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ORIGINAL PAPER

Plasma level of beta endorphin in seborrheic dermatitis patients

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ABSTRACT

Introduction and aim. Defects in the epidermal barrier, changes in sebum secretion and its composition, *Malassezia* spp. overgrowth, endocrine, immune, and neurological disorders are the main pathogenesis items of seborrheic dermatitis (SD). "The opioid system of the skin" was considered a new target in the diagnosis and treatment of SD. The study aimed to determine beta-endorphin (BE) levels in adult patients with seborrheic dermatitis and correlate them with the severity of symptoms and itching.

Material and methods. 26 healthy and 62 SD people were examined. SEDASI scale were used to estimate the severity of symptoms and intensity of itching. The determination of the beta-endorphin level was carried out by the ELISA method with the test system Human BE NBP2 (78774 Novus Biologicals).

Results. BE in the SD group was higher compared to the control group (35.5 pg/mL, 22. pg/mL, p<0.001). The level of BE in seborrheic patients did not depend on age and sex but was rising with severity of symptoms. Positive correlations were found between the level of BE and the SEDASI was 0.42 (p<.001), between the level of BE and itching was 0.332 (p=0.009).

Conclusions. SD patients have an increased level of BE that positively correlates with itching and disease severity.

Keywords. beta-endorphin, itch, seborrheic dermatitis

Introduction

The development of seborrheic dermatitis (SD) includes three main prerequisites: hypersecretion of sebum, overgrowth of *Malassezia* fungus, and an immune system response. Pathogenesis is described in the following phases. Sebaceous glands secrete lipids onto the skin surface. Then *Malassezia* funguses colonize areas covered with lipids and consume saturated fatty acids, leaving behind skin-irritating unsaturated fatty acids (like oleic). Those acids induce desquamation and skin barrier disruption. Because of the increased growth and activity of funguses, T-lymphocytes produce cytokines that stimulate keratinocyte proliferation and differentiation. Immune response and products of fungal metabolism damage the skin surface, resulting in erythema, pruritus, and scaling.[1] This pathogenic chain gives a logical explanation of the inflammatory process, but some facts are contradictory. Nearly 80% of SD patients are colonized with *Malassezia spp.*,[2] and hyphae are more invasive than yeast forms, but they colonize a third of all inflamed sites in SD patients.[3] *Staphylococcus* and *Streptococcus spp.* are cultivated from skin specimens more often than pathogenic fungi.[4] Also, patients with SD may not have oily skin.[5] Additional skin regulatory mechanisms should be considered to provide a more flexible pathogenesis theory (Fig. 1).

Neuroendocrine regulation of derma metabolism and the immune response are combined into the term "opioid system of the skin". Opioid receptors and their ligands are part of this system, allowing the skin to respond to various biological, chemical, and physical stresses.[6] The cutaneous opioid system is mainly responsible for nociception and inflammation, playing an essential role in

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skin homeostasis, regeneration, wound healing, and aging.[7] Unmyelinated nerve fibers in the derma, epidermis and keratinocytes express μ-opiate receptors that respond specifically to β-endorphin (BE). BE may be absorbed from the blood or synthesized directly by nerve endings, keratinocytes, and sebaceous glands. The keratinocytes that produce BE are clustered around the terminal ends of the unmyelinated nerve fibers and can influence nerve fibers directly. On the other hand, nerve fibers also secrete BE and influence the differentiation, migration, and cytokine production of keratinocytes.[8]

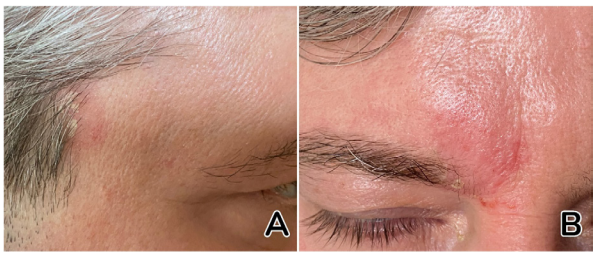

Fig. 1. Mild form of seborrheic dermatitis: the temple area (A); forehead area (B). Patient washed away the flakes of dry skin and used skin moisturizer to conceal the inflamed areas

