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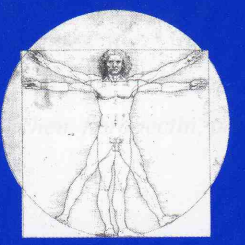

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ANALYSIS OF PLASMA FIBRONECTIN LEVELS IN PATIENTS AFFECTED BY ORAL LICHEN PLANUS

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Previous studies have demonstrated that patients affected by Oral Lichen Plauns (OLP) show lower levels of salivary fibronectin when compared with normal controls. Similarly, tissutal fibronectin expression is lost in epidermal basal layer and papillary dermis of OLP patients. To date, no data exist on the potential role of Plasma Fibronectin(PFn) in OLP pathogenesis, diagnosis and treatment. The objectives of the present study are: a) to determine the PFn levels in OLP patients; b) to evaluate a possible association between OLP clinical form and PFn levels; and c) to determine the PFn levels in relation to OLP signs and symptoms treatment. Twenty consecutive patients affected by OLP were enrolled. All patients were treated for eight weeks with topical clobetasol 0.05%. OLP signs and symptoms were scored before and after treatment. PFn level was determined by a nephelometric system. OLP signs and symptoms significantly improved after treatment. The mean levels of PFn were 31.84mg/dL at the beginning and 26.76mg/dL at the end of the study. The difference was not statistically significant (p=0.60). PFn in OLP patients remains in normal value range. OLP clinical form does not influence the PFn levels. Amelioration of symptoms and signs of atrophic-erosive and reticular OLP are induced by clobetasol treatment and the PFn seems not to interfere in the healing processes induced by topical corticosteroid. In contrast to what is observed in traumatic or diabetic wound healing, levels of PFn do not promote OLP lesion healing. PFn is not to be considered as a marker of OLP disease activity and its role in OLP pathogenesis still remains unclear.

Two major forms of fibronectin are produced and secreted by human cells *in vitro* and *in vivo* (1). The cell-associated fibronectin is relatively insoluble and supports cell adhesion, wound healing, cell differentiation and phagocytosis. The plasma fibronectin (PFn), produced primarily in the liver, is a soluble serum protein with biological properties similar to those of cell fibronectin. It mediates a variety of cellular interactions with the extracellular matrix and plays important roles in cell adhesion, migration, growth and differentiation (2).

Oral lichen planus (OLP) is a T-cell-mediated autoimmune disease but its cause is still unknown. Activated T cells are attracted and migrate towards the oral epithelium (homing), as the result of intercellular adhesion molecules (ICAM-1 and

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VCAM) and the up-regulation of epithelial basement membrane extracellular matrix proteins, including collagen types IV and VII, laminin and integrins, and CXCR3 and CCR5 signalling pathways. Cytokines, produced by keratinocytes such as TNF-alpha and interleukins (IL)-1, IL-8, IL-10, and IL-12, play a pivotal role as chemotactic factors for lymphocytes. The T cells bind to keratinocytes and IFN-gamma and the subsequent up-regulation of p53, matrix metalloproteinase 1 (MMP1) and MMP3 induces cell apoptosis (3-5). Two different clinical forms of OLP are recognized: atrophic-erosive OLP and reticular OLP. They have different biological, clinical and prognostic significance.

To date, there are no studies on a possible association between OLP and PFn. The aim of this study is a) to determine the PFn levels in OLP patients, b) to evaluate a possible association between OLP clinical form (reticular and atrophic-erosive) and PFn levels, and c) to determine the PFn levels in relation to OLP signs and symptoms treatment and the possible clinical implications.

MATERIALS AND METHODS

Patients recruitment

Consecutive patients referred to the Odontoiatric Clinic of the "Policlinico of Bari" affected by histologically confirmed OLP were enrolled from September 2009 to September 2010. Demographic characteristic, personal medical history, underlying diseases and duration of mucosal lesions, were collected at the time of the initial visit. Evaluation of mucosal sites involved by the lesions and assessment of the clinical form of OLP according to Andreasen classification (6) were performed by a single operator. Patients affected by C or B hepatitis were excluded.

