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DANDRUFF AETIOLOGY AND THE EFFECTS OF EDIBLE LIPIDS ON THE GROWTH OF ISOLATES

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ABSTRACT

Background: Dandruff is caused by *Malassezia* species, a lipophilic fungus. Information on the aetiology of the fungi associated with this condition and profile of affected persons are sparse in our locality. This prospective study was designed to determine the aetiology of dandruff causing fungi and to assess the effects of some edible lipids on the growth of predominant isolates in our locality. **Methods:** The participants enrolled for the study were 245 in number. Those with dandruff-like lesions were 145 and those without lesions (controls) were 100. Ethical approval was obtained. Questionnaires were administered for biodata. Scalp scrapings were obtained from subjects visiting hair and beauty salons into sterile paper envelopes and transported to the Microbiology Laboratory, UCTH, for analysis. Samples were subjected to culture, microscopy and physiological tests. Growth response of *Malassezia* species to lipids was assessed using common edible oils including groundnut oil. **Results:** Out of the 145 subjects with dandruff-like lesions, 90.3% were positive for different *Malassezia* species. The most prevalent *Malassezia* species among subjects with dandruff lesions was *M. furfur* (70.2%) while *M. globosa* (51.9%) was the most prevalent among the controls. *Malassezia pachydermatis* was the least prevalent species among the two groups. *M. furfur* grew exceptionally on SDA overlaid with groundnut oil. **Conclusion:** Groundnut oil could be used as an alternative for the cultivation of *M. furfur* especially in a resource poor setting like ours and should not be used in the preparation of hair ointment or creams.

KEYWORDS: *Malassezia pachydermatis, M. globosa.*

INTRODUCTION

Dandruff, medically known as Pityriasis capitis is a condition, characterized by small white flakes of skin scales that separate and fall from the scalp. It is a condition that causes social or self esteem problems to those affected.[1] Symptoms include: itching, reddening and flaking in areas with a rich supply of sebum production. Lesions are red and covered with greasy scales. There are more chances of hair lose on scalp with dandruff than non-dandruff scalps which shows the relationship with *Malassezia* infection.[1]

Dandruff affects more than 50% of adult population and represents 25% of all scalp disorders.[2-4] A USA survey reported that about 50 million people suffer dandruff infections yearly and about $300 million is spent annually on different treatment options.[5-6]

Malassezia is the only known fungi implicated in Dandruff. The different species include; *M. furfur, M. globosa, M. pachydermatis, M. sympodialis, M. obtuse, M. slooffiae* and *M. restricta*. However, the dominant species on human scalp are *Malassezia furfur, Malassezia globosa* and *Malassezia restricta.*[2] *Malassezia* are lipophilic fungi.[7-8] People with over active sebaceous glands suffer from repeated attack of dandruff because of their oily scalp.[9] Malassezia proliferates more and deregulates keratinization.[10] The keratinized cells clump together resulting in large flakes of scales. The scales can be washed away with agents that lyse keratinized cells, example, selsun blue and salicylic acid.[10]

There is a suggestion that the presence of androgens in males play a role in their higher susceptibiity to dandruff than females. People who are bald do not suffer this condition because the hair follicle is necessary for fungal colonization.[11]

Malassezia colonies are cream colored, yellow to brown, orange or beige depending on the species. They are raised and smooth initially but become dry and wrinkled with age. This organism is a fast grower and mature in 5 days at 30-37°C. Growth is poor at 25°C incubation.[12] Dandruff predisposing factors in immunocompetent and immune-compromised individuals are multifactoral, they may be Intrinsic, Extrinsic or Pathogen related.[13] Intrinsic factors include; Genetic predisposition, emotional component, increase in the alkalinity of the skin and neurological diseases.[14] Extrinsic factors

include; too much application of oils on hair with scalp, use of wrong hair conditioners, not rinsing hair properly after application of conditioners and poor hair hygiene.[15] Pathogen factor include; lipase production, metabolism of triglycerides and oleic acid synthesis.[14]

There is paucity of information on pityriasis capitis aetiology, and the profile of persons affected in our locality. This study was carried out to determine the aetiology and types of dandruff-like causing fungi among individuals visiting hair salons in Calabar and the prevention and management of this condition.