After the skin damage, the opioid system is activated via a pain stimulus. BE has a strong antinociceptive effect.[9] In low dosages, it's excreted immediately after the skin damage. Concentration rises in the first two hours and reaches its peak in the fourth hour. BE level rises due to the high threshold of sensitivity of the opioid receptors (OR). Typically, action lasts no longer than a day.[10] The decrease in sensitivity of OR due to constant exposure to BE makes its action dosage-dependent. Chronic exposure to BE opioid use leads to tolerance, defined as a decrease in the drug response.[9] It is possible to reproduce in vitro such a phenomenon when cellular models expressing OR are exposed to agonists; in that situation, a decrease in signaling is observed and is designated as OR desensitization.[11] Some reports distinguish the OR desensitization from the cellular tolerance. When rats are chronically exposed to morphine, examination of μ-OR activity on the outward potassium current shows a reduction compared to naive animals, which is not reversible even after 6 hours in morphine-free medium.[12]

Aim

The aim of the study was to determine the levels of beta-endorphin in adult patients with seborrheic dermatitis and correlate them with the severity of symptoms and itching.

Material and methods

Patients with seborrheic dermatitis were recruited from the Transcarpathian regional clinical dermato-venereological center and the Center of Family Medicine of Uzhgorod city during the 2020–2022 years. The study was carried out within the framework of "Alternative methods of treatment of opportunistic infections using medicinal and non-medicinal means," subject code 12A-2021, state registration number 0121U110174, and "Health and recreation. Peculiarities of the clinical and epidemiological courses of infections and parasitosis characteristic of the Transcarpathian region", state registration number 0117U00283, subject code 02070832. The Ethics Committee of the Medical Faculty at Uzhgorod National University approved the scientific evaluation and study protocol (No. 318/4-6, dated September 16, 2020). Before the examination, the patients were informed about the research design, developed within the framework of the Helsinki Declaration of the World Medical Association "Ethical Principles of Medical Research with the Participation of a Person as an Object of Research," the Convention of the Council of Europe on Human Rights and Biomedicine, and the legislation of Ukraine, and signed the informed consent. People with mild-to-moderate seborrheic dermatitis were asked to participate in the study. Patients were included according to the following criteria: age between 18 and 55 years and diagnosis of SD based on the symptom scale of seborrheic dermatitis (SSSD). The control group was chosen from healthy medical workers with no skin diseases. Participants were excluded if they were diagnosed with viral hepatitis, HIV+, atopic dermatitis, acne, psoriasis, autoimmune, oncological, or rheumatological diseases that require corticosteroid medication use. The following diseases were taken as most commonly associated with SD to exclude their impact on the severity of SD.[13]

The seborrheic dermatitis area and severity index (SEDASI)[14] scale was used to estimate the severity of symptoms: 1–14 mild; 15–29 moderate; 30–44 severe; 45–60 very severe. A visual analog scale where zero is no itch (or sleeplessness) and 10 is the worst imaginable itch (or sleeplessness) was used to represent the intensity of itching. 0 = no pruritus, > 0-< 4 points = mild pruritus, ≥ 4-< 7 points = moderate pruritus, ≥ 7-< 9 points = severe pruritus, and ≥ 9 points = very severe pruritus.[15]

The determination of the beta endorphin level was carried out by the ELISA method with the test system Human BE NBP2 – 78774 Novus Biologicals (sensitivity of the test: 9.38 pg/mL. detection range 15.63 -1000 pg/mL)

Fasting blood samples were collected between 8 and 9 a.m. Blood was collected in a Vacuette tube with a plasma coagulation activator (CAT serum cloth activator) and left to rest at room temperature for 2 hours. The tubes were centrifuged for 20 minutes at 1000xg at 8°C. Frozen plasma was stored at -18°C for 2 weeks. Defrosted samples were tested by given protocol of enzyme-linked immunosorbent assay for quotative detection.

Statistical analysis was carried out by Jamovi v. 1.6 (Sydney, Australia). The chi-square test was used to de-

termine the difference in gender distribution between the SD and control groups. The Mann-Whitney t-test for independent samples was used to determine the difference in age between groups. The normality of the distribution of quantitative data was analyzed by the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare levels of BE in SD and control groups. The Dwass-Steel-Critchlow-Flinger pairwise comparison test was used to compare levels of BE at different severity levels. Spearman's correlation was used to determine the relationship between the level of BE, SEDASI and itching scores. The strength of the correlation was evaluated using the Chaddock scale. α=0.05 was considered the critical level of reliability.

