Clinical Plasma Medicine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Plasma Medicine

journal homepage: www.elsevier.com/locate/cpme

Cold atmospheric plasma (CAP) as a promising therapeutic option for mild to moderate acne vulgaris: Clinical and non-invasive evaluation of two cases

Dr. Arisi Mariachiara *, Venturuzzo Anna, Gelmetti Alessandra, Guasco Pisani Edoardo, Bassissi Stefania, Rossi Mariateresa, Calzavara-Pinton Piergiacomo

Department of Dermatology, University of Brescia, ASST Spedali Civili di Brescia, Piazzale Spedali Civili 1, 25123 Brescia, Italy

ARTICLE INFO

Keywords:
Cold atmospheric plasma
Acne vulgaris
Therapy

ABSTRACT

Background: Acne vulgaris is a multifactorial skin disease that may be triggered by the presence of the bacteria Cutibacterium acnes and is characterized by an increase in the sebum excretion. Cold atmospheric plasma (CAP) represents an innovative technological device that uses plasma, the fourth state of matter, to treat several skin conditions, considering its bactericidal potential and its efficacy in promoting tissue proliferation.

Objectives: In the light of its well-known antibacterial properties and its promising regenerative properties, we assumed that CAP could be highly effective to treat acne vulgaris both in its acute phase and in the long-term cicatricial period.

Methods: We reported the cases of two young patients affected by mild-moderate acne vulgaris of the face treated with CAP. In addition to the evaluation of inflammatory skin lesions, treatment efficacy was assessed by sebometry and trans epidermal water loss (TEWL) evaluation.

Results: In both patients, acne lesions reduced after treatment. Both sebum excretion parameters and TEWL improved considering basal values.

Conclusion: Despite the small number of patients enrolled, our data support CAP effectiveness in patients with mild to moderate acne vulgaris poorly responsive to other conventional treatments.

1. Introduction

Acne vulgaris is a common chronic skin disease with a complex pathogenesis. Several factors play an important role in its onset and perpetuation: follicular epidermal hyperproliferation leading to follicular plugging, dysregulation in sebum production, proliferation and overactivity of the commensal bacteria Cutibacterium Acnes are the most important factors among the several ones involved. Acne vulgaris usually manifests itself with non-inflammatory (i.e. open and closed comedones) and inflammatory lesions (i.e. papules, pustules, nodules) and its clinical grading may vary from mild to severe.

Anti-acne drugs mostly act by reducing inflammation, sebum excretion and bacterial load. The drugs most commonly used topically are antibiotics, benzoyl peroxide and retinoids. As for systemic treatment, oral antibiotics such as minocycline and doxycycline, are frequently used in the clinical practice. To treat moderate to severe acne vulgaris isotretinoin is often required in addition to topical drugs. In the presence of a hormonal disregulation, an estrogen/progestogen therapy can be useful [1].

Notwithstanding, in case of systemic contraindications or drug-resistance, acne treatment becomes challenging. New therapeutic options are currently being studied, among which peroxisome proliferator-activated receptor (PPAR) antagonists represent a new and promising therapeutic option [1].

Cold atmospheric plasma (CAP) is a partly ionized gas created by electric discharges. The so-called "plasma –medicine" has become an innovative field over the last years; leading applications in dermatology are wounds and skin cancer care [2–5].

CAP is constituted by electrons, ions, neutral atoms and photons and it has then a final net neutral charge. It works by affecting biochemical processes of the organism mainly through its active components, reactive oxygen and nitrogen species (ROS and RNOS) [6,7]. Taking into account that an overwhelming release of these species may exert a deleterious effect on human tissues, CAP sources are able to produce

* Corresponding author.
E-mail address: mariachiara.arisi@unibs.it (Dr.A. Mariachiara).

https://doi.org/10.1016/j.cpme.2020.100110
Received 6 August 2020; Accepted 8 October 2020
Available online 9 October 2020

"controllable" RONS which act positively as part of the immune response and intracellular signaling cascades [8–11]. Furthermore, many trials highlighted the huge bactericidal potential of plasma [12] so it was successfully used to treat even infectious diseases [12,13]. In the face of Cutibacterium acnes role in acne pathogenesis, CAP may represent an innovative therapeutic option for the disease and its feasibility and efficacy should be investigated.

