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RESEARCH ARTICLE

A CLINICO INVESTIGATIVE AND MYCOPATHOLOGICAL PROFILE OF FUNGI CAUSING SUBCUTANEOUS INFECTIONS IN A TERTIARY CARE HOSPITAL

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Abstract

Background & Objectives: Subcutaneous mycoses comprise a heterogeneous group of fungal infection. They are characterized by development of lesions at the site of inoculation of fungi in the subcutaneous tissues. Immunocompromised patients are at increased risk of infections. The main subcutaneous mycoses are Mycetoma, Chromoblastomycosis, Phaeohyphomycosis, Sporotrichosis followed by Rhinosporidiosis, Subcutaneous phycomycosis and lacaziosis. This study was planned with the aim of determining the prevalence, etiological agents of subcutaneous mycoses and their Clinicopathological profile.

Materials And Methods: A total of 40 consecutive patients with clinically suspected subcutaneous mycoses attending various departments of Govt. Stanley Medical College and Hospital were included in this Cross sectional study. Fungi were identified by Direct microscopy in 10 and 40 % KOH, histopathological examination of biopsied tissue, colony characteristics on Sabourauds dextrose agar media both at 25 and 37 °C and detailed morphology of each fungus on Lactophenol cotton blue mount.

Results: The percentage of Subcutaneous mycoses among fungal infections was 2.11%. Of the 40 cases of subcutaneous mycoses, 16 cases of Phaeohyphomycosis (40%), 12 cases of Mycetoma (30%), 10 cases of Chromoblastomycosis (25%) and 2 cases of Rhinosporidiosis (5%) were identified. In this study, 14 different species of fungus were found to be responsible for subcutaneous infections.

Interpretation &Conclusion: The prevalence of Subcutaneous mycoses in this region is 2.11% of total fungal cases. Higher incidence of infections were noticed in the age group of 41-60. Comorbidities plays a crucial role in disease progression. Clinical suspicion, early laboratory confirmation of diagnosis and appropriate treatment is crucial in these infections. Clinicians must be aware of the clinical presentations of subcutaneous mycoses. Timely diagnosis will prevent chronic morbidity in patients.

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Introduction:-

Fungal infections are important cause of mortality and morbidity because of changing patient profiles and environmental factors[2]. There are many studies of bacterial, viral and protozoal infections but only limited studies in fungal infections. In 1677, Hooks study described yellow spots on Demark rose leaves and implicated filamentous organism as a causative agent. This was the initial study about fungi before the bacteriology was well defined.

Though some fungi are pathogenic to man, some of them are beneficial to mankind. They breakdown organic substrate to recyclic carbon and other elements. So they are a component of our ecosystem. In Atharva veda, they described Mycetoma as 'padavalmikam' meaning "anthill foot". It was observed by John Gill in 1842 in Madurai district of Tamilnadu (India), so the disease was designated by Henry Carter in 1860 as "Madura foot".

Subcutaneous mycoses have many clinical, economical and social impacts on patients, families and the community. In endemic areas, local health facilities and health education are inadequate [16].The causative agents also vary among different areas, hence this study was conducted to know the prevalence of Subcutaneous mycoses, the causative agents in our region and their clinical manifestations with mycopathological pattern. This can help the clinician to identify the lesion early and for treatment.

Materials And Methods:-

The study was conducted after obtaining approval from the institutional ethical committee of Government Stanley Medical College, Chennai. Permission to conduct this study was obtained from the department of Dermatology, Surgery and ENT in Govt. Stanley medical college and hospital. Informed consent was obtained from the patients before enrolment in this study.

Study Design And Duration

Cross sectional study conducted from Feb 2019-Aug 2020**.**

Inclusion criteria:

1. All patients attending Dermatology, Surgey and ENT department showing lesions suggestive of Subcutaneous mycoses as diagnosed by the clinician were included in the study.
2. All ages and both sexes.