Results

88 people participated in the study, including 62 people with SD (the main group) and 26 people without SD (the control group). There were 35 (56.5%) men and 27 (43.5%) women in the main group, 10 (38.5%) men, and 16 (61.5%) women in the control group. The BE level of one of the participants in the main group exceeded the average group level by more than 10 times and therefore was evaluated as an outlier, being excluded from further analysis. The average age of the main group participants was 32.9±1.55, and the average age of the control group participants was 34.88±1.79 years; the groups did not differ in age (p>0.05). Men and women in the main group did not differ in age (34.57±2.49 vs. 30.65±1.42; p>0.2).

Table 1. Comparison of BE level in healthy individuals and seborrheic dermatitis patients*

BE (pg/mL)	SD group	control group	χ^2	W	p
Men under 30 years	36.5±10.1	22±2.8	8.44	4.11	0.004
Men over 30 years	31.9±9.78	21.1±6.07	5.03	3.17	0.025
Men of all ages	34.1±10.1	22.6±9.61	12.3	4.95	0.001
Women under 30 years	36.6 ±8.3	18.4±6.58	8.58	4.14	0.003
Women over 30 years	39.1±8.03	24.1±10.5	8.54	4.12	0.003
Women of all ages	37±6.4	18.5±3.15	17.5	5.95	0.001
All people under 30 years	35±6	21.1±5.53	17.8	5.96	0.001
All people over 30 years	34.7±9.67	23.3±5.44	12.8	5.05	0.001
All men	34.1±10.1	22.6±9.61	12.3	4.95	0.001
All women	37.0±6.92	18.5±2.98	17.5	5.95	0.001
Group total	35.0±5.37	22.0±3.69	29.8	7.72	0.001

* under 30 – age from 18 to 29 years; over 30 – age from 30 to 55 years

The level of BE in the main group was significantly higher compared to the control group (35.5 pg/mL, 22 pg/mL, p<0.001). BE in SD patients did not differ significantly between males and females and did not depend on age (Table 1). A comparison of males and females under and over 30 showed a significant difference between SD patients and the healthy control group. The SEDASI score did not depend on sex (χ^2=1.2756, p=0.982) or age (χ^2=0.2367, p=0.627). Itching score also had no signific difference between males and females (χ^2=0.8100, p=0.235) and did not depend on age of the patients (χ^2=0.4496, p=0.503).

The level of BE depended on the severity of the SD. The average of BE in plasma of patients with mild forms of SD (n=13) was 26±6.13 pg/mL (the amount of BE is rounded to whole numbers); average of SEDASI score in mild form group was 11±2.0; itch intensity 3±0.725. BE in moderate form group (n=29) was 35±3.97 pg/mL, SEDASI 20±3.5, pruritus 6±1. BE in severe SD group (n=20) was 43±5.24 pg/mL, SEDASI 36±3, pruritus 6±1. Very severe SD patients (n=4) had the average of BE of 46±1.22 pg/mL, pruritus 6±1.5. A statistical difference was found in BE level of mild and severe form (p=0.006) (Table 2).

Table 2. BE pairwise comparisons between different degrees of severity of SD

Severity of SD	W	p
Mild vs moderate	4.014	0.024
Mild vs severe	4.598	0.006
Mild vs very severe	3.538	0.060
Moderate vs severe	2.619	0.249
Moderate vs very severe	2.290	0.368

Correlation analysis showed that the level of endorphins increases with SEDASI score, intensity of itching and does not depend on age in patients with seborrheic dermatitis (Table 3).

Table 3. Correlation between BE, intensity of itching, severity of disease and age of SD patients*

	BE		Pruritus vs BE*		SEDASI vs BE*		SEDASI vs pruritus *	
	R	p	R	p	R	p	R	p
All patients (n=67)	-0.074	0.569	0.332	0.009	0.42	<0.001	0.533	<0.001
All patients under 30 years (n=32)	0.078	0.671	0.307	0.087	0.415	0.018	0.612	<0.001
All patients over 30 years (n=29)	-0.039	0.842	0.362	0.054	0.421	0.422	0.41	0.027
Man of all ages (n=35)	-0.192	0.27	0.35	0.039	0.424	0.011	0.511	0.002
Man under 30 years (n=17)	0.098	0.709	0.3	0.242	0.546	0.023	0.563	0.019
Man over 30 years (n=18)	-0.025	0.920	0.412	0.09	0.202	0.423	0.443	0.065
Woman of all ages (n=26)	0.219	0.282	0.277	0.171	0.391	0.048	0.573	0.002
Woman under 30 years (n=15)	0.041	0.884	0.326	0.235	0.221	0.43	0.695	0.004
Woman over 30 years (n=11)	0.205	0.546	0.254	0.45	0.6	0.601	0.44	0.176

