

Morphological changes of gums in occlusal trauma

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Abstract

Aim: Occlusal trauma causes major modifications of the coverage periodontium, which in turn reflect on the dental unit. The aim of the present study is to evaluate some of the morphological modifications occurred in the marginal periodontium surrounding teeth affected by occlusal trauma. **Materials and Methods:** Fragments of marginal periodontium coming from 51 patients with occlusal trauma were processed using classical histological techniques (formalin fixation and paraffin embedment) and stained with Hematoxylin–Eosin (HE), Masson's trichrome and anti-CD34 antibody immunostaining, in order to highlight the epithelial and connective tissue changes of gingival mucosa. A set of epithelial and connective tissue morphological parameters were assessed individually and based on three topographical criteria concerning the affected tooth. **Results:** The epithelium and especially its superficial compartment presented changes depending on the tooth type and the dental arch. Epithelial thickness had the tendency to decrease as the fibrosis in both corium compartments and vascular density in the deep corium compartment were increasing. Leukoplakia present around the affected teeth but not always was related with the tooth type and was more obvious as the superficial compartment of the epithelium was thicker and as fibrosis was more reduced in the papillary compartment of the corium. Vascular density reduced when fibrosis process increased in the corium. **Conclusions:** Lesions determined by occlusal trauma and their topography can and are influencing locally the different structures of the surrounding periodontium.

Keywords: occlusal trauma, gingival mucosa, morphology, histology.

Introduction

Any modification that occurs in the dental system reflects on each dental unit. The tooth, which is made of two components that closely relate and interact with each other, can suffer from alterations that, in time, disrupt the functionality of the dental system. The odontium, which is made of enamel, dentin and pulp, is in a close relationship with the periodontium, which in turn is comprised from the sustainment periodontium and the coverage periodontium.

Dysfunctional occlusion causes a variety of modifications in both components. The coverage periodontium is made out of the gingival mucosa and supraalveolar ligaments. The gingival mucosa covers the alveolar processes and surrounds the cervical region of the teeth. It is a well-known fact that occlusal trauma causes major modifications of the coverage periodontium, which in turn reflect on the dental unit. The consequences are determined mostly by the intensity of the force that acts on the tooth, the degree of implantation and, most of all, dental occlusion [1–4].

Specialty literature includes, as risk factors, either general dysmetabolic factors that alter the trophicity of the marginal periodontium, local disrupting or precipitating factors such as dental plaque, bacterial infiltration, as well as factors that alter regional trophicity following dysfunctional occlusion caused by premature contact and occlusal interferences.

Occlusal trauma causes and aggravates epithelial retraction by breakage of fibrous collagen structures, accelerating thus the vascular modifications of the respective tooth [5–8].

This present study tries to assess some of the morphological changes of the marginal periodontium determined by occlusal trauma.

Materials and Methods

The basis of this study is represented by a group of 51 patients with occlusal trauma, from which marginal periodontium was recovered and investigated.

The study material was represented by two categories of data sources: the medical documents of the patients

from the dentistry and the biological material consisting of fragments from marginal periodontium obtained from patients that were treated for occlusal trauma.

The study was a prospective one and consisted of histological examination of marginal periodontium fragments on slides stained with Hematoxylin–Eosin (HE), Masson's trichrome and anti-CD34 antibody immunostaining.

The investigated parameters of the morphological status of the marginal periodontium included:

- Epithelial compartment (E T) with:
 - Superficial epithelium (E Sup);
 - Papillary epithelium (E Pap);
 - E Pap/E Sup ratio.
- Leukoplakia (LkP);
- Interstitial connective tissue compartment with the percentages of:
 - Papillary fibrosis (F Pap);
 - Deep interstitial fibrosis (F Prof);
 - Papillary vascular density (VD Pap);
 - Deep vascular density (VD Prof).

where:

- “Papillary” meant the areas of connective tissue placed between epithelial spurs and
- “Deep interstitial” meant the areas of connective tissue placed below the epithelial spurs.

Each of the morphological parameters was assessed in relation with three topographic parameters of the lesions determined by occlusal trauma, namely:

- Affected tooth, meaning incisive (I), premolar (PM) and molar (M);
- Location of the tooth on the dental arch, meaning maxillary (MAX) and mandible (MDB);
- Location of the tooth according to the mediosagittal plane, meaning left side (L) and right side (R).

Acquisition, processing and morphometric determinations were done using specialized software analysis Pro, ACDSee 4.0, Aperio ImageScope [v12.3.2.8013] and a morphometry module designed in the MATLAB (Mathworks) programme.

All the data obtained was introduced and processed using Microsoft Excel module of the Microsoft Office 2010 Professional software along with the XLSTAT add-in program for MS Excel.

The algorithm for statistical analysis contained, for numerical parameters, determination of the minimum (VMIN), maximum (VMAX) values, and the mean value (AV), the standard deviation (STDEV) and variance (Var).

The numerical values of the evaluated parameters were stratified in classes, thus obtaining score scales for each parameter (Tables 1–4).

The statistical tests were Student *t*/Wilcoxon, Pearson's and “ χ^2 ”/Fisher. Diagrams (graphs) illustrating evolution tendencies of different assessed parameters as well as statistical comparisons between them have been done with the help of the “Graph” instrument from “Word” and “Excel” modules of the Microsoft Office 2010 Professional software suite and the XLSTAT 2009 add-on for the Excel module.

Table 1 – Epithelial score by classes of values

Epithelial score	Description
E1	$E < 100 \mu\text{m}$
E2	$100 \mu\text{m} < E < 200 \mu\text{m}$
E3	$200 \mu\text{m} < E < 300 \mu\text{m}$
E4	$300 \mu\text{m} < E < 400 \mu\text{m}$
E5	$400 \mu\text{m} < E < 500 \mu\text{m}$
E6	$500 \mu\text{m} < E$

Table 2 – Leukoplakia score by classes of values

Leukoplakia score	Description
L0	Absence of leukoplakia
L1	$0 \mu\text{m} < L < 10 \mu\text{m}$
L2	$10 \mu\text{m} < L < 20 \mu\text{m}$
L3	$20 \mu\text{m} < L < 30 \mu\text{m}$
L4	$30 \mu\text{m} < L$

Table 3 – Fibrosis score by classes of values

Fibrosis score	Description
F1	$F < 20\%$
F2	$20\% < F < 40\%$
F3	$40\% < F < 60\%$
F4	$60\% < F$

Table 4 – Vascular density score by classes of values

Vascular density score	Description
VD1	$VD < 50/\text{mm}^2$
VD2	$50/\text{mm}^2 < VD < 100/\text{mm}^2$
VD3	$100/\text{mm}^2 < VD < 150/\text{mm}^2$
VD4	$150/\text{mm}^2 < VD < 500/\text{mm}^2$
VD5	$200/\text{mm}^2 < VD$

Results

Topographical assessment

The most affected tooth of the studied series was the molar. At the tooth subtype level, the second molar, the second premolar and the lateral incisor were the most affected teeth, in a decreasing order (Figure 1a).

The teeth with lesions caused by occlusal trauma belonged more often to the mandibular arch (Figure 1b) and had no obvious predilection for one of the sides of the dental arches (Figure 1c).

There was no significant difference between the teeth distributions on the two dental arches in the sense that molars were the most affected teeth both on maxillary and mandible (around half of the cases), followed by premolars with around one third of the cases (Figure 2a).

In turn, there was a difference between the distributions of affected teeth in relation to the mediosagittal plane. Thus, molars represented almost 60% of the affected teeth of the left side hemiarches while the premolars represented almost half of the affected teeth on the right side hemiarches (Figure 2b).

Finally, the affected teeth were slightly predominant on the left side of the maxillary whereas there was no predilection for the left or the right side of the mandible arch (Figure 2c).

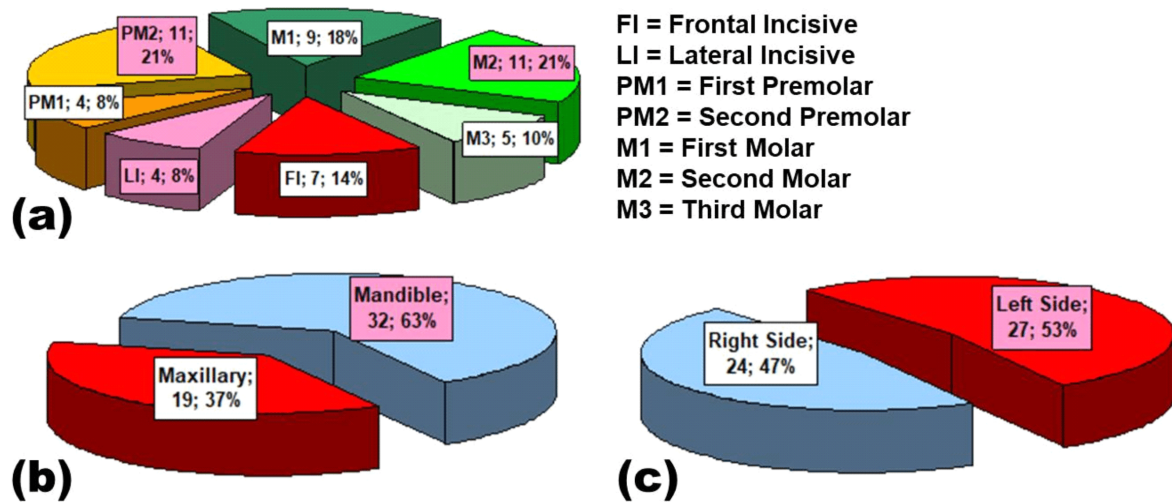


Figure 1 – Distribution of the teeth with lesions caused by occlusal trauma.