Moreover, a significant role of CAP in favouring tissue proliferation [8–10,14] was also reported: modification of cytokines levels involved in cell proliferation and epidermal thickening, and improvement of dermal microcirculation by nitric oxide, might influence fibroblasts proliferation [10,15]. Hence it has to be considered the actual possibility of a beneficial effect of CAP in reducing post-acne scars.

On the basis of these assumptions, herein we present two cases of mild-moderate acne effectively treated with CAP.

2. Materials and methods

Two adult Caucasian patients affected by mild to moderate acne of the face were selected at the outpatient clinic of the Dermatology Department of Brescia University from September to December 2019. The study was approved by the Local Ethic Committee (protocol number 4195) and patients signed the informed consent before treatment.

Both patients had shown resistance and/or contraindications to common topical and systemic acne treatments.

Any topical or systemic therapy for acne had been discontinued at least 3 months before enrolment.

Argon-CAP (Adtec SteriPlas; Adtec Plasma Technology, Adtec Europe, Hunslow, UK) was used to irradiate affected skin areas of the face in each patient. The device consists of a torch operated at microwave excitation and production of an argon plasma [16] (Fig. 1a-b). The emission spectrum peak in the UVA and UVB range was 308 and 336 nm respectively, measured by using a StellarNet Silver Nova Spectrometer, (StellarNet Inc. Tampa, Florida, USA).

In all patients, the treatment procedure was performed under standard conditions (environmental temperature and humidity ranged respectively between 20°−23 °C and 40–60% and the patient remaining in a lying position for at least 10 min before examination). Considering that tissue moisture (but not wet) proved to improve efficacy of plasma [17], the skin area to be treated has been appropriately cleansed with physiological solution and dried with a sterile dressing before CAP application.

As no standard protocols for the use of CAP in acne patients have been validated so far, single session per week was performed for every patient until complete remission or no further amelioration after 2 consecutive sessions. Treatment response was assessed based on the percentage of cleared lesions compared to baseline: patients with a reduction of more than 60% were considered as responders to treatment.

At each session CAP was applied on the skin for 5 min. Considering that the diameter of the plasma handle is 227.4 mm and a single application of plasma consequently encloses a field of 12 cm^2, in every session multiple applications of plasma were needed to treat the whole affected areas. The device used is in a "spot style" and designed to fixate few millimeters (about 5 mm) over the lesion to treat and stay in place during the irradiation period.

For both patients, at baseline (T0) and at 1 month from the end of the treatment (T1), clinical photographs were taken (VISIA® camera, Canfield, Fairfield, NJ, USA).

Additionally, at the same timepoints, sebometry and trans epidermal water loss (TEWL) evaluation were also performed by using the Sebumeter® SM 810 PC (Courage and Khazaka GmbH, Koln, Germany) and the Tewameter TM210 (Courage and Khazaka GmbH, Koln, Germany) respectively. [18].

At each visit, measurements were taken according to established methods [19,20] on the same skin area by the same physician (M.A), in order to avoid possible intraindividual variations of biophysical parameters.

Patients were followed-up at three months after the last CAP application. At this time point only clinical evaluation and lesion count were performed.

During the whole treatment course and the 3-months follow-up period (T2), local and systemic adverse events, if any, were graded according to Common terminology Criteria for Adverse Events (CTCAE) scale [21] and patients were allowed to apply only emollient cream containing olus oil and pentylene glycol on the treated skin, twice daily.

