32
Age range	44–85	44–78
M/W ratio	18/22	17/23

The subjects were selected according to the following criteria:
- An established diagnosis of Diabetes Mellitus type II,
- Oral hypoglycemic therapy for at least 1 year, with routine checks on the level of glycemia,
- Levels of glycated hemoglobin between 6.3% and 7.1% (indicative of a good glycemic control),
- Absence of other pathological conditions that could cause alterations to the peripheral microcirculation of oral mucous such as hypertension, rheumatoid arthritis, Sjogren's syndrome, oral lichen planus, pemphigus, pemphigoid, scleroderma, Hashimoto's thyroiditis,
- Absence of exposure to risk factors such as cigarette smoke and alcohol,
- Absence of exposure to radiologic and chemotherapeutic agents.

All the subjects underwent an odontostomatological examination. A complete objective examination of the soft tissues and hard tissues of the oral cavity were carried out for each subject. Subjects with red or white lesions in the oral cavity were excluded. Every subject received an examination to ascertain their periodontal status. All the subjects (health subjects and diabetic subjects) were enrolled in the study if they had a healthy periodontal condition [8]. The subjects with evident clinical signs of periodontal disease were excluded from the study. The subjects with insufficient oral hygiene were preventively submitted to professional dental cleaning sessions. The state of oral hygiene was meticulously rechecked before the capillaroscopic examination. For every subject the following indexes of periodontal health were considered: bleeding on probing (BOP); plaque index (PII); and clinical attachment level (CAL). At the time of the capillaroscopic examination, all the enrolled subjects had a plaque and bleeding index equal to 0 and CAL was not significantly different between healthy subjects and diabetic subjects in a healthy periodontal condition.

All the subjects, both diabetic and healthy, were submitted to a videocapillaroscopic examination of the mucous of the oral cavity. The examination was conducted through the Videocap 200 videocapillaroscope produced by DS Medica S.r.l., Milan, Italy.

The videocapillaroscope used for this study was made up of:
- a central body that includes a light source consisting of a cold halogen light emitted by a 100 W lamp fitted with an automatic or manual control device to regulate luminosity and white balancing (Figure 1),

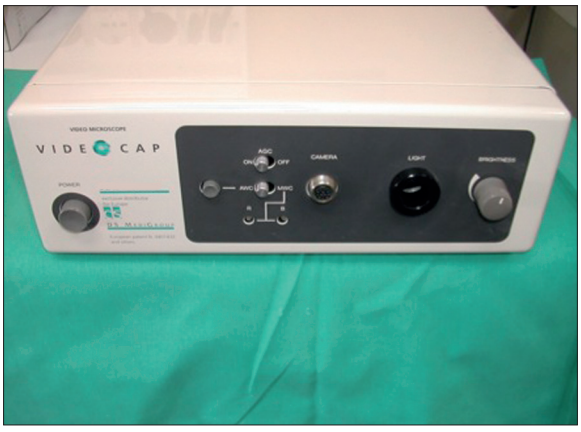

Figure 1. Central body, of videocapillaroscope, that includes a light source consisting of a cold halogen light emitted by a 100 W lamp fitted with an automatic or manual control device to regulate luminosity and white balancing.

Figure 2. Probe with an optic terminal connected to the central body by a fibreoptic cable 2 metres in length; the optic terminal is in turn constituted by a colour high definition micro television camera (420.000 pixels), a support for holding the different lenses, a ferrule for fine focussing regulation, a radial ferrule at the tip with annular illumination (for a uniform illumination without shadows). The lenses which can be applied can be either contact or non-contact types with varying enlargements: 20×, 50×, 100×, 200×, 500×, 1000×.

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- a personal computer with dedicated graphics card connected to the central body of the videocapillaroscope through an S-video cable. *VideoCap Software release 8.0* capillaroscopic imaging software is installed on the PC,
- a high resolution color cathode ray tube monitor.

