Original Article

Detection Of Some Immunological And Biochemical Markers In Seborrheic Dermatitis Patients

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Abstract

Background: Seborrheic dermatitis (SD) is a common skin disease in Iraq, which is due to various causes, the most important of which are fungal infections. The current study aims to isolate Malassezia spp., which may be a risk factor for SD, with the evaluation of some cellular parts of those yeasts, such as GM and BDG, that may play a role in the severity of this disease or its development, as well as evaluating the role of some immunological indicators, such as IL-17A, IL-8, IL-1alpha, histamine, and Cathepsin.

Methods: The current study included the collection of 50 samples from patients with SD, the Malassezia species were diagnosed laboratory by microscopic examination, culture media and biochemical tests. BDG and GM were determined by Fungitell assay while histamine, cathepsin S, IL-17A, IL-8, and Il-1alpha were determined by ELISA.

Results: Our results showed that most of the patients were female (68%), and they were within the age group of 16-30 years. We also found that the highest rates of infection were 34%, 22% and 20% due to the Malassezia globosa, Malassezia sympodialis and Malassezia furfur. Moreover, we determined an increase serum level of IL-8, IL-17A and IL-1alpha in patients (78.31,789.22 and 97.42 pg/ml, respectively) compared with healthy control group (0.823, 1.09 and 5.41 pg/ml, respectively) and we also found statistical differences as a result of an increase in some biochemical compounds in patients represented by histamine and cathepsin S (156 and 1190 ng/ml, respectively) and we also found an increase level for fungal compounds such as BDG and GM in SD patients where their average was 500 and 4.91 pg/ml, respectively.

In conclusion: Malassezia globosa is one of the most important causes of SD, so BDG, GM, histamine, cathepsin S, IL-17A, IL-8, and IL-1alpha indicators can be relied for diagnosis and response to treatment of SD.

Keywords: seborrheic dermatitis ,Malassezia species, BDG, GM, histamine, cathepsin S, IL-17A, IL-8, and IL-1alpha

INTRODUCTION

Seborrheic dermatitis is affects About 3-5% of adults [1, 2] suffer from sebum-rich areas like the scalp when they have seborrheic dermatitis, a chronic dermatosis marked by dandruff, itching, and occasionally mild to severe erythema. Despite its great incidence, the pathophysiology of SD is poorly known [3]. Various predisposing variables, such as fungus colonization and Sebaceous gland activity plus a number of other variables that determine an individual's vulnerability, have been discovered in investigations. Several lines of evidence point to Malassezia yeasts playing a pathogenic role in SD [4,5]. Malassezia are lipophilic yeasts found mostly in seborrheic areas of the body. Malassezia spp. have been found on the scalps of dandruff sufferers in studies [6,7], and larger Malassezia (M. globosa and M. restricta) numbers correspond to SD appearance/severity. Furthermore, antifungal activity is the only common mechanism of action one of the several chemical substances that helpful in treatments such as Azole, hydroxypyridone, allylamine, selenium, and zinc treatments for SD and dandruff [8-10]. Malassezia also has lipase activity, which hydrolyzes human sebum triglycerides and produces unsaturated fatty acids including oleic and arachidonic acid [11]. These compounds produce improper keratinocyte development, which results in stratum corneum defects as parakeratosis, intracellular lipid droplets, and uneven corneocyte envelope [12]. Such modifications affect the cutaneous barrier action and activate an immune reaction, whether or not apparent Localized discomfort. Furthermore, these metabolites stimulate keratinocytes to release pro-inflammatory cytokines such IL-1, IL-8, and IL-17A, extending the inflammatory response [13,14]. Arachidonic acid can also be a source of prostaglandins, which are inflammatory mediators that increase inflammation by attracting neutrophils and dilatation of the blood vessels. [15].

Antigen-presenting cells such as macrophages, B lymphocytes, dendritic cells, and microglia express cathepsin S. Some epithelial cells express cathepsin S [16]. Its expression is significantly raised in human keratinocytes after stimulation with interferon-gamma, and it is also increased in skin keratinocytes after stimulation with proinflammatory stimuli. Because of a difficulty with their stability, many lysosomal proteases are imprisoned inside the lysosome [17, 18]. Cathepsin S, on the other hand, is stable and has a physiological purpose outside of the lysosome. In response to

inflammatory mediators such as lipopolysaccharides, proinflammatory cytokines, and neutrophils, immune cells such as macrophages and microglia release cathepsin S, which may be measured during SD. Histamine, as a component of the immune system, may have a role in immune system diseases and allergies. Mastocytosis is an uncommon condition in which mast cells proliferate and release excessive histamine [18-20]. Furthermore, as is well documented, there is a link between cathepsin S and histamine, since capsin production causes a rise in histamine levels as a defense against numerous disorders, including skin diseases such as psoriasis and eczema [16-19].
The current study aims to isolate Malassezia spp., which may be a risk factor for SD, with the evaluation of some cellular parts of those yeasts, such as GM and BDG, that may contribute to the severity of this disease or its development, as well as evaluating the role of some immunological indicators, such as IL-17A, IL-8, IL-1alpha, histamine, and Cathepsin, which may open the door to future studies about its relevance to the diagnosis and treatment of SD

