Isolation and Identification of Fungus Associated with Nail Scalps of Patients in Betul District of Madhya Pradesh

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Abstract - A dermatophyte, yeast, or mould species invading the nail plate causes onychomycosis, a common nail condition that can manifest clinically in a variety of ways. The purpose of the present study was to isolate and identify the causative fungi of onychomycosis in the population of Betul District of Madhya Pradesh. Nail samples from 50 patients with prediagnosis of onychomycosis during 2021 were examined both by direct microscopic observation of fungal elements in KOH preparations and in culture for the identification of the causative agent. A sufficient number of fungal species were isolated and identified.

Keywords: onychomycosis, causative agent, prediagnosis, microscopic.

1 - Introduction
A common chronic fungal nail infection is called onychomycosis, by invading the nail plate with hyphal or pseudohyphal growth and enzymatic activity, fungal growth progressively destroys the nail plate (Syall et al., 2019). It is one of the common nail disorders and accounts for up to 30% of all superficial infections of the skin. The prevalence of onychomycosis is rising globally, and a number of variables including diabetes, poor peripheral circulation, immunodeficiency, drug treatment, nail damage, and infrequently genetic defects are contributing to this growth (Mercantini et al., 1996). The successful definitive therapy of onychomycosis requires extended treatment courses. Even after initial responses that were clinically sufficient, recurrences are frequently observed. The selection of the first treatment regimen is crucial for a stable long-term cure without relapses due to this and the delayed assessability of the therapeutic effect after the start of treatment (Gupta et al., 2019). The WHO estimates that 20% to 25% of people globally have superficial mycotic infection. More than 50% of patients visiting dermatology outpatient departments in South India have dermatophytic infections (Grumpt et al., 2013). While tinea infections are widespread around the world, they are particularly prevalent in tropical regions and places with high levels of humidity, population density, and unhygienic living circumstances. Clinical manifestations and the types of superficial parasite infection that cause them change according to geographic location, economic conditions, and behavioural tendencies. (Havlickova et al., 2008). Either direct contact or indirect contact (fomites) can spread it. In the current investigation, dermatophytes were isolated and identified using potassium hydroxide (KOH) and sabouraud dextrose agar (SDA) media from nail samples of clinically suspected cases of oncomycosis.

2 - Material And Methods
In the year 2021, 50 samples of nail scrapings, consisting of 39 males and 11 females, were collected from the Betul district of Madhya Pradesh.

3 - Culture Media
SDA media were employed for the isolation and identification of the causative agents. The culture medium for the growth of fungi contained mycological peptone and had a high content of either glucose or dextrose (20 gm/L, Himedia, Mumbai). The medium had a pH of 5.0, which prevented most bacteria from growing.

4 - Process of materials for culture
Clinical materials were collected from the patients suffering from various types of

5 - Nail scrapings
Nail scrapings, especially those close to the nail bed, were gathered in a sterile petri plate for
6 - Microscopic examination
The clinical material that was so collected was dissolved in two drops of 10% KOH on a glass slide. After applying the coverslip, the slide was briefly heated over a spirit lamp while taking care to prevent the material from boiling.

7 - Culture of the clinical specimens
Dermatophytes were isolated from the clinical sample using Sabouraud dextrose agar with gentamicin medium. With the use of a sterile needle, nail scrapings were applied to the slant surface of the medium to inoculate the agar slants. For three to four days, all of the infected tubes were cultured at 30°C room temperature. The slide preparation was examined under a high power microscope to obtain a comprehensive microscopic morphology of the etiologic agent in clinical material, and the observations were noted.

The clinical specimens were included microscopic analysis and culture. The culture every day for any changes in the medium's colour and signs of fungal development. Expected cultural responded on dermatophyte test medium at 30°C after 2–7 days.

8 - Identification of the growth of the dermatophytes (microscopic after culture)
A small amount of the fungus was taken from the cultured tubes and placed on the clean slide. After 2-3 drops of lactophenol cotton blue had been applied, the materials were spread out across the slide with the aid of a botanical needle, so that a clear examination was possible. After separating the entire, they were covered with coverslips and placed under a microscope to identify the fungi.

9 - Results
A total of 50 samples were obtained from patients with suspected dermatophytes (superficial mycoses). Diagnosis was confirmed by microscopic examination (KOH mounting) was in 27 cases (54%). From the total isolates identified by culture growth Trichophyton rubrum and Candida albicans were the most common accounting for 4 cases each (14.8%) of all fungal infections. Microsporum gypseum, Alternaria alternate, Scopulariopsis flava and Cladosporium sphaerospermum were the next most commonly isolates agents with 3 cases each (11%). 7.4% of the infections were found to be caused by Fusarium oxysporum and Acremonium blochii. Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Penicillium Chrysogenum, Cladosporium brevicaulis and Rhizopus microsporus were found to cause least infection (3.7%).

● Percentage of male and female infected

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<tr>
<td>Male</td>
<td>11%</td>
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<td>Female</td>
<td>78%</td>
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proportion of samples infected by various Fungal species

Fig.1-Showing isolation of infected nail sample from patient

Fig.2-microscopic finding of Rhizopus microsporus

10 - Conclusion
Onychomycosis is a challenging fungal infection with a high recurrence rate that is related to the anatomical and pathophysiological conditions in the nail organ and the prolonged duration of treatment that is necessary. Infections with yeast and mould strains that are not dermatophytes play a significant and potentially growing role in the epidemiology of onychomycosis, according to data from clinical studies. Therefore, in order to choose an efficient treatment plan for all patients, a mycological diagnosis of the causal fungal pathogens should be regarded obligatory. Clinical studies demonstrate that the use of combination therapy combining locally applied and orally administered antifungals increases the complete cure rates (mycological and clinical) of severe onychomycosis. For a treatment to be successful, it must be followed for the long term, until a clinical cure is obtained, therapy should be continued.

11 - Conflict of Interest
There are no conflicts of interest
REFERENCES:
3. Brilhante, R. S. N., Cordeiro, R. A., Medrano,
12. Sylla, K., Tine, R. C., Sow, D., Lelo, S., Dia,