DISTRIBUTION OF MALASSEZIA SPECIES IN PATIENTS WITH PITYRIASIS VERSICOLOR IN KOLAR REGION

By

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Under the guidance of

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I hereby declare that this dissertation titled **"DISTRIBUTION OF MALASSEZIA SPECIES IN PATIENTS WITH PITYRIASIS VERSICOLOR IN KOLAR REGION"** is a bonafide and genuine research work carried out by me under the guidance and supervision of **Dr. BEENA P. M** MD, Professor & Head, Department of Microbiology and Co-guidance of **Dr. SHIVAKUMAR V.** MD, DNB, Professor, Department of Dermatology, Sri. Devaraj Urs Medical College, Tamaka, Kolar.

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LIST OF ABBREVIATIONS

KOH	→	Potassium Hydroxide
SDA	→	Sabouraud's Dextrose Agar
DMSO	→	Dimethyl Sulphoxide
LPCB	→	Lacto Phenol Cotton Blue
RFLP	→	Restriction Fragment Length Polymorphism
PCR	→	Polymerase Chain Reaction
MLEE	→	Multilocus Enzyme Electrophoresis
PFGE	→	Pulse Field Gel Electrophoresis
RAPD	→	Random Amplified Polymorphic
AFLP	→	Amplified Fragment Length Polymorphism
ELISA	→	Enzyme Linked Immuno Sorbent Assay
TE Slant	→	Tween Esculin agar slant

ABSTRACT

BACKGROUND AND OBJECTIVES

Pityriasis versicolor is a superficial, chronically recurring fungal infection of stratum corneum. The etiological agents of this superficial surface infection belong to the genus Malassezia. Malassezia is a member of normal skin flora of human beings. Under the influence of certain exogenous and endogenous factors, the commensal yeasts transform into filamentous pathogenic forms. Recent taxonomic revision of the genus Malassezia, is classified into 14 species, in that only seven species have been well studied in relation to pityriasis versicolor.

This study aims to find out the a) distribution of Malassezia species in Kolar region, b) distribution in patients of different age groups and c) to find out any correlation between the species with clinical presentation.

MATERIALS AND METHODS

Hundred clinically diagnosed cases of pityriasis versicolor were included in the study. The clinical specimens were collected under aseptic precautions and subjected to culture on SDA overlaid with olive oil and mDixon agar. The isolates were identified by biochemical tests.

RESULTS

Of the 100 cases 73% were males, 26% were females. Predominant age group was 21-30 years (57%) followed by 31-40 years (27%). Out of 100 samples 70 yielded growth. The most common isolate was M. sympodialis (50%), followed by M. furfur (32.86%), M. globosa (14.28%) and M. sloofiae (2.86%). Among 100 cases 74% had hypopigmented and 26% had hyperpigmented lesions. M. sympodialis and M. furur were predominantly isolated from hypopigmented lesions and M. globosa and M. slooffiae were found to be more common in hyperpigmented lesions. The distribution of different species in patients with hypo or hyperpigmented lesions did not show any statistical significance which may be due to lower sample size.

CONCLUSION

M. sympodialis was the most common isolate, followed by M. furfur, M. globosa and M. slooffiae. Adult males between 21-30 years were most commonly affected. Hypopigmentation was the most common clinical presentation. There was no significant difference in distribution of different species in patients with hypo or hyper pigmented lesions.

KEYWORDS:

Pityriasis versicolor; Malassezia

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1. INTRODUCTION

Pityriasis versicolor is a superficial, chronically recurring fungal infection of stratum corneum. The lesions are characterised by scaly, hypo or hyper pigmented irregular macules, most often seen on upper part of trunk, neck, face and upper aspect of arms.[1] It is more common in young adults than children with a peak incidence at 20 years. It has a worldwide distribution, though it is more frequent in tropical region (40%) due to relatively high temperature and humidity.[2,3]

The etiological agents of this superficial surface infection belong to the genus Malassezia. Malassezia is a member of normal skin flora of human beings.[4] Under the influence of certain exogenous and endogenous factors, the commensal yeasts transform into filamentous pathogenic forms.[5] Mycelial phase of the organism predominates in lesion of Pityriasis versicolor.

Clinically, the disease is asymptomatic, usually patient seeks medical attention for cosmetic purposes.[1] Cutaneous infection with Malassezia can manifests either as papulosquamous lesions, folliculitis, inverse tinea versicolor or rarely as pityriasis versicolor rubra.[1]

Initially, the mycelial phase of organism was designated as Malassezia and yeast phase was divided into two species based on morphology: Pityrosporum ovale and Pityrosporum orbiculare. But later it was recognised that both were variants of same species and designated them as Malassezia furfur. Prior to 1990 only three species were

recognised. [6,7] With the development of molecular techniques, 14 species have been described. Nine of the fourteen species, M. furfur, M. sympodialis, M. globosa, M. restricta, M. slooffiae, M. obtusa, M. dermatis, M. japonica and M. yamatoensis are associated with normal human flora and can cause skin lesions. Five species M. pachydermatis, M. nana, M. equina, M. caprae and M. cuniculi are associated with animals.[6,8]

Malassezia species have been associated with diverse dermatological lesions like Pityriasis versicolor, Seborrheic dermatitis, Atopic dermatitis, Pityrosporum folliculitis, Psoriasis, onychomycosis and blepharitis. Though generally associated with very mild superficial infections, it is emerging as an opportunistic pathogen causing systemic infections in immunocompromised patients and those receiving intravenous lipid emulsion.[6]

Diagnosis of Pityriasis versicolor is mainly based on clinical history, physical examination and simple tests such as Wood's lamp examination of lesions, which shows yellowish fluorescence of the involved skin. Laboratory confirmation can be made by 10% potassium hydroxide (KOH) examination of skin scraping, which reveals characteristic "spaghetti and meat ball" or "banana and grape appearance" and the isolation of Malassezia species from clinical sample by inoculation on Sabouraud's Dextrose Agar (SDA) overlaid with olive oil. Special medias such as Leeming Notman agar and modified Dixons agar can also be used for isolation.[3,9,10] Species can be identified by biochemical tests such as esculin hydrolysis, cremophor EL, glycine assimilation and tween assimilation.

2. OBJECTIVES OF THE STUDY

- To find out the distribution of Malassezia species in Kolar region.
- To find out the distribution of Malassezia species in patients of different age groups.
- To find out whether, there is any correlation between the species with clinical presentation

3. REVIEW OF LITERATURE

3.1 HISTORY

The Genus Malassezia has been recognised as member of normal skin flora as well as organisms involved with superficial cutaneous infections.[11] Eichstedt in 1846 and Sluyter in 1847 first observed and described the fungus causing Pityriasis versicolor.[12,13] They named the disease Pityriasis versicolor, but did not propose the name of fungus. In 1853 Robin described the organism in scales of the patients with pityriasis versicolor. He thought the fungus was similar to dermatophyte, Microsporum audounii and named it Microsporum furfur.[12] Revolta in 1973 reported that similar type of yeasts isolated from Psoriasis patients. In 1874 Malassez described typical round and oval budding yeast cells and called them "spores". Bizzozero in 1884 observed spherical as well as elliptical yeast like cells and named them Saccharomyces sphaericus and Saccharomyces ovalis respectively.[13,14] Baillon in 1889 proposed the name M furfur to describe furfuraceous nature of skin lesions. Sabouraud's in 1904 named the budding yeast cells without hyphal elements as Pityrosporum malassezii.[12,14] Castellani and Chalmers in 1913 termed this organism as Pityrosporum ovale.[13,14] In 1927 Acton and Panja from India considered Pityrosporum to be a synonym of Malassezia, but this significant finding did not receive much attention.[12]

Benham in 1939 described the lipophilic nature of Malassezia organisms.[12] Gordon in 1951 isolated a spherical to oval yeast from scales of pityriasis versicolor. He named the organism Pityrosporum orbiculare.[12,14] Sternberg and Keddie in 1960 detected same antigenic components in both P orbiculare and M furfur, by using fluorescent

antibody technique and proposed that both are synonymous. Burke in 1961 was able to produce clinical pityriasis versicolor by inoculating P. orbiculare on the skin of person with high plasma cortisol levels.[12] Dorn and Roehnert in 1977, succeeded in showing the invitro conversion of globose cells into hyphal forms using variety of substances.[15] In 1981 Redline and Duhm reported that M furfur not only causes superficial infection but also invades deep tissue. Later Hassal et al reported the same in young children receiving intravenous lipid therapy and Shek et al reported the disseminated infection in premature infants.[12]

3.2 PHYLOGENY AND TAXONOMY

The Genus Malasezia was recognised as Basidiomycetes, although no sexual reproduction was observed. The data supporting the classification include laminar ultrastructure of cell wall as revealed by electron microscopy, unipolar sympodial budding, resistant to cell wall lysis by beta(1,3) D glucurase and capacity to hydrolyze urea, positive reaction to the staining with Diazonium Blue B (DBB). On the basis of molecular studies, confirmed that the genus Malassezia belongs phylogenetically to phylum Basidiomycota.[3] They are now classified as:

Kingdom: Fungi

Phylum: Basidiomycota

Class: Basidiomycetes

Order: Malasseziales

Genus: Malassezia

The genus Malassezia has undergone several taxonomic revisions till 1990. The taxonomic revision by Guillot and Gueho in 1995 based on G+C content of chromosomal DNA, sequencing of the large subunit rRNA and nuclear DNA complementarity studies defined seven species of Malassezia: M. furfur, M. symopodialis, M. obtusa, M. globosa, M. restricta, M. slooffiae, M. pachydermatis.[3]

In the last few years on the basis of DNA relatedness, few more species have been isolated from human skin namely M. dermatis, M. yamotoensis and M. japonica and from animal skin, namely M. nana, M. caprae and M. equine, M. cuniculi thereby increasing the number of species to fourteen.[8,16-19]

3.3 NOMENCLATURE

The designation of Malassezia was previously used to describe the mycelia phase of organism where as yeast phase was divided into distinct species based on microscopic morphology namely P. orbiculare and P. ovale.[9]

Much debate had arisen over nomenclature of these yeast like organisms and till recently the designation M. furfur, P. orbiculare and P. ovale were used universally and interchangeably.[9]

Table 1: Shows the various species of genus Malassezia, with both new and old nomenclatures[9]

New Nomenclature	Old Nomenclature
Malasszia furfur	Malasszia ovalis, P. malassezii, P ovale
Malasszia pachydermatis	P. pachydermatis
Malasszia sympodialis	M. furfur – serovar A, M ovalis
Malasszia globosa	M. furfur – serovar B, P. Orbiculare
Malasszia restricta	M. furfur – serovar C
Malasszia obtusa	No previous nomenclature
Malasszia slooffiae	No previous nomenclature
Malasszia dermatis	No previous nomenclature
Malasszia yamatoensis	No previous nomenclature
Malasszia japonica	No previous nomenclature
Malasszia nana	No previous nomenclature
Malasszia caprae	No previous nomenclature
Malasszia equi	No previous nomenclature

3.4 MYCOLOGY

The Malassezia is lipophilic dimorphic fungus, occurs as yeast and mycelia forms.[20] The normal human skin flora consists predominantly the yeast phase where as mycelia phase predominates in skin lesions.[21] Yeasts cells morphologically variable occur as spherical, cylindrical or oval forms. The mycelial form comprises short, septate hyphae, arrange at an angle or end to end with occasional branching.[11,14] Pathological specimens consists predominantly hyphae with clusters of spherical yeasts described as 'spaghetti and meat ball' appearance.[22]

3.5 PHYSIOLOGY AND BIOCHEMISTRY

The cell wall of Malassezia species is relatively thick and multilaminar with characteristic invagination of the innermost layer. They reproduce by budding on a broad base. The daughter cell separate by fission.[23]

Malassezia require addition of preformed fatty acids for their growth due to inability of organism to synthesise long chain fatty acids.[24] Malassezia species elaborate range of enzymes and metabolites. They have lipolytic activity both in vitro and in vivo indicating the production of lipase. Also produce phospholipase, lipooxygenase etc. Lipoperoxides produced by the action of lipooxygenase may damage cell membrane and interfere with cellular activity – a mechanism which results in alteration in skin pigmentation.[24,25]

All species assimilate glucose and mannitol but not lactose, lactic acid, succinic acid.[26] All Malassezia species hydrolyse urea.[9]

3.6 EPIDEMIOLOGY

The Pityriasis versicolor is worldwide in distribution, but more common in tropical and sub tropical with incidence of 40% than temperate climate.[17,27] It is more common in summer than in winter.[27] The lesions are restricted to anatomical sites covered by clothing suggesting the role of increased heat and moisture in the pathogenesis of lesions.[21]

