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# Clinico-mycological Study of Dermatophytosis at a Tertiary Care Centre-A Cross-Sectional Study

**Ananya Verma[1], Anju Mahor[2], Sanjay Khare[3], Suneel Kumar Ahirwar[4*], Sadhna Sodani[4]**

[1]Post Graduate Student, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, MP, India
[2]Assistant Professor, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, MP, India
[3]Professor & Head, Department of Dermatology, Mahatma Gandhi Memorial Medical College, Indore, MP, India
[4]Associate Professor, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, MP, India

***Address for Correspondence:** Dr Suneel Kumar Ahirwar, Associate Professor, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India
**E-mail:** drsk2311@gmail.com

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## ABSTRACT

**Background:** Dermatophytes, hyaline septate molds, are divided into *Trichophyton, Epidermophyton,* and *Microsporum*, which produce protease enzyme, enabling skin, hair shaft, and nail colonization and infection. This study is undertaken to study clinical presentation and laboratory identification of fungal species in suspected cases of dermatophytosis.

**Methods:** The study involved 404 patients suspected of dermatophytosis at Maharaja Yashwantrao Hospital, Indore, Madhya Pradesh, who were studied after receiving samples from the Department of Microbiology from December 2022 to January 2023. The affected area was cleaned with 70% ethanol before sample collection, and materials were used to isolate dermatophytes from hair, nails, and skin from the lesion's active zone.

**Results:** Out of 404 clinically suspected cases, only 205 were culture-positive for dermatophytes. The age group of 31–40 years old accounts for the bulk of patients (33.6%), followed by 21–30 years old (26.5%), and the age group of above 71 years old (0.24%). The most prevalent species was *Trichophyton rubrum* 112 (54.3%), followed by *Trichophyton mentagrophytes* 93 (45.1%), primarily found in *Tinea corporis* 75 (66.9%) and *Tinea cruris* 25 (22.3%).

**Conclusion:** Dermatophytes are prevalent in tropical and subtropical India, with *T. corporis* being the most common clinical form. *T. rubrum, mentagrophyte,* and *M. gypseum* are predominant species. Laboratory diagnosis is crucial for appropriate therapy.

**Key-words:** Dermatophytosis, Trichophyton, Tinea corporis, KOH, SDA

## INTRODUCTION

Hyaline septate moulds, or dermatophytes, are categorised into three primary taxa based on physical traits. *Trichophyton, Epidermophyton,* and *Microsporum* are among them [1]. These fungi are capable of producing protease, an enzyme that breaks down keratin and permits infection, invasion, and colonisation of the nail, hair shaft, and stratum corneum [2].

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Dermatophytosis is the term for the infection caused by dermatophytic fungus in keratinised tissues [3].

In clinical terms, dermatophytosis is also referred to as "Tinea" or "Ringworm," and it can be categorised based on the affected place. These include *T. manuum* (palms), tinea pedis (athlete's foot), *T. capitis* (scalp), *T. faciei* (face), *T. barbae* (beard parts of the face and neck), *Tinea corporis* (glaborous skin of the body), tinea cruris (groin), and *T. ungium* (nail infection) [4]. Based on their native habitat, these fungi can be divided into three groups: geophilic, zoophilic, and anthropophilic.

According to [5], these groupings are descended from animals, people, and soil, respectively. Clinical symptoms can be asymptomatic or limited to pruritus, and they can linger for months or years depending on the causal agent and the host's immunological response [6]. Twenty to

 

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twenty-five percent of people worldwide suffer from these cutaneous mycoses. Recent research indicates that the incidence of dermatophytosis has increased nationwide over the last ten years, and it has increased even more over the last 5 to 6 years. India is currently experiencing conditions similar to an epidemic as a result of this alarming rate of increase. There is a very large range in the current reported prevalence in India (6.09%–61.5%). Studies from south India have reported a prevalence ranging from 6.09% to 27.6%, while north India has recorded a high prevalence of 61.5% [7].

