Plant Archives

ELICITATION A RATING SCALE FOR PITYRIASIS VERSICOLOR AND CHARACTERIZATION OF *MALASSEZIA* AND DERMATOPHYTES ISOLATED FROM HUMAN SKIN

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ABSTRACT

Pityriasis versicolor symptoms are characterized by fluctuating seriousness and inconstancy. Determine both of patches appearance's scale and the pathogen(s), will contribute in the selection of the best treatment types and decrease the effort, cost and patient affliction. The aim of the study to characterize the suspected Pityriasis pathogens and elicitation a novel rating scale define the disease severity. Study period from November to May 2018. A total of 140 specimens were collected (105 specimens by tape stripping technique and 35 swabs) from skin sites of the different gander in Babylon province/Iraq. The results shown 125 positives out of 140 samples diagnoses by means of phenotypic and molecular methods. The majority of the patients were males 52.8% (66/125) while, 47.2% (59/125) specimen from females. Elicitation a novel rating scale of Pityriasis versicolor disease based on patches number and nature. Conclusion: Malassezia is the main pathogens Dermatophytes, Candida spp. and mold are co-exist at the same infection site.

Keyword : Disease rating scale, *Malassezia* , Dermatophytes, Characterization.

Introduction

Malassezia yeasts are dimorphic Basidiomycetes, there are more than 100 of *Malassezia* spp. It caused Pityriasis versicolor, considered as should be expected vegetation on the skin of human and creature, and it ends up pathogenic under specific conditions. Be that as it may, it very well may be refined from practically all body region. Pityriasis versicolor is more typical in men than female, might be because of additional consideration regarding excellence and skin cleanliness (Al-Khadim *et al*., 2019).

In the fields of pathogenicity, finding and treatment, there is a significant issue incorporate careful recognizable proof of reasons for skin pathogens was permitted a particular, coast, the best treatment and decrease patients severity (Kwon-Chung 1992) This undertaking especially imperative to check for single or mixed infections, making focused on differential treatment fundamental.

Making rating scale for assessment the earnestness of the harm and the taking of preventive and preventive gauges together is critical for both the patient and the master. To decide the medication, recognizing confirmation of solution and treatment and incidental study, the nature of treatment and draw the thought of the patient to envision the section of the harm to the edge change the nearness of the skin. Along these lines, the improvement of a measure to overview the earnestness of the issue is huge, scientific composition, which has conveyed reports and research on the ailment, has not developed rating scale.

Material and Methods

Total of 140 clinical samples were collected from private clinics and 10 samples apparent healthy as control, targeted skin body sites: chest and neck regain, face, upper hands, thigh, shoulders, chest, neck by tape stripping technique(TS) and swabs (Hasan and Al-Shibli 2015, 2016). The TS culture on SDA and Modified Dixon's Agar (MDA), both media supported with to cyclohexomide (500 mg/L). A sterile Olive oil was added to SDA for their lipid requirement for lipophilic *Malassezia* spp. (Aspiroz *et al*., 2002).

The characterization of dermatophytes (Al-khafajii, 2019), yeasts and mold were performed by cultured pure colony on stander culture media for each fungal group; Malassezia on MDA, SDA and pre-identified on CHROMO agar. Dermatophytes cultured on SDA for characterize the shape, texture and pigments based on (Levin, and Delano, 2011). A loop full of colonies were mountain with drop of lactophenole cotton blue stain and examined under magnification power (X40-X100) for each. The Malassezia microscopic criteria cell shape, dimensions and budding mods following up based on Astrid, (1999). Molecular techniques were tested on these yeasts based on Hasan, (2016); Al-Khadem *et al*., (2019).

Results and Dissuasion

Prevalenceof Malassezia among Pityriasis versicolor sufferers:

A 125 of the 140 clinical samples were positive cases. The majority of the patients were males 52.8% (66/125) while, 47.2% (59/125) specimen from females. The average

of patients' age was ranged from (9-66 years) in both genders. With youngest age in total specimen is 9 years and older age is 66 years. With high infection percentage 52% of the total specimen occur between (9-30 years) and 48% occur between (30-70 years) Table 1.

