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Analysis of Fungal Causes Onychomycosis and Its Risk Factors in Farmers at South Sulawesi, Indonesia

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Abstract: Onychomycosis is a fungal infection of the nail caused by dermatophytes, non-dermatophyte, mould (NDM), and yeast. The purpose of the research is to determine the cause of onychomycosis and its risk factors in farmers' nails in Pacciro Village, South Sulawesi, Indonesia. This is an observational research, where macroscopically and microscopically examination used to determine the type of fungus, and questionnaire used to determine the risk factors. By using purposive sampling, there were 47 subjects of farmer with suspected of nails onychomycosis. Most of participants were middle aged and elderly (75%), and had medical history of both or one of hypertension and diabetes mellitus (72%). The result show that 31.08% have poor hygiene, and 27.03% have fairly good hygiene. There were only 14.86 % samples succesfully cultured and determined caused by non-dermatophyte fungi (Aspergillus sp). The Chi-Square test shows a correlation between extrinsic factors (environmental condition and personal hygiene) and Onychomycosis (p = 0.001). There were no correlation between type of fungi (p = 0.21), age (p = 0.30) and medical history (p = 0.44) with Onychomycosis. It concluded that Onychomycosis in study area is caused by Aspergillus sp, with the risk factors of environmental condition and personal hygiene.

Keywords: fungi, risk factors, onychomycosis, nails.

INTRODUCTION

Indonesia is a country known as an agrarian nation, and the majority of its population utilizes Indonesia's natural resources in the agricultural sector. The vast expanse of land dedicated to rice cultivation creates a workforce that turns this activity into a business venture for farmers. However, as a tropical country, Indonesia provides a favorable environment for fungi to thrive.[1,2] Fungi are eukaryotic organisms; most fungi are non-motile, possess a rigid cell wall, and are non-photosynthetic. Less than 50 species cause more than 90% of the fungal infections of humans and other animals. The large population of fungi and the habit of farmers with poor hygiene practices and non-compliance in using personal protective equipment (PPE) can increase the risk of health problems in the workplace in rice fields. Onychomycosis is an infectious disease that affects the nails caused by fungi such as Trichophyton rubrum or other dermatophyte fungi such as Trichophyton mentagrophytes and Epidermophyton floccosum [3,4,5]

Nails are one of the smallest units on the body located at the distal part of the phalanx, surrounded by ligaments and interphalangeal joints that assist in holding the nail and performing its mechanical functions.[6] Onychomycosis is a fungal infection that gradually affects the nails and can involve the surrounding skin, causing dystrophic changes in the infected nails.[7,8] The global population experiencing onychomycosis is estimated at 5.5% and is predicted to increase due to more modern footwear and lifestyle changes affecting individual physical health.[9] Indonesia's tropical climate with high temperature and humidity levels provides a

conducive environment for fungal growth. It places Dermatophytosis as the second most common occurrence after *Pityriasis versicolor*, accounting for approximately 52%.[10]

Intrinsic risk factors of Onychomycosis originate from the individual's condition, such as age. Onychomycosis often affects the elderly, typically between the ages of 45-64 years. However, other studies indicate that onychomycosis can also occur in individuals aged 22-44.[11,12,13] Besides age, gender also influences onychomycosis, with women being more frequently affected than men. Comorbidities of onychomycosis patients, including diseases such as diabetes, immunosuppression, and malignancy, are considered risk factors.[11,13] In patients with a history of diabetes, in addition to finding blood sugar levels > 200, symptoms such as excessive thirst, polyuria, blurred vision, and weight loss may also be present. Some literature also suggests that trauma makes it easier for fungal invasion, supported by a family history of similar complaints.[13]

Extrinsic risk factors in the occurrence of onychomycosis are related to the environment and habits of the individuals. Habits such as walking barefoot and lacking hygiene can influence an increased risk of onychomycosis. It is because Indonesia, as a tropical country, has high humidity, which provides the most favourable conditions for fungal growth.[10]

Fungal infection of the nails begins with fungi on the nail, with a growth pattern from the fingertip through the hyponychium and continuing towards the nail bed, specifically the distal-lateral portion of the nail. The tip of the nail serves as the entry point for the fungus. When the fungus successfully penetrates the nail bed, hyperkeratosis occurs, followed by the role of the nail plate as a barrier against fungal infection at its site.[11]

