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Correlation of Malassezia species with clinical characteristics of pityriasis versicolor

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DEDICATION

To God Almighty, with whom all things are possible.

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LIST OF ABBREVIATIONS

PV	-	Pityriasis versicolor
M	-	*Malassezia*
CDLQI	-	Children dermatology life quality index
QoL	-	Quality of life
RFLP	-	Restricted fragment length polymorphism
HRQoL	-	Health related quality of life
TEWL	-	Trans epidermal water loss
UV	-	Ultraviolet
AD	-	Atopic dermatitis
IgE	-	Immunoglobulin E
SC	-	Stratum corneum
PCR	-	Polymerase chain reaction
RNA	-	Ribonucleic acid
DNA	-	Deoxyribonucleic acid
RAPD	-	Random amplification of polymorphic DNA
AFLP	-	Amplified fragment length polymorphism
tFLP	-	terminal fragment length polymorphism
SSCP	-	Single strand conformation polymorphism
DGGE	-	Denaturing gradient gel electrophoresis
FDA	-	Federal drug regulation agency
FCT	-	Federal capital territory
LMU	-	Ludwig Maximilian University
NIMR	-	Nigerian Institute for Medical Research
SS	-	Senior secondary
GSS	-	Government senior secondary
ANOVA	-	Analysis of variance

ABSTRACT

Background: Pityriasis versicolor (PV) is a superficial mycosis, highly prevalent among teenagers living in the tropics. *Malassezia* is the etiological agent of PV. Fourteen species have been identified worldwide. So far, the debate is on which species is more prevalent and how the clinical presentations of PV differ geographically.

Objective: To investigate the most prevalent *Malassezia* causing PV in Nigerian students, describe its clinico-epidemiological characteristics and estimate its effect on health related quality of life (HRQoL).

Methodology: Students were recruited from senior secondary schools within the period of May 2012 to May 2013. Their clinical characteristics were described and scales obtained from PV lesions and non-lesions. The Children's Dermatology Life Quality Index questionnaire was used to assess HRQoL. *Malassezia* species were identified using a molecular method. Results were analysed using SPSS package version 16.

Results: The students recruited were 304. They had an average age of 16 years. The age of onset of PV was influenced by a positive family history, socioeconomic status, the daily use of petrolatum and concomitant presence of dandruff. The recurrence of PV and the color of the lesions which was majorly hypopigmented, were not related to differences in personal hygiene or family history of PV. The location of the lesions was predominately on face while the extent of distribution of the lesion was significantly linked with pruritus and dysesthesia. A moderate effect (25% impairment) on the students' HRQoL was observed. Only three species were identified. The most prevalent was *Malassezia furfur*. Finally, no distinct clinical feature was linked with a specific species.

Conclusion: PV has negative effect on the life of teens. Apart from climate, genetics and hyperhidrosis, its presentation could also be influenced by dandruff, routine application of petrolatum and socioeconomic status. *Malassezia furfur* is the main causative species of PV in Nigerian students.

Keywords: pityriasis versicolor; *Malassezia* species; senior secondary school students; quality of life; tropical environment

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CHAPTER ONE

INTRODUCTION

1.1 Background

Pityriasis versicolor (PV) is a superficial fungal infection of the skin. It is also known as tinea versicolor, previously as dermatomycosis furfuracea, tinea flava and achromia parasitica.(1) In Nigeria, the common names for PV include “eczema” in English language,(2) “Ifo” in Yoruba language, “Makenkero” in Hausa language and “Ngwo” in Ibo language; the three major languages spoken by Nigerians.

It is a chronically recurring fungal infection despite the easy availability of antifungal medications; however, there are no guidelines acceptable for long term treatment. It is majorly asymptomatic but cosmetically not acceptable. Many affected individuals usually do not seek medical attention, thus its prevalence might be more than those inferred in the literature. PV occurrence is worldwide and covers most geographic locations. It is most prevalent in hot, humid climate(3) where it is seen all year round. In temperate regions, it is noticed more easily during summer when the skin tends to tan in light-coloured individuals. The disease affects males and females alike, most commonly young adults.

It is non-contagious and highly prevalent in individuals with genetic predisposition, hyperhidrosis, poor general health, and immune-compromised states but not in pregnancy and diabetes mellitus.(3) The lesions are described characteristically as multiple hypopigmented and hyperpigmented macules; these may coalesce into large, irregular patches, and have a fine scaly appearance(4) which can become obvious by stretching the skin (Zeliri’s sign). Although the lesions may be asymptomatic, some patients complain of pruritus and tingling sensation. Meanwhile, in a majority of patients, the lesions are disfiguring, embarrassing and restrict choice of clothing.

Malassezia species are the causative organisms of PV. They are lipophilic yeasts which are of the normal skin microflora. They convert from their saprophytic yeast form to the pathologic mycelial morphology to cause disease.(5) They are richly located in the sebum-rich body areas such as the face, chest, back and upper arm due to their requirement of lipid to grow.(6) Diagnosis of PV is essentially clinical. Examination with Wood’s light may reveal a yellowish or golden fluorescence. Direct microscopy of scales with potassium hydroxide shows hyphae and spores; the characteristic “spaghetti and meatballs” appearance. Culture of *Malassezia* requires a lipid rich media while molecular analysis as well as physiological characteristics can be used to identify the various species.

There are various treatment options for PV which could be applied via topical and oral routes; however, relapse is common due to the importance of both exogenous and endogenous factors that aid the development of PV.

1.2 Literature review

1.2.1 PV and climate

Pityriasis versicolor (PV) occurs worldwide. However, its prevalence is influenced both by environmental and endogenous factors. An important environmental factor is the climate. PV affects more people living in the tropics than those in temperate regions. The tropical environment is rich in heat and moisture which are important elements that attribute to the development of PV.(7)

In order to assess the effect of climate on the prevalence of PV; a quick literature search using “prevalence of PV” as the search object with focus on studies from various regions of the world was carried out. The regions were grouped according to the updated Köppen-Geiger climate classification by Peel et al.(8) which classified world climate into 5 types; namely type A – tropical, type B – arid, type C – temperate, type D – cold and type E – polar climate. This search, however, revealed only a handful of prevalence studies on PV. Selecting randomly 3 countries each from the same climate type showed the following: In tropical countries of Brazil, Central African Republic and Thailand; prevalence of PV were documented as 13.2%,(9) 16.6%(10) and 17%,(11) respectively. In arid countries of Tunisia, Saudi Arabia and Libya, prevalence of PV were 21.6%,(12) 25.8%(13) and 27.8%,(14) respectively.

In Malawi, a country in southern Africa with a subtropical climate type A, 8% of the people examined had extensive PV which the authors described as PV involving 3 or more regions of the body while 9% was the prevalence for mild disease among the people who were aged 15 - 24 years.(15) Also in the subtropical climate of Santo Andre in Brazil where the average all year high temperature is below 30^0 C, a prevalence of 3.1%(16) was documented.

Available data from temperate climate are quite limited. In Italy a prevalence of 2.1% was observed in 1024 young sailors(17) while the famous study from Sweden by Hellgren et al. showed a prevalence of 0.5% in males and 0.3% in females.(18) There were no studies of PV documented in type E polar climate regions of the world such as Norway or Siberia. It is obvious from the above statistics that highest prevalence of PV is observed in arid countries, areas which have high temperatures all year round.

1.2.2 PV and Age

The common endogenous factors linked with PV include age, poor immune status and genetic predisposition. The age factor contributes significantly to the onset and duration of PV. The age distribution of individuals with PV in most epidemiological studies falls within the puberty period, 12 to 40years.(10, 11, 15, 19, 20) These are usually students and young adults. The high prevalence of PV in this age group is linked with the increased presence of androgen which stimulates sebum production. Also, the rise in the level of physical activity leading to increased sweating and the

probability of harbouring the causative yeast as part of skin flora is higher in this age group. However, studies assessing factors known to hasten or delay the age of onset of PV in these predisposed individuals are few. Apart from genetic predisposition (21), data is lacking on possible associated factors that may affect the age of onset of PV. Another research question yet to be adequately answered is with regards to the natural course of the infection. Although death has not been associated with PV, the degree of morbidity suffered by patients with PV as a result of chronicity and/or recurrence of the infection is unknown. Also are age and genetics the only factors that influence the course of PV?

PV has rarely been reported in infants less than 2 years of age and in most of these cases the infants were premature and placed in intensive care following birth. In one report of PV in a 2-week-old infant; the authors suggest that the hot, humid environment of the incubator may have been a contributing factor,(22) although the sebum level was not assessed. Premature neonates are more often found to have the causative yeast on their skin, regardless of whether they develop PV lesions. These yeasts as part of their skin microbiota may have been picked up via nosocomial route because these infants are handled more frequently by health care personnel than babies born at term.(23) In an epidemiological study of PV in the pediatric age group by Jena et al.(24) conducted in Cuttack, a tropical region in India, PV in children aged up to 14 years accounted for about 31% of the total cases of PV seen in a 2-year period with about 4.8% cases presenting in infancy. This high prevalence may suggest that hot humid climate or environment increases the prevalence of PV in children and even infants.(24)

Furthermore, it is unusual for elderly individuals to suffer from PV. This could be due to the reduction in sebum production that occurs with increasing age(25) although studies on PV in this age group is sparse. Similar to the prevalence of PV in the pediatric age group, environmental factors play a significant role in the prevalence of PV in the geriatric age group. Thus, the study by Di Silverio et al(26) showed the presence of *Malassezia* yeasts and hyphae in about 40.4% of hospitalized elderly patients aged over 60 years. They had the scaling and hyperpigmented patches of PV. The possible predisposing factors included heat and sweat, especially as their clothes were not changed frequently, and the frequency of bath taken may have been reduced in some cases.(26) There was no relationship observed between PV and underlying illness of these hospitalized patients, thus the presence of this skin disorder was not related to the immunocompromised state of the individuals.

1.2.3 PV and skin hydration/sebum

The combination of hydration and sebum contribute to the cause of PV by providing a conducive growth milieu for the etiological yeasts.(7) Studies measuring the difference in the stratum corneum humidity status and sebum excretion rate in PV lesions as compared with non-lesional skin or healthy individuals are quite limited. Investigation of the skin characteristics of PV patients as compared with healthy

controls using the non-invasive MPA-5 by Park et al. showed higher skin humidity, increased sebum excretion rate and increased trans-epidermal water loss (TEWL) values in PV patients(7). The authors deduced that higher humid status and sebum excretion of the skin form part of the factors that encouraged the growth of the etiological yeast of PV while the increase in TEWL is a consequence from skin barrier disruption caused by the interaction between the yeasts and skin barrier materials. They also noted no significant difference in these measured factors between the hypopigmented and hyperpigmented skin lesions.

This result is in contrast with a study by Lee et al.(27) who observed reduced skin hydration status in lesional skin compared with adjacent infection-free skin. They argued that the reduced hydration level of the PV lesions was as a result of the alteration of the skin biophysical properties by the etiological yeasts on the body. The study unlike Park et al. did not measure the sebum excretion rate nor did they compare skin features of the affected individuals with healthy subjects. On the other hand, Burke's study done many years(28) measured lipid levels in PV lesional skin and healthy skin (through a different methodology), observed a higher skin lipid level in PV skin but this was not a consistent feature on all lesions. Meanwhile, Nazzaro-Porro et al. observed higher levels of specific lipoperoxide values derived from the oxidation of different skin lipid classes in PV lesions than in normal controls and deduced that these lipoperoxides may play an important role in the pathogenesis of PV(29) particularly in hypopigmented PV lesions; this was confirmed by De Luca et al.(30)

In neonates, sebum secretion by the skin is elevated due to maternally transferred androgen; this decreases significantly during childhood but starts to rise again during puberty and reaches its maximum in young adults.(25) Sebum production decreases in menopausal females but remains stable with increasing age in males.(25) The role of skin hydration and sebum production in PV is clear and the above studies have shown that this role may not be due to a combined effect; however, studies are needed to determine which of these two factors is more important and also the minimum level of sebum production and skin hydration at which PV is expected to develop. This could possibly influence the use or non-use of emollient in predisposed individuals.

1.2.4 PV and immune status

The relationship between the immune status and PV is not linear. For example, PV is not particularly more prevalent in patients with human immunodeficiency virus (HIV) and the clinical presentations also do not differ when compared with non-HIV patients.(31, 32) An individual with a low immune status does not necessarily develop the infection. The main factor predisposing an immunocompromised individual to PV remains unknown; probably genetics and hormone status driving sebum production play a determining role. Only a few studies of some diseases have shown a clear relationship between PV and a low immune state. An example is the

increased PV prevalence when compared to controls observed in renal transplant patients(33, 34) and chronic kidney disease patients;(35, 36) while the contrast is observed in diabetic patients in which case-control studies show a relatively low prevalence of PV.(37-39) Probably, the development of PV in chronic kidney disease patients may be related to the accumulation of or failure to excrete an unknown endogenous factor. It could also be due to a disruption in skin barrier mechanism or changes in the skin milieu. Since studies on hydration status of the stratum corneum in patients on dialysis revealed a low hydration state and increased TEWL which in one study correlated with the complaints of pruritus in these patients.(40, 41) Sebum excretion rate in these patients are yet to be documented in the literature.

According to the minireview by Tragiannidis et al.(42) *Malassezia* yeasts, that is etiological agents of PV, found in immunocompromised patients such as diabetics, patients with haematological malignancies, bone marrow transplantation, and solid organ transplantation, Crohn's disease and AIDS are known more often to cause severe skin conditions and systemic diseases. These severe skin conditions included *Malassezia* folliculitis, seborrheic dermatitis, catheter-related fungaemia and sepsis and a variety of deeply invasive infections.(42) PV was clearly not mentioned. This suggests the ability of these yeasts to invade the body to a deeper level when the individual is immunocompromised as compared to its very superficial state in PV.

Meanwhile, PV infection is not dependent on the cellular or humoral immune status of the affected individual. Data from Saadatzadeh et al.(43) which assessed the cell-mediated immune response to the active (mycelial) phase of *Malassezia* in PV patients as compared with age- and sex-matched controls revealed a similar immune response between the two study groups. There was no deficiency in cell-mediated immune response observed in the diseased patients. On the humoral immune response of PV patients compared with age- and sex-matched controls study conducted by Ashbee et al.(44) there was no statistically significant difference in measured total immunoglobulins produced in response to *Malassezia furfur* by both groups. The result of both studies supports the hypothesis that PV infection is not directly linked to the patient's immune status.(43, 44)

1.2.5. PV and genetic predisposition

PV is not a contagious disease and the lack of report of conjugal cases(23) among discordant couples who have been living together for a prolonged length of time combined with the tendency of PV to occur in at least one other member of the family unit lay credence to genetic predisposition being one of the important endogenous factors that contribute to the development of PV. Genetic models described by Hafez and later confirmed by He et al. revealed that PV patients fulfil the criteria for multifactorial (genetic-environmental) mode of inheritance.(21, 45) Meanwhile, inconsistencies with single-gene recessive or dominant mode of inheritance were shown as well as rejection of the environmental and non-transmitted genetic models of inheritance.(21)

Available literature from various studies have revealed very strong positive family history of PV among patients, ranging from 21.1% to 38.3%(21, 45-47) families. He et al calculated the heritability of PV in first-degree relatives to be 48.13%, second-degree to be 40.11% and third-degree to be 27.2%.(21) This shows a risk reduction with distance in family ties, thus justifying the multifactorial nature of the disease. Additionally, the role of environmental factor cannot be over-emphasized since the heritability of PV is by and large lower than 70%.(45) In general, these studies also agreed with the finding that patients with a positive family history of PV had an earlier onset and a longer duration of the disease. A chance of recurrence was also higher, compared with individuals without a family history.

1.2.6 PV and other predisposing factors

Other factors that may predispose individuals to PV include hyperhidrosis, use of oral contraceptives, malnutrition, pregnancy, skin occlusion and prolonged use of systemic corticosteroid. Evidence supporting the relationship between oral contraceptives use, malnutrition and PV is quite weak and not properly documented. Although hyperhidrosis is generally accepted as one of the predisposing factors to PV(23), studies have produced conflicting results. While profuse sweating was considered on the one hand, in the studies by Tarazooie and He et al. as one of the strong endogenous factors mediating the development of PV;(21, 48) on the other hand, this was not supported in the studies by Burke and Ingordo et al.(17, 49) were no significant association between PV and hyperhidrosis was observed.

Even though the skin colonization by *Malassezia* yeasts is increased in the third trimester and postpartum period, the frequency of PV in pregnancy has been shown not to differ significantly from that of the general population.(50)

PV studies in patients on prolonged steroid use are sparse. The incidence of PV in corticosteroid-treated patients as observed a long time ago by Boardman et al. was 16%.(51) Similarly a significant relationship was observed by Burke(49) between increased systemic cortisol levels that are found in Cushing's syndrome patients as well as those on systemic glucocorticoid therapy and PV development. The possible explanation for this interaction is not yet proven by research especially as this relationship with PV was not observed among patients applying topical glucocorticoids. An experimental work on steroids and *Malassezia* yeasts did not show any cause and effect relationship. Steroids did not encourage or increase the growth of the yeasts in culture. Moreover, topical glucocorticoids can induce a negative nitrogen balance and reduction in pH when applied on the skin.(49) This could in turn impede the growth of the fungi.

Some studies could not relate personal hygiene with the development of pityriasis versicolor(46, 47) although this is in contrast to a general perception by most people that PV results from an inadequate personal grooming of the patients.(52) This perception could increase stress and feeling of being stigmatized in these patients. A

recent work on skin infections and infestations among Nigerian prison inmates showed that PV formed 27% of all 178 infections, second only to infections by dermatophytes.(53) Skin infection was significantly associated with the frequency of bath, frequency of soap usage and frequency at which their clothing was changed. These confirm a possible relationship between personal hygiene and skin infections and infestations of which PV was included. They also observed a significant influence caused by accommodation arrangements namely overcrowding and poor ventilation on skin infections. It would have been interesting to know if the prison environment (hot and humid) was considered also as a possible predisposing factor. Meanwhile, this study differs with a similar study of PV among Indian prisoners in which no significant difference in PV prevalence and clinical features was observed when compared with the general population.(54)

1.2.7 Prevalence of pityriasis versicolor in Nigeria.

Studies on PV in Nigeria are quite limited but documented prevalence of PV range from 6.7% in Northwestern region,(55) a region closest to the Sahara desert and higher temperatures all year round to 3.7%(56) in Southern region of the country which has a cooler climate and closest to the ocean. However, both studies had limitations. The first study was hospital-based which does not usually give a true prevalence of skin diseases in the community and the second was conducted in a primary school where majority of the pupils were below the adolescent age, the peak age of PV development.

A prevalence of 4.6% was observed by Uneke et al. in an epidemiological survey of tinea capitis and PV infections among school children in South-east Nigeria.(57) While in the Southwestern region, a prevalence of 4.7% was observed, also among school children.(58) In both studies, an epidemiological comparison of PV was made with dermatophyte infections, in which the prevalence of dermatophytosis was significantly higher than PV (51.8% vs 4.6% and 15.2% vs 4.7%) while the children with PV were significantly older than those with tinea capitis. The peak age group for tinea was 3 - 7 years while for PV was 14 - 17 years.(57, 59)

In neighbouring countries of Nigeria, few prevalent studies of PV have been conducted. Examples include Mali which is located to the north of the country. There Faye et al.(60) observed a group of children aged 15 years and below in rural communities of Mali for non-leprous hypochromic patches. A prevalence of 4.1% was documented, of which PV had the highest frequency of 39.4%. Studies from other West African countries like Ghana, Togo, Benin and Liberia could not be found in the literature.

1.3 Aetiology of pityriasis versicolor

PV is a superficial cutaneous infection caused by the fungus of the genus *Malassezia*, one of the numerous dimorphic fungi that infect humans and animals. This microorganism form part of the normal skin flora when it exists in the

saprophytic or yeast state. It causes PV most times when it changes to the hyphal or mycelial state; the mechanism by which this change of state is conducted has remained unclear.

Meanwhile, epidemiological study of *Malassezia* yeast found on normal skin of individuals from tropical environment has shown increased chance of hyphal formation. This leads to the conclusion that the mycelial state of *Malassezia* per se may not always indicate the presence of a disease.(3) Another group argues the possibility of “overt” infection since these hyphae were isolated from non-lesional skin scales of same patients who had PV infection. Additionally, the percentage of hyphae isolated from skin of healthy individuals is negligible in comparison to samples obtained from PV lesions.(61) The reason why *Malassezia* yeasts have been implicated as aetiological agent of PV is the increased likelihood of obtaining a positive fungal culture from skin specimens taken from PV lesions than from clinically unaffected skin areas of either the same individual or matched healthy controls.(48, 61, 62)

1.3.1 History and taxonomy of *Malassezia*

After several decades of continuous reclassification, *Malassezia* yeasts are presently placed in the Phylum *Basidiomycota*, subphylum *Ustilaginomycotina*, class *Exobasidiomycetes*, order *Malasseziales* and family *Malasseziaceae.*(61) There were disagreements as to when the organism was first grown, the optimal culture medium, the relationship between the different morphological and colonial variants of the organism, the genus to which it should be assigned and with what name, and the role it plays in a variety of cutaneous diseases.

The pathological agent of PV was first described in 1846 by Eichstedt.(63) This fungus was later named *Microsporum furfur* by Robin Baillon in 1853, as he thought it was identical to the dermatophyte *Microsporum auduoinii*. In 1874, Malassez further described the shape (round, oval) and budding nature of this organism and differentiated it from dermatophytes. Robin Baillon in recognition of this work and the furfuraceous (consisting of or covered with flaky particles) nature of the PV lesions proposed the first nomenclature *Malassezia furfur* in 1889.(64)

However, 15 years later Sabouraud described a group of budding yeast cells without hyphal elements observed in and isolated from normal skin and scalp. He proposed the genus Pityrosporum.(65) Thus two genera were established: *Pityrosporum*, as the yeast form found on normal skin flora and *Malassezia* as the mycelia form found in disease state.

Panja in 1927 was able to successfully isolate the organism from PV lesions and he also suggested that both yeast and mycelia forms may be the different expression of one organism by proposing the merging of both genera into one.(64) This suggestion was not generally accepted as there were no scientific ways of proving that this relationship exists. Most important was the difficulty in culturing the organism in vitro.

The reason for the difficulty was later explained by Benham in 1939. She observed that the organism required a “fatty substance” to grow.(64) Once this lipid requirement was established it paved way for the formulation of various culture media that reliably recovered and maintained the organism thus enabling much work to be done on the taxonomy, physiology and biochemistry of these yeasts especially in the discovery of new species for each genus.

By 1984 mycologists restricted the genus to just 2 valid species: a human one, *M. furfur*, and an animal one, *M. pachydermatis*. The old name *Pityrosporum* was rejected (*nomen rejiciendum*) because it was considered a synonym of *Malassezia*, a term already in use and so given priority according to the rules of taxonomic nomenclature.(66)

Despite all these studies and more, the taxonomy and nomenclature of *Malassezia* species was marred with confusion and chaos until 1995 when the advancement in molecular techniques allowed physiological and ultrastructural studies describing the characteristics of each species. Using these techniques Guillot and Gueho analyzed 104 different isolates of *Malassezia*, and subsequently defined seven species of *Malassezia: M. furfur, M. sympodialis, M. obtusa, M. globosa, M. restricta, M. slooffiae, and M. pachydermatis.*(67) In recent years, using the combination of biochemical, physiological, morphological and molecular techniques 7 additional species have been identified*: M. dermatis, M. japonica* (isolated from humans with atopic dermatitis), *M. yamatoensis* (in seborrheic dermatitis), *M. equina, M. caprae, M. nana* and finally *M. cuniculi* (last 4 isolated from various animals).(68)

The current members of the genus Malassezia with their dates of identification as adapted from the review by Crespo-Erchiga et al.(66) are highlighted in Table 1.1 below.

Table 1.1 Members of the Genus *Malassezia* and dates of identification(66)

First Studies (3spp)	1996 Review (7spp)	The Genus in 2014 (14spp)
M. furfur 1889	*M. furfur* 1889	*M. furfur* 1889
P. ovale 1913	*M. pachydermatis* 1935	*M. pachydermatis* 1935
P. orbiculare 1951	*M. sympodialis* 1990	*M. sympodialis* 1990
		M. globosa 1996
	M. globosa 1996	*M. obtusa* 1996
*M. pachydermatis*1935	*M. obtusa* 1996	*M. restricta* 1996
*P. pachydermatis*1925	*M. restricta* 1996	*M. slooffiae* 1996
P. canis 1955	*M. slooffiae* 1996	
		M. dermatis 2002
		M. japonica 2003
M. sympodialis 1990		*M. nana* 2004
		M. yamatoensis 2004
		M. caprae 2007
		M. equine 2007
		M. cuniculi 2010

1.3.2 Other diseases associated with *Malassezia*

PV is not the only mycosis caused by *Malassezia* yeasts. There are other diseases, both cutaneous and systemic, in which they have been implicated and a discussion on *Malassezia* and PV will not be complete without a brief mention of the role played by these yeasts in these diseases. In some, the association remains controversial and the pathogenesis unclear.

The systemic infections caused by *Malassezia* include intravenous catheter induced fungemia, endocarditis, interstitial pneumonia and peritonitis in patients undergoing continuous ambulatory peritoneal dialysis.(61) Most of these systemic infections were said to be as a result of the fungus being introduced into the blood stream or

the peritoneum via the skin during these procedures. The rate of infection correlates with the length of time the catheters were kept in place. *Malassezia* can cause systemic infection in people of all ages particularly those with immunosuppression and venous catheter either central or peritoneal.(42) Affected individuals present with persistent fever, no response to antibiotics and a high degree of clinical suspicion is required for timely management.(61)

For cutaneous diseases such as seborrheic dermatitis, pityriasis capitis and psoriasis, the role played by *Malassezia* is not completely clear. Controversial roles of *Malassezia* has been associated with also the following: neonatal cephalic pustulosis, atopic dermatitis, onychomycosis, sinusitis, confluent and reticulated papillomatosis of Gougerot-Carteaud and otitis externa.(61)

Seborrheic dermatitis is a recurring disease commonly seen in sebum-rich areas of the skin, such as scalp, eyebrows, paranasal folds, upper chest and back. It is characterised by erythema and scaling.(61) It commonly affects neonates and infants, Parkinson's disease patients and also immunocompromised individuals.(42) Current data available do not sufficiently define the pathological features of *Malassezia* that leads to the development and exacerbation of seborrheic dermatitis, but it has been considered as a deeper level of infection in comparison with PV(61). The yeasts were isolated from the dermatitis lesions by various studies (69, 70) and anti-fungal medications form part of the treatment plan for seborrheic dermatitis.

Malassezia (Pityrosporum) folliculitis is a benign cutaneous disorder, characterised by follicular papules and subcorneal pustules most commonly located on the face, chest, back and upper arms. It is often pruritic and could be misdiagnosed as acne.(3) Direct microscopy of pustules usually reveals an abundance of *Malassezia* yeasts and the absence of other microorganisms. Histology is also helpful in diagnosis where a periodic acid-schiff (PAS) or methenamine silver staining technique will show the budding yeasts within the keratinous material of dilated hair follicles. A study in Korea showed a predominance of *M. restricta* in patients with *Malassezia* folliculitis.(71)

Confluent and reticulate papillomatosis of Gougerot-Carteaud is a rare cutaneous disorder characterised by confluent, grayish-brown, hyperkeratotic papules often symmetrically located on the trunk.(3) It responds to topical and systemic anti-fungals, although in combination with other modes of treatment. Studies on this skin disorder are few. However, *M. furfur* and *M. sympodialis* have been isolated from the lesions.(3)

Neonatal cephalic pustulosis is a benign disorder commonly seen in neonates. It is characterised by non-follicular papulopustules found on the face and neck of newborns. Direct microscopy of the pustules have shown *Malassezia* yeasts, although the exact pathogenesis is not well known, this disease responds well to topical ketoconazole therapy.(61)

Pityriasis capitis (dandruff) also known as mild seborrheic dermatitis is a subclinical inflammatory condition of the scalp associated with episodic, recurrent or constant scaling of the scalp. It is more prevalent in young adults and may be aggravated by environmental factors such as dust, hair cosmetics, UV irradiation, and airborne irritants. *Malassezia* yeasts have been isolated from dandruff scalp(69) and the condition is known to improve on reducing the population of *Malassezia* on the scalp with proper antifungal treatment(72). Meanwhile, a study has demonstrated the contribution of other fungi apart from *Malassezia* such as *Filobasidium* and *Acremonium* species in the aetiopathogenesis of dandruff.(73)

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterised by severely itchy, red, dry, and crusted skin affecting all age groups but most commonly the paediatric age group. Pathogenetically, it results from a combination of defective skin barrier and inappropriate immune responses against both genetic and environmental factors. *Malassezia* yeasts exacerbate AD especially in those with dermatitis lesions involving mainly the head and neck regions.(61, 64, 74) These patients have been shown to have a higher IgE titre to *Malassezia* and also a more positive skin prick test to *Malassezia* allergen than healthy subjects.(64, 74, 75) *M. furfur, M. globosa, M. restricta* and *M. sympodialis* are the most common species isolated from atopic dermatitis skin lesions.(75, 76) Likewise, the addition of antifungals to patients' management has resulted in rapid treatment response. In the interim, exact roles played by the fungi in atopic dermatitis remains under intense investigation.