It is more common in young adults with the peak incidence at 20yr age group. This may be because of hormonal changes and excessive sebum secretion.[21] It is rare in childhood, as healthy children below one year age group do not carry Malassezia on their skin. It occurs in both sexes and ratio of male to female is either nearly equal or higher in males.[28] There is no evidence to indicate racial predilection in the incidence of the disease.[9]

3.7 ECOLOGY

The Malassezia yeasts are most commonly isolated from the skin of warm blooded animals because of two physiological peculiarities: They have an absolute requirement for lipids to grow and survive. They are mesophilic with optimum temperature requirement of $30\text{-}35^{0}$c.[3]

The presence of Malassezia species in healthy human skin had been detected in the second half of nineteenth century. The frequency and density of colonization are related to the subject age and to sebaceous gland activity in area.[3] 95% of clinically normal people carry Malassezia on the scalp and 92% carry on their trunk. Malassezia pachydermatis is mainly associated with otitis externa in dogs.[29] Recently, some case have been reported where M. furfur and M. Obtusa were isolated as agents of otitis externa in dogs and M. Sympodialis in a similar condition in cats.[30] Many studies have already demonstrate that Malassezia lipid dependent species also colonize the skin of many other animals like monkeys, pigs, rhinoceros, bears and birds.[31]

Crespo Erchiga et al reported that M. Sympodialis appears as the predominant species in healthy skin, especially on the trunk and on face at lower percentage along with M. Globosa. M. restricta predominant in scalp.[3] Aspiroz et al. also reported that M. restricta was associated particularly with scalp, M. sympodialis with the back, M. globosa was evenly distributed on scalp, forehead and trunk. These data are similar to those published by Midbley in UK.[3,32]

There are various studies which shows different rate of distribution of different species. Differences encountered among these studies could be due to the different sampling technique, culture media and possibly also by ethnic and geographical factor. But all these studies confirm that M. sympodialis, M. restricta, M. globosa and M. furfur are common inhabitants of human skin.

There are very few studies on epidemiology and ecology of Malassezia species from India. Kindo et al. from southern India showed M. sympodialis as the commonest agent (58.3%) followed by M. globosa (39.6%).[4] Dutta et al from northern central India showed that the M. globosa(54%), followed by M. furfur (30%).[33] Chaudhary et al. from central India showed M. Globosa (58%), followed by M. sympodialis (25%), and M. furfur (7%). They also isolated M. obtusa (7%) and M. restricta (3%).[7]

3.8 IMMUNITY

The immunological response to Malassezia yeasts in Pityriasis vericolor patients have been investigated with equivocal results. Wu et al found an increase in antibodies in patients compared to control subjects, but other studies did not found any significant difference between two groups.[3,24,34]

Earlier studies showed deficient cell mediated immunity in Pityriasis versicolor patients.[35] The patients with Pityriasis versicolor showed diminished lymphocyte transformation response to Malassezia compared with normal controls.[13] In addition larger numbers of langerhans cells are found in the epidermis and an increase in T cells in both dermis and epidermis of skin lesions.[36] Sometimes an inflammatory response with perivascular inflammation may be present in association with Pityriasis versicolor. The perivascular infiltrate contains lymphocytes, plasma cells and histiocytes, which are involved in immune reaction.[37] Malassezia can also activate complement by both the classical and alternative pathways, contributing to the inflammation often seen in Pityriasis versicolor patients.[13]

3.9 PATHOGENESIS

The occurrence of clinical disease by Malassezia depends on the factors permitting conversion of the saprophytic yeast phase of the organism to mycelia phase.[38] The factors contributing for conversion can be classified as endogenous and exogenous.[9,13,22,39]

Endogenous

- High sebum levels at puberty
- Excessive sweating
- Malnutrition
- Pregnancy
- Hyperhidrosis
- Cushing's disease
- Corticosteroid therapy
- Immune deficiency disorders

Exogenous

- High temperature
- Application of oil

The disease is initiated by alteration in usual relation between host and resident yeast flora that facilitates mycelia conversion or by the transmission from a source patient. Genetic factors and subtle variation in cell mediated immunity also predispose to disease.[40]

The Malassezia produce a lipid soluble low molecular weight substance which acts as chemotactic for leucocytes and induces inflammation.[41] Clinically, patient presents with macular, erythematous, hyperpigmented (chromic) or hypopigmented (achromic) lesions with fine scaling.

The process of lipoperoxidation by Malassezia accounts for hypopigmentation by causing damage to cell membrane and interfere with cellular activity.[25] Hypopigmentation is also explained by azelaic acid, a dicarboxylic acid produced when Malassezia grown in the presence of oleic acid, which inhibits enzyme DOPA tyrosinase, involved in production of melanin causing hypopigmentation.[9] Another explanation is damage to melanocytes and by blocking of the UV light by lipid like material accumulated in stratum corneum. Hyperpigmentation is by abnormally large melanocytes, a thick stratum corneum and a hyperaemic inflammatory response.[42]

Electron microscopic studies have revealed abnormally large melanosomes in hyperpigmented and smaller than normal in hypo pigmented lesions.[9]

The variety of disease states linked to infection or colonization by Malassezia

Table 2: Diseases associated with Malassezia[22]

Conditions where Malassezia has definite role	**Other conditions where the role of Malassezia has been proposed**
Superficial cutaneous: Pityriasis versicolor **Systemic**: • Intravenous catheter associated fungemia • Endocarditis • Interstitial pneumonia • Peritonitis in chronic ambulatory peritoneal dialysis patients	• Seborrheic dermatitis • Atopic dermatitis • Folliculitis • Neonatal cephalic pustulosis • Confluent and reticulate papilomatosis • Guttate psoriasis • Onychomycosis • Balanitis • Lacrimal canaliculitis and dacryolith • Sinusitis • Nipple discharge • Otitis externa

3.10 CLINICAL FEATURES

The yeasts of genus Malassezia are associated with number of human diseases, basically affecting the skin, although in the last two decade a number of opportunistic systemic infections have also been implicated.[3]

Pityriasis versicolor is the most common skin disease caused by various species of genus Malassezia. In the past it was known as Tinea versicolor under the misconception that it is caused by dermatophytes. Therefore, the term 'pityriasis' is preferred nomenclature and 'tinea' is reserved for various clinical types of dermatophytes. Recently, variants with different colours have been given various names like with red macule (Pityriasis versicolor rubra) and another with black ones as (Pityriasis versicolor nigra).[9]

Pityriasis versicolor is a asymptomatic, mild, chronic, recurrent superficial fungal infection of stratum corneum, characterised by slightly scaly patches of variable discoloration ranging from hypo to hyper pigmentation. As implied in name versicolor means significant variation in colour as lesions may be hypopigmented, hyperpigmentad, leukodermal, erythematous, or dark brown (versi means several).[9]

Patients usually presents with multiple well defined, non inflammatory, maculopapular lesions with fine scaling which are discrete and vary in appearance from hypo to hyperpigmented depending on degree of pigmentation of surrounding skin and seeks medical advice only for cosmetic purpose. Mycelial phase of fungus predominates in lesions. Lesions most commonly appear on the upper part of trunk, neck, back, upper

arms and abdomen. Extension to the thighs, neck and forearm may occur , but lesions of scalp, palm and feet are rare.[13] Lesions can also occur on penis, most commonly with the involvement of the trunk as well, although occasionally as the only area of outbreak.[43,44] Lesions have been reported on other unusual locations, without any involvement of the trunk: groin, nipple and periareolar area (in a male patient), the groin and axillae, the groin and perineum, the webbing of the left hand and the antecubital fossae and forearms.[45] The colonization of the ear by Malassezia has been linked to ear wax and may play a role in tinea versicolor of the ear canal.[46]

In Southern Spain, Crespo Erchiga et al (2000) performed epidemiologic studies, comparing isolation of Malassezia species from Pityriasis versicolor lesions. In that survey, M. globosa 60% followed by M. sympodialis 29% and M. slooffiae 7% were isolated. Studies carried out by Aspiroz et al (2002) also supported the earlier findings.[12,32] Midgley concluded that the species responsible for Pityriasis versicolor could vary with the body site and different regions of the globe.

OTHER CLINICAL CONDITIONS ASSOCIATED WITH MALASSEZIA INFECTION

1. **Seborrheic dermatitis:** Is a chronic, relapsing, inflammatory disorder of skin, characterized by greasy, scaly, reddish patches localised in sebum rich areas such as the scalp, eyebrows, paranasal and middle thoracic regions. Commonly affects children and young adults. Dandruff which affects 5-10% of the adult population is considered to be the mildest or initial from of the disease. Seborrhoea is combination of Latin word ‘sebum’ meaning ‘grease’ and Greek word ‘rhoea’

used for ‘flow’. The term refers to oily appearance of skin and not to increased secretion of sebum.[3,9] AIDS patients have an increased incidence of Seborrheic dermatitis, may be due to immunosuppression. The higher incidence is also seen in patients with neurological diseases, multiple sclerosis and Parkinson’s disease.

Guptha et al found that Malasezia globosa was predominant species in Seborrheic dermatitis.[21] Nakabayashi et al. found both M. globosa and M. restricta on diseased skin.[47]

Studies have shown the levels of Malassezia specific antibodies against yeast antigen are consistently raised in seborheic dermatitis patients.[14]

2. Atopic dermatitis

It is a chronic intensely pruritic, inflammatory disorder of skin which affects genetically predisposed individuals.[48] The lesions are localised to scalp, face and neck. The earliest clinical signs are erythema, papules and pruritis. Secondary excoriation and lichenification occurs on scratching and rubbing. Skin prick test for type I hypersensitivity is positive in patients with atopic dermatitis. Tengvall et al found that specific IgE in serum of patients with atopic dermatitis and they concluded that Malassezia can trigger an eczematous reaction in sensitized patients.[48,49]

3. Malassezia folliculitis

Malassezia folliculitis is chronic inflammatory skin disorder characterized by florid, acneform, pruritic eruptions. It was first reported in 1969 by Weary et al. The lesions localised mainly to back, chest and to lesser extent to shoulder and arms.[3,7] Typically lesions begin as inflamed hair follicle that progress to papules or pastules and are puritic. In immunocompromised patients, lesions spread rapidly and accompanied by high fever.[10] The conditions which predispose to Malassezia folliculitis include diabetes mellitus, pregnancy, Cushing's syndrome, chronic renal failure, renal transplantation, bone marrow transplantation, malignancy and administration of broad spectrum antibiotics or corticosteroids.[22]

4. Systemic Malassezia infections

Systemic infection by Malassezia species have been reported in premature neonates, low birth weight, hospitalized neonates or adults receiving infusion of intravenous lipid preparation as part of parenteral alimentation.[3,9,14] The source of the fungus in such cases is usually patients own skin flora, the hands of health care workers, contaminated disinfectant or contaminated hub. The long chain fatty acids in intravenous lipid solution facilitate the growth of the organism along the lumen of the indwelling catheter and depending on the host immune status there is systemic spread. Examination of the catheter in such cases reveals adherent fungi, maximally along the distal lumen, often visible as white clumps.[44]

Two groups of patients most commonly affected; neonates with cardiovascular disease and immunosuppressed host. The lung is most frequently involved and patient usually presents with lipid deposits in pulmonary arteries. The patient may also have positive blood culture and few develop multiple cutaneous pustules.[9]

5. HIV INFECTION AND MALASSEZIA

The growth of Malassezia is known to be enhanced in immunocompromised patients. In a recent study from India, found that 13.5% incidence of Malassezia infection in HIV infected patients. The incidence of pityriasis versicolor, Malassezia folliculitis and seborrheic dermatitis was 40%, 16% and 56% respectively.[50]

The HIV infected state increases the level of serum interferon and tumour necrosis factor α, which are known to alter lipid metabolism, increasing serum triglycerides and cholesterol levels. This increases patient's sensitivity to inflammatory mediators released by Malassezia.[41]

In addition Malassezia species also have been found to be associated with variety of dermatoses like confluent and reticulate papillomatosis, psoriasis, otitis externa, obstructive dacryocystitis and onychomycosis.