Over the past ten to twelve years, dermatophytosis incidence has increased in India. There also seems to be a change in the primary causing organism, with more and more recent research reporting *T. mentagrophytes* as the principal culprit [8]. The current gold standard for dermatophyte identification is a microscopic analysis of clinical specimens, which is followed by fungus culture to determine the particular type of fungus [9]. This study is undertaken to study clinical presentation and laboratory identification of fungal species in suspected cases of dermatophytosis. To identify the causative fungal species from all clinically suspected cases of Dermatophytosis in a tertiary care hospital.

## MATERIALS AND METHODS

**Study design and place-** The present study was a cross-sectional study conducted on clinically suspected cases of dermatophytosis. A total of 404 patients with suspected dermatophyte infection visited the Outpatient Department of the Department of Dermatology, Venereology, and Leprosy at Maharaja Yashwantrao (M.Y.) Hospital, attached to Mahatma Gandhi Memorial Medical College (MGMMC), Indore, Madhya Pradesh, was studied. Samples were received in the Department of Microbiology from December 2022 to January 2023. The study was conducted after approval from the scientific and ethical committees.

**Inclusion Criteria-** All age groups of either sex have clinical features of dermatophytosis.

**Exclusion Criteria-** Nil

**Sample Collection-** As per standard protocols [10]. The affected area was thoroughly cleaned with 70% ethanol before sample collection. The samples were sufficiently collected and captured from the lesion's active zone, which is the boundary of the affected area. The following materials were used to isolate the dermatophytes from the hair, nails, and skin.

**Skin-** Skin lesions that exhibit an active inflammatory border were scraped from the periphery; if not, they were scraped completely. The most effective method for collecting was to use a sterile scalpel blade to collect epidermal scales from the area close to the advancing edges of the rings.

**Nails-** Samples were taken from the nail bed and lower nail plate, especially around the lesion's edge, using a small curette or scalpel blade after the nails were clipped in the case of distal subungual onychomycosis. The healthy top plate of the nail was pared when proximal subungual onychomycosis was suspected, and material from the infected lower plate of the nail was then collected. By scratching the white areas, infected material for white superficial onychomycosis was gathered.

**Hair-** Hair roots and crusts were plucked from the infected area or its edge for large lesions so that the root was included and suppurating lesions were swabbed. If hair fragility prevented this, as in "black dot" tinea capitis, a scalpel was used to scrape scales and excavate small parts of the hair root [11].

### Microbiological methods

**Direct microscopy of the specimen-** Two sections of the collected specimens were separated. The specimens were initially inspected under a microscope with a 10% potassium hydroxide (KOH) solution placed on a glass microscope slide, followed by the addition of a small specimen piece and a cover slip. Skin and hair samples were left in a petri dish for 20–30 minutes, while nail samples were soaked in a 20% KOH solution overnight. Dermatophytes were identified under the microscope by observing branching and septate hyphae with angular or spherical arthroconidia (arthrospores), usually in chains. All skin and nail specimens of ringworm fungi displayed similar characteristics.

**Specimen culture-** The fungus was isolated using Sabouraud dextrose (SDA) agar containing chloramphenicol (0.05%) without cycloheximide or Dermatophyte Test Media (DTM)**.** The second portion of samples of skin, hair, and nails were directly inoculated

 

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onto the medium and then examined every alternate day for evidence of growth, with SDA and DTM plates being observed for 4 weeks and 2 weeks, respectively. Further evaluation was performed based on colony morphology, microscopic characteristics, and relevant biochemical tests for the detection of dermatophyte species.

**Identification of cultural growth**

**Tease mount preparation**- Put a tiny drop of the mounting medium, lactophenol cotton blue, on a sanitised, grease-free microscopic slide. A tiny amount of growth should be aseptically removed halfway between the colony's perimeter and middle. Put that colony on a cotton blue lactophenol drop. With a pair of dissecting needles, tease the fungus until it becomes thin and spreads out. A cover slip should be carefully placed at the edge of the mounting fluid drop. You can remove excess lactophenol with a tissue or blotting paper. Use varnish or nail paint to coat the coverslip's edges to protect the mount.