Psoriasis is a chronic inflammatory immune-mediated disorder that affects the skin and joints. The skin lesions are characterised by erythematous scaly papules and plaques. Although the aetiology of psoriasis remains elusive, *Malassezia* yeasts have been reported as one of the microorganisms that can exacerbate this disease condition. Some studies have isolated *M. furfur*, *M. globosa*, *M. restricta* and *M. japonica* from surfaces of psoriatic lesions.(77, 78) One of these studies was able to demonstrate pseudohyphae in active psoriatic lesions and none was observed in stable lesions.(77) The mechanism is still not clear since it is difficult to understand if the pseudohyphae developed as a result of the reduced barrier function and low immune defence observed in exacerbated lesions or if the presence of the yeasts contributed directly to the exacerbation. Nevertheless, these patients are known to respond more quickly when topical antifungals are included in their management.(78)

Malassezia yeasts have been demonstrated to induce onychomycosis in normal and immunocompromised patients.(79, 80) This association remains contentious especially as these lipophilic yeasts are known not to normally colonize nails and besides nails are poor sources of lipids. Also these yeasts do not have keratolytic properties as dermatophytes. Zhao et al. attributed the process whereby *Malassezia* can cause onychomycosis to a change in the microenvironment induced by the local application of steroids.(79)

1.4 Pathogenesis of pityriasis versicolor

The pathogenesis of PV is yet to be completely explained. However, various studies on PV development concur on the interdependence of the following factors: *Malassezia* skin colonization, sebum production and individual predisposition. PV infection is caused by the lipophilic dimorphic yeasts named *Malassezia.* These yeasts colonize the stratum corneum (SC) and are among the organisms that make up the skin microbiota.(1, 72) The microorganisms of the skin flora are quite diverse and are made up of bacteria, viruses, protozoa and fungi. Some are transient that is they are detected as a result of transient hand/body carriage from contamination or transmission events (examples include adenovirus and rotavirus) while others are resident. The resident flora are persistent and cannot be completely washed away; examples of these group of organisms include *Malassezia, Staphylococcus epidermidis, Corynebacterium, Demodex folliculorum,* just to mention a few.(81)

The skin becomes colonized by microbes soon after birth. These microbes are usually from the environment and they subsequently interact with the epithelial cells of the newborn leading to microbial colonization and co-existence.(82) Most of these microbes are beneficial, commensals or neutral, however, a few do become pathogenic and harmful to the skin or body in general.(82) The commensal or pathogenic nature of an organism is argued to depend on the immune system of the individual rather than the inherent properties of the microbe.(82) Of equal importance is the interaction among species. In order to survive, a microbe has to compete with other microbes of the normal flora for available nutritional elements and space. By so doing, they try to keep the skin flora in a stable state and resist abrupt changes in the community structures. This process in the long run also protect against the growth of pathogenic microbes that may cause disease.(81, 82)

Various skin structures may harbor their own type of microbes. Such that the stratum corneum, keratinocytes, hair shaft and follicle, sweat glands, apocrine glands and sebaceous glands may each have their own type of unique microorganisms which colonize there. *Malassezia* yeasts reside mainly in the stratum corneum, though some authors suspect their presence in the hair follicles which could act as a reservoir and thus accounts for the recurrence of PV after treatment.(47, 73) Meanwhile, the stratum corneum provides nutrients in form of lipids secreted by the sebaceous glands. Sebum production is increased in humans at puberty and so is PV. This also explains the reason for the predilection of the yeasts for sebum-rich regions of the body. Sebum lipids are postulated as essential ingredients that support the growth of these yeasts while the sebum triglycerides are degraded by *Malassezia*-derived lipases to produce proinflammatory unsaturated fatty acids, an antimicrobial property that limits the types of microorganisms that can co-exist on the skin.(83)

Since these *Malassezia* yeasts exist as skin flora on healthy skin, the development of PV in certain individuals indicates an interactive role played by other factors which

could have altered the ecosystem of the skin, and thus a resultant change in the yeasts' microbial state. This interactive process was well described in the conceptual framework developed by Rosenthal et al.(82) Most important of these factors that could predispose individuals to develop PV is the presence of high temperature and high relative humidity. There are also behavioral characteristics of the individual such as cosmetic use and host demographic characteristics such as seborrhea and hyperhidrosis. Of equal importance is the genetic susceptibility of the individual.(21) All of these driving factors interact to some degree to influence the pathogenesis of PV.

Although PV is a cutaneous infection, it is associated with little or no inflammation unlike other *Malassezia*-associated diseases such as *Malassezia* folliculitis and seborrheic dermatitis. *Malassezia* are dimorphic yeasts, that is, they are able to exist in both yeast and mycelial (hyphal) forms. The mechanism by which individual's endogenous as well as environmental factors influence the yeasts to convert to the hyphal state, thus causing PV is also not well established. Moreover, studies investigating the absence of an active immune response by the host to the presence of *Malassezia* and possible factors that mediate the immune balance in the host between the commensal and pathogenic state of the yeasts are lacking in the literature. A more likely hypothesis has been linked to the lipophilic nature of the yeasts cell wall. Lipids reduce phagocytosis of *Malassezia* by neutrophils. Phagocytosis is an important nonspecific immune mechanism for removal of microorganisms from the body. The uptake of the yeasts by these inflammatory cells is efficiently higher when they are killed than when they are alive.(64)

Presently there is little information on the relative pathogenicity or virulence factors of *Malassezia* species. The limitation by the body's neutrophils in killing the organism may account for its persistence in predisposed individuals. A study described by Ashbee and Evans(64) specified that only 5% of the *Malassezia* cells taken up by neutrophils were actually killed in an *in vitro* complement-dependent process, and this killing ability by the same neutrophils was increased to 23% when the yeasts were pretreated with ketoconazole. This was in contrast with the killing of 30% - 50% *Candida albicans* yeast cells and 80% of the cells of other fungal genera. Another hypothesis proposed for this limitation was attributed to the production of azelaic acid by *Malassezia* when it is grown in the presence of oleic acid or olive oil. Azelaic acid scavenges oxygen radicals, thus preventing oxidative killing of the organism by the phagocytes. Likewise is the protective function of lipids richly found within the cell wall of the organism; this reduces their uptake by and subsequent activation of neutrophils and so protecting them from being phagocytosed.(64) *M. furfur* produces pityriarubins which is protective against neutrophilic activity. The lack of inflammation seen in PV has been attributed to pityriarubin through its role in down regulation of the immune response.(84)

Immunomodulatory properties of *Malassezia* have been demonstrated in mice and in vitro. In mice, *Malassezia* through the up-regulation of macrophages protect against

infection and malignancy(85) while *in vitro* it reduces the production of cytokines such as interleukin 1, interleukin 6 and tumour necrosis factor-alpha.(86) Cellular and humoral immune reactions to *Malassezia* have been measured in healthy individuals despite their having no evidence of *Malassezia*-associated diseases; these activities were age-related with higher levels observed in young adults and lower levels in children and the elderly.(43, 44, 87) This poses some difficulties in studies on the immune pathogenesis of *Malassezia*-associated disorders and it will be necessary to conduct these studies as age- and sex-matched case-control studies in order to appropriately interpret the results. Meanwhile studies have not shown any deficiency in cell-mediated immunity to *Malassezia* in patients with PV.(43, 64)

1.4.1 Stratum corneum (SC) and PV

The human skin is made of the epidermal, dermal and subcutaneous layers. The stratum corneum (SC) is the final differentiated product of the epidermis.(88) In its healthy state, the SC forms a protective barrier against various environmental insults including pathogenic microbes, damaging irradiation, and potentially toxic xenobiotics and it also maintains skin/body hydration by preventing water loss.(83, 88) A disruption of the skin barrier structure and function has been observed in PV.(27) These disruptions include an increase in transepidermal water loss (TEWL) and a significant reduction in skin hydration. The resultant effect of these disruption processes leads probably to the pathologically increased fragility of PV lesional skin surface.(61)This can be demonstrated in the “evoked-scale” sign(61) also called “Zeliri’s sign”(89). This sign is elicited when on stretching or scraping the surface of a PV lesion, scales become more visible. The precise pathogenetic mechanism by which *Malassezia* contributes to this characteristic feature of PV has several explanations. Some hypotheses are discussed below.

The SC is the most superficial layer of the skin that is solely responsible for the characteristic features of PV which is scaling and dyspigmentation. To fully understand the pathogenesis of PV, it is important to understand the structure and organization of the SC and how these properties are altered during PV. The SC is a multilayered tissue composed of anucleated, flattened cells which are surrounded by multiple lamellar sheets of lipids.(83) It has also been described as a “unique sophisticated biosensor” that signals the underlying epidermis to respond to external stresses.(90) SC has been likened, in the simplest terms, to a brick wall (these are the terminally differentiated keratinocytes also called corneocytes) linked by intercellular cement (this is composed of a continuous matrix of specialized lipids).(90) The corneocytes provide protection against injuries while the intercorneocyte lipids which are mainly broad sheets of ceramides produced from lamellar bodies in the granular layer, serve as water barrier.(88) SC is formed during the process of keratinization of which the final act is desquamation. This is the orderly release of single corneocytes at the skin surface. Desquamation is facilitated by several proteolytic enzymes that degrade corneodesmosomes which are the

protein structures that fasten neighboring corneocytes together both in the plane of the SC layer and adjacent layers.(90) These proteolytic enzymes are controlled in the end by water activity and the pH of the SC.(83) Another possible facilitator of the desquamation process is sebum. The presence of triglycerides and short-chain fatty acids, as structural barrier lipids of the SC may disrupt the organization in the superficial layer of the intercellular lipid (intercellular cement).(83) Triglycerides and short-chain fatty acids are produced and released primarily by the sebaceous gland.

Desquamation process can be facilitated by the presence of yeasts in the SC. Three distinct forms of *Malassezia* yeasts were recognized in the SC by Piérard et al.(91) These include round spores or conidia, budding spores and mycelium. Meanwhile, the mycelial elements are regularly observed to be located within the corneocytes. They are usually the invasive form of most yeast. Additionally, the keratinous content (mainly the tonofilaments) of these yeasts invaded cells are replaced by “amorphous, moderately electron-dense material” which were observed by Borgers et al.(92) as lipid in nature. Similar amorphous material was observed in between keratinocytes (extracellular compartment); most often in form of several sized spheres. Its keratinocyte origin is supported by the certainty of frequent membrane ruptures of swollen invaded keratinocytes. The degradation of the tonofilaments in the keratinocytes is speculated to be via the activity of *Malassezia* yeast enzymes. The replacement by lipids serve as nutritive material, which were demonstrated to be essential for growth initiation and maintenance of this lipophilic yeast in the SC.(92) Meanwhile this work done by Borgers et al. is yet to be confirmed as a similar study by del Palacio-Hernanz et al.(93) who studied the ultrastructure of PV lesions before and after treatment with cyclopiroxolamine could not observe these intracellular amorphous materials but rather severe necrosis of the cytoplasm. The reason was attributed possibly to the short fixation time by the later authors (2 weeks vs 2-3 days).

In conclusion, although the exact structural alterations of the SC are still unknown, the possible combination of frequent membrane ruptures of invaded keratinocytes and the activities of the *Malassezia* yeasts enzymes may contribute to the increased fragility of the lesional SC which is evident in the “Zeliri’s sign” or “evoked-scale” sign of PV.

1.4.2 Mechanism for hypo- and hyperpigmentation of PV lesion

Some studies have been done to explain the reason why PV lesions are either hypopigmented or hyperpigmented. Some have tried to answer this question by observing the relationship between *Malassezia* and melanocytes. Study by Karaoui et al.(94) confirmed earlier research in which no difference in number of melanocytes between PV skin whether hypopigmented or hyperpigmented and normal skin was found; but rather observed a difference in the dispersion and arrangement of melanosomes by the melanocytes.

In hypopigmented skin, the melanosomes were individually dispersed and fewer in number while in hyperpigmented skin, they were sequestered in most cells.(94) This was later collaborated by Galadari et al.(95) but refuted by Dotz et al.(96) who examined biopsy specimens from lesions of hyperpigmented PV involving a vitiliginous skin and found absence of both melanosomes and melanocytes. This study increased the doubt of a possible role play by melanocytes in the dyspigmentation of PV lesions.

The explanation behind the interaction between *Malassezia* causing PV and melanocytes was further attempted by Krämer et al.(97) who suggests an indole alkaloid produced by *M. furfur* from tryptophan called melassezin as the likely cause of the hypopigmented lesions. Melassezin induces melanocytes to undergo apoptosis and thus reduces melanin production but this may not explain the hyperpigmentation of PV lesions. Other reasons for the hypopigmentation of some PV lesions have been connected to the production of lipoperoxides from oxidation of skin lipid by the yeast. Since a higher lipoxygenase and lipoperoxidase activity was measured in hypopigmented lesions positive for fungal hyphae and spores than controls.(29, 30)

Another speculation in view of the hypopigmentation of PV lesion worthy of note to mention is the role of the lipid-like material produced by the Malassezia yeast in the stratum corneum. The hypochromia is said to be as a result of filtering of the ultraviolent light provided by the “lipid” screen.(92) This “lipidification” of the SC is clearly documented only with *Malassezia* yeast infection and thus differentiates it from other fungal infections. Also this lipid process is not influenced by antifungal medications such as itraconazole. For this reason the hypopigmentation of PV lesions persists for 3 – 4 weeks after disappearance of viable fungi from the skin since normal epidermal regeneration takes about 4 weeks.(92)

Meanwhile, another school of thought has attributed the color change of PV to the thickness of the stratum corneum. In both pigmentary states, the thickness of the stratum corneum differs with hyperpigmented skin being thicker than hypopigmented skin and both being thicker than that of uninvolved skin.(94)

1.4.3 Histology of PV lesions

Electron microscopy of skin scales from PV lesions has revealed the clustering of yeast cells and short hyphae in nest-like cavities.(98) The hyphae may be seen perforating the outer layer of the stratum corneum perpendicularly.(93) *Malassezia* species reproduce asexually by monopolar, enteroblastic budding from a characteristic broad or narrow base depending on the species. Separation leaves a prominent bud scar through which successive daughter cells emerge.(64) In lesional skin, particularly around hair follicles and acrosyringium, the clustering of yeast cells and mycelial hyphae is larger in the superficial part of the stratum corneum than

deeper layers. Furthermore in healthy skin, the clusters are mainly made up of yeast cells and are fewer in number (98, 99).

Histology of skin biopsy from PV patients can be accessed via the Hematoxylin-eosin stain or the PAS stain. Basically, the histology has shown a slight to moderate hyperkeratosis with “basket weave” stratum corneum, acanthosis, and vacuolization of epidermal cells.(99) There is also increased or normal basal layer pigmentation. Occasionally, melanin incontinence may be seen.(61) Gaitanis et al.(61) describe two types of PV that may be observed histopathologically from lesional skin biopsy; these are the inflammatory and non-inflammatory PV. The non-inflammatory PV is characterized by mild or almost absent infiltration of the dermis by superficial perivascular inflammatory cells while in the inflammatory PV, there is a moderately dense infiltration in the upper dermis of perivascular inflammatory cells. These mononuclear infiltrates are characteristically Langerhans cells, T lymphocytes and occasionally plasma cells.(95) Meanwhile, numerous budding yeast cells and short hyphae can be viewed in the stratum corneum and only the hyphal and not the spore form of the yeast are observed within the cells.(1) Unusual histological features include the presence of dilated blood vessels in relation to the extremely rare erythematous PV and papillomatosis like acanthosis nigricans.(99)

1.4.4 *Malassezia* and healthy skin

The presence of *Malassezia* yeasts on healthy skin of humans was described since the second half of the 19th century, and it soon became evident that colonization density was associated with age and differences in the activity of sebaceous glands in different areas of the body. However, there are no differences observed between the *Malassezia* species identified from healthy and diseased skin.(74)

Malassezia, as part of the body flora can be isolated from the sebum-rich areas of the skin, particularly the chest, back, and head regions. A study done by Leeming(6) examined clinically normal skin at 20 different sites over the entire body surface. *Malassezia* species were recovered from every subject from the chest, midline back, scalp, ear, and upper inner thigh. The highest mean population densities occurred on the chest, ear, upper back, forehead, and cheeks. Some differences in carriage rates were noted between females and males, with higher population densities from the lower trunk and upper thigh of males.

In a study by Lim et al. on the distribution of the different *Malassezia* species on normal human skin,(100) *M. restricta* was seen to predominate on the scalp, *M. sympodialis* on the trunk and *M. globosa* was widely and equally distributed in all seborrheic areas. When the number of colonies isolated from healthy individuals was compared with those from PV, seborrheic dermatitis, AD and psoriasis patients; the number was comparable with PV patients but significantly smaller in seborrheic dermatitis, AD and psoriasis.(72, 74)

1.4.5 *Malassezia* in animals

Malassezia is rarely isolated from the environment because it is mostly confined to the skin of warm blooded animals. This is probably because its distribution in nature has not been well researched.

Veterinarians' investigation of these yeasts on the skin of several animals demonstrates the role of *M. pachydermatis* (and to a lesser extent, *M. furfur, M. obtusa*, and *M. sympodialis*) as the causative agents of otitis externa in cats and dogs. *M. pachydermatis* is also known to cause *Malassezia* dermatitis in dogs. This chronic skin disorder with similar clinical features to seborrheic dermatitis in humans is characterized by relatively demarcated scaly erythematous patches located at the sebum-rich regions of the animal.(101) Other clinical features that could be associated with *Malassezia* dermatitis in animals include pruritus, lichenification, excoriations and alopecia.

Meanwhile, studies in many other animals have shown that specific species of *Malassezia*, just as in humans, also form parts of their skin microbiota. Such that *M. equine* is commonly isolated from the skin of monkeys, pigs, bears, birds, and horses. *M. caprae* specifically colonises goats while *M. nana* colonises cattle and cats.(66) The most recent addition to Malassezia species, *M. cuniculi* was isolated from rabbits.(68)

1.4.6 Predominant *Malassezia* species in PV

The predominant causative *Malassezia* species of PV have been demonstrated from different countries. Studies in Spain by Crespo Erchiga et al.(102) were the first to show *M. globosa* as the most prevalent species in PV lesions. *M. globosa* was also the most prevalent species observed in Japan by Nakabayashi et al.(70) in Spain by Aspiroz et al.(103) and in India by Dutta et al.(104) Also in Iran by Tarazooie et al.(48) and Bosnia-Herzegovina by Prohic et al.(105) In particular, Aspiroz et al.(103) demonstrated greater lipase and esterase production in *M. globosa* strains than in other species of the genus, a fact that may explain the greater level of pathogenicity associated with this species on human skin, and may also support its hypothetical role in the etiology of PV. However, contrasting results were obtained in Canada by Gupta et al. where a predominance of *M. sympodalis* was observed although noted is the important difference in culture medium and methodology used when compared with aforementioned authors.(3) Other countries where *M. sympodialis* was observed to predominate in PV lesions include Brazil(106, 107) and Argentina.(20)

It is also noteworthy to state that some PV studies conducted in tropical or subtropical climates regions have shown a clear predominance of *M. furfur*. Examples are studies in Madagascar, Panama, Brazil and later Indonesia by Kristanty et al.(108) These results may confirm the over eighty-year-old hypothesis which postulates that differences in species of *Malassezia* which infect different individuals are based on distinct geographic distribution.

1.5 Clinical features of pityriasis versicolor

PV lesions are characterised by discrete confluent, scaly, dyspigmented, irregular macules.(3) These lesions may be hypopigmented or hyperpigmented and an individual may have both types of lesions. The hyperpigmented lesions vary in colour from pink or tan to dark brown or black.(4)

The distribution of the lesions generally parallels the density of sebaceous glands and common sites of affectation include the chest, face and back; flexural involvement of PV is rare.(7, 46) Morphologically the common patterns of PV lesions could be macular with dyspigmented areas, follicular which is usually multiple hypopigmented macules surrounding hair follicles, confluent which are composed of multiple macules arranged very close together and in an irregular pattern and finally the macules could be tear-drop like which is known as the guttate pattern.(47)

In literature, unusual and rare forms PV lesions have also been described. These include:

a. Inverse tinea versicolor(5) in which the characteristic PV lesions are mainly located in the axillae, groin or perineum. Differential diagnosis of this form of PV will include dermatophyte infections, erythrasma and seborrheic dermatitis.

b. Pityriasis versicolor atrophicans in which the lesions are atrophic, erythematous and asymptomatic. Some lesions may have minute surface teleangiectasia. The topography of PV atrophicans generally follows that of common PV and it partially or completely resolves with appropriate antifungal therapy. The possible differential diagnosis of this form of PV includes anetoderma, acne scars, and macular atrophy. Histology is required to make diagnosis.(109)

c. Pityriasis versicolor pseudoatrophicans in which the typical hypo- and hyperpigmented PV lesions coexist with atrophic patches. These atrophic patches are iatrogenic and secondary to prolong topical corticosteroid therapy. The lesions resolve with the suspension of the steroid use.(109)

d. Blaschkoid pityriasis versicolor(110) was described as a rare variant in which the PV lesions are distributed along Blaschko's lines.

e. Pityriasis versicolor rubra is a red variant of PV in which the lesions are erythematous and has overlying teleangiectasia which can be seen through a capillaroscopy. Lesions are distributed in sebum-rich body areas and improve with antifungal treatment.(111)

1.6 Differential diagnosis of pityriasis versicolor

Pityriasis versicolor is relatively easy to diagnose especially in dark skinned individuals although the varying clinical presentation of the lesions may be confusing

to an inexperienced physician.(4) Possible differential diagnosis of PV will include pigmentary disorders such as vitiligo, idiopathic guttate hypomelanosis and melasma; scaling is usually absent in these disorders. Other diseases to exclude are pityriasis alba, Hansen's disease, pityriasis rosea, pityriasis rotunda, hypo- or hyperpigmented mycosis fungoides, secondary syphilis, lentigo solaris, piebaldism and post-inflammatory hyperpigmentation.(23) Pityriasis rotunda is a rare disorder of keratinization characterized by a persistent, hyperpigmented or hypopigmented, geometrically perfect circular patches of dry ichthyosiform scaling with no inflammatory changes.(112) Pityriasis rotunda lesions may be associated with malignancies and liver diseases and it can be misdiagnosed as PV. In addition, some of the diseases also caused by *Malassezia* such as seborrheic dermatitis and confluent and reticulated papillomatosis of Gourgerot and Carteaud may co-exist and or resemble PV; thus making diagnosis more difficult.(70)

1.7 Diagnostic investigations in PV management

1.7.1 Direct examination

On direct examination of the lesions, Zeliri's sign may be elicited. The sign is positive if stretching of the skin reveals the fine desquamation of the lesions. This is due to the slight collapse of the keratin.(89) However, this sign has a low efficacy in clinical diagnosis with high false negative results.(89)

Wood's light examination may also help in the diagnosis of PV. Wood's light is produced with a high pressure mercury lamp emitting radiation between 320nm and 400nm (ultraviolet A). It is absorbed by melanin and it produces characteristic fluorescence in pathologic conditions, thus making it a useful diagnostic tool in pigmentary and some cutaneous disorders. The examination should be conducted in a dark environment so the fluorescence can be well appreciated. The fluorescence of PV lesions tends to be bright yellow or golden yellow.(23) A positive response has been observed in only about a third of cases.(113) This examination does not confirm PV diagnosis.(26)

Most recently added is a possible bedside test in the PV diagnosis called "in vivo gram staining of tinea versicolor".(114) The technique involves the use of gentian violet to stain PV lesions. PV is present when the infected area becomes more prominent on cleaning off the stain with alcohol. This effect has not been observed in other pigmenting disorders. It was noticed that gentian violet forms a covalent bond with thioredoxin reductase 2 which is produced by *Malassezia*. The accentuation could also be due to alteration in either the host or fungal lipids by the stain.(114) A validation study is needed to assess the level of efficacy of this method.

1.7.2 Microscopic evaluation

Microscopy of the PV scales adds a distinguishing feature to its diagnosis and helps differentiate it from the various skin disorders that may have similar clinical features

to PV. The scales for light microscopy are usually taken from the centre of lesions, where a large number of the yeasts are expected to be obtained.(113) Skin scraping may be collected directly onto a glass slide with a blade or the edge of a second glass slide.

Alternatively, a scotch tape stripping technique can be used.(76) The tape is placed on a glass slide and examined under the microscope. The keratin and debris of the skin scales are first dissolved with the use of 10-20% potassium hydroxide and then stained with methylene blue, parker ink or lacto-phenol blue to encourage clear viewing of the fungal element.(102) The characteristic “spaghetti and meat balls” appearance of the fungus is usually observed.

1.7.3 Culture characteristics

Malassezia is lipophilic and requires lipid medium to grow *in vitro*; cultures have to be grown on selective media, except for *M pachydermatis* which can also grow on standard media (Sabouraud dextrose).(102, 115) The 2 media commonly used to isolate *Malassezia* are modified Dixon agar (mDixon agar), first described by Van Abbe in 1964 and LN medium described by Leeming and Notman in 1987.(68)

Modified Dixon Agar medium comprises 36 g malt extract, 10 g bacteriological peptone, 20 g desiccated ox bile, 10 ml Tween 40, 2 ml glycerol, 2 g oleic acid, 0.5 g chloramphenicol and 0.5 g cycloheximide(116) while the LN medium comprises 10 g bacteriological peptone, 0.1 g yeast extract, 5 g glucose, 8 g desiccated ox bile, 1 ml glycerol, 0.5 g glycerol monostearate, 0.5 g Tween 60, 10 ml whole fat cow milk, 0.5 g chloramphenicol and 0.5 g cycloheximide.(68) These are added in 1 L of demineralized water. The pH is adjusted to 6.0 and 12 - 15 g of agar is added before the mixture is sterilized by autoclaving at 115^0 C for 15 min, and aliquot as required.

Other media used in the culture of *Malassezia* include Sabouraud agar plus olive oil, CHROM agar(117). Growth of the yeast on these agars is observed on an average of 5-10 days. The mDixon medium permits better visualization and isolation of the colonies, which is very important when, as is common, 2 or more species are found in the same lesion(118). Incubation temperature is also an important factor, since several species (among them *M. globosa* and *M. restricta*) do not grow above 36^o C. Thus, cultures should be incubated in ovens between 30^o C and 35^o C, and the dishes wrapped in plastic bags to ensure suitable humidity and prevent the medium from drying out.(63) The yeast form of the fungus predominates in culture though hyphae may also be seen.

The morphology of the grown colony varies according to the species of *Malassezia*. Its surface could be dull or glistening, smooth or rough, convex or flat with slightly folded or grooved edges. Texture could be friable, coarse or hard. The colour varies from cream to white. Sometimes a slight central elevation could be visualized.

Microscopy of the cultured yeasts that is a colony also varies with species. The cells are usually unipolar in appearance with a broad or narrow base bud. The cells could be large or small, spherical or cylindrical, ovoid or globose. These variable macroscopic and microscopic features have been implored in the identification of the different species of the organism.

1.7.4 Biochemical tests

These are usually carried out on the cultured specimen and not directly on the scales.

1. Catalase reaction: A positive reaction is seen when there is production of gas bubbles on adding a drop of hydrogen peroxide on a tiny part of the cultured specimen. Only *M. restricta* gives a negative reaction.

2. Tween assimilation test: *Malassezia* species grow differently around Tween compounds, basically Tween 20, 40, 60 and 80.

3. Esculin Test: The splitting of Esculin reveals the presence of β-glucosidase activity of *Malassezia* species. This is positive with *M. furfur*, *M. sloofiae* and *M. sympodialis*.

4. Assimilation of glycine: This is positive in *M. furfur* only.

1.8 Identification of *Malassezia* species

The *Malassezia* species can be identified based on their macroscopic/microscopic features, and physiological characteristics and also the use of molecular techniques. The macroscopic features of the predominant colonies include the shape, size, colour, texture, and the characteristics of the medium around them. While the microscopic features of the cultured yeast after staining include the morphology, size and nature of the budding base. To assess physiological properties of *Malassezia* species; catalase and β-glucosidase reactions (esculin test), and Tween test using 10%, 0.5%, 0.5% and 0.1% of Tween 20, 40, 60, and 80, respectively, are done. This method is usually time-consuming, though it is cheap and does not require sophisticated equipment.

1.8.1 Morphological and physiological identification methods

The macro-/microscopic characteristics as well as the physiological features of some *Malassezia* species as described by Gueho et al(63) are summarized below:

Table 1.2. Morphological characteristics of *Malassezia* species

Species	Nature of colony	Microscopy
M. furfur	Dull, smooth or slightly folded with convex elevations (average	Large, oval, cylindrical or spherical cells, broad base

	diameter 5mm); soft/friable texture	bud
M. pachydermatis	Dull, convex sometimes umbonate, cream in colour (average diameter 5mm); soft/friable texture	Small ovoid cells, broad base bud leaving a prominent budding scar
M. sympodialis	Glistening smooth, flat or with slight central elevation (average diameter 5mm); soft texture	Ovoid to globose cells, narrow base bud. Repetitive or sympodial budding occur.
M. globosa	Raised, folded and rough (average diameter 4mm); coarse and brittle texture	Spherical cells, narrow base bud
M obtuse	Smooth and flat (average diameter 4mm); sticky texture	Large cylindrical cells
M restricta	Dull surface; smooth to rough edges (average diameter 3mm); hard and brittle texture	Spherical or oval cells, narrow base bud
M slooffiae	Rough surface with fine grooves (average diameter 3mm); coarse texture	Short cylindrical cells with buds formed on a broad base.

