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Actuality Of Skin Scales Microscopy In Patients With Seborrheic Dermatitis

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Abstract:

Background. Fungal infection of the scalp (tinea capitis) and seborrheic dermatitis (SD) of the scalp are sometimes difficult to distinct because of absence the exact clinical symptoms. In such cases physician may use the result of skin scales microscopy. Aim of this study was the estimation of results of skin scales microscopy for the presence of yeast and dermatophyte fungi among SD patients. Materials and Methods. Two groups of SD patients – the first with clinical diagnosis "SD of scalp" (n = 21) and the second with the same diagnosis which needed accurate definition because of excluding of mycosis (n = 39). Microscopy of skin scales was carried out after refining of specimens with alkali solution. Results. The dermatophyte fungi were frequently detected in patients of both groups: in the first group 90,5% of patients had spores and 47,6% had mycelium, with that in the second group - 97,4% and 46,2% correspondingly. Yeast blastospores, predominantly of Malassezia genus, were find out with frequency of 23,8% in the first group and 25,6% in the second. Conclusions. It is obvious that microscopic research in general should lead to updating of diagnosis: "seborrheic dermatitis, mycosis of the scalp".

Keywords: *microscopy, seborrheic dermatitis, dermatophytes, yeasts.*

Introduction: seborrheic dermatitis (SD) is a chronic recurrent skin disease associated with high secretion and altering of composition of sebum and localized in areas of sebum glands accumulation – scalp, face, upper part of body, creases of the skin [1]. SD of scalp should be differentiated from the mycosis of the locus - tinea capitis – in the cases of usual SD symptoms associated with scalp pruritus, occipital adenopathy, and singular or multiple zones of alopecia, or diffuse al[1]opecia [2]. The characterization of skin mycrobiota in such patients is usually restricted with use of ultraviolet irradiation at which areas with disseminated fungi are distinctive shining. Sowing of skin dermatophytes is not a routine practice because of their long period of cultivation and identification. In this case the microscopy of skin scales is good support for physician, although the results of the method are for the most extent depended from the level of analyst` proficiency and quality of microscope.

Yeasts of Malassezia genus are the only members of mycrobiota, which are confessed as etiologic agent of SD, at that they distinct from other opportunistic yeast because of their lipophilic nature. The yeasts are normal residents of human skin [3] however in SD patients owing to abundant sebum secretion (especially on the scalp, back, chest etc.) their metabolism became visual. Tactics of medical treatment of such patients include antimycotic shampoos, however for a long time was observed that positive effect may be reached not each time. The fact has two-way explanation: firstly, the shampoos as a rule contained

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azoles, terbinafine, zinc piritionate etc., to which fungal cells in the course of time could develop the resistance [4]; secondly, the mycrobiota per se may not be limited only with Malassezia yeasts. In research literature we found two articles concerning the composition of fungal microbiome in SD patients. In facial skin scales obtained from 24 patients with SD 30 specia of micromycetes were found by PCR method, including 4 specia of Malassezia genus, 17 specia of other yeasts and 19 specia of mycelial (not dermatomycetes) fungi [5]**.** The authors did not put the problem to find dermatophyte fungi and not refuse their possible presence. In the other study skin scales from scalp of 7 volunteers with usual dry dandruff and without it were inspected by PCR method [6]. Result was the similar – many specia of yeast and mycelial fungi were found, but not dermatophytes. Obviously the authors were not considered necessary to take into account controversial results in which dermatophyte fungi were found. Thus the aim of present study was the estimation of results of microscopy investigation of SD patients for the detection of yeast and dermatophyte fungi.

Materials and Methods: Group of patients with SD of scalp included 60 individuals – 32 women and 28 men – age 18 – 66 years (median 32 year) in exacerbation stage. SD was diagnosticated on the base of typical clinical picture. The group was divided on two subgroups - I - 21 patients with clinical diagnosis, which gave not rise to doubt, and II – 39 patients with the same diagnosis, but with necessity of accurate definition due to suspicion of mycosis. All the examined patients signed the agreement of their personal data processing.