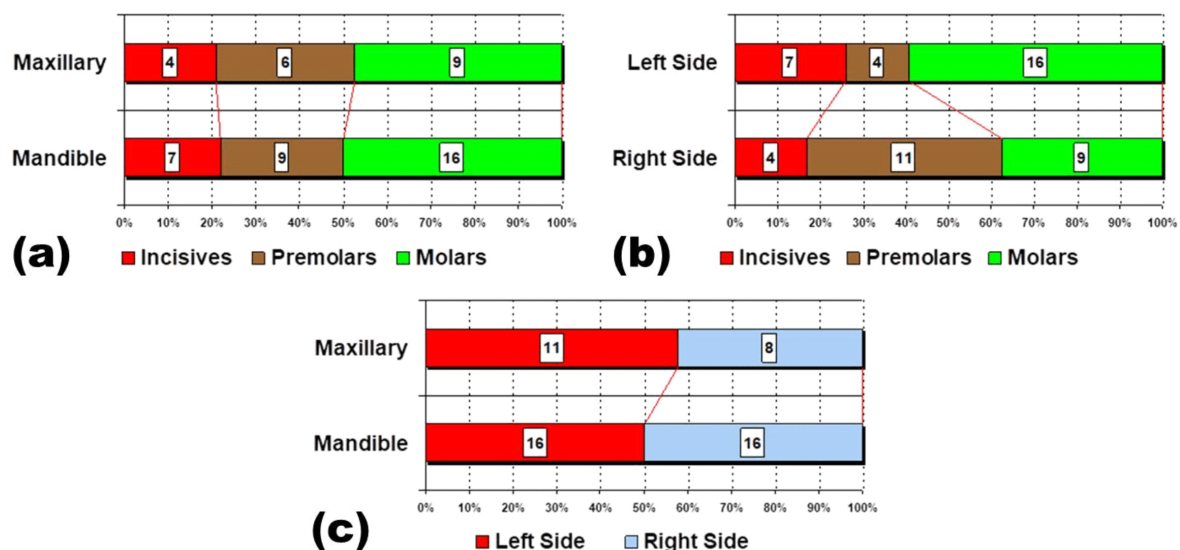


Figure 2 – Topographical distribution of the teeth with lesions caused by occlusal trauma: (a) According to the dental arch; (b) According to sagittal plane; (c) According to both dental arch and sagittal plane.

The epithelial compartment

The thickness of the gingival mucosa epithelial compartment has varied within a wide range of values (0.25–1.32 mm) but the large majority of determinations grouped in a much narrower range defined by a STDEV of 0.19 around an average value of almost 0.5 mm. Even the whole range was quite large, the range of most values was pushed to the lower limit of the interval by the above-mentioned mean value (Figures 3 and 4).

The thickness of the epithelial superficial compartment represented usually one third of the whole epithelium thickness and the thickness of the deep, papillary compartment represented the other two thirds of the whole gingival epithelium.

The range of values was narrow for the superficial compartment but very large for the papillary compartment.

However, in both cases, the range that comprised most of the values, established by the standard deviation (STDEV) around the mean value (AV) was narrower and pushed to the lower limit of the whole range of values by the mean values of the thickness of both epithelial compartments.

The average value of the ratio between the deep and the superficial compartments of the gingival epithelium was around “2” (Figure 3).

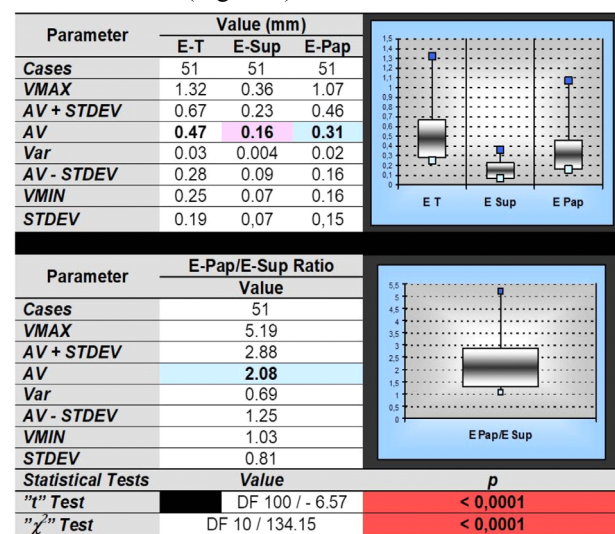


Figure 3 – Statistical assessment of different compartments of the gingival mucosa epithelium.

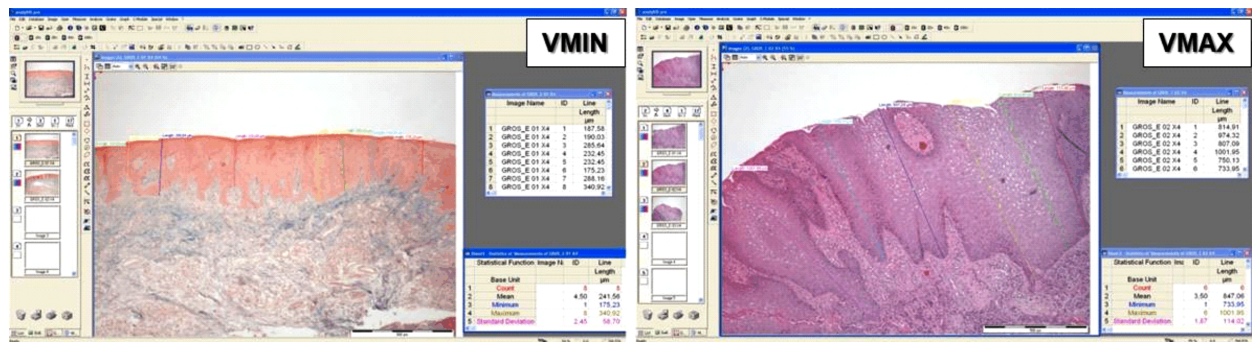


Figure 4 – Assessment of the thicknesses of different compartments of the gingival mucosa epithelium using analySIS Pro software.

The differences between the thickness of the two compartments were confirmed by the “ χ^2 ” test, after the dividing into classes of values of the thickness following the score scale described above.

Thus, while superficial compartment had in most of the cases a mean thickness between 100 μ m and 200 μ m, papillary compartment had in around 40% of the cases a mean thickness between 200 μ m and 300 μ m, and in one quarter of the cases a mean thickness between 300 μ m and 400 μ m (Figure 5).

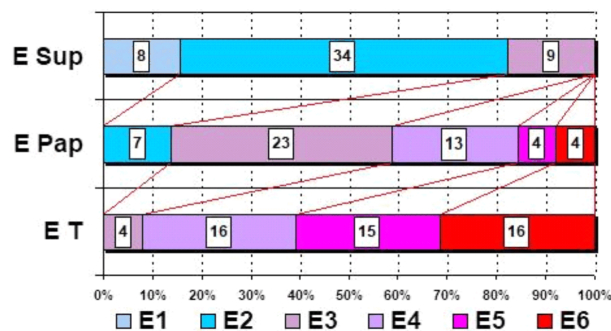


Figure 5 – Weight of the thickness classes in the two compartments of the gingival epithelium.

Assessment based on tooth type

The assessment of gingival epithelium thickness showed that both as a whole and at the compartment level the epithelium had a tendency to thicken from the front group to the lateral groups of teeth, keeping an over “1” value of the E Pap/E Sup ratio in each group of teeth (Figure 6).

Assessment based on dental arch

On both dental arches, the value of the E Pap/E Sup ratio was over “1” and, both as a whole and at the compartment level, the epithelium had a tendency to be more thickened on the mandible than on the maxillary, trend not confirmed by the “ χ^2 ” test (Figure 7).

Assessment based on sagittal plane

The value of the E Pap/E Sup ratio was also over “1” on left and right side of the sagittal plane but with no differences when compared the epithelial compartments of the same type (Figure 8).

“Leukoplakia” phenomenon

The phenomenon of squamous metaplasia or leukoplakia was present in the gingival mucosa epithelium in most of cases but not in all. In many cases, the superficial

layer of anucleate, keratinized cells had between 10 μ m and 20 μ m, however one quarter of the patients had metaplastic areas thicker than 20 μ m and even than 30 μ m (Figures 9 and 10).

The thickness of the layer of leukoplakia has varied within a wide range of values (0–40 μ m) but the large majority of determinations grouped in a much narrower range defined by a STDEV of 6 μ m, around an average value of almost 15 μ m.

Although the whole range was quite large, the range of most values was pushed to the lower limit of the interval by the above-mentioned mean value (Figure 10).

The leukoplakia layer mean value revealed a direct correlation with the mean values of both epithelial compartments (superficial and papillary) but this correlation was statistically validated by Pearson’s test only in the case of the superficial compartment of the gingival mucosa layer (Figures 10 and 11).

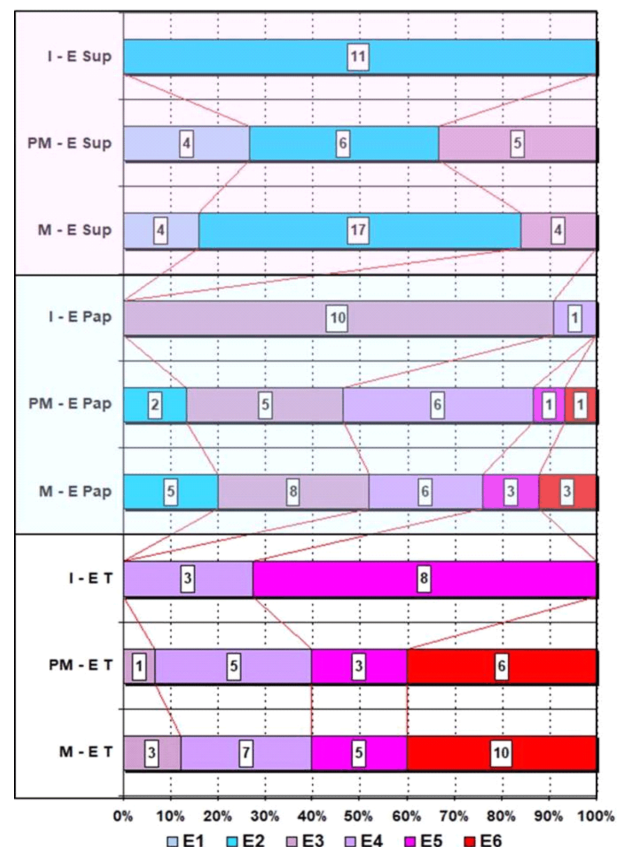


Figure 6 – Weight of the thickness classes in the two compartments of the gingival epithelium based on tooth type.