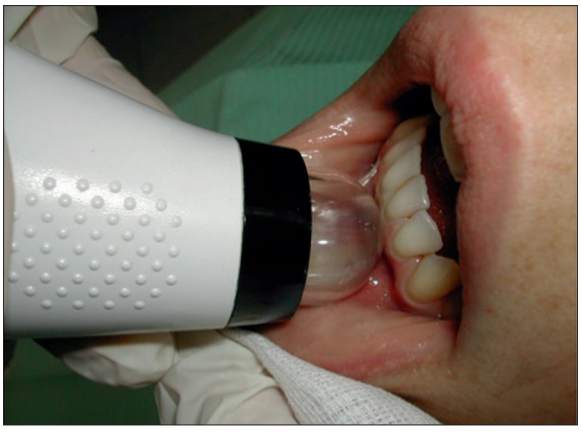

Figure 3. Capillaroscopic examination on periodontal mucosa of lower arch.

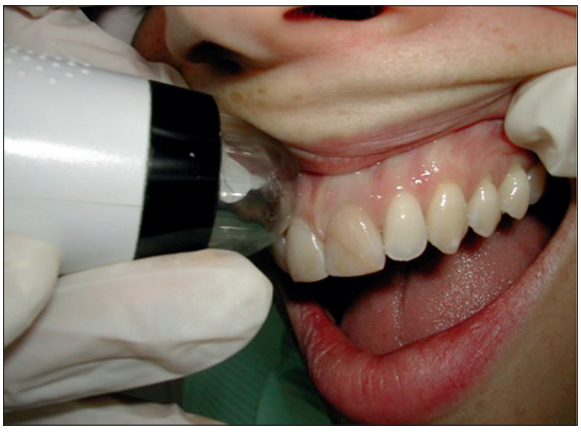

Figure 4. Capillaroscopic examination on periodontal mucosa of upper arch.

The capillaroscopic examination was conducted under standardized conditions of temperature and illumination:

- room temperature of 24±1°C was maintained constant through air-conditioning systems,
- illumination was through neon light for medical use with a white point equal to 6500°K.

The investigated site was selected according to criteria of accessibility and legibility of the capillaroscopic data: vestibular masticatory/gingival mucous of the III and V sextant.

A minimum number of 5 images were taken for every site. All the acquisitions were obtained in the morning, with the subjects in a seated position.

The lens chosen was the one with a constant enlargement of 200× and a varying focal spot (from 0 to 2 mm). This lens was chosen from those available for the good quality of its images in terms of the details offered (good reading of the capillary bed) and the size of the examined area (1.818 mm^2).

All the acquisitions and the subsequent elaboration of the capillaroscopic images were conducted by the same operator (GAS) (Figures 3, 4).

The capillaroscopic parameter evaluated was the following:

Table 2. Loop Density on periodontal mucosa in diabetics and healthy subjects.

	Loop density (n°/mm²)
Type II diabetics (mean ±SD)	35.62±10.40
Healthy subjects (mean ±SD)	17.55±3.88
Significance*	HS
P	0.000000986

* Significance (S) is setted in p<0.05. Values of p<0.001 are expression of High Significance (HS).

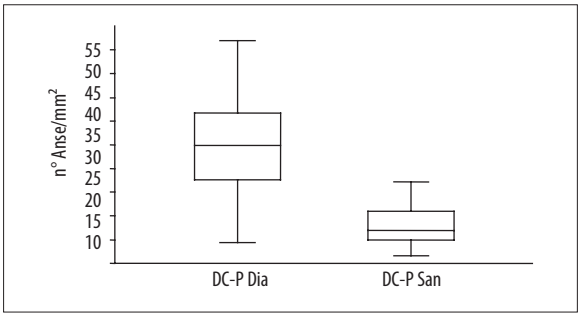

Figure 5. Box plot of capillary loop density on periodontal mucosa in diabetics and healthy subjects. * DC-P Dia (Capillary Loop Density in diabetics). ** DC-P San (Capillary Loop Density in healthy subjects).

density of the capillary loops (DC), defined as the number of loops per surface unit; this parameter, as has emerged in previous capillaroscopic studies on oral mucous, is the one most directly correlated to the inflammatory state of the tissues and to angiogenetic activity.

Results

Over 800 capillaroscopic images were examined in total (2 sites of investigation for each of the 80 subjects and a minimum of 5 images taken for each site investigated), and for each picture or capillaroscopic image a minimum of 5 measurements were taken, for a total of over 4000 measurements.