* data represents result of correlation between level of beta endorphin and intensity of itching or SEDASI score in a group divided by age and sex

The correlation in the whole group of SD patients (n=67) between the level of BE and the SEDASI score

was 0.42 ($p<0.001$), between the level of BE and itching was 0.332 ($p=0.009$). Positive correlation was found between BE level and SEDASI score in a group of SD patients younger than 30 years ($p=0.018$), males younger than 30 years ($p=0.023$) and man and woman of all ages ($p=0.011$, $p=0.048$). Positive correlation of mild intensity between SEDASI score and intensity of itching was found in all most all age groups of SD patients ($p=0.027$, $p<0.001$). There was found no significant correlation between the age of SD patients and level of BE ($p>0.27$). No significant correlation between BE and age was found in a control group ($R=0.141$, $p=0.491$).

Discussion

In this work, we first determined that seborrheic dermatitis patients have increased levels of BE in the blood compared to healthy individuals. The average level was 35 (28.3; 43.8) pg/mL. To accurately determine a difference in the level of BE at different ages, the amount of patience needs to be increased. Males and females older than 30 years had different mean levels of BE (31.9±9.78; 39.1±8.03) compared to a group level 34.7±9.67, but no signific statistical difference was found ($p=0.52$). The level of BE rises with the severity of seborrheic dermatitis and is strongly associated with the intensity of itching. BE level correlates with the area of skin damage. There is a significant increase in plasma BE in burned patients that correlates positively with the extent of the burn areas.[10] Classical itching mechanisms involve histamine release by IgE activation of mast cells in response to allergens. SD patients have a significant increase in histamine[16] and cathepsin S in the blood.[17] High levels of histamine increase BE in cerebrospinal fluid,[18] but the direct influence of BE on histamine release remains unclear. Although opioid analgesics like codeine provoke mast cell degranulation,[19] morphine was reported to have dose-dependent itching intensity.[20]

Histamine-unrelated theories of skin itch are based on a decrease in BE sensitivity. The unmyelinated nerve fibers in the epidermis are stretched and therefore thinner and less sensitive to itching stimuli. The opiate receptors on nerve endings in inflamed skin are downregulated, which suggests dosage-dependent mechanisms of chronic itch development.[21] In addition to this theory, it was described that intravenous administration of naloxone, an opioid receptor antagonist, significantly reduces chronic itching.[22]

Psoriasis patients have twice the normal levels of BE, and about 50% have both atopic dermatitis (AD) and systemic sclerosis, compared with healthy individuals. BE rises following long-lasting and actively spreading psoriatic plaques and decreases after treatment and in the remission stage. The study reports that men with psoriasis and itch have comparatively lower amounts of BE than non-itch males, but there is no difference in female patients.[23] The other study compared the level of BE in children with AD. They found that kids with aggravation of atopic dermatitis have higher levels of BE and more intense itch. Kids in the remission stage had similar to control group levels of BE.[24]

A new trichoscopic sign of SD was described. The vascular conglomerate "Dandelion," which looks like a yellow dot, is surrounded by glomerular and comma-shaped vessels. The cumulation of sebum and keratin in the hair infundibulum forms the yellow center.[25] Interestingly, opioid growth factor (the met-encephalin molecule) inhibits angiogenesis, including mesenchymal and endothelial vessels.[26] According to these data, endocannabinoids enhance lipid synthesis and apoptosis of human sebocytes via cannabinoid receptor-2-mediated signaling.[27] Keratinocytes produce BE, which binds to opioid receptors, and this has been associated with the intensity of subjective itch in patients with AD.[28] Also, activation of the cannabinoid receptor-2 (CB2R) in skin keratinocytes by endocannabinoids is the mechanism underlying circulating BE elevation in patients with obstructive jaundice.[29] The "itchscriptome" analysis was made via RNA sequencing to identify itch-related mediators and receptors in patients with AD and psoriasis. Cytokines such as IL-17A, IL-23A, IL-31, and BE had elevated gene transcript levels in both itchy atopic and psoriatic skin. However, the administration of BE may dose-dependently enhance scratching, which can be inhibited by μ-opioid peptide antagonists.[30] The effect of BE was studied on nonhuman primates. Administration of BE intensified the itch and attenuated inflammatory pain by binding to the mu-opioid peptide receptor. An anti-itch effect was achieved after dynorphin A blockage on kappa-opioid peptide receptors.[31]