Table 1 : Prevalence of *Malassezia* among of Pityriasis versicolor Patients:

Age in year	No. specimen of male	No. specimen of females	Total/ Percentage (%)
0-10	0	1	1/0.8
11-20	13	12	25/20
21-30	22	17	39/31.2
31-40	11	9	20/16
41-50	11	13	24/19.2
51-60	7	6	13/10.4
61-70	2	1	3/2.4
Total	66	59	125

Elicitationanewscaleof Pityriasis versicolor

The importance of determining a scale of Pityriasis versicolor disease helps both the researcher and the doctor to characterize the infection and determine these variety. Pityriasis versicolor symptoms ranged color (pink, tan, brown, flaky white patches); and distribution of skin per square centimeter (CM^2): sparser, scatter, neighbor and merger patches. Each person customarily has only one dual disease symptom include colors or pigmentation and one scale of patches distribution. This disease properties enable both researcher and physician to make the right decision.

Based on our Pityriasis versicolorscale, the coverage of pigmentation percentage through the patient bodies was classed into five categories:1-patches spars, pigmentation covered more 1%, 2- patches scatter, pigmentation covered more than 10% of the body surface of patients shown in 64 samples. 3-patches neighbor, pigmentation pigmented was covered more than 20% of the body of patients included 33 specimens. 4- patches nested, pigmentation covered more than 30% occur in 21 specimens. 5- patches merged, pigmentation cover more than 40% occur in 7 specimens covered more Figure (1).
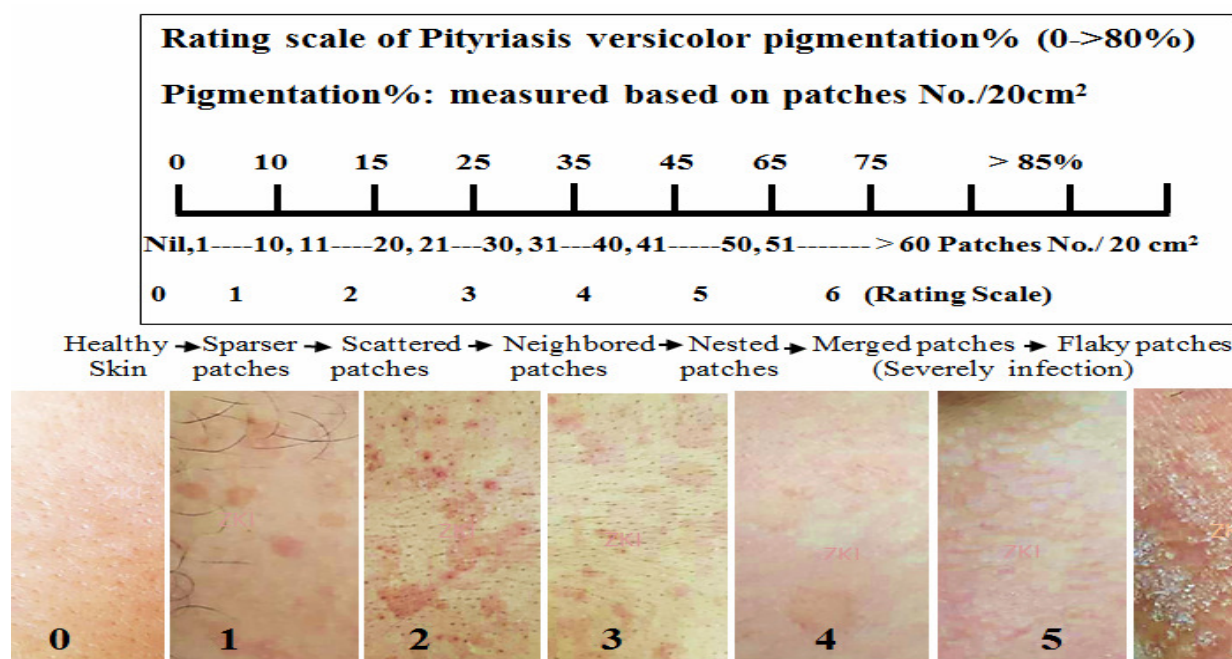
Fig. 1: Elicitation a new scale of Pityriasis versicolor pigmentation based on patches number and natured patterns: Sparser, Scattered, Neighbored, Nested, Merged and Flaky patches.