Exclusion Criteria:

Patients, who were already on antifungal therapy within 3 months prior to the commencement of the study, were excluded.

Specimen Collection, Transport And Processing*:*

Specimens were collected under strict aseptic conditions to optimize fungal yield. Specimens like Pus, Exudates, Aspirations, Curettage, Punch biopsy from subcutaneous growth were collected in a sterile container[1, 2].In a case of suspected Mycetoma, after cleaning the affected area with 70% ethanol, gauze piece moistened with normal saline were occluded over the sinuses for 24 hours and then examined for grains[21,22].

Macroscopic and Direct Microscopic Examination were done using KOH mount, Gram stain, Modified acid fast stain. Pathological features were identified by H & E stainand GMS stain. Fungal culture properties were studied using Neutral Sabouraud's Dextrose agar (SDA) with antibiotics (Gentamycin, Cycloheximide), Potato Dextrose Agar, Cornmeal agar, Chrom agar. Culture media were incubated at 25°C and 37°C. Macroscopic pictures like Rate of growth, General topography, Texture, Obverse pigmentation and Pigmentation on the reverse were noted. Fungal culture was kept incubated for six weeks. Culture was examined daily for first week and two times a week for subsequent period. Microscopic features like mycelia arrangement and conidial morphology were identified by Lactophenol cotton blue mount, Scotch tape technique and slide culture.

Results:-

Table I:- (Age Wise Distribution (n=40).

Age group (years)	Mycetoma (%)	Chromoblasto mycosis(%)	Phaeohypho mycosis(%)	Rhinosporidiosis (%)	Total (%)
1 - 20	0	1(10%)	2(13%)	0	**3(8%)**
21 - 40	3(25%)	1(10%)	1(6%)	2(100%)	**7(18%)**
41 - 60	8(67%)	6(60%)	11(68%)	0	**25(62%)**
61 - 80	1(8%)	2(20%)	2(13%)	0	**5(12%)**
Total	**12(100%)**	**10(100%)**	**16(100%)**	**2(100%)**	**40(100%)**

Table II:- (List Of Subcutaneous Mycoses In The Study Population (n=40).

S.NO	CASES	NO. OF CASES	PERCENTAGE
1	Mycetoma	12	30%
2	Chromoblastomycosis	10	25%
3	Phaeohyphomycosis	16	40%
4	Rhinosporidiosis	2	5%
Total	**CASES**	**40**	**100%**

Table III:- (Dematiaceous And Non Dematiaceous Fungi Isolated (N=14).

FUNGI		TOTAL	PERCENTAGE
DEMATIACEOUS	Bipolaris hawaiiensis(1) Curvularia lunata(1) Cladosporium carrionii(2) Fonsecaea pedrosoi(1) Exophiala jeanselmei(1)	**6**	**43%**
NON DEMATIACEOUS	Aspergillus nidulans(1) Acremonium falciforme(1) Madurella mycetomatis(2) Trematosphaeria grisea(1) Paecilomyces variotii(1) Candida parapsilosis(1) Trichosporon spp.(1)	**8**	**57%**

Fig 1:- (Gender Wise Distribution).