3.11 DIFFERENTIAL DIAGNOSIS

The differential diagnosis of pityriasis versicolor includes other common entities associated with cutaneous depigmentation. Vitiligo and pityriasis alba are distinguished by complete absence of scaling. Erythrasma and tinea corporis are differentiated from

pityriasis versicolor by wood's lamp examination in which erythrasma fluoresce coral red and tinea corporis does not fluoresce at all. Seborrheic dermatitis, pityriasis rosea, secondary syphilis show more inflammatory response than pityriasis versicolor.[11]

3.12 LABORATORY DIAGNOSIS

The diagnosis of Malassezia infections is based on:

1. Clinical examination
2. Wood's lamp examination
3. Direct examination
4. Fungal culture
5. Biochemical reactions
6. Serological tests
7. Molecular methods
8. Animal pathogenicity

1. **Clinical examination**: Usually presents as asymptomatic patches of hypo or hyperpigmented macules of varying size, shape and colour.

2. **Wood's lamp examination**: Woods light is filtered UV light with a peak of 365nm. Pityriasis versicolor usually gives a golden yellow fluorescence. Wood's lamp examination also helps in identification of subclinical cases.[27]

3. **Direct examination**

Specimen collection: The skin from the affected area is thoroughly cleaned with 70% alcohol. After drying active edges of lesions are scrapped by using sterile scalpel blade or edge of glass slide.[8]

Another effective and reliable method of specimen collection, particularly in children is by applying a piece of scotch tape with adhesive side down, followed by pressing tape firmly to recover the scales.

The skin scrapings are examined using 10% potassium hydroxide (KOH) with or without dimethyl sulphoxide (DMSO). The KOH helps to dissolve the keratin and debris, facilitating examination of fungal elements. Gentle heating of glass slide speeds dissolution of keratin. If DMSO is used, heating step can be avoided. Microscopic examination reveals both hyphae and yeast cells. Yeast cells are round, measuring about 2-7um in size with occasional budding. The hyphae are blunt, short, stout that may be curved with infrequent branching. The characteristic "banana and grapes" or "spaghetti and meat balls" appearance.[9,14,51] The appearance can also be enhanced if parker's blue or black ink is incorporated in KOH.

Albert's stain can also be used as an alternative to KOH. It is less time consuming and effective as KOH wet mount. It stains yeast cells and hyphae purple, clearly delinate the details of fungi against a background of surrounding keratinocytes.[9,52]

Calcoflour white staining may also be used to avoid any confusion of artifacts. It is a rapid, simple, sensitive and a highly reliable method for identifying fungi, as it provides a good definition of the fine fungal structure and better contrast from background debris, cells and tissue fragments.[53]

If sample is collected in scotch tape, adhesive side of tape is placed over 1-2 drops of KOH on the glass slide and examined with light microscope to confirm the presence of characteristic appearance.[54]

4. **Fugal culture**: For clinical diagnosis culture is not necessary. But culture is essential to establish final diagnosis of Malassezia infection in routine clinical practice. As Malassezia is lipophilic yeast, lipids such as oleic acid, olive oil, glycerol monostearate and tweens are incorporated into the culture media. The skin scrapings from the infected area are inoculated on Sabourads Dextrose Agar (SDA) with chloramphenicol, cycloheximide and a film of strile olive oil in concentration of 10ml per litre. The inoculated media should be incubated aerobically at 32-35^0 c for 2 weeks. M. pachydermatis is not an obligate lipophilic species hence does not require lipid as growth factor and can grow on ordinary media like SDA.[9]

Colonies are small 3-6mm, cream to yellow in colour, with slightly raised irregular edges. The Lacto Phenol Cotton Blue (LPCB) mount/ Gram's stain of colonies show 2-7µm round to oval yeasts with many small 2-3µm bottle shaped narrow to broad

based budding yeast cells. Hyphae are occasionally seen in fungal cultures.[9] Two complex media were introduced to isolate all Malassezia species.

a. Modified Dixon (mDixon) Agar

This medium was originally described by van Abbe in 1964. Its modified formula mDixon is widely use now. This media provides substantial growth and yields colonies with typical features which help in identification. The modified media contains: 3.6% malt extract, 0.6% peptone, 2% dessicated ox bile, 1% tween 40, 0.2% glycerol, 0.2% oleic acid and 1.2 % agar with 0.5% chloramphenicol and 0.05% cycloheximide. Dixons broth can also be used for growth of Malassezia species.[3]

Colony characteristics on mDixon agar medium[3]

Malassezia furfur: Thick convex or umbonate colonies, 4-5mm in diameter, with a smooth to rough surface and cream colour. The texture is soft and the colonies are easy to emulsify.

Malassezia pachydermatis: Thick, cream coloured, convex colonies with mat surface and a brittle texture which makes it difficult to emulsify.

Malassezia sympodialis: colonies are 5-6mm in diameter, cream to buff in colour, flat with slight central elevation, smooth shiny surface, homogenous texture ad very easy to emulsify.

Malassezia globosa: 4mm in diameter, rough with a deeply folded surface, cream to buff colour and a very brittle texture difficult emulsify.

Malassezia obtusa: colonies are morphologically similar to M furfur, but smaller in diameter with sticky nature.

Malassezia restricta: irregular, small 2mm in diameter, cream colour with hard texture and difficult to emulsify.

Malassezia slooffiae: cream to buff coloured, 3mm in diameter, brittle texture with finely folded margins.

b. Leeming Notman media

This media was described by Leeming and Notman in 1987. This media contains whole fat cow's milk which gives better results that is high recovery rate and has longer shelf life than poured plates.[3,9]

But mDixon media is preferred over Leeming and Notman media because of its dark coloration which makes colony counting easy especially when several species are mixed, and a better recognition of their morphology.[3,9]

Alternative culture media like Glucose Yeast Extract (GYP-S) agar can also be used. which provides growth and quatification of colonies within 3-4days. The medium contains glucose, yeast extract, peptone, agar, olive oil, tween 80 and glycerol monostearate.[3,9]

Another minimal medium consisting of L-tryptophan and lipid source induces formation of brown pigmentation which diffuses into agar, used for isolation of M. furfur. However few strains of M. sympodialis and M. pachydermatis fail to grow on this media.

5. **Biochemical tests**: Speciation of Malassezia is done by routine physiological and biochemical tests like lipid dependence, maximum growth temperature, catalase reaction, esculin hydrolysis, urea hydrolysis, utilization of tween 20, 40, 60, 80 and cremaphor EL as sole source of complex lipid.

   a) **Lipid dependency**: All Malassezia species require supplementation of lipids for their growth except M. pachydermatis.

   b) **Temperature tolerance**: Optimum temperature for growth of Malassezia is 32 to 35^0 C. But M. pachydermatis, M. sympodialis, M. slooffiae, M. furfur and M. dermatis can also grows at 40^0 C, where as other species fails to grow.[3]

   c) **Urease test**: All Malassezia species hydrolyse urea.

   d) **Catalase test**: All lipid dependent Malassezia species produce the enzyme catalase except M. restricta.

   e) **Esculin splitting**: M. sympodialis, M. obtusa and M. japonica are able to split esculin due to the production of enzyme beta glucosidase there by producing black zones on the media.[55]

f) **Tween assimilation test**: M. pachydermatis, M. furfur and M. dermatis utilize all tweens (tween 20,40, 60 and 80). M. sympodialis utilize all tweens except tween 20, M. sloofiae utilize all tweens except tween 80, M. obtusa, M. globosa and M. restricta do not utilize any tween.[3,9,56]

g) **Cremophor EL**: M. furfur grows in the presence of cremophor EL where as all other species shows variable results.

i) **Glycine assimilation**: M. furfur is the only species which assimilate glycine as nitrogen source.[57]

6. **Serological tests**: Serological tests are not of much diagnostic importance. A transferable solid phase ELISA was developed for determination of antibody titres specific to M. furfur serovars A, B and C in human sera. The patients with poor cellular immune response can be assessed by lymphocyte blastogenesis to specific fungal antigen.

7. **Molecular methods**: The molecular methods like restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), and multilocus enzyme electrophoresis (MLEE) are used to identify isolates and also for epidemiological studies. Pulse field gel electrophoresis (PFGE) may be useful to identify species of this medically important yeast and Random amplified polymorphic DNA (RAPD) and Amplified fragment length polymorphism (AFLP) are useful for screening epidemiological surveys.[9] Terminal fragment length polymorphism analysis, for rapid and reliable identification of Malassezia species.[58,59]

Table 3: IMPORTANT CHARACTERISTICS OF MALSSEZIA SPECIES[3, 35]

Species characteristics	M.furfur	M.pachydermatis	M.sympodialis	M.obtusa	M.restricta	M.slooffiae	M.globosa
Colony morphology	Umbonate usually smooth soft friable	Pale, convex, smooth soft friable	Flat, smooth, shiny, soft	Dull smooth, hard and brittle	Finely folded brittle	Finely folded brittle	Rough, coarse brittle
Colony colour	Cream	Cream	Cream to buff	Cream	Cream	Cream to buff	Cream to buff
Cell shape and size	Elongated oval or spherical 6µm	Cylindrical 2.5-4 µm long	Ovoid to globose 2.5-5 µm long	Cylindrical 4-6 µm	Spherical oval 2-4 µm	Cylindrical 1.5-3.5 µm long	Spherical 6-8 µm diameter
Budding pattern	Broad base	Broad bud base	Some sympodial budding	Broad bud base	Narrow bud base	Broad bud base	Narrow bud base
G+C content (%)	66.4	55.6	62.2	60.7	59.9	68.8	53.3
Catalase	+	Variable	+	+	-	+	+
Urease	+	+	+	+	+	+	+
Growth at 37^0C	Good	Good	Good	Poor	Poor	Good	Poor
Maximum growth temperature	40-41	40-41	40-41	38	38	40-41	38
Utilization of Tween 20	+	+	-	-	-	+	-
Utilization of Tween 40	+	+	+	-	-	+	-
Utilization of Tween 60	+	+	+	-	-	+	-
Utilization of Tween 80	+	Variable	+	-	-	-	-
Cremphor El	+	Variable	-	-	-	-	-
Esculin hydrolysis	Weak	Variable	+	+	+	-	-
Assimilation of glycine	+	-	-	-	-	-	-
Precipitation on Dixon's agar	-	ND	+	-	ND	-	+

ND – Not Determined

8. **Animal pathogenicity:** The common laboratory animals appear immune to this fungus when scales are applied. Studies conducted to infect guinea pig and swiss mice showed experimental dermatitis with hyperkeratosis, particularly at follicular ostia and pilous bulbs.[9]

3.13 TREATMENT

Antifungal treatment for pityriasis versicolor can be divided into topical and oral medications.

Topical treatment: is less expensive and is preferred therapy in children.[60] They are available in the form of solutions, paints, creams, ointments, solutions spray and shampoos. Compliance is sometimes low for variety of reasons, including odour, difficulty in applying the solution to back, or the extended periods of time the solutions must be applied before or after shower.[13]

A variety of topical preparations were also reported to be equally effective including sodium thiosulfate 25% with salicylic acid 1%, selenium sulphide 2.5%, Whitfield ointment, many topical azoles like cotrimazole 1%, ketoconazole 2% etc. Topical application of terbinafine available as 1% cream or 1% solution spray applied once or twice daily for 1-2 weeks was reported to be successfully used for treating pityriasis versicolor.[61] Studies had shown that topical terbinafine has a cure rate of 70% by third week and 80-100% cure rate overall.[9,13]

Systemic medication is preferred in patients with severe disease, frequent relapses or in whom topical agents have failed. Ketoconazole in a dose of 200 mg/day for 10 days was widely used. A study done by Fernandez et al 1997 compared a single dose of 400mg Ketoconazole to 10 days 200mg daily dose of Ketoconazole and did not report any significant difference in their outcome.[62,63] Itraconazole and fluconazole could also be used as systemic medications. Itraconazole in a dose of 200mg/ day or 5-6 days was reported to prevent relapses, and more specific. Fluconazole could be used along with antacids and exhibits more selectivity to fungal cytochrome p 450 than other azoles at antifungal doses.[6]

4. METHODOLOGY

4.1 SOURCE OF DATA

This study comprises of 100 clinically diagnosed cases of pityriasis versicolor attending Dermatology Outpatient Department during the period January 2011 to June 2012 at R. L. Jalappa Hospital and Research Centre, Kolar. A detailed history was taken with reference to name, age, sex, place and clinical details like site of lesion and whether hypo or hyperpigmented were recorded on a predesigned proforma.

4.2 INCLUSION CRITERIA

All patients clinically diagnosed as pityriasis versicolor and skin scraping showing positive in KOH mount.

4.3 EXCLUSION CRITERIA

- Patients who have already received topical antifungal therapy within last 3 months and oral therapy within the last 6 months.
- Patients with hypopigmented lesions like leprosy, vitiligo, pityriasis alba etc.
- Patients with hyperpigmented lesions like lichen planus, psoriasis etc.