MATERIALS AND METHODS

Study area

The study area was Calabar, Nigeria, the capital of Cross River State. She is located at (4^{o}57N, 8^{o}19E). The state falls within the Tropical Zone. There are two seasons, the rainy season from May-October with a mean annual rainfall of 243-336mm and dry season from November-April. This period may change from year to year. It has a daily temperature which varies between 27^{o}C-31^{o}C with small annual range of 4^{o}C and 5^{o}C. Relative Humidity is high for the rainy season (80-90%) and low in the dry season (45-50%).[16]

Subject selection/Ethical approval

Subjects for this study were enrolled after obtaining due approval from the Ethical Research committee. Informed consents were also obtained from the subjects. A pre-designed protocol was administered to all the subjects for information on biodata, treatment options and presenting complaints.

Sample collection

Convenient samples were obtained from subjects of both gender visiting hair salons and barber shops located in the two Local Government Areas in the State capital, between December 2012 and May 2013. Control subjects were male and female students who reside in the University of Calabar hostels. Human scalp scrapings were obtained from 245 subjects; 145 subjects with dandruff-like lesions and 100 with no sign of dandruff-like lesions (controls) into sterile paper envelops. Samples were transported to the Microbiology Laboratory, University of Calabar Teaching Hospital for analysis.

Microscopic examination

Scalp scrapings were examined as wet mounts in 10% KOH for thick-walled round, yeast-like cells alongside short angular hyphae which are characteristic features of *Malassezia* species.[17]

Culture

Samples were cultured on Sabouraud Dextrose Agar containing 0.5mg chloramphenicol overlaid with sterile olive oil.[18] Plates were incubated for 7 days at a temperature of 37^{0}C. The agar plates were examined every other day for fungal growth.[18]

Identification of isolates

Pure cultures of every isolate were prepared before performing any physiological test. Isolates were identified by gross and microscopic morphology alongside physiological tests. Microscopic examination of isolates was in Lactophenol cotton blue stain. Identification and speciation of isolates was carried out using the following test; catalase test, Lipid test and Tween assimilation test.[19]

Physiological tests

Catalase Test

Catalase production was used to differentiate *M. restricta* from *M. obtuse, M. globosa and M. furfur*. All *Malassezia* species produce catalase except *M. restricta*. *Malassezia pachydermatis* have variable reaction.[20-21]

Lipid test

The yeast isolates from pure cultures were inoculated on SDA containing 0.5mg chloramphenicol in duplicates. One was overlaid with sterile olive oil and the other was not. The plates were incubated for 7 days at 37^{o}C. Growth rate was observed to determine lipid dependent and non-lipid dependent species of *Malassezia.*

Tween Assimilation Test

This test was performed to differentiate *Malassezia furfur* from *M.globusa* and *M. obtuse*. The test was evaluated according to Guillot *et al*.[22] methods. A 24hr culture of the isolates was used to prepare the inoculum. The organisms suspension was constituted in 5ml of sterile normal saline. The turbidity was adjusted to 0.5 McFarland standard which is an equivalent of 1 x 10^{6} to 5x10^{6} CFU/ml. One ml of the McFarland adjusted culture was inoculated on Sabouraud Dextrose Agar plate. After the surface has dried, four holes were made with sterile 2mm diameter punch and filled with 5µl of Tween 20, 40, 60 and 80 (Shreeji Pharma International, India) respectively. The plates were incubated for 7 days at 37°C. Tweens utilization was assessed by the degree of growth and/or precipitation of the lipid loving yeasts around the wells and their rate of growth.[21-22] Plates with visible growth were incubated at 40°C for 48 hours, only *M. furfur* can survive this temperature.

Urease test

Urease test was positive only for *M. furfur* and *M. pachydermatis*.

Effect of selected oil on the growth of *M. furfur*

Commonly available edible oils were selected to assess the growth rate of *M. furfur* when overlaid with them. The oils were palm oil, margarine, coconut oil, groundnut oil, olive oil, castor oil and palm kernel oil. All the oils were procured in the market and sterilized at 160^{o}C in hot air oven for 60 minutes. Sterile swab sticks were dipped into sterile Tween 80 and used to pick pure distinct colonies of the yeast to suspend in 3ml of sterile normal saline and vortex. The suspension turbidity was adjusted to 0.5 McFarland standards by visual inspection.