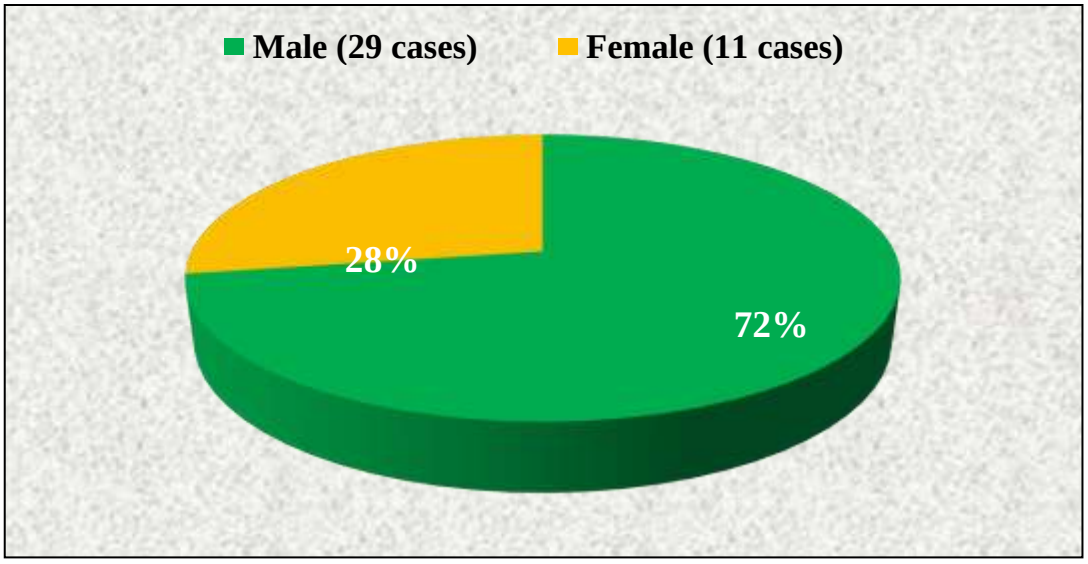


Fig II:- Occupational Pattern (n=40).

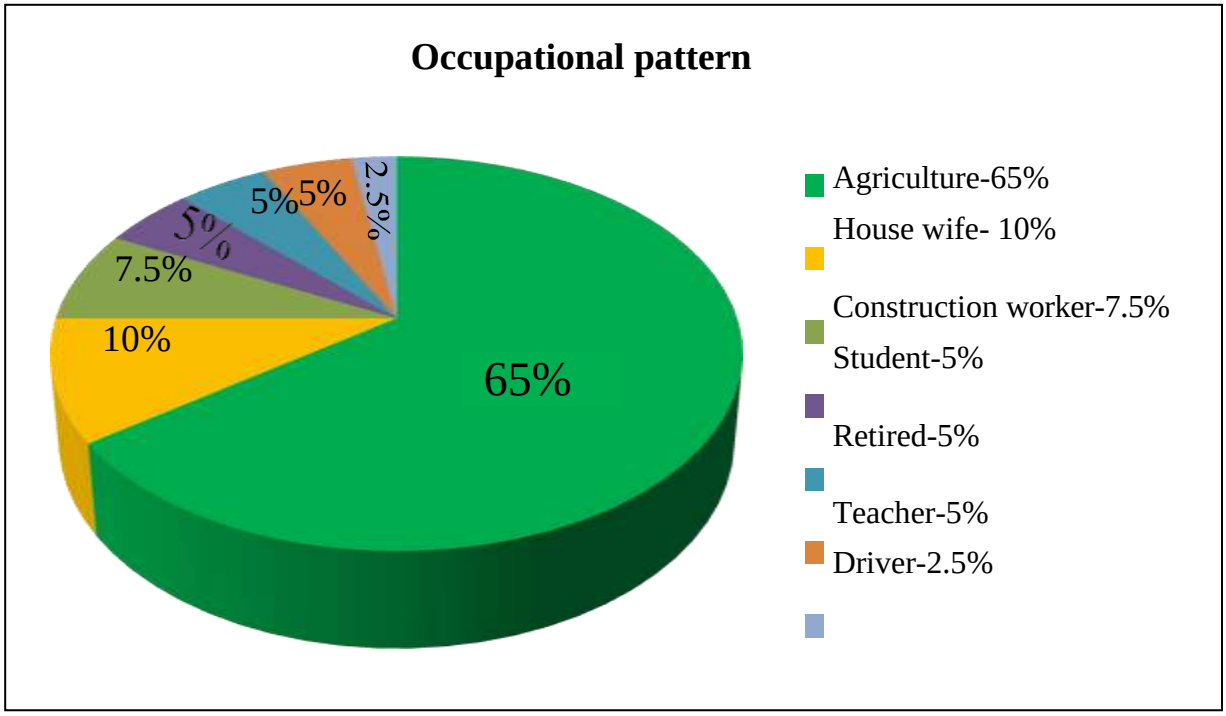


Fig III:- (Distribution Of Diabetes Mellitus (n=40).