Although parakeratosis rarely occurs in cases of onychomycosis, when there is infiltration of inflammatory cells towards the nail bed and epithelium, neutrophils will accumulate. It occurs when the fungus grows proximally. Additionally, onychomycosis patients may experience dermatophytoma, which is the compression of thick-walled fungi, resulting in the appearance of longitudinal yellow streaks at the nail base. Furthermore, subungual hyperkeratosis, leukonychia, and thickening and dystrophy of the nails will be observed.[9]

Examination of fungi can be conducted macroscopically (colonial) by culturing on Sabouraud Dextrose Agar (SDA) media for 14 days and microscopically using potassium hydroxide (KOH) solution. Macroscopic to assess the shape of the colony, surface, and color of the colony. Microscopic examination to assess the structure of the fungus, such as spores and hyphae.[15]

The definition of farmers has been regulated in the Republic of Indonesia Law Number 19 of 2013 concerning the Protection and Empowerment of Farmers in Article 1 paragraph 3, which states that farmers are individual Indonesian citizens and/or their families who engage in farming activities in the fields of food crops, horticulture, plantations, and/or animal husbandry.[16]

Relevant research was written by [17] researchers, which was conducted with a descriptive design where the researchers aimed to identify whether there were *Candida albicans* and *Trichophyton rubrum* fungi on the nails of rice farmers in Candimulyo Village, Jombang. The sampling technique used was total sampling, with 15 persons as the sample. This research identified the presence of fungi by culturing using Sabouraud Dextrose Agar media. The results showed 100% negative for Candida albicans and Trichophyton rubrum, but positive for Rhizopus and Aspergillus fungi.[17]

Another study was conducted by [18], which had a sample of fingernails from chicken meat traders. Samples taken were cultured using Potato Dextrose Agar (PDA), which was then identified through macroscopic and microscopic morphology. The results found non-dermatophyte fungi, namely *Aspergillus flavus* and *Aspergillus niger*.[18] Fungi, often referred to as fungi, consist of eukaryotic organisms that typically absorb nutrients from the organisms they inhabit, whether living or dead. The most common types of mycoses are Candidiasis and Dermatophytosis, which are usually caused by the normal flora of the body.[15]

MATERIAL AND METHOD

This research was conducted using an analytical observational design. Chi-Square test was performed to determine whether the types of fungi causing onychomycosis in rice farmers' nails were related with risk factors (intrinsic and extrinsic). This research has received ethical approval from the Ethics Committee Faculty of Medicine and Health Scieces Universitas Muhammadiyah Makassar with number of 20230926100.

This research collects the data using structured interviews with a standardized questionnaire designed by the research team. The questionnaire to assess both intrinsic (age, medical history) and extrinsic (personal hygiene pattern, work environment) risk factors associated with onychomycosis. The age of participant was

collected as numerical data and categorized based on the Indonesian Ministry of Health Regulation No. 25 of 2016 concerning the National Action Plan for Elderly Health: 19–44 years: *Adult*, 45–59 years: *Pre-elderly*, ≥60 years: *Elderly*. Medical history were asked they had ever been diagnosed by a physician with hypertension, diabetes mellitus, or both. A checklist was used to record: no history, one condition (either diabetes or hypertension) and both conditions. Personal hygiene pattern based on five items in the questionnaire covering: frequency of foot washing after working in rice fields, frequency of changing socks or footwear, use of personal protective equipment (boots), nail cleaning habits, tendency to work barefoot. Each item was scored 0 (inadequate) or 1 (adequate).

The total score (range: 0–5) was then used to classify hygiene patterns: poor hygine: total score 0-2 and fair hygine: total score 3-5. The criteria of clinical symptoms are nail thickening (hyperkeratosis), wavy or uneven nail surface, black or yellow discoloration, partial nail destruction or fragmentation and subungual debris or detachment. Participants had two or more of these symptoms were considered clinically suspected of onychomycosis and qualified for laboratory testing (KOH and culture). All participants were rice farmers work in the same geographic and occupational conditions (Pacciro Village, Ajangale District), this variable was treated as constant and not subjected to statistical comparison. All data obtained through the questionnaire were manually, verified during clinical inspection, and subsequently entered into the SPSS software for statistical analysis using the Chi-Square test.