Table 1.3. Physiological features of *Malassezia* species

Species	on SDA	Growth on mDixon at			10 % Tw 20	0.5 % Tw 40	0.5 % Tw 60	0.1 % Tw 80	Esculin	Catalase reaction
		32 °C	37 °C	40 °C						
M. pachydermatis	+	+	+	+	+	+	+	+	±	+
M. sympodialis	_	+	+		+	_	+	+	+	+
M. globosa	_	+	±		_	_	_	_	_	+
M. dermatis	_	+	+		+	+	+	+	_	+

M. furfur	_	+	+		+	+	+	+	_	+
M. slooffiae	_	+	+		+	±	+	_	_	+
M. obtusa	_	+	±		_	_	_	_	+	+
M. restricta	_	+	+		_	_	_	_	_	_
M. japonica	_	+	+		_	_	+	_	+	+

mDixon (modified Dixon agar); Tw (Tween); +, positive; _, negative; ±, weakly positive; SDA (Sabouraud dextrose agar).

1.8.2 Molecular identification methods

Molecular techniques in *Malassezia* species identification was developed in the mid-1990s particularly the rRNA sequencing analysis. This has accelerated investigations into *Malassezia* epidemiology and pathobiology. It also encouraged research on the association of *Malassezia* species with regard to specific geographic locations(4). The probe used in these techniques to identify *Malassezia* could be culture and non-culture based that is, samples can be collected from skin and cultured before performing the molecular analysis or the analysis can be done directly from patient skin scales.(119) The difference is that the yeast yield when done directly may be insufficient for the molecular analysis while the drawback for the culture based analysis is the possibility of missing out some species especially slow growing species or those that are outgrown in culture by contaminating fungi.

The four molecular techniques already in use to detect and identify *Malassezia* species include:

1. DNA sequence analysis. This is first method implored in *Malassezia* species identification. It involves the nucleotide sequence analysis of the obtained ribosomal DNA gene of the yeast and the result is phylogenetically compared with distant or closely related species.

2. Biotyping using Api 20 NE and ApiZym enzymes. This has received very little attention(120) and has so far being used to identify six of the 14 species of *Malassezia*. It includes the use of enzyme activity profiles of the different species. Common enzymes studied include catalase, esculin, lipase, phospholipase, proteinase, hyaluronidase and chondroitin-sulfatase.(120)

3. Chromosomal analysis using pulsed field gel electrophoresis (PFGE). This is the use of the heterogeneity in chromosomal number and patterns to identify *Malassezia* species. The method is time consuming,

methodologically demanding and the recently described species karyotypes are yet to be analysed.(120)

4. Polymerase chain reaction (PCR) – based methods. These methods require the development and use of species-specific probes and are very common methods implored in *Malassezia* species identification. They consist of the Random Amplification of Polymorphic DNA (RAPD) and DNA fingerprinting; Restriction Fragment Length Polymorphism (RFLP) analysis and the Amplified Fragment Length Polymorphism (AFLP) analysis. In these methods a band pattern is generated per strain which is compared with available band patterns. Other PCR – based methods are the Terminal Fragment Length Polymorphism (tFLP) analysis in which amplicon lengths are compared; Denaturing gradient gel electrophoresis (DGGE) and single strand conformation polymorphism (SSCP) analysis in which the migration nature of single stranded DNA of species are compared. Also included is the species identification by the Luminex Technology whereby the probes are bound to latex fluorescent beads and species detected with the use of the Luminex analyser. The Real-time and multiplex PCR(120, 121) are additional PCR-based methods used in the identification of *Malassezia* species.

1.8.3 Restriction Fragment Length Polymorphism (RFLP) PCR method.

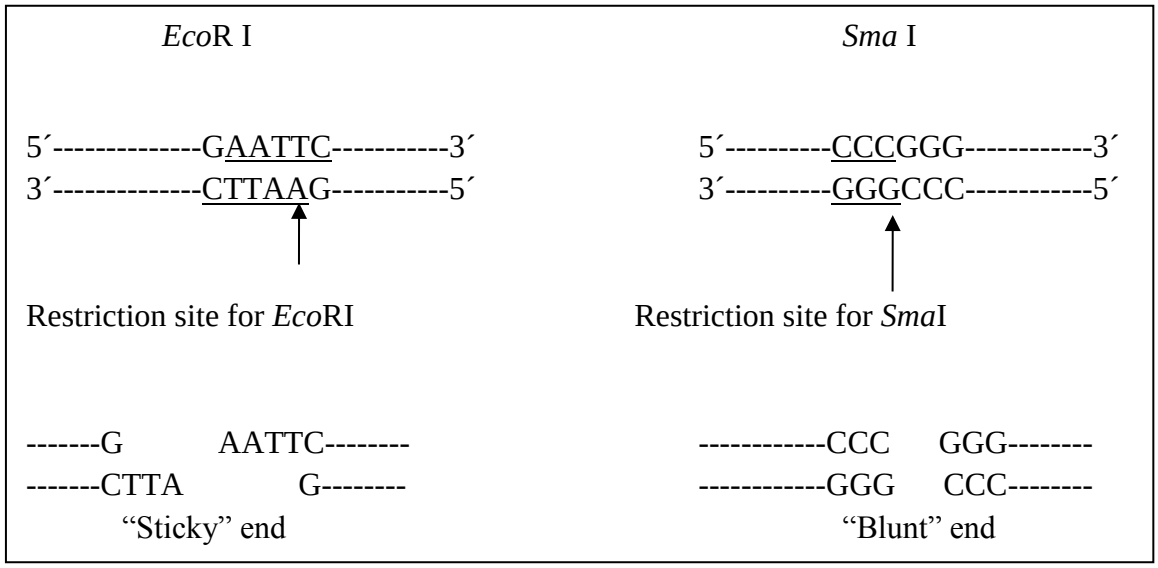
RFLP is a molecular biology technique that exposes the dissimilarity in homologous DNA sequences by identifying the presence of fragments of different lengths following digestion of this same DNA sample with specific restriction enzymes. Basically in RFLP analysis, the DNA sample is digested by restriction enzymes and the products of this digestion called restriction fragments are separated according to their lengths by gel electrophoresis. This method apart from identification of strains/species of microorganisms, has been used in various genetic studies for the location of mutations, generation of human linkage maps, identification of disease genes and in DNA fingerprinting where solutions to paternity cases, criminal justice can be applied.(122)

Restriction enzymes are bacterial derived enzymes that recognize and cleave specific sequences in DNA to produce fragments. A common example of a restriction enzyme is *Eco*R I which is isolated from the bacterium ***E****scherichia* ***co****li.* This enzyme recognizes the sequence 5´-GAATTC-3´. Whenever *Eco*R I find this sequence in a DNA molecule, it cuts between the G and the first A. Another example is *Sma* I isolated from ***S****erratia* ***ma****rcescens* which recoqnizes 5´-CCCGGG-3´sequence and cuts between the last C and the first G. See figure 1.1.

Since each enzyme recognizes one specific DNA sequence, each enzyme will digest a particular DNA molecule only at that specific point. Thus a DNA molecule that has just one 5´-GAATTC-3´sequence and is to be digested by *Eco*R I will be cut once

and if the same molecule has three 5´-CCCGGG-3´sequences that is recognized by *Sma* I, it will be cut thrice, producing 3 DNA fragments. These 3 fragments may have different length, that is base pairs, and they usually add up to the original size of the molecule.

Figure 1.1 showing a DNA palindrome of the recognition sequence for *Eco*R I and *Sma* I with arrows indicating cut sites



Studies in the identification of *Malassezia* species from PV lesions using PCR-RFLP method have used specific primers to amplify the 26S rDNA and the 5.8S rDNA-ITS2 regions of the fungi. These amplified DNA sequences were then digested with specific restriction enzymes.(119, 123) The digested fragments were observed to give species-specific patterns which were comparable with known DNA sequences of the different species. Seven species can be identified with the 5.8S rDNA amplification product using Alu I and Hinf I restriction enzymes while 11 species can be identified with the 26S rDNA amplification product using *Cfo* I and *Bst*F 51 enzymes. See Table 1.6 and 1.7 below

Table 1.4. Size of PCR products before and after restriction digestion with *Alu* I and *Hinf* I

***Malassezia* species**	**5.8S rDNA-ITS2 amplification product (bp)**	***Alu* I digest (bp)**	***Hinf* I digest (bp)**
M. furfur	509	268, 241	483, 18, 8
M. globosa	430	241,173,16	412, 10, 8
M. obtusa	506	No restriction site	480, 18, 8

M. slooffiae	486	368,118	403, 83
M. restricta	410	No restriction site	392, 18, 8
M. sympodalis	374	No restriction site	356, 10, 8
M. pachydermatis	483	374,109	457, 18, 8

Table 1.5. Size of PCR products before and after restriction digestion with *Cfo* I and *Bst*F 51

***Malassezia* species**	**26S rDNA amplification product (bp)**	***Cfo* I digest (bp)**	***Bst*F 51 digest (bp)**
M. slooffiae	580	250, 109, 87, 64, 74	No restriction site
M. furfur	580	250, 113, 109, 59, 30,19,2	400, 180
M. obtusa	580	250, 153, 109, 29, 19, 18, 2	No restriction site
M. pachydermatis	580	250, 221, 97,	500, 70
M. globosa	580	457, 127	480, 100
M. restricta	580	No restriction site	500, 70
M. dermatis	580	359, 199,21	No restriction site
M. nana	580	250, 197, 62, 47, 21	No restriction site
M. yamatoensis	580	165, 113, 108, 85, 59, 30, 19,2	No restriction site
M. sympodialis	580	357, 197, 21	400, 180
M. japonicum	580	250, 220, 104, 5	No restriction

1.9 Treatment of pityriasis versicolor

1.9.1 Goal of treatment

The goal of treatment of PV is to achieve both clinical and mycological cure. Clinical cure is the disappearance of the scaling and dyspigmented lesions of PV while mycological cure is achieved when the mycelial state of the causative yeasts,

Malassezia can no longer be identified in the lesions. Consequently, they have returned to their skin flora status. This is the state in which they are not associated with disease. However, assessment of an effective treatment is challenging since the yeasts remain on the skin surface a few weeks after cell death while the reversal of pigmentation takes a slow course up to several weeks therefore giving the impression of no response.

Treatment of PV could be via the topical and/or systemic route. Almost all the drugs available are not specifically developed to treat PV but can also be used to treat other superficial dermatomycosis both *Malassezia* and non-*Malassezia* associated. These medications are largely safe with minimal side effects and some are cost effective. However the high recurrence of the infection and patients' adherence to treatment could affect management.

1.9.2 Topical treatment

There are numerous topical medications available to treat PV; they are classified into the non-specific and the specific topical anti-fungal agents. The application of topical medications is easy although patient compliance may affect their effectiveness. The non-specific drugs are the oldest and first available treatment for PV and they do not have any direct antifungal activity but generally act by removing infected superficial epidermis and prevent invasion of newly formed ones.(124) There are also non-fungal specific forms of treatment that have been added most recently in the literature. Examples of some of the non-specific topical antifungal agents are highlighted below; most were reviewed by Gupta et al.(99)

1. 2.5% Selenium sulphide which could be used as a lotion, cream or shampoo is one of the first and oldest treatment options of PV. It is also effective though associated with a high relapse rate.

2. There is 5% or 10% benzoyl peroxide gel which contains propylene. This agent is effective after 3 weeks. Its mode of action may be due to the propylene glycol which is a keratolytic agent.

3. Whitfield's ointment consists of 3% salicylic acid and 6% benzoic acid. The combination of these two components gives the ointment its keratolytic property.

4. The effectiveness of the shampoo containing 1% zinc pyrithione in PV treatment is usually noticeable after 2 weeks.

5. 50% propylene glycol in water is also a non-specific antifungal medication used in the treatment of PV. The lesions are said to clear when used alone, twice daily for 2 weeks.

6. Sulphur and salicylic acid shampoos or creams have shown good efficacy in the treatment of PV.

7. Nitric oxide liberating cream has also been used in PV treatment. A 10 day twice daily application is effective.(125)

8. 0.2 mol/liter aqueous cycloserine solution was used in a 3-patient study. It was observed to give complete healing and rapid pigment correction.(126) More studies with a larger study population are required to confirm this observation.

9. 5-aminolevulinic acid photodynamic therapy has also been used in PV treatment but it is limited to regionally confined lesions,(127) thus cannot be used for extensive PV.

10. Adapalene gel was used recently in a PV treatment clinical trial and it showed no clinically effective difference with ketoconazole.(128)

The specific anti-fungal agents used in PV treatment are mainly the azoles and allylamines. They work by inhibiting enzymes involved in the synthesis of the fungal cell membrane. Specifically, they inhibit the biosynthesis of ergosterol, the main sterol in the fungal cell membrane. The azole derivatives also distort the synthesis of triglycerides and phospholipids which are components of enzymes involved in oxidative and perioxidative enzyme activities. The accumulation of highly toxic levels of hydrogen peroxide may contribute to the deterioration of subcellular organelles and cell necrosis commonly observed when these medications are used.(129) Some of the specific topical antifungal medications used in the treatment of PV highlighted below include the following, all of which were reviewed by Gupta et al.(124)

1. 2% ketoconazole shampoo or cream. This is quite effective and is retained for a long time in stratum corneum.

2. 1% bifonazole cream, spray, solution and gels produces irreversible changes in the yeasts. A 3-day treatment is highly effective.

3. 1% clotrimazole cream or solution is a broad spectrum imidazole used commonly in superficial fungal infections.

4. 2% miconazole cream is effective when used twice a day for 2 weeks.

5. 1% econazole foaming solution, cream or shampoo is effective when used once daily.

6. 1% or 2% sertaconazole cream is also an effective PV medication.

7. 2% fenticonazole lotion is effective in PV.

8. 1% tioconazole lotion or solution is a 1-substituted imidazole with shown effectiveness in PV treatment.

9. 2% fluconazole shampoo is considered effective in PV

10. 1% terbinafine solution, emulsion and cream also contains propylene glycol. It is highly lipophilic and has long lasting effect.

11. 1% ciclopirox-olamine solution or cream is a substituted pyridine, unrelated to the imidazole derivatives but more effective than clotrimazole. It also has anti-inflammatory properties.

1.9.3 Systemic treatment

Systemic treatment of PV is known to increase patient compliance since the treatment is more convenient and less time consuming than topical medications. This is the preferred mode of treatment for extensive PV which is PV affecting large skin surface areas. Systemic treatment has been used as prophylaxis in recurring cases.

Common drugs used include:

1. Ketoconazole 200 mg/day for 5, 10 and 28 days.(124) It is the first significant oral imidazole developed to treat many fungal infections. Some studies have recommended a single dose of 400 mg.(130, 131) Ketoconazole can be excreted through the sweat glands and this is the major route by which it rapidly reaches the stratum corneum. Thus, patients should not bathe for 12 hours after taking the drug.(130) It is a cytochrome P-450 enzyme inhibitor. Although no side effects have been documented with its use in PV treatment, oral use of ketoconazole is being discouraged by the United States Federal Drug regulation Agency (FDA). This is why there is need to discover a more accessible and affordable replacement.

2. Terbinafine used orally has not shown good results in treating PV as the level of its antifungal properties are quite low in the stratum corneum in patients with PV since it is not excreted through the sweat glands as ketoconazole or sebum as itraconazole.(4)

3. Itraconazole has been shown in vitro to be active against *Malassezia* by inhibiting its transformation into the mycelial form from the yeast form and also induces cell wall abnormalities.(132) It is extensively excreted in the sebum and persists in the basal layer of the epidermis (the reservoir effect). Most fungal pathogens respond to a plasma concentration of 100 ng/ml of itraconazole(133) which translates to 5 days of 200 mg daily. However, some clinical trials have shown an effective treatment of PV with itraconazole at a single dose of 400 mg/day and 200 mg/day for 7 days.(132)

4. Fluconazole is used in the systemic treatment of PV at an effective weekly dose of 400 mg, 300 mg and 150 mg for as long as 2 to 4 weeks.(124) This is because of its slow elimination rate from the skin. It selectively inhibits fungal cytochrome P-450 enzymes than other azoles and thus has a better safety profile.(134)

5. Pramiconazole, a broad-spectrum triazole antifungal has been shown to be a convenient short duration therapy for PV. It is effective at a 200 mg or 400 mg taken once or a 200 mg daily dose for 2 or 3 days.(135)

1.9.4 Herbal treatment of pityriasis versicolor

This involves the use of plant extracts. Some have been studied in vitro and shown to be effective for PV treatment; however evidence of efficacy is yet to be evaluated by randomized controlled clinical trials. Examples include Xanthorrhizol extracted from the rhizome of Curcuma xanthorrhiza and found to kill 100% of *M. furfur* and *M. pachydermatis* inoculated cells.(136)

Australian tea tree oil is an essential oil of Melaleuca alternifolia that has shown good in vitro activity against six common *Malassezia* species.(137)

Other plant extracts shown to have therapeutic properties in PV include Acalypha wilkesiana,(138) Cassia alata,(139) Ilex paraguariensis(140) and Artemisia abrotanum.(141)

1.9.5 Prophylactic treatment of pityriasis versicolor

The importance of above mentioned endogenous and exogenous predisposing factors increases the tendency of PV to recur especially in individuals living in tropical environment and who have a genetic predisposition. This could be quite distressing and frustrating to both the patients and their physicians(142) necessitating the need for the development of an effective guideline for prophylactic treatment.

A study from Israel in which the patients with PV were followed up for up to 2 years showed a relapse rate of 60%(143) although this rate could be as high as 90% in some cases.(4) Patients with PV can have 4 or more relapsing episodes within 12 months despite adequate treatment.(144) Also the number of relapsing episodes is not significantly linked to the extent of distribution of the lesions.

Oral therapy has been used in clinical trials at variable doses and methods as a form of prophylaxis in PV patients. Patients were followed-up for variable length of time. Examples include the use of ketoconazole at 400 mg single dose per month and followed up for a year or 200 mg per day for 3 consecutive days per month and followed up for up to 15 months; both studies showed low rate of recurrence.(144, 145) Also is the use of 400 mg single dose per month for 6 months of itraconazole which was shown to significantly delay recurrence than placebo.(146) However, there are no consensus guidelines available for the prophylactic treatment of PV.

CHAPTER TWO

Rationale and Objectives

2.1. Rationale

Pityriasis versicolor is a common superficial fungal infection of the skin caused by *Malassezia*. Nigeria is one of the tropical countries with its main latitude and longitude lying 10 degree North of the Equator and 8 degree East of the Greenwich Meridian respectively. It is located in the western region of Africa and considered as one of the most populous areas of black population.

Figure 2.1 Map of Nigeria in Africa showing the latitude and longitude

The climatic condition in the northern part is arid while the south is tropical in nature. PV has a higher prevalence rates in tropical and arid regions. It is most prevalent in adolescents and young adults and is chronically recurring such that affected individuals can have a prolonged course of the disease. It has various clinical presentations and a strong familial tendency.

Fourteen species of this organism have been identified and studies have shown significant differences in response to treatment by these species. The distribution of the different *Malassezia* spp. recovered from PV lesions have been speculated to be determined by the differences in geographic or climate conditions, ethnic origin of the

population, body site, specimen collection and identification methods.(108) And yet, studies from the tropical regions of the world are very few in number.

A summary of several studies done to determine the causative species in most PV lesions have shown *M*. *globosa, M. sympodialis and M. furfur* as the three dominant *Malassezia* species.(108) There is also a major geographical location difference among these species with *M globosa* being more prevalent in temperate regions and *M furfur* in tropical regions of the world, although the other *Malassezia* species do exist. The predominant species in West Africa has not well been studied. The clinical characteristics of PV among Africans need to be updated.

The rationale for this study is to see if there is any association between the clinical features and causative species of *Malassezia* in this environment which may influence treatment.

2.2 Research question(s) and/ or hypotheses

1. Which of the *Malassezia* species isolated is more prevalent in Nigeria?
2. Is there an association between the species and clinical characteristic?
3. How does PV affect the quality of life of affected individuals?

2.3 Objectives

To identify the existing *Malassezia* species in Nigerian students with pityriasis versicolor and to correlate the various species with the clinical characteristics of the lesions

Aim of study:

1. To identify the prevailing species of *Malassezia* causing PV.
2. To describe the clinical characteristics of PV.
3. To describe attitude and health related quality of life effect of PV on affected subjects.

Objectives:

1. To identify the most prevalent species of *Malassezia* in Nigerian subjects.
2. To determine if the clinical morphology of PV lesions is species specific.
3. To identify possible predisposing factors contributing to development of PV.

CHAPTER THREE

Subjects, Materials and Methods

3.1 Study design

The research was a descriptive/cross-sectional survey, carried out over an eighteen month period (January 2012 to October 2013) with ethical approval obtained both from the Ethics Review Committee of National Hospital Abuja, Nigeria and Ludwig Maximillian University Munich, Germany.

3.2 Study location

The subjects and sample collection part of the study was conducted within the Federal Capital Territory (FCT) of Nigeria while a part of the laboratory work namely PCR-RFLP was done in the Department of Dermatology Ludwig Maximilian University (LMU) Munich, Germany and later for logistics reasons at the Department of Molecular Biology, Nigerian Institute for Medical Research (NIMR) Lagos state, Nigeria.

The Federal Capital Territory of Nigeria, Abuja is located at the center of the country, Nigeria. It is a recent capital city and was designed to signify neutrality where all ethnic groups can live in a sense of national unity. It officially became the seat of power on 12 December 1991, replacing Lagos, which remains the city with the highest population of Nigerians. Abuja as a planned city is still expanding and undergoing various construction.

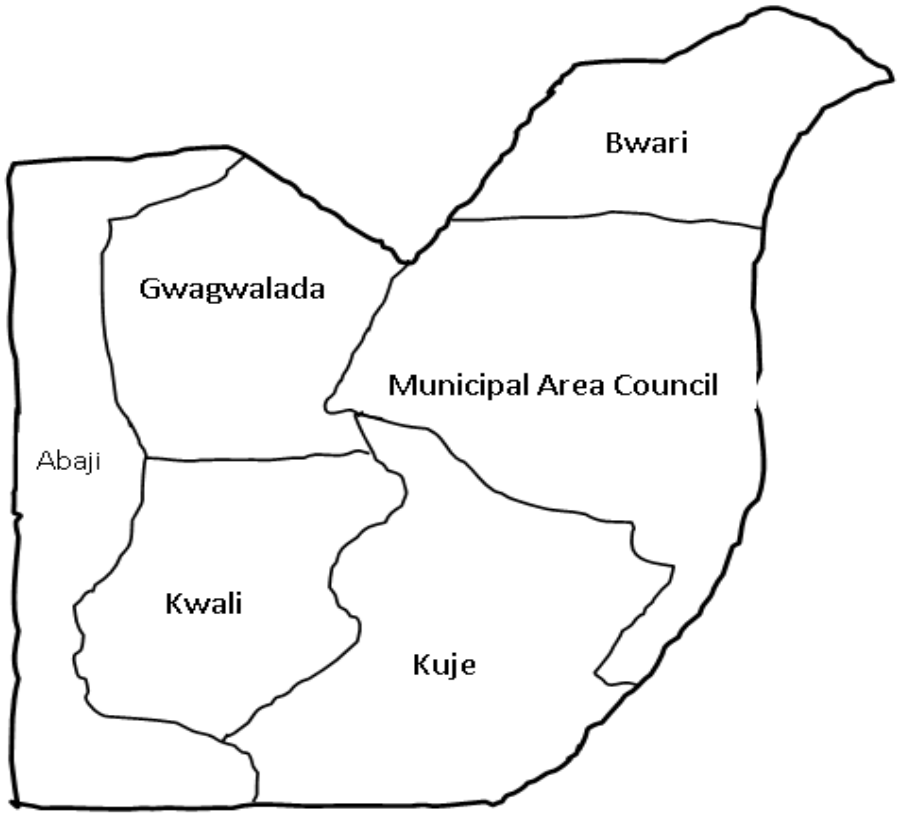
Figure 3.1 Showing a Map of Nigeria with its 36 states and FCT

It is divided into six area councils. The area councils include Bwari, Gwagwalada, Municipal (city center) Area Council, Kuje, Abaji and Kwali. The estimated population of Abuja for 2013 by the 9th edition of the Demographia World Urban Areas of March 2013 is about 2.36 million making it the fourth largest city after Lagos, Kano and Ibadan. Unlike other cities in Nigeria, Abuja is not dominated by any tribe or ethnicity and the native people who lived there before it became the country's capital are considered a minority group.

Abuja's climate is tropical wet and dry under the Köppen climate classification.(8) It experiences three seasonal weather conditions annually. The first is the rainy season from April to October, when the atmospheric temperatures range from 22^0 C to 30^0 C. The second is called the dry season (without any rains) from November to March, when the temperatures vary from as low as 12^0 C to as high as 40^0 C. Within the dry season, in December/January there is usually a short period of the harmattan season caused by the northeast trade winds which blows in fine dust particles from the Sahara into the Gulf of Guinea. It is characterized by dust haze and an intensity of cold as well as dry weather. According to Wikipedia, the dust haze can impair visibility and sun exposure for several days; atmospheric temperature can be as low as 5^0 C while humidity may drop to 15 percent.

Most of the laboratory work for the study namely the culture media preparation, incubation of the fungi, DNA extraction and storage of extracted culture were done at the National Hospital Abuja one of the tertiary hospitals located in Abuja.

Figure 3.2 Showing Abuja Area Councils



3.3 Study population

The study population consisted of senior secondary (SS) students from year one to three (final year) who had clinical features of PV as well as positive culture growth of the fungus from the lesions. The students were consecutively selected from the Government Secondary Schools (GSS) in Abuja. They included a wide range of Nigerians from different ethnic groups and social backgrounds.

For a student to be in the senior secondary school, he or she must have spent at least 6 years in primary school, passed the National Common Entrance Examination to enter the Junior Secondary School and after at least 3 years of junior secondary education, must pass the Junior Secondary School Certificate Examination in order to begin his or her senior secondary education. The minimum time spent in this education is three years. Due to the above reasons, the expected age range of the students in this study population (SS one to three) should lie between 13 up to 16 years. However, this may not be the case as the age range of the students in each class varied widely with some students being as old as 22 years. Some students may have commenced their education later than others or took a break from school or are slow in learning. The reasons are usually socioeconomically related. Meanwhile, the rate limiting step used in the students' placements include mainly the School Entrance, Certificate and Class Examinations. Therefore, students are placed in classes based on the level of their intellectual capacity as shown by their success at these examinations. Age, especially when older, does not usually play a major role.

The schools selected were randomly distributed within the six area councils of Abuja. Permission was obtained from the FCT Secondary School Management Board in order to gain access to the schools (appendix V). The Board controls 57 GSS distributed randomly in all the area councils. Some area councils have more schools than the others and a bigger student population. For example Abaji and Kwali have 3 and 4 senior secondary schools, respectively, while Municipal Area council has 22. These schools are attended by both girls and boys alike and comprise mostly of Day schools while the Boarding schools are just 5 in number.

3.4 Sample size determination

The sample size calculation was done based on the prevalence rate of 6.7% (prevalence of PV in Northwestern Nigeria).(55) Using the formula

$n = 2zpq/d^2$

n = sample size

p = 0.067 (prevalence of PV in Northwestern Nigeria)

q = 1 - p

d = 5% (degree of accuracy desired)

The calculated sample size was 96.1

Samples could only be collected when the schools were open. Basically most of the first year senior secondary students are registered and admitted late into the first term since they have to await the result of their junior secondary school certificate examinations and some may have to change/transfer schools. Also from the second term of the session, the third year students commence their senior secondary school certificate examinations. Therefore at any point in time about 20 to 30% of the students may not be assessable for the research. Furthermore, there were students who could refuse to participate. To compensate for the above reasons, a new sample size using 30% was calculated as

corrected sample size = 96.1 divided by (1-0.30). Thus new sample size is 137.3

Considering that we had to analyze the identification of *Malassezia* species in relation to the clinical features of PV, and using the culture based technique, a growth rate of 50% is expected.(100) This increases the minimum sample size to 275 [that is calculated from 137.3 divided by (1-0.5)].

Students were recruited into the study based on their satisfaction of the inclusion criteria (shown below). A total of 304 students were included in this study.

3.4.1 Inclusion Criteria

1. Students with clinical features of pityriasis versicolor and whose skin scraping's culture result showed growth of the *Malassezia* fungus.

2. Students whose parents and self, voluntarily consented to participate in the study.

3.4.2 Exclusion Criteria

1. Students who claim to have had PV but do not currently have clinical evidence of PV.

2. Students with PV who have used any topical antifungal medications in the previous 2 weeks or systemic antifungal agents in the previous 1 month.