4.4 SPECIMEN COLLECTION

After taking informed consent, skin from the affected area is thoroughly cleaned with 70% alcohol to remove surface contaminants. After drying, scrapings from active edges of lesion are taken using disposable sterile blade. Samples were collected in a butter paper and further processing was done as early as possible.

PROCESSING OF SAMPLE

4.5 DIRECT MICROSCOPY: The scrapings collected were examined for the presence of fungal elements using 10% potassium hydroxide (KOH).

KOH Wet Mount

The skin scraping was added to a drop of 10% KOH on a clean glass slide and cover slip was applied. The mount was gently warmed by passing it over the flame two to three times to hasten keratolysis. After 10 minutes the slide was examined, first under low power objective and later under high power objective of the microscope. Each slide was thoroughly examined for the characteristic “banana and grapes” or “spaghetti and meat balls” appearance.

Calcofluor white staining

The skin scraping was added to a drop of 20% KOH supplemented with calcofluor. The preparation was observed under fluorescent microscope for apple green fluorescence of yeast cells and hyphae suggestive of Malassezia species.

4.6 CULTURE

Those samples that showed the typical "spaghetti and meat balls" appearance on 10% KOH examination were subjected to culture. The samples were inoculated into two SDA slants, one overlaid with sterile olive oil and the other without olive oil. The specimen was also inoculated into modified Dixon agar (annexure) and all the cultures were incubated at 37^0 c. The cultures were examined every day for growth and culture negatives were discarded only after 3 weeks of incubation.[64]

4.7 IDENTIFICATION OF MALASSEZIA SPECIES

Gram staining: A smear was prepared from the cream to buff coloured, smooth or rough colonies and stained by Gram's Method. The morphological features like budding pattern, shape of yeast cell were observed under microscope.

Colonies from both tubes of SDA were studied to rule out yeasts other than Malassezia.

Biochemical tests used for identification

a) **Catalase test**: one drop of 3% hydrogen peroxide (H_20_2) was placed on slide. A small portion of the colony with wooden applicator was transferred on to the drop of H_2O_2. Production of gas bubbles indicated positive reaction. All Malassezia show positive catalase reaction except M. restricta.[3,9]

b) **Urease test**: Christensen's Urease medium (Himedia) was used to assess the production of the enzyme urease. The colonies were streaked on to the slant and incubated overnight at 37^0 C. All Malassezia species are urease positive and change the colour of the medium to pink on incubation for 24-48 hours.[26]

c) **Esculin splitting or test for β-glucosidase activity:** The β-glucosidase activity of different Malassezia species was assessed by using Tween Esculin agar slants (TE slant).

Colonies were streaked on the TE slant and incubated for 5 days at 37^0 C. Malassezia species showing β glucosidase activity will produce black zones due to esculin hydrolysis. This test helps to distinguish M. sympodialis and M. obtusa from other Malassezia species that are negative.

d) **Tween assimilation test**: Ability to utilize different Tween compounds as a unique lipid supplement by Malassezia species was evaluated according to method reported by Kindo et al.[4]

Sterile SDA (16 ml) was melted and allowed to cool to about 50^0 C. A suspension of yeast was made by inoculating 5 ml of sterile distilled water with a loopful of actively growing yeasts and vortexed for 15 min. The turbidity of suspension is then adjusted with 5 Mc farland standard to have about 10^5 yeasts/ml. 2 ml of this suspension is added to 16 ml of molten SDA and poured into 90 mm diameter petridish. Once the medium had solidified, 4 wells were made on the agar surface. 5 µl of Tween 20, 40, 60 and 80 were

added on to respective wells. The plates were then incubated for 3-4 days at 37^0 C. Utilization of Tweens was assessed by degree of growth or reaction (precipitation) of the lipophilic yeasts around individual wells.

4.8 METHOD OF STASTICAL ANALYSIS

Done with descriptive tools like Proportion and mean.

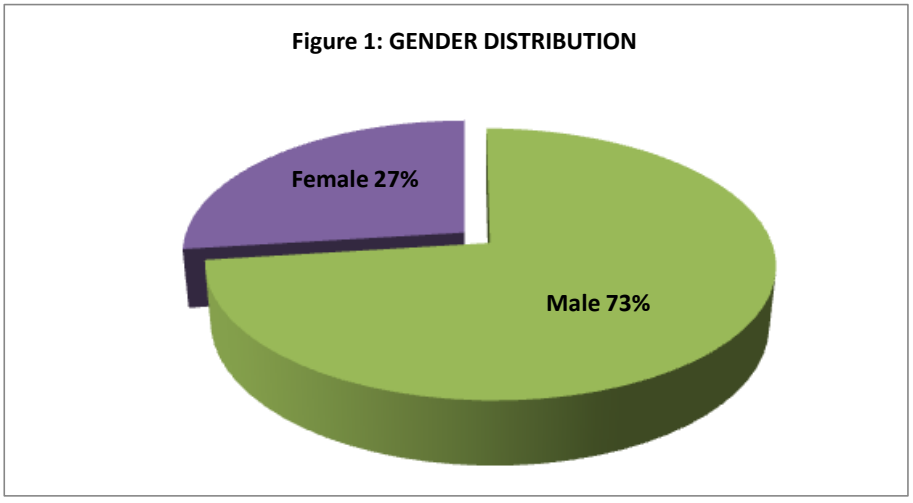
Proportional difference observed within groups was be compared using Chi-square test.

5. RESULTS

A total of 100 samples from clinically diagnosed cases of pityriasis versicolor were processed. All 100 samples from patients with pityriasis versicolor were positive for the typical “spaghetti and meat ball” appearance in 10% KOH preparation.

Table 4: GENDER DISTRIBUTION OF PATIENTS WITH PTYRIASIS VERSICOLOR

Gender	Total	Percentage
Male	73	73%
Female	27	27%

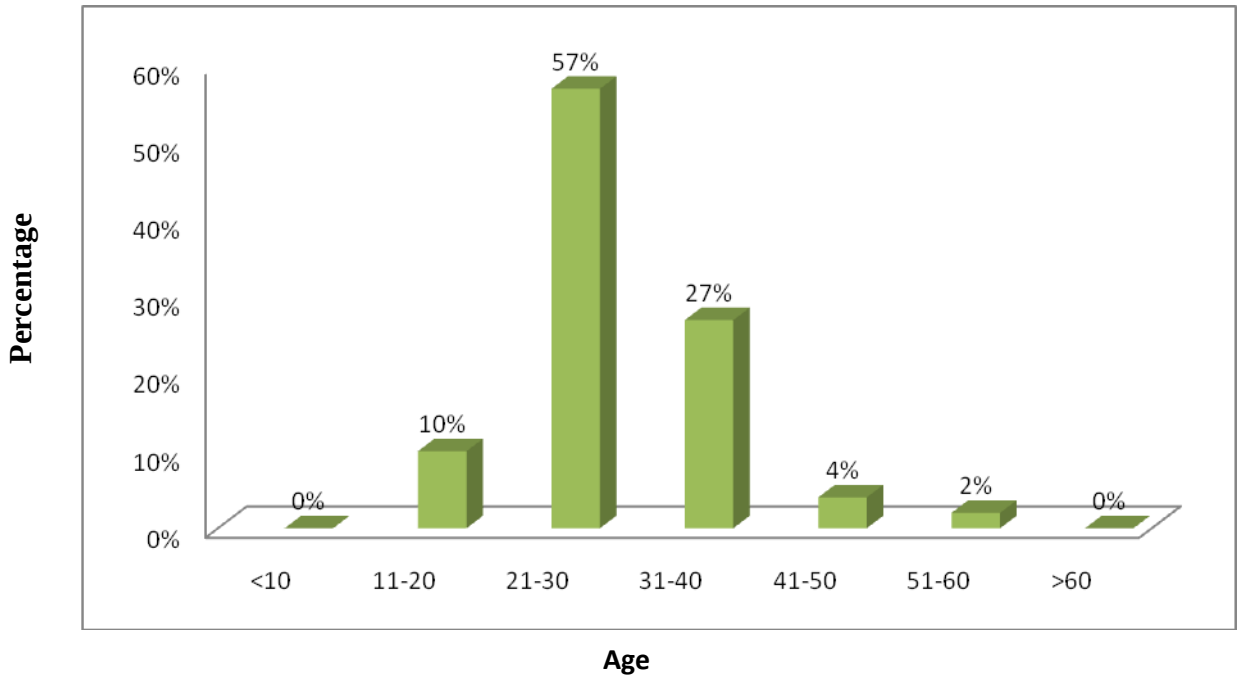


Out of the 100 cases of pityriasis versicolor, 73(73%) were male and 27(27%) were females, with a high male preponderance.

Table 5: DISTRIBUTION OF PITYRIASIS VERSICOLOR IN PATIENTS WITH DIFFERENT AGE GROUPS (n=100)

Age	No. of patients	Percentage
< 10	-	-
11 - 20	10	10%
21 – 30	57	57%
31 - 40	27	27%
41 – 50	4	4%
51 - 60	2	2%
> 60	-	-

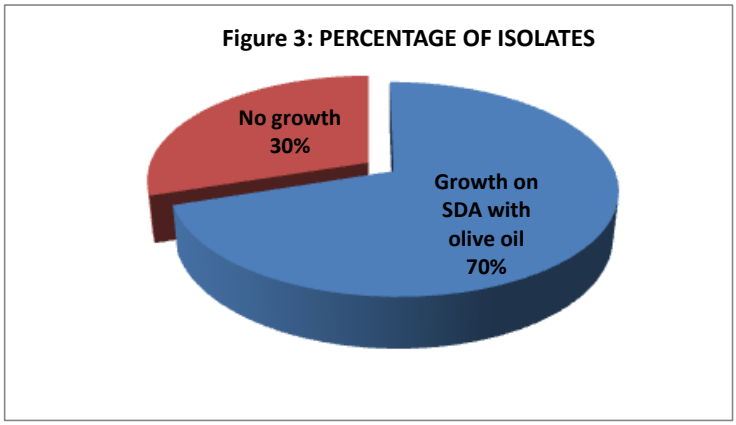
Figure 2: DISTRIBUTION OF PITYRIASIS VERSICOLOR IN PATIENTS WITH DIFFERENT AGE GROUPS (n=100)



The age of the patients with pityriasis versicolor ranged from 15-60 years. Majority of them were in the age group of 21-30 years (57%), followed by 31-40 years (27%), 11-20 years (10%), 41-50 years (4%) and 51-60 years (2%). None of them were seen below 10 years and above 60 years of age. The association of pityriasis versicolor in the patients between 21-30 years age group was statistically significant with $p<0.001$.

Table 6: PERCENTAGE OF ISOLATES

Total No. Sample	Positive KOH mount	Growth on SDA with olive oil	Growth on plain SDA
100	100	70 (70%)	_

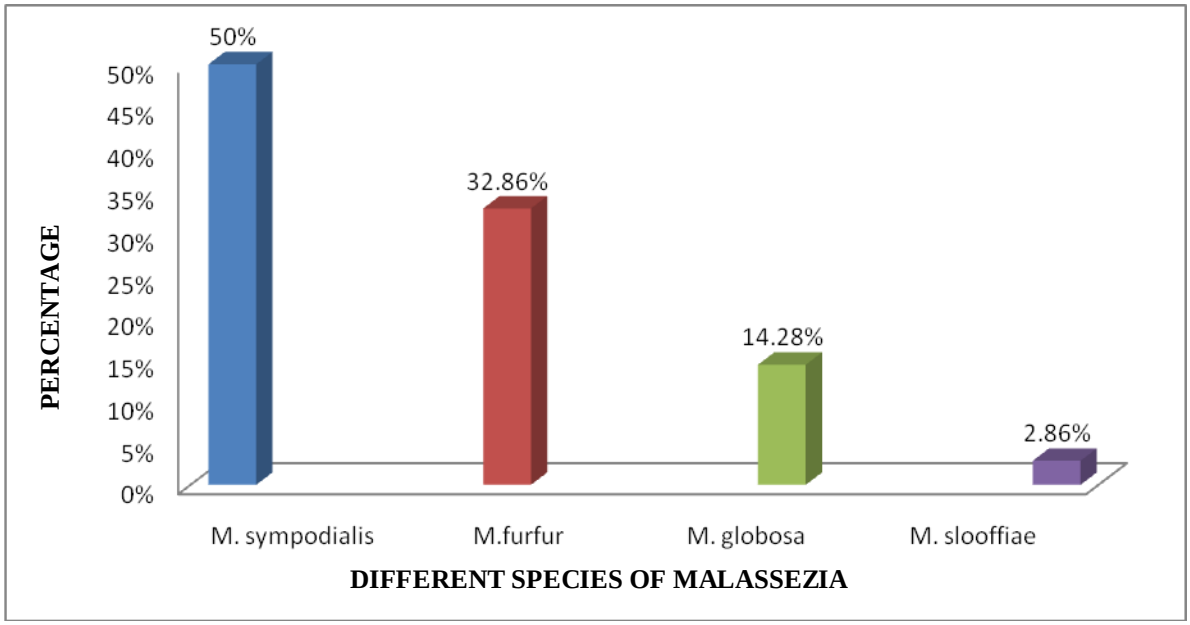


Among 100 samples only 70 yielded growth on SDA with olive oil. Fungal growth was entirely absent on plain SDA. The growth on SDA with olive oil appeared after 8 to 10 days of inoculation. All 70 samples also yielded growth on mDixon agar within 5 days of inoculation.

