Dedication

To My mother who gave me her life and lightined to me the way……..

To My wife who is sharing with me the dream…….

To My lovely kids Ibrahim and Ahmed……

To My father, brothers, sisters, extended family and friends ……..

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ABSTRACT

Pityriasis versicolor is a chronic superficial fungal disease that is characterized by the appearance of round to oval lesions, most commonly found on the trunk and upper aspects of the arms. The objectives of this study were to isolate and characterize the cutaneous *Malassezia* in patients with pityriasis versicolor and healthy individuals, as well as to study the antifungal activity of common antifungal agents against the isolated *Malassezia* species.

A total of 370 scale samples were collected (140 by moist cotton swabs and 230 by Opsite™ transparent dressing to lesional and non-lesional sites) from patients suspected of having Pityriasis versicolor and healthy individuals in Khartoum and Gedarif States. Swabs samples (n=140) were cultured on Sabouraud's Dextrose agar with Olive oil and the isolated colonies were identified on the base of colonial morphology, microscopic examination and biochemical tests. DNA was extracted from the yielded colonies and directly from the Opsite tapes, then amplified by PCR, sequenced and analyzed by real-time PCR using a TaqMan probe. Antifungal susceptibility of the isolates to nine antifungal including azoles and other antifungal agents (amphotericin B, micafungin, anidulafungin, fluconazole, itraconazole, 5-flucytosine, voriconazole, posaconazole and caspofungin) was tested via the Sensititre Yeast One system. Obtained data were analysed using SPSS programme.

Twenty eight isolates (20%) were identified according to colonial morphology, microscopic examination and biochemical tests as *Malassezia* species while other 112 (80%) showed negative growth. After PCR amplification and DNA sequencing 18 (64.3%) out of 28 isolates were found to be *Malassezia furfur*, and 10 (35.7%) ruled out of *Malassezia* which were found as *Pichia mandshurica*, *Cryptococcus albidus* and *Clavispora lusitaniae*. DNAs of *Malassezia globosa* and *Malassezia restricta* were detected in 71.7% and 21.8%, respectively in P.V patients. The distribution of *M. globosa* and *M. restricta* in P.V patients were higher (60.9%) in 20-30 age group and lower (1.1%) in 61-70 age group. On the other hand, the distribution of *M. globosa*

and *M. restricta* in healthy subjects, males and females were higher (38.6% and 56.2%) in 20-30 age, and lower (8.6% and 6.8%) in 61-70 age. The overall level of colonization by *Malassezia* at the lesional sites was higher than that at the non-lesional sites for all body sites, including the face, neck, cheeks, and trunk (2.7- to 6.0-fold increase) ($P< 0.05$).

Posaconazole (POS), Voriconazole (VOR) and Itraconazole (ITZ) were highly active against isolates of *M. furfur* since all isolates were inhibited by 0.06-0.12 µg/mL, 0.015-0.03 µg/mL and 0.25 µg/mL respectively. Amphotericin B and caspofungin have high MICs and low *in vitro* activities against *M. furfur* isolates, since most isolates were inhibited by 8µg/ml of amphotericin B, 0.5µg /ml of caspofungin and 1 µg/mL of fluconazole.

This study indicates that *M. furfur* strains were susceptible to ITZ, VOR and POS. The Sensititre Yeast One seems to be a suitable commercial tool for the antifungal susceptibility of *Malassezia*. Moreover, the study confirmed that both DNAs of *M. globosa* and *M. restricta* can be detected in healthy subjects, although *M. restricta* was predominant in both sexes with slight difference in colonization level between males and females. *M. furfur* was susccefully isolated from scale samples of both patients and healthy subjects while other species, *M. globosa* and *M. restricta* were not isolated on cultures and found only after molecular characterization by real- time PCR directly from the specimen. *M. globosa* was predominant in Sudanese patients with pityriasis versicolor. Further studies are required in different part to establish a solid base for research on the basic molecular biology of *Malassezia* fungus in Sudan.

الخلاصة

مرض النخالية المبرقشة (Pityriasis versicolor) من الامراض الفطرية السطحية التى تتميز بظهور طفح جلدى دائرى او بيضاوى غالبا ما يصيب المناطق العلوية من الجسم خاصة العنق والايدى. تهدف هذه الدراسة إلى عزل ودراسة التمييز الجزيئي لفطر الوبيغاء من مرضى يشتبه في إصابتهم بمرض النخالية المبرقشة ومجموعة من الأشخاص الاصحاء, كما تهدف إلى دراسة مدى فعالية بعض مضادات الفطريات الشائعة ضد عزلات فطر الملاسيزية (الوبيغاء).

جمعت 370 عينة سطحية من الجلد (140 باستخدام مسحة القطن المبلل و 230 بالشريط اللاصق الشفاف من الأماكن التي يظهر بها الطفح الجلدي وأماكن خالية من الطفح الجلدي) من مرضى مصابين بمرض النخالية المبرقشة واشخاص اصحاء في ولاية الخرطوم و ولاية القضارف.حُ قنت جميع العينات (140 عينة مسحة جلدية)في الأوساط الغذائية ثم دُ رست المستعمرات المتكونة حسب شكل النمو الظاهر ي, الأشكال المجهرية والتفاعلات البيوكيميائية. تم عزل الحمض الريبى النووي منزوع الأكسجين (DNA) من المستعمرات ومن الشريط اللاصق الشفاف مباشرة, ثم تمت دراسته باستخدام طريقة الإنزيم مجمع البلمرة التسلسلى (PCR) و التسلسل الجيني (sequence) و الإنزيم مجمع السلسلة الكمي حقيقي الزمن (real time PCR). تم قياس ودراسة مدى حساسية عزلات فطر الملاسيزية لتسعة أنواع من المضادات الفطرية تشمل مجموعة الازولات وانواع اخرى من المضادات الفطرية باستخدام طريقة Sensititre Yeast One. كل النتائج المتحصلة تم تحليلها احصائيا" باستخدام برنامج SPSS .