The explanation of the occurrence of skin color changing (pigmentation case) by Pityriasis versicolor, may be attention to melanin degradation, and this explains, agree with many reports, Most alteration occurred based on the of cytotoxicity of dicarboxylic acids (Nazzaro-Porro and Passi, 1978) or due to elevated of the thick of the keratin, and leakage of inflammatory cells is more pronounced in these individuals as a catalyst for melanocytes (Chehoud *et al.*, 2013)

**Survey of dermatophytes and other opportunistic fungi**

A total of 15 fungi were isolated included two Trichophyton: *Trichophyton ementogrophyte* and *T. versicolar*; seven *Malassezia* species : *Malassezia furfur*, *M. pachydermatis*, *M. sympodialis* and *M. restricta*, *M. slooffiae*,

M. globosa, *M. obtusa*; *Candida* spp: *Candida albicans*, *C. glabrata*, *C. parapsilosis*; *Aspergillus* spp.; *Penicillium* spp., and *Alternaria* spp.

The powerful of tape stripping technique (TS)

The results of imprint of tape stripping technique (TS) shown powerful results compare with swabs and scraping techniques. Its successful methods for isolation and differentiation directly between different *Malassezia* spp.; some of *Malassezia* shown white colonies other creamy color, but not forever, because, some tape samples gave negative planting yeasts, Figure (2).
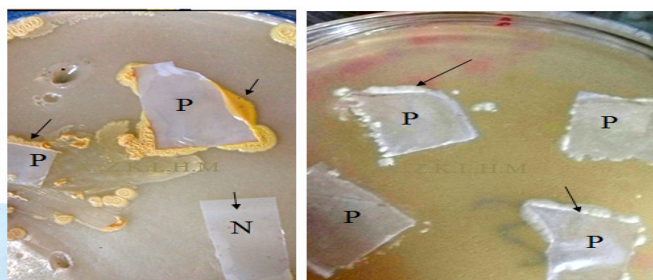
Fig. 2 : Imprint of cello-tape technique, the colonies of *Malassezia* sprouted from the margin of tape, this important method for differentiating isolation and purification of *Malassezia* isolates.

Coexistence of *Malassezia* spp.

This study observed different shape and size of the colonies in each culture plate. Most patients, severing from infection by more than one *Malassezia* species, could be isolating them from the same niche in the human body. The results showed that *more* than one*Malassezia*spp. were identified most frequently grown media Figure (3), this coexistence of more than one species of *Malassezia* in one site of human body may be referred to a synergistic relationship between *Malassezia* spp., Our result also agrees with previous studies reported the same event (Jang *et al.*, 2009).
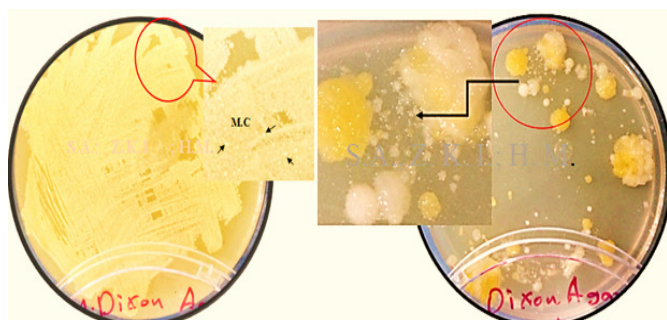

Fig. 3 : Co-existence of *Malassezia* spp. Was recovering from single patient body site incubated on Modified Dixon Agar, 32°C for 5days. M. C=Mixed colonies.

## Microscopic characterization of *Malassezia* spp.:

This study successfully in identification of some *Malassezia* spp. microscopic evidence such as: buds: shape, size and number; cell shape and size.

