Epidemiological and Molecular Characterization of *Malassezia* species from Patients with *pityriasis versicolor* in Erbil Province

Zuber Ismael Hassan¹, Dindar Sharif Qurtas²

¹Department of Medical Laboratory, Erbil Technical Health & Medical College, Erbil Polytechnic University, Kurdistan Region of Iraq’s
²College of Medicine, Hawler Medical University-Erbil, Kurdistan Region of Iraq

**ABSTRACT**

**Background:** *Pityriasis versicolor* (PV) is the most common chronic superficial infection of the stratum corneum. *Pityriasis versicolor* is the prototypical skin disease etiologically connected to *Malassezia* species. *Malassezia furfur* is the primary causative agent of *pityriasis versicolor* which causes either hyperpigmentation or hypopigmentation of the skin.

**Material and Methods:** Sixty patients suffering from *pityriasis versicolor* disease who attended Erbil Dermatological Center, from August 2021 up to July 2023. Clinical diagnoses were done by consultant dermatologist. Thirty nine were males and Twenty-six were females. DNA has been extracted from skin scraps isolated from various body areas using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and it was amplified using specific primers for *Malassezia* strains. The amplified PCR products were sequenced commercially in both directions (Macrogen Inc. South Korea).

**Results:** The largest proportion of infections was reported by hyperpigmentation (64.6%), followed by hypopigmentation (44.5%). The rosy-coloured lesions were present only in 8 (12.3%) of patients. Applying of polymerase chain reaction is extremely critical to verify the diagnosis of *Malassezia* species. Ribosomal region of sequence analysis revealed that, the sequences of 14 isolates under the accession number (MT000715 and MT000716) were (99.9%-100%) homologous to *M. furfur* under the accession number NG_057730 and 6 isolates under the accession number (MT000717) were (100%) homologous to *M.globosa* (AY743604 and AJ249951). Phylogenetic analyzes were performed to assist investigate the relationship of *M.furfur* and *M.globosa* to support these results in Erbil province.

**Conclusions:** Phylogenetic analysis of the fourteen isolates (*M. furfur*) under the accession (MT000715 and MT000716) and the remaining six isolates belonging to the *M. globosa* (MT000717) were analyzed by MEGA 5.05 and compared with sequences of different *Malassezia* species available in Gen Bank database, the data showed a clear convergence between our *Malassezia* isolates from Erbil patients and that of the Gen Bank database.

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The taxonomy of the genus Malassezia to a considerable extent [10]. Sequence variability between Malassezia species has been documented in rRNA genes. In 1996, Gueho et al. [11], classified the genus of Malassezia in seven distinct species, namely: M. furfur, M. pachydermatis, M. sympodialis, M. globosa, M. obtusa, M. restricta and M. slooffiae. Recently, based on DNA analysis, six new species have been introduced: M. dermatis, M. nana, M. japonica, and M. yamatoensis, M. equine, M. caprae. Of these M. caprae, M. equine and M. nana have only been isolated from domestic animals [4]. As well as, molecular techniques like Polymerase Chain Reaction (PCR) of ribosomal 26S rDNA gene that have been used to identify Malassezia spp [12-15] and its simple, reliable, cost-effective and accurate method [4, 16, 17]. As a result, the categorization of the Malassezia genus was drastically changed [4, 18].

Nine of the thirteen genus species, M. furfur, M. sympodial, M. globosa, M. restricta, M. slooffiae, M. obtusa, M. dermatis, M. japonica, and M. yamatoensis, are related to human normal flora and pathogens. As well as, four species that are related to animals which include M. pachydermatis, M. nana, M. equina, and M. caprae [19-21]. The study's
objective is to determine the prevalence of specific Malassezia species using PCR technique among Pityriasis versicolor patients in the province of Erbil.

2- Methodology

2.1- Collection of Samples:
The research was carried out at the Erbil Dermatological Center, from August 2021 up to July 2023. Randomly 65 cases with pityriasis versicolor are involved. The patients diagnosed clinically by dermatologist upon their visit to the out-patient clinical center. Data were collected by direct interviewing with patient regarding socio-demographic information, disease history and symptoms. Then detailed physical examination is completed, and recorded. Later, the sample from the patients’ skin lesions (5–10 mg) by scraping the skin with a sterile Forceps and surgical blades was collected in a clean Petri-dish, and it’s preserved in 70% of ethanol for molecular analysis [12, 22].