ثمانية وعشرون عزلة (20%) وجدت مشابهة لفطر الملاسيزية بينما كانت بقية العزلات 112 (80%) مشابهة لفطريات اخرى . بعد إجراء اختبار تفاعل الإنزيم مجمع البلمرة التسلسلي (PCR) وتحليل تركيب التسلسل الجيني (sequence analysis) وجد أن 18 بنسبة (64.3%) من مجموع 28 عزلة عبارة عن فطر الملاسيزية النخالية (*M. furfur*) بينما أعطت 10 عزلات بنسبة (35.7%) نتائج لفطريات أخرى مختلفة (*Pichia mandushurica*, *Cryptococcus albidus* and *Clavispora lusitaniae*). باستخدام طريقة الإنزيم مجمع البلمرة التسلسلى الكمي حقيقي الزمن (real time PCR) وجد أن الملاسيزية الدائرية (*M. globosa*) و الملاسيزية المحددة (*M .retricta*) تنمو بنسبة (71.4% , 21.8%) على التوالي في المرضى, وبنسبة (46.7%, 54.4%) في الأشخاص الأصحاء على الرغم من عدم عزلها في الأوساط الغذائية مما يدل على أن طرق التصنيف الجزيئي أكثر دقة من طريقة

العزل وربما يعود ذلك أيضا لنوع الوسط الغذائي المستخدم **(** Sabouraud dextrose agar with olive oil**)** والذي لايدعم نمو كل أنواع هذا الفطر. وجد أيضا" إن نسبة انتشار هذه الأنواع من فطر الملاسيزية عالية في الأعمار من 20-30 سنة بنسبة (60.9%) في المرضى وبنسب (56.2% , 38.1%)في الأصحاء ذكور وإناث , بينما كانت النسبة منخفضة في الأعمار من 61-70 سنة **(**P>0.05**).** كما وجد أن النسبة العامة لنمو هذا الفطر في مواقع الطفح الجلدي **(**lesional site**)** أعلى منه في الأماكن الخالية من الإصابة **(** non lesional site**)** بما فيها الوجه والعنق والأكتاف بمعدل زيادة بلغ 2.7 إلى 6 مرات **(**P< 0.05**).** بعد اجراء اختبار الحساسية للمضادات الفطرية وجد أن البوساكونازول والفوريكونازول والاتراكونازول عالية الفعالية ضد عزلات فطر الملاسيزية النخالية **(***M. furfur***)** حيث إن اقل تركيز يثبط النمو **(**MIC**)** هو (0.12-0.6, 0.03-0.015, 0.25 mg/dl**)** على التوالي. هذه الدراسة أوضحت بشكل عام أن فطر الملاسيزية يستجيب لبعض المضادات الفطرية مثل الفوريكونازول والبوساكونازول والاتراكونازول بينما يقاوم البعض الأخر مثل الكاسبوفنقين و الامفوترايسن (ب), وقد اتضح ان طريقة (Sensititre Yeast One) تعتبر مناسبة لاجراء هذا الاختبار. هذه الدراسة اكدت أن الملاسيزية الدائرية **(***M. globosa***)** و الملاسيزية المحددة **(***M. retricta***)** توجد فى الاشخاص الاصحاء والمرضى مع اختلاف نسبة انتشارها حسب الجنس. كما اوضحت ايضا" امكانية عزل فطر الملاسيزية النخالية (*M. furfur***)** بينما تختفى الاجناس الاخرى والتى تم التعرف عليها باستخدام طرق التمييز الجزيئى. وجد فى هذه الدراسة **ان** نوع الملاسيزية الدائرية **(** *M .globosa*)هو الأكثر شيوعا" في المرضى السودانيين المصابين بمرض النخالية المبرقشة **(**Pityriasis versicolor**).**

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LIST OF ABBREVIATIONS

EDTA	Ethylinediaminetetracetic acid
PCR	Polymersae Chain Reaction
RT-PCR	Real – time Polymersae Chain Reaction
TBE	Tris Boric EDTA
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphate
PV	Pityriasis versicolor
TV	Tinea versicolor
SD	Sebrrhoeic dermatitis
AD	Atopic dermatitis
LSU	Large subunit
rDNA	Ribosomal Deoxyribonucleic acid
rRNA	Ribosomal Ribonucleic acid
ITS1	Iinternal transcribed space 1
IGS	Iintergenic spacer
D1/ D2 LSU	The large subunit of spacer sequence Domain 1 and Domain 2
RAPD	random amplified polymorphic DNA
DGGE	denaturing gel electrophoresis
SDA	Sabouraud's dextrose agar
ATCC	American Type culture collection
UV	Ultra violet
Trp	Tryptophan
AEDS	Atopic eczema/dermatitis syndrome
MF	*Malassezia* folliculitis
PS	Psoriasis
NCCLS	National Committee for Clinical Laboratory Standards
CLSI	Clinical and Laboratory Standards Institute

EUCAST	European Committee for Antimicrobial Susceptibility Testing
mLNA	Modified Leeming-Notman Agar
CBS	Centraalbureau voor Schimmelcultures
MIC	Minimum inhibitory concentration
SPSS	Statistical Package for Social Science
UPGMA	Unweighted pair-group method using arithmetic mean
ICU	Intensive care unit