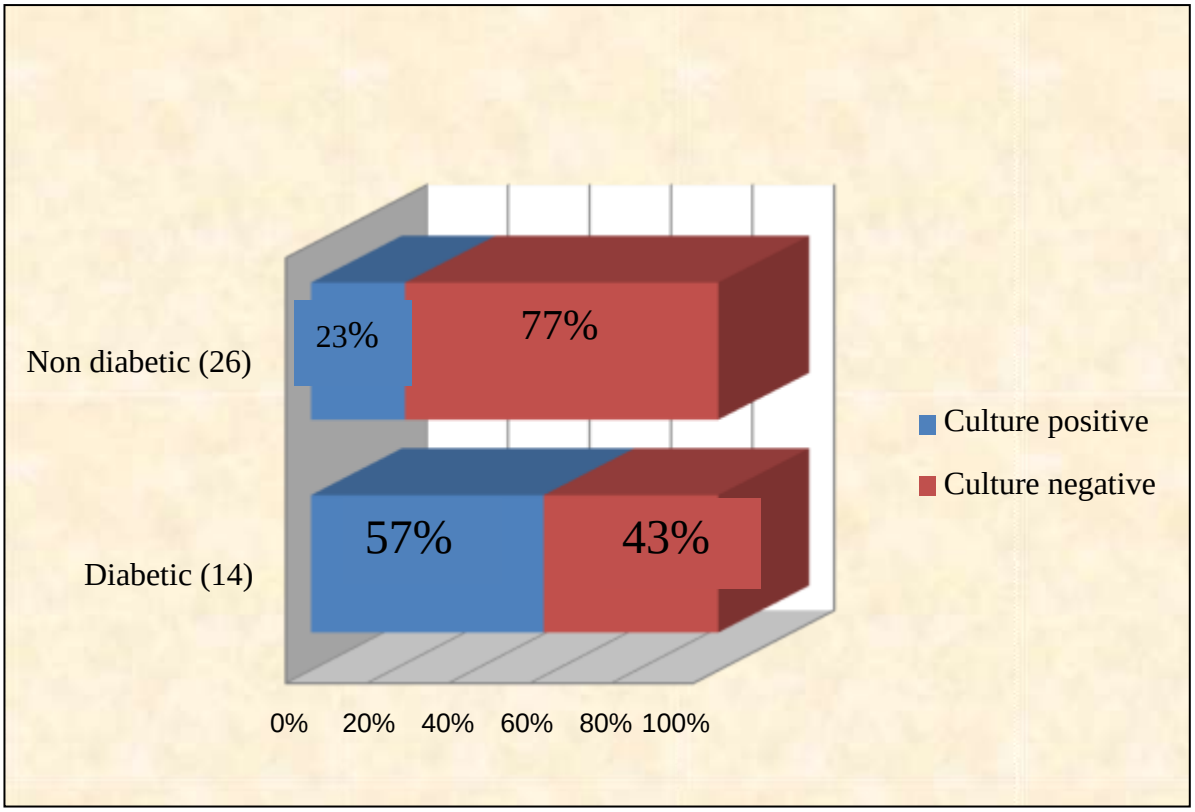


Fig IV:- (Microscopic picture of Bipolaris hawaiiensis shows septate hyphae with multi- septate conidia (distosepta) sympodially attached to conidiophores.