3. Case reports

3.1. Case 1

A 24-year-old man, affected by moderate acne vulgaris of the face for many years, was previously treated with several topical drugs (i.e. erythromycin cream, adapalene gel, benzoyl peroxide cream), oral antibiotics (i.e. minocycline, azithromycin) resulting only in a mild clinical improvement. Isotretinoin assumption was stopped 5 months earlier due to creatine kinase increase. The patient presented many comorbidities, including ulcerous rectocolitis and persistent truncus arteriosus. At the clinical evaluation, active papulo-pustular lesions and open comedones (Fig. 2a, Table 1) of chin and cheeks were evident. A swab was performed on a pustular lesion of the chin resulting positive for

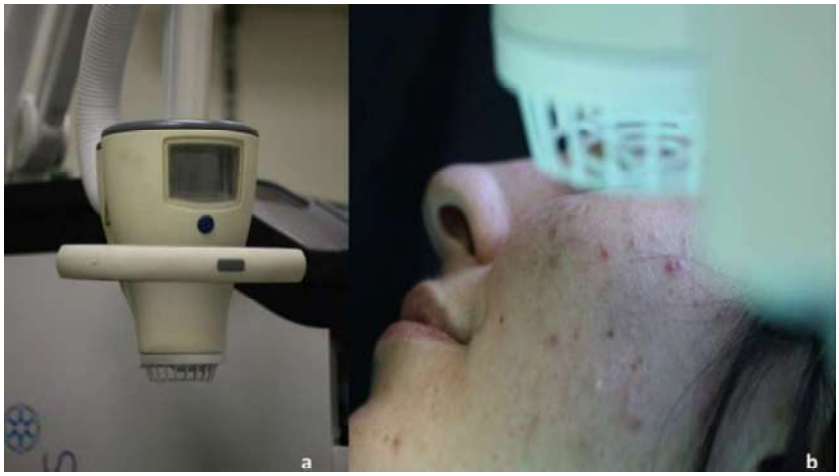

Fig. 1. Argon-CAP device used to irradiate affected skin areas of the face in each patient (a). The device consists of a torch operated at microwave excitation and production of an argon plasma (b).

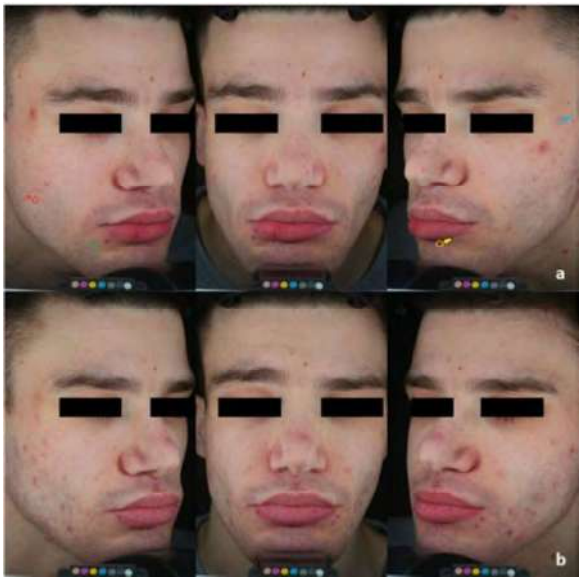

Fig. 2. 3D pictures of patient n° 1 before (a) and after (b) plasma therapy. Pustules (yellow arrow line), papules (blue arrow line), open comedones (red star), close comedones (green star).

Enterobacter cloacae as sign of acne- associated Gram-negative folliculitis probably induced by long term oral tetracycline assumption. Argon-CAP treatment was started and 5 sessions were performed. Patient stopped the treatment for personal reasons. Every treatment session was well-tolerated with no adverse effects reported. At the end of the treatment, the clinical response was good, with a significant decrease of the number of the inflamed lesions and presence of post-inflammatory erythematous macular lesions (Fig. 2b, Table 1). A cutaneous swab was repeated at the end of the treatment on the same skin area, resulting negative for bacteria and fungi. Both sebum excretion parameters and TEWL improved considering basal values (Table 1).

Clinical features remained essentially stable at 3-months follow-up (T2).