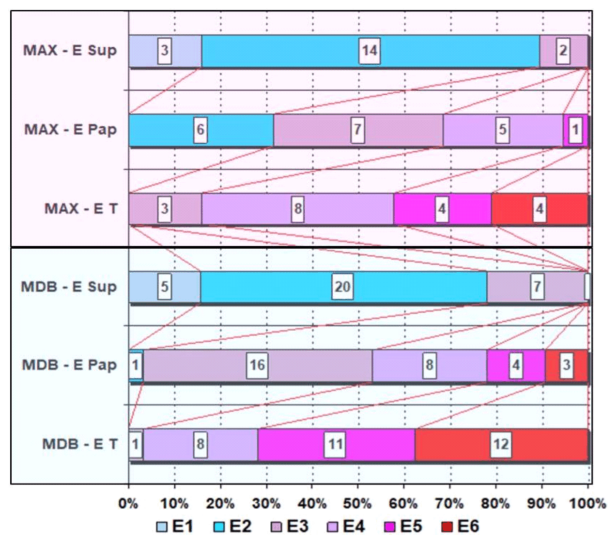


Figure 7 – Weight of the thickness classes in the two compartments of the gingival epithelium based on dental arch.

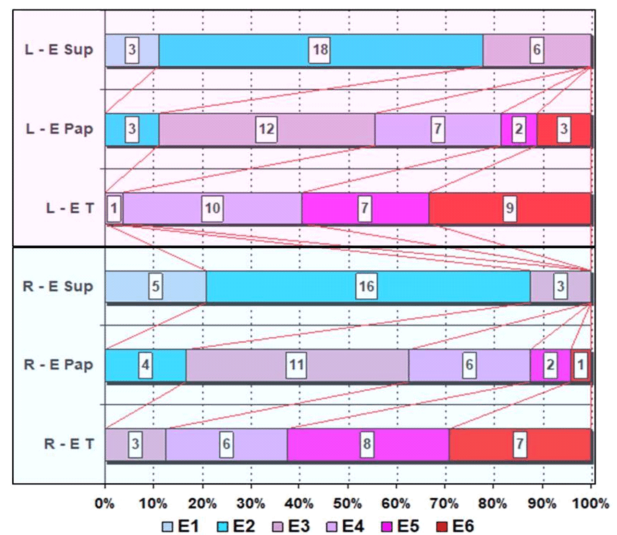


Figure 8 – Weight of the thickness classes in the two compartments of the gingival epithelium based on mediosagittal plane.

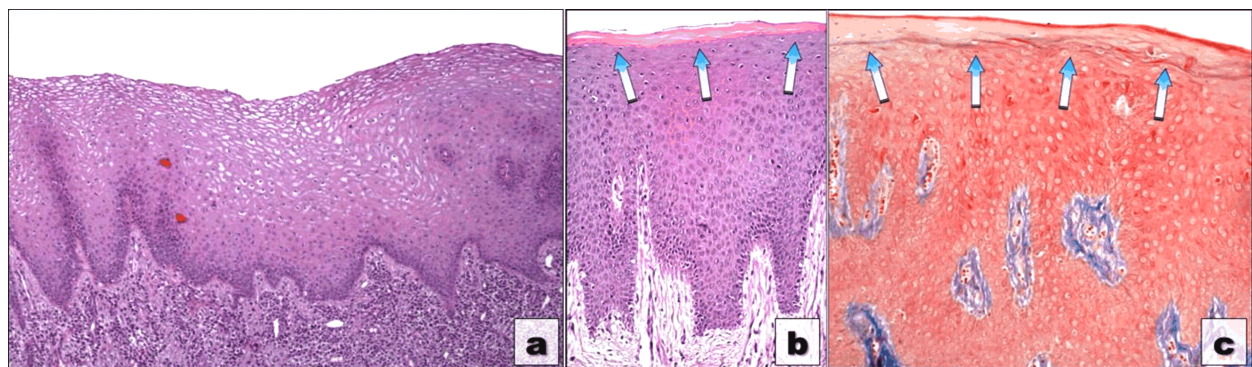


Figure 9 – Gingival mucosa epithelium: (a) Without leukoplakia; (b and c) With evident leukoplakia – blue arrows. HE staining: (a) $\times 40$; (b) $\times 100$. Masson's trichrome staining: (c) $\times 100$.

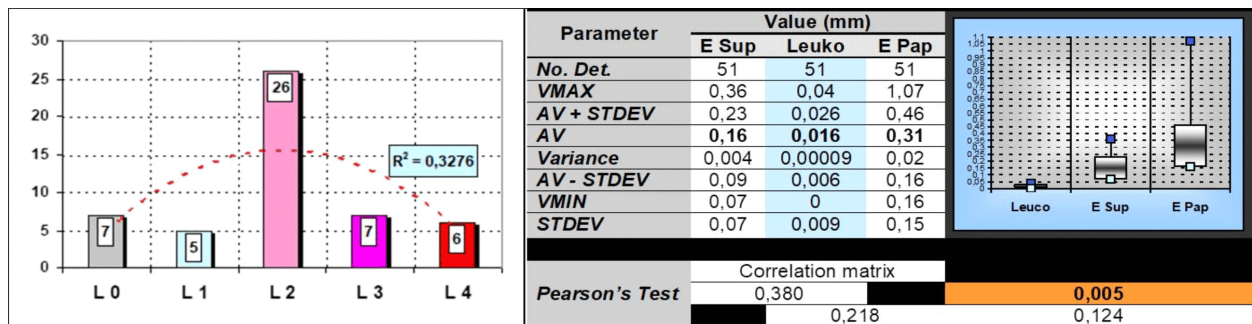


Figure 10 – Value distribution and statistical assessment of leukoplakia layer thickness.

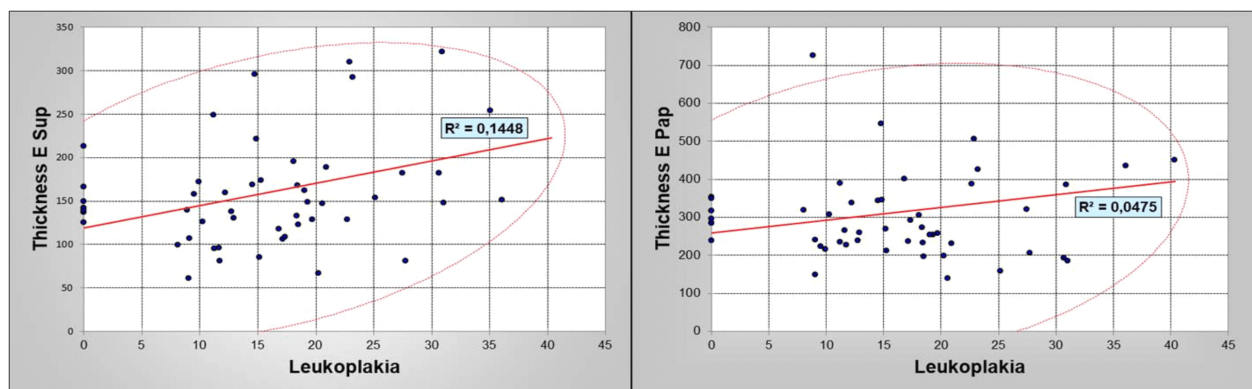


Figure 11 – Graph representation of Pearson's correlation tests between mean values of leukoplakia layer compartments of gingival mucosa epithelium.

Assessment based on tooth type

The presence of squamous metaplastic lesions became more obvious from the front group, where one third of the patients did not present it to the lateral groups, where, at the molar group level, for instance, the lesion was more severe, with thickness higher than 20 μm (Figure 12).

This trend was statistically validated by the " χ^2 " test.

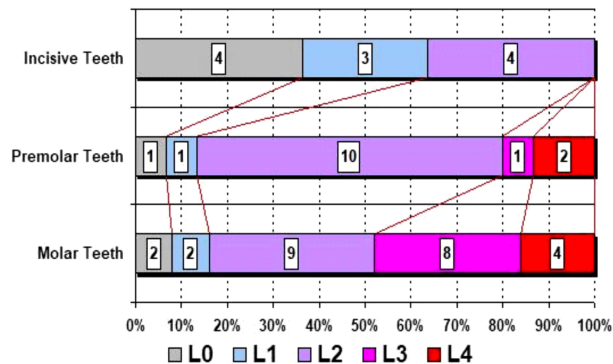


Figure 12 – Weight of the thickness classes in of the leukoplakia layer based on tooth type.

Assessment based on dental arch

The distribution patterns of mean values of the metaplastic layer were almost similar to the two dental arches with a slight predominance of the mild lesions (less than 20 μm) to the mandibular arch (Figure 13).

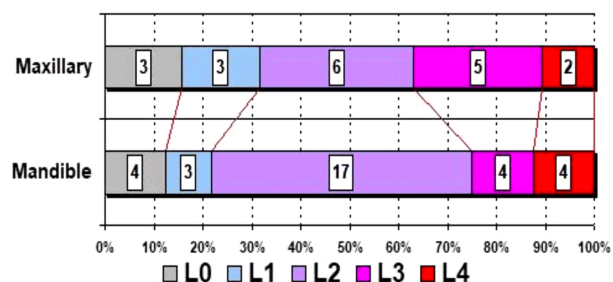


Figure 13 – Weight of the thickness classes in of the leukoplakia layer based on dental arch.