The measurements of the density (expressed in the number of loops/mm²) of the capillary loops presented differences between the healthy subjects and the diabetic subjects.

The data obtained was grouped together and submitted for statistical analysis to verify the significance of the differences between diabetic and healthy subjects. The test selected was Mann-Whitney, a test of statistical comparison for non-parametric ordinal data. The significance level selected was p<0.05. The differences between the groups with a value of p below 0.05 were considered statistically significant. The differences with p <0.001 were considered highly significant.

For all the measurements and the statistical analyses, we were aided by the computer software P.A.S.T., a *freeware* developed by Øyvind Hammer, D.A.T. Harper and P.D. Ryan in 1995 and updated to the latest version, *release* 1.97, in January 2010. The results of the statistical analysis were collected and are reproduced in Table 2.

The statistical analysis of the data selected presented highly significant variations in capillary density at the periodontal mucous level.

The average periodontal capillary density (DC-P) was clearly superior in diabetic subjects (35.62±10.40 n°loop/mm²) compared to healthy subjects (17.55±3.88 n°loop/mm²). The value of P, well below the level of significance (p=0.000000986), demonstrates the high significance of the result obtained (Table 2) (Figure 5).

Discussion

Diabetes mellitus has important effects on macrocirculation and on peripheral microcirculation, as demonstrated by the frequent cardiovascular pathologies and by diabetic microangiopathy, with its organ-specific and local effects [7–9]. The dysfunctions in microcirculation involve the arterioles and the capillaries of the retina and of the kidneys, the *vasa nervorum* and the *vasa vasorum*. They often lead to irreversible consequences such as blindness, renal dysfunction with the need for dialysis or organ transplant, peripheral neuropathy with alterations in sensitivity and, finally, peripheral vasculopathy with damage from altered trophism.

Periodontal disease has been recognized as the sixth-most important complication of diabetes mellitus. It has been demonstrated that the severity of periodontal disease increases with the duration of diabetes, and the chronic nature of periodontal infection may contribute to worsening of diabetes status leading to more severe diabetes complications [10]. The periodontal vasculature is profoundly affected during the progression of diabetes mellitus, and there is evidence that inflamed tissues enhance the expression of the various cytokines and growth factors that may regulate angiogenesis. Angiogenesis is defined as the process by which new blood vessels are produced by sprouting from established vessels. It occurs under physiological and pathological conditions, and can contribute to the degree of inflammation as a result of the ability of new blood vessels to transport proinflammatory cells to the lesion and to supply oxygen and nutrients to the inflamed tissues. Vascular endothelial growth factor (VEGF), an angiogenic growth factor, potently increases microvascular permeability, stimulates endothelial cell proliferation, and induces proteolytic enzyme expression and the migration of endothelial cells, monocytes and osteoblasts, all of which are essential for angiogenesis. A meta-analysis of data (Keles et al.) has demonstrated that diabetes mellitus increases the risk for periodontal disease 2-fold, independent of the effect of age and local factors.

Although type 2 diabetes has a significant genetic component, pre-existing obesity, and concomitant elevation in free fatty acids and pro-inflammatory cytokines increase risk. The impact of diabetes mellitus is multifold. Glycation of proteins (eg, hemoglobin, collagen) and accumulation of sorbitol and fructose (eg, in nerves, lens) contribute to tissue toxicity and oxidative stress.

The biological link between diabetes and periodontal disease has been established in a number of studies. Several

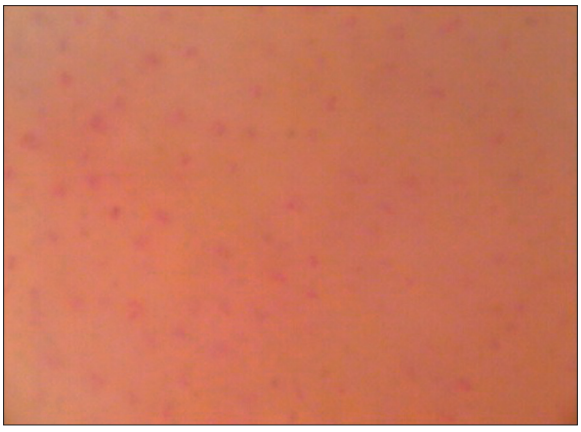

Figure 6. Capillaroscopic pattern of periodontal mucosa in healthy subject. 200× magnification.