3. Those who are under 18 years of age whose parents did not give consent to the study.

4. Students who are immunocompromised or chronically ill.

3.5 Selection Technique

The study was conducted from January 2012 to October 2013. The students were recruited from May 2012 to May 2013 during the period the schools were in session. There are three terms of 2 to 3 months period within a school year. Recruitment was not done during the holidays.

Average number of students per class was estimated at 40. Average number of classes per school-year was estimated as 6. Assuming that within the study period, second year students and either first or third year students will be available for the study; the average student population per school who may be available for the study was then estimated as 480.

Using a prevalence of 6.7%, expected average number of students with PV per school is 32. Therefore, visit to a minimum of 10 schools was required in order to attain the calculated sample size. These schools were selected from the pool of 57 schools by simple random sampling. On visit to the schools, the Principal was informed about the study and the letter from the FCT board presented to him or her. The Principal then directed the head teacher in charge of health and the school nurse to assist with the study. A convenient date when the study could begin was usually selected by the school authorities. The study period in each school (this includes timing for students' education, collection of consent and skin scrapping of PV lesions) was on average seven days and it was dependent on the student population.

The students were addressed and information about pityriasis versicolor provided to all the students who were present in their classes and on the Assembly Ground. The students with PV who voluntarily presented themselves were given the consent form and parents/patients information form (appendix I) for subsequent presentation to their parents/guardians. The following days, the students whose parents have already signed the consent form and who agreed to participate in the study as well as those who did not have any of the exclusion criteria stated above were examined at the School Clinic.

They were examined by the dermatologist and allotted a number. They filled the questionnaire and skin scraping was collected and grown. Questionnaires not properly or completely filled by the student especially with regards to key aspects of the study were discarded.

Figure 3.3 Picture of one of the schools used in the study

Figure 3.4 Picture of the school clinic where samples were collected

3.6 Data Collection Instrument

Information was obtained from the students using a pre-tested questionnaire (appendix II). The questions were simple and easy to understand. The questionnaire was divided into four sections: section A for collection of demographic data, section B on history related to PV infection including personal grooming habits, after school activity and family history of PV. Section C was questions from the Children's Dermatology Life Quality index (CDLQI). All ten questions of the CDLQI were obtained with the permission of copyright holders, Prof. Andrew Finlay and Dr. Lewis-Jones of Cardiff University, United Kingdom. It consisted of questions structured under 6 headings: symptoms and feeling (questions 1 and 2), leisure (questions 4, 5 and 6), school or holiday (question 7), personal relationship (questions 3 and 8), sleep (question 9) and treatment (question 10). Section D contained records of the clinical pattern and distribution of the PV lesions as well as the result of laboratory investigations. This section was completed by the principal investigator. The questionnaire was administered in English language which is the language spoken and also used for teaching in the schools. Knowledge of the English language is a prerequisite for admission into any of the schools. Nevertheless, since there was a possibility that some students may not have a clear understanding of the questions, provision was made to assist them with easier interpretations. This, however, was rarely utilized.

3.6 Clinical Evaluation

All the eligible students recruited into the study filled in the questionnaire. Thereafter a detailed physical examination was conducted in a well lit room with particular focus on the skin lesions. Photographs of some lesions were also taken.

PV was diagnosed as macules of different shapes which may coalesce into large irregular patches. They may have overlying thin scales which can be made more obvious by stretching the skin (Zileri's sign). The size of the lesions varied from a few millimeters to large confluent areas.

The colours of the skin lesions were hypopigmented or hyperpigmented. Some subjects had a combination of the two. The lesions were distributed on the face, upper trunk, upper arms and back.

The sites of involvement were divided into regions: face (perioral, around the nose, behind ears, cheeks, and forehead), chest, back, upper arm and others which includes lower limbs, scalp, and perineum. The number of regions involved were counted and grouped as localized when one region was involved and extensive when three or more regions were involved. This was used to grade the severity of infection.

3.7. Skin Scrapings

Skin scrapings were collected from the clinical lesions with the use of OpSite transparent dressing (3 by 4 cm; Smith and Nephew Medical Ltd, Hull, United Kingdom) as described by Sugita and Saad et al.(76, 147) This was then placed on a glass slide for onward transfer to the laboratory. The site of scrapings was documented. Scrapings from adjacent healthy skin from the same student (as controls) were also collected. This was also collected with Opsite dressing. In subjects with PV involvement of more than two regions, samples were collected from the most accessible. In schools where a room with a dark background was available, wood's light examination was also done. A part of the collected scrapings was used for direct microscopic examination with 20% potassium hydroxide plus lactophenol blue.

A piece of the dressing was placed immediately on modified Dixon agar media consisting of 3.6% malt extract (Sigma-Aldrich Ltd), 0.6% peptone (Sigma-Aldrich), 2% desiccated ox bile (Sigma-Aldrich), 1% Tween 40 (Sigma-Aldrich), 0.2% glycerol, 0.2% oleic acid (Sigma-Aldrich), 1.2% agar (Sigma-Aldrich), 0.05 g/dL chloramphenicol and 0.05 g/dL cycloheximide (Sigma-Aldrich)(63). The medium was used within a week of preparation and the cultures incubated at 34°C for a maximum of seven days. The macroscopic feature of the fungal growth was compared with pictures of *Malassezia* species found in textbook written by Crespo Erchiga et al.(3)

In some culture plates, there was contamination by other fungi species. Sometimes these species grew quite fast (within two days) and completely covered up the culture plate, preventing growth of the *Malassezia* yeasts. In these cases sub-culture of the growing *Malassezia* yeasts was done on to a fresh culture plate. Catalase test and microscopy of culture was also matched to confirm *Malassezia* species growth. The morphology of the colonies was documented and two or three of these were selected and placed in microtubes and stored in -80^0 C freezer until use. The storage period varied from one to 10 months.

Figure 3.5 OpSite transparent dressing used for scale collection

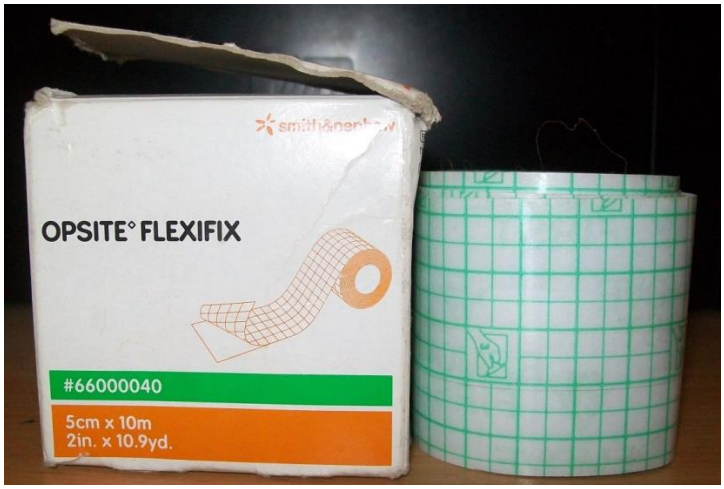


Figure 3.6 “Spaghetti and meatball” microscopy (x4) of PV scales

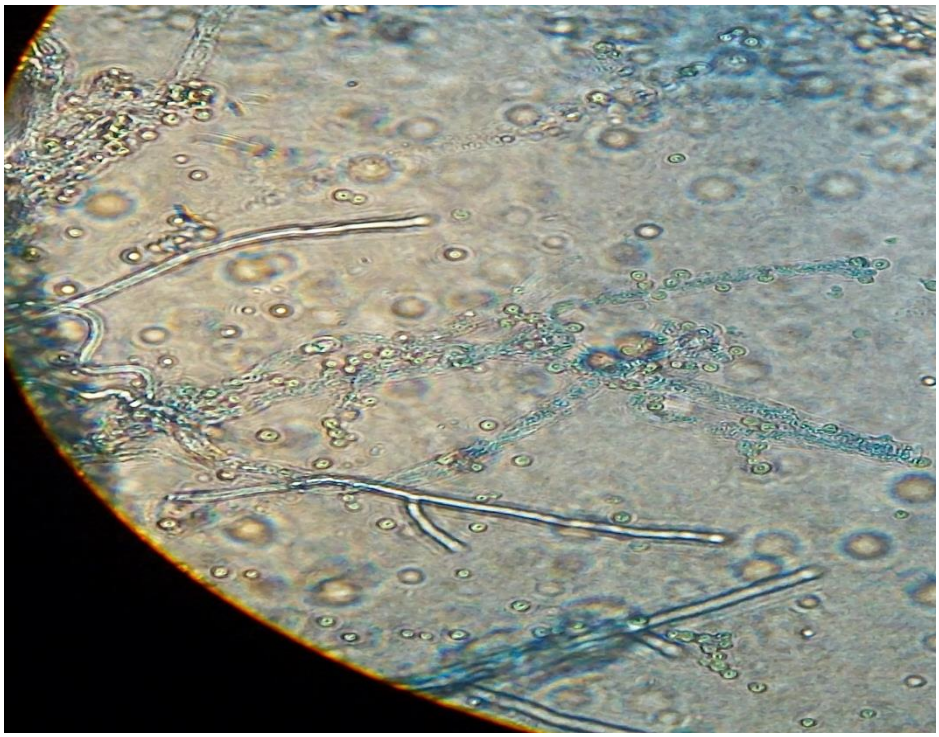
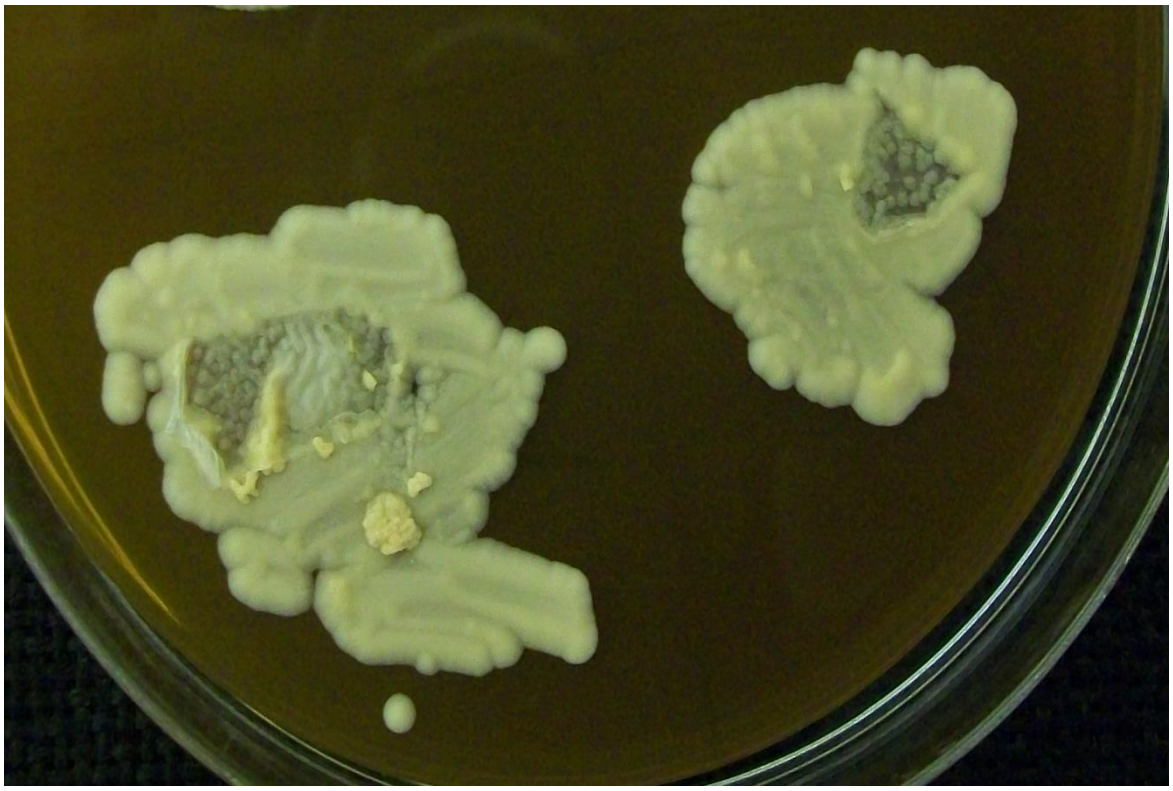


Figure 3.7 Culture of *Malassezia* on mDA



3.8. Isolation and identification of colonies compatible with *Malassezia*

This was done using the PCR-RFLP identification method as described by Mirhendi et al.(123) There was some modification with regard to the extraction of genomic DNA from the culture. This was extracted using the heating and freezing method.

3.8.1 DNA preparation: Heat-freeze method

One ml of sterile water was added to microtubes containing *Malassezia* colony which was obtained from the -80^0 C freezer. It was then heated to 95^0 C in a dry heat block for 10 minutes. It was immediately transferred to a bowl containing liquid nitrogen and frozen. This heating and freezing sequence was repeated three times and the samples were allowed to thaw at room temperature and homogenized by vortex for 10 seconds. There was no further DNA extraction, samples were used as prepared.

3.8.2 PCR Procedure

Primers that aim to amplify the 26S rDNA of *Malassezia* which has been verified to identify at least 11 species with the use of RFLP-PCR was used.(7, 71, 123, 148) Their sequences were: forward, 5' – TAACAAGGATTCCCCTAGTA and reverse, 5' – ATTACGCCAGCATCCTAAG. A final volume of 50 µL for each sample was used to perform PCR amplification. Each reaction contained 1 µL of the above prepared DNA sample, 10 µL of 5X Herculase buffer, 0.5U Herculase Hotstart DNA Polymerase (Agilent Technologies, Stratagene), 100mM each deoxynucleoside triphosphate (dNTPs), 10µM of each primer and 1µL of 100% DMSO. The rest of the volume was made up with DNA - free water. The mixture was done on ice with the help of a master-mix.

The amplification carried out at the Department of Dermatology Ludwig Maximilian University Munich, Germany was performed with the Stratagene RoboCycler 40 PCR machine while that done at the Department of Molecular Biology Nigerian Institute of Medical Research Lagos, Nigeria was carried out with the Eppendorf Mastercycler PCR machine using the same program. The program consisted of an initial denaturation step at 94^0 C for 5 minutes, followed by 40 cycles of denaturation step at 94^0 C for 45 seconds, annealing step at 55^0 C for 45 seconds and an extension step at 72^0 C for 60 minutes, thereafter a final extension at 72^0 C for 7 minutes.

Amplified products were visualized with the use of 1.5% (w/v) agarose (Sigma-Aldrich) gel electrophoresis in TBE (Tris HCl, boric acid, EDTA) buffer and stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology).

The agarose was first boiled in microwave until the crystals were completely dissolved. On cooling to a level when the bottom of flask can be comfortably touched, 5 µL of RedSafe was added and mixed before pouring into tray. Pouring was done from the edge to avoid bubbles. Formed bubbles were quickly removed. A comb was placed at one end and middle of the tray, then the agarose was left to solidify.

Following the solidification of the agarose, the combs were carefully removed. Fifteen microliter of each product plus three microliter of 6X gel loading buffer were then loaded into the pockets created by the combs in the gel. The electrodes were attached and left to run for one and half hours at a voltage of 100 V. Thereafter, the DNA bands were visualized and photographed under UV transillumination. The products successfully amplified by the primers were all expected to produce a single band of approximately 580 base pairs.

3.8.3 Enzyme digestion

Digestion of products with positive band was done with the *Cfo* I (Roche Applied Science) and *BtsC* I (New England BioLabs) restriction enzymes as described by other workers.(7, 71, 123) Digestion was performed with a final reaction volume of 20 µL with 10 U and later 1.25 U of the enzymes. Incubation was at 37^0 C for 3 hours. Digested products were viewed with the use of 2.2% agarose gel electrophoresis in TBE buffer. The electrophoresis was done also at 100 V but for one hour only. Subsequently the gel was placed in a chamber containing one µg/ml of ethidium bromide. This was left for 20 minutes, then, placed in distilled water chamber for 5 minutes before being read under UV transillumination.

The bands generated were of variable predictable sizes depending on the *Malassezia* species. The base pair length used for identification is as in Table1.7 above. Nine species can be clearly distinguished from one another using the *Cfo* I enzyme. These include *M. furfur, M. pachydermatis, M. globosa, M. obtuse, M. restricta, M. slooffiae, M. nana, M. japonica,* and *M. yamatoensis*; however, *M. sympodialis* and *M. dermatis* were shown to produce a similar pattern, which are 357bp, 197bp, 21bp and 359bp, 199bp and 21bp respectively. Therefore, these two species were further differentiated using the enzyme *Bts*C I which is an isoschizomer (recognizes the same sequence) of *Bst*F 51.

Figure 3.8. In preparation to commence PCR showing the ice tray, microtubes, DNA-free water and the polymerase and enzymes

Figure 3.9. The Stratagene RoboCycler 40 PCR machine and the loaded gel operating at 100 V

Figure 3.10. Staining with Ethidium Bromide and the Gel Documentation System where the DNA bands were visualized and photographed under UV transillumination

3.9 List of materials

3.9.1 List of equipment:

1. Sellotape
2. Opsite flexi transparent adhesive 10cmx10cm roll (Smith and Nephew GmbH)
3. Microscope slides. Cat. Number 7104
4. Petri dishes 100 mm x 15 mm
5. Autoclave machine
6. Measuring scale
7. Binder Incubator
8. Thermocool Fridge
9. -80^0 C freezer
10. Vortex machine
11. Dry heat block
12. Liquid Nitrogen
13. 2ml Eppendorf tubes
14. 1.5 Eppendorf tubes
15. Tube racks
16. Filtered pipette tips
17. Unfiltered pipette tips
18. Pipettes 20 µL, 200 µL, 1000 µL
19. PCR tubes
20. Stratagene RoboCycler 40 PCR Machine
21. Eppendorf Mastercycler
22. Gel electrophoresis apparatus
23. Bio-Rad Gel Doc 2000 System
24. Conical flask
25. Siemens Microwave
26. Microscope

27. Coverslips
28. Camera
29. Disposable nitrile gloves
30. Disposable latex gloves

3.9.2 List of Supplies and reagents:

1. 3.6% malt extract (Sigma-Aldrich; Product Number: 70145)
2. 0.6% peptone (Sigma-Aldrich; Product Number: 18332)
3. 2.0% desiccated ox bile (Sigma-Aldrich; Product Number: 70168)
4. 1.0% Tween 40 (Sigma-Aldrich; Product Number: P1504)
5. 0.2% glycerol
6. 0.2% oleic acid (Sigma-Aldrich; Product Number: O1008)
7. 1.2% agar with pH of 6.0 (Sigma-Aldrich; Product Number: 05039)
8. 0.5% chloramphenicol
9. 0.5% cycloheximide (Sigma-Aldrich; Product Number: 18079)
10. Distilled water
11. Sterile water
12. DNA – free water
13. 20% potassium hydroxide
14. Lactol phenol blue
15. Herculase Hotstart DNA Polymerase (Agilent Technologies, Stratagene; Product Number: 600310)
16. 10X Herculase buffer (Agilent Technologies, Stratagene; Product Number: 600310)
17. Herculase dNTPs (Agilent Technologies, Stratagene; Product Number: 600310)
18. DMSO (Agilent Technologies, Stratagene; Product Number: 600310)
19. Forward primer, 5’ – TAACAAGGATTCCCCTAGTA (Eurofins MWG Operon)
20. Reverse primer, 5’ – ATTACGCCAGCATCCTAAG (Eurofins MWG Operon)
21. Agarose (Sigma-Aldrich; Product Number A9539)

22. RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology; Product Number: 21141)
23. Ethidium bromide solution
24. 6X gel loading buffer (Sigma-Aldrich; Product Number: G7654)
25. DNA SmartLadder – 200 to 10000 base pairs (Eurogentec; Product Number: 08A31-2)
26. Low molecular weight DNA ladder – 25 to 766 base pairs (New England BioLabs; Product Number: N3233S)
27. Cfo I (Hha I) enzymes and buffer (Roche Applied Science; Product Number: 10688541001
28. BtsC I enzymes and buffer (New England BioLabs; Product Number: R0647S)
29. Icebox
30. TBE buffer: Tris base 54 g, boric acid 27.5 g and EDTA 3.72 g in 1 L

3.10 Statistical analysis

Data generated from the study were entered, coded, and analyzed using the Statistical Package for Social Sciences (SPSS Inc, Chicago, IL) software version 16.0. Descriptive variables were presented using frequency tables, pie charts and bar charts as appropriate. The student's t-test was used to test for statistical difference between the age of the students and gender. It was also used between age of onset of PV and some clinical variables which included residence of the students, presence of PV in a nuclear family member, daily use of petroleum jelly, daily use of body cream, episodes of PV infection (first episode vs recurring), presence of dandruff and regular animal contact.

Chi-square and Fisher exact tests as appropriate were used to test for independence between age group and the use of bleaching creams, having dandruff, recurrence of PV, residence of the students and presence of a family member with PV. Odds ratio was used to analyze the relationship between having a positive family history of PV and recurring PV. Chi-square was also used to test for independence between genders and some personal grooming habits as well as some clinical features. Odds ratio was used to analyze the relationship between genders and routine use of Vaseline and bleaching/toning creams.

For the analysis of the health-related quality of life (HRQoL), a statistical difference between the total CDLQI score in relation to some clinical variable was analysed with the use of the independent student's *t*-test. The clinical variables included age, gender, episodes of PV infection, family history of PV, site affected (exposed vs unexposed), number of body regions affected by PV, color of PV lesions and the presence of additional symptoms. Also used were the Spearman's rank coefficient, one way analysis of variance ANOVA and the Fisher's least significant difference

(LSD) post hoc analysis as appropriate to find the correlation between the students' year in school and number of regions affected by PV.

Chi-square test and Fisher exact test as appropriate were used to test for the association between the identified *Malassezia* species and the clinical variables mentioned above. A *P*-value of <0.05 was considered significant.

3.11 Ethical Certificate

Approval was obtained from the National Hospital Abuja Ethics Review Committee (appendix III) and the Ethics Commission of the Ludwig Maximilian University Munich Germany (appendix IV). Permission was also obtained from the FCT Secondary School Education Board Abuja (appendix V). Only students who were adequately informed and whose consent was obtained were included in the study.

CHAPTER FOUR

Results

4.1 Clinical characteristics of subjects

A total of 304 students with clinical features of pityriasis versicolor (PV) were recruited from 10 senior secondary schools into the study between May 2012 and May 2013. Out of these, 175 skin samples of PV grew from the mDA culture medium. This produced a 57.6% growth rate. Mode of distribution is as shown in Table 4.1

Table 4.1. Distribution of schools selected in the study

The table shows the number of students recruited in each school and the number whose skin scraping had a positive culture growth. Government senior secondary (GSS) Kwali had the highest number of students with PV while GSS Wuse had the lowest number. There was no statistically significant correlation between the number of students recruited and number with positive culture growth.

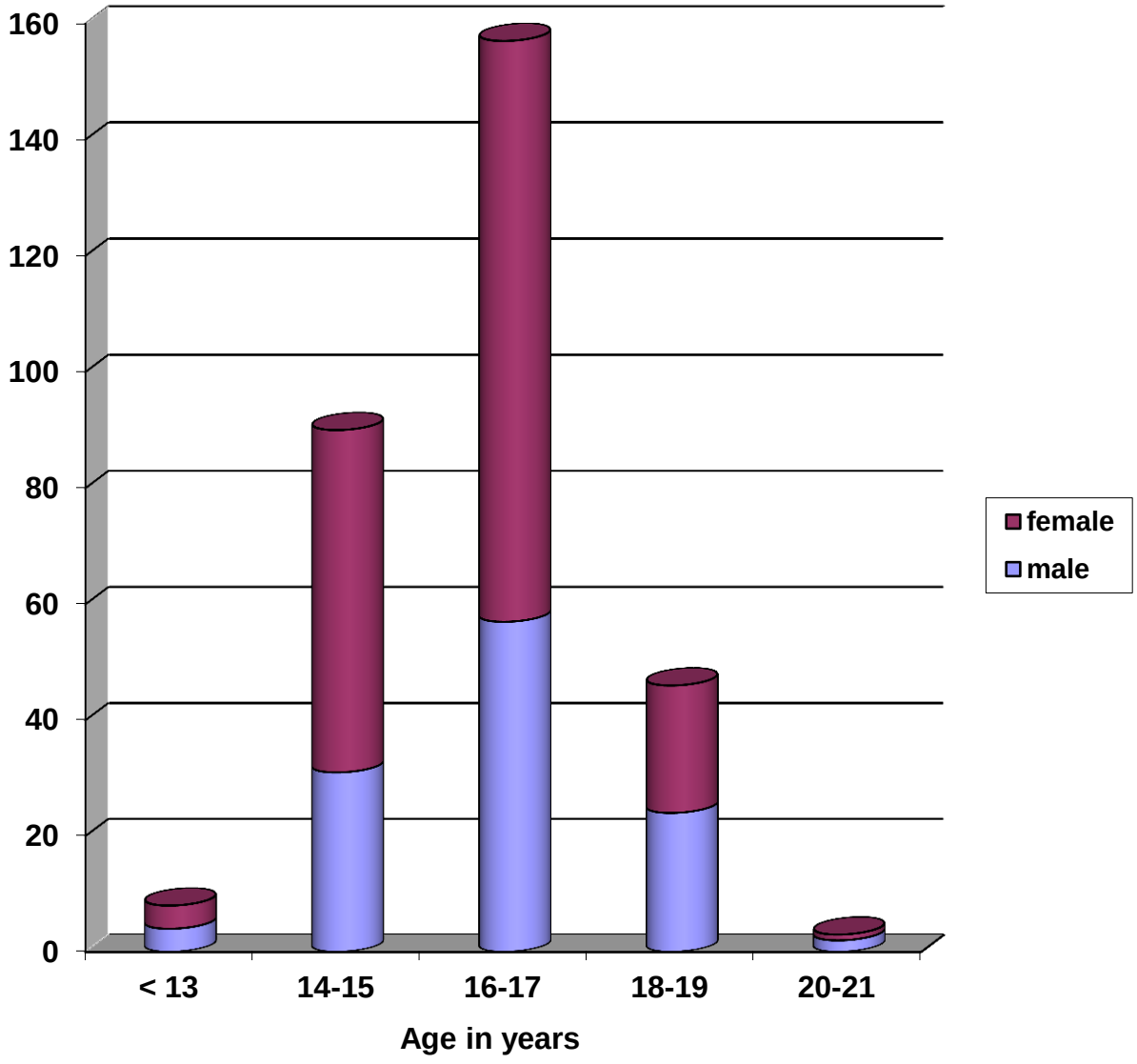
Schools	Number of Students with PV recruited	Number with positive culture growth
GSS Gwarinpa	9	4
GSS Garki	20	12
GSS Jabi	18	10
ADSS Maitama	18	8
GSS Nyanya	42	24
GSS Lugbe	53	35
GSS Kuje	27	20
GSS Gwagwalada	50	27
GSS Wuse	8	5
GSS Kwali	59	29
Total	304	175

4.1.1 Age characteristics of the students

There were 118 males and 186 females (M:F 1:1.6). The age distribution is as shown in figure 4.1. The mean age of students with PV was 16.13 (95% Confidence Interval (CI) 16.03 - 16.30) years. The age group with the highest number of PV was 14 - 18 years. The mean age of the male students with PV was 16.31 years (95% CI 16.09 – 16.59) while the mean age of the females was 16.02 (95% CI 15.82 – 16.22). There was no statistically significant difference between the ages; *P* = 0.08.

Figure 4.1. Age distribution of the students

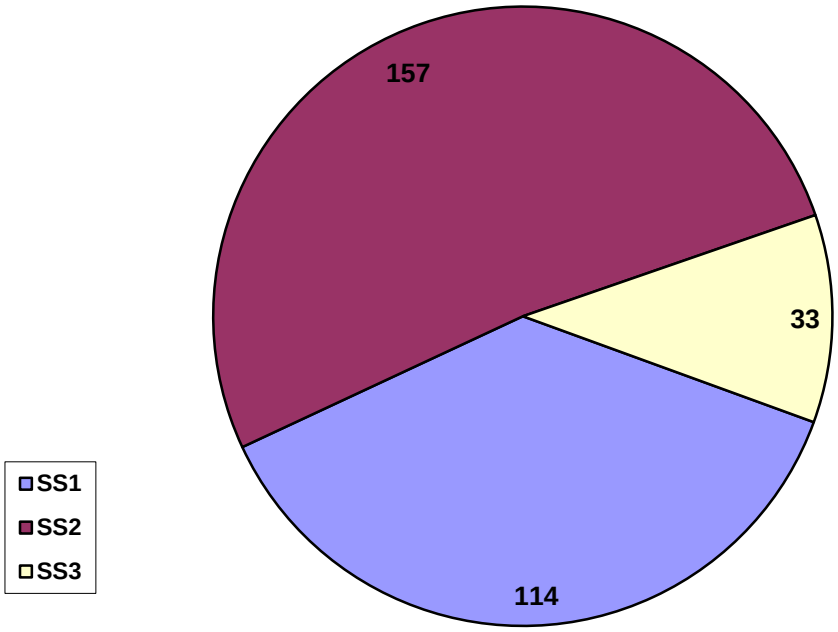
This figure shows that a majority of the students with PV fell within the age group 14 – 18 years. Most of the female students with PV were within the 16 to 17 age group. Both genders were equally distributed in those less than 13 years and 18 - 19 age groups.