MATERIALS AND METHODS

Samples collection: The current study is a case-control study that included the collection of swabs and blood samples from 50 patients with seborrheic dermatitis, where the pathological cases were diagnosed clinically and in a laboratory at Al-Diwaniyah General Teaching Hospital between 3/4/2022 and 1/7/2022, and 50 samples were collected from healthy people as a control group, and consent was obtained from all participants during the collection of the questionnaire and samples.

Clinical assessment: Qualified dermatologists conducted the clinical evaluation. Patients' ages, genders, general health, sickness onset, and course were all reported. Anomalies in the nails were documented. The Degree of Alopecia Tool (SALT) [21] was used to assess the severity of hair loss in the scalp.

Microbial study: We examined the main isolates under the microscope to identify and choose distinct morphotypes consistent with Malassezia spp. These were grown on modified Dixon agar and incubated at 33 ° C for 4 to 5 days. The following factors were evaluated to determine the Malassezia species: (a) biochemical traits such as catalase, urease, and -glucosidase activities; (b) the capacity to ingest cremophor EL and Tween 20, 40, 60, and 80; (c) the production of pigment on tryptophan-based medium; and (d) the capacity to grow on Sabouraud agar, Sabouraud plus 10% Tween 20, Sabour [22].

Biochemical and immunological tests: Assaying fungitell (Associates of Cape Cod, Cape Cod, MA) was used to assess GM and BDG in a range of values from >500 pg/mL to non-detectable (31 pg/mL); more than 500 pg/mL, samples were retested after being diluted in reagent-grade water. get reliable findings. Histamine and cathepsin S concentrations were measured in samples using an enzyme-linked immunoabsorbent test (ELISA) (DuoSet, R&D Systems, Minnesota, USA). ELISA was used to quantify IL-8, IL-1 alpha, and IL-17A using specialized kits (DuoSet, ref. DY208, DY200, DY280, KGE0068) [23].

Statistical analysis: Statistical analysis for social sciences program and Excel 2010 version were used for statistical analysis, and average values were relied on to obtain significant differences, which were mathematically translated using the probability equation, and comparisons smaller than 0.05 were considered statistically useful.

RESULTS

The current study is a case-control study that included 50 patients ranging in age from 16 to 64 years, with an average age of 9 34.22 9.62 years, and the majority of them were within the age groups of 16-30 years (30%) and 45-55 years (28%) as shown in Table 1, as we discovered 68% of patients are female, and gender differences lead to the emergence of a statistical difference between patients (p = 0.031). The control group, on the other hand, had 50 healthy adults who did not have infections or skin injuries, and their ages varied from 16 to 65 years, with an average age of 36.01 8.59 years, and 32%, 26%, and 26% of them were in the 30-45, 16-30, and 16-30 age categories, respectively.

Table (1): Demographical presentation of studied groups

Properties	Patients	Control	P value
Age range /year	16 – 64	16-65	
Mean	34.22	36.01	0.351
SD	9.62	8.59	
SE	1.36	1.20	
Age groups	N (%)	N (%)	
16-30	15 (30)	13 (26)	0.082
30 -45	12(24)	16 (32)	0.074
45 - 55	14 (28)	8 (16)	0.047
≥55	9 (18)	13 (26)	0.073
Total number	50	50	
Gender	N (%)	N (%)	
Male	16 (32%)	13(26%)	0.095
Female	34 (68%)	37(74%)	0.077
P value	0.031	0.029	

During the evaluation of patients and clinical and analytical investigations of samples, we discovered that seborrheic dermatitis is a superficial fungal illness that affects the skin and occurs in regions with a high concentration of sebaceous glands. Malassezia yeasts are thought to be linked to seborrheic dermatitis. This might be because of an aberrant or inflammatory immunological response to these yeasts. Laboratory diagnosis revealed that Malassezia globosa and Malassezia sympodialis caused 34% and 22% of the infections, respectively, while Malassezia furfur and Malassezia restricta caused 20% and 16% of the infections, respectively, and mixed infections emerged in 8% of cases, as shown in Figure (1) .According to this study, 58% of seborrheic dermatitis patients have recurring infections with the yeasts listed in Table 2.