The increase in BE levels in SD patients is associated with the ability of CD4+ T lymphocytes to produce opioids.[32] The skin of SD patients is overpopulated with *Staphylococcus epidermidis* and *Cutibacterium acnes*.[33] Excessive bacterial growth causes CD4+ T lymphocytes to produce BE upon antigen priming in draining lymph nodes[34] and correlates with the stimulatory potency of antigen-presenting cells.[35] BE-induced B-lymphocyte suppression was observed too. Exposition of *S. aureus*-stimulated peripheral blood-derived mononuclears to BE resulted in a dose-dependent inhibition of immunoglobulin-secreting cell (ISC) formation. It was found that IgG-ISC was suppressed more than IgA-ISC or IgM-ISC. In contrast to these results, BE was found to be unable to suppress *S. aureus*-induced immunoglobulin secretion.[36]

Endogenous opioid BE inhibits the transcription of IL-2 in T lymphocytes and activates the transcription factors AP-1, NFAT, and NF-kappaB, which transactivate IL-2. Incubation of T-cells with opioids causes a marked increase in cAMP and further enhancement of the ton-

ic inhibition of the leukocyte-specific protein tyrosine kinase, thereby blocking the initiation of T-cell receptor signaling.[37] Also, there is a strong induction of interleukin 4, a cytokine that induces differentiation of naive helper T cells.[38] On the other hand, BE, acting through a nonopioid beta-endorphin receptor, may modulate immunocompetence by stimulating T-cell proliferation and counteracting the inhibitory effects of prostaglandin E1.[39] The strain-dependent opposing effects of BE on inflammation are mediated through delta and kappa opioid receptors and involve changes in the production of reactive oxygen species by inflammatory cells.[40]

T-lymphocytes were described as being able to switch on analgesia by accumulating near the sites of injured nerves and utilizing BE to activate local antinociception receptors. This effect was demonstrated in wild-type and severe combined immunodeficiency (SCID) mice. In wild-type mice, T-lymphocytes that infiltrated the injured nerve expressed BE and receptors for corticotropin-releasing factor (CRF), which associate with the release of opioids from leukocytes. In SCID mice, T-cells expressing BE and CRF receptors were absent. The decreased antinociception was fully restored after transferring T-lymphocytes from wild-type mice. Also, antinociception was reversed after BE-antibodies injection.[41] In mice, CD4+ T-lymphocytes lose analgesic opioid-mediated activity when there is an encephalin deficiency.[32]

BE enhances NK cell activity. It is, however, not known whether it influences NK cell activity by recruiting effector cells, increasing adhesion (the number of effector cell-target cell conjugates), or enhancing the lytic step.[42] The IL-1 family directly influences the anterior pituitary cells and thereby induces the production of BE.[43] IL-31 is an inflammatory cytokine that triggers cell-mediated immunity against pathogens. IL-31 stimulates BE production by keratinocytes and correlates with the intensity of pruritus in inflamed skin.[44]

Conclusion

To conclude, both keratinocytes, sebaceous glands, and nerve endings possess μ-opioid receptors and can be activated by blood or self-produced BE. Dosage-dependent opioid receptors become less sensitive after prolonged exposure to high dosages of BE, which leads to an addiction mechanism and chronization of inflammation in the skin. Normally, opioid peptide is needed no longer than 4 hours after wounding to start antinociception and inflammation. Increased opioid receptor tolerance involves higher dosages of BE. Instead of activating the immune response in a wound, a high BE level inhibits T-cell maturation and IgG production, stimulates sebaceous glands to produce more fat, provokes pathological itching as well as new vessel growth and hyperkeratosis, and enhances NK cell activity. A better understanding of SD pathogenesis leads to a personalized treatment approach, including pharmacological or biological via antibodies regulation of epidermal μ-opioid BE specific receptors.