4.1.2 Demography of students

About half of the students recruited (51.6%) were in their second year (SS2) while the percentage of students in third and final year was the lowest at 10.9%. There was no significant difference between the male and female according to their classes, *P* = 0.51. The distribution is shown in Figure 4.2

Figure 4.2. Class distributions of the students
The least number were students in senior secondary class 3 (SS3).



The tribe of students recruited numbered up to 46. The tribes were from the different ethnic groups and state of origin found in Nigeria. However, the majority were Ibo (26.9%), next was Yoruba (15.8%) then Igala (7.6%) and there were about 15% of the students who did not fill in their ethnic origin.
On the area of residence which was evenly divided into urban and rural areas, most of the students were recruited from Kwali and Gwagwalada Area Councils; see Table 4.1 above. One of the schools had only 8 students with PV. This school GSS Wuse is located right in the city centre.
Most of the students lived with their parents (78.9%) while the rest were residing with a Guardian. There were about 16% of the fathers and 26% of the mothers who had no formal education.
The occupation of the parents was varied; however, trading was the occupation with the highest frequency by both parents.
The students had diverse and multiple afterschool activities. These included household chores, playing football, cooking, reading, watching television, dancing, and singing. Others helped in the farms and shops of their parents. A few loved to play volleyball, basketball, table tennis, jog, swim and or visit friends.

4.1.3 Personal grooming of the students

Table 4.2. The frequency of respondents with regard to their personal grooming

Personal grooming of the students	Frequency in Percentage (%)
Daily bath	100%
Use Tap water daily	75.7%
Use toilet soap	48%
Use medicated antibacterial soap	51%
Use body creams	67.8%
Use petrolatum (Vaseline® jelly)	51%
Use bleaching and toning creams	14.5%
Use talcum powder	32.2%
Share body towel	34.2%
Sweat excessively during the day	39.8%
Sweat excessively at night	32.2%
Notice worsening of PV and thinks PV is related to the climate or seasons	46%

Toilet soaps were the ordinary bathing soaps commonly used included “Lux”, “Joy”, “Premier”, Johnson baby soap etc. The medicated antibacterial soaps frequently used were “Dettol”, “Tetmosol”, “Delta” and “Lifebouy”. The common body creams used by the students included “Familia”, “Jergens”, “Nivea”, “Cocoa butter”. The beaching/toning creams are body moisturizers containing hydroquinone, highly potent steroids and/or mercury. These included “Tura”, “Fair and white”, “Funbact A”, “Dermovate”, “Rapid white”, “Skin light” and “Maxi tone”

All the students took daily baths but not all used Tap water as routine source of water. More students used body creams than petrolatum, commonly called Vaseline® as their regular body moisturizer. Almost 40% of the students sweat excessively during the day while 14.5% use toning/bleaching creams. About half of the students recruited believed their PV infection is climate and seasons related especially when the atmospheric temperature is at its peak during the dry season.

Table 4.3. Differences in personal grooming and clinical features among genders.

Grooming habits and clinical features	Number of boys (%) Total number = 118	Number of girls (%) Total number = 186	*P* value
Use toilet soap	64 (54.2)	83 (44.6)	0.56
Use medicated soap	71 (60.2)	86 (46.2)	0.13
Use talcum powder	19 (16.1)	79 (42.5)	**0.01**
Use body cream	65 (55.1)	141 (75.8)	**<0.001**
Use bleaching creams	11 (9.3)	33 (17.7)	**0.04**
Use Vaseline®	70 (59.3)	85 (45.7)	**0.02**
Sweat during the day	50 (42.4)	71 (38.2)	0.47
Family history of PV	40 (33.9)	71 (38.2)	0.47
Symptomatic PV	46 (39)	83 (44.6)	0.34
Associated dandruff	12 (10.2)	55 (29.6)	**<0.001**

The percentage of boys who used toilet soap and or medicated soaps daily was higher than that of the girls but this difference was not statistically significant. Meanwhile, more girls than boys used talcum powder, body creams and bleaching/toning creams and the differences were found to be significant. There was a significant difference between the genders with regards to the use of Vaseline®. Exactly 1.7 times more male students used Vaseline® as routine body moisturizers when compared with the female students (95% CI 1.09 – 2.76). The presence of a positive family history of PV in a member of the nuclear family, sweating during the day and associated symptoms of pruritus or tingling sensation was not significantly different between the two genders. Also observed as insignificant is the difference in having recurring episodes of PV and the number of body sites involved. The female students with PV were also observed to have dandruff 2.7 times more than their male counterparts (95% CI 0.14 – 0.53).

4.1.4 Family relations with PV

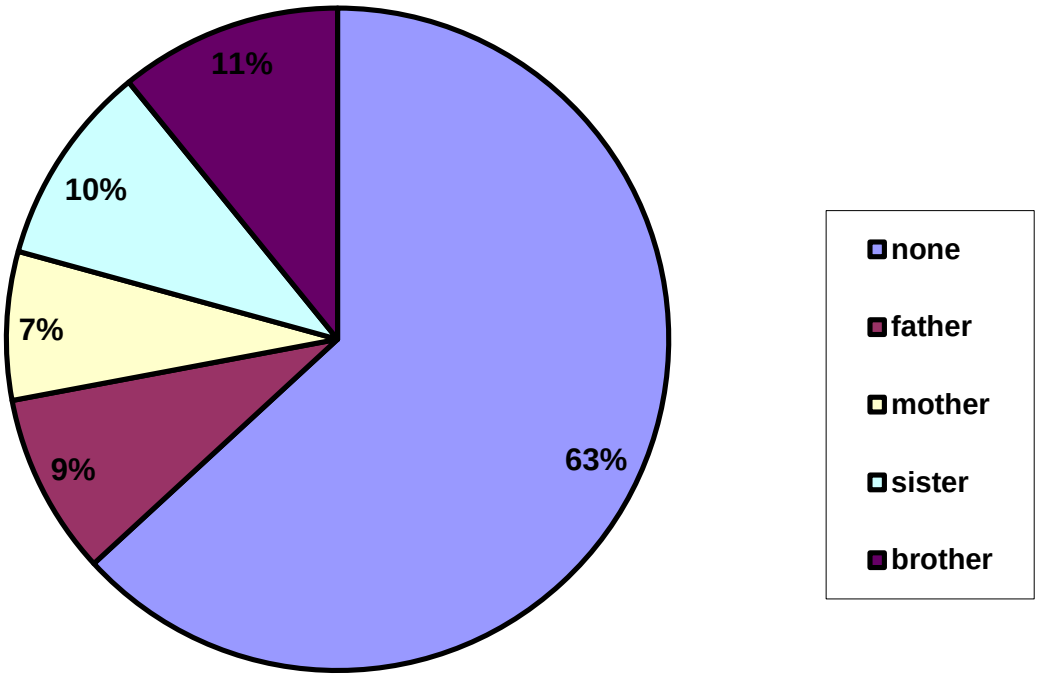
A little above one third of the students (36.5%) had at least one member of their nuclear family who also suffers from PV infection. The distribution is as shown in the figure 4.4 below. The siblings were more affected than the parents (21% vs 16%).

The relationship between the age of onset of PV and the presence of a family (within the nuclear family) member with PV was further analysed. The mean age of onset of PV in those with a positive family history of PV was younger at 13.3 (CI 12.9 – 13.7) years while the mean age of those without a family history of PV was 13.9 (CI 13.5 – 14.2) years. The difference between these groups of students was statistically significant at *P* = 0.03. (Shown in Table 4.4 below)

There was no significant association between the presence of a family member with PV and the possibility of having recurring PV (odds ratio for family history [yes/no] 1.5; 95% CI 0.94 – 2.55).

Also, there was no significant relationship between the area of residence of the students (urban vs rural) and the presence of a family history of PV (*P* = 0.4).

Figure 4.3. Showing distribution of family relations with PV



4.2 Clinical features and characteristics of PV

4.2.1. Age of onset of PV

The average age of onset of PV among the students was 13.7 years (95% CI 13.4 – 13.9 years). The median was 14 years while the minimum age of onset was 5 years and the maximum was 19 years. The mean age in males was 13.9 years (C.I. 13.5 – 14.4 years) and the females were younger at 13.5 years (13.1 – 13.9 years). The difference in the means was not statistically significant, *P* = 0.15.

The area of residence by the students was divided into rural and urban. The rural areas are regions more likely to have untarred roads and lower cost of housing as well as food. People with lower income usually reside here thus they are more populated than the urban region. These areas are also known as satellite towns. The rural regions selected in this study included Gwagwalada, Kwali, Nyanya, Kuje and Mararaba. The urban areas are close to or within the city centre. They are less populated because of the higher cost of housing and living expenses. About 157 (53.6%) students were from the satellite towns while those from the city centre were 147 (46.4%). The students who resided in the rural part of Abuja developed PV at a younger age (mean age of onset was found to be 13.3; 95% CI 12.8 – 13.6 years) compared with students living in the city centre [mean age was 14.2 (95% C.I. 13.8 – 14.6) years]. The difference between the 2 means was statistically significant, *P* = 0.001. See Table 4.4.

A large number of the students (69.1%) had recurring episodes of PV infection while for 94 students (30.9%); there was no history of recurrence. There was no statistically significant difference between the students who had recurring and first episode of PV with regards to age, sex, age of onset, family history, symptoms, colour and locations of lesions. See Figure 4.4.

Figure 4.4. Number of students with recurring and first episode of PV

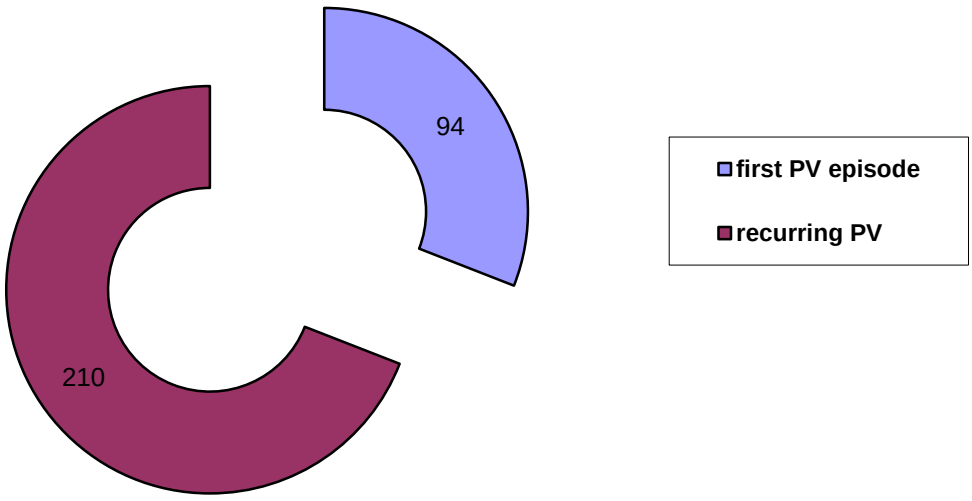


Table 4.4. The clinical characteristics in relations to the age of onset of PV

	Mean age of onset of PV in years (95% CI)	**Mean age of onset of PV in years (95% CI)**	***P* value**
Residence of student	Satellite towns 13.2 (12.8 – 13.6)	City centre 14.2 (13.8 – 14.6)	**0.001**
Presence of nuclear family member with PV	Positive 13.3 (12.9 – 13.7)	Negative 13.9 (13.5 – 14.2)	**0.03**
Daily use of Vaseline® jelly	Yes 13.3 (12.8 – 13.7)	No 14.1 (13.7 – 14.5)	**0.004**
Daily use of body cream	Yes 13.6 (13.2 – 13.9)	No 13.8 (13.3 – 14.3)	0.43
Difference between first and recurring episodes	First episode of PV 13.8 (13.3 – 14.3)	Repeat episode of PV 13.6 (13.3 – 13.9)	0.5
Difference between those with/without dandruff	Those with dandruff 12.6 (11.9 – 13.2)	Those without dandruff 14.0 (13.7 – 14.3)	**0.001**
Regular animal contact	Yes 13.4 (12.9 – 13.95)	No 13.7 (13.4 – 14.04)	0.37

Also shown in this table is that students who routinely used Vaseline® or had associated history of dandruff developed PV at a significantly younger age than their counterpart who did not use Vaseline® or did not have dandruff. Meanwhile the younger age of onset of PV in students who used body creams, or had repeated episodes of PV or regular animal contact was not statistically significant.

4.2.2. PV and possible predisposing factors

Only 44 students (14.5%), majority of which were females admitted the use of skin toning/bleaching creams. A little over half (54.5%) of this group of students were either 16 or 17 years. Also the most frequent age of onset of PV in students who used bleaching/toning creams was observed in the 13 to 16 age group which was not different from the general student population (*P* = 0.18). Overall, the average age of students who used bleaching/skin toning products were older (16.3 vs 16.1 years) and the average age of onset was also older when compared with students who did not use bleaching/toning creams (14.3 vs 13.5 years).

About 20% of the students had regular contact with animals either as house pets or kept livestock, however, this did not affect the age of onset of PV when compared with those without regular animal contact.

Also 22% of the respondents had dandruff (pityriasis capitis) in addition to PV. PV in this group of students appeared at a younger age than those without dandruff (*P* =

0.001). Meanwhile, there was no significant association observed between the students who had dandruff and those who had recurrence of PV infection (*P* = 0.55).

4.2.3 Symptoms and Signs of PV

Table 4.5. Showing the percentage distribution of complaints experienced by the students.

Complaints	Number of students	Percentage (%)
Skin lesions	304	100%
Symptomatic: Pruritus Tingling sensation	130 106 24	42.8% 34.9% 7.9%
Asymptomatic	174	57.2%

Symptomatic complaints were documented for those students who said their PV lesions were pruritic or gave them tingling sensation. Interestingly, it was observed that those who sweat excessively during the day were twice likely to be symptomatic than those who did not have hyperhidrosis. This was statistically significant. The odds ratio for not symptomatic/symptomatic was 0.58, 95% CI 0.37 – 0.93. Statistically significant differences when compared to other clinical characteristics between patients who were symptomatic and asymptomatic were not observed.

Figure 4.5. Showing the percentage distribution of the complaints experienced by the students.

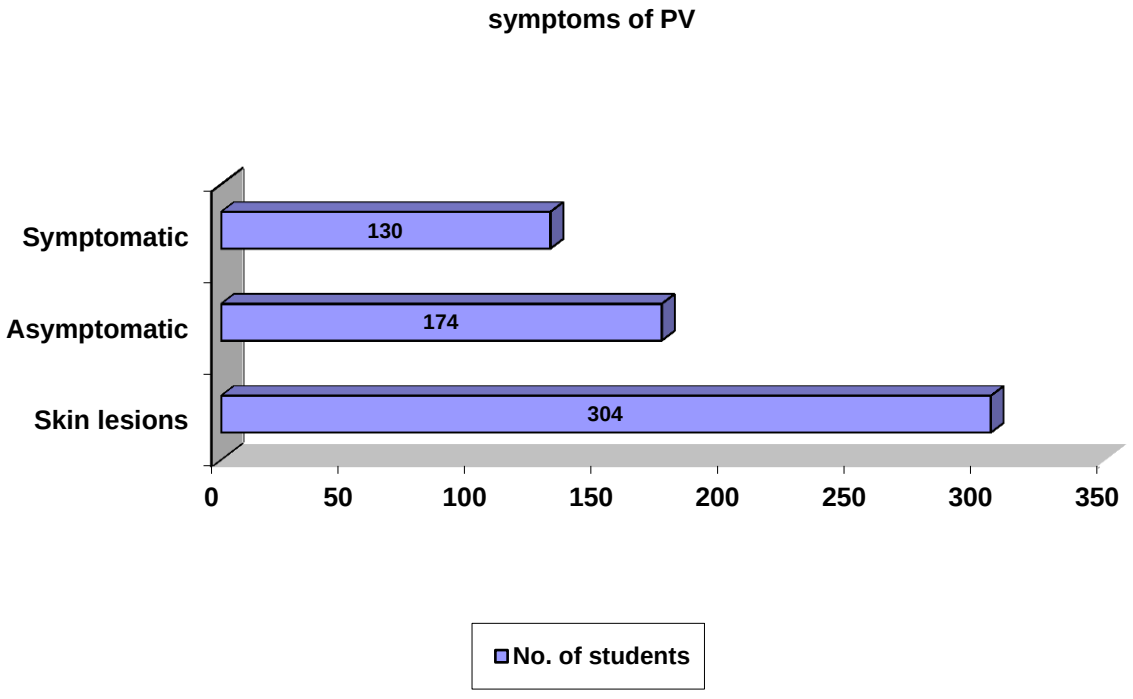
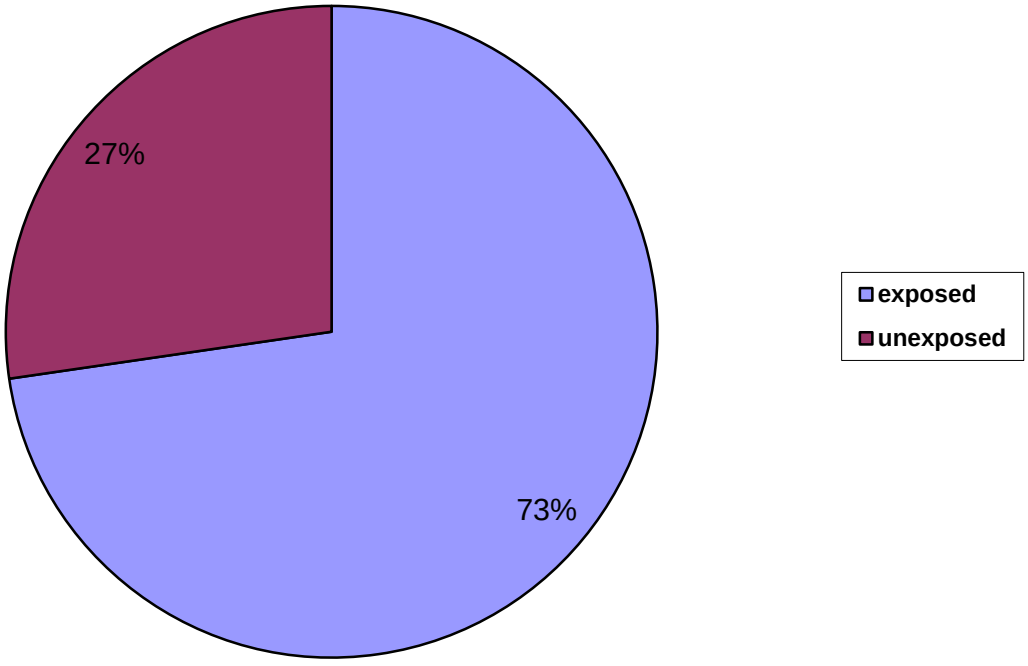


Table 4.6. Location of PV lesions

Location	Number of students	Percentage (%)
Back	101	24.2
Face	189	45.3
Chest	57	13.7
Upper arm	27	6.5
Neck	18	4.3
Others	25	6.0

This was categorized by body regions such as face, back, etc and not by the number or count of PV lesions. Affectation of the face had the highest frequency at 45.3% while the neck had the lowest at 4.3%. “Others” included scalp, nasal creases, lower limbs and abdomen. Some students had involvement of more than one body region.

Figure 4.6. Exposed and unexposed PV location



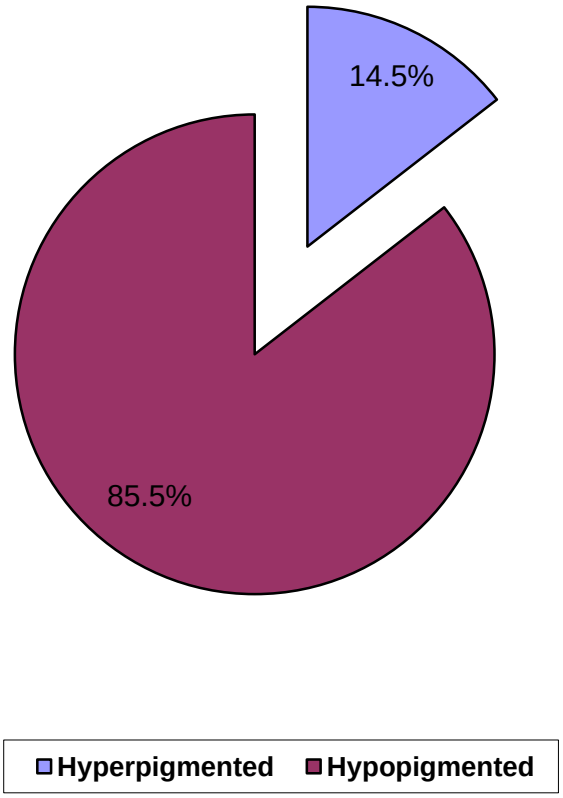
A chart showing the number of students with PV lesions located at an exposed part of the body mainly face, neck, scalp, nasal creases and unexposed part of the body mainly chest, back and upper arms. About a third of the students had the PV lesions on an unexposed part of the body.

Table 4.7. Number of body regions infected with PV

Number of body region involved with PV	Frequency	Percentage (%)
1	208	68.4
2	71	23.4
3	21	6.9
4	4	1.3

Exactly 8.2% of the students had extensive PV (involvement of 3 or more body regions). A complaint of pruritus or tingling sensation was statistically associated with students who had extensive PV (χ^2 = 8.237, *P* = 0.02). Most of the students had just one body region involved (68.4%).

Figure 4.7. Colour of PV



The pigmentations of the lesions observed were hypo- and hyperpigmented. Only 5 students were observed to have a combination of both.

Table 4.8. Patterns of PV lesions observed

Pattern	Frequency	Percentage (%)
Macules	167	85.2
Guttate	7	3.6
Follicular	17	8.7
Confluent	5	2.6

Most of the lesions were observed as dyspigmented macules with no specific pattern of arrangement. Only 3.6% appeared in a tear-drop (guttate) pattern and 8.7% had the follicular pattern of PV presentation.