And we discovered that all patients with mixed infection (100%) have repeated infections, as do 80%, 59%, and 55% of patients with Malassezia furfur, Malassezia globosa, and Malassezia sympodialis, respectively, but Malassezia restricta yeast infection was 12.5% less common. As shown in Table 2, we found evident significant differences when comparing the percentage of recurring infections to the percentage of new (first) infections based on Malassezia species.

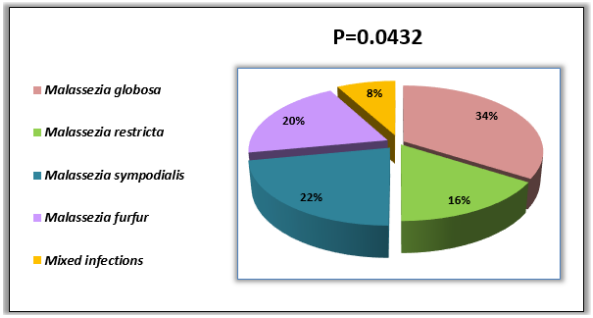

Figure (1): Frequency of *Malassezia spp* in SD patients

Table (2): Distribution of *Malassezia spp* according to recurrent infections

Infectious agents	N	Recurrent infections	First infection	OR	P value
Malassezia globosa	17	10(59)	7 (41)	4.01	0.045
Malassezia restricta	8	1 (12.5)	7 (87.5)	16.81	0.003
Malassezia sympodialis	11	6 (55)	5 (45)	3.77	0.048
Malassezia furfur	10	8 (80)	2 (20)	29.6	0.009
Mixed infections	4	4(100)	0(0)	84.3	0.0001
Total number	50	29 (58)	21 (42)	3.39	0.047

*OR= Odd Ratio

The immunological and biochemical analyses revealed that seborrheic dermatitis is accompanied by an immune response and chemical alterations that might be caused by infection with the aforementioned yeasts. In any event, we found an increase in certain interleukins (IL-8, IL-17A, and IL-1alpha) in patients (78.31, 789.22, and 97.42 pg/ml, respectively) as compared to healthy persons in the control group (0. 823, 1.09, and 5.41 pg/ml, respectively). We also discovered statistical differences due to an increase in some chemical compounds in patients represented by histamine and Cathepsin S (156 and 1190 ng/ml, respectively), as well as an increase in fungal compounds such as BDG and GM in SD patients, where the tested average was 500 and 4.91pg/ml, respectively. As seen in Table 3.
We also discovered that the serum concentrations of the examined variables alter when distributed by age group, with strong statistical differences (p0.05) as shown in Table 4. Furthermore, patients aged 16 to 30 years had the greatest incidence of IL-8, IL-17A, cathepin S, BDG, and GM (88.58 pg/ml, 900.21 pg/ml, 1300 ng/ml, 611.8 pg/ml, and 5.41 pg/ml, respectively). While patients aged 55 had the greatest rates of IL-1alpha and histamine, with rates of 110.8 pg/ml and 170.1ng/ml, respectively.

Table (3): Evaluation studied biomarkers in case- control groups

Immunological markers	Mean of biomarkers		X^2	P value
	Patients	Control		
IL-8 (pg/ml)	78.31	0.823	44.72	<0.0001
IL-17A (pg/x)	789.22	1.09	56.81	<0.0001
IL-1alpha (pg/ml)	97.42	5.41	34.83	<0.0001
Biochemical markers				
Histamine (ng/ml)	156	141	2.00	0.047
Cathepsin S (ng/ml)	1190	1233	1.89	0.045
BDG	500	31	29.7	0.0001
GM	4.91	0.06	0.16	0.049

*X^2 = chi square

Table (4): Distribution of studied biomarkers according to patients age groups

Immunological markers	Patients age groups/ years				X^2	P value
	16 -30	30-45	45-55	≥55		
IL-8(pg/ml)	88.58	44.30	56.55	70.47	3.64	0.031
IL-17A (pg/ml)	900.21	795.4	502.6	779.91	4.46	0.0293
IL-1alpha (pg/ml)	99.29	59.30	67.11	110.8	3.55	0.033
Biochemical markers						
Histamine (ng/ml)	166.9	125.8	147.51	170.1	3.41	0.036
Cathepsin S (ng/ml)	1300	993	1199	1293	5.88	0.029
BDG (pg/ml)	611.8	593.6	401	444.8	2.09	0.041
GM (pg/ml)	5.41	3.01	1.02	1.51	1.49	0.048