Skin scales were collected from erythematous /squamosal foci by two methods, depends on the type of substrate. Large scales gathered with thin pincer, but in the case of very small scales the plug logged with Tween 80 buffer was used. After that the plug was suspended in the same buffer and obtained suspension was centrifuged during 5 min at 14000 g for scales precipitation. Scales were treated with 10% KOH during 17-24 hours [7], then microscopy of the lysate was carried out at sum magnification of 1750. Abundance of detected fungal morphotypes in 10 fields of view was expressed as numbers: 0 – means the absence; 1 – presence on average of 1-2 cells in the field; 2 - from 3 to 5 cells; 3 – more than 6 cells. Quantity of bacterial morphotypes estimated also in 10 fields of view (numbers): 0 - means the absence; 1 – presence on average of 1-10 cells in the field; 2 – from 10 to 40 cells; 3 - more than 40 cells.

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to microorganisms.

Results Group of SD patients consisted from the people only with scalp problems therefore skin scales were collected from this locus. During the examination patients were subjected to diagnostic procedure of trichoscopy with professional program Tricho Sciene Pro © and trichoscope Aramo SG (magnification x 60).

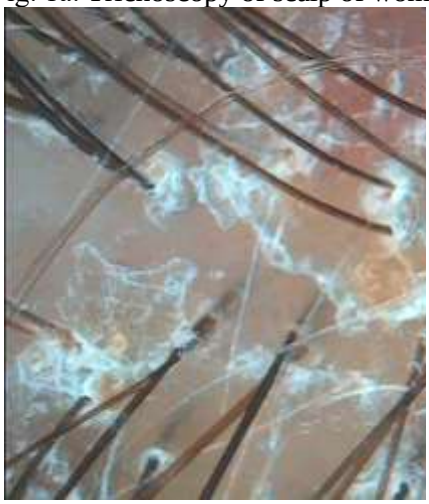
Results of trichoscopy are presented at Fig. 1a and 1b by the example of women from subgroups I and II. In both cases exfoliation took place, however the second case characterize with abundancy of scales and scalp hyperemia. As may be seen from microscopy of skin scales from patient of subgroup II (Fig. 2) the fungal morphotypes belong to dermatophytes and strike with diversity of forms – from spore cells to mycelial ones. Most of patients were hosts of dermatophytes at that the mycelial forms were detected with the same frequency in both subgroups.

Trichoscopy with professional program Tricho Sciene Pro © and trichoscope Aramo SG (magnification x 60, x 200) (figure1 a,b).

1. а (at the magnification x 60)

1. а (at the magnification x 200)

Fig. 1a. Trichoscopy of scalp of woman with SD from the subgroup I.



1. б (at the magnification x 60)

1. б (at the magnification x 200)

Fig. 1 b. Trichoscopy of scalp of woman with SD from the subgroup II:

Fig. 2. Detection of dermatophyte fungal elements – spores and mycelium in KOH preparation of scalp scales of patient with SD from the subgroup II.

Table. 1. Detection of microbial morphotypes in skin scales of patients with seborrheic dermatitis.

Diagnosis	Parameters of carriage	Bacteria		Dermatophyte fungi		Yeast fungi	
		Coccoidal	Bacillary	Mycelium	Spores	Mycelium	Spores
Seborrheic dermatitis N = 21	Detection frequency, %	4,8	90,5	47,6	90,5	9,5	23,8
	Abundance (numbers),	0,10 ± 0,18	2,38 ± 0,83	0,90 ± 0,95	2,57 ± 0,65	0,19 ± 0,34	0,57 ± 0,87
	Correlation of detection frequency with sex*	0,194	-0,281	0,248	0,047	-0,047	-0,194
	Correlation of detection frequency with age*	0,534	0,111	-0,238	-0,276	0,276	-0,141
Seborrheic dermatitis with necessity of accurate definition N = 39	Detection frequency, %	2,6	87,2	46,2	97,4	0	25,6
	Abundance (numbers),	0,08 ± 0,15	2,40 ± 0,82	0,85 ± 0,82	2,60 ± 0,57	0,08 ± 0,15	0,48 ± 0,72
	Correlation of detection frequency with sex*	0,194	0,008	0,396	0,135	-0,135	-0,132
	Correlation of detection frequency with age*	-0,227	0,103	0,044	0,072	-0,072	-0,176

Pirson`s correlation coefficients

We detected presence of only yeasts in 9,5% of patients from the I subgroup and in 2,6% of patients from the II group, however combined dermatophytic-yeast mycrobiota was found in 14,3% and 23,1% of patients correspondingly. Dermatophyte mycelium was not detected without dermatophyte spores, but spores without mycelium – in 42,9% (I) and 46,2% (II) of cases accordingly. At that frequency of dermatophyte spores detection did not correlate with that of dermatophyte mycelium (Pirson coefficients: $r_I = 0,309$ and $r_{II} = 0,166$). Any correlations were not revealed between sex/age and frequency of detection of different bacterial or fungal morphotypes (Table 1). The aim of present study was the estimation of results of microscopy investigation of patients with seborrheic dermatitis for the detection of yeast and dermatophyte fungi.