**Slide culture-** Using aseptic methods, cut CMA or PDA agar blocks into squares about 1 cm in size. Then, place the agar block onto a slide. Spoon a tiny bit of colony onto each of the agar block's four edges. Position a coverslip over the inoculated agar block using sterile forceps. To keep the agar block from drying out, add 1 to 1.5 mL of sterile water to the petri dish to produce a humid environment. To avoid moisture condensation on the slide, it is optional to incorporate 5–20% glycerine into the sterile water. Slide culture should be put on a petri dish and left to grow in the dark. When mature conidia or spores are visible, the slide culture is prepared for observation. On a tiny slide, apply a tiny drop of mounting media (LPCB). Without pushing or tugging, carefully remove the coverslip from the agar block using forceps. Heat-fix the fungus and its spores by passing the coverslip swiftly through the blue part of a flame (overheating might cause hyphae breakdown). To prevent trapped air bubbles, gently place the coverslip on the mounting media. Wipe off any extra mounting medium, then use nail polish to seal the borders of the coverslip. By taking off the agar block from the microscope slide used for the slide culture setup, adding a coverslip and a drop of lactophenol, and sealing the coverslip with nail polish, one can produce a second mount. Before adding lactophenol blue, immerse the colony in 95% alcohol or ethyl acetate to prevent air bubbles.

**Urease test-** We inoculated the slant surface of Christensen's urea agar with a pure culture of the test fungi in a test tube and then incubated it at room temperature (27°C) for 7 days to assess the urease activity of the isolated dermatophyte species. A positive outcome was signified by the medium changing from straw to pink, whilst no colour shift was interpreted as a negative outcome.

**Statistical Analysis-** The SSPS version 22 was used to analyse the data. The study's categorical variables were described using percentages and frequencies. With a 95% confidence interval, the proportion ratios representing the results were displayed. A p-value of less than 0.05 indicated statistical significance.

**RESULTS**

Table 1 shows the distribution of patients according to age. Most of the patients belong to the age group of 31–40 years (33.6%), followed by 21–30 years (26.5%), and the least from the age group of more than 71 years (0.24%). Out of 404 clinically suspected cases, only 205 were culture-positive for dermatophytes. The most common age group showing culture positivity was 31–40 years.

**Table 1:** Distribution of patients according to age (n=404)

| Age (Years) | Patients (%) | Culture-positive for Dermatophytes | Culture positive (%) |
|---|---|---|---|
| 0-10 | 16(3.96) | 8 | 3.90 |
| 11-20 | 49(12.10) | 21 | 10.24 |
| 21-30 | 107(26.48) | 56 | 27.32 |
| 31-40 | 136(33.6) | 78 | 38.05 |
| 41-50 | 68(16.83) | 29 | 14.15 |
| 51-60 | 19(4.70) | 11 | 5.37 |
| 61-70 | 8(1.98) | 2 | 0.98 |
| >71 | 1(0.24) | 0 | 0.00 |
| Total | 404 | 205 | 100.00 |

*Mean±S.D=37.49 ± 5.76 years*

 

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Table 2 indicates that there is a significant association between clinical diagnosis and age group. *T. corporis* and *T. cruris* were more common in the age group of 31–40 years. While tinea unguium was more common in the age group of 21–30 years.

**Table 2:** Age-wise distribution of microscopically confirmed dermatophytosis cases

| Provisional diagnosis | Number (%) | Age of patients (years) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | >71 |
| *T. cruris* | 99 | 3(18.8) | 10(20.4) | 24(22.4) | 33(24.2) | 22(32.3) | 3(15.8) | 4(50) | 0 |
| *T. corporis* | 218 | 6(37.5) | 21(43) | 60(56) | 82(60.2) | 36(52.9) | 10(52.6) | 2(25) | 1(100) |
| *T. faciei* | 14 | 1(6.2) | 5(10.2) | 5(4.6) | 3(2.2) | 0 | 0 | 0 | 0 |
| *T. pedis* | 8 | 0 | 0 | 6(5.6) | 1(0.7) | 1(1.4) | 0 | 0 | 0 |
| *T. unguium* | 24 | 1(6.2) | 5(10.2) | 8(7.5) | 5(3.6) | 3(4.4) | 1(5.2) | 1(12.5) | 0 |
| *T. capitis* | 17 | 5(31.3) | 6(12.2) | 2(1.9) | 3(2.2) | 1(1.5) | 0 | 0 | 0 |
| *T. barbae* | 4 | 0 | 1(2) | 1(0.9) | 1(0.7) | 1(1.5) | 0 | 0 | 0 |
| Combination pattern | 20 | 0 | 1(2) | 1(0.9) | 8(5.9) | 4(5.9) | 5(26.3) | 1(12.5) | 0 |
| Total | 404 | 16 | 49 | 107 | 136 | 68 | 19 | 8 | 1 |