*X^2 = chi square

DISCUSSION

The findings of our study revealed that seborrheic dermatitis was a common effect among young people aged 16 to 30 years, with infections being more common in females. This may be due to hormonal changes during that age period, particularly in females, and studies have been contradictory regarding the rate of infection. Oily skin varies by gender and age of patients, and this may be related to the varied study regions that originate from different races and diverse habitats, including variances in the species of Malassezia species, which is one of the primary causes of seborrheic dermatitis [24, 25]. We also isolated Malassezia species to learn more about their function in seborrheic dermatitis, and discovered that M. globosa is one of the most prevalent kinds in the incidence of recurring infection, as well as mixed infections from more than one type of contact. The Malassezia species that causes seborrheic dermatitis has been determined by different studies, and these discrepancies may be attributed to the nature of the patient's skin, which reflects the Malassezia food addition to hygiene and environmental circumstances [25-27]. Malassezia species' ecological diversity has been examined all around the world. According to reports M. is a citizen of France, Spain, Japan, Korea, and other nations. M. globosa and limiting are the most prevalent different species from SD patient samples [28-31]. And, to determine the precise role of those yeasts in the development of SD, we examined GM and BDG, two of the most important cellular components of these yeasts, and found an increase in their rates in patients compared to controls, indicating a role for these yeasts in the development and severity of SD infection, as previously observed in studies evaluating GM and BDG from pathogenic isolates of different fungi [32, 33]. On the same pathogenic pathway, GM may have a role in stimulating the immune response, as we discovered a high proportion of IL-17A, IL-8, and Il-1alpha, in addition to an increase in some immunologically active chemical components like histamine and Cathepsin S. Furthermore, the high serum percentage of these indicators indicates a high rate of immune cells such as lymphocytes, macrophages, neutrophils, monocytes, and so on, which may have a role in the severity of skin inflammation caused by Malassezia infection [33-35].

Cathepsin S is a lysosomal enzyme that belongs to the cysteine protease family that includes papain-like proteases. While cathepsin S has long been known to have a function in antigen presentation, it is now known that it also plays a role in itch and pain, or nociception. Cathepsin S, a proteinase-activated receptor-2 activator (PAR2) [36], was newly discovered to be elevated in subjects with SD, along with PAR2 and histamine, and to be correlated with clinical parameters related to the severity of SD and itching, implying that cathepsin S could be used as a biomarker of pruritus. Pruritus biomarkers [37], Recently, it was discovered that Skin barrier function, hyperproliferation, and inflammation are altered in SD and can be therapeutic action has returned. Histamine is a key modulator of itching and is involved in the inflammatory response [17]. Basophils and mast cells in the surrounding connective tissues produce histamine as part of an immune response to invasive infections. Histamine increases the permeability of capillaries to specific proteins and white blood cells, enabling them to interact with pathogens in diseased tissues [38].

Schwartz et al. [39] addressed the structural and biomolecular anomalies that characterize SD in 2013. They observed that the SD condition disrupted biomarkers reflecting hyperproliferation, barrier activity, and inflammationall of which reacted strongly to treatment resolution. A variety of biomarkers, including IL-1RA, were shown to be substantially linked with the primary SD symptom of flaking, including IL-1a, IL-8, histamine, involucrin, and keratins. Various research employing other biomarkers, including cathepsin and a clinical scoring approach that focuses focusing more on erythema and pruritus than desquamation, sheds fresh light using biomarkers as novel clinical assessment goals in SD clinical trials [23]. The use of biomarkers in the assessment of medicinal products has been proposed by the FDA in a recent white paper titled "Innovation or Stagnation?" [40]. Furthermore, Schwartz et al. [39] stated that biomarkers, because they represent illness processes underlying clinical pathophysiology, might be effective instruments for early diagnosis of aberrant conditions. Finally, studies on the assessment of some immunological and chemical substances in the pathogenesis of SD were limited, and we in Iraq need further research on the bacteria that cause this dermatitis, adding us to immunological and molecular studies for patients and pathogens such as Malassezia spp.

CONCLUSION

The current study found that SD is a prevalent dermatitis illness in Iraq, especially when the culprit is Malassezia, particularly M. globosa. We also discovered a distinct immune response in SD patients, as seen by higher levels of immunological markers such as IL-17A, IL-8, IL-1alpha, histamine, and Cathepsin S, which may be utilized to diagnose

SD. On the other hand, we discovered several Malassezia cellular components, particularly GM and BDG that might be employed as a therapy response indicator in future trials.

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