КЛІНІЧНІ СПОСТЕРЕЖЕННЯ

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CHRONIC INFLAMMATORY SKIN DISORDERS: INTERACTIONS BETWEEN ANGIOGENESIS AND ENDOTHELIAL CELL PROLIFERATION

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Objective. Determination of morphological features of skin lesions in patients with widespread psoriasis, study of the level of expression of immunohistochemical markers of vascularization.
Materials and methods. 80 patients with psoriasis were under observation. The control group consisted of 20 practically healthy people (control group) of the same age. All patients underwent a skin biopsy with a histological evaluation of the biopsy material to determine the form and severity of the course of psoriasis in accordance with the requirements of the morphological section of the modern classification.
Results and discussion. Applying the scale of intensity of skin vascularization according to Amin M.M. et. al. (2012), it was detected that damaged dermal areas due to psoriasis, on average, had 17.25±5.34 micro vessels at magnification (×400), which corresponds to the level of moderate vascularization (11-20 capillaries). Normal skin in control group had mean index 4.32±2.01 at (×400) at the level of weak vascularization (4-10 capillaries), which statistically reliably differs from general group ($p<0.05$). Analysis of the condition of vascular bed at different levels of severity of psoriasis course showed that a number of cells at moderate degree of severity (22.65±5.87) was considerably higher than at mild psoriasis (10.09±3.22), and even more numerous than in CG (4.32±2.01). Statistically reliably both groups differ between them ($p<0.05$), and with CG ($p<0.05$). According to Fisher's exact test, distribution of absolute meanings of monitoring of intensity of cytoplasmic staining with VEGF marker in all groups between them had a reliable difference ($p<0.05$). We detected a moderate correlation connection between increased intensity of VEGF expression and amplification of the severity of psoriasis course ($r=+0.430$). According to Fisher's exact test, distribution of monitoring of intensity of cytoplasmic staining with MMP-9 marker in all groups between them had a reliable difference ($p<0.05$); a moderate correlation connection between increased intensity of MMP-9 marker expression and amplification of the severity of psoriasis course was detected ($r=+0.532$).
Conclusions. The results of conducted clinical, morphological and immunohistochemical investigations enable to consider importance of neoangiogenesis processes in pathogenesis of this dermatosis and need in elaboration of therapeutic measures with direct influence on this aspect of pathogenesis.
Key words: psoriasis, immunity, neoangiogenesis.

Introduction

Nowadays a marked increase in morbidity of chronic dermatoses in the structure of skin diseases is observed. Considering evident worldwide spread of psoriasis (3.5-5% of population) in general structure of skin diseases (9%), among those admitted to hospital (35-38%), as well as long-lasting, relapsing, incurable feature of dermatosis, which affects the most active, working individuals, increased