Table 7: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES IN PATIENTS WITH PTYRIASIS VERSICOLOR (n=70)

Malassezia species	No. of isolates	Percentage
Malassezia sympodialis	35	50%
Malassezia furfur	23	32.86%
Malassezia globosa	10	14.28%
Malassezia slooffiae	02	2.86%

Figure 4: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES IN PATIENTS WITH PITYRIASIS VERSICOLOR n=70



Of the 70 culture positives, 35 (50%) yielded M. sympodialis, 23(32.86%) M. furfur, 10(14.28%) M. globosa, 2(2.86%) M. slooffiae. Other species like M. obtusa, M. restricta and M. pachydermatis were not isolated in our study population. The isolation of M. sympodialis is significantly ($p<0.001$) higher than the isolation of other species in our study.

Table 8: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES IN PATIENTS WITH DIFFERENT AGE GROUP

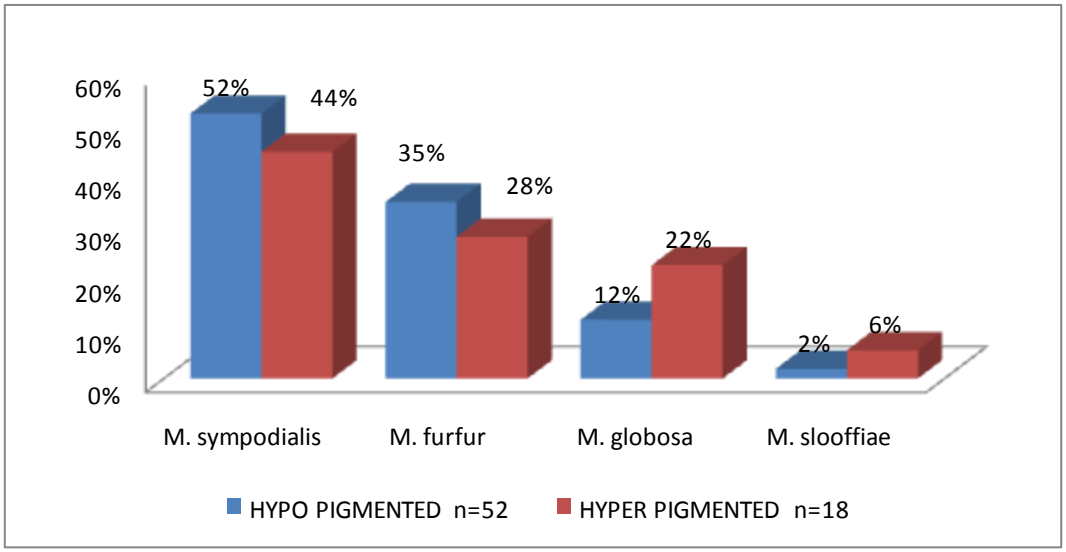
SPECIES	AGE					TOTAL
	11-20	21-30	31-40	41-50	51-60	
M. sympodialis	5(14.28%)	16(45.71%)	13(37.14%)	1(2.76%)	-	35
M. furfur	2(8.69%)	14(60.87%)	4(17.39%)	3(13.04%)	-	23
M. globosa	2(20%)	6(60%)	1(10%)	-	1(10%)	10
M. slooffiae	1(50%)	1(50%)	-	-	-	2

Distribution of different species in patients with different age group showed all species are more common in the age group of 21-30 years. But this association is statistically not significant. This may be because of lower sample size.

Table 9: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES IN PATIENTS WITH HYPO AND HYPERPIGMENTED LESIONS

Type of lesion	No. of cases	No. Isolates	M sympodialis	M furfur	M globosa	M slooffiae
Hypopigmented	74(74%)	52 (74.27%)	27 (51.92%)	18(34.61%)	6 (11.53%)	1 (1.92%)
Hyperpigmented	26(26%)	18 (69.23%)	8 (44.44%)	5 (27.77%)	4 (22.22%)	1 (5.55%)

Figure 5: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES IN PATIENTS WITH HYPO AND HYPER PIGMENTED LESIONS



Among 100 patients with pityriasis versicolor, 74 (74%) had hypopigmented lesions and 26 (26%) had hyperpigmented lesions. Predominance of hypopigmentation in this study was statistically significant with p value < 0.001.

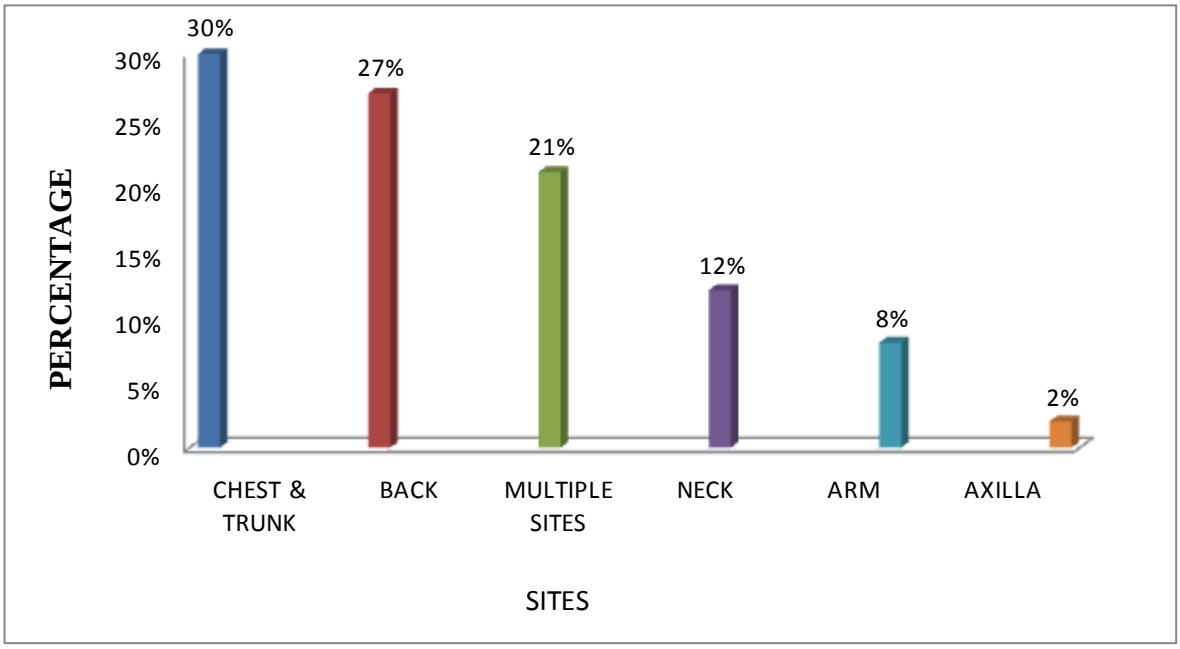
Of the 70 culture positive patients, 52(74.27%) had hypopigmented lesions and 18(25.71%) had hyperpigmented lesions. Of the 52 hypopigmented lesions, 27(51.92%) yielded M. sympodialis, 18(34.61%) M furfur, 6(11.53%) M. globosa and 1(1.92%) M. slooffiae. Of the 18 hyperpigmented lesions, 8(44.44%) yielded M. sympodialis, 5(27.77%) M.furfur, 4(22.22%) M.globosa and 1(5.55%) M. slooffiae.

M. sympodialis and M. furur were predominantly isolated from hypopigmented lesions and M. globosa and M. slooffiae were found to be more common in hyperpigmented lesions. The distribution of different species in patients with hypo or hyperpigmented lesions did not show any statistical significance which may be due to lower sample size.

Table 10: DISTRIBUTION OF LESIONS ON DIFFERENT SITES OF THE BODY

Site	No. of cases	Percentage
Chest & trunk	30	30%
Back	27	27%
Multiple sites	21	21%
Neck	12	12%
Arm	8	8%
Axilla	2	2%

Figure 6: DISTRIBUTION OF LESIONS ON DIFFERENT SITES OF THE BODY

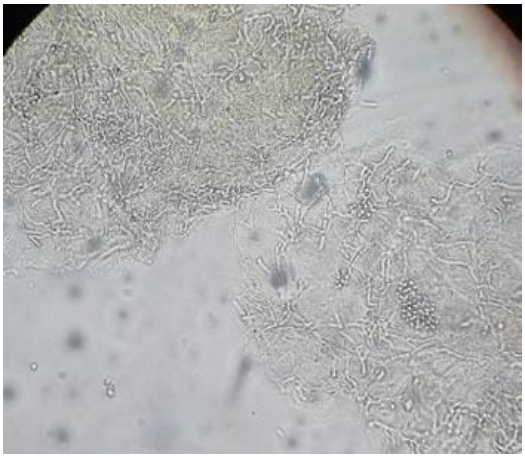


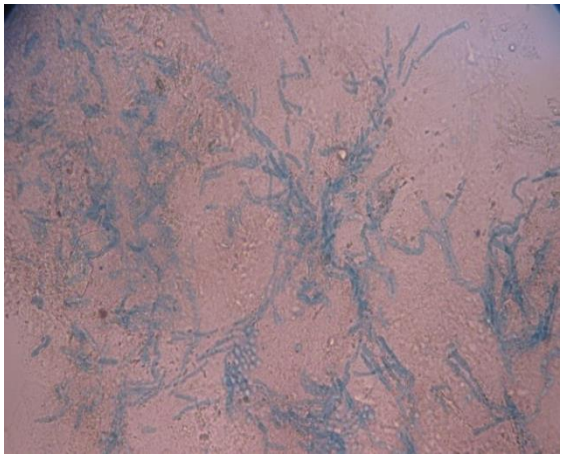
Distribution of pityriasis versicolor lesions on diferent sites of the body showed that, chest and trunk (30%) was the most common site involved, followed by back (27%), multiple sites (21%), these patients showed involvement of two or more sites, neck (12%), arm (8%) and axilla (2%).

Table 11: DISTRIBUTION OF DIFFERENT MALASSEZIA SPECIES ON DIFFERENT SITES OF THE BODY

SPECIES	SITE						TOTAL
	CHEST TRUNK	BACK	MULTIPLE SITES	NECK	ARM	AXILLA	
M.sympodialis	6(17.14%)	14(40%)	8(22.85%)	2(5.71%)	5(14.28%)	-	35
M. furfur	5(21.74%)	8(34.78%)	5(21.74%)	5(21.74%)	-	-	23
M. globosa	6(60%)	1(10%)	1(10%)	1(10%)	-	1(10%)	10
M. slooffiae	1(50%)	-	1(50%)	-	-	-	2

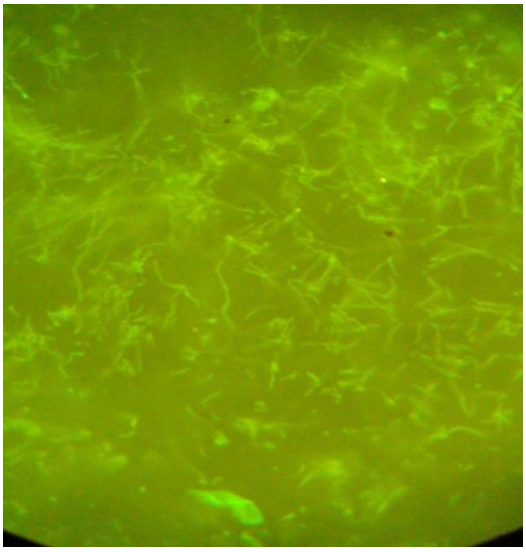
Distribution of Malassezia species in different sites of the body showed that, M. sympodialis and M. furfur was isolated predominantly from lesions on the back, M. globosa from the chest and trunk, M. slooffiae was isolated from chest and trunk and also from lesions on neck and arm.