Figure 7. Capillaroscopic pattern of periodontal mucosa in healthy subject. 200× magnification.

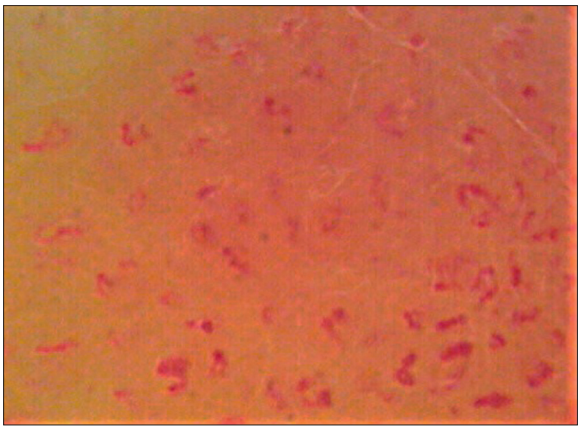

Figure 8. Capillaroscopic pattern of periodontal mucosa in diabetic patient. It's possible to see the characteristic leopard spot' morphology, with diffused microhaemorrhages and capillaries with a cockade pattern. 200× magnification.

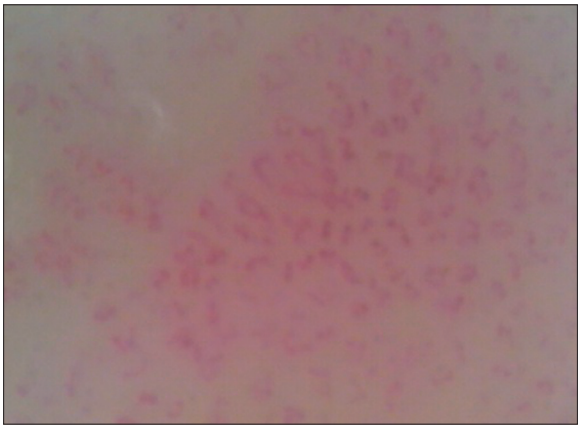

Figure 9. Capillaroscopic pattern of periodontal mucosa in diabetic patient. It's possible to see the increased loop density. 200× magnification.

different mechanisms have been proposed to explain this link: increased oxidative stress, advanced glycation end-products, altered immune function, and changes in collagen [11]. Oxidative stress occurs through a number of pathways, including the effects of hyperglycemia and of increased circulating free fatty acids. Hyperglycemia increases generation of superoxides in the mitochondria and increased levels of glucose increase the proton gradient across the inner mitochondrial membrane. When the gradient exceeds a threshold, electron transfer is blocked, leading to leakage of electrons from ubiquinone, with formation of superoxide. The formation of superoxide in turn mediates activation of the polyol pathway, the hexosamine pathway, protein kinase C, and the formation of advanced glycation end-products. Increased free fatty acid delivery to peripheral tissues seen in diabetes can activate inflammatory processes through activation of toll-like receptors. Although this mechanism of oxidative stress has been demonstrated in other tissue (eg, muscle), very little work has been done relating free fatty acids to increased inflammation in periodontal disease [12,13].

On the basis of these recent studies, aggressive periodontitis is now recognized as the sixth-most common complication of diabetes; according to Loe, multiple epidemiologic studies have demonstrated that both insulin-dependent diabetes mellitus (IDDM; type I) and noninsulin-dependent diabetes mellitus (type II) are predictors of periodontal disease when the systemic disease is poorly controlled. The study by Tsai confirms earlier epidemiologic studies.

Several mechanisms have been proposed that may explain how diabetes produces alterations in the organs and tissues, including the periodontium. Early studies demonstrated that the advanced glycation end-products (AGE), synthesized due to hyperglycemia, can convert macrophages into cells with a destructive phenotype, producing high levels of interleukin-1β, interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) [14,15].