Figure 4.8. Hyperpigmented PV on mandibular region of the face

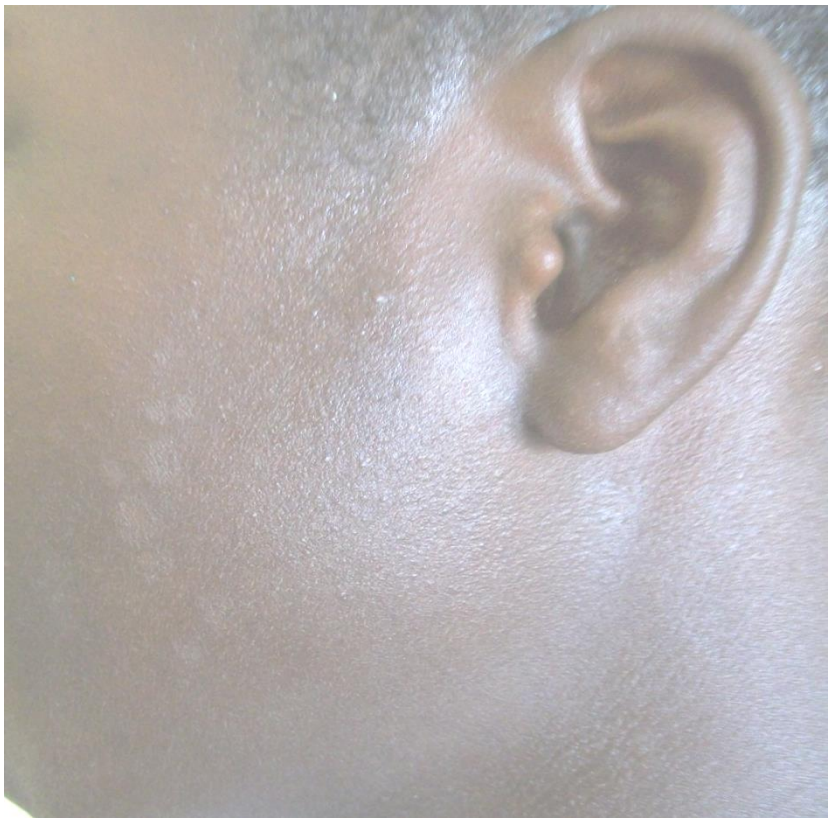


Figure 4.9. Hypopigmented PV on shoulders/upper arm

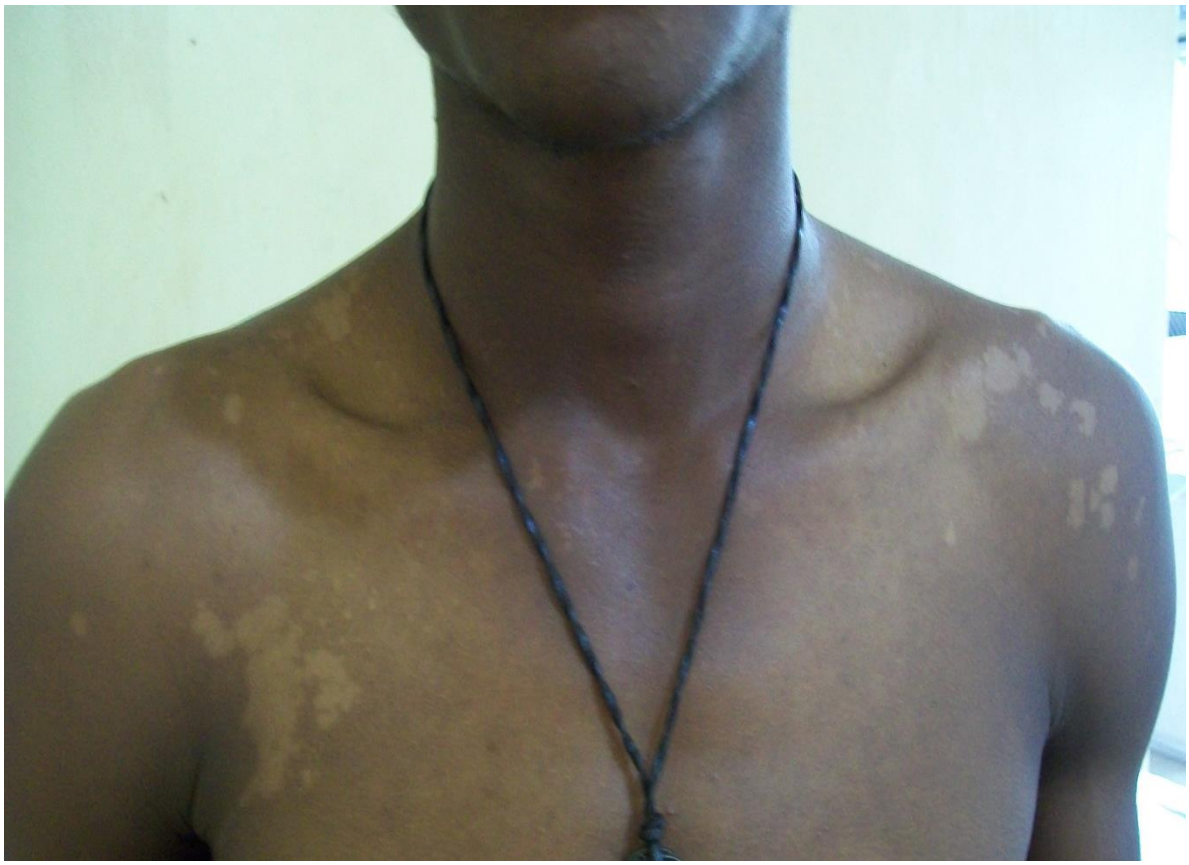


Figure 4.10. Hyperpigmented PV located at the back

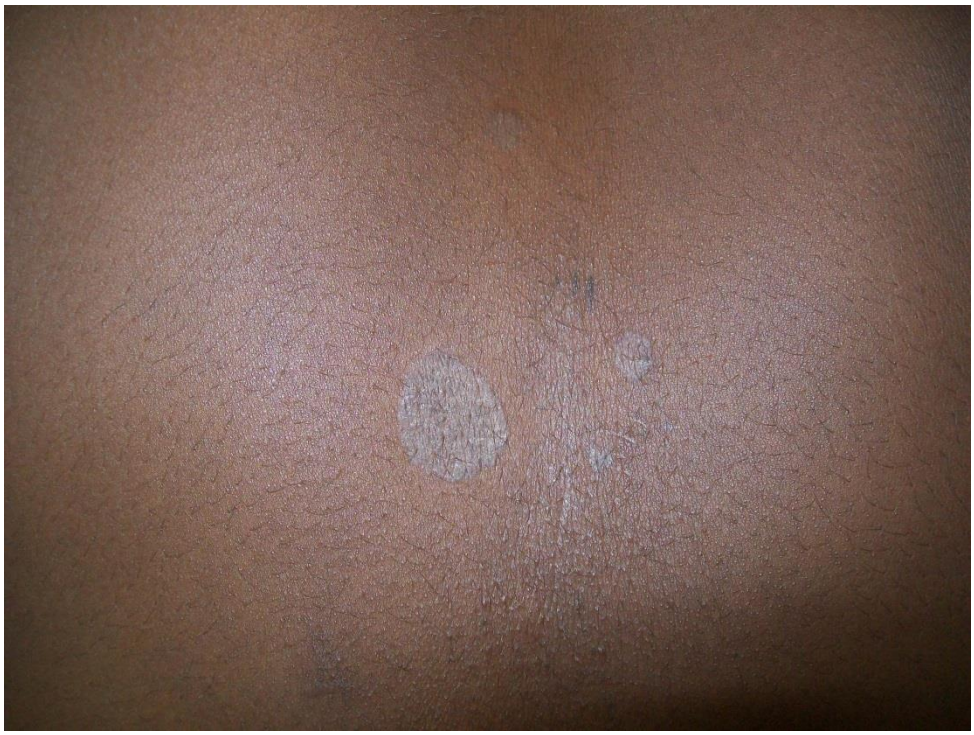


Figure 4.11. Extensive PV involving the face, neck and upper arm

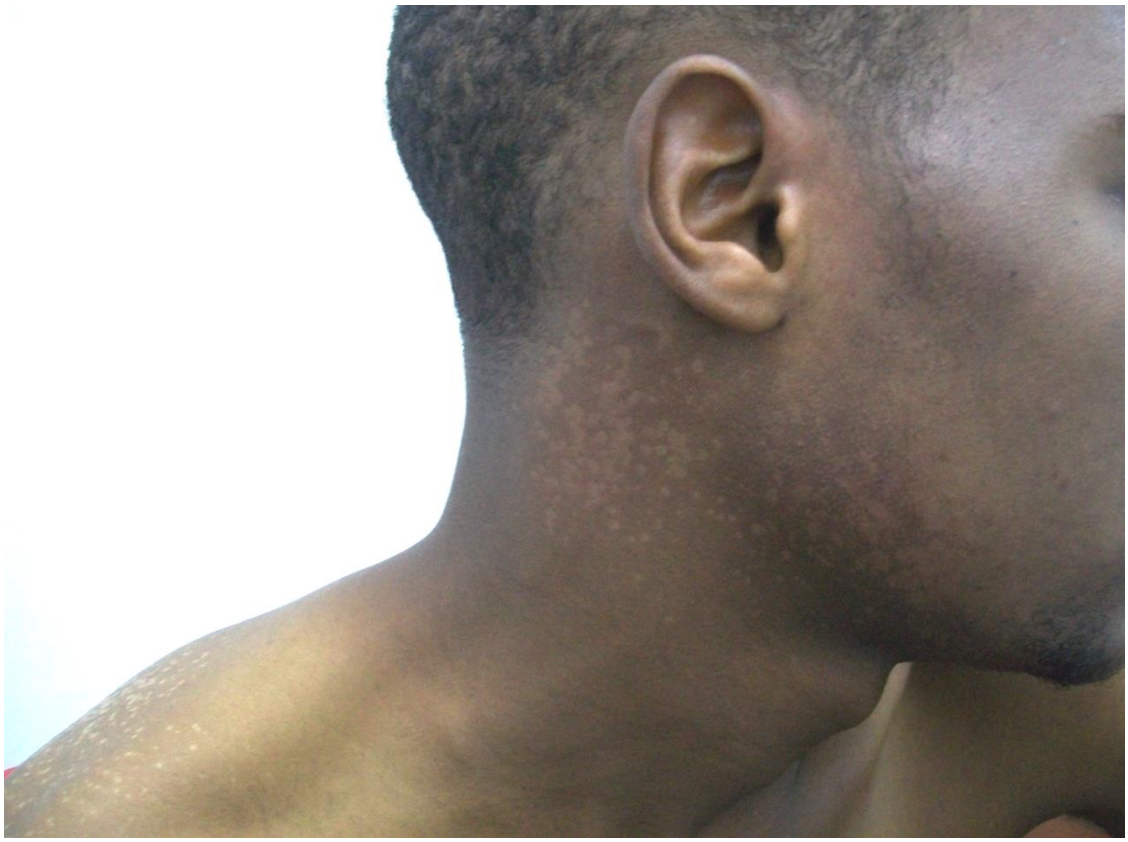


Figure 4.12. Follicular PV at the shoulders

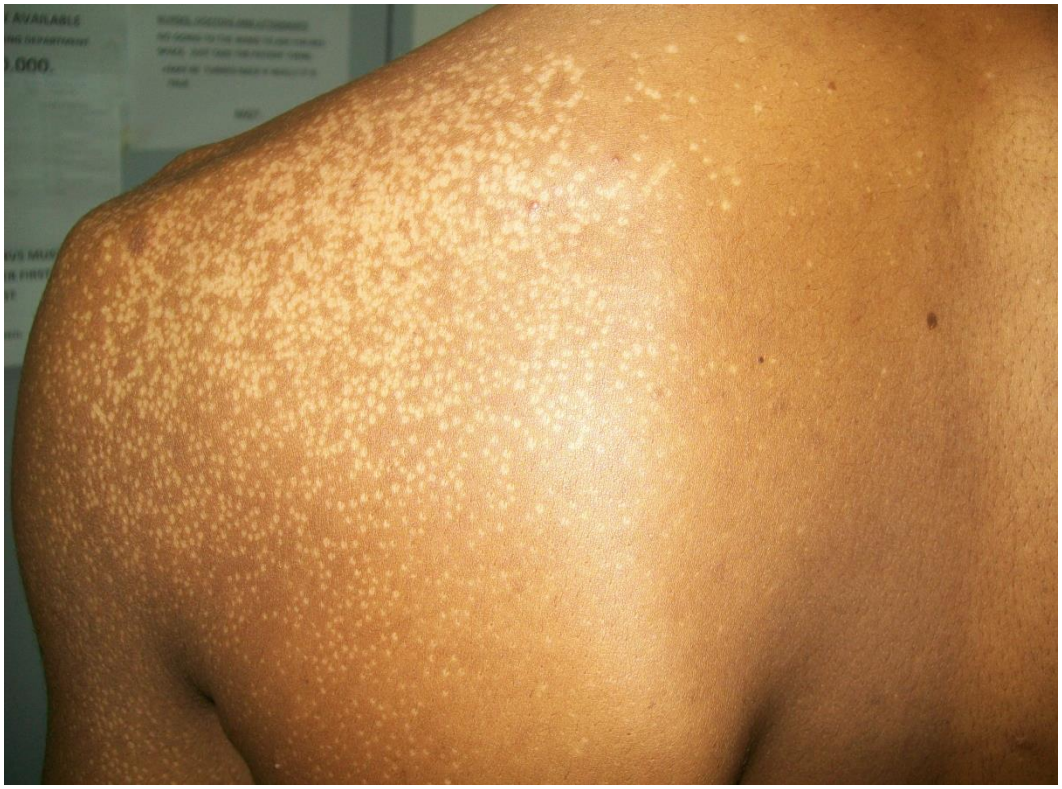
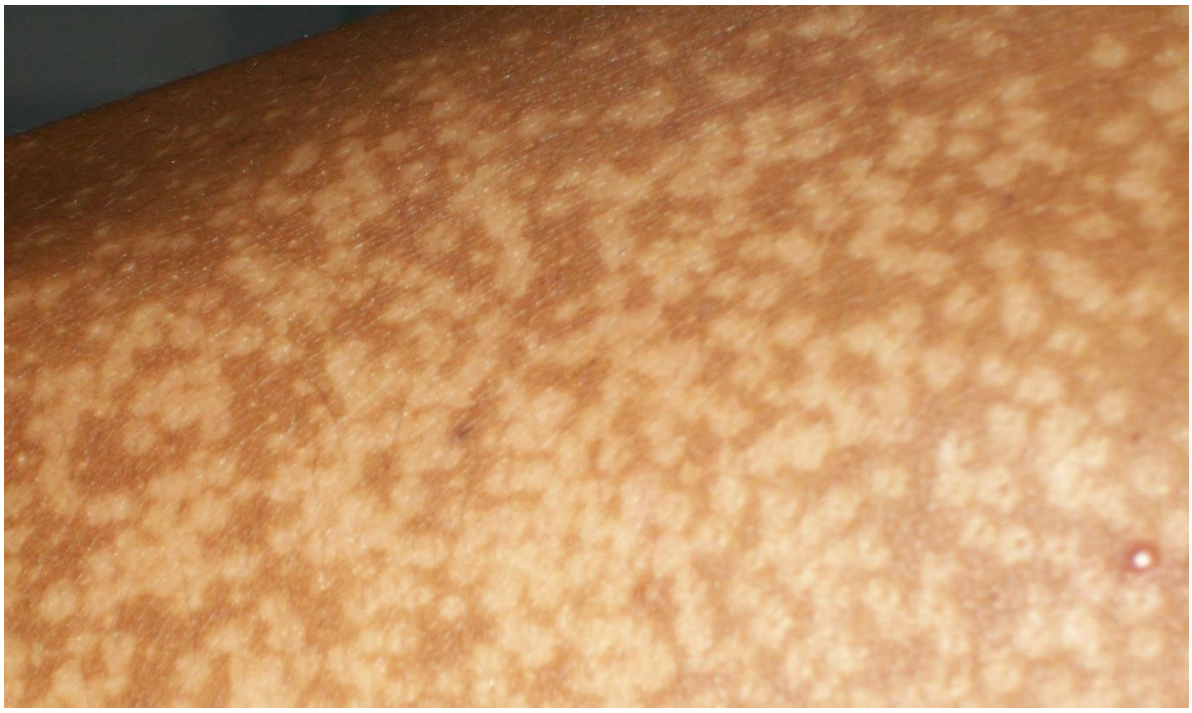
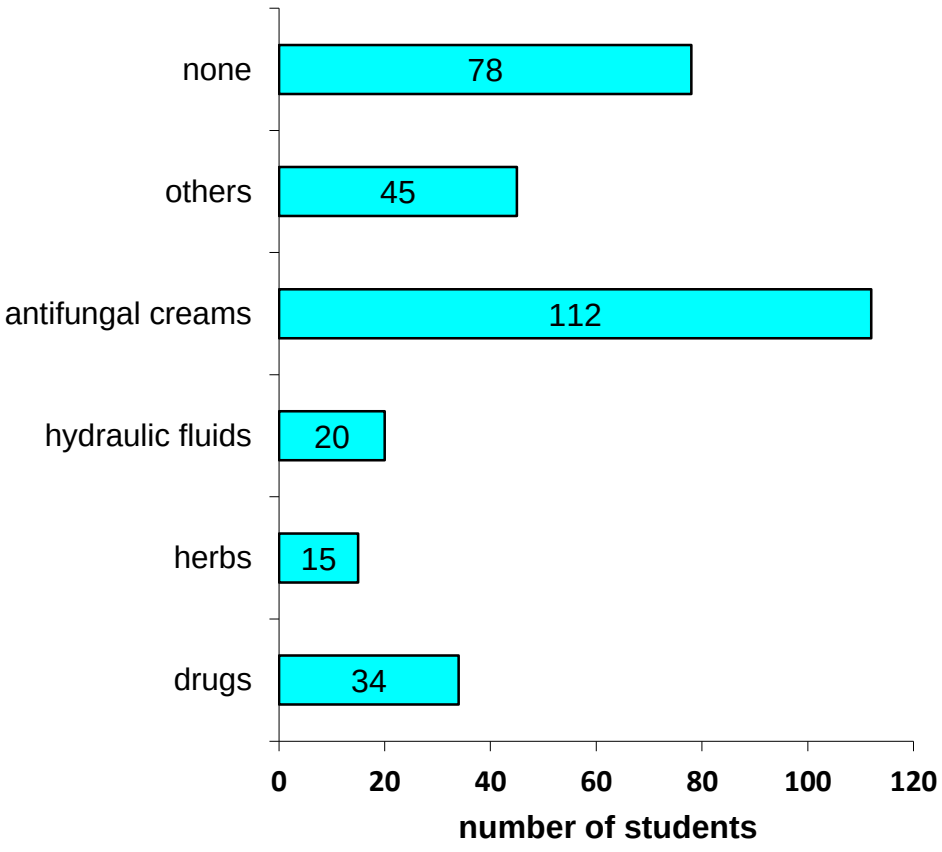


Figure 4.13. Close-up of the follicular PV lesions, notice how each hair shaft is surrounded by hypopigmented macule



4.2.4 Mode of treatment implored by the students

Figure 4.14. Mode of treatment utilized by the students



Most of the students (36.8%) used antifungal creams to treat their PV infection while 25.7% of the students did not try any form of treatment. 6.6% of the students used hydraulic fluids, mainly brake fluids and 4.9% used herbs as a form of treatment. The drugs used by the students were mainly antifungal medications taken via oral route such as ketoconazole, fluconazole and itraconazole. The “others” mode of treatment implored by the students were classified as inappropriate for PV management. They included antibacterial medications both topical and systemic, antiseptic soaps, “Nixoderm”, “Skin light” body cream, “Biocoten”, “Funbact-A”.

4.3 Health related quality of life (HRQoL) of affected students with PV

Table 4.9. Socio-demographic and clinical variables in relation to the mean total CDLQI score

Variables	**Mean total CDLQI (95% CI)** **Total score: 7.39 (6.73-8.06)**	***P* value**
Mean CDLQI by gender		
Male: n=118 (38.8%)	7.36 (6.23 - 8.49)	0.95
Female: n=186 (61.2%)	7.41(6.58 - 8.23)	
Mean CDLQI by age group		
≤13 years old: n=8 (2.6%)	4.75 (3.35 - 6.15)	**0.002**
>13years old: n=296 (97.6%)	7.46 (6.78 - 8.14)	
Mean CDLQI by residence		
Urban: n=141(46.4)	7.11(6.14 - 8.08)	0.433
Rural: n=163 (53.6%)	7.64 (6.72 - 8.56)	
Mean CDLQI by school year		
First year: n=114(37.5%)	7.27(6.17-8.37)	
Second year: n=157(51.6%)	7.32 (6.39 - 8.25)	0.505
Third year: n=33 (10.9%)	8.12 (6.07 - 10.17)	
Mean CDLQI by age of onset		
<13 years: n=91(29.9%)	7.41(6.18 - 8.83)	0.977
≥13 years: n=213 (70.1%)	7.38 (6.59 - 8.18)	

Mean CDLQI by episode of PV First episode: n=94 (30.9%) Recurring PV: n=210 (69.1%)	 8.85 (7.57 - 10.14) 6.74 (5.98 - 7.50)	**0.006**
Mean CDLQI by family history Positive: n=111(36.5%) Negative: n=193 (63-5%)	 8.41(7.27 - 9.55) 6.80 (5.99 - 7.62)	**0.021**
Mean CDLQI by site Exposed region: n=221 (72.7%) Unexposed: n=83 (27.3%)	 7.26 (6.47 - 8.05) 7.73 (6.48 - 8.99)	0.534
Mean CDLQI by number of body region affected One site: n=208 (68.4%) More than one site: n=96 (31.6%)	 6.78 (6.05 - 7.51) 8.72 (7.35 - 10.08)	**0.014**
Mean CDLQI by color of lesions Hypopigmented: n=260 (85.5%) Hyperpigmented: n=44 (14.5%)	 7.24 (6.54 - 7.94) 8.30 (6.26 - 10.33)	0.271
Mean CDLQI by associated symptoms with PV None: n=175 (57.6%) Present: n=129 (42.4%)	 6.34 (5.53 - 7.15) 8.81 (7.73 - 9.89)	**<0.001**

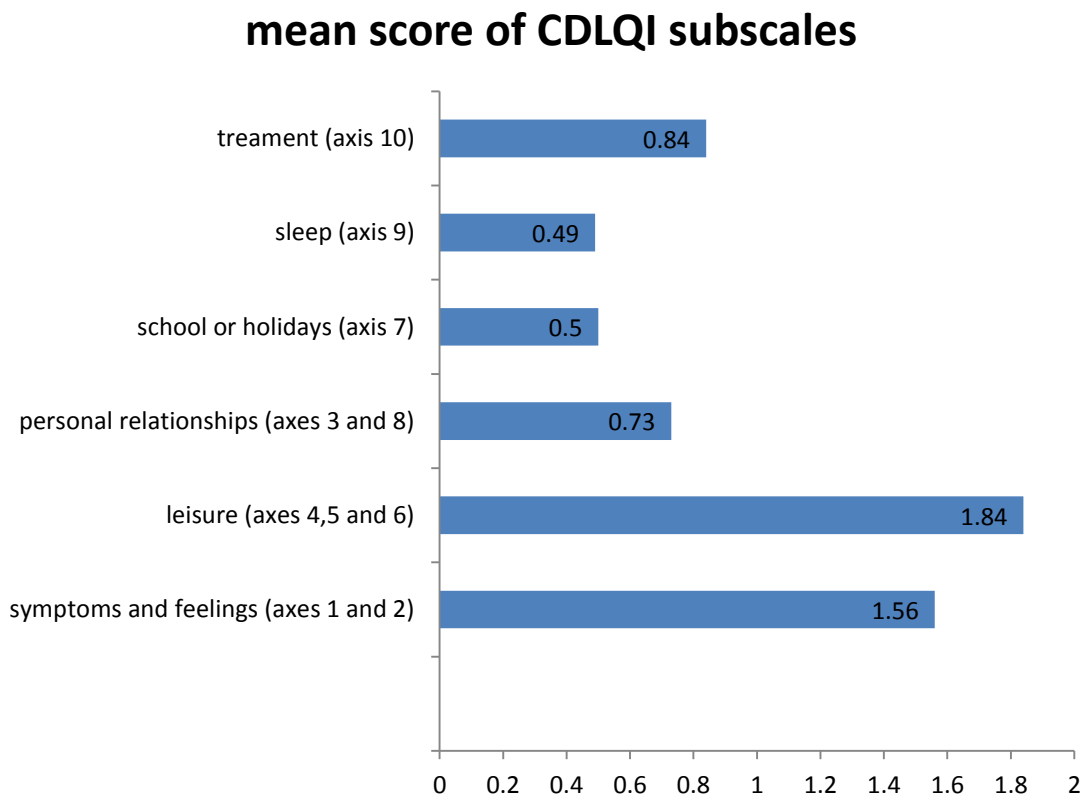
In this study, the overall mean of total CDLQI was 7.39 (95% CI 6.73 - 8.06). In relation to some socio-demographic and clinical variables (Table 4.8.), the mean was significantly higher in those older than 13 years (*P* = 0.002). Girls had higher mean scores than boys and those who live in rural parts of Abuja had also higher mean scores but both differences were not statistically significant. Students with first episode of PV and a positive family history of PV had higher mean total CDLQI which was significantly different from the students with recurring episodes of PV as well as a negative family history (*P* = 0.006; *P* = 0.021 respectively). Although the mean total CDLQI increased with higher classes, those in third and final year senior

secondary school had the highest score and those in first year the lowest. No statistically significant correlation was observed ($r_s = 0.04$, $P = 0.505$).

Interestingly, the mean total CDLQI scores were higher in those whose PV first appeared when they were less than 13 years old and in those whose PV were located on a frequently unexposed body region such as chest, back or upper arm. Students with hyperpigmented PV lesions also had higher CDLQI scores (Table 4.8.). These differences were, however, not statistically significant when compared with students whose PV infection began when they were older than 13 years or had the lesions on exposed body region such as face and neck or who had hypopigmented PV lesions respectively. Further on the mean total score of CDLQI, the students who had PV on more than one body region and who had additional symptoms associated with PV had statistically significant higher scores. Further bivariate analysis showed the number of body regions affected by PV correlated positively with the mean total CDLQI ($r_s = 0.1$, $P = 0.021$). The one way analysis of variance (ANOVA) revealed that students with three or more body regional involvement had significantly impaired QoL when compared with those who had just one body region affectation (F = 5.293, df = 2, $P = 0.006$)

The mean of the CDLQI subscales are shown in Figure 4.16. The leisure axis had the highest score (1.70), followed by the symptoms and feeling axis (1.07) while holiday or school and sleep were closely tied as the axis with the least scores.

Figure 4.15. The mean of the subscales of CDLQI among the students.



The significant results from the mean total CDLQI were further analysed at the level of the subscales in order to know which axis contributed significantly to the quality of life score. This is shown in Table 4.9. The leisure category had the highest CDLQI among the subscales. It was significantly higher in those who were having the first episode of PV infection, who had more than one body region affected, who had a positive family history of PV and who had additional symptoms associated with the lesions. Further bivariate analysis showed the number of body region affected by PV correlated positively with the leisure subscale ($r_s = 0.1$, $P = 0.043$). However, the one-way ANOVA was not significant (F = 2.806, df = 2, $P = 0.06$).

Essentially, the mean score of the students whose PV was pruritic or gave tingling sensation was significantly higher in all subscale except the treatment category when compared with those without additional symptoms. The mean score of those older than 13 years was significantly higher in the personal relationship and also symptoms and feeling category however, not in the other subscales. The score of those whose PV lesions were located on an unexposed body part was significantly higher in the sleep category but not in other subscales. Meanwhile there was no statistically significant difference observed in the treatment category.

Table 4.10. Relationship of the mean scores of the CDLQI subscales and some variables

Variable (no of students)	**S and F Mean**	**Leisure Mean**	**PR Mean**	**S or H Mean**	**Sleep Mean**	**Tx Mean**
Sex:						
Male (118)	1.07	1.77	0.58	0.47	0.47	0.82
Female(186)	1.07	1.66	0.46	0.52	0.51	0.85
	P=0.468	*P*=0.574	*P*=0.413	*P*=0.628	*P*=0.743	*P*=0.774
Age group						
≤ 13 years(8)	0.73	1.10	0.08	0.25	0.25	0.62
>13 years(296)	1.08	1.72	0.52	0.50	0.50	0.85
	P=0.177	*P*=**0.049**	*P*=**<0.001**	*P*=0.420	*P*=0.382	*P*=0.522
Family history						
Yes (111)	1.20	2.04	0.57	0.58	0.56	0.92
No (193)	0.99	1.51	0.47	0.45	0.46	0.80

	P=0.097	*P*=**0.015**	*P*=0.222	*P*=0.254	*P*=0.280	*P*=0.295
Age onset:						
<13 years (91)	1.33	1.75	0.46	0.57	0.46	0.80
≥13 years(213)	0.96	1.69	0.53	0.46	0.51	0.86
	P=**0.004**	*P*=0.786	*P*=0.501	*P*=0.331	*P*=0.649	*P*=0.640
PV Episode						
First (94)	1.20	2.18	0.62	0.61	0.61	0.91
Recurring(210)	1.01	1.49	0.46	0.45	0.44	0.81
	P=0.103	*P*=**0.002**	*P*=0.085	*P*=0.171	*P*=0.130	*P*=0.382
Body parts:						
Exposed (221)	1.06	1.60	0.48	0.50	0.42	0.86
Unexposed(83)	1.08	1.97	0.59	0.49	0.69	0.80
	P=0.615	*P*=0.111	*P*=0.660	*P*=0.973	*P*=**0.019**	*P*=0.606
No. of sites						
One site(208)	1.04	1.57	0.48	0.43	0.46	0.79
>one site(96)	1.14	1.99	0.58	0.65	0.57	0.96
	P=0.072	*P*=**0.041**	*P*=0.274	*P*=0.065	*P*=0.237	*P*=0.156
PV symptoms						
No (175)	0.86	1.42	0.40	0.39	0.34	0.78
	1.35	2.09	0.64	0.64	0.70	0.93
Yes (129)	*P*=**0.001**	*P*=**0.002**	*P*=**0.017**	*P*=**0.014**	*P*=**<0.001**	*P*=0.174

S and F = Symptoms and feeling; PR = Personal relationship;

S or H = School or Holiday; Tx = Treatment

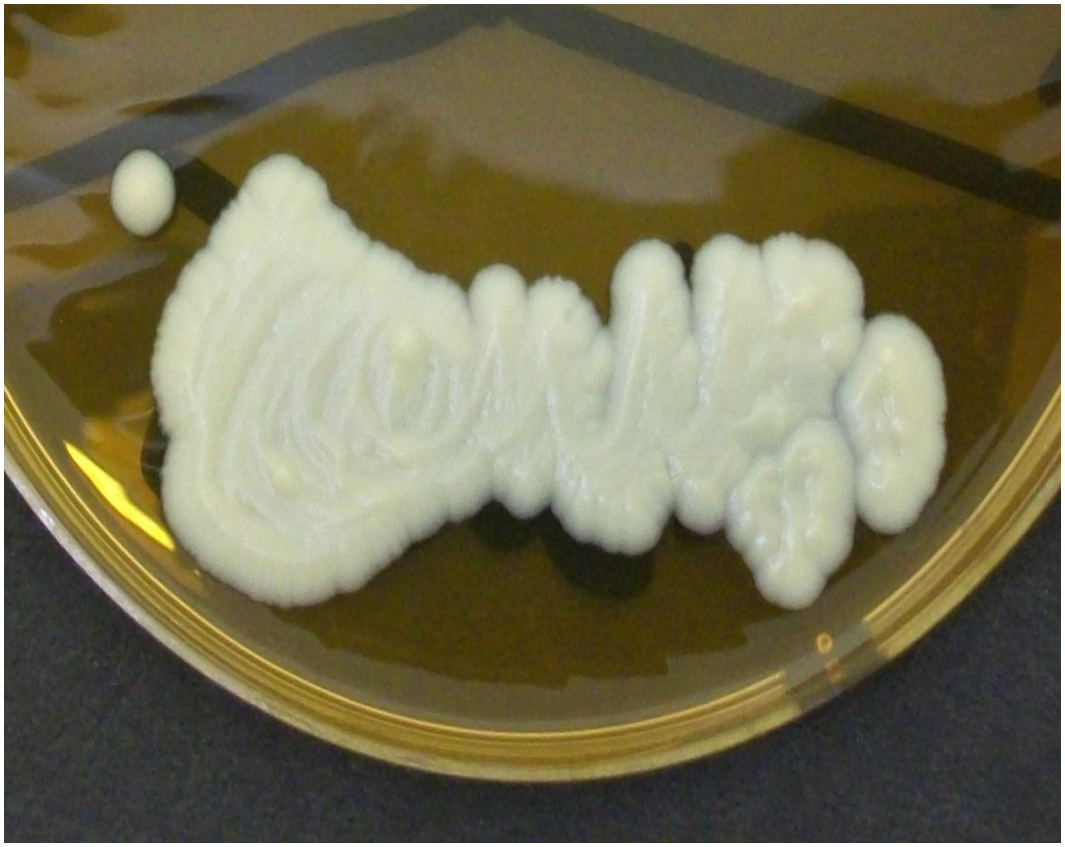
Questions were asked on how the students with PV perceived themselves when compared with other students. These questions included if they felt ashamed having PV, if they have low self-confidence and if having PV has prevented them from making friends. Their response was analysed with the mean of the total CDLQI. The results showed that slightly less than two-third of the students felt ashamed (64.0%) and acknowledged having low self-confidence (60.4%) while about one-sixth of the students (16.2%) agreed that PV did prevent them from making friends. The mean total CDLQI scores were higher in these three categories; however, the difference was only significant in those with low self-confidence and those who had difficulty making friends (Table 4.11 below.).