Assessment based on sagittal plane

The same situation was observed with regard to the sagittal plane too, the mild lesions having a slight predominance on the right side (Figure 14).

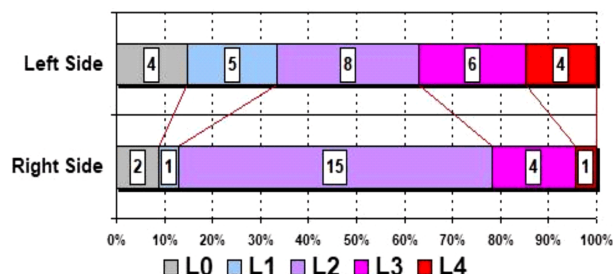


Figure 14 – Weight of the thickness classes in of the leukoplakia layer based on mediosagittal plane.

Gingival fibrosis

The assessment of the amount of mature collagen fibers within the corium of gingival mucosa revealed

that the fibrosis process was more pronounced in the deep compartment of the corium, difference validated by the Student's t -test (Figures 15, 18 and 19).

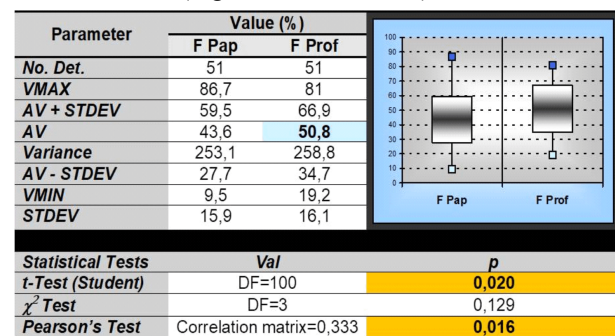


Figure 15 – Statistical assessment of fibrosis score in different compartments of the interstitial connective tissue.

The ratio between deep fibrosis and papillary fibrosis had, in general, a mean value higher than "1". However, almost one third of the cases had a ratio value lower than "1".

That meant that in the majority of cases the fibrosis process was more pronounced in the deep compartment of the epithelial corium, there were situations where fibrosis was more pronounced in the papillary compartment of the corium (Figure 16).

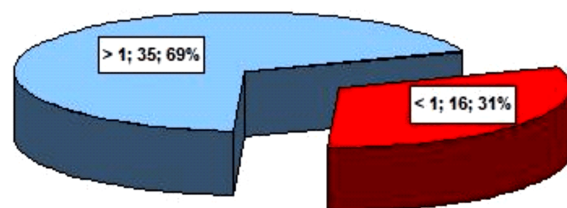


Figure 16 – Distribution of F Prof/F Pap ratio values.

Another interesting aspect was the result of the assessment of the correlation between the fibrosis amounts in the two compartments of the epithelial corium of the gingival mucosa using Pearson's correlation test. The statistical test revealed that there is a direct correlation between the ways the fibrosis process evolves in the two compartments of the gingival epithelium corium (Figure 17).

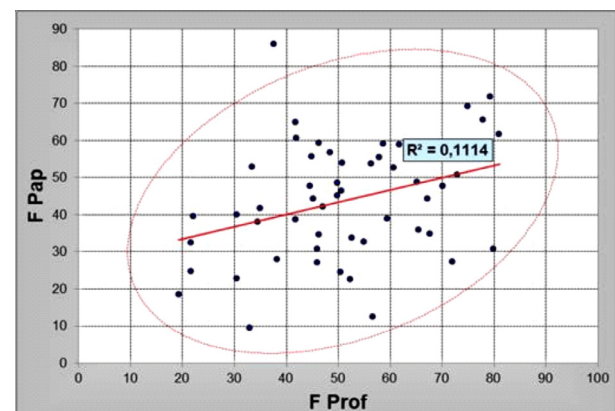


Figure 17 – Graph representation of Pearson's correlation test between deep and papillary fibrosis.

In other words, fibrosis becomes more or less pronounced in both compartments in the same time.

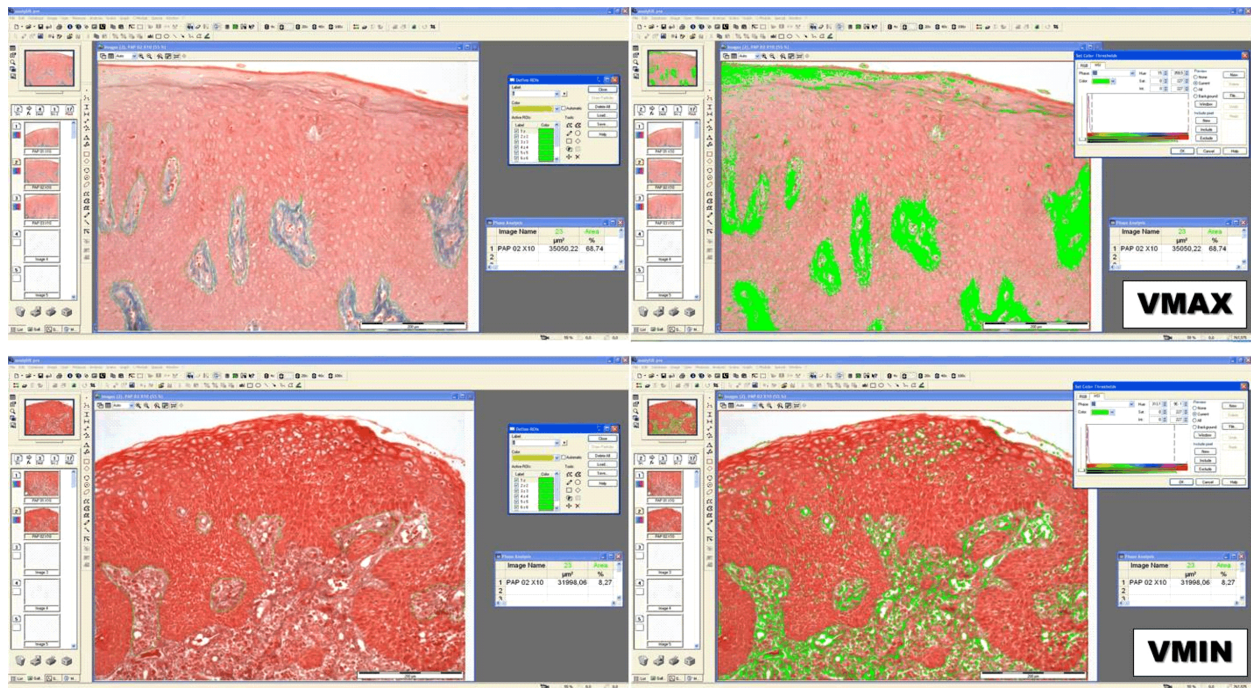


Figure 18 – Determination of papillary fibrosis score using analySIS Pro software on images acquired with $\times 10$ objective from histological samples stained with Masson's trichrome.

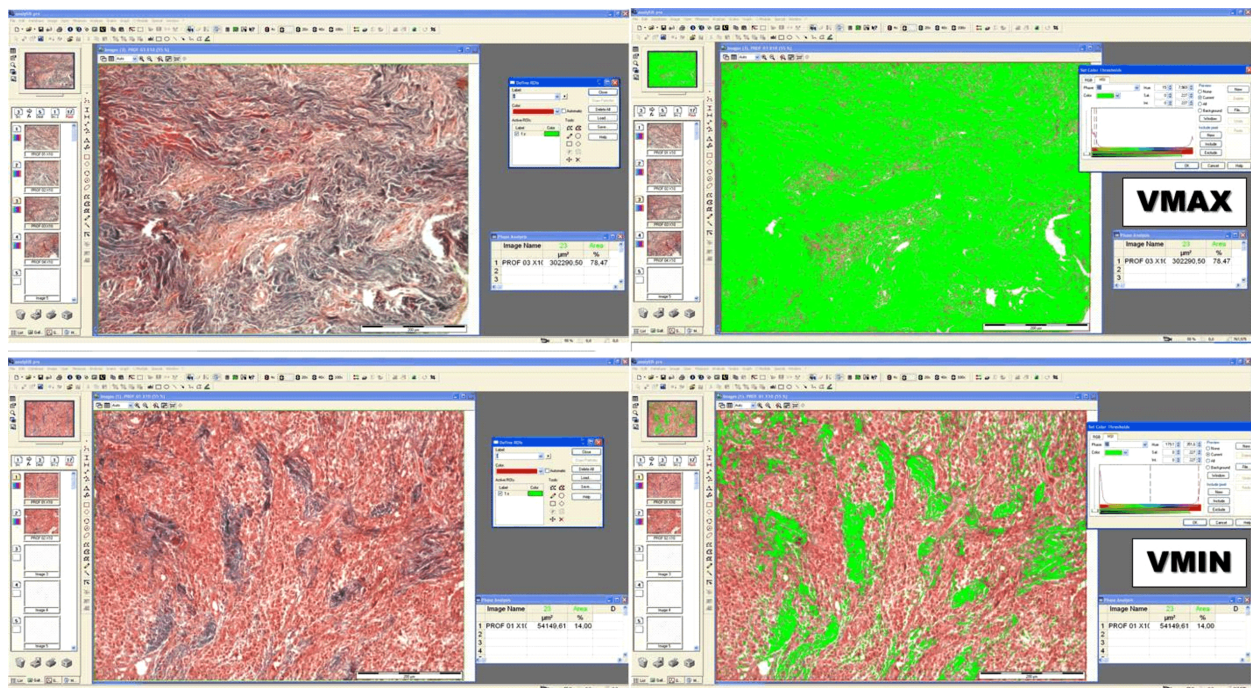


Figure 19 – Determination of deep fibrosis score using analySIS Pro software on images acquired with $\times 10$ objective from histological samples stained with Masson's trichrome.