3.2. Case 2

A 21-year-old woman addressed to our department for a long story of a mild to moderate acne vulgaris of the face already unsuccessfully treated with topical drugs (adapalene gel and benzoyl peroxide cream) and oral tetracycline. At clinical examination, she presented multiple inflamed papules on the cheeks and chin (Fig. 3a). Considering the young age of the patient and previous therapeutic failures, it was decided to start CAP treatment. After 8 sessions, a significant reduction of the inflamed lesions count was seen (Table 1; Fig. 3b). Every treatment session was well-tolerated by the patient with no adverse effects reported. Clinical improvement was accompanied by the reduction of sebum excretion and TEWL. (Table 1). No clinical relapse was seen at 3-months follow-up (T2).

4. Discussion

Acne vulgaris is a multifactorial skin disease that may has a significant impact on patients' quality of life. Among the several factors that may influence the disease onset and severity, Cutibacterium acnes plays a significant role.

Cold atmospheric plasma (CAP) represents an innovative and easy-to-use treatment option for several skin conditions considering its high anti-inflammatory and bactericidal properties exerted through its active components: reactive oxygen and nitrogen species [6,7].

A previous study investigated CAP efficacy in treating acne with positive outcome [17]. The authors described a non-invasive exfoliative effect of their non thermal plasma device which was able to induce a 75% improvement of acne lesions along with a reduction of 80% sebum secretion at sebometry and 75% of fluorescence emission by C. acnes measured by a UV camera. Another technique, the plasma skin regeneration (PSR) system, has been tested in the treatment of acne scars [22] with a good efficacy. The procedure is based on the delayed ablation of the epidermis and controlled thermal modification for the underlying

Fig. 3. 3D pictures of patient n° 2 before (a) and after (b) plasma therapy. Pustules (yellow arrow line), papules (blue arrow line), open comedones (red star), close comedones (green star).

Table. 1
Clinical and laboratory improvement before (T0) and after treatment with CAP (T1=1 month follow-up; T2= 3 months follow-up).

		Sebum excretion (*mcg/cm2*)		TEWL (*g/m2h*)	Swab	Papules			Pustules			Open comedones			Closed comedones		
		Right cheek	*Chin*	*Left cheek*	*Chin*	*Right cheek*	*Left cheek*	*Chin*	*Right cheek*	*Left cheek*	*Chin*	*Right cheek*	*Left cheek*	*Chin*	*Right cheek*	*Left cheek*	*Chin*
Patient 1	**T0**	32	43	42,6	Pos (Ent. Cloacae)	7	6	3	3	2	2	7	6	4	1	1	1
	T1	21	25	32,1	Neg	3	3	1	0	0	1	3	2	1	0	0	0
	T2	NP	NP	NP	NP	3	4	0	0	0	0	3	2	1	0	0	0
Patient 2	**T0**	32	50	22,8	NP	8	6	1	5	5	2	5	4	3	2	3	2
	T1	16	43	20,6	NP	4	3	0	1	1	0	2	0	1	0	0	0
	T2	NP	NP	NP	NP	4	3	0	0	0	0	3	0	0	0	0	0

NP: not performed.

dermis and it is therefore not superimposable to CAP as mechanism of action. We decided then to study the effect of CAP on inflamed and non-inflamed skin lesions in two young patients with mild to moderate acne vulgaris of the face, which had shown to be resistant to other several topical and systemic conventional treatments.

At the end of the treatment with CAP, the clinical improvement was evident for both patients with a reduction of about 60% of skin lesions. Clinical improvement was less evident in patient n°1 but we believe that this was due to fewer sessions and early discontinuation of treatment.

No adverse effects or skin reactions were reported either during the treatment nor at 3-months follow-up [22]. Treatment was completely painless and well tolerated. Patients did not report itching or burning sensation during plasma application and in the following days.

Clinical improvement was also associated to a general improvement of non-invasive investigations. In both our patients, TEWL values reduced after treatment. As TEWL reflects skin barrier functionality, our results suggest that CAP does not induce dehydration when applied on the skin and could help in restoring the impaired epidermal barrier in agreement with previous results [23].