1. *M. furfur* ; cells shape ovals to cylindrical, average dimensions: 2x4.5 µm, buds emerge polar from a relatively narrow base, small, when reaching mature be long with ratio equal 3/4 about the size of it is mother cell, consider to be a diagnostic character. Figure (4). The description consistent with (Guého, *et al.*, 1996 and Ashbee, (2007).
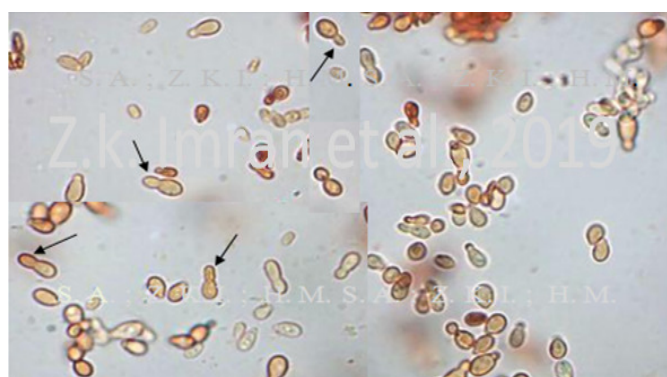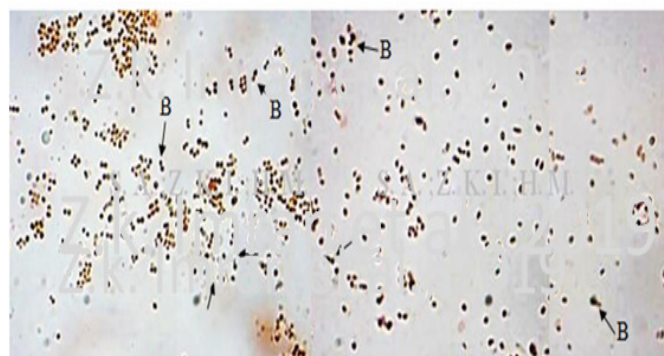

Fig. 4 : *Malassezia furfur* ; microscopic characters, shown the bud emergent mature buds, and yeast cell characters, magnification power = X100.

2. *Malassezia globosa*: Cells shape large to spherical, typically wrinkled average dimensions:1.8x4.0µm, buds emerge polar from a relatively narrow base, small, when reaching mature be long with ratio equal 1/4 about the size of it is mother cell, consider to be a diagnostic character. Figure (5).
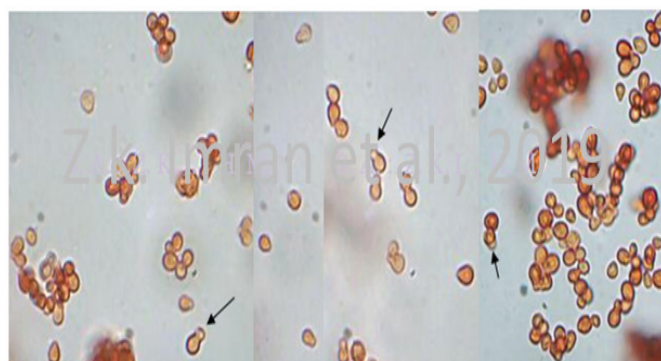

Fig. 5 : *Malassezia globosa*; Conidia globes –spherical shape, budding produce sort buds Magnification power= X 100.

3. *M. restricta* : Cells shape small, spherical, average dimensions:1.5×2.5µm, buds emerge polar from a relatively narrow base, small, when reaching mature be long with ratio equal 1/4 about the size of its mother cell, consider to be a diagnostic character. Figure (6).

Fig. 6 : *Malassezia restricta* microscopic characters, shown the budding in yeast cells under 100X power.