*Fishers exact test = 0.001, p value = <0.0001.*

Table 3 shows culture-positive cases in different clinical diagnoses. Here, *T. corporis* (63.2%) shows the maximum culture positivity, followed by *T. corporis* (23.2%), and the least positivity was seen in tinea capitis and tinea pedis. This shows very small p-value indicates that there is a significant association between the clinical type of tinea infection and the culture results.

**Table 3:** Culture positivity in different clinical types

| Clinical type | No. of cases | Culture positive | Percentage |
|---|---|---|---|
| *T. cruris* | 99 | 50 | 23.2 |
| *T. corporis* | 218 | 136 | 63.2 |
| *T. faciei* | 13 | 6 | 3 |
| *T. pedis* | 8 | 1 | 0.46 |
| *T. barbae* | 24 | 2 | 0.93 |
| *T. unguium* | 17 | 10 | 4.6 |
| *T. capitis* | 5 | 1 | 0.46 |
| Combination pattern | 20 | 9 | 4.2 |
| Total | 404 | 215 | 100 |

*Chi-square=35.63 D.F.=7, p-value<0.0001*

Table 4 shows that *T. rubrum* 112 (54.3%) was the most common species isolated. Most of them were isolated from cases of tinea corporis 75 (66.9%), followed by *T. cruris* 25 (22.3%). The second most common species isolated was *T. mentagrophytes* 93 (45.1%). Only 1 species of *M. gypseum* was isolated from *T. cruris*.

 

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**Table 4:** Dermatophyte isolates in different clinical types of *Tinea*

| Fungal isolates | *T. corporis* | *T. cruris* | *T. unguium* | *T. capitis* | *T. pedis* | *T. barbae* | *Tinea facie* | Combination pattern | Total |
|---|---|---|---|---|---|---|---|---|---|
| *T. rubrum* | 75 | 25 | 0 | 0 | 1 | 0 | 5 | 6 | 112 |
| *T. mentagrophytes* | 61 | 24 | 0 | 1 | 0 | 1 | 1 | 4 | 92 |
| *M. gypseum* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total isolates | 136 | 50 | 0 | 1 | 1 | 1 | 6 | 10 | 205 |

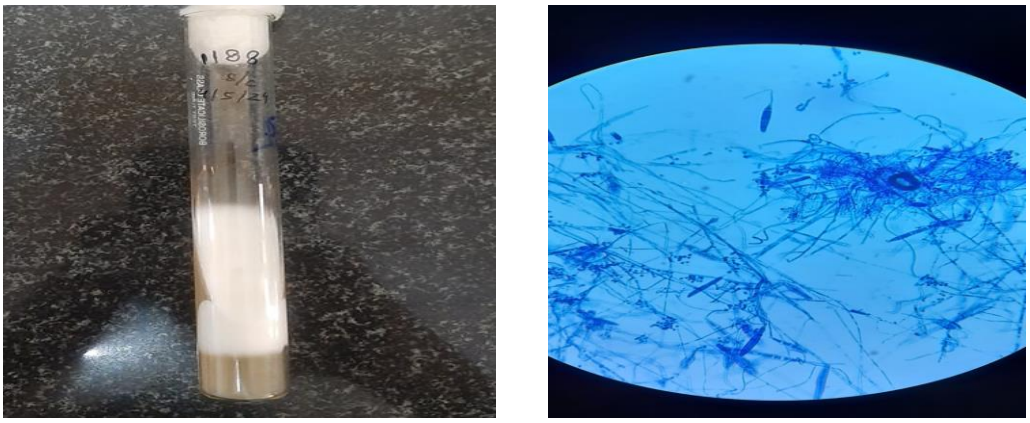
*Chi-square=18.61 Degree of freedom = 14; p-value = 0.180*

*As the p-value >0.05, this indicates that there was no significant association between fungal species and clinical diagnosis.*

The most common species isolated from skin samples is *T. rubrum* 112 (54.6%), followed by *T. mentagrophytes* 92 (44.9%). In 18 hair samples, only 1 dermatophyte, i.e. *T. mentagrophyte*, was isolated. No dermatophyte was isolated from nail samples (Table 5).