Assessment based on tooth type

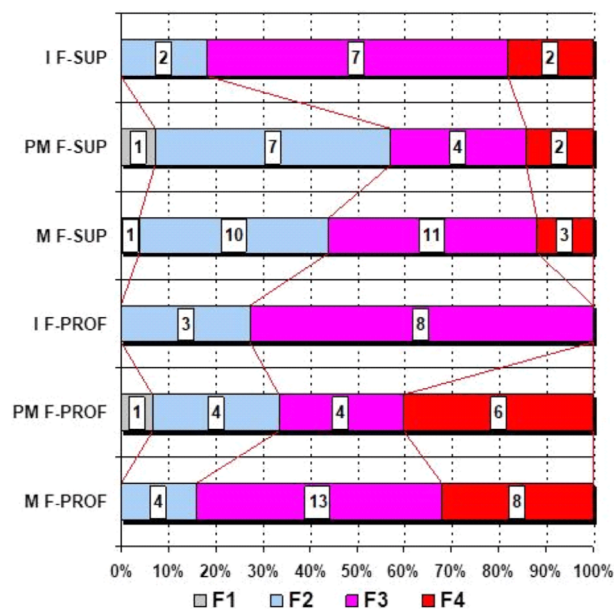
The assessment of the fibrosis process based on the tooth type revealed its tendency to become more pronounced from superficial compartment towards deep compartment and from frontal groups of teeth towards lateral groups of teeth. Thus, fibrosis was more pronounced in deep compartment of corium of the gingival mucosa surrounding the premolars and molars as compared with that in the papillary compartment. On the other hand, fibrosis was more pronounced both superficially and deeply in the corium of gingival mucosa surrounding

the molars as compared to the premolars (Table 5; Figure 20).

In the corium of gingival mucosa surrounding the incisive teeth, fibrosis had a different behavior. Thus, it was more pronounced in the superficial compartment as compared to the deep one. In the same time, the mean value of fibrosis amount in the superficial compartment of the corium of the gingival mucosa surrounding the incisive teeth was almost equal to that of the deep compartment of the corium of the gingival mucosa surrounding the premolars, which was the highest from the entire group of patients (Table 5; Figure 20).

Table 5 – Statistical assessment of fibrosis values based on tooth type

Parameter	Value (%)					
	Incisives		Premolars		Molars	
	F Sup	F Prof	F Sup	F Prof	F Sup	F Prof
No. Det.	11	11	15	15	25	25
VMAX	86	58,6	71,7	79,9	69,3	81
AV + STDEV	68,8	52,5	56,9	70	55,5	69,9
AV	53,9	44,4	39,6	49,9	41,4	54,2
Variance	223	64,2	298,9	401,9	196,7	247
AV - STDEV	39	36,4	22,4	29,9	27,4	38,5
VMIN	30,7	30,38	9,5	19,24	12,4	21,7
STDEV	14,9	8	22,4	29,9	14	15,7
Statistical Tests						
Student Test	0,078		0,144		0,004	
Pearson Test	0,739		0,076		0,009	
χ^2 /Fisher Test	0,635 (F)		0,425		0,090 (F)	

**Figure 20 – Weight of the fibrosis classes in the two compartments of the corium based on tooth type.**

The Pearson's correlation test revealed a trend of parallel evolution between the way the fibrosis process evolves in the two compartments of the gingival epithelial corium and, moreover, this trend was more obvious

from the frontal groups towards the lateral ones, being statistically validated, however, only for the gingival mucosa surrounding the molars (Figure 21).

Assessment based on dental arch

The assessment of the fibrosis process based on dental arch revealed, on one hand, that the fibrosis was more pronounced in deep compartment of corium of the gingival mucosa surrounding affected teeth as compared with superficial, papillary compartment on both dental arches and, on the other hand, that fibrosis was more pronounced in both compartments of the maxillary as compared with the mandible (Table 6, left).

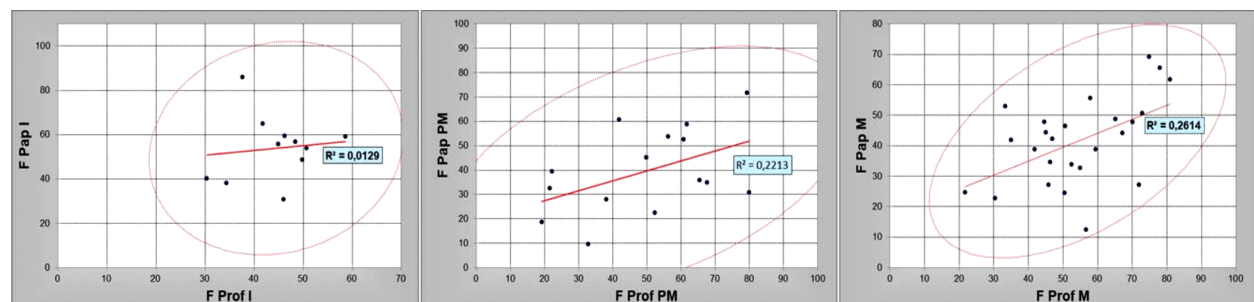
The Pearson's correlation test revealed a trend of parallel evolution between the way the fibrosis process evolves in the two compartments of the gingival epithelial corium on both dental arches but this trend was not statistically validated (Figure 22).

Assessment based on sagittal plane

The assessment of the fibrosis process based on sagittal plane revealed also, on one hand, that the fibrosis was more pronounced in deep compartment of corium of the gingival mucosa surrounding affected teeth as compared with superficial, papillary compartment on both sides of the sagittal plane.

On the other hand, the comparative analysis of fibrosis process between the two compartments of corium of the gingival mucosa surrounding affected teeth showed a dissociated pattern in the sense that while in the superficial compartment fibrosis was more pronounced on the left side, in the deep compartment, fibrosis was more pronounced on the right side (Table 6, right).

The Pearson's correlation test revealed also a trend of parallel evolution between the way the fibrosis process evolves in the two compartments of the gingival epithelial corium on both sides of the sagittal plane but this trend too was not statistically validated (Figure 23).

**Figure 21 – Graph representation of Pearson's correlation test between papillary and deep fibrosis based on tooth type.****Table 6 – Statistical assessment of fibrosis value: left – based on dental arch; right – based on sagittal plane**

Parameter	Value (%)				Parameter	Value (%)			
	Maxillary		Mandible			Left Side		Right Side	
	F Sup	F Prof	F Sup	F Prof		F Sup Stg	F Prof Stg	F Sup Dr	F Prof Dr
No. Det.	19	19	32	32	No. Det.	27	27	24	24
VMAX	86	81	69,27	79,9	VMAX	86	81	71,7	79,9
AV + STDEV	66,1	68,6	55,19	66,1	AV + STDEV	62,1	64,5	56,3	69,7
AV	47,3	52	41,4	50,17	AV	46,6	49,1	40,3	52,8
Variance	354,5	227,5	189,17	255	Variance	240,9	236,4	256	288
AV - STDEV	28,5	35,3	27,68	34,2	AV - STDEV	31	33,7	24,3	35,8
VMIN	9,5	21,7	12,4	19,2	VMIN	9,5	22,1	12,4	19,2
STDEV	18,8	16,6	13,7	15,9	STDEV	15,5	15,4	16	16,9
Statistical Tests	Val		p		Statistical Tests	Val		p	
Student Test	0,423		0,022		Student Test	0,547		0,011	
Pearson Test	0,060		0,176		Pearson Test	0,150		0,028	

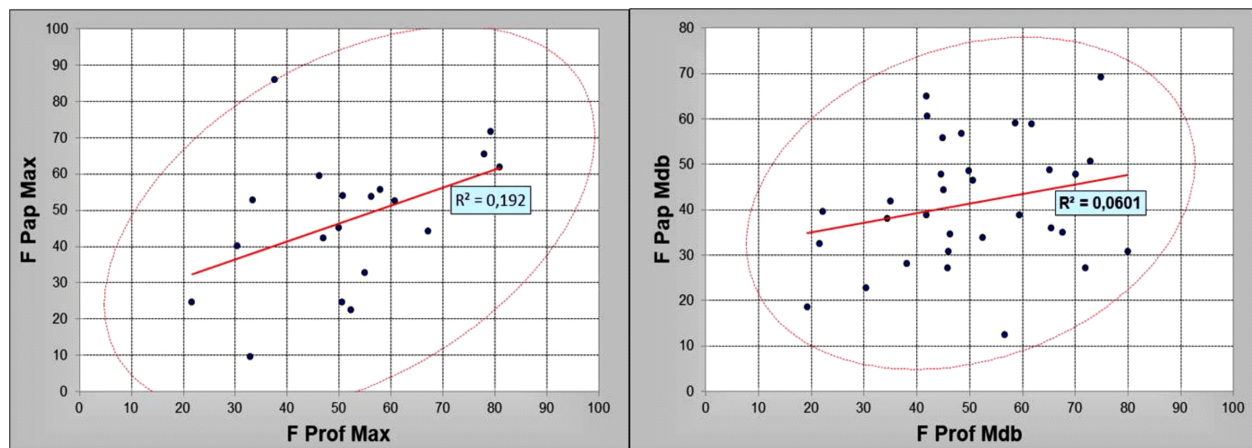


Figure 22 – Graph representation of Pearson's correlation test between papillary and deep fibrosis based on dental arch.