4. *Malassezia sympodialis*: Cells shape large, ovoid-globosa, average dimensions: 2.5×5µm, buds rounded, emerge sympodial from a relatively narrow base, small, when reaching mature be long with ratio equal 1/4 about the size of it is mother cell, consider to be a diagnostic character. Figure (7).
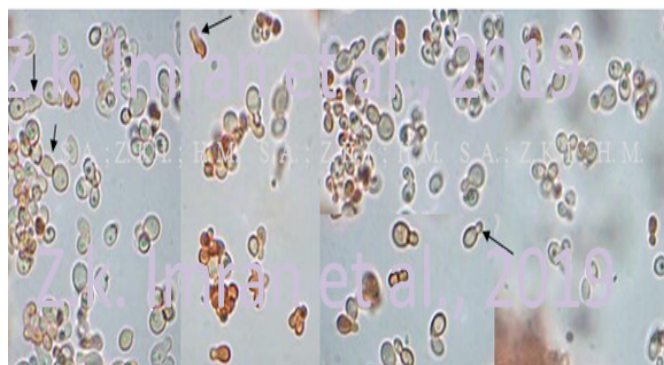
Fig. 7 : *Malassezia sympodialis* sometimes emerged sympodial from a relatively narrow base, the arrow pointed to the sympodial bud type. Microscopic characters of Malassezia pychidermitis, shown the budding mode of yeast cells, 100X power.

5. *Malassezia slooffiae* **:** Cell cylindrical to slightly elongated yeasts with a broad budding site cell shape cylindrical, average dimensions: 1.5×3µm buds that may emerge polar from a relatively narrow base, Figure (9).
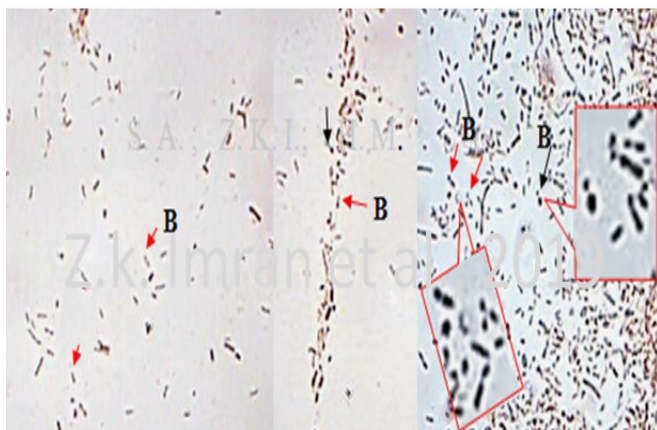
Fig. 9: *Malassezia slooffiae* microscopic characters, shows the budding in yeast cells under 100X power.

F-Identification of *Malassezia* spp. based on CHROMOagar:

The results shown, that the streaking of the inoculums of *Malassezia* spp. appearing pink color, when grown in modified CHROMOagar medium, *M. furfur* shown pale pink colony with margin wrinkled, M.sym.=*M.sympodialis* (large colony and pink color), *M. sloffiae* shown small colony and pale-pink color with smooth margin as in Figure (10).
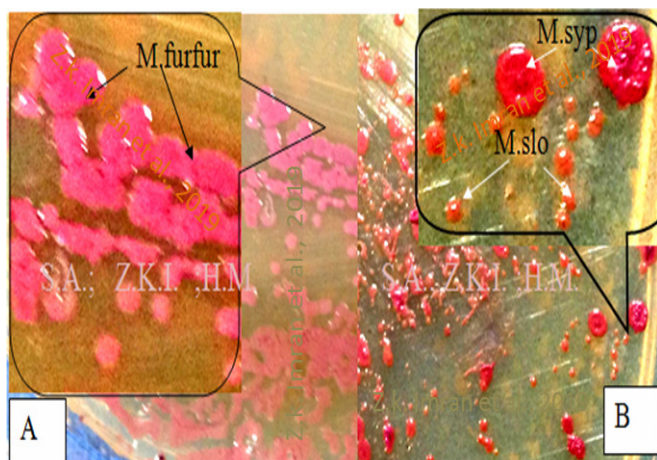
Fig. 10 : Colony characters of *Malassezia* spp. on modified CHROMO agar. A: *M. furfur* (pale pink colony with margin wrinkled), B:M.sym.= *M.sympodialis* (large colony and pink color), M.slo =*M. sloffiae* with small smooth colony and pale-pink color.

The results shown that the pre-identification of *Malassezia* spp. Based on Modified CHROMOagar medium, all isolates shown high purity isolates of *Malassezia* and none mixed culture or contamination with *Candida* spp. Our results consistent with many results (Kaneko *et al.*, 2006; 2007).

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Ethical approval

All authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Conflict of interests

The authors did not declare any conflict of interest.

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