2.2- Molecular Analysis:
DNA has been extracted from skin scraps isolated from various body areas using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and it was amplified using specific primers for Malassezia strains. Both primers [forward 5'-TAACAAAGATTCCCTAGTA-3') and the reverse (5'ATTACGCCAGCATCCTAAG-3') [23] are used. The mixture of amplification consist of 25μl which included (2X) Go-Tag Master Mix (12.5μl), 2μl of each primer (forward and reverse), DNA template (2μl) and nuclease-free water (6.5μl). The following conditions were used to carry out the amplification reaction: initially denatured (94°C for 5min), Following, 30 cycles of denaturation (94°C for 45s), annealing (55°C for 45s), and elongation (72°C for 1min), with a final extension step (72°C for 7min). The results of amplification were examined by electrophoresis on a 1.5% agarose-gel and staining with ethidium bromide (0.5 μg/ml) in Tris-Acetate EDTA (TAEBuffer) 1X. DNA ladder (100 bp) makes by Qiagen, Germany, was used as the marker (molecular weight marker), and photographed under UV transillumination. The amplified PCR products (~580bp) were sequenced commercially in both directions (Macrogen Inc. South Korea).

3.2- Nucleotide sequence accession number:
The species of Malassezia was confirmed by analyzing the nucleotide sequences and the sequences were aligned through the ClustalW algorithm [24], provided by BioEdit v7.2.5 [25], with sequences available in the GenBank (NCBI) database. Nucleotide sequences were deposited in the GenBank database under accession numbers MT000715, MT000716 & MT000717 through the use of BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST).

4.2- Phylogenic analysis:
Malassezia species under the accession number (MT000715 - MT000717) were compared with reference sequences (AJ249955, AY387131, AY743602, EU815319, EU815319, KJ425391, KX721515, KY108384, KY108389, NG_057730, AJ249951, AY387132, AY387133, AY743604, AY387136, AB070359, AB105064, AB105862, AB125263, AJ249950, AJ249952, AJ249953, AJ249954, NR_126107, KP825367, KP825375, KP825376, KP825377 and KT239958) in the GenBank database was supported in the species determination by utilizing the BLAST Algorithm (https://blast.ncbi.nlm.nih.gov/). Phylogenetic analyses were performed on individual partial gene sequences using MEGA-7 software (Molecular Evolutionary Genetics Analysis 3.1; (http://www.megasoftware) and the neighbor joining were used to build the tree.

4.2- Statistical analysis:
Through scatter plot software (GraphPad Prism v.7, CA, USA), data was analyzed in order to understand characteristics of the examined population with the prevalence of Malassezia species.

3- Results and Discussion
Out of 65 patients with Pityriasis Versicolor, (39/65) were males and (26/65) were females, it means high frequency found in males than females (Table 1). These concurred with Ahmed et al., [26] in Bangladesh, who showed that, the highest infection rate was discovered in males (89.11%) as compared to females (10.85%). Also, Jaffer et al., [27] who represented that, the highest prevalence rate occurred in males more than females (M:F ratio is 2:1:1). While the result disagreed with Nikpoor et al., [28], this infection incidence was higher in women. These indicated, the significant participation of men in outside activities with maximal exposure to heat and moisture, favoring the growth of Malassezian yeasts. Table 1 expressed that most of our patients were occupied as a government employee (33.85%) and students (32.31%). These agreed with Morais et al., [29] who showed that the students are predominated 37.1% (43/116), liberal professionals 16.4% (19/116) and home professionals 12.9% (15/116) was second and third sectors in frequency. These could include the use of oil on the surface of the body, excessive sweat, low hygiene, malnutrition and systematic steroid medication. As well as, The results explained that, the frequency of Pityriasis versicolor is more predominant in patients with an adverse history of the disease by 78.46%.The result agreed with Jabry and Alsudani [30] in Iraq, which showed that the majority of patients (82.8%) with PV do not have a positive history of the disease (negative history), compared to patients with a positive history of the disease at 17.2%. There are little indications that the disease is highly contagious, since few cases are present in couple’s cohabitation. Thus the varied factors due to the disease's existence within the family are variable [27].
The disease mostly was asymptomatic among our patients (58.5%). Only 27 (41.5%) patients had mild itching during physical activities. Objectively, the skin lesions mostly affected the neck region (75.4%). The chest was second place in frequency (64.6%) of localization of *Pityriasis versicolor* lesions. Both head (face) and abdomen regions were least (18.5%) affected areas of the body on our study sample (figure 1). These agreed with [15, 27, 30], who showed that the majority cases were asymptomatic and for cosmetic concern went to the hospital. The neck is the first section of the body to be affected, followed by the chest and then the back. The reason of these disease being more common in the upper body, which has a higher percentage of fat than the lower body parts, may be related to the pathogen's lipophilic nature, as these regions are characterized by thick and active sebaceous glands. The numerous of the skin lesions at the time of presentation were hyperpigmented (64.6%). The rosy-coloured lesions were present only in 8 (12.3%) of patients (Table 3). The result agreed with Talae et al., [4] who revealed that 50% of patients with PV had hyperpigmentation lesion, while 37% of them had hypopigmentation lesion and 13% had both hyperpigmentation and hypopigmented lesion. Shah et al., [31] revealed that, the shape, size, and color of the infection spots might vary from person to person, and they can be circular, oval, or irregular in appearance. Its color may range from yellow to brown or reddish-brown, but it usually takes a stain paler than the rest of the complexion. These spots are covered with a superficial layer of scales that resembles bran, making them appear light in color to those with dark skin and brown in those with white skin [30].