Impairment of water barrier function in acne vulgaris has been proved by several previous studies [24] compared with healthy skin. An important pathogenetic role seems to be played by altered ceramide (CER) levels [25,26]. The decreased proportion of linoleate in ceramide 1 caused by the dilutional effect of sebum might induce the characteristic responses of hyperkeratosis and decreased barrier function [24,27,28]. Furthermore, it has been observed a pronounced seasonal variation in skin ceramides in acne skin; TEWL was higher in winter months than in summer ones, reflecting the reduction in ceramide levels during winter [25,29]. In addition to the essential role of ceramides in comedogenesis, inflammation due to C. acnes colonization could deteriorate skin barrier homeostasis [27,28,30]. To note, our patients were both treated in autumn and TEWL was assessed under standard conditions without any environmental factors affecting it.

Presence of C.acnes and hyperkeratosis contribute to sebum hyperproduction and retention.

In literature a correlation between acne vulgaris and the increase of sebum secretion was reported [13,31], although some authors claim that the level of sebum in the skin is not a decisive factor in determining the onset of acne lesions [32]. By the way, sebometry has a significant relevance in monitoring the efficacy and safety of a treatments for acne vulgaris [31,33]; in fact, a clinical improvement after treatment is often associated with a significant decrease in the level of sebum contents [31]. In our series, sebum excretion was significantly reduced after the treatment in both patients albeit this result does not allow us to fully support a CAP reducing effect on sebum production.

The effectiveness of CAP in the treatment of acne can be explained by considering its well known in vivo effects mediated by ROS and NOS.

More recently, C. acnes was found to be highly sensitive to different concentrations of nitric oxide in nanoparticles (NO-np). NO-np significantly suppressed IL-1b, tumor necrosis factor alpha (TNF-alpha), IL-8 and IL-6 from human monocytes, and IL-8 and IL-6 from human keratinocytes and peripheral blood mononuclear cells. These data suggest that NO-np can effectively prevent C. acnes-induced inflammation by both clearing the organism and inhibiting microbial stimulation of the innate immune response and cytokine release by keratinocytes. [34,35]. Reduction of C. acnes load would contribute to sebum reduction and renewal of skin barrier function.

Our study has however some limits, the main one having carried out a culture examination limited to a skin swab and not on a bioptic sample, which would have allowed us to better investigate the presence of C. acnes. In fact, this bacterium, being anaerobic and placed deeply in the pilosebaceous unit, is notoriously difficult to isolate. However, the antibacterial effect of CAP was confirmed by Enterobacter cloacae disappearance in the cutaneous swab performed after treatment on patient 1. Another limit of our study is the small number of patients enrolled; however, it has to be considered the difficulty in recruiting adult patients for this treatment in consideration of the large amount of therapies available for acne and the compliance requested in terms of time and costs of the procedure. However, our study offers interesting preliminary clinical data, supported by instrumental results.

5. Conclusions

Our preliminary results demonstrate that CAP could represent an effective and well-tolerated therapeutic option for patients with mild to moderate acne vulgaris poorly responsive to other conventional treatments. Nevertheless, CAP cannot be considered a first-line therapy for the treatment of acne vulgaris yet due to the high cost of the device and the consequent limited availability in dermatology research centres.

Funding sources

I confirm that the research has not been funded by any company.

Ethics

I confirm that the research has been held according to ethical roules.

Declaration of Competing Interest

I confirm that all the authors have no conflict of interest to declare.

Acknowledgements

None.