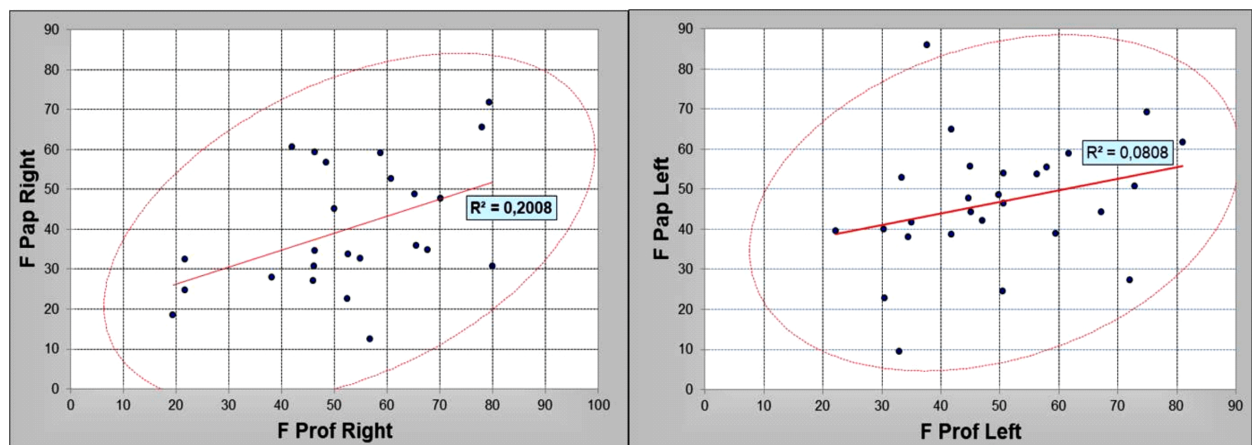


Figure 23 – Graph representation of Pearson's correlation test between papillary and deep fibrosis based on sagittal plane.

Vascular density

Vascular density was, on the whole, more reduced in papillary compartment of the gingival mucosa corium, with one quarter of the patients having values less than 50 capillaries/mm² than in the deep compartment where more than 40% of the patients had values bigger than 100 capillaries/mm² (Figures 24 and 25).

In the majority of cases, the ratio between vascular densities of the deep and papillary compartment had a value higher than "1". However, in less than 15% of the patients, the ratio had a value lower than "1", meaning a higher vascular density in the superficial, papillary compartment (Figure 26).

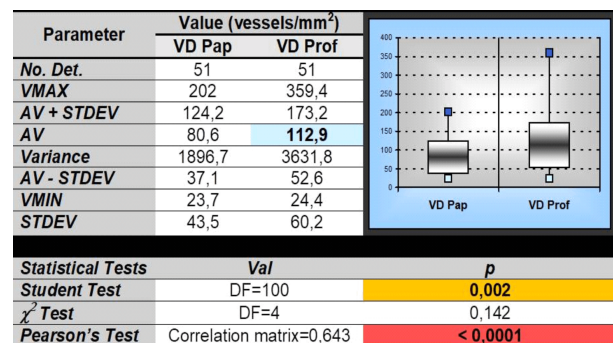


Figure 24 – Statistical assessment of vascular density values in different compartments of the interstitial connective tissue.



Figure 25 – Determination of vascular density score using a morphometry module designed in the MATLAB (Mathworks) program on images acquired with ×10 objective from histological samples immunostained with anti-CD34 antibody.

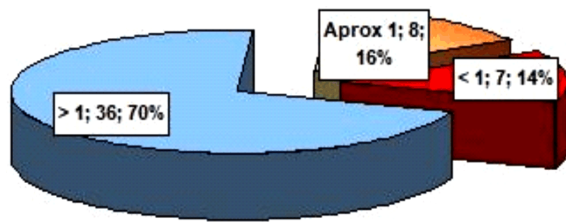


Figure 26 – Distribution of VD Prof/VD Pap ratio values.

Pearson's correlation test revealed a direct correlation between the way the vascular density evolves in the two compartments of the gingival epithelium corium, correlation also validated from statistical point of view. In other words, vascular density becomes more or less pronounced in both compartments in the same time (Figure 27).

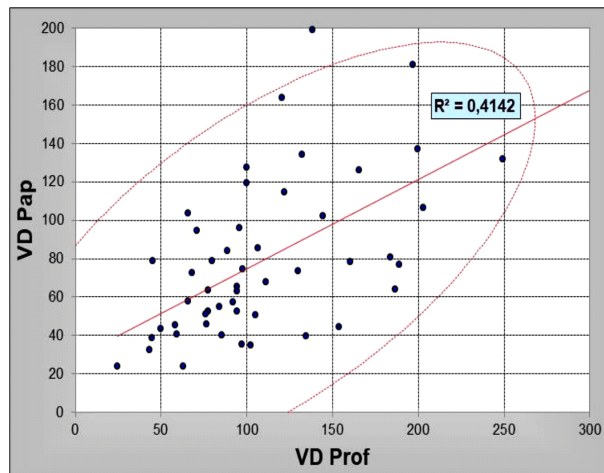


Figure 27 – Graph representation of Pearson's correlation test between papillary and deep vascular densities.

Assessment based on tooth type

The assessment of the vascular density based on the tooth type revealed a tendency to become more pronounced from superficial compartment towards deep compartment of the gingival mucosa corium surrounding each type of tooth. This trend was however statistically validated only for the premolar group (Table 7). One can talk also here, like in the case of the fibrosis process, about a general decreasing trend of the vascular density values from the frontal towards the lateral groups of teeth. In the case of vascular density too, the papillary compartment of gingival mucosa corium surrounding the incisive teeth had

a more rich vascular density than the similar compartments surrounding the lateral groups of teeth.

Despite this, there is also an increasing trend towards the lateral groups of teeth but it is more attenuated than in the case of the fibrosis process (Figure 28).

The Pearson's correlation test revealed also in the case of vascular density a trend of parallel evolution between the way it evolves in the two compartments of the gingival epithelial corium and, moreover, this trend was more obvious from the frontal groups towards the lateral ones, being statistically validated, however, only for the gingival mucosa surrounding the molars (Figure 29).

Table 7 – Statistical assessment of vascular density values based on tooth type

Parameter	Value (vessels/mm ²)					
	Incisive teeth		Premolars		Molars	
	VD Sup	VD Prof	VD Sup	VD Prof	VD Sup	VD Prof
No. Det.	11	11	15	15	25	25
VMAX	134,1	188,9	163,9	199,4	202	359,4
AV + STDEV	119,5	155	121,8	161,7	127,5	186,5
AV	89,1	109,7	78,7	115,9	78	112,5
Variance	926,3	2057,5	1854,5	2094,7	2441,8	5476,4
AV - STDEV	58,7	64,3	35,7	70,2	28,6	38,5
VMIN	38,9	44,6	24	58,2	23,7	24,4
STDEV	30,4	45,3	43	45,7	49,4	74
Statistical Tests						
Student Test	0,225		0,029		0,058	
χ ² Test	0,632 (F)		0,030 (F)		0,784	
Pearson's Test	0,196		0,182		< 0,0001	

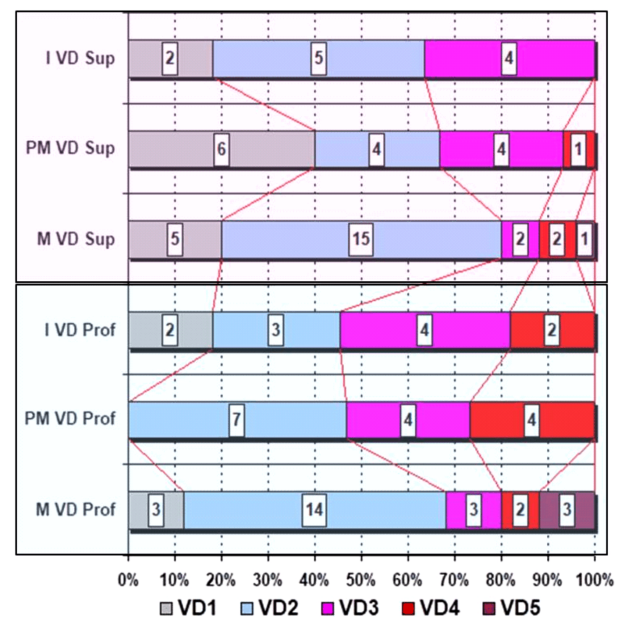


Figure 28 – Weight of the vascular density classes in the two compartments of the corium based on tooth type.

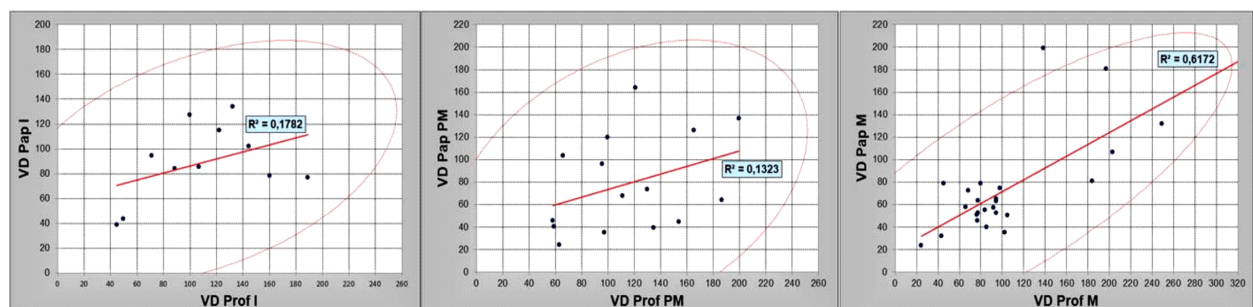


Figure 29 – Graph representation of Pearson's correlation test between papillary and deep vascular densities based on tooth type.

Assessment based on dental arch

The assessment of the vascular density based on dental arch revealed that it was more pronounced in deep compartment of corium of the gingival mucosa surrounding affected teeth as compared with superficial, papillary compartment on both dental arches.

However, the comparative analysis of vascular densities between each type of compartment of corium of the gingival mucosa surrounding affected teeth on the two dental arches showed a dissociated pattern in the sense that while in the case of the superficial compartment, the vascular density was more pronounced on the mandible, in the case of deep compartment, the vascular density was more pronounced on the maxillary (Table 8).