incidence of low sensitivity to therapy that leads to social de-adaptation of patients, temporary disability, and, sometimes, invalidity. Psoriasis is still an absolute "leader". Besides being one of the most widespread diseases, psoriasis is one of the severest dermetoses. Besides, more cases of torpid, malignant and resistant to treatment course of dermatosis have become one more negative tendency. These factors undoubtedly make psoriasis not only medical, but also social issue. Taking into account actuality of psoriasis morbidity problem, specialists point out differentiated individual approach to diagnostic and therapeutic stages as a key aspect in the management of patients [8; 9; 10]. Investigation of etiopathogenesis and elaboration of target influence on its key aspects will enable to achieve a set goal. Psoriasis is largely an immune system-mediated disease with both genetic and environmental predisposing factors. The prevalence is 3.5-5% of population in general structure of skin diseases (9%), among those admitted to hospital (35-38%), as well as long-lasting, relapsing, incurable feature of dermatosis, which affects the most active, working individuals, increased incidence of low sensitivity to therapy that leads to social de-adaptation of patients, temporary disability, and, sometimes, invalidity. The key pathophysiology is immune cell-triggered keratinocyte hyperproliferation [1]. Although psoriasis is largely T-cell-driven [2], the pathophysiology is greatly modulated by abnormalities of the papillary dermal vasculature. The Auspitz is a visible, characteristic, vascular abnormality that is pathognomonically diagnostic of psoriasis [3]. It appears as pinpoint bleeding that occurring when the scale of psoriatic plaque has been removed, reflecting vascular dilation and elongation with increased blood vessel permeability and a tortuosity specific for psoriasis [4]. Importantly, these vascular changes precede epidermal hyperplasia of psoriatic lesions. Psoriasis improvement (on appropriate treatment) is accompanied by normalization of the vascular structure [5; 6], suggesting that psoriasis-associated microvascular abnormalities play functionally important roles in terms of the primary psoriasis pathogenesis. Angiogenesis of psoriatic lesions ("inflammatory angiogenesis") is characterized by significant vasodilation, vessel elongation, and increased vascular permeability [7, 8]. In healthy skin, the dermal vessels exhibit principally the arterial phenotype, whereas the vessels of psoriasis evidence venous capillaries characterized by a single- or multi-layered basement membrane and a fenestrated endothelium that enhances vascular permeability [9]. Normal functioning of the tissues depends on regular transport of oxygen by blood vessels. Angiogenesis is the process of formation of new blood vessels from the existing vascular system, which plays an important role in normal tissue functioning (ovulation, wound healing, etc.) and in pathological processes (tumors, rheumatoid arthritis, hyperproliferation, among which is psoriasis). Secretion of angiogenic factors, the source of which may be endothelial ones, macrophages, etc., is an important mechanism of neoangiogenesis regulation. Growth of new vessels is determined by balance between its stimulants and inhibitors. In low meaning of correlation between stimulants and inhibitors of vessel formation, neoangiogenesis is blocked or just slight, and vice versa, in high meanings of this correlation, active launching of neoangiogenesis occurs. Growth factors are polypeptides with molecular weight 5-50 kDa, united in group of trophic regulatory substances. Similar to hormones, these factors have a wide spectrum of biological activity on various cells, and unlike hormones, growth factors are produced by non-specific cells, located in all body tissues. The most powerful and dominant stimulant of angiogenesis is vascular endothelial growth factor (VEGF), which is heterodimer glycoprotein growth factor, a potential mitogen for epithelial cells of the vessels. Among non-specific stimulants of angiogenesis, matrix metalloproteinase (MMP) should be singled out, which is a group of matrix-destructing enzymes, the source of which are fibroblasts, macrophages, neutrophils and other cells that play an important role in tissue remodeling, including neoangiogenesis pro-

cesses. Both rapid epidermal proliferation and dermal inflammatory infiltration are accompanied by numerous formations of new blood vessels, which start during the early changes of psoriasis and vanish after skin lesion clearance. These observations highlighted that angiogenesis is the chief distinguishing feature during the pathogenesis of psoriasis. The aim of our research was to determine morphological peculiarities of skin lesions in patients with common psoriasis, investigation of the levels of expression of immunohistochemical markers of vascularization.

Materials and methods

Eighty examined patients (47 males and 33 females) aged 24-58 years with psoriasis (general group) and 20 individuals – without this pathology (in general, healthy) for control group (CG) were included in the investigation. Skin biopsy with histological evaluation of biopsy materials was performed for all patients to establish form and severity of the course of psoriasis according to requirements of morphological chapter of contemporary classification [10, 11, 12].

Results and their discussion

Early changes, which can be observed on light optic level, are dilatation and tortuosity of the vessels of derma papillae and moderate perivascular lymphocyte infiltrates with slight swelling of the neighboring stroma. Marker CD34, giving specific membranous reaction with vascular endothelium helps to more thoroughly evaluate qualitative and quantitative changes in vascular structure. Applying the scale of intensity of skin vascularization according to Amin M.M. et. al. (2012), it was detected that damaged dermal areas due to psoriasis, on average, had 17.25±5.34 micro vessels at magnification (×400), which corresponds to the level of moderate vascularization (11-20 capillaries). Normal skin in control group had mean index 4.32±2.01 at (×400) at the level of weak vascularization (4-10 capillaries), which statistically reliably differs from general group ($p<0.05$). Analysis of the condition of vascular bed at different levels of severity of psoriasis course showed that a number of cells at moderate degree of severity (22.65±5.87) was considerably higher than at mild psoriasis (10.09±3.22), and even more numerous than in CG (4.32±2.01) (table 1). Statistically reliably both groups differ between them ($p<0.05$), and with CG ($p<0.05$).