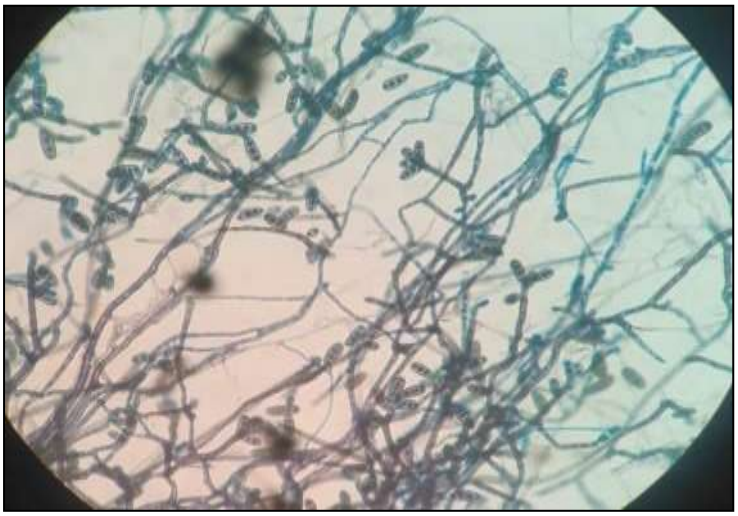
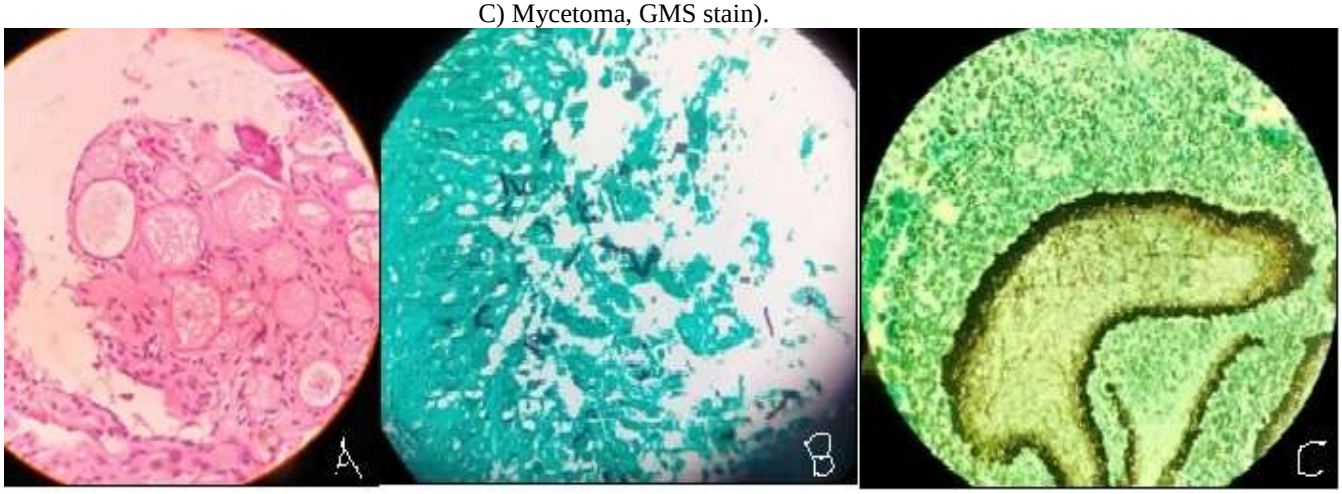


Fig.V (CHROMOBLASTOMYCOSIS- Verrucous plaques of chromoblastomycosis and cultural pictures of Fonsecaea pedrosoi)

Fig.VI:- (Madurella Mycetomatis- Macroscopically glabrous, folded, leathery, greenish colonies and microscopically septate hyaline hyphae with flask shaped phialides)

Fig.VII:- (Histopathological features of A) Rhinosporidiosis ,H&E stain B) Phaeohyphomycosis, GMS stain C) Mycetoma, GMS stain).