Declarations

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Author contributions

Conceptualization, V.V. and G.M.; Methodology, V.V.; Software, V.V.; Validation, V.V.; Formal Analysis, V.V.; Investigation, V.V.; Resources, V.V.; Data Curation, V.V.; Writing – Original Draft Preparation, V.V.; Writing – Review & Editing, G.M.; Visualization, V.V.; Supervision, G.M.; Project Administration, V.V.; Funding Acquisition, V.V.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

The study was carried out within the framework of "Alternative methods of treatment of opportunistic infections using medicinal and non-medicinal means," subject code 12A-2021, state registration number 0121U110174, and "Health and recreation. Peculiarities of the clinical and epidemiological courses of infections and parasitosis characteristic of the Transcarpathian region", state registration number 0117U00283, subject code 02070832. The Ethics Committee of the Medical Faculty at Uzhgorod National University approved the scientific evaluation and study protocol (No. 318/4-6, dated September 16, 2020). All participants were provided with a study protocol and signed the informed consent.

References

1. Adalsteinsson JA, Kaushik S, Muzumdar S, Guttman-Yassky E, Ungar J. An update on the microbiology, immunology and genetics of seborrheic dermatitis. *Exp Dermatol*. 2020;29(5):481-489. doi: 10.1111/exd.14091
2. Hamdino M, Saudy AA, El-Shahed LH, Taha M. Identification of Malassezia species isolated from some Malassezia associated skin diseases. *J Mycol Med*. 2022;32(4):101301. doi: 10.1016/j.mycmed.2022.101301
3. Li J, Feng Y, Liu C, et al. Presence of Malassezia Hyphae Is Correlated with Pathogenesis of Seborrheic Dermatitis. *Microbiol Spectr*. 2022;10(1):e0116921. doi:10.1128/spectrum.01169-21