Table 1 Quantitative characteristic of the density of micro vessels according to meanings of CD34 marker expression depending on severity of the course of common psoriasis, M±m

Expression of CD34 marker	***Control group*** **(n=20)**	***Severity of the course of common psoriasis***	
		Mild degree (n=21)	**Moderate degree (n=59)**
Density of micro vessels per 1 mm (×400)	4.32±2.01	10.09±3.22*	22.65±5.87 *,**

Note: * – difference is reliable in relation to control group at 5% level of significance ($p<0.05$);
** – difference is reliable in relation to psoriasis of mild degree at 5% level of significance ($p<0.05$).

Angiogenesis is always accompanied by increased expression of vascular endothelial growth factor (VEGF) involved in pathological process of tissues. Immunohistochemical investigation of biopsy skin materials with VEGF marker even at the beginning of disease development showed a considerably increased expression of vascular endothelial growth factor in epidermal keratinocytes (besides horny layer) and in the vascular endothelium and perivascular dermal infiltrates of affected areas due to psoriasis. Intensity of

staining of psoriatically changed areas of the skin commonly corresponded to the 3rd category – strong reaction, whereas normal epidermis in CG with VEGF always showed moderate or weak reaction (2, 1 categories of staining) ($p<0.05$). VEGF expression also considerably increased with the worsening of pathological process (table 2).

Table 2 Meaning of VEGF expression depending on psoriasis, n, (%)

Expression of VEGF marker	Control group (n=20)	Severity of common psoriasis severity	
		Mild degree (n=21)	Average degree (n=59)
Negative (0)	-	-	-
Weak (1)	16 (80%)	-	-
Moderate (2)	4 (20%)	6 (29%) *	18 (30%) *,**
High (3)	-	15 (71%) *	30 (51%) *,**
Excessive (4)	-	-	11 (19%) *,**

Note: * – difference is reliable in relation to control group at 5% level of significance ($p<0.05$);
** – difference is reliable in relation to psoriasis of mild degree at 5% level of significance ($p<0.05$).

According to Fisher's exact test, distribution of absolute meanings of monitoring of intensity of cytoplasmic staining with VEGF marker in all groups between them had a reliable difference ($p<0.05$). We detected a moderate correlation connection between increased intensity of VEGF expression and amplification of the severity of psoriasis course ($r=+0.430$), indicating the importance of neoangiogenesis processes in pathogenesis of this disease and necessity of treatment measures with direct influence on this aspect of pathogenesis. It has significant perspectives for target therapy with humanized monoclonal antibody against vascular endothelial growth factor (VEGF) of patients with such diagnosis. On investigation of distribution of meanings of MMP-9 [13, 14] marker expression depending on the severity of common psoriasis course according to recommendations of Aronson Peter J. (2008), a significant increase in expression of this marker in comparison with control group was established (table 3), especially in psoriasis of moderate degree of severity in regions of intracorneal abscesses Munro-Sabouraud, areas of Kogoj pustulosis and perivascular dermal infiltrates having numerous neutrophil infiltration [15, 16].

Table 3 Distribution of meanings of MMP-9 marker expression depending on severity of common psoriasis course, n, (%)

Expression of MMP-9 marker	***Control group*** **(n=20)**	***Severity of common psoriasis course***	
		Mild degree (n=21)	**Moderate degree (n=59)**
Negative (0)	-	-	-
Weak (1)	20 (100%)	-	-
Moderate (2)	-	10 (48%) *	6 (10%) *,**
High (3)	-	11 (52%) *	39 (66%) *,**
Excessive (4)	-	-	14 (24%) *,**

Note * – difference is reliable in relation to control group at 5% level of significance ($p<0.05$);
** – difference is reliable in relation to psoriasis of mild degree at 5% level of significance ($p<0.05$).