**Table 5:** Distribution of dermatophyte species in skin scraping.

| Species | Number of isolates | Percentage (%) |
|---|---|---|
| *T. rubrum* | 112 | 54.6 |
| *T. mentagrophytes* | 92 | 44.9 |
| *M. gypseum* | 1 | 0.5 |



**A** **B**

**Fig. 1 (A)** Growth Trichophyton mentagrophytes on SDA **(B)** Microconidia, macroconidia and spiral hyphae *Trichophyton mentagrophytes*

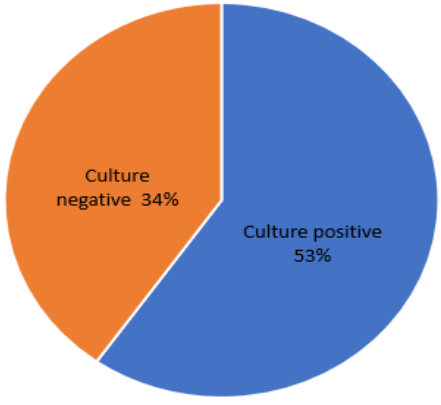



**Fig. 2:** Pie diagram showing culture isolates.

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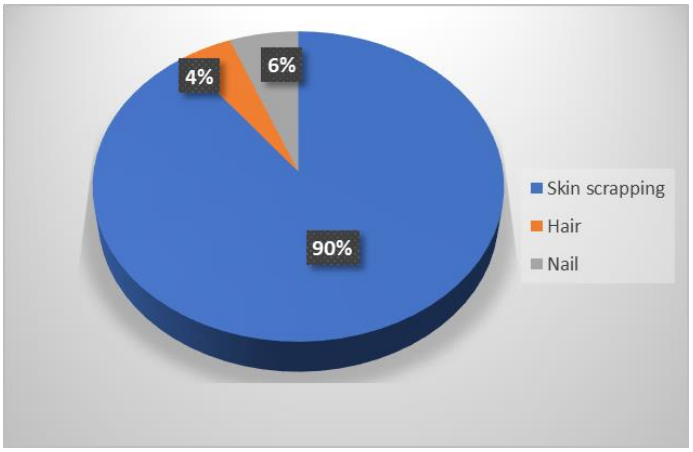


**Fig. 3:** Pie diagram showing the percentage of clinical samples.

## DISCUSSION

Worldwide, fungal infections of the skin, known as dermatophytosis, have emerged as a significant health issue. Fungi called dermatophytes are among the most prevalent in regions with warm climates, including tropical and subtropical areas [12]. This study indicates that the age range of 31–40 years has the highest prevalence of dermatophytes at 33.6%, closely followed by 21–30 years at 26.5%. These findings align with those from previous research, including studies by Veer P et al., Sahebrao Mundhe et al., Farooq et al., and Araya et al. The peak occurrence in adults aged 31–40 years might be attributed to factors such as heightened physical activity, greater exposure to outdoor environments, and warmer climates, which elevate the risk of contracting the infection. Additionally, adults in this age group tend to socialize more with diverse individuals, which plays a role in the transmission of the infection [13-16]. This study indicates that there is a significant association (Fisher's exact test= 0.001, p=<0.0001.) between clinical diagnosis and age group. *T. corporis* and tinea cruris were more common in the age group of 31–40 years. While tinea unguium was more common in the age group of 21–30 years.