4. Dityen K, Soonthornchai W, Kueanjinda P, et al. Analysis of cutaneous bacterial microbiota of Thai patients with seborrheic dermatitis. *Exp Dermatol*. 2022;31(12):1949-1955. doi: 10.1111/exd.14674
5. Yoon JS, Shim J, Lim JM, Park SG. Biophysical characteristics of dandruff-affected scalp categorized on the basis of sebum levels. *J Cosmet Dermatol*. 2021;20(3):1002-1008. doi: 10.1111/jocd.13626
6. Slominski AT, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM, Steketee JD. Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. *Adv Anat Embryol Cell Biol*. 2012;212:v-115. doi: 10.1007/978-3-642-19683-6_1
7. Bigliardi PL, Dancik Y, Neumann C, Bigliardi-Qi M. Opioids and skin homeostasis, regeneration and ageing - What's the evidence? *Exp Dermatol*. 2016;25(8):586-591. doi: 10.1111/exd.13021
8. Bigliardi-Qi M, Sumanovski LT, Büchner S, Rufli T, Bigliardi PL. Mu-opiate receptor and Beta-endorphin expression in nerve endings and keratinocytes in human skin. *Dermatology*. 2004;209(3):183-189. doi: 10.1159/000079887
9. Yaksh TL, Henry JL. Antinociceptive effects of intrathecally administered human beta-endorphin in the rat and cat. *Can J Physiol Pharmacol*. 1978;56(5):754-759. doi: 10.1139/y78-120
10. Xue JZ. Changes in plasma immunoreactive beta-endorphin in burn and its clinical significance. *Chinese journal of plastic surgery and burns. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi*. 1991;7(4):253-6, 317.
11. Allouche S, Noble F, Marie N. Opioid receptor desensitization: mechanisms and its link to tolerance. *Front Pharmacol*. 2014;5:280. doi: 10.3389/fphar.2014.00280
12. Levitt ES, Williams JT. Morphine desensitization and cellular tolerance are distinguished in rat locus ceruleus neurons. *Mol Pharmacol*. 2012;82(5):983-992. doi: 10.1124/mol.112.081547
13. Wikramanayake TC, Borda LJ, Miteva M, Paus R. Seborrheic dermatitis-Looking beyond Malassezia. *Exp Dermatol*. 2019;28(9):991-1001. doi: 10.1111/exd.14006
14. Micali G, Lacarrubba F, Dall'Oglio F. A new proposed severity score for seborrheic dermatitis of the face: SEborrheic Dermatitis Area and Severity Index (SEDASI). *J Am Acad Dermatol*. 2017;76(6):AB18. doi: 10.1016/j.jaad.2017.04.088
15. Reich A, Heisig M, Phan NQ, et al. Visual analogue scale: evaluation of the instrument for the assessment of pruritus. *Acta Derm Venereol*. 2012;92(5):497-501. doi: 10.2340/00015555-1265
16. Vijayaraghavan S, Vedula SK, Bourokba N, et al. Relationship between scalp histamine levels and dandruff within an Indian population: A confirmation study using LC/MS/MS method. *Exp Dermatol*. 2022;31(5):814-818. doi: 10.1111/exd.14539
17. Viodé C, Lejeune O, Turlier V, et al. Cathepsin S, a new pruritus biomarker in clinical dandruff/seborrhoeic dermatitis evaluation. *Exp Dermatol*. 2014;23(4):274-275. doi: 10.1111/exd.12357
18. Kjaer A, Larsen PJ, Knigge U, Møller M, Warberg J. Histamine stimulates c-fos expression in hypothalamic vasopressin-, oxytocin-, and corticotropin-releasing hormone-containing neurons. *Endocrinology*. 1994;134(1):482-491. doi: 10.1210/endo.134.1.8275963
19. Babina M, Wang Z, Roy S, et al. MRGPRX2 Is the Codeine Receptor of Human Skin Mast Cells: Desensitization through β-Arrestin and Lack of Correlation with the FcεRI Pathway. *J Invest Dermatol*. 2021;141(5):1286-1296.e4. doi: 10.1016/j.jid.2020.09.017
20. Nakasone T, Sugimoto Y, Kamei C. The interaction between histamine H1 receptor and μ- opioid receptor in scratching behavior in ICR mice. *Eur J Pharmacol*. 2016;777:124-128. doi: 10.1016/j.ejphar.2016.03.005
21. Bigliardi-Qi M, Lipp B, Sumanovski LT, Buechner SA, Bigliardi PL. Changes of epidermal mu-opiate receptor expression and nerve endings in chronic atopic dermatitis. *Dermatology*. 2005;210(2):91-99. doi: 10.1159/000082563
22. Wang Z, Jiang C, Yao H, et al. Central opioid receptors mediate morphine-induced itch and chronic itch via disinhibition. *Brain*. 2021;144(2):665-681. doi: 10.1093/brain/awaa430
23. Glinski W, Brodecka H, Glinska-Ferenz M, Kowalski D. Increased concentration of beta-endorphin in sera of patients with psoriasis and other inflammatory dermatoses. *Br J Dermatol*. 1994;131(2):260-264. doi: 10.1111/j.1365-2133.1994.tb08502.x
24. Georgala S, Schulpis KH, Papaconstantinou ED, Stratigos J. Raised beta-endorphin serum levels in children with atopic dermatitis and pruritus. *J Dermatol Sci*. 1994;8(2):125-128. doi: 10.1016/0923-1811(94)90006-x
25. Ruiz-Arriaga LF, Arenas R, Vega-Sánchez DC, Asz-Sigall D, Martínez-Velazco MA. Seborrheic Dermatitis: Three Novel Trichoscopic Signs and Its Correlation to *Malassezia* sp. Colonization. *Skin Appendage Disord*. 2019;5(5):288-292. doi: 10.1159/000497782
26. Blebea J, Mazo JE, Kihara TK, et al. Opioid growth factor modulates angiogenesis. *J Vasc Surg*. 2000;32(2):364-373. doi: 10.1067/mva.2000.107763b
27. Dobrosi N, Tóth BI, Nagy G, et al. Endocannabinoids enhance lipid synthesis and apoptosis of human sebocytes via cannabinoid receptor-2-mediated signaling. *FASEB J*. 2008;22(10):3685-3695. doi: 10.1096/fj.07-104877
28. Ádám D, Arany J, Tóth KF, Tóth BI, Szöllősi AG, Oláh A. Opioidergic Signaling-A Neglected, Yet Potentially Important Player in Atopic Dermatitis. *Int J Mol Sci*. 2022;23(8):4140. doi:10.3390/ijms23084140
29. Tao K, Zhu J, Wei K, et al. Cannabinoid Receptor-2 Activation in Keratinocytes Contributes to Elevated Peripheral β-Endorphin Levels in Patients With Obstructive Jaundice. *Anesth Analg*. 2021;133(1):251-262. doi: 10.1213/ANE.0000000000005405