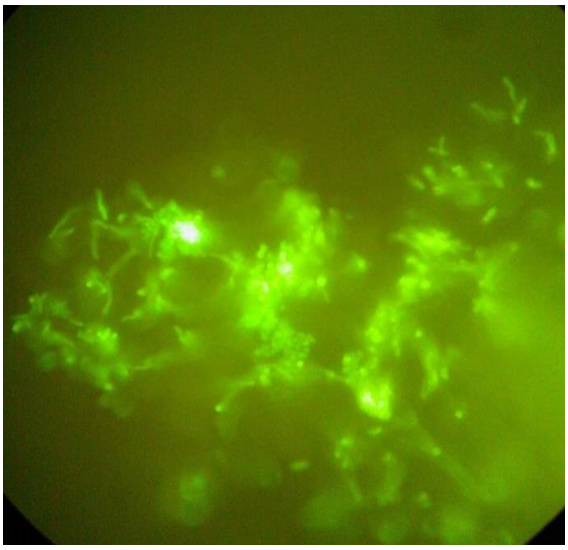




KOH MOUNTING **KOH + PARKER INK**

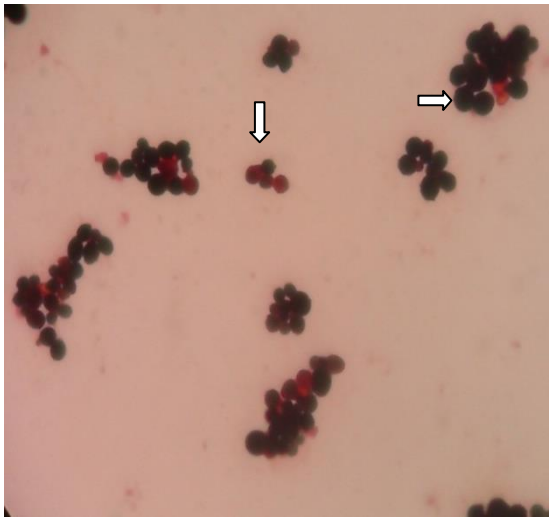
Figure 7: KOH MOUNTING SHOWING 'SPAGHETTI & MEAT BALLS' APPEARANCE





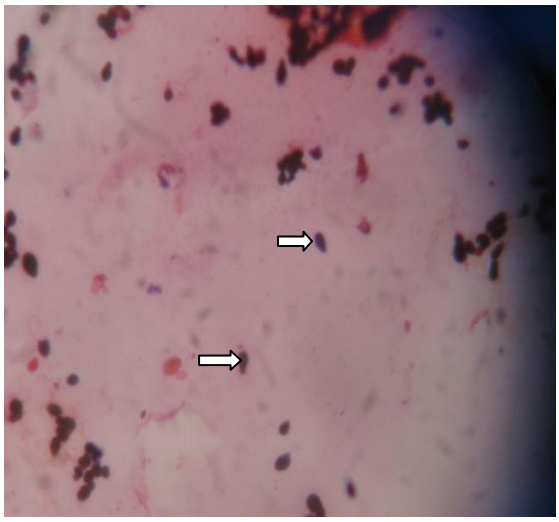
Under 20X magnification **Under 40X magnification**

Figure 8: Calcofluor white staining



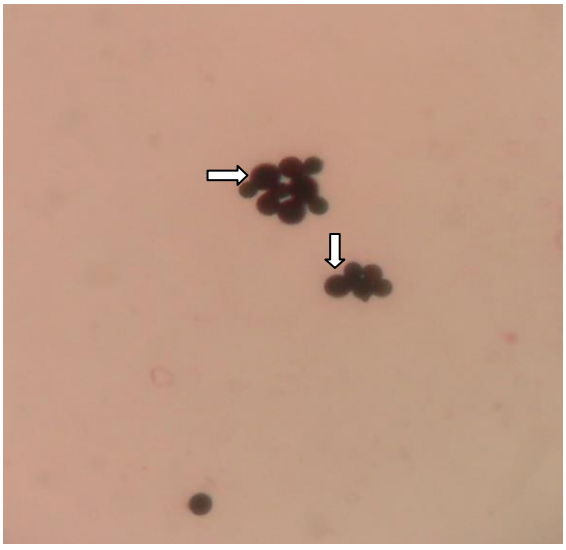
M. sympodialis

Ovoid yeast cells with base of the bud narrower than mother cell but equal in width to the bud



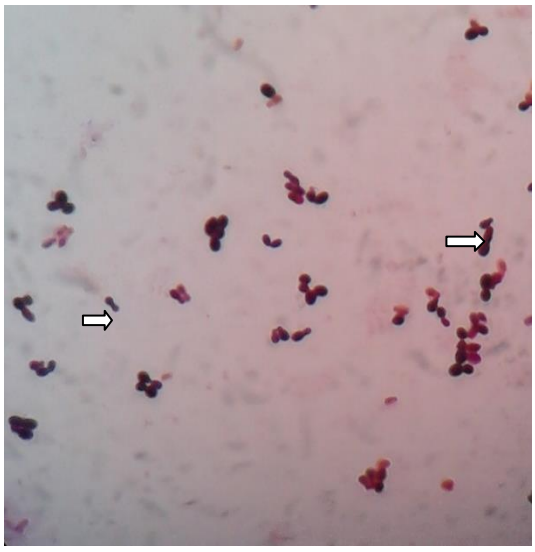
M. furfur

Yeast cells are short and ovoid. Buds are formed on a very broad base



M. globosa

Yeast cells are spherical with buds formed on a narrow base. Some daughter cells elongate but the bud base remains narrow



M. slooffiae

Yeast cells are small and cylindrical with buds formed on a broad bud base

Figure 9: Gram's Staining

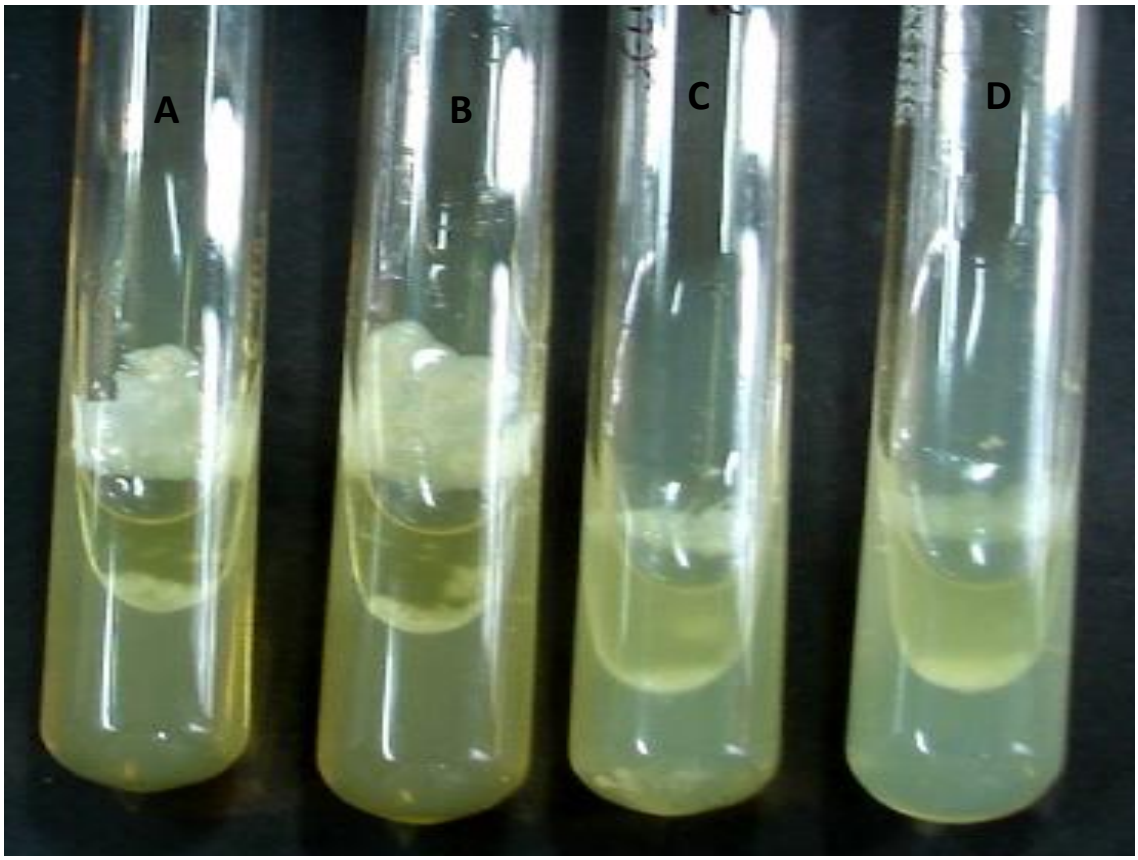
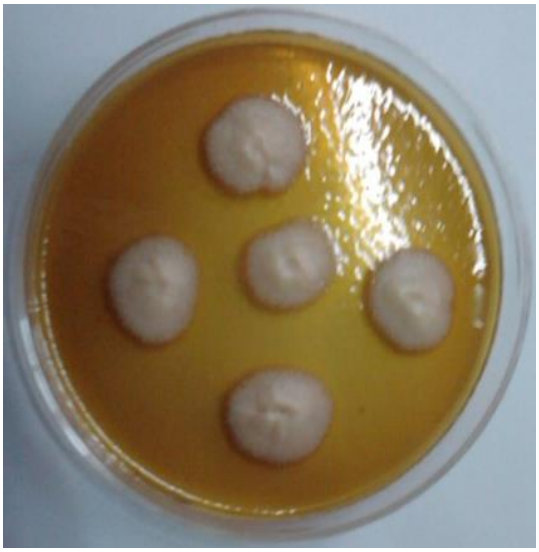
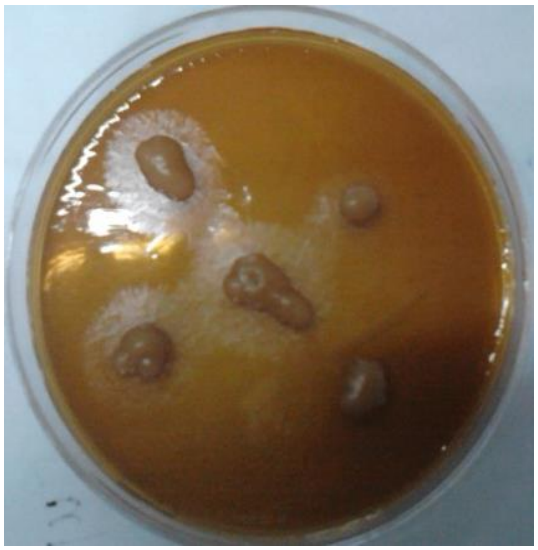


Figure 10: Growth on SDA with olive oil

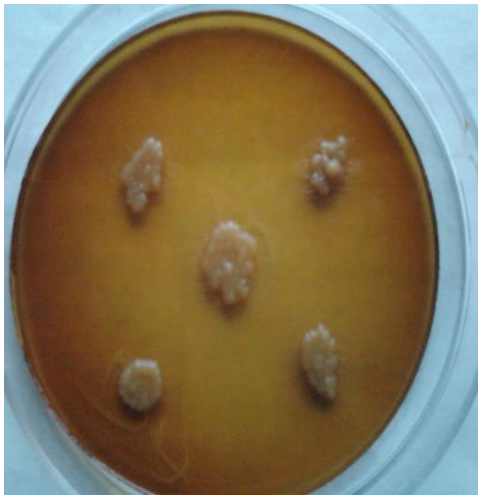
A. M. sympodialis
B. M. furfur
C. M. globosa
D. M. slooffiae