In our research, we discovered that tinea corporis (63%) was the most prevalent form, with *T. cruris* (23%) coming in second, consistent with findings by Salahudeen *et al.* [17] and Vijay Nanoty *et al.* [18], who reported rates of 54.4% and 65%, respectively, for tinea corporis. The high prevalence of tinea corporis may be due to its characteristic symptom of itching, prompting individuals to seek medical attention. Additionally, factors such as wearing tight, poorly fitting clothing, complex body structures, and excessive humidity can create an environment conducive to the growth of dermatophytes, leading to conditions that increase body dampness and warmth, thereby favoring the development of tinea corporis and tinea cruris. These conditions are associated with a higher likelihood of tinea corporis and tinea cruris. A divergence in culture isolation ranging from 33% to 77% has been found in the Indian subcontinent [19].

In the present study, 53% of cases were culture-positive. However, a study by Das et al. [20]. done in Kolkata showed a culture positivity rate of 81.55%, which was much higher, and a study done in Bihar showed a low rate of culture positivity of 20.6%. Socioeconomic and cultural differences could be the cause of this discrepancy. The current study showed a very small p-value (Chi-square=35.63, D.F.=7, p<0.0001) indicating that there is a highly significant association between the clinical type of tinea infection and the culture results. The results of this investigation showed that there was a high frequency *of T. rubrum* and *T. mentagrophytes* in instances involving *T. corporis* and *T. cruris*. Whenever these species live among humans, their presence, environmental adaptation, and proximity to humans facilitate the fungal invasion [21, 22]. *T. mentagrophytes* 92 (44.9%) was the leading etiological agent in most clinical types in the current study, followed by *T. rubrum* 112 (54.6%).

The high prevalence of this species is due to its virulence and easy colonization of hard keratin, and its adaptability to the Indian environment accounts for its higher isolation rates [23]. However, a change in the pattern was seen in some states of India over the past five years due to an increase in the incidence of *T. mentagrophytes* [7,24]. In various studies like Das *et al*. Bihar, 2022 [25], and Salahudeen *et al.* Kerala, 2023 [17] showed a higher

 

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prevalence of *T. mentagrophytes*, i.e., 53% and 50%, respectively. Nonetheless, *T. rubrum* and T. *mentagrophytes* were reported to have about identical incidence in two studies [26,27]. The most prevalent species from Sikkim was found to be *T. mentagrophytes* [28]. With 94% of cases coming from *T. interdigitale*, it was the most prevalent isolate from Delhi [29]. Although the exact reasons behind the shift in the causative organisms are unknown, several theories have been put out, such as the misuse of steroids, the indiscriminate use of oral and topical antifungals, modifications to the host and agent variables, and environmental changes [30].

## CONCLUSIONS

The most frequent cause of cutaneous fungal infections in tropical and subtropical regions, including India, are dermatophytes. Each region of India has a varying level of species isolation. We have determined that *Tinea corporis* was the most often identified clinical type in this study, with Tinea cruris coming in second. *T. rubrum* was the most common species, followed by *M. gypseum* and *T. mentagrophyte*. Laboratory diagnosis is crucial for all clinically diagnosed fungal diseases to ensure appropriate therapy and determine the prevalence of specific dermatophytes.

## CONTRIBUTION OF AUTHORS

**Research concept-** Ananya Verma, Suneel Kumar Ahirwar, Anju Mahor, Sanjay Khare
**Research design-** Ananya Verma, Anju Mahor, Sanjay Khare, Suneel Kumar Ahirwar
**Supervision-** Suneel Kumar Ahirwar, Sadhna Sodani
**Materials-** Ananya Verma, Anju Mahor, Sanjay Khare
**Data collection-** Ananya Verma, Anju Mahor
**Data analysis and Interpretation-** Ananya Verma, Anju Mahor, Suneel Kumar Ahirwar
**Literature search-** Ananya Verma, Anju Mahor
**Writing article-** Suneel Kumar Ahirwar, Anju Mahor
**Critical review-** Suneel Kumar Ahirwar, Sadhna Sodani
**Article editing-** Anju Mahor, Suneel Kumar Ahirwar
**Final approval-** Ananya Verma, Anju Mahor, Sanjay Khare, Suneel Kumar Ahirwar, Sadhna Sodani

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