30. Nattkemper LA, Tey HL, Valdes-Rodriguez R, et al. The Genetics of Chronic Itch: Gene Expression in the Skin of Patients with Atopic Dermatitis and Psoriasis with Severe Itch. *J Invest Dermatol*. 2018;138(6):1311-1317. doi: 10.1016/j.jid.2017.12.029
31. Lee H, Ko MC. Distinct functions of opioid-related peptides and gastrin-releasing peptide in regulating itch and pain in the spinal cord of primates. *Sci Rep*. 2015;5:11676. doi: 10.1038/srep11676
32. Basso L, Boué J, Mahiddine K, et al. Endogenous analgesia mediated by CD4(+) T lymphocytes is dependent on enkephalins in mice. *J Neuroinflammation*. 2016;13(1):132. doi: 10.1186/s12974-016-0591-x
33. Fournière M, Latire T, Souak D, Feuilloley MGJ, Bedoux G. *Staphylococcus epidermidis* and *Cutibacterium acnes*: Two Major Sentinels of Skin Microbiota and the Influence of Cosmetics. *Microorganisms*. 2020;8(11):1752. doi: 10.3390/microorganisms8111752
34. Boué J, Blanpied C, Brousset P, Vergnolle N, Dietrich G. Endogenous opioid-mediated analgesia is dependent on adaptive T cell response in mice. *J Immunol*. 2011;186(9):5078-5084. doi: 10.4049/jimmunol.1003335
35. Boué J, Blanpied C, Djata-Cabral M, Pelletier L, Vergnolle N, Dietrich G. Immune conditions associated with CD4+ T effector-induced opioid release and analgesia. *Pain*. 2012;153(2):485-493. doi: 10.1016/j.pain.2011.11.013
36. Morgan EL, McClurg MR, Janda JA. Suppression of human B lymphocyte activation by beta-endorphin. *J Neuroimmunol*. 1990;28(3):209-217. doi: 10.1016/0165-5728(90)90014-e
37. Börner C, Warnick B, Smida M, et al. Mechanisms of opioid-mediated inhibition of human T cell receptor signaling. *J Immunol*. 2009;183(2):882-889. doi: 10.4049/jimmunol.0802763
38. Börner C, Lanciotti S, Koch T, Höllt V, Kraus J. μ opioid receptor agonist-selective regulation of interleukin-4 in T lymphocytes. *J Neuroimmunol*. 2013;263(1-2):35-42. doi: 10.1016/j.jneuroim.2013.07.012
39. Hemmick LM, Bidlack JM. Beta-endorphin stimulates rat T lymphocyte proliferation. *J Neuroimmunol*. 1990;29(1-3):239-248. doi: 10.1016/0165-5728(90)90167-l
40. Stanojević S, Mitić K, Vujić V, Kovacević-Jovanović V, Dimitrijević M. Beta-endorphin differentially affects inflammation in two inbred rat strains. *Eur J Pharmacol*. 2006;549(1-3):157-165. doi: 10.1016/j.ejphar.2006.08.012
41. Labuz D, Schreiter A, Schmidt Y, Brack A, Machelska H. T lymphocytes containing β-endorphin ameliorate mechanical hypersensitivity following nerve injury. *Brain Behav Immun*. 2010;24(7):1045-1053. doi: 10.1016/j.bbi.2010.04.001
42. Mathews PM, Froelich CJ, Sibbitt WL Jr, Bankhurst AD. Enhancement of natural cytotoxicity by beta-endorphin. *J Immunol*. 1983;130(4):1658-1662.
43. Făgărăsan MO, Axelrod J, Catt KJ. Interleukin 1 potentiates agonist-induced secretion of beta-endorphin in anterior pituitary cells. *Biochem Biophys Res Commun*. 1990;173(3):988-993. doi: 10.1016/s0006-291x(05)80883-6
44. Lee CH, Hong CH, Yu WT, et al. Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide β-endorphin, via STAT3/calcium axis in atopic dermatitis. *Br J Dermatol*. 2012;167(4):794-803. doi: 10.1111/j.1365-2133.2012.11047.x