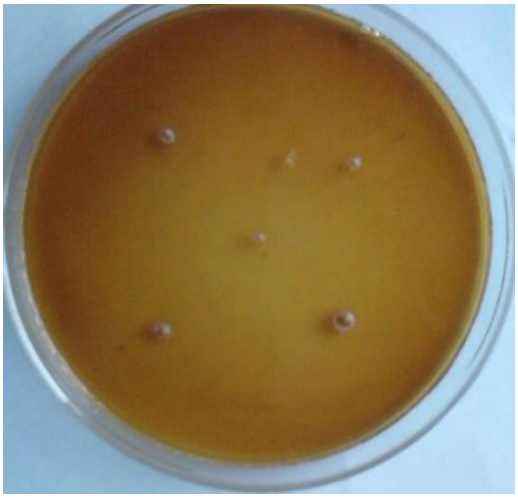
M. sympodialis



M. furfur



M. globosa



M. slooffiae

Figure 11: Growth on mDixon agar

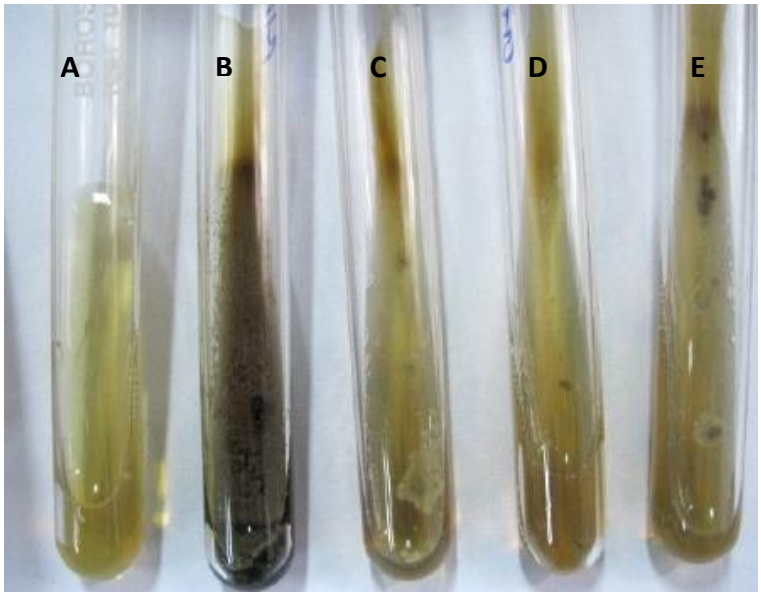


Figure 12: Esculin hydrolysis

A. Uninoculated medium

B. M. sympodialis

C. M. furfur

D. M. globosa

E. M. slooffiae

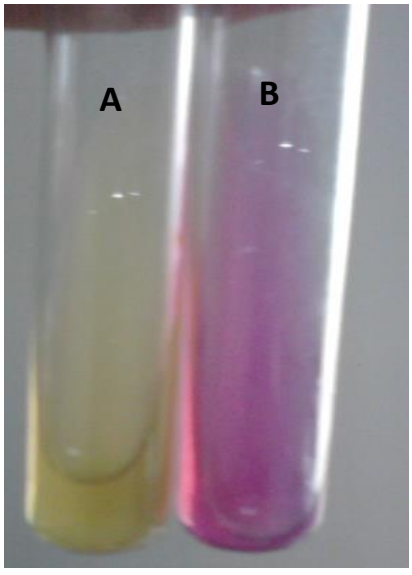
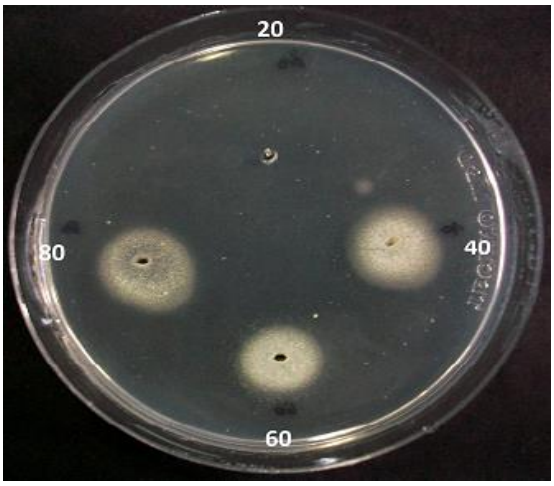


Figure 13: Urease Test

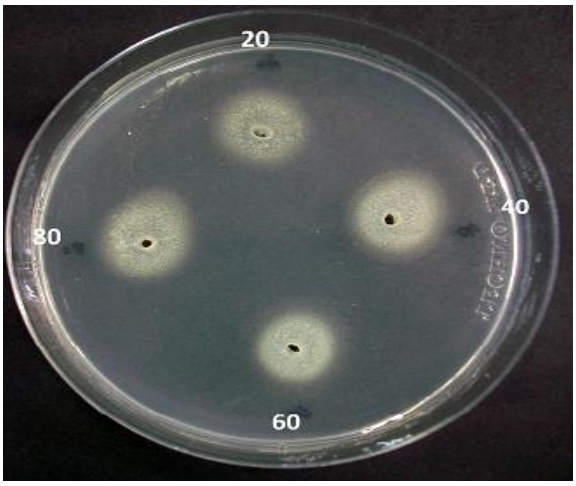
A. Uninoculated medium

B. Positive reaction



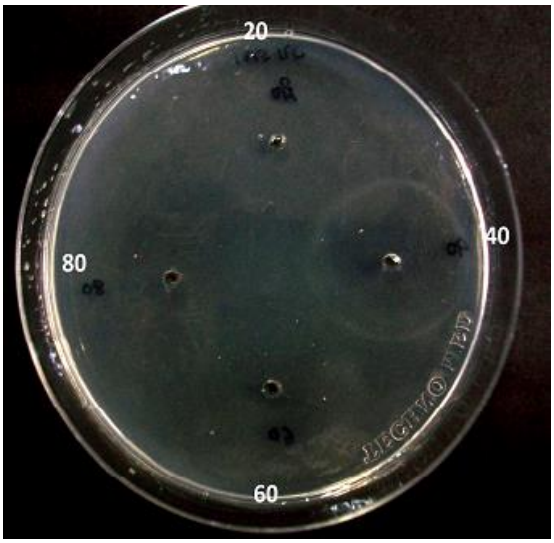
M. sympodialis

Assimilation of Tween 40, 60, 80 and not 20



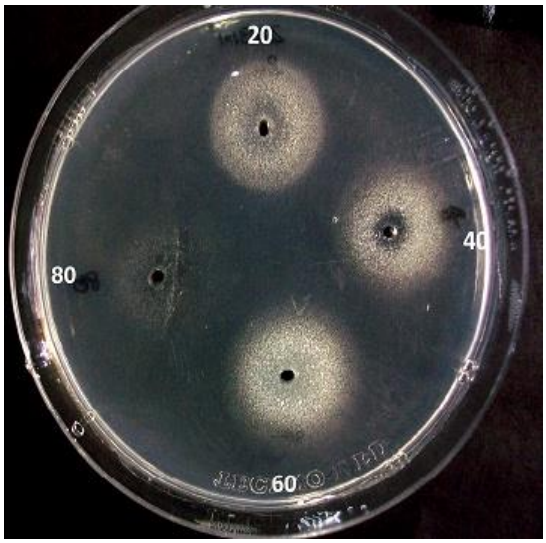
M. furfur

Assimilation of Tween 20, 40, 60 and 80



M. globosa

Tween assimilation negative



M. slooffiae

Assimilate Tween 20, 40, 60 and not 80

Figure 14: Tween assimilation test

6. DISCUSSION

Yeasts of the genus Malassezia causes various skin lesions, among which pityriasis versicolor is the commonest and world wide in occurence. The genus Malassezia has undergone several taxonomic revisions. In the last reclassification 14 distinct species are recognised, in that only seven species have been well studied in relation to pityriasis versicolor.

In this study, a total of 100 samples from clinically diagnosed cases of pityriasis versicolor were studied and the isolates were identified on the basis of morphological and cultural characteristics. All the 100 skin scrapings from patients with pityriasis versicolor showed the typical ‘ spaghetti and meatball’ appearance on 10% KOH preparation.

Out of 100 patients with pityriasis versicolor 73% were male, 27% were female, with high male preponderance. Similar to our study Gosh et al, Rao et al and Krishnan et al also found male predominance.[1,2,42]

The occurrence of pityriasis versicolor was more common in adult age group than in the paediatric age group. In adults it was found to be more common in 21-30 year age group (57%), followed by 31-40 years (27%), 11-20 years (10%), 41-50 years (4%) and 51-60 years (2%). The association of pityriasis versicolor in the patients between 21-30 years age group was statistically significant with $p<0.001$. A similar finding was also reported by Rao et al (21-30yr), Krishnan et al (15-29yr) and Dutta et al (11-30yr).[2,33,42] In the study by Akapata et al found that majority of cases occurred during

adolescence.[65] This was believed to be due to hormonal changes and increase in sebum secretion duing this period.

The isolation rate of Malassezia species in patients with pityriasis versicolor in the current study is 70%, which is comparable to that of Kindo et al (68.57%) from south India. In contrast to our study it is higher in Chaudhary et al (96.66%) from central India and Shokoshi et al (88.4%) from Iran.[4,7,10] The growth on ordinary media like SDA with olive oil appeared after 8 to 10 days of inoculation and growth on special media like mDixon agar appeared as early as, within 5 days of inoculation.

The distribution of Malassezia species varies with different geographical locations. In the present study, the most common Malassezia species isolated was M. sympodialis 35(50%), followed by M. furfur 23(32.86%), M. globosa 10(14.28%) and M. slooffiae 2(2.86%). The isolation of M. sympodialis is significantly ($p<0.001$) higher than the isolation of other species in our study. Kindo et al from South India also reports M. sympodialis as the predominant species.[4] Similar reports also available from other parts of world such as Canada and Indonesia, where they found M. sympodialis as predominant species in pityriasis versicolor.[3,7,56] In contrast Chaudhary et al from Central India and Dutta et al from North India reported M. globosa as the most common isolate.[7,33] M. furfur was the second most frequent species isolated from pityriasis versicolor in this study, which is comparable to Dutta et al.[33] Our study showed M. globosa as third common isolate, which is contrary to other studies from India (Chaudhary et al, Dutta et al) which report it as the most common species causing pityriasis versicolor.[7,33] In our study M. slooffiae was isolated from 2(2.86%) cases of

pityriasis versicolor which is almost similar to report of Tarazooic et al (4%) from Iran, however none of the Indian studies have isolated M. slooffiae, from pityriasis versicolor.[66]

Distribution of different species in patients with different age group showed all species are more common in the age group of 21-30 years. But this distribution is not statistically significant possibly because of lower sample size.

Of the 100 patients 74% had hypopigmented lesions and 26% had hyperpigmented lesions. Other studies from India, Gosh et al (81% hypopigmented), Rao et al (75% hypopigmented) and Krishnan et al (84% hypopigmented) also showed predominance of hypopigmented lesions in pityriasis versicolor.[1,2,42] Hypopigmentation in pityriasis versicolor is mainly due to damage to the melanocytes, inhibition of tyrosinase by azelaic acid, a dicarboxylic acid produced by Malassezia, melanosomes of small size and by blocking of ultraviolet light by lipid like material accumulated in the stratum corneum.[9] Hyperpigmentation is due to abnormally large melanosomes, a thick stratum corneum and a hyperemic inflammatory respose.[42] In general, pityriasis versicolor is thought to have a tendency to be hypopigmented in dark skinned individuals and hyper pigmented in fair skinned individuals. However Aljabre et al. who studied pigmentary changes in patients with pityriasis versicolor concluded that pityriasis versicolor is not necessarily hypopigmented in dark skinned individuals and there is no correlation between pigment variation and the type of skin, sex and age of patient and site of lesion.[67]

In our study M. sympodialis and M. furur were predominantly isolated from hypopigmented lesions whereas M. globosa and M. slooffiae were found to be more common in hyperpigmented lesions. But this distribution is statistically not significant, because of low sample size.

The most common site of involvement was chest and trunk, followed by back, multiple sites involving 2 or more sites, neck, arm and axilla. Similar findings are also noted by Dutta et al, Rao et al and Gosh et al with chest and trunk, back and neck as most commonly involved sites. Possibly because of the distribution of sebaceous glands is higher in these areas.[1,2,33]

Distribution of Malassezia species in lesions from different sites of the body showed that, M. sympodialis and M. furfur was more common in lesions on the back, M. globosa from lesions on the chest and trunk, M. slooffiae was isolated from chest and trunk and also from lesions on neck and arms.

Usually, the diagnosis of pityriasis versicolor is based on clinical findings and microscopic examination of skin scrapings. Use of special media like mDixon agar and Leeming and Notman agar can yield a good growth in shorter period. But preparation of these media is difficult and they are expensive when compared to SDA. Use of simple media like SDA overlaid with olive oil for isolation of lipid dependent Malassezia species is cost effective and can be used in any routine diagnostic laboratory.[3,9]

Speciation of Malassezia is based on simple biochemical tests such as catalase reaction, esculin hydrolysis and tween assimilation. These tests are simple to perform and cost effective and can be used in routine diagnostic laboratory.