Table 4.11. Relationship between the mean total CDLQI scores and perception of the students

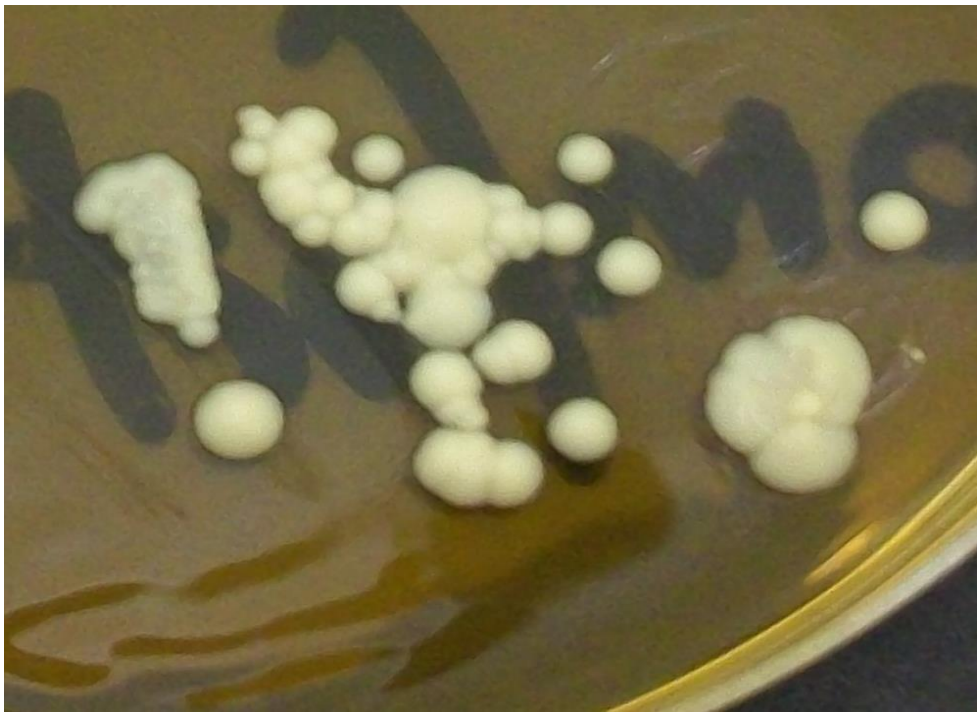
Variables	**Mean total CDLQI**	***P*-value**
Feel ashamed having PV Yes: n=71(64.0%) No: n=40 (36.0%)	 8.85 (7.5 - 10.18) 6.82 (4.89 - 8.76)	 0.80
Has low self-confidence Yes: n=67 (60.4%) No: n=44 (39.6%)	 9.16 (7.81 - 10.52) 6.52 (4.72 - 8.33)	 **0.019**
PV prevents from making friends Yes: n=18 (16.2%) No: n=93 (83.8)	 14.78 (11.93 - 17.63) 6.83 (5.81 - 7.84)	 **<0.001**

4.4. Culture and microscopy results

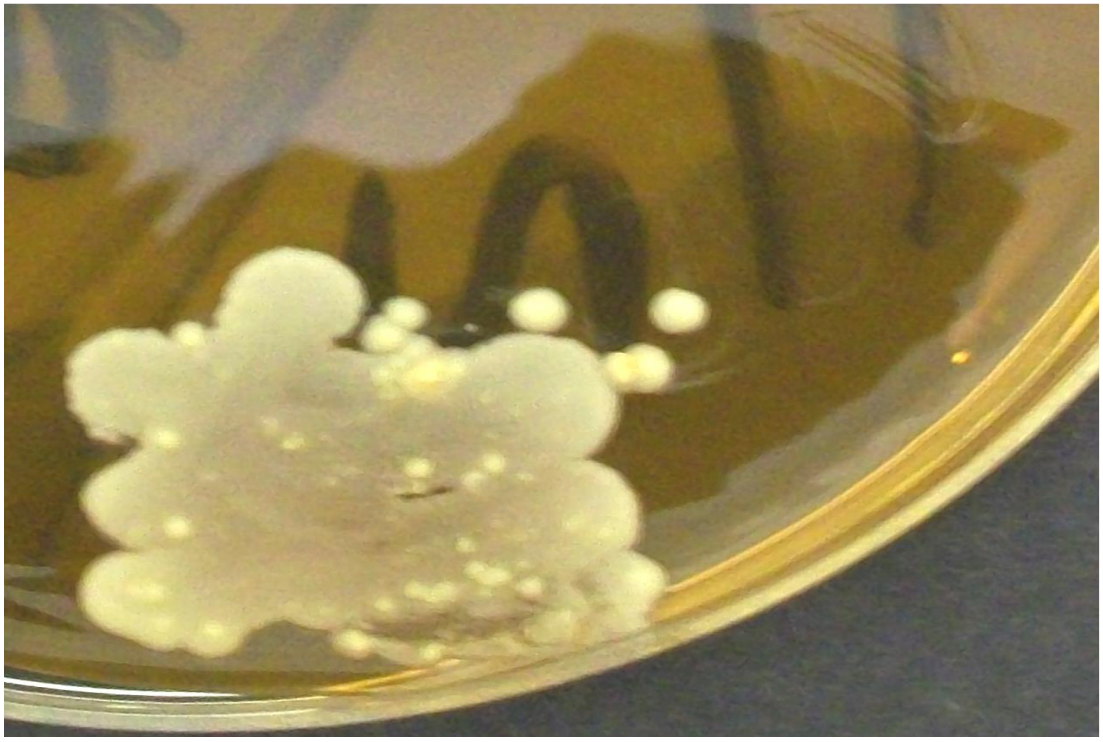
Figure 4.16. (a) Culture results of *Malassezia* species showing white glistening smooth surface and grooved edges. The texture was soft



(b) Showing cream coloured glistening smooth surface colonies and slight central elevation which is characteristic of *M. sympodialis*



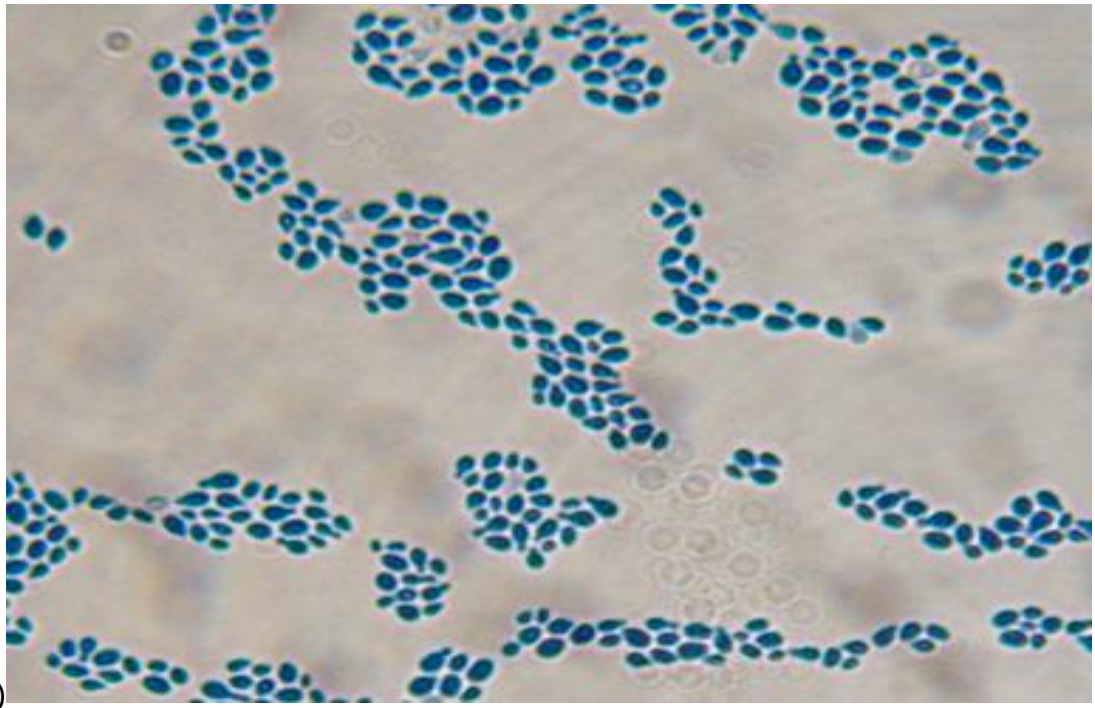
(c) This is in keeping with *M. restricta* showing colonies with dull surfaces, smooth edges and average diameter of 3mm. The texture was hard and brittle.



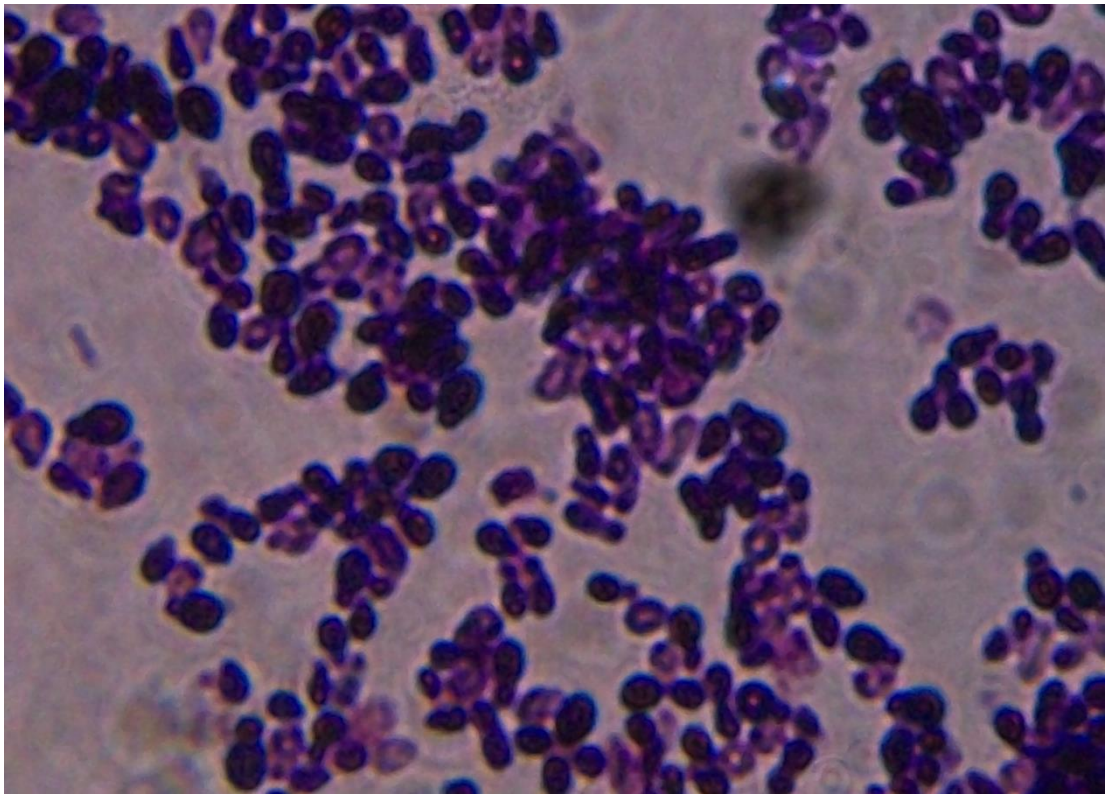
(d) These colonies are characteristic of *M. furfur* with smooth surface, convex elevations, average diameter of 5mm and soft texture

Figure 4.17. Microscopy of *Malassezia* culture showing the unipolar, enteroblastic budding from a characteristic broad base of large, oval cells. Separation leaves a prominent bud scar through which successive daughter cells emerge. (x400 magnification)

(a)



(b)



4.5. *Malassezia* species identified

Only three species were identified in this study; namely *M. furfur, M. sympodialis* and *M. restricta*. There were 175 samples collected from PV lesions which grew on the modified Dixon agar. Out of this number 143 (81.7%) were identified as *M. furfur*, 9 (5.1%) were *M. restricta* while 4 (2.3 %) were *M. sympodialis*. Exactly 10.9% of the cultured samples did not show an identifiable band on PCR despite several repeats done on DNA extraction.

The species distribution according to body site is as shown in Table 4.12 below. The PV lesions on the face had a 50% species identification rate while the back lesions had a higher yield at 61.2%. These two were the only sites where the three identified species were all isolated. The site with the highest positive species identification from culture was the scalp (80%) followed by the chest (68.6%) but just two species, *M. furfur* and *M. restricta,* could be isolated from these locations. Meanwhile, only one species, *M. furfur* was isolated from the upper arms lesions. Further, analysis did not find any significant correlation between the species identified and clinical features of affected students.

Skin samples were also collected from normal (non-lesional) skin. The scales collected were limited to the sebum-rich body regions. A total of 110 samples were collected, out of which growth was established in 58 (52.7%) cultures. *M. furfur* was identified in 45 (77.6%), *M. restricta* in 6 (10.4%) while no PCR band was seen in 7(12%). The species distribution according to body site is shown in Table 4.13 below. *M. restricta* was isolated only from the head and neck region and similar with species isolation from PV lesions, the face had about 50% species identification rate. This was lower than the percentage yield obtained from other body regions.

Table 4.12. Distribution of *Malassezia* species isolated from PV lesions according to body sites

Body site	***M. furfur***	***M. sympodialis***	***M. restricta***	**No growth**
face	79	1	3	82
back	25	2	3	19
chest	23	0	1	11
Upper arm	8	0	0	12
neck	6	1	0	5
scalp	2	0	2	1
Total	143	4	9	129

Table 4.13. Distribution of *Malassezia* species isolated from normal skin according to body sites

Body site	*M. furfur*	*M. restricta*	No growth
face	25	4	32
back	10	0	6
chest	9	0	6
Upper arm	9	0	7
neck	1	2	0
Total	45	6	53

Figure 4.18. 26S rDNA PCR giving a product of approximately 580bp before digestion by restriction enzymes shown with a DNA SmartLadder (200 to 10000 bp)

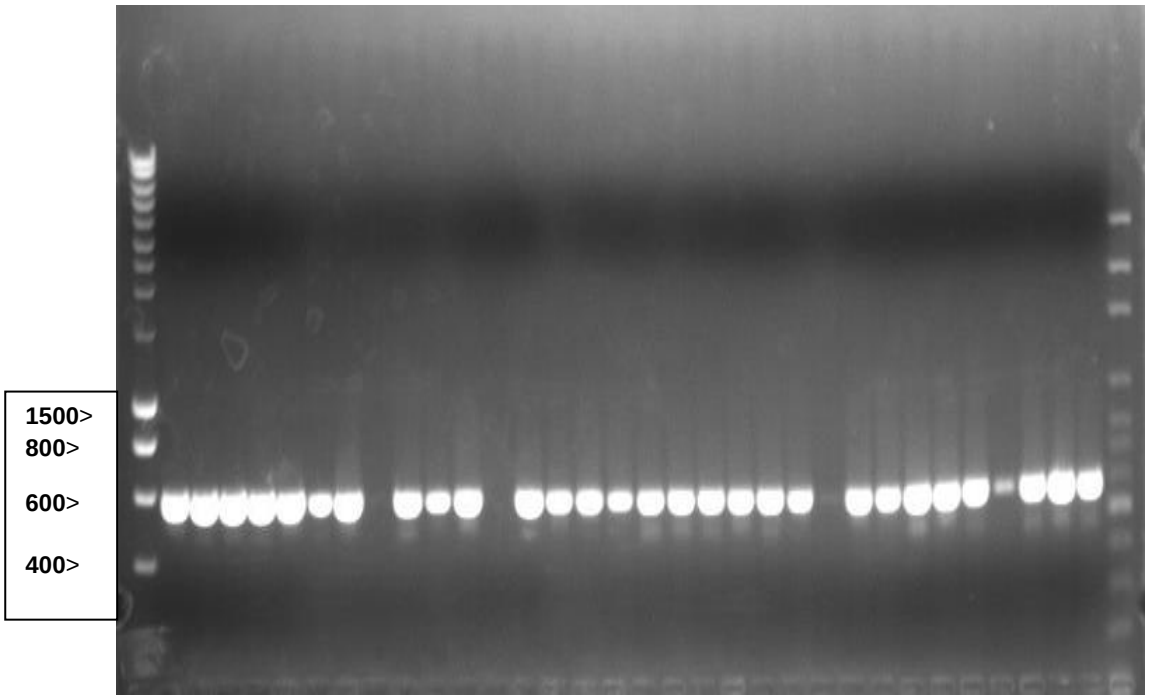


Figure 4.19. 580bp digestion with *Cfo* I showing 2 species identified: mf = *Malassezia furfur*; mr = *Malassezia restricta*, M = low molecular weight DNA ladder (25 to 766 bp)

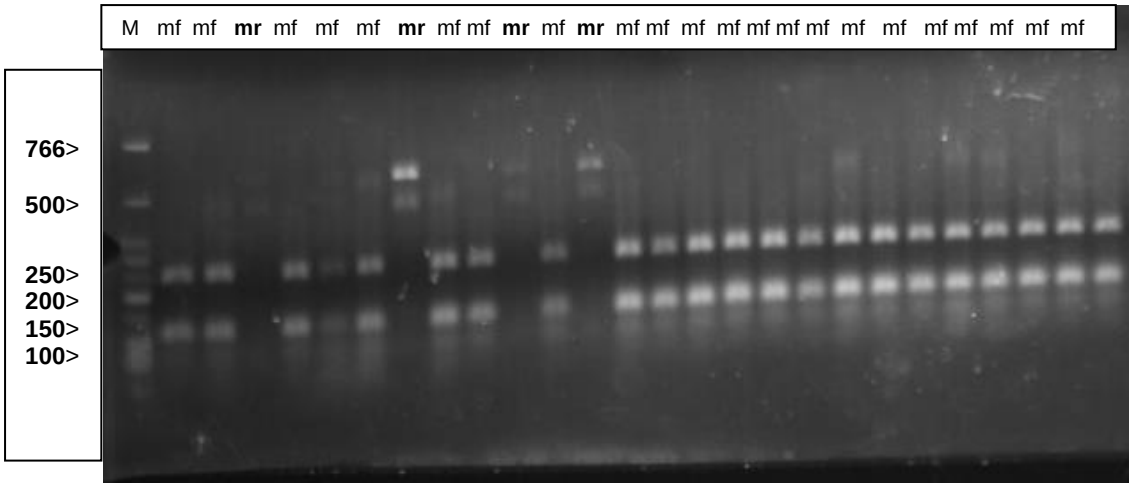


Figure 4.20. 580bp digestion with *Cfo*I showing 2 species identified: mf = *Malassezia furfur*; ms = *Malassezia sympodialis*, M = low molecular weight DNA ladder (25 to 766 bp)

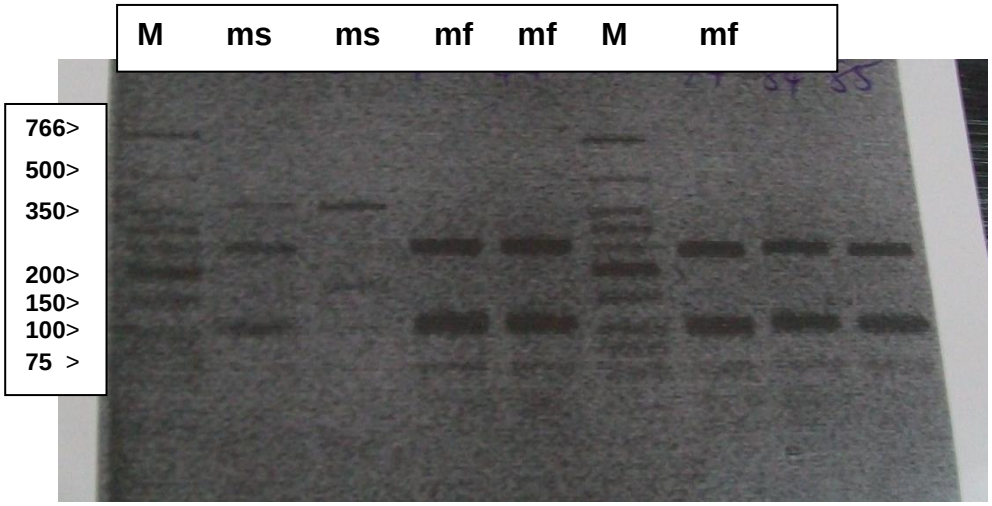
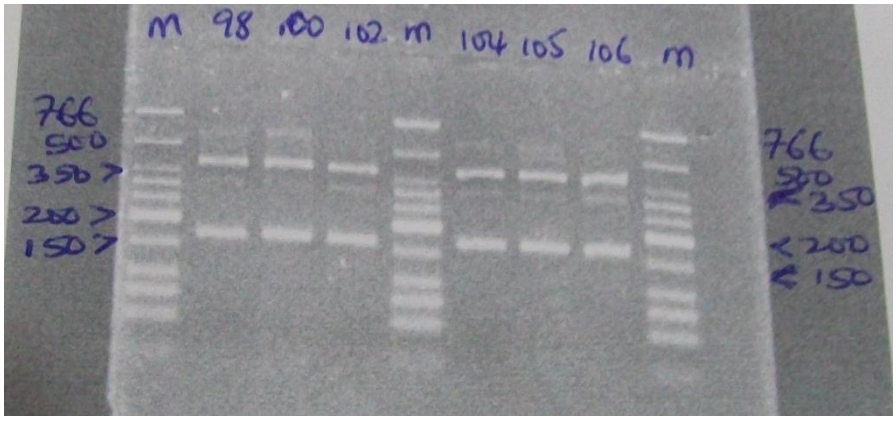


Figure 4.21. 580bp digestion with *BstC* I showing only *Malassezia furfur* at 400bp and 180bp, M = low molecular weight DNA ladder (25 to 766 bp)



CHAPTER FIVE

Discussion

5.1. Clinical characteristics of the participants

The average age of students with PV observed in this study was 16 years and the age group with the highest number of students was 14 up to 18 years. This is similar to previous studies conducted in Brazil, Dar es Salaam and Bangkok, where the highest prevalence of PV was observed in patients aged between 10 and 19 years, 16 and 19 years and 12 and 21 years respectively.(10, 11, 19, 56, 89) It also agrees with past observation of PV as an infection of older teens/adolescents when compared with dermatophyte infections.(4, 14, 57, 89) The reason can be attributed to the increase in activity of sebaceous glands(15, 25) which is linked with increased release of hormones especially androgens in this age group.(10, 149)

There was no significant difference between age and gender among the participants. Also more females than males were observed with PV in this study (M:F 1:1.6). Some studies have shown increased prevalence of PV in females;(11, 57, 89) while other reports suggest that there is no sex predilection.(3, 4) A major factor that could have contributed to the higher prevalence of PV in females in this study was the eagerness of the females to be participants. The other contributing factors may be the higher use by the female population of occlusive cosmetics namely talcum powders as well as skin lightening creams, some of which may also contain corticosteroid which is known to impair the integrity of the skin immune surveillance against superficial infections. Daily use of cosmetics has been observed to increase concentration of carbondioxide in the epidermis thus inducing a change in the microflora, producing low skin pH and encourage growth of the *Malassezia* yeast.(89)

This study showed that personal grooming habits such as having a daily bath did not prevent the development of PV in this environment. All of the students had at least one daily bath, about two-third used tap water (running water) and more than half of the students used soap to wash daily. This is always expected from school children and is a rule usually enforced by their teachers and the Principal of the school. Studies from India and the Central African Republic did not observe, similar to this study, any direct association with regards to personal hygiene and the development or spread of PV infection.(10, 46, 47) However, this is in contrast to a study in this environment on prevalence of skin infections and infestations among prison in-mates where a significant association was observed with poor hygiene.(53) Meanwhile, the humid and hot conditions of the prisons compared to the community may have been a confounding factor.

There was a higher prevalence of PV among those living in low income residential areas of Abuja where overcrowding, resulting in poor ventilation and higher environmental temperatures could be quite common. Majority of the parents were traders which can be regarded as a low income occupation. Some studies have

documented a higher prevalence of PV in patients from low socioeconomic background; however, similar with results from this study there was no statistically significant difference observed by Belec et al. in Central African Republic(10) and Jena et al. in India.(24) According to Belec et al, the clinical characteristics of PV did not differ distinctly within country or by the type of urbanization in which the patient lived.(10, 150)

This study suggested hyperhidrosis as an important factor in the development of PV. Although most of the students after school activity were not sports related, some of them were involved in other activities that could lead to excessive sweating such as farming and street trading. Most importantly, sweating is common in our environment as gadgets that cool the temperature such as air-conditioning and fans are not readily available. Presently electricity is also erratic. The average atmospheric temperature recording in the time course of the study was as high as 38^0 C which is a strong predisposing environmental factor to PV development.(3, 17, 48, 53, 149) None of the students had associated chronic systemic diseases or were in any immunocompromised state.

A family history was associated with the development of PV in this study. About one third of the students had at least one member of the nuclear family with a history of PV. This was observed more among siblings than parents. A slightly higher result was obtained by Rao et al. (38.3%) in India(47) while lower percentages of 22.2% and 25%, respectively, were observed by Hafez et al.(21) and Ghosh et al.(46) Genetic factor has been linked to increased sebum production in those infected with PV.(1, 7, 149) This combines with environmental factors to predispose individuals to PV. Meanwhile students with the presence of a family history of PV were significantly younger than those without a family history (13.3 vs 13.9 years). This was expected and also agrees with previous observations.(21, 45, 47) Unpredictably, the relationship between recurring PV and having a positive family history was not observed in this study unlike in above mentioned studies. It is worthy to note that having a family history of PV was not influenced by socioeconomic status or gender.

5.2 Clinical features of PV

The average age of onset of PV in this study was 13.7 years. This is the mean age when an increase in sebaceous activity is observed.(25) Although females with PV developed the disorder at a slightly younger age than males, this difference was not significant. Besides, study by Luebberding et al. observed a significant difference in sebum production in both genders, with the production always higher and stable with increasing age in the males and progressively decreases over lifetime in the females.(151) Meanwhile females begin puberty at an earlier age than males.

The area of residence of participants from this study had an effect on the age of onset of PV. The students who lived in the low income residential part of Abuja with poor availability of amenities including housing and increased population were

observed to develop PV at a younger mean age of 13.3 years in contrast to those living in the more expensive city centre region of Abuja. Belec et al.(10) compared the characteristics of PV patients and non-PV patients with their place of residence and found no significant difference between the two groups. In contrast, this study compared place of residence within PV patients. The earlier age of onset in this group of students could be related to poor living conditions and possible persistence of poor ventilation and high environmental temperatures; however, further research would be needed to confirm this finding. Meanwhile, studies looking at factors that could affect the age of onset of PV need to be included in the literature.

There are four main classes of moisturizers based on their mechanism of action. They include the occlusives, humectants, emollients and protein rejuvenators. Occlusives provide exogenous barrier for the skin and marked reduction in transepidermal water loss (TEWL). A well know prototype of occlusive moisturizers is petrolatum. Petrolatum was shown to have an occlusive effect on the skin, stimulate skin lipid biosynthesis and permeate all levels of the stratum corneum in a research by Ghadially et al.(152) These processes were observed on normal skin and have been confirmed by Patzelt(153) and Stamatas et al.(154) However, studies with regard to the relationship of petrolatum and *Malassezia* infection are sparse. Koyama et al. was able to demonstrate a florid growth of *Malassezia furfur* but not of *Malassezia globosa* in a culture plate containing petrolatum.(155) Humectants increase water content of the skin by enhancing water absorption from the dermis into the epidermis. The absorbed water can easily evaporate into the environment, thus producing increased TEWL. The most common humectant is glycerol or glycerine which is a major component of most of the body creams used by the participants. In this study, about 51% of the students mainly males applied petrolatum namely Vaseline® on a daily basis. These students developed PV at a younger age than those who did not use Vaseline® (13.3 vs 14.1 years). This difference was found to be statistically significant $P = 0.004$. Meanwhile, a statistically significant difference was not observed among those who applied body creams routinely.

Dandruff also known as pityriasis capitis or mild seborrheic dermatitis is a non-inflammatory scaling condition of the scalp(73) which commonly affects postpubertal individuals and has been associated with *Malassezia, Acremonium, Penicillium and Cryptococcus* species hence the good results obtained after treatment with antifungals. Non microbial causes of dandruff include dust, heat, cosmetic use etc.(73) Studies on the pathogenesis and management of this very common scalp disorder are few and inconclusive. This study observed a very high percentage (22%) of PV students with concomitant pityriasis capitis; higher than were observed by Ghosh and Rao et al. who observed 10% and 11.6%, respectively.(46, 47) Generally, not all individuals with *Malassezia* on their skin have dandruff(156) and also the level of *Malassezia* on the normal scalp tends to be doubled that on dandruff scalp.(73) In this study the students who had pityriasis capitis were found to have an

earlier age of onset of PV infection (12.6 vs 14.0 years; p=0.001). The reason for this association is, however, unclear and may be related to non-studied endogenous factors that could predispose these individuals at an earlier age to fungal infections. Although the information on the age of onset of pityriasis capitis by this group of students was not obtained, it would be interesting to know if there is any relationship with the age of onset of PV. Meanwhile, there were no significant differences observed between those who had pityriasis capitis and recurring PV; thus the fact that an individual has dandruff does not predispose him/her to recurring episodes of PV. Probably this could be because the etiological agent of the pityriasis capitis was not necessarily the source of the PV. Further studies are needed to clarify this.

Animals have been observed to have *Malassezia* infections although the species differ slightly from those that infect man,(66, 68) a study have shown that the possible transfer of zoophilic *Malassezia pachydermatis* to human skin is quite rare.(157) A small number of the students had regular contact with animals (20%) and there was no relationship between having animal contact and PV infection from this study.

On the use of bleaching products by the students, 14.5% of them acknowledged their use. These were mainly female students. This is predictable as females are known to be more beauty conscious, thus use more cosmetic products than males. It is also known that females tend to be disturbed by blemishes on the skin. However, the clinical features of PV in this group of students did not differ significantly from the other students in the study. According to case-control study reports by some authors, pityriasis versicolor is not as common as dermatophyte infections in population who use bleaching products.(158, 159)

Pruritus and tingling sensation are major symptoms associated with PV. Slightly less than half of the students in this study had pruritus/tingling sensation (42.8%). Similar percentages were also reported by Ingordo et al. in Italian sailors, Rao et al. in India and Morais et al. in Brazil.(17, 46, 160) It was also observed in this study that the students who sweat excessively are more likely to have associated symptoms of pruritus and tingling sensation. Although this association was not found in literature, since research in this area is sparse, excessive perspiration could also be associated with pruritus as seen in disorders of keratinization. It could also act as a confounding factor in PV studies. Another reason for the pruritus could be due to the effect of dehydration of the stratum corneum from the increased perspiration. This is corroborated by reviews that have shown PV to be more pruritic when lesions are exposed to high temperatures. Meanwhile a higher skin humidity provides a better growth environment in the skin for *Malassezia* yeasts.(7) A study by Santana et al. correlated pruritus in PV patients with skin type and observed that pruritus was more frequent in patients with dehydrated epidermis.(89)

Recurrence of PV is commonly reported. About two-third of the students had recurring episodes. This is similar to reports by Framil and Morais et al.(144, 160)

who noted recurrence rate of 67.4% and 52.6%, respectively. However, this study did not find significant differences among these students when analysed with regard to their use of petrolatum and presence of excessive sweating, as well as having a positive family history of PV which had been previously linked to recurrence in PV. It is possible that endogenous factors may predispose individuals to recurrent/relapse episodes of PV and not only the presence of environmental factors such as hyperhidrosis, application of body oils and occlusive dressing.(4) Rao et al. attributed the reason for the high recurrence rate in PV infection to the presence of *Malassezia* in individual's hair follicles.(47) There is need for studies investigating the actual factors that predispose individuals to PV recurrence. This is grossly not explored in the literature.