Proposed treatment

All patients were treated with topical clobetasol propionate 0.05% cream (2 daily applications) for eight weeks. They were also trained to apply the cream: finger application on dried lesions after meals without eating, drinking or speaking for at least half an hour afterwards. Miconazole was used in association to prevent the onset of opportunistic mycoses.

OLP signs score and symptoms

Oral mucosal lesions were scored before (*t0*) and after eight weeks (*tf*) of treatment according to Piboonniyom et al. (7). In brief, the oral cavity of each individual was divided into 10 sites. The severity of the lesions in each site was scored according to the presence of reticular/hyperkeratotic, erosive/erythematous, and/or ulcerative lesion(s) as follows: reticular/hyperkeratotic lesions were scored from 0 to 1 (0 = no white striations, 1 = presence of white striations or keratotic papules); erosive/erythematous areas were scored from 0 to 3 by area of involvement (0 = no lesion, 1 = lesions less than 1 cm^2, 2 = lesions from 1 to 3 cm^2, 3 = lesions greater than 3 cm^2); ulcerative areas were scored from 0 to 3 by area of involvement (0 = no lesion, 1 = lesions less than 1 cm^2, 2 = lesions from 1 to 3 cm^2, 3 = lesions greater than 3 cm^2). For each of the 3 clinical signs, a score is derived from the sum of the scores of all 10 areas: reticular score = ΣR, erythema score = ΣE, and ulcerative score = ΣU (REU score) with a total weighted score of $\Sigma R + \Sigma (E \times 1.5) + \Sigma (U \times 2.0)$.

Symptoms were recorded at (*t0*) and after eight weeks (*tf*) of treatment using a 0-10 Visual Analogical Scale (VAS) score.

Fibronectin dosage

The PFn assay was used to determine the fibronectin levels in serum specimens of patients and controls. Blood samples were taken using standard sampling tubes. Serum contents of blood samples were separated at 2000 g for 10 minutes by centrifugation. PFn dosage was determined by a nephelometric automatic system (BN ProSpec™ – Siemens). Normal value of PFn was considered in a range of 25-40mg/dL. The dosage was carried out at *t0* and at *tf*

Statistical analysis.

Data were imputed in excel (Microsdoft 2011 for Mac®) spreadsheet and then elaborated using STATA® 10 software (StataCorp, Texas USA - http://www.stata.com). Descriptive, bivariate and multivariate analyses were performed. Oral mucosal lesion score was treated as a discrete continuous variable, VAS score as an ordinal variable.

Mean, Standard error (SE) and 95% Confidence Interval for each parametric variable were calculated. The difference recorded at *t0* and at *tf* was statistically analysed using Student's *t* test for paired couples. Furthermore, a stepwise logistic regression model using the presence of atrophic- erosive OLP as dependent variable, was used. The statistical significant values were expressed at $p<0.05$.

RESULTS

The vital statistic data and clinical record collected at *t0* in the included sample are reported in Table I. Patients mean age was 59.6 years (SD, 8.7 years). Only four patients did not present any

coexisting pathologies, while the remaining were affected by several systemic diseases, such as high or low blood pressure, thyroiditis, diabetes, hypercholesterolaemia, osteoporosis, allergy and hepatic steatosis. The mean duration of OLP was 10.4 months (SD,10.2). Twelve patients presented the reticular form of OLP while the eight remaining patients showed the atrophic-erosive form.

PFn levels, VAS score and lesion score of twenty OLP patients enrolled in the present study are summarized in Table II. The mean of lesion score at *t0* was 4.18 while after eight weeks of treatment, the score was 1.50 (p=0.03). Furthermore, the symptoms showed a significant improvement: the mean of VAS score at *t0* was 4.30 while at *tf* it was 2.35 (p=0.02). The mean levels of PFn in the studied cohort was 31.84 mg/dL at *t0* and 26.76 mg/dL at the end of the study. The difference was not statistically significant (p=0.60). Moreover, the mean values at the beginning and at the end of the study were in the normal PFn range values.