In the present study period, out of 59525 cases of skin diseases, 1892 cases of fungal etiology were noticed. It was 3.17% of total skin diseases. From the 1892 patients with fungal etiology, 40 patients were clinically diagnosed as subcutaneous mycoses. It was 2.11% of total fungal cases. Higher incidence of infections were noticed in the age group of 41-60 (25), followed by the age group, 21 – 40 (7)[Table.I]. In this study male patients outnumbered (29) female patients (11)[Fig.I]. Of the 40 cases of subcutaneous mycoses, 16 cases of Phaeohyphomycosis (40%), 12 cases of Mycetoma (30%), 10 cases of Chromoblastomycosis (25%) and 2 cases of Rhinosporidiosis (5%) were identified[Table.II].

In Phaeohyphomycosis, Cladosporium carrionii was the common infecting agent and it was isolated in 2 cases (33%). Compared to phaeohyphomycosis, Mycetoma is less prevalent in this area. 12 cases have been reported during the study period (30%). Fungal isolation was obtained in five cases. The important fungal isolate was Madurella mycetomatis in two cases[Fig.VI]. T.grisea, A.falciforme and A.nidulans were isolated in one case each.

In this study, two rare species of fungi (Bipolaris hawaiiensis and Paecilomyces variotii) were associated with Chromoblastomycosis. The other fungal isolate was Fonsecaea pedrosoi.

Discussion:-

The present study was undertaken to determine the prevalence of subcutaneous mycoses and fungal species responsible for the subcutaneous mycoses. The age group of the patients were from 5 to 72 years, the mean age being

46.625 years(Table.I), same as that studied by *Alexandro Bonifaz et al (2014)* in 7 to 92 years[3]. The common age groups affected were between 41 and 60, which was similar to *Shahindokht Bassiri et al (2018)* who found in more than 40 years [16]. This may be due to that the infection may have started many years ago, suggesting that these individuals may have acquired the disease in younger age [3].

In this study male patients outnumbered (29) female patients (11).The increased incidence in male patients in India is most probably due to the greater outdoor exposure of men, being the sole earning members of the family. In this study, 14/40 patients (35 %) had comorbid conditions (Fig.III). Ten of them presented a single comorbidity and others had two comorbidities. Comorbidities like Diabetes, Hypertension, Tuberculosis, renal failure and asthma were found. The similar data were found in *Bassiri et al (2018),* 10/35 (28.6%).In the presence of underlying immunodeficiency, traumatic inoculation with fungus may result in rapid local spread and systemic disease, often with fatal outcome [16].

Of the 40 cases of subcutaneous mycoses, the incidence was high in rural population i.e. 28 cases (70%) and in the urban population it was 12 cases (30%) (Fig.3).The reason for high incidence of subcutaneous mycoses in rural population is that they were involved in various types of agricultural work and also they did not use any protective measures to prevent injuries during their agricultural works. The similar results were found in *Hirak Jyoti raj et al (2015)*, 32/50 (72%) [9, 12].Of the 40 cases of subcutaneous mycoses, 16 cases of Phaeohyphomycosis (40%), 12 cases of Mycetoma (30%), 10 cases of Chromoblastomycosis (25%) and 2 cases of Rhinosporidiosis (5%) were identified. So according to the present study, the commonest subcutaneous mycoses prevalent in this area is Phaeohyphomycosis followed by Mycetoma.

To conclude, Clinical suspicion, early laboratory confirmation of diagnosis and appropriate treatment is crucial in subcutaneous infections. Eventhough, Sabouraud dextrose agar with antimicrobials needs incubation for upto 8 weeks, it is the routinely used media for isolation. KOH wet mount which gives presumptive identification of fungal elements is also necessary. Early full course of treatment gives mycological cure but resistant strains are also occurring. So it is necessary to monitor cases and their etiological agents, epidemiology, and to establish intervention for treatment, prevention and rehabilitation.

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