References

[1] M. Gupta, V.K. Mahajan, K.S. Mehta, P.S. Chauhan, R. Rawat, Peroxisome proliferator-activated receptors (PPARs) and PPAR agonists: the 'future' in dermatology therapeutics? Arch. Dermatol. Res 307 (2015) 767–780.
[2] L. Gan, S. Zhang, D. Poorun, D. Liu, X. Lu, M. He, X. Duan, H. Chen, Medical applications of nonthermal atmospheric pressure plasma in dermatology, J. Dtsch. Dermatol. Ges. 16 (2018) 7–13.
[3] G. Isbary, S. Tetsuji, L. Yang Fang, W. Stolz, H.M. Thomas, G.E. Morfill, J. L. Zimmermann, Cold atmospheric plasma devices for medical issues, Expert. Rev. Med. Devices 10 (2013) 367–377.
[4] P.C. Friedman, V. Miller, G. Fridman, A. Lin, A. Fridman, Various cold plasma devices for the treatment of actinic keratosis, J. Eur. Acad. Dermatol. Venereol. 32 (2018) 445–e446.
[5] M. Wirtz, I. Stoffels, J. Dissemond, D. Schadendorf, A. Roesch, Actinic keratoses treated with cold atmospheric plasma, J. Eur. Acad. Dermatol. Venereol 32 (2018) e1–e40.
[6] S. Arndt, P. Unger, E. Wacker, T. Shimizu, H.M. Thomas, G.E. Morfill, S. Karrer, J. L. Zimmermann, A.K. Bosserhoff, Cold Atmospheric Plasma (CAP) changes gene expression of key molecules of the wound healing machinery and improves wound healing in vitro and in vivo, PLoS ONE 8 (2013) e79325.
[7] S. Arndt, M. Landthaler, J.L. Zimmermann, P. Unger, E. Wacker, T. Shimizu, Effects of Cold Atmospheric Plasma (CAP) on β-defensins, inflammatory cytokines, and apoptosis-related molecules in keratinocytes in vitro and in vivo, PLoS ONE 10 (2015), e0120041.
[8] S. Fathollah, S. Mirpour, P. Mansouri, A.R. Dehpour, M. Ghoranneviss, N. Rahimi, Investigation on the effects of the atmospheric pressure plasma on wound healing in diabetic rats, Sci Rep 6 (2016) 19144.
[9] J. Holcomb, K.J. Kent, D.E. Rousso, Nitrogen plasma skin regeneration and aesthetic facial surgery: multicenter evaluation of concurrent treatment, Arch Facial Plast Surg 11 (2009) 184–193.
[10] S. Babossalam, F. Abdollahimajd, M. Aghighi, et al., The effect of nitrogen plasma on the skin and hair follicles: a possible promising future for the treatment of alopecia, Arch Dermatol Res (2019), https://doi.org/10.1007/s00403-019-02020-w.
[11] P. Babington, K. Rajjoub, J. Canady, et al., Use of cold atmospheric plasma in the treatment of cancer, Biointerphases 10 (2015) 29401–29403.
[12] R. Pompl, F. Jamitzky, T. Shimizu, B. Steffes, W. Bunk, H.U. Schmidt, M. Georgi, K. Ramrath, W. Stolz, R.W. Stark, T. Urayama, S. Fujii, G.E. Morfill, The effect of low-temperature plasma on bacteria as observed by repeated AFM imaging, New. J. Phys. 11 (2009), 115023.
[13] G. Isbary, T. Shimizu, J.L. Zimmermann, J. Heinlin, S. Al-Zaabi, M. Rechfeld, G. E. Morfill, S. Karrer, W. Stolz, Randomized placebo-controlled clinical trial showed cold atmospheric argon plasma relieved acute pain and accelerated healing in herpes zoster, Clin. Plasma Med. 2 (2014) 50–55.