The Pearson's correlation test revealed a trend of parallel evolution between the ways the vascular density

evolves in the two compartments of the gingival epithelial corium on both dental arches, trend that was also statistically validated (Figure 30).

Table 8 – Statistical assessment of vascular density values based on dental arch

Parameter	Value (vessels/mm ²)			
	Maxillary		Mandible	
	VD Sup	VD Prof	VD Sup	VD Prof
No. Det.	19	19	32	32
VMAX	136,9	249,2	202	359,4
AV + STDEV	110,7	178,5	131,7	170,5
AV	77,1	119,9	82,8	108,8
Variance	1126,1	3437,1	2393,1	3814,1
AV - STDEV	43,5	61,3	33,8	47
VMIN	35	44,6	23,7	24,4
STDEV	33,5	58,6	48,9	61,7
Statistical Tests				
Wilcoxon/t-Test	p		p	
Pearson's Test	0,009		0,003 (W)	
	0,002		< 0,0001	

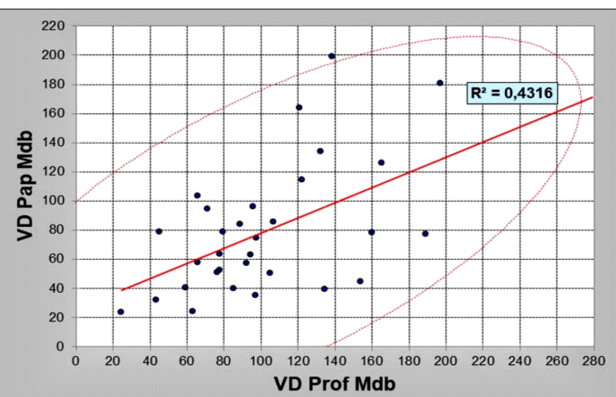
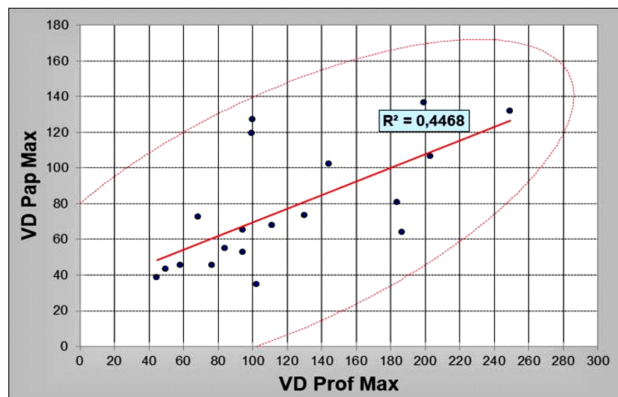


Figure 30 – Graph representation of Pearson's correlation test between papillary and deep vascular densities based on tooth type.

Assessment based on sagittal plane

The assessment of the vascular density based on sagittal plane revealed also, on one hand, that the vascular network was more pronounced in deep compartment of corium of the gingival mucosa surrounding affected teeth as compared with superficial, papillary compartment on both sides of the sagittal plane. Also, the vascular network was more dense in both compartments of corium of the gingival mucosa surrounding affected teeth placed on the left side as compared with those placed on the right side (Table 9).

The Pearson's correlation test revealed a trend of parallel evolution between the ways the vascular density evolves in the two compartments of the gingival epithelial

corium on both sides of the sagittal plane, trend that was also statistically validated (Figure 31).

Table 9 – Vascular density values according to the dental arch

Parameter	Value (vessels/mm ²)			
	Left side		Right side	
	VD Sup	VD Prof	VD Sup	VD Prof
No. Det.	27	27	24	24
VMAX	202	359,4	163,9	199,4
AV + STDEV	132,6	195	114,4	143,3
AV	82,6	121,5	78,4	103,2
Variance	2498,5	5392,9	1289,5	1612,5
AV - STDEV	32,6	48,1	42,5	63
VMIN	23,7	24,4	32,3	43,3
STDEV	49,9	73,4	35,9	40,1
Teste statistice				
Test-t/Wilcoxon	Val		p	
Test Pearson	0,0003 (W)		0,029	
	< 0,0001		0,036	

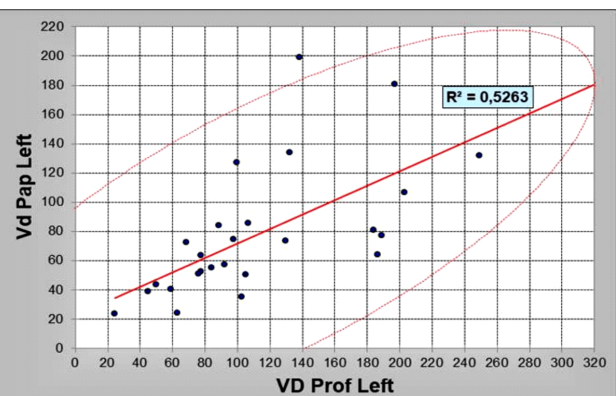
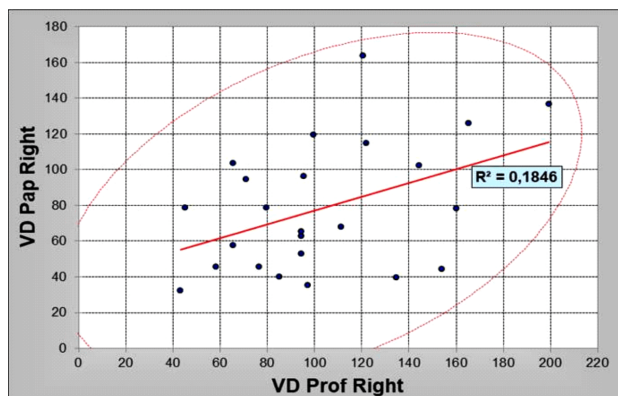


Figure 31 – Graph representation of Pearson's correlation test between papillary and deep vascular densities based on sagittal plane.

Discussions

Studies on humans and animals (dogs) demonstrated that the gingival thickness varies according to the dental arch, gender, and age [9, 10].

The concept of gingival phenotype, encompassing gingival thickness and width, was recently recognized as having a crucial importance in the assessment of mucogingival conditions [11]. The gingival biotype is unique for a patient [12]. Different gingival biotypes respond differently to inflammation, restorative, trauma and parafunctional habits [13, 14]. Almost 10 years ago, De Rouck *et al.* developed a new classification of gingival biotype, identifying the following classes: thin scalloped, thick scalloped, and thick-flat scalloped [15].

Thick gingival tissue is associated with a broad zone of the keratinized tissue, with low or high gingival scalloping and flat gingival contour suggestive of thick bony architecture. The tissue here is dense and fibrotic with a large zone of attached gingiva. It is usually associated with periodontal health, being more resistant to inflammation and trauma [15, 16].

Thin gingival biotypes are delicate, associated with a thin band of the keratinized tissue, highly scalloped and translucent in appearance, suggestive of thin bony architecture. The soft tissue appears delicate and friable with a minimal amount of the attached gingiva. It is more sensitive to inflammation and trauma, therefore patients are considered at risk [16, 17–23].

The separate lesions of occlusal trauma and inflammation are thought to act on distinct anatomical structures: first on apical periodontium; the second on gingival mucosa. In other words, the lesion of occlusal trauma is an injury limited to the ligament region (the cementum, the periodontal ligament proper and the associated alveolar bone) and not the gingiva. Gingival mucosa in turn is not affected directly by the occlusal trauma but it is primarily affected by inflammatory processes [24].

In this respect, there are many studies in the literature demonstrating that trauma from occlusion does not initiate nor alter gingival inflammation, nor does it have an effect on subgingival bacterial compositions [25–35].

In our study, we focused on microscopic changes of some main morphological elements of gingival mucosa around the teeth affected by occlusal trauma like the epithelium and its compartments, the areas of squamous metaplasia, the fibrosis process and the vascular network within the corium of gingival mucosa. We did not consider the connective tissue cells and the possible inflammatory cells. Correlations targeted the relationship between these morphological changes and the topography of affected teeth and the relationship between each other of these changes.

Correlations between morphological parameters and topographical criteria

There are few references in the literature concerning the epithelium of the gingival mucosa and most all of them are talking about the ageing process. The most obvious changes related with ageing are thinning and decreasing in the keratinization of the gingival epithelium and they are supposed to indicate an increase in tissue

permeability and a decrease in its resistance to infection and trauma [36–38]. More detailed descriptions of these changes are: flattening of the epithelial invaginations toward the conjunctive tissue, a high vacuolization of interstitial spaces and the invasion of fat cells [38, 39]. Also, the apical migration of the junctional epithelium, with consequent gingival recession is more frequently discussed but the loss of insertion caused by aging alone may not seem to have clinical significance [40]. The only study identified where occlusal trauma and epithelium are mentioned together is the one of Wentz *et al.*, published almost 60 years ago, who reported that despite the traumatic lesions noted in the periodontal ligamentum, no pathological changes were noted in the gingival tissues or the epithelial attachment due to occlusal trauma [41].

In our study, the epithelium of the gingival mucosa taken as a whole presented change of the thickness with respect to the type of affected tooth, having a thickness between 400 µm and 500 µm on the mucosa around the affected incisive teeth and over 400 µm and even above 500 µm in the lateral groups.

The epithelium of the gingival mucosa around the affected teeth did not however present significant variations with respect to the arch or hemi-arch on which the affected teeth were located.

The outer layer of the gingival mucosa epithelium around the affected teeth had a different behavior, presenting a tendency to thicken from the frontal towards the lateral groups of teeth.

The papillary compartment of the epithelium, however, did not present significant variation with respect to the affected tooth or the hemi-arch on which the tooth was located, but with respect to the dental arch on which the affected tooth was located, showing the tendency of the mandible to thicken in comparison with the maxillary (Table 10).