![Fig. 1: Pityriasis versicolor (Hypopigmentation and Hyperpigmentation) over the body surface.](image)

<table>
<thead>
<tr>
<th>General characteristics of patients</th>
<th>Gender</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>%</td>
</tr>
<tr>
<td>occupations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government employee</td>
<td>14</td>
<td>21.54</td>
</tr>
<tr>
<td>Private employee</td>
<td>9</td>
<td>13.85</td>
</tr>
<tr>
<td>Student</td>
<td>14</td>
<td>21.54</td>
</tr>
<tr>
<td>Retired</td>
<td>2</td>
<td>3.08</td>
</tr>
<tr>
<td>Family history of <em>pityriasis versicolor</em></td>
<td>Yes</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 1: General characteristics of patients

Table 2: Incidence of Isolated *Malassezia Species* According to Symptoms

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itchy</td>
<td>27</td>
<td>41.5</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>38</td>
<td>58.5</td>
</tr>
<tr>
<td>Location of Lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head (face)</td>
<td>12</td>
<td>18.5</td>
</tr>
<tr>
<td>Neck</td>
<td>49</td>
<td>75.4</td>
</tr>
<tr>
<td>Chest</td>
<td>42</td>
<td>64.6</td>
</tr>
<tr>
<td>Back</td>
<td>36</td>
<td>55.4</td>
</tr>
<tr>
<td>Shoulders</td>
<td>19</td>
<td>29.1</td>
</tr>
<tr>
<td>Abdomen</td>
<td>12</td>
<td>18.5</td>
</tr>
<tr>
<td>Colour of Lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>29</td>
<td>44.5</td>
</tr>
<tr>
<td>Dark (hyperpigmented)</td>
<td>42</td>
<td>64.6</td>
</tr>
<tr>
<td>Rose</td>
<td>8</td>
<td>12.3</td>
</tr>
</tbody>
</table>