The interviews to the rice farmers, by asking a series of questions. Following the interviews, the participants were observed directly to examine their fingernails for clinical symptoms consistent with onychomycosis. Samples were collected by scraping the farmers' fingernails, after which KOH staining was performed to identify the type of fungus. Additionally, a culture was performed using Sabouraud Dextrose Agar medium to identify the fungal culture types by looking at the colony shape, edges, surface, elevation and color. The determination of the type of fungus is based on matching the morphology of the fungus found in the culture with that described in the literature [4], both macroscopically and microscopically.

Population and sample

The population in this study consisted of all rice farmers in Pacciro Village, Ajangale District, Bone Regency, South Sulawesi. The sampling technique used in this study was probability sampling, where samples were taken in accordance with the principles of probability to control the risk of bias in the study. Therefore, the minimum sample size in this study was 47. Data obtained were collected through interviews with the samples using questions found in the questionnaire.

Data Analysis

The analysis technique used is bivariate analysis. These data were then entered and analyzed using the Statistical Package for the Social Sciences (SPSS) to identify the most common risk factors for onychomycosis occurrence. The risk factors both intrinsic (age, comorbid medical history) and extrinsic (work environment, personal hygiene practices). The correlation test was used to determine whether there was a relationship between the independent variable (type of fungi and risk factors) and the dependent variable (Onychomycosis) using the Chi Square test. This research uses a significance level of 0.05, meaning that a hypothesis is considered significant if the p-value is ≤ 0.05.

RESULT

The study was conducted in Pacciro Village, Ajangale District, Bone Regency, South Sulawesi. This village the majority of the population works as rice farmers in high-humidity environments. Total sample are 74 rice farmers who exhibited clinical signs of suspected onychomycosis were recruited for this study through purposive sampling. Data were collected through structured interviews and direct clinical observation. The characteristics of this study are most respondents were female farmers, and the age majority 49% were elderly (≥60 years). Medical history assessment revealed that 16% had both hypertension and diabetes mellitus, 56% had one of the two conditions, and 28% reported no chronic illness. Hygiene patterns classified 23 participants as having poor hygiene and 20 as having fairly good hygiene practices

The samples treated with the culture method using Sabouraud Dextrose Agar (SDA) media and examined microscopically with potassium hydroxide (KOH) solution yielded only about 11 samples that grew successfully. During the culture process, the fungi grew over 14 days. Subsequently, analysis was conducted under a microscope with a magnification of 10×40. The results revealed the presence of Aspergillus species,

which belong to the non-dermatophyte fungi. Below is the morphology of the Aspergillus fungi found in the samples:

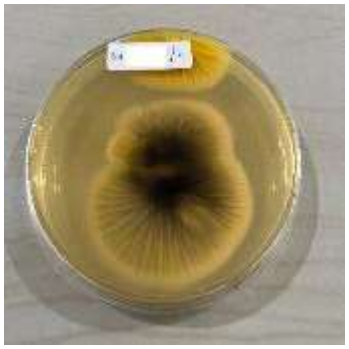

Figure 1. The macroscopic appearance of *Aspergillus sp*.

Culture on Sabouraud Dextrose Agar, diameter 50 mm on day 3 for large colonies and 41 mm for small colonies – Flat topography with firm boundaries – Cotton-like texture with a surface full of folds – Large colonies are greenish with a darker color approaching black in the central part, and small colonies are yellow

Figure 2. The microscopic appearance of *Aspergillus sp*.

In this study, 10 samples were obtained that grew on the third day and 1 sample that grew on the sixth day. Samples that grew on day 3 were easier to assess from both macroscopic and microscopic aspects. Macroscopically, the colonies that grew on the third day had topographies that had characteristics according to the type of fungus. Likewise, microscopically, morphology is easier to identify with a magnification of 10×40. After identifying 11 samples, the type of fungus that causes Onychomycosis was found in the nails of rice farmers. The type of fungus is Aspergillus sp., which is classified as a non-dermatophyte fungus.