Molecular methods such as RFLP, nested-PCR or PCR- REA are being developed to hasten the identification process and resolve the difficulty in interpretation of some physiological patterns. Although molecular techniques are most sensitive methods for identification, they are expensive and technically demanding.[7] The present study indicates that the culture based system is an inexpensive and effective method for isolation and routine identification of clinically important Malassezia species.

7. CONCLUSION

In our study, pityriasis versicolor infection was found to be most common in adults between the age group of 21 to 30 years and was predominantly seen in male patients. Hypopigmentation was the most common clinical presentation. Chest and trunk were the sites predominantly involved.

M. sympodialis was the most common species isolated in our study followed by M. furfur, M. globosa and M. slooffiae. There was no significant difference in distribution of different species in patients with different age group and also with clinical presentation i.e. hypo or hyperpigmented lesions.

SDA overlaid with olive oil is a simple, inexpensive media for isolation of Malassezia species. However, special media like mDixon agar may also be used, in which growth occurs, within 5 days. Speciation of Malassezia can be done by using simple biochemical tests like catalase, urease, esculin hydrolysis and tween assimilation.

This study indicates that the culture based system is an inexpensive and effective method for isolation and routine identification of clinically important Malassezia species, which can be employed in any routine diagnostic laboratory.

8. SUMMARY

Hundred clinically diagnosed cases of pityriasis versicolor were subjected to clinico-mycological study. Skin scrapings from patients were screened for fungal elements by 10% KOH and those showing positive were subjected to culture on SDA with olive oil and mDixon agar.

Of the 100 patients, 73% were males and 27% were females. The most affected age group was 21-30 years (57%), followed by 31-40 years (27%), 11-20 years (10%), 41-50 years (4%) and 51-60 years (2%). None of the patients were below 10 years and above 60 years of age.

Out of 100 cases 70% yielded growth on SDA overlaid with olive oil and mDixon agar medium. However, growth occurs earlier in special media like mDixon agar (within 5 days) when compared to SDA, which takes about 8-10 days. M. sympodialis was the most common isolate accounting for 50%, followed by M. furfur 32.86%, M. globosa 14.28% and M. slooffiae 2.86%. All species were found to be more common in the age group of 21-30 years. But this distribution was not statistically significant possibly because of lower sample size.

Of the 100 cases 74% of patients had hypopigmented lesions and 26% had hyperpigmented lesions. M. sympodialis and M. furur were predominantly isolated from hypopigmented lesions and M. globosa and M. slooffiae were found to be more common

in hyperpigmented lesions. But this distribution was statistically not significant, because of lower sample size.

The most common site of lesion was chest and trunk (30%), followed by back (27%), multiple site involvement (21%), neck (12%), arm (8%) and axilla (2%). M. sympodialis and M. furfur was more common in lesions on the back, M. globosa from lesions on the chest and trunk, M. slooffiae was isolated from chest and trunk and also from lesions on neck and arms.

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10. ANNEXURES

ANNEXURE I: PROFORMA: CLINICAL DETAILS

1. NAME OF THE PATIENT:

2. AGE/SEX:

3. ADDRESS AND CONTACT NO.:

4. OCCUPATION:

5. CHIEF COMPLAINTS:

6. PAST HISTORY:

WHETHER RECEIVED ANTIFUNGAL

a. ORAL WITHIN THE LAST 6 MONTHS:

b. TOPICAL WITHIN THE LAST 3 MONTHS:

7. ON EXAMINATION:

   a. DISTRIBUTION OF LESIONS:

   b. COLOUR OF THE LESIONS: HYPOPIGMENTED/ HYPERPIGMENTED

8. DIAGNOSIS:

9. TREATMENT GIVEN:

10. KOH MOUNTING:

11. SPECIES GROWN:

ANNEXURE II

I. Sabouraud's Dextrose Agar (Himedia)

Peptone:	1gm
Dextrose:	4 gm
Agar:	2 gm
Cycloheximide:	0.05 gm
Chloramphenicol:	0.005 gm
Distilled water:	1000 ml

Autoclave the above mentioned ingredient at 121^0 C for 15 min and adjust pH at 5.6. Dispense in tubes allowed it to cool in slanted position.

II. Christensen's Urease Medium (Himedia)

Peptic digest:	0.1 gm
Dextrose:	0.15 gm
NaCl:	0.5 gm
Disodium phosphate:	0.12 gm
Monopotassium phosphate:	0.08 gm
Phenol red:	0.0012 gm
Agar:	1.5 gm
Distilled water:	100 ml

Final pH is adjusted to 6.8.

Mix properly all the ingredients in 100 ml of distilled water and autoclave at 121^0 C for 15 min. Dispense in tubes allowed it to cool in slanted position.

III. **Tween Esculin Agar**

Peptone:	1 g
Glucose:	1 g
Yeast extract:	0.2 g
Ferric ammonium citrate:	0.05 g
Esculin:	0.1 g
Agar:	1.5
Distilled water:	100 ml

It was sterilized by autoclaving at 121^0 C for 15 min. The media was then allowed to cool to 45^0 C to which 500 µl 0f tween 60 was added. It was then poured into tubes and kept in slanting position to obtain TE slants.

IV. Modified Dixon agar[64]

Malt extract:	3.6 g
Mycological peptone:	0.6 g
Dessicated ox bile:	2g
Tween 40:	1 ml
Glycerol:	0.2 ml
Oleic acid:	0.2 ml
Agar:	1.2 g

Chloramphenicol:	0.5 g
Distilled water:	100 ml

All ingredients were dissolved in distilled water and autoclaved at 121^0 C for 15 min. Then poured into petridishes.

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
1	SRINIVAS	60	M	HYPOPIGMENTED	CHEST, BACK, UPPER LIMBS	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
2	NAGENDRA	23	M	HYPOPIGMENTED	NECK, TRUNK, ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
3	NARENDRA	21	M	HYPOPIGMENTED	TRUNK, NECK, ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
4	CHALAPATHI	23	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
5	SHARANYA	22	F	HYPOPIGMENTED	TRUNK	KZ Lotion, tab AF	+	-	-								NO GROWTH
6	JASKEL	30	M	HYPERPIGMENTED	NECK, TRUNK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
7	RAJANNA	45	M	HYPOPIGMENTED	NECK, TRUNK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
8	JUNAID	42	M	HYPOPIGMENTED	NECK, BACK, TRUNK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
9	KRISHNAPPA	35	M	HYPOPIGMENTED	NECK, ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
10	BHARATHI	18	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
11	VENKATESH	33	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
12	NARAYAN SWAMY	26	M	HYPERPIGMENTED	TRUNK	KZ Lotion, tab AF	+	-	+	+	+		+	+	+	-	M. SLOOFFIAE
13	VENKATAKRISHNAIAH	32	M	HYPOPIGMENTED	TRUNK	KZ Lotion, tab AF	+	-	-								NO GROWTH
14	SHEK NIZAMUDDIN	18	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
15	YOGISH	18	M	HYPERPIGMENTED	CHEST, NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
16	SHRUTHI	20	F	HYPOPIGMENTED	NECK, ARMS	KZ Lotion, tab AF	+	-	+	+	+		+	+	+	-	M. SLOOFFIAE
17	PRABHAKAR	23	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
18	NAGARAJ	25	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
19	ANAND	30	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	-								NO GROWTH
20	SHANKAR	25	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	-								NO GROWTH
21	SRINATH	16	M	HYPERPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
22	CHALAPATHI	32	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
23	ABIDHA	40	F	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
24	MOHAMMED IRFAN	28	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
25	AMRUTHA	26	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
26	NIKHIL	32	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
27	ANIL	23	M	HYPOPIGMENTED	ARMS, NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
28	YESHWANTH	28	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
29	SUNIL	23	M	HYPOPIGMENTED	BACK,ARM	KZ Lotion, tab AF	+	-	-								NO GROWTH
30	FOUZIA	32	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
31	YOHAN	34	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
32	CHAITRA	23	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
33	GANESH	34	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
34	VENKATARAMAPPA	55	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
35	CHOWDANATH	23	M	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
36	SHIVKUMAR	26	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
37	SUNITHA	21	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
38	SUDHA	30	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
39	MALLIKA	15	F	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
40	DEVARAJ	21	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
41	SURESH	19	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
42	RAMESH	21	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
43	VENKATESH	28	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
44	AMRUTHA	24	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
45	LAKSHMAN	26	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
46	RAJESH	31	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
47	VIJAY	28	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
48	RAJENDRAPPA	26	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	-								NO GROWTH
49	RANGANATHAN	28	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
50	SHRUTHI	23	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
51	VENUGOPAL	27	M	HYPOPIGMENTED	CHEST, ARMS	KZ Lotion, tab AF	+	-	-								NO GROWTH
52	CHARAN	24	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
53	DHANANJAY	35	M	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	-								NO GROWTH
54	DEEPAK	34	M	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
55	SHANTHAMMA	36	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
56	MUNIYAPPA	26	M	HYPOPIGMENTED	AXILLA	KZ Lotion, tab AF	+	-	-								NO GROWTH
57	LAKSHMI	32	F	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
58	LAKSHMANAPPA	31	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
59	PUSHPALATHA	24	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	-								NO GROWTH
60	NAGARAJA	38	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
61	AVINASH	23	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
62	SRINIVAS	30	M	HYPERPIGMENTED	AXILLA	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
63	SHANKAR	19	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
64	MANJULA	26	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
65	ROHAN	22	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
66	VENKATESH	22	M	HYPOPIGMENTED	CHEST, SHOULDER	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
67	RAJESH	27	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
68	SAVITHRI	24	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
69	MAHESHWARAPPA	26	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
70	RAMESH	32	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
71	VENKATESHWARAPPA	28	M	HYPOPIGMENTED	ARMS, CHEST, NECK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
72	SARASWATHI	23	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
73	REVANNA	36	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
74	GAYATHRI	35	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
75	MADAN	27	M	HYPERPIGMENTED	NECK	KZ Lotion, tab AF	+	-	-								NO GROWTH
76	SHRUTHI	28	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
77	DHANUJA	35	F	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
78	ISMAIL	33	M	HYPOPIGMENTED	NECK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
79	MAHADEV	42	M	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
80	SHASHANK	26	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
81	MANJUNATH	23	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
82	NADEEB	25	M	HYPERPIGMENTED	CHEST, NECK, ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
83	NAGAMMA	36	F	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
84	VENKATAMMA	28	F	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	-								NO GROWTH
85	RAVI	24	M	HYPERPIGMENTED	CHEST, BACK, NECK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
86	SRINIVASALU	50	M	HYPOPIGMENTED	BACK, CHEST, ARMS	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
87	PRASHANTH	15	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
88	GURUMURTHI	26	M	HYPOPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	-								NO GROWTH
89	NAGESH	23	M	HYPERPIGMENTED	BACK, NECK, ARMS	KZ Lotion, tab AF	+	-	-								NO GROWTH
90	RAGHUPATHI	28	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA

MASTER CHART

SL. NO	NAME	AGE	SEX	TYPE OF LESIONS	SITE	TREATMENT (KZ lotion – Ketoconazole lotion), Tab AF - Tablet Fluconazole	KOH	SDA	SDA WITH OLIVE OIL	CATALASE	UREASE	ESCULIN	TWEEN 20	TWEEN 40	TWEEN 60	TWEEN 80	ISOLATE
91	NANDINI	21	F	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
92	LAKSHMAN	32	M	HYPOPIGMENTED	SHOULDER, NECK, BACK	KZ Lotion, tab AF	+	-	-								NO GROWTH
93	FATHIMA	28	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
94	SUBRAMANI	17	M	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
95	DAYANAND	32	M	HYPOPIGMENTED	ARMS	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS
96	YOGISH	25	M	HYPERPIGMENTED	CHEST	KZ Lotion, tab AF	+	-	+	+	+	-	-	-	-	-	M. GLOBOSA
97	MANJULA	23	F	HYPERPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
98	ZABI	18	M	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	-	+	+	+	+	M. FURFUR
99	MUNIRAJ	31	M	HYPOPIGMENTED	SHOULDER	KZ Lotion, tab AF	+	-	-								NO GROWTH
100	LEELAVATHI	23	F	HYPOPIGMENTED	BACK	KZ Lotion, tab AF	+	-	+	+	+	+	-	+	+	+	M. SYMPODIALIS

Key words

KZ lotion – Ketoconazole lotion
Tab AF - Tablet Fluconazole