[14] M.M. Cals-Grierson, A. Ormerod, Nitric oxide function in the skin, Nitric Oxide 10 (2004) 179–193.
[15] M.S. Kim, H.J. Song, S.H. Lee, K. Lee, Comparative study of various growth factors and cytokines on type I collagen and hyaluronan production in human dermal fibroblasts, J Cosmet Dermatol 13 (2014) 44–51.
[16] A. Hemkle, T. Gerling, K.D. Weltmann, Plasma sources for biomedical applications. Comprehensive Clinical Plasma Medicina, Springer Nature Ed, 2018, pp. p23–p41.
[17] C. Chutsirimonkol, D. Boonyawan, N. Polnikorn, et al., Non-thermal plasma for acne and aesthetic skin improvement, Plasma Med 4 (2014) 79–88.
[18] A.G. Ovhal, H.R. Jerajani, R.S. Dhurat, A comparative study of sebum secretion rates and skin types in individuals with and without acne, Medpulse Res. Publ. 7 (2018) 88–92.
[19] J. Pinnagoda, R.A. Tupker, T. Agner, J. Serup, Guidelines for transepidermal water loss (TEWL) measurement. A report from the standardization group of the european society of Contact dermatitis, Contact Derm. 22 (1990) 164–178.
[20] M.K. Kim, S.Y. Choi, H.J. Byun, C.H. Huh, K.C. Park, R.A. Patel, A.H. Shinn, S. W. Youn, Comparison of sebum secretion, skin type, PH in humans with and without acne, Arch dermatol Res 298 (2006) 113–119.
[21] https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf (last access 17th April 2020).
[22] M.J. Gonzalez, W.H. Sturgill, E.V. Ross, N.S. Uebelhoer, Treatment of acne scars using plasma skin regeneration (PSR) system, Lasers Surg. Med. 40 (2008) 124–127.
[23] G. Daeschlein, S. Scholz, R. Ahmed, et al., Cold plasma is well-tolerated and does not disturb skin barrier or reduce skin moisture, JDDG 10 (2012) 509–515.
[24] A. Yamamoto, K. Takenouchi, M. Ito, Impaired water barrier function in acne vulgaris, Arch. Dermatol. Res 287 (1995) 214–218.
[25] A. Pappas, A.C. Kendall, L.C. Brownbridge, N. Batchvarova, A. Nicolaou, Seasonal changes in epidermal ceramides are linked to impaired barrier function in acne patients, Exp Dermatol 27 (2018) 833–836.
[26] S. Meguro, Y. Arai, Y. Masukawa, et al., Relationship between covalently bound ceramides and transepidermal water loss (TEWL), Arch Dermatol Res 292 (2000) 463–468.
[27] D.T. Downing, M.E. Stewart, P.W. Wertz, S. Strauss, Essential fatty acids and acne, J. Am. Acad. Dermatol 14 (1986) 221–225.
[28] M.E. Stewart, M.O. Grahek, L.S. Cambier, P.W. Wertz, D.T. Downing, Dilutional effect of increased sebaceous gland activity on the proportion of linoleic acid in sebaceous wax esters and in epidermal acylceramides, J. Inv. Dermatol. 87 (1986) 733–736.
[29] K. Meyer, A. Pappas, K. Dunn, G.O. Cula, I. Seo, E. Ruvolo, N. Batchvarova, Evaluation of Seasonal Changes in Facial Skin With and Without Acne, J Drugs Dermatol 14 (2015) 593–601.
[30] R.M. Lavker, J. Leyden, K.J. McGinley, The relationship between bacteria and the abnormal follicular keratinization in acne vulgaris, J. Inv. Dermatol. 77 (1981) 325–330.
[31] B.A. Khan, N. Akhtar, Clinical and sebumetric evaluation of topical emulsions in the treatment of acne vulgaris, Postepy Dermatol Alergol 31 (2014) 229–234.
[32] S.W. Youn, E.S. Park, D.H. Lee, C.H. Huh, K.C. Park, Does facial sebum excretion really affect the development of acne? Br J Dermatol 153 (2005) 919–924.
[33] T. Kesseler, K. Enderer, G.K. Steigleder, [Quantitative analysis of skin surface lipids using a sebumeter method. Random lipid levels on the skin surface during therapy with 13-cis-retinoic acid, minocycline hydrochloride and UVA rays], Z Hautkr 60 (1985) 857–865.
[34] E. Ingham, E.A. Eady, C.E. Goodwin, J.H. Cove, W.J. Cunliffe, Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris, J. Inv. Dermatol. 98 (1992) 895–901.
[35] B. Dréno, What is new in the pathophysiology of acne, an overview, J. Eur. Acad. Dermatol. Venereol. 31 (2017) 8–12.