The PCR products are separated and electrophoresed to obtained ~580 bp band size on 1.5% agarose gel after staining with ethidium bromide as shown in Figure (2) which were the same bands generated by primer measured for DNA size marker 100bp DNA ladder [23]. As well as, the alignment of a partial nucleotide sequence of *Malassezia* species as compared with the previously published sequences under accession number NG_057730, AY743604 and AJ249951 [32, 33]. The result showed that the *Malassezia species* under the accession number MT000715 (8/20) was 100% and MT000716 (6/20) were 99.9% homologous to *M. furfur* under the accession number (NG_057730) due to nucleotide substitution (G → A) at the position of 514 as shown in Figure (3). On the other hand, Figure (4) revealed that *Malassezia species* under the accession number MT000717 (6/20) were 100% homologous to *Malassezia globosa* under the accession number AY743604 and AJ249951. The present study showed that; *M. furfur* 14/20 as the major species followed by *M. globosa* 6/20 from P.V. lesions in Erbil Province (Table 3). The outcome supported by Honnavar et al., [34] in India who showed that; the commonly isolated species is *M. furfur* (50%), followed by *M. globosa* (27.3%), both of *M. furfur* and *M. globosa* (15.9%), *M. sympodialis* (4.5 %), and *M. slooffii* (2.3 %) and Elshabrawy et al., [35] in Egypt. They revealed that, out of 98 samples, six species was isolated which include (44 (44.9%) *M. furfur*, 24 (24.5%) *M. globosa*, 12 (12.2%) *M. sympodialis*, 10 (10.2%) *M. restricta*, *M. obtusa* and 4(4.1%) *M. pachydermatis*). As well as, Diongue et al., [14] in Senegal, showed that, the only
M. furfur was discovered in 100% (39/39). Several factors play a major role in M. furfur pathogenicity such as high level of sebum production, stress, hormonal change, illness, allergic food, deficiency of vitamin B, unusual shampooing, curlers hair and blow dryers [21]. Other studies Talaee et al., [4] from Iran, opposite to our results which is showed that, the higher infection rates is M. globosa (66%) as the major causes of pityriasis versicolor lesions followed by M. furfur(26%), M. restricta(3%), M. sympodial(3%), and M. slooffii (2%), respectively.

On the other hand, Shokohi et al., [36] revealed that, M. globossa 29(47%) is the most prevalent while M. furfur 25 (41%) is the second most frequent agent. The molecular method used for isolation of Malassezia species to resolve the time consuming and the difficulties in interpreting some morphological, physiological patterns and confirmation of strains [13, 18, 34, 36, 37]. The different culture mediums and probably the ethnical, climatic and geographical elements and features of patients are responsible for factors determining this variation [14].

<table>
<thead>
<tr>
<th>Malassezia Species</th>
<th>Accession number</th>
<th>Neck (6)</th>
<th>Chest (5)</th>
<th>Back (3)</th>
<th>Shoulders (2)</th>
<th>Face (2)</th>
<th>Abdomen (2)</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. furfur</td>
<td>MT000715</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>MT000716</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>M. globassa</td>
<td>MT000717</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 2: Agarose gel electrophoresis of Malassezia species DNA isolated from human samples. DNA marker size (L: 100bp DNA ladder), lanes (1, 4, 5, 7, 8, 10, 12, 14, 17, 18, 20, 21, 22, 40 and 42–specific product for Malassezia species isolated from skin.

Table 3: Isolated Malassezia species from Different Lesion Area
Fig. 3: Compared parts of the sequence results of cloned *Malassezia furfur* positive samples in humans with published sequences available in the NCBI database (NG_057730).
Fig. 4: Compared parts of sequence results of cloned *Malassezia globosa* positive samples in humans with published sequences available within the NCBI database (AY743604 and AJ249951).

The phylogenetic tree was diagrammatic by (MEGA) software version 7.0 is shown in Figure (5). Fourteen isolates under the accession (MT000715 and MT000716) were *M. furfur* that had 99.5-100% similarity with the *M. furfur* under ID: AY743602, KX721515, KJ425391 and KY108389 [19, 20, 33, 38, 39]. The remaining samples (six isolates) belonging to the *M. globosa* was found, which showed 99.7-100% similarity with the sequence recorded for *M. globosa* under ID: AJ249951, KT239958, AY743604, KP825367, KP825376 and kp825375) sequence [19, 20, 33, 38, 39]. The phylogenetic tree confirms our results and the identity of the isolates are associated with *Pityriasis versicolor* which is the first report in Erbil Province, whereas *M. restricta* and *M. globosa* are generally related with seborrhoeic dermatitis/dandruff [40, 41].
4- Conclusion

Pityriasis versicolor was more prevalent among males than female in our study sample. The most frequent locations of the lesion were within the neck and chest. The hyperpigmented lesion was predominant presentation among the studied sample. M. furfur is dominant and M. globosa was the second species which are prevalent in our patients. Sequencing and phylogenetic analysis showed that, M. furfur and M. globosa in Erbil was not different from the pathogens in other humans from geographically distinct regions. These data provide important information about the incidence of Malassezia in humans and its benefit’s for managing and controlling programs of the disease.

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References