According to Fisher's exact test, distribution of monitoring of intensity of cytoplasmic staining with MMP-9 marker in all groups between them had a reliable difference ($p<0.05$); a moderate correlation connection between increased intensity of MMP-9 marker expression and amplification of the severity of psoriasis course was detected ($r=+0.532$). For substantiation of possible effective target therapy, it is expedient to use matrix metalloproteinase MMP-9 on investigation of skin biopsy materials of patients with psoriasis, taking into consideration existence of inhibitors of matrix metalloproteinase, which as a component of combined therapy will lead to a significant decrease in severity of psoriasis course [17].

Conclusions

Need in the study of additional prognostic signs exists; it will enable to substantiate peculiarities of psoriasis course and efficacy of the conducted therapy. The results of conducted clinical, morphological and immunohistochemical investigations enable to consider importance of neoangiogenesis processes in pathogenesis of this dermatosis and need in elaboration of therapeutic measures with direct influence on this aspect of pathogenesis.

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РЕЗЮМЕ
ХРОНІЧНІ ЗАПАЛЬНІ ДЕРМАТОЗИ: ВЗАЄМОЗВЯЗОК МІЖ АНГІОГЕНЕЗОМ І ПРОЛІФЕРАЦІЄЮ ЕНДОТЕЛІОЦИТІВ

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Вступ. Псоріаз – це захворювання шкіри, яке супроводжується системним запаленням і уражає приблизно від 1 до 5% населення в усьому світі. Найбільш обґрунтованою теорією

етіопатогенезу вважається імунна, згідно з якою вирішальну роль відіграють порушення в клітинній ланці імунітету з більш вираженими змінами Т-системи.

Мета роботи. Визначення морфологічних особливостей ураження шкіри у хворих на розповсюджений псоріаз, дослідження рівня експресії імуногістохімічних маркерів васкуляризації.

Матеріали та методи. Під спостереженням перебували 80 хворих на псоріаз. Контрольну групу склали 20 практично здорових людей (контрольна група) того ж віку. Усім хворим проводили біопсію шкіри з гістологічною оцінкою біопсійного матеріалу для встановлення форми та тяжкості перебігу псоріазу відповідно до вимог морфологічного розділу сучасної класифікації.

Результати та їх обговорення. Застосовуючи шкалу інтенсивності васкуляризації шкіри за Амін М.М. et. al. (2012), виявлено, що пошкоджені ділянки шкіри внаслідок псоріазу в середньому мають 17,25±5,34 мікросудин при збільшенні (×400), що відповідає рівню помірної васкуляризації (11-20 капілярів). Нормальна шкіра контрольної групи мала середній показник 4,32±2,01 (×400) на рівні слабкої васкуляризації (4-10 капілярів), що статистично достовірно відрізняється від загальної групи ($p<0,05$). Аналіз стану судинного русла при різних ступенях тяжкості перебігу псоріазу показав, що кількість клітин при середньому ступені тяжкості (22,65±5,87) була значно більшою, ніж при легкому псоріазі (10,09±3,22), і навіть більше, ніж у КГ (4,32±2,01). Статистично достовірно обидві групи відрізняються як між собою ($p<0,05$), так і КГ ($p<0,05$). За точним критерієм Фішера розподіл абсолютних значень моніторингу інтенсивності забарвлення цитоплазми маркером VEGF у всіх групах між ними мав достовірну різницю ($p<0,05$). Виявлено помірний кореляційний зв'язок між підвищенням інтенсивності експресії VEGF та посиленням тяжкості перебігу псоріазу ($r=+0,430$). За точним критерієм Фішера розподіл моніторингу інтенсивності забарвлення цитоплазми маркером MMP-9 в усіх групах між собою мав достовірну різницю ($p<0,05$); виявлено помірний кореляційний зв'язок між підвищенням інтенсивності експресії маркера MMP-9 та посиленням тяжкості перебігу псоріазу ($r=+0,532$).

Висновки. Результати проведених клінічних, морфологічних та імуногістохімічних досліджень дозволяють врахувати значення процесів неоангіогенезу в патогенезі даного дерматозу та необхідність розробки терапевтичних заходів з безпосереднім впливом на цю сторону патогенезу.

Ключові слова: псоріаз, імунітет, неоангіогенез.

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