Moreover, AGE have the capacity to increase the endothelium permeability and express high levels of molecular adhesion receptors. These changes could explain the greater susceptibility to infections and the delayed wound healing in diabetic subjects. This depressed immune response could explain why it may not be possible to eradicate periodontal infection totally in diabetics after conventional periodontal therapy. This might be one of the reasons why antibiotics may be suggested with mechanical therapy for diabetic

subjects, especially for uncontrolled cases. By contrast, in trying to determine the capacity of periodontal disease to adversely affect the control of diabetes by influencing glycemia levels, it has been hypothesized that chronic low-grade inflammations such as this might result in insulin resistance.

At a microscopic level, diabetic damage occurs in the endothelial cells that constitute the lining of the small and large vessels. The principal function of vasal endothelium is homeostasis through the synthesis and the release of a great variety of molecules and through pro-coagulative, vasoconstrictive and vasodilatatory activity; among these are found coagulation factors, prostacyclin, endothelin, prostaglandin and nitric oxide (NO). Together, these substances contribute to modulating vascular tone, permeability, coagulation and, in a wider sense, the reparative processes of the small vessels. An intact and perfectly functioning endothelium protects against the development of atheromatous plaques and constitutes the basis for the health of tissues. This protective barrier is strengthened by the maintenance of a smooth and homogeneous vasal surface that prevents thrombogenesis, the adhesion of monocytes, macrophages and platelets and the transportation of lipoproteins through the cell wall. In the presence of hyperglycemia, this barrier is destroyed and the reactive mechanisms become dysfunctional, resulting in micro- and macro-vascular complications. The dysfunction of vasal endothelium is also reflected at a capillaroscopic level, as seen in the results obtained.

Diabetes mellitus is a chronic pathology that causes progressive damage to peripheral microcirculation, rendered explicit by alterations in vascular patterns, as widely demonstrated by previous studies on conjunctival and periungual capillaroscopy and on retinal fundus examinations.

With regards to the vascular bed of the superficial periodontium (masticatory/gingival mucous), at a capillaroscopic level there is an increase in the number of capillary loops per mm^2, which is synonymous of strong angiogenic activity. The density of the vessels in diabetic subjects is approximately double that of healthy subjects.

Another characteristic of the periodontal capillary pattern in diabetic subjects is that there appears a 'leopard spot' morphology, with diffused microhemorrhages and capillaries with a cockade pattern. The capillaries, which are usually seen in capillaroscopic images of the site of masticatory mucous as pinheads, seem broader, almost forming little circles close to one another, (Figures 6–9). The increase in capillary density could suggest the presence of active inflammatory phenomena or, more probably, a tendency to a greater susceptibility to inflammatory phenomena. Several authors have stressed a greater propensity for periodontal disease in diabetic subjects, linking it to more or less visible subclinical signs and symptoms (eg, spontaneous or provoked bleeding, an increase in the flow of sulcular fluid, alteration of the composition of the sulcular fluid). Until now, the periodontal microcirculatory pattern in diabetic subjects has not been focussed on and objectified *in vivo* in the absence of a diagnosis of periodontal disease. It is evident that videocapillaroscopy applied to the study of the superficial periodontium is important for early diagnosis and monitoring of periodontal disease.

Conclusions

Several studies in recent years have been carried out to appraise *in vivo* the morphological alterations of the capillary bed in diabetic subjects using videocapillaroscopy, a sign of the need of the medical community to be able to 'stage' diabetic illness, monitor it, and make early diagnosis the damages correlated to it [16–19]. In the past this method was used to investigate the conjunctival bed and the ungual bed, and in both cases it succeeded in directly revealing alterations correlated to diabetes. The results obtained by the present study appear to be in accordance with the literature and add a new point of view to it.

The mouth has numerous advantages in terms of accessibility, non-invasivity and visibility in comparison to conjunctival mucous and the nailfold [20–23].

Ultimately, this study shows that there is some peripheral damage to microcirculation at the masticatory mucous level in diabetic subjects, and that such alterations are instrumentally objectifiable and quantifiable through the videocapillaroscopic method.

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