Sabouraud dextrose agar plates prepared in duplicates for each isolate were overlaid with 2ml of the various oils. All plates were inoculated with 1ml of 0.5 McFarland adjusted culture suspension by the use of an automatic pipette. All cultures were incubated for 48hrs at 37^{o}C or until good growth was seen in the control. Olive oil plates were used as control because it is the standard recommended oil for the growth of *M. furfur*. Growth on the plates were compared to that of the control (olive oil plate) and scored by visual inspection. They were graded as shown follows;

++++ - Excellent growth
+++ - growth same as control
++ - Fair growth (less than that of control)
+ - Poor growth

RESULTS

Out of the 245 subjects enrolled for the study, 185/245(75.5%) were positive for *Malassesia* infection. The prevalence rate was 90.3% (131/145) among subjects with dandruff-like lesions and 54.0% (54/100) among those without dandruff lesion (Table 1).

TABLE 1: Prevalence of *Malassezia* isolates among subjects.

Status of *Malassezia* infection	No. (%) of Subjects with Dandruff-like lesions (n=145)	No. (%) of Subjects without dandruff-like lesion (n=100)	Total (n=245)
Malassezia positive	131(90.3)	54(54)	185(75.5)
Malassezia negative	14(9.7)	46(46)	60(24.5)

Figure 1 shows the distribution of *Malassezia* species among subjects. *Malassezia furfur* 92(70.2%) and *M. globosaI* 31(23.7%) were more predominant species among subjects with dandruff-like lesions while *M. globosa* 28(51.9%) and *M.restricta* 14(25.9%) were more predominant among subjects without lesions.

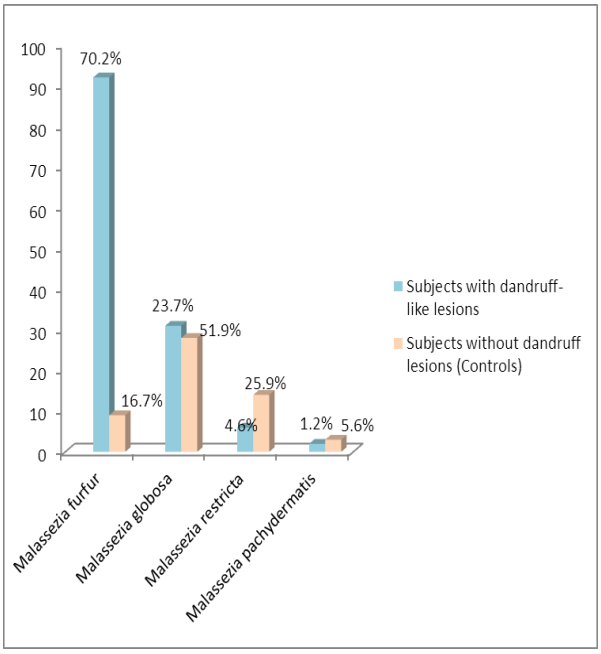

Fig 1 Distribution of *Malassezia* species among subjects

Plate 1 shows a photograph of a human scalp in health and with dandruff. Plate A shows a healthy scalp while B shows a scalp with Pityriasis capitis lesions alongside white flaky scales on the hair.

B

A

PLATE 1 Photograph of a healthy and dandruff scalp

Table 2 shows the distribution of *Malassezia* infection among subjects by age. The highest *Malassezia* infection rate 51.9% (96/109) was seen in age 25-34 years followed by 27.6%(51/63) among age 15-24 years while the least 0.5%(1/5) was seen among age 55-65 years.

TABLE 2: Distribution of *Malassezia* species among subjects by age

Age (Years)	Total No. examined		No. (%) positive for Malassezia
15-24	63		51(27.6)
25-34	109		**96(51.9)**
35-44	36		26(14.1)
45-54	32		11(5.9)
55-65	5		1(0.5)
Total	245		185

Figure 2 shows the symptoms and complaints from subjects with Pityriasis capitis. The commonest presenting symptoms were itching (28.9%), flaking or scaling (23.5%), social/emotional discomfort (16.6%) and alopecia (15.2%). Other less frequent complaints were wound on the scalp (7.6%), headache (2.1%) and erythema (6.2%).