Table 10 – Correlations between morphological parameters and topographical criteria

Correlated Parameters	Tooth type (I, PM, M)	Dental Arch (Max, Mdb)	Sagittal plane side (L, R)
	χ^2 /Fisher Test – "p" Value		
Total Epithelium	0,030 (F)	0,151	0,274 (F)
Superficial Epithelium	0,028 (F)	0,578	0,485
Papillary Epithelium	0,109 (F)	0,046 (F)	0,895
Leucoplakia	0,021	0,546	0,116
Papillary Fibrosis	0,621	0,264	0,208
Deep Fibrosis	0,124	0,879 (F)	0,308 (F)
Papillary VD	0,225 (F)	0,343 (F)	0,708 (F)
Deep VD	0,174 (F)	0,863	0,573 (F)

There is no consensus in the literature regarding epithelium–connective tissue interface namely the shape of the rete pegs. An increase in the height of the epithelial ridges associated with ageing was also demonstrated [9, 10].

Moreover, with advanced age, a shift from ridges to papillae is producing which, in turn, involves the formation of epithelial cross-ridges [42].

In our study, the ratio between the papillary epithelium and superficial epithelium compartment varied in a very wide range of values, from almost "1", meaning almost equality between the two compartments or, in other words,

the flattening of the epithelial ridges, to more slightly more than “5”, meaning a significant increase of the papillary compartment.

The metaplastic lesion of leukoplakia, and important lesion for its premalignant nature, showed a higher degree of association with lesions caused by occlusal trauma located in the lateral groups and even an increase of severity from the lateral or frontal groups. Its presence did not correlate however with the position of affected teeth on the arches or hemi-arches.

We have to point again the absence of any other study in the literature for comparison concerning this epithelial morphological change in relation with occlusal trauma.

The same situation encountered when we assessed the epithelium of the gingival mucosa was for the analysis of the fibrosis process of the gingival mucosa corium. The scarce studies in the literature addressed also to the process of ageing.

Thus, with ageing, in the connective tissue of the gingival mucosa the number of total cells is reducing with a consequent reduction in collagen synthesis but, paradoxically, with a higher collagen amount in the tissue due to an increase in the conversion of soluble collagen to insoluble collagen, as well as to an increase in the denaturation temperature and mechanical resistance of fibers [37–39, 43]. Old fibroblasts also present an increased rate of collagen intracellular phagocytosis and alterations in the composition of extracellular matrix proteoglycans secretion [44, 45].

In turn, regarding the vascular network of gingival corium, there was one study more than 60 years old where the authors stated that occlusal forces had no significant effect on the blood supply to the gingival tissues [46].

In our study, fibrosis and vascular density did not present significant variations with respect to the topography of teeth affected by occlusal trauma injuries, neither in the superficial papillary compartment nor in the deep compartment of the gingival mucosal line around the affected teeth (Table 10).

Correlations between morphological parameters

The epithelium of the gingival mucosa tends to be thicker especially if the fibrosis from the papillary lining is lower and vascular density of the deep lining is smaller (Table 11; Figure 32, a and b).

Table 11 – Correlations between morphological parameters

Correlated Parameters	Pearson's correlation Test	
	Correlation matrix	"p" Value
Total Epithelium / Papillary Fibrosis	-0,206	0,146
Total Epithelium / Deep VD	-0,148	0,297
Superficial Epithelium / Papillary Fibrosis	-0,209	0,139
Papillary Epithelium / Papillary Fibrosis	-0,171	0,228
Papillary Epithelium / Deep VD	-0,206	0,146
Leucoplakia / Superficial Epithelium	0,380	0,005
Leucoplakia / Papillary Fibrosis	-0,441	0,001
Papillary Fibrosis / Papillary VD	-0,223	0,114

In the papillary epithelial compartment, the same tendencies of inverse correlation as the ones described on the total thickness of the epithelium were observed, even more pronounced in the papillary epithelial compartment/papillary fibrosis and the papillary epithelial compartment/deep vascular density relations (Table 11; Figure 32, c–e).

The metaplastic pathological process of leukoplakia appears to be influenced as a whole by the thickness of the gingival mucosal epithelium, especially due to its outer compartment. The keratinized, anucleated layer has a reduced thickness and it may even disappear as the amount of fibrosis within the papillary lining increases (Table 11; Figure 32, f and g).

As the fibrosis percentage increases, the vascular density decreases, a process which seems logical at first sight. The tendency was more obvious in the superficial compartment located in apposition with the epithelium (Table 11; Figure 32h).

Finally, it is worth mentioning that, although there were tendencies, generally of inverse correlation between the studied morphological parameters, these were not statistically validated with one exception namely fibrosis that influences the presence and extent of the leukoplakic layer.

Conclusions

The thickness of the gingival mucosa epithelium and especially its superficial compartment tended to increase from the frontal group towards the lateral groups of teeth and the modifications to the thickness of the epithelium and to its layers showed an obvious inverse correlation with the quantitative modifications of fibrosis from both compartments of the gingival mucosal lining.

Modifications of the density of the vascular network from the gingival mucosal lining sketched and inverse correlation with the modifications of the thickness of the gingival mucosal epithelium and those of its superficial layer but not with those deep papillary epithelial layer.

Quantitative changes of the fibrosis process sketched a logical report of inverse correlation with the modifications of the vascular density in both compartments of the gingival mucosal lining.

The mean size of the superficial keratinized acellular layer showed an increase of severity from the frontal group towards the lateral groups. The same value was in direct correlation with the evolution of the size of the gingival mucosal epithelium as a whole and its superficial compartment. It was also in an inverse correlation with the evolution of the quantity of mature collagen fibers from the papillary compartment of the gingival mucosal lining and not with that of the deep compartment of the gingival mucosal lining.

Unfortunately, the correlations shown above were only suggested by diagrams of the utilized tests, but were not however statistically validated, the explanation being the small size of the studied group.

Finally, one can sustain that the morphological changes that take place in the different compartments of the gingival mucosa are influenced to a certain extent by the occlusal trauma lesions and their topography.

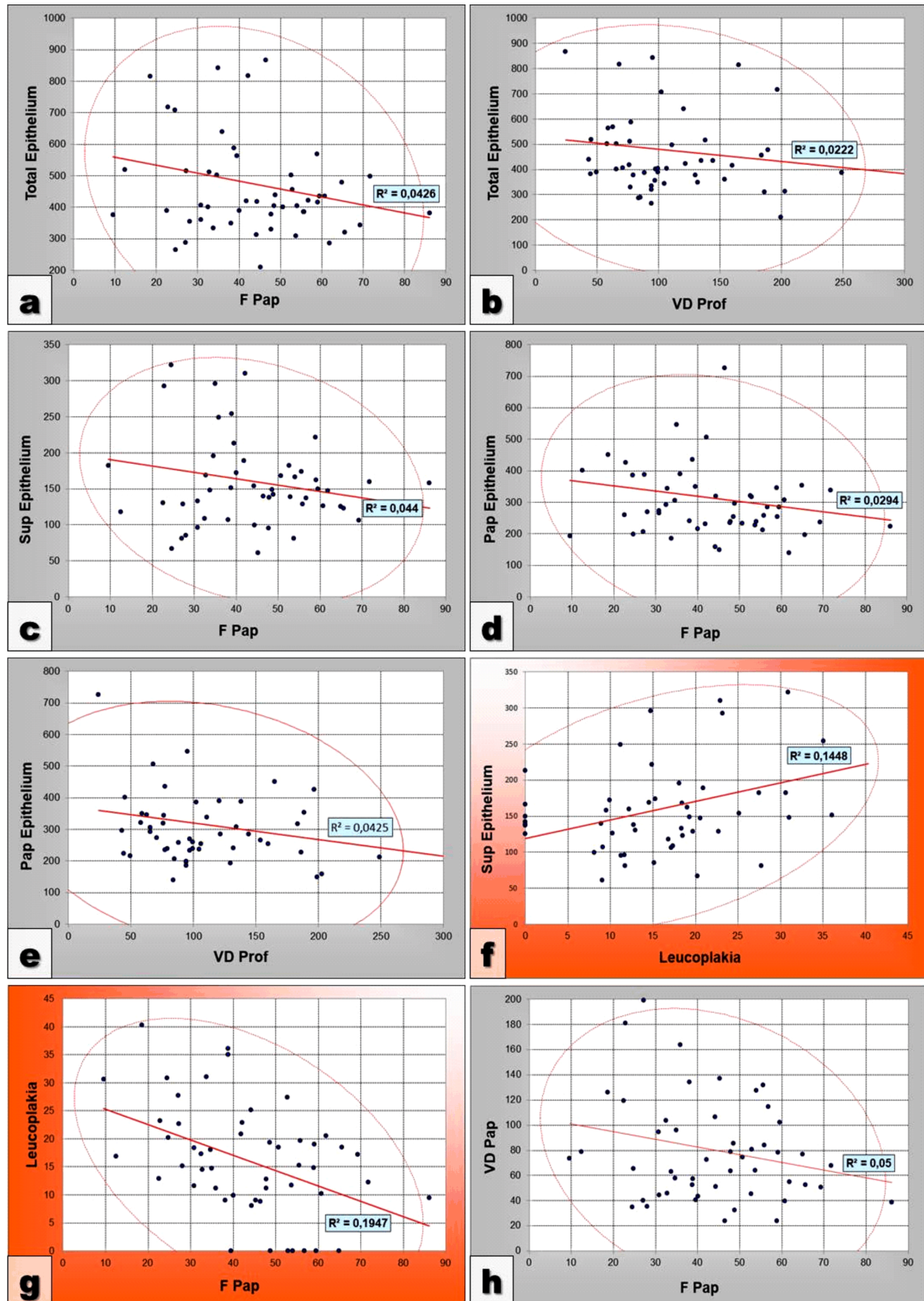


Figure 32 – Graph representation of Pearson's correlation test between morphological parameters.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: January 20, 2018

Accepted: November 1, 2018