The data of PFn levels, VAS score and lesion score considered in patients affected by atrophic-erosive and reticular OLP subgroups are reported in Table III.

The eight patients affected by atrophic-erosive OLP showed a significant decrease of lesion score (*t0*=5.81 - *tf*=2.00; $p<0.01$) and VAS score (*t0*=6,62 - *tf*=3.37; $p=0.01$). A not significant decrease of PFn mean levels was recorded (*t0*=32.42 mg/dL - *tf*=30.14 mg/dL; p=0.28).

The twelve patients affected by reticular OLP

Table I. *Demographic and clinical data of OLP patients enrolled in the present study.*

Patients	Sex	Age°(years)	Underlying diseases	Duration of OLP lesions* (months)	Type of OLP
1	M	65	hypertensive cardiomyopathy	24	reticular
2	F	50	Hypotension	2	reticular
3	F	60	hypertension, diabetes, allergic rhinitis, thyroid nodules	12	reticular
4	F	69	hypotension, osteoporosis	3	atrophic-erosive
5	F	75	hypertensive cardiomyopathy, hepatic steatosis	4	atrophic-erosive
6	M	59	Hepatic steatosis	4	reticular
7	F	64	Hashimoto's thyroiditis, hypercholesterolaemia	24	atrophic-erosive
8	F	49	vasomotor rhinitis, previous thyroid carcinoma	2	reticular
9	F	63	previous ovarian carcinoma	4	atrophic-erosive
10	F	53	hypothyroidism, osteoporosis	2	reticular
11	M	46	no diseases	9	atrophic-erosive
12	F	39	no diesases	3	reticular
13	M	62	Diabetes	36	reticular
14	M	61	no diseases	9	atrophic-erosive
15	M	70	Diabetes	11	reticular
16	F	54	nichel allergy	24	reticular
17	F	62	lupus erythematosus , thyroiditis, rheumatoid arthritis, hypotension	24	atrophic-erosive
18	F	63	no diseases	7	reticular
19	F	64	hypercholesterolaemia, hypothyroidism	4	atrophic-erosive
20	F	65	hypertension, hypercholesterolaemia	1	reticular

*M:F=6:14; °Mean ±SD=59.6±8.7 years; *Mean ±SD=10.4±10.2 months*

Table II. *Clinical scores and PFn level before and after the proposed treatment.*

	Lesion score *Mean (SE) 95%CI*	VAS Score *Mean (SE) 95%CI*	PFn level (mg/dL) *Mean (SE) 95%CI*
t0	4.18 (0.56) 3.00–5.35	4.30 (0.86) 2.50–6.10	31.84 (2.12) 27.41–36.27
tf	1.50 (0.23) 1.02–1.98	2.35 (0.62) 1.05–3.65	26.76 (1.90) 22.78–30.74
p value	***0.03***	***0.02***	*0.60*

Table III. *Scores and PFn levels in atrophic-erosive and reticular OLP subgroups patients.*

a - atrophic-erosive OLP.

	Lesion score *Mean (SE) 95%CI*	VAS Score *Mean (SE) 95%CI*	Fn dosage (mg/dL) *Mean (SE) 95%CI*
t0	5.81 (0.96) 3.54–8.08	6.62 (0.80) 4.73 – 8.51	32.42 (3.21) 24.84 – 40.00
tf	2.00 (0.23) 1.29–2.70	3.37 (1.03) 0.93 – 5.82	30.14 (2.12) 25.11 – 35.16
p value	***<0.01***	***0.01***	*0.28*

b - reticular OLP.