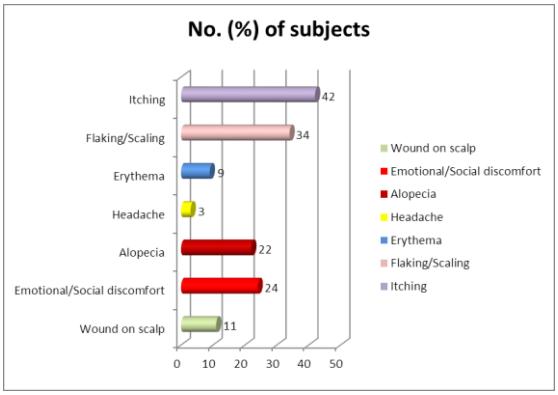

Fig 2 Symptoms associated with Pityriasis capitis

Table 3 shows the characterization of *Malassezia* species by physiological and biochemical tests. The tests carried out were; Tween assimilation test, Catalase test, Urease test, Growth at 40^{o}C and Growth with or without the addition of olive oil.

Table 4 shows the growth assessment of *M. furfur* using seven edible lipids in the locality. These include: groundnut oil, margarine, castor oil, palm oil, palm kernel and coconut oil. Olive oil was used as a control. Among the seven lipids, *M. furfur* grew exceptionally on SDA overlaid with groundnut oil. Good growth was also observed on SDA overlaid with coconut oil and margarine but growth was poor on SDA with palm kernel oil and palm oil.

Table 3 Characterization of *Malassezia* species.
(Soo-Jung *et al.*[23]

Malassezia Species	Urease test	No Lipid supplement	Lipids supplement	Tween 20,40,80	Catalase reaction	Growth at 40oc
M. furfur	+	-	+	+	+	+
M. globosa	-	-	+	-	+	-
M. restricta	-	-	+	-	-	-
M. pachydermatis	+	+	-	+	+	-

KEY
+ = presence of growth
- = absence of growth

TABLE 4: Growth assessment of *Malassezia furfur* on Sabouraud Agar overlaid with edible oils in the locality.

Edible oils	Growth rate
Groundnut oil	++++
Coconut oil	+++
Olive oil	+++
Margarine	+++
Castor oil	+++
Palm oil	+
Palm Kernel oil	+

++++ = Excellent Growth, +++ = Good Growth, ++ = Fair Growth, + = Poor Growth.

DISCUSSION AND CONCLUSION

Discussion

Information on the aetiology of dandruff causing fungi and the profile of affected persons are sparse in Calabar, Nigeria. This study has provided useful information on these areas.

Malassezia was isolated from most (90.3%) of the subjects with dandruff-like lesions however, 54% of the control subjects were colonized. This suggests that *Malassezia* may be a normal flora on healthy scalp. On the other hand, *Malassezia furfur*, the most predominant species among the test subjects may have caused dandruff-like lesions and its associated symptoms.

Although sebum production was not investigated in our study, the highest prevalence of dandruff-like lesions was seen in subjects aged 25-34 years which correspond with the age of the peak production of sebum which is usually at post-puberty.[23-24] An evaluation of sebum production may be needed to assess its role in the production of dandruff and growth of *Malassezia*.

Subjects with dandruff-like lesions presented more than one symptom and complaints. The commonest complaint/symptom were itching, scaling and loss of hair, this agrees with the reports of Nematian *et al.*[1] who reported flaking and hair loss as the predominant symptom of dandruff.

It is interesting to note that the species *M. pachydermatis* also isolated in our study are known to be found in pet animals which suggest a zoonotic transmission. However data on pet reference was not obtained from our subjects.

CONCLUSION

Malassezia furfur and *M. globosa* could jointly cause dandruff. Groundnut oil enhances the growth of *M. furfur* and could be used as an alternative for the cultivation of *M. furfur* especially in resource poor settings like ours. On the other hand it should not be used in the preparation of hair ointment or creams. Further investigations should be done to evaluate sebum production and its association with *Malassezia* and dandruff formation.

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Competing Interest: None.

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