Discussions In terms of ecological mycology, the mycelium formation indicated the unfavorable conditions for fungus, and its followed attempt to "leave" this ecosystem [8]. Usually the unfavorable ecological factors are following: limit of nutrient substrate, suboptimal pH and occurrence of antagonistic substances, which are produced by host or normal microbiota. From the abundancy of dermatophyte elements in the field of view we may be sure in absence of substrate deficit. It is known that pH of skin in SD patients change from acid to neutral values, which are more comfortable for fungal growth [9]. One may reasonably suppose that in this case the main cause of frequent mycelium formation are protective substances of host, for example antimicrobial peptides, or killer substances of bacteria (bactericins) and Malassezia fungi (mycocins).

Yeast microbiota in observed patients` skin represented mainly with Malassezia genus: yeast cells had typical "collars" and buds on the broad base. In these cases the detection of yeasts is quite logical, because increased production of scalp sebum should accompanied with intensive growth of lipophilic yeasts.

Comparing the results of abundance of different microbial types one can see that most plentiful in both subgroups were dermatophytes, but the least – coccoid bacteria. Bacillary forms of bacteria were detected by microscopy noticeably more often and their abundance were higher than this of coccoid forms in both subgroups of patients. Among skin bacillary bacteria predominated is the genus Propionibacterium, which is lipophilic, i.e. well growing on lipid substrates – this fact may explain the obtained data.

Its known that most often detected species of dermatophyte fungi at tinea capitis is Trichophyton rubrum. Physiology of the fungus growth on lipid sources of carbon was already described in two studies [10]. The researchers noted that growth of T. rubrum on lipids, particularly on olive oil as the only carbon source, followed not by acidulation of medium as in the case of glucose consumption, but with increasing of pH as in the case of keratin use. The 14 genes were identified responsible for the synthesis of proteins, which are promoted the adaptation of fungus growth on lipid substrates [11]. Therefore the dermatophytes, which are often detected in skin scales are physiologically adapted to sebum consumption.

Conclusion For our opinion most intriguing fact obtained in this study is the following: during the examination of patients with clinically determined SD diagnosis, dermatophyte fungi were detected with high frequency both in subgroup with exact diagnosis and in subgroup with scrutable diagnosis. In this connection at once two questions appear: a) how rightful patients were diagnosed?; b) can dermatophytes take part in sebum utilization together with Malassezia and Propionibacterium?

Based on the obtained data the relevancy of SD diagnostics without microscopy gives rise to doubt. Obviously microscopic research in general should lead to updating of diagnosis: "seborrheic dermatitis, mycosis of the scalp". In terms of the study results microscopy has been and still is the "gold standard" in diagnostics of skin diseases suspected the infectious reason.

Data Availability
The relevant data generated and (or) analyzed in the current study is available from the corresponding author upon reasonable request.

Conflicts of Interest
The authors declares that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions
V. Arzumanian contributed to the conceptualisation, software, writing—original draft preparation, writing—review and editing, project administration. I. Il`ina and M. Khaldina contributed to the formal analysis and investigation. L. Agasarov contributed to the writing—review and editing and visualization. V. Lim contributed to the validation, resources, writing—original draft preparation, funding acquisition. N. Barunova contributed to the conceptualisation, methodology, software, validation, formal analysis, investigation and funding acquisition. A. Antishin contributed to the resources, visualization and funding acquisition. Y. Dronina contributed to the conceptualisation, validation, formal analysis and supervision. V. Gavrilov contributed to the data curation and visualization.V. Zaborova contributed to the methodology, data curation, writing—review and editing, supervision and funding acquisition.

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Supplementary Materials
There is no supplementary materials.

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