The lesions seen in our study participants were hypopigmented macules which usually start perifollicularly as seen in Figure 4.13. This corroborates Rao et al. speculation on the presence of *Malassezia* in individual's hair follicles. The lesions then coalesce to form larger macules. Some of the lesions had a brawny scaly appearance especially at areas which were not accessible to scrubbing during a bath. Lesions on the face had minimal scaling. Hypopigmentation of PV lesions is a common presentation in a darker-skinned population. Akin findings were reported in studies conducted in Africa, Brazil, India and Bangkok.(10, 11, 46, 47, 60, 89, 160) The reason still remains unclear. This study, comparable with other authors did not observe any correlation between the colour of the lesions with personal hygiene, clinical features and presence of a family history.

Most of the lesions were located on the face. This confirms literature reports describing facial distribution of PV lesions as common mostly in children and black people,(10, 24, 56, 89, 149, 161) in contrast to the rarity of facial involvement in the white skinned population. In this study, frequent sites involved by PV were the face, back and chest. Parallel findings were noted in studies by Ghosh and Rao et al.(46, 47) Extensive PV which can be described as PV involvement of 3 or more body regions was observed in 8.2% of the students. This is comparable with the study by Pönnighaus et al. in Malawi who observed 8.0%(15) and Terragni et al. in Italy who observed 8.9%(149) but lower than figure obtained by Jena et al. in India who observed extensive PV in 45 (16.6%) children.(24) In this study, students with extensive PV were significantly associated with PV symptoms of pruritus and tingling sensation. No known similar study has compared PV symptoms with extent of distribution of lesions. Macular pattern of PV lesions as well as follicular, confluent and guttate patterns was observed in this study as were observed by other authors.(46, 47) Inverse distribution of PV was not detected.

Medications used by the students were mostly over-the-counter antifungal products. Most of these products are sold in combination with highly potent steroids. Meanwhile 10.5% of the students used folk remedies to treat PV, particularly hydraulic (brake) fluid which has been observed by some studies in Nigeria and Botswana as a common means utilized by less educated individuals to treat most

skin diseases at home.(52, 162) The students acknowledged clearance of the lesions on application of hydraulic fluid, though, there was usually a recurrence. This clearance could be due to propylene glycol a common base stock for hydraulic fluids. Propylene glycol is one of the several non-specific topical antifungal agents available for PV treatment. However, due to the other chemical compounds also found in hydraulic fluids, these students need to be informed that dermal exposure to these fluids could lead to signs of skin irritation.

5.3 Health related quality of life (HRQoL) of students with PV

The total quality of life score observed in this study was 7.39 (95%CI 6.73-8.06) which is approximately 25% impairment in HRQoL. This figure is close to the total CDLQI score (7.17±3.03) observed in a Nigerian community cross-sectional study of children with tinea infection.(163) Comparing this effect with the comparative study done by Beattie et al.(164) the level of impairment (25%) caused by PV was higher than urticaria (20%) and acne (18%) but lower than psoriasis (30.6%) and atopic dermatitis (30.5%).

According to the health behavior in school aged children (HBSC) 2009/2010 report, age, gender, socioeconomic, and environmental differences are significant social determinants of health and well-being among young people.(165) In this study, there was a significant increase in HRQoL impairment among the students with PV who were older than 13 years and those who had PV among member(s) of their nuclear family. This was similarly observed in the study by Akinboro et al. where the CDLQI score was significantly higher in children with tinea capitis older than 13 years.(163) The increase in impairment observed in the students with PV who also had a family member with PV could be due to the additional stress they felt having someone they care about with a skin disorder. In this study, the difference in gender, social environment and level of education did not have a significant effect on their quality of life.

A large percentage of students had recurring PV. They had a lower HRQoL impairment than those having PV for the first time. This was unpredictable since chronic or recurring skin diseases are expected to produce a negative impact on individual's psychosocial well-being as seen in the study on hand dermatitis and psoriasis.(166, 167) Perhaps, since the comparison is between individuals with recurring disease and first timers, the anxiety and sensitivity to peoples' opinion felt by the recurring disease individuals had waned with time. More studies are needed to further evaluate this aspect because the recurring nature of PV has been generally assumed to contribute to the psychosocial well-being of patients.(142)

It is interesting to observe that students with PV lesions on unexposed parts of the body had lower quality of life than those who had on exposed body parts although this difference was not statistically significant. This observation was similarly documented in the paper of Ludwig et al.(168) in which no significant difference in

quality of life was found associated with the sites of skin lesions. Correspondingly, quality of life did not differ significantly between students having hypopigmented PV and hyperpigmented PV. This could be because the skin is regarded as a whole and indivisible organ; hence no matter the region of the body affected or the colour of the lesion, the presence of a skin disorder is a cause of embarrassment and anxiety for the patient.(167, 169)

Although the percentage of students with PV who had reduced self-confidence and were ashamed of the lesions was high, the significant impairment in quality of life associated with low self-confidence and reduced ability to make friends is, however, inconclusive as a comparative study with students without PV would be required.

The HRQoL impairment correlated positively with the number of body regions afflicted with PV. The increase in number of involved sites could be related with the disease severity which has been shown to correlate with patients' quality of life.(163, 168, 169)

Students who had pruritus or tingling sensation associated with PV had significantly higher CDLQI scores. Pruritus is an important factor affecting QoL,(170) thus it is expected that it would impair quality of life and this should be taken into consideration when treating patients with PV.

The leisure category contributed mostly to the quality of life impairment observed in this study. Since the burden of skin disease encompasses psychosocial and financial consequences on the patients, their families and the society,(171) it is obvious from this study that PV was a contributor to the psychosocial burden of the students. The treatment category was third in rank as factors that impair quality of life but it did not make a statistically significant impact. Generally, skin diseases that itch, have unacceptable appearance or not easily treatable tend to significantly impair social relations, psychological status and day-to-day activities of patients.(167, 171) Unfortunately, a lot of individuals underestimate the effect that PV has on the quality of life of adolescents in the Tropics. This should be noted and addressed in its management.

In summary, quality of life determinants in students with PV observed in this study included age of the patient, number of episodes of PV infection, existence of a family member with PV, number of body regions affected and the presence of associated pruritus or tingling sensation. The gender, level of education and the type of neighborhood where the students reside did not significantly contribute to their quality of life. Overall, this study has demonstrated the negative impact which PV has on the quality of life among adolescents and more studies are being called especially from areas with high PV prevalence as this knowledge can lead to improved patient management.

5.4 Identification of *Malassezia* species

In this study, three species were identified from PV lesions namely, *Malassezia furfur, Malassezia restricta* and *Malassezia sympodialis*, of which 81.7% was composed of *M. furfur*. A higher percentage of *M. furfur* (77.6%) was also observed on non-lesional skin samples. Two species only were identified from non-lesional skin (*M. furfur* and *M. restricta*). This observation is quite different from most studies on the identification of *Malassezia* species from PV. These studies have indicated *M. globosa* as the most prevalent causative species.(62, 70, 102, 105, 172-175) They were studies from temperate and mediterranean climates of the world, namely, Spain, Japan, Bosnia-Herzegovina, Israel, Tunisia and Italy. The few investigations where *M. furfur* have been most prevalent were from tropical and subtropical climates such as Thailand, Indonesia, Brazil and Venezuela.(11, 108, 176, 177) Furthermore, the method of identification used by these studies from tropical climates was based on the physiological and biochemical characteristics of the cultured yeast while this study used the molecular analysis method. This difference should not affect results obtained just as a study by Romano et al. did not observe differences in the identified *Malassezia* species when comparing conventional culture-based with the molecular based methods.(173)

Further studies from Brazil(106, 107) and Argentina(20) observed *M. sympodialis* as the most prevalent species in PV and this was consistently followed by *M. furfur*. Yet, Crespo-Erchiga et al.(66) agree with the more than 80 years old hypothesis which postulates that a second species other than *M. globosa* (mostly observed in temperate climates) could be predominant in warmer and more humid climates. This study is subject to their call for larger and reliable studies from a tropical climate to test this hypothesis. Not only is *M. furfur* the most prevalent species in PV lesions, it was also the most prevalent on non-lesional skin. By this result, we can conclude that *M. furfur* is the causative agent of PV in Nigerian students. It is possible to observe so very little number of *Malassezia* species per study as was seen in a report from Israel where *M. globosa* was mostly detected in the lesional and non-lesional skin of patients with PV at a rate of 97.3% and 80.8%, respectively.(175)

Prevalent studies on identification of *Malassezia* species from sub-Saharan Africa are sparse. The study in Sudan on the molecular epidemiology of *Malassezia* in patients with PV was restricted to the identification of *M. globosa* and *M. restricta.*(147) Saad et al. looked at the level of colonization of only these 2 species using a molecular-based, culture-independent method while comparing diseased with healthy individuals. Further studies are required to observe the possible prevalence of all *Malassezia* species and how they compare with the above mentioned species and thus determine the most prevalent causative species of PV in sub-Saharan Africa.

In this study, the second most prominent species identified though at very low percentage was *M. restricta* which is in contrast also with most studies where *M.*

sympodialis is, in order of frequency, the second most common species isolated from culture.(102, 108, 176) Also different in this study is the failure to identify *M. sympodialis* from healthy skin. *M. sympodialis* has been observed to predominate on healthy skin of the trunk, both in non-lesional areas in PV patients and healthy controls.(66, 72, 157) The reason could be related to the absence of *M. globosa* which is known to be associated almost always with *M. sympodialis.*(20, 66)

A comparable report in this study with other authors on the identification of *Malassezia* species is the higher culture and isolation rate of *Malassezia* obtained from the scalp (80%) and chest (68.6%) in contrast with other regions of the body. Also similar is the more common recovery of *M. restricta* from the upper regions of the body (scalp, face and neck) than the lower body.(76, 178, 179) The correlation of the different species isolated with the clinical characteristics of the participants as well as the PV lesions did not reveal any significant difference. This is in line with all reported studies on the identification of *Malassezia* species from patients with PV. No distinct feature has been linked with a specific species.

In this study, the recovery rate of *Malassezia* species from cultures both from lesional and non-lesional sites were 57.6% and 52.7%, respectively, which was most comparable to the study by Lim et al.(100) but lower than the yield observed by Tarazooie et al.(48) and Nakabayashi et al.(70) This was not predicted as the study participants were within the age group when the highest positive culture rate was expected. (76, 179) The difference could be related to the sampling technique used and the growth conditions of the culture. Similar to the study conducted by Tarazooie et al.(48) only single species per culture were identified in this study unlike many other studies where more than one species could be recovered from a sample. Some of the cultures were covered up by faster growing species of other fungi. It was difficult to maintain a pure culture and to discriminate a species from a mixed sample, thus, some *Malassezia* species might have been lost after several subcultures.

5.5 Conclusion

PV was more prevalent in older teenagers and females. The clinical presentation was similar to those of other studies with the mean age at first presentation of infection observed at 13.7 years. The age of onset of PV was significantly affected by a positive history of PV in genetically related family members, socioeconomic status, and the daily use of petrolatum and concomitant presence of pityriasis capitis (dandruff). The recurrence of PV among the students and the colour of the lesions were not linked with inadequate personal grooming methods, clinical features or family history of PV. The facial location of the lesions was more prevalent, a location similar with those of other studies of children and black population while the extent of distribution of the lesion was associated significantly with presence of pruritus and dysesthesia.

The quality of life determinants in students with PV observed in this study included age of the patient, number of episodes of PV lesions, presence of a family member with PV, number of body regions affected and the presence of associated pruritus or dysesthesia. The gender, level of education and the type of neighborhood where the students lived did not significantly contribute to their quality of life while a moderate effect on the students' HRQoL was observed.

Only three species were identified from this study with the main prevalent species being *Malassezia furfur* which was observed in both lesional and non-lesional skin samples. Finally, as observed in other studies, no correlation was found between the species and clinical features of PV lesions.

5.6 Limitations of the study

1. This was a cross-sectional study. Most of the information used were as related by the students.
2. Some students did not come forward because they did not want to be recognized as having PV.
3. Poor culture yield of the samples; this could have affected the number in species identified as the RFLP-PCR was done on culture results.
4. The skin scales yield even with the use of the Sellotape was poor in some cases. Most were affected by sweat which caused poor Sellotape - skin adhesion as well as absent yield on skin scrapping as samples were collected in the heat of the afternoon. Most of the lesions especially the facial PV had little or no scales. Also the use of sponge and daily bath of the students could have resulted in the poor skin scales yield.
5. There were several contaminations from fast growing species of other fungi which covered up the *Malassezia* species and their subsequent loss after several subcultures.

CHAPTER SIX

Recommendations

1. RFLP-PCR can be used even in tropical climate to identify the different species of *Malassezia.*
2. Since the culture based PCR yields a little above 50% of species identification, and the direct skin scale based PCR requires a good amount of scales which could be affected by sweat and personal hygiene practices, a better method of sample collection needs to be developed to adequately study the presence of PV *Malassezia* species in a tropical environment.
3. PV had a negative effect on the quality of life of these students; an effective management guideline should be developed and if possible with focus on how the factors affecting onset of PV can be controlled. This could reduce its prevalence.
4. Most of the students took daily body bath. There is need for public awareness to remove the general believe that PV was associated with poor personal hygiene and thus reduce the self-stigmatization of patients.
5. There is prerequisite for more studies to look into other factors that could affect the age of onset of PV. This may probably explain why the infection affects certain individuals and spare others even when they share similar environment.

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APPENDIX I

Patient information on the study: "Correlation of *Malassezia* species with clinical characteristics of pityriasis versicolor"

Principal investigator:
Dr. Perpetua Ibekwe [MBBS (Ib), FMCP]
National Hospital,
Abuja
Nigeria
Tel: +2348033192661
Email: perppy_u@yahoo.com

Abuja, February 05 2013

Dear Participant,

You have volunteered to participate in the above named study. Please read the patient information carefully. You can also request for more information from your doctor.

Pityriasis versicolor known commonly in Nigeria as “eczema” or “Ugwa” in igbo, “Ifo in yoruba”, “Makenkero” in hausa is that light- or dark- colored, flat, usually asymptomatic rash found on ones face, upper chest and upper back. It can easily return after treatment.

This rash is actually caused by a fungus called *Malassezia* and it resides normally in the outer part of your skin. This fungus can be found in everyone but for some unconfirmed reason it causes this skin infection in only some people. There are 13 types, also called species of *Malassezia* which are distributed based on climatic conditions, ethnic origin, and body site of the affected individual. There are studies conducted in a few countries showing the different species of *Malassezia* available there but none yet from our country Nigeria.

The species of *Malassezia* found on one’s skin lesion are known to play a role in the clinical characteristics of pityriasis versicolor. Our aim is to describe the clinical characteristics of your skin lesion, that is the pityriasis versicolor and to identify the associative *Malassezia*.

To this end, we wish to obtain skin scale samples from the region which has the rash by the use of swabs/sellotape. I assure you that this method is safe and does not bring you to any bodily harm.

The scales are cultured in a special medium where we expect this fungus to grow. These grown fungal cells are further analyzed by observing their physical characteristics through the microscope. These will be done at the laboratory of the Department of Microbiology, National Hospital, Abuja. Afterwards, we will carry out molecular analysis of the same fungal cells to identify their species. This part will be done at the laboratory of the dermatology hospital of the Ludwig Maximilian University, Munich, Germany.

The data samples (skin scrape/cultured fungus/isolated material) will be destroyed not later than one year after their evaluation. Despite the isolation of genetic material from the fungi, no genetic studies of human genes are being made.

Participation is voluntary and you may withdraw your consent at any time and without notice.

We will greatly appreciate your voluntary participation in this research as it will support our efforts to broaden our knowledge on pityriasis versicolor and *Malassezia* from an environment such as ours and thus improve on better patient management. For inquiries, please contact us at any time.
Dr. Perpetua Ibekwe

Informed consent on the study: "Correlation of *Malassezia* species with clinical characteristics of pityriasis versicolor"

Patient Name:

Patient Address:

I have been informed verbally and in writing about the above named study by the physician named below. I have read through the written patient information. I also understand the voluntary nature of my participation and am aware of my right to revoke my consent at any time without prejudice.
My consent is being requested to collect skin samples from the pityriasis versicolor lesions on my body. The nature of the investigations to be carried out on the samples has been explained to me.
I have noticed that genetic material will be isolated and analyzed only from the fungal cells grown from the skin sample. The extracted biological material from fungi on my skin is destroyed completely in a year.

I have a copy of the full Patient Information for this study and a signed copy of this consent form.

_____ _____
Date, signature of participant Signature of the doctor

_____
Date, signature of parent/guardian

Data protection statement

In this study, the rules on medical confidentiality and privacy are respected. The collected data will not include personal details (i.e., neither your name nor your initials or date of birth will be used), just some medical details related to pityriasis versicolor. An identification of the participant from the fungal sample will be impossible (all data will be irreversibly anonymized). For this reason, in case you wish to withdraw your consent after anonymization, the data cannot be identified and destroyed any more.
Only the principal investigator, Dr. Perpetua Ibekwe will have access to the original medical data. The documents will be in paper form at the Department of Microbiology, National Hospital, Abuja, Nigeria. It will be kept for up to 10 years after completion or termination of the study in a room inaccessible to unauthorized persons. In the case of publications of the study results, the confidentiality of personal information is guaranteed by the anonymization.

I hereby agree for my medical data to be collected in this study and to be stored in paper form in the Department of Microbiology, National Hospital, Abuja. They may be used according to the information given in the patient information above. I hereby agree that the medical data collected from my medical information and skin sample can be reported in accordance with the agreed patient information.

_____ _____
Date, signature of participant signature of the doctor

_____
Date, signature of parent/guardian

APPENDIX II

QUESTIONAIRE ON PITYRIASIS VERSICOLOR
(please fill up or tick where appropriate)

1. ID No._____
2. Sex M__ F___
3. Age_____
4. Where do you **live**? _____
5. Do you live in a Flat, Bungalow, Duplex, Face-me-I-face-you, or_____(circle one only)
6. How many rooms are in your house _____
7. How many people in total live in your house _____
8. Tribe _____ State of origin_____
9. Class in the school _____
10. Do you live with your parents_____ or guardian_____
11. Level of education of parents/guardian: University/college of ducation/polytechnic
    Secondary school
    Primary school
    None
12. **Occupation (please be specific)** of
    Mother:_____
    Father:_____
    Guardian:_____
13. What do you normally do after school: Do sports___, Do household chores____
    Watch TV____, Go to the farm_____
    Visit friends__, Have afternoon naps__
    Read novels___ Sell things in market___
    Others (pls specify)_____
14. Personal habits: **Tick** the appropriate answers (**you can tick more than one**)

I take my **bath** everyday:	YES	NO
I use **tap water** to bath:	YES	NO
I use **sponge** to scrub my body:	YES	NO
I use ordinary **toilet soap** to bath**:**	YES	NO
I use **medicated soap** to bath:	YES	NO
I use **talcum powder** everyday:	YES	NO
I use **body cream** after bathing:	YES	NO
I use **skin toning/bleaching** body creams:	YES	NO
I use **vaseline:**	YES	NO
I **share** my towels and cloths:	YES	NO
I sleep **alone** on my bed:	YES	NO
I **sweat** a lot **during the day**:	YES	NO
I **sweat** a lot **at night**:	YES	NO
The cloths I wear **make** me sweat:	YES	NO

I have PV during the **hamattan** season____ and/or **rainy** season____

15. My PV is **worse** during the **hamattan** season____ and/or **rainy** season_____
16. I noticed the rash when I was _____ **years old**
17. Which **parts of your body** do you have PV_____
18. Are you **ashamed of** the lesions? Yes____ No____
19. Does the presence of the lesions **reduce your self-confidence**? Yes___ No___
20. Has the lesions **prevented** you from **making friends**? Yes____ No_____
21. Should the lesions be treated? Yes___ No___
22. What did you use to treat your PV: drugs__, herbs__,
    battery water__, engine oil__
    antifungal creams___ others____
23. Course of the lesions. Please **tick** the approriate **answers** (you can tick more than one)
    The lesions **continue** to stay **without treatment**_____
    The lesions **continue** to stay even **with treatment**_____
    The lesions **returns after treament**____
    The lesions **disappears without treatment** and then **returns**_____
24. The lesions cause **itching sensation**___ **tingling sensation** ___ **no sensation**___
25. There is someone in my **family who has PV**. Yes____ No_____
    If yes, is it your: Father / Mother / brothers / sisters (**circle the appropriate ones**)
26. Do you have any other sickness? Yes / No If yes _____
27. Do you have **dandruff**? Yes____ No____
28. Do you have a **House pet**? Yes / No If yes Dog/Cat/Others_____
29. Do you have regular **contact** with **animals** Yes____ No____
30. Over the last week, how **itchy**, **"scratchy"**, **sore** or **painful** has your skin been?
    Very much □
    Quite a lot □
    Only a little □
    Not at all □
31. Over the last week, how **embarrassed** or **self-conscious**, **upset** or **sad** have you been because of your skin?
    Very much □
    Quite a lot □
    Only a little □
    Not at all □
32. Over the last week, how much have your skin affected your **friendships**?
    Very much □
    Quite a lot □
    Only a little □
    Not at all □
33. Over the last week, how much have you changed or worn **different** or **special clothes** because of your skin?
    Very much □
    Quite a lot □
    Only a little □
    Not at all □

34. Over the last week, how much have your skin trouble affected **going out**, **playing**, or **doing hobbies**?	Very much Quite a lot Only a little Not at all	□ □ □ □
35. Over the last week, how much have you avoided **football** or **other sports** because of your skin trouble?	Very much Quite a lot Only a little Not at all	□ □ □ □
36. Last week, was it **school time?** ⇨ **If school time**: Over the last week, how much did your skin problem affect your **school work**?	Prevented school Very much Quite a lot Only a little Not at all	□ □ □ □ □
OR		
was it **holiday time?** ⇨ **If holiday time**: How much over the last week, have your skin problem interfered with your enjoyment of the **holiday**?	Very much Quite a lot Only a little Not at all	□ □ □ □
37. Over the last week, how much trouble have you had because of your skin with other people **calling you names**, **teasing**, **bullying**, **asking questions** or **avoiding you**?	Very much Quite a lot Only a little Not at all	□ □ □ □
38. Over the last week, how much have your **sleep** been affected by your skin problem?	Very much Quite a lot Only a little Not at all	□ □ □ □
39. Over the last week, how much of a problem has the **treatment** for your skin been?	Very much Quite a lot Only a little Not at all	□ □ □ □

Please check that you have answered EVERY question. Thank you.

EXAMINATION (This will be done by me)

40. Colour of rash Hypopigmented_____ Hyperpigmented_____
41. Pattern of lesion: Macular___
    Follicular___
    Confluent___
    Guttate_____
42. Location of rash _____

43. Other Dermatological disorders present_____

44. Skin scraping location: Lesion_____
    Nonlesion_____

45. Microscopy: predominant morphology_____
    size_____
    budding base_____
46. Wood'lamp: Positive_____ Negative_____
47. Culture Macroscopy: Shape_____
    Size_____
    Color consistency_____
    Characteristics of surrounding media_____
48. Species identified _____

APPENDIX III

NATIONAL HOSPITAL
The Presidency
(Established by Act No 36 of 1999).

ABUJA

NHA/ADMIN/236/V.VII/

28th March, 2012

Re: A Cross-sectional study to Correlate Malassezia species with clinical characteristics of pityriasis versicolor

Health Research Ethics Committee (HREC) Assigned number: **NHA/EC/167/2012**

Name of Principal Investigator: Dr. Perpetua Ibekwe

Address of Principal Investigator: Block 3, Flat F, Road 45
First Avenue, Gwarinpa
Gwarinpa II Abuja.

Date of Receipt of Valid Application: 31st January, 2012

Notice of Approval

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and *given full approval by the Health Research Ethics Committee, National Hospital Abuja.*

This approval dates from **28th March, 2012** to **27th March, 2014.** If there is delay in starting the research, please inform the HREC National Hospital Abuja so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study.*

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

Dr. O. Ekumankama
Chairman, HREC, National Hospital Abuja

Plot 132 Central District (Phase II) P.M.B. 425, Garki - Abuja Nigeria
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E-mail: info@nationalhospitalabuja.net www.nationalhospitalabuja.net

APPENDIX IV

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

ETHIKKOMMISSION BEI DER LMU MÜNCHEN

Ethikkommission · Pettenkoferstr. 8 · 80336 München

Herrn
Dr. Dr. M. Sárdy
Klinik f. Dermatologie u. Allergologie
Frauenlobstr. 9-11
80337 München

Vorsitzender:
Prof. Dr. W. Eisenmenger
Telefon+49 (0)89 5160 - 5191
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Ethikkommission@
med.uni-muenchen.de

www.ethikkommission.med.uni-muenchen.de

Postanschrift:
Pettenkoferstr. 8a
D-80336 München

Hausanschrift:
Pettenkoferstr. 8
D-80336 München
München, 05.11.2013 Hb /sc

Titel:	**Correlation of Malassezia species with clinical characteristics of pityriasis versicolor**
Antragsteller	**Dr. P. Ibekwe**
Projekt- Nr	**077-13**

Sehr geehrter Herr Kollege Sárdy,
sehr geehrte Frau Kollegin Ibekwe,

besten Dank für Ihr Schreiben (Eingang 05.11.2013) mit der Beantwortung unserer Fragen bzw. Erfüllung der Auflagen und den noch ausstehenden bzw. überarbeiteten Unterlagen (Schreiben Dr. P. Ibekwe, Patient /parents' information and informed consent forms, EK- Antrag, Originalbescheide).

Die Ethikkommission (EK) kann Ihrer Studie nun die ethisch-rechtliche Unbedenklichkeit zuerkennen.

Vorsorglich möchte ich darauf hinweisen, dass auch bei einer positiven Beurteilung des Vorhabens durch die EK die ärztliche und juristische Verantwortung für die Durchführung des Projektes uneingeschränkt bei Ihnen und Ihren Mitarbeitern verbleibt.

Änderungen des Studienprotokolls sind der EK mitzuteilen. Für Ihre Studie wünsche ich Ihnen viel Erfolg.

Mit freundlichen Grüßen

Prof. Dr. W. Eisenmenger
Vorsitzender der Ethikkommission

APPENDIX V

FCT - SECONDARY EDUCATION BOARD
EDUCATION SECRETARIAT

P.M.B. 151, Garki, Abuja. Tel: 09-2341148

Block 3, Area 3,
Garki, Abuja.

Our Ref:.....

Your Ref:.....

Date:..11th April, 2013....

The Principal

Sir/Ma,

CONVEYANCE OF AP0PROVAL

I am directed to convey the approval of the Board for **Dr. Perpetua Ibekwe** to conduct her Ph.D research in your school on the topic "**Correlation of Malassezia Species in patients with Pityriasis Versicolor**".

2. She wish to obtain skin scale samples from the region which has the rash by the use of a transparent plaster. This method is safe and does not bring any bodily harm.

3. This study has the ethical approval of the National Hospital Ethical Committee.

4. Your assistance will be highly appreciated.

Olasoji Vincent
CEO (Student Affairs)
For: Director (SEB).

ABUJA
The Heart of Nigeria

LIST OF PUBLICATIONS

1. Ibekwe P. U (2012). Textbook on Prevalence of HHV8 in AIDS Patients with Kaposi's sarcoma", ISBN 978-3-8473-3610-5, Lambert Publishers Germany
2. Ibekwe, P.U. (2012). Social stigmatization of two sisters with Lamellar ichthyosis. Int. J. Dermatol. Jan;51(1):67-8. doi: 10.1111/j.1365-4632.2011.05060x
3. Ibekwe P.U (2011). Kaposi's Sarcoma in HIV-infected men and women in Nigeria. AIDS Patients Care and STDs. Nov;25(11):635-7.
4. Ibekwe P.U (2011). Dermatological manifestations of HIV in Nigerian Patients. Access Dermatology. October 2011
5. Ibekwe P.U (2011). Immunohistochemical detection of Human Herpes virus-8 in epidemic Kaposi's sarcoma. Presented at the World Congress of Dermatology Korea(Seoul) 2011) and published in the book of Abstracts
6. Ibekwe, P.U (2010). Psoriasis co-existing with vitiligo in a female patient with human immunodeficiency virus infection. *Kosmetische Medizin* ; Vol 31:68-72
7. Ibekwe, P.U (2008). Migraine and Meniere's disease: two different phenomena with frequently observed concomitant occurrences. J NatlMed assoc; Vol 100(3):334-338
8. Ibekwe, P. U (2007). Paediatric Otorhinolaryngology Emergencies: A Tropical Country's Experience. Emerg Med Australasia; Vol. 19(1):76-77.
9. Ibekwe, P. U (2005). Spectrum of Otorhinolaryngology Emergengies in the Elderly in Ibadan Nigeria. Journal of Medicine; Vol 114 (4): 411-414