	Lesion score *Mean (SE) 95%CI*	VAS Score *Mean (SE) 95%CI*	Fn dosage (mg/dL) *Mean (SE) 95%CI*
t0	3.08 (0.45) 1.98–4.18	2.75 (1.15) 0.22–5.28	31.45 (2.92) 25.03–37.87
tf	1.17 (0.30) 1.51–1.82	1.67 (0.74) 0.03–3.30	24.51 (2.70) 18.55–30.47
p value	***<0.01***	*0.22*	*0.05*

showed a significant decrease of lesion score (*t0*=3.08 - *tf*=1.17; *p<0.01*) but not a significant reduction of VAS score (*t0*=2.75 - *tf*=1.17; p=0.22). A not significant decrease of PFn mean levels was also recorded (*t0*=31.45 mg/dL - *tf*=24,51 mg/dL; p=0.05).

Logistic regression showed that only the lesion scored at *t0* were significantly associated with atrophic/erosive OLP (p=0.04).

DISCUSSION

According to the present findings, OLP patients do not show any significant alteration in PFn levels. Even if reticular and atrophic-erosive OLP patients are considered separately, PFn levels remain in the

normal range values. Our results confirm clobetasol propionate 0.05% as an efficacious treatment in signs of remission in both reticular and atrophic-erosive OLP. Furthermore, the symptoms of atrophic-erosive OLP benefit from clobetasol treatment, while the PFn levels are not associated to improvement of signs and symptoms.

PFn seems not to interfere in the healing processes induced by topical corticosteroid treatment similarly to what is observed for non-steroidal anti-inflammatory drugs and interferon (8, 9). Although PFn plays a critical role in wound healing by contributing to haemostasis and phagocytosis, assisting in the control of infection, promoting fibroblast migration and proliferation, enhancing epithelialization and organisation of granulation tissue and modifying the tensile strength of scar tissue, its role in autoimmune-mediated lesions still remains unclear. Conflicting data exist in medical literature regarding PFn concentration in patients affected by autoimmune diseases. Elevated concentrations of PFn occur in psoriatic patients, especially in those affected by erythrodermic psoriasis. In patients with psoriasis vulgaris the increased levels of PFn seem to be related to the area of involvement, and not to the activity of the disease or the duration of relapse. However, in patients with lesions affecting less than 20% of the skin surface, the level of PFn appears normal (10). PFn values of patients affected by Systemic Lupus Erythematosus (SLE) are significantly higher than in the control group. Moreover, active form of SLE shows significantly higher values when compared with the PFn of non-active SLE patients (11). No previous studies have analysed the PFn levels in OLP patients. Zahn et al. demonstrated that salivary fibronectin level was lower in saliva of OLP patients when compared with a normal control group (12). They hypothesized that recurrent and non-healing erosions of oral mucosa may be related to a deficit of fibronectin salivary level. Some authors support the hypothesis that PFn is a reservoir for fibronectin in tissue, and suggest that there is a movement of fibronectin from plasma into the tissues (13). The studies on cutaneous lichen planus and cell-associated fibronectin revealed fibronectin loss in the basal layer and papillary dermis (14).

Although the results of the present study are limited to twenty patients, they suggest that PFn is probably not involved in OLP pathogenesis and tissue reparation/healing. Amelioration of erosive and atrophic lesions do not correspond to PFn level decrease as we expected according to the "PFn reservoire theory". On the contrary, we have paradoxically recorded *quasi*-significant values (p=0.05) observing the PFn level variations in reticular OLP patients in whom there are no chronic wounds. These unexpected data could reflect the differences observed in epithelial activity, apoptosis and peripheral immunocompetent cells in reticular and atrophic-erosive OLP (15-18).

This study has implications for clinical practice and particularly research: no studies have ever simultaneously compared PFn and cell-associated fibronectin in patients affected by skin/mucous autoimmune diseases. This type of study may reveal the eventual presence of a real interaction between the two fibronectin isoforms. Moreover, the topical application of fibronectin on mucosal lesions induced by OLP could be useless, contrary to what was observed in rats by Kwon et al. in full thickness skin induced wound (19). Further clinical trials and large cohort studies are necessary to better evaluate the role of PFn in OLP and in other oral autoimmune diseases.

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