Based on the intrinsic risk factor, the majority of farmers in Pacciro Village, Ajangale District, Bone Regency are women. Based on the interviews conducted regarding the risk factors of Onychomycosis in farmers, the results obtained are presented in Figure 4.

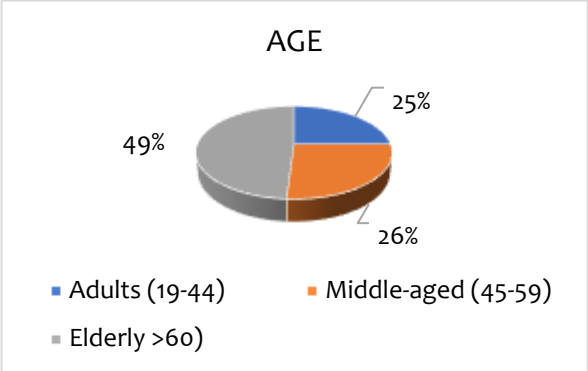

Figure 3. Characteristics of nails scraping based on age

Based on the intrinsic risk factor, namely age, the age classification in the Regulation of the Minister of Health of the Republic of Indonesia No. 25 of 2016 concerning the National Action Plan for Elderly Health for 2016-2019, which states that those aged 19-44 years are classified as adults, those aged 45-59 years are classified as pre-elderly, and those aged 60 years and over are classified as elderly. Thus, of the 43 positive samples during this study, 11 samples (25%) were classified as adults, 14 samples (26%) were classified as pre-elderly (middle age), and 18 samples (49%) were typically elderly.

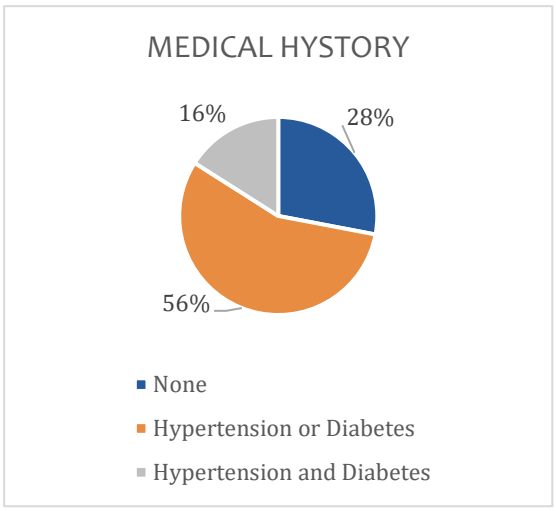

Figure 4. Characteristics of nails scraping based on medical history

According to the disease history, 7 (16%) samples had both hypertension and diabetes, 25 (56%) samples had either hypertension or diabetes, and 11 (28%) samples had no disease history. It shows that majority people with nails scraping are suffering from hipertension or diabetes.

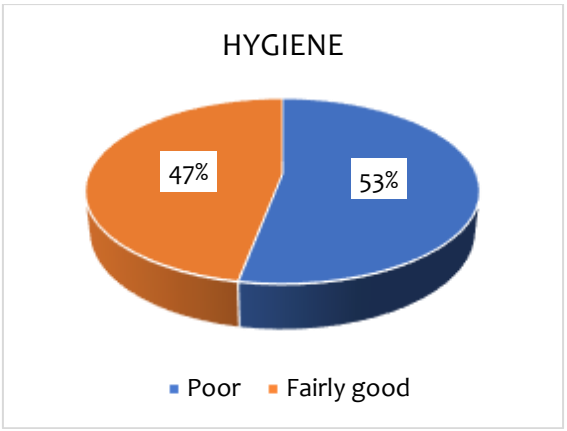

Figure 5. Characteristics of nails scraping based on hygiene patterns

Extrinsic risk factors are the environment and the quality of the sample's hygiene. The environmental parameters are continuous exposure to moist soil, prolonged barefoot activity, high ambient temperature, and frequent contact with decomposing organic matter conditions conducive to fungal proliferation such as *Aspergillus* spp. Based on their environment, all samples work as farmers, which requires them to spend a long time in rice fields with high humidity, which is the best place for fungi to grow. Meanwhile, based on the hygiene patterns found in the samples, 23 samples had poor hygiene patterns and 20 samples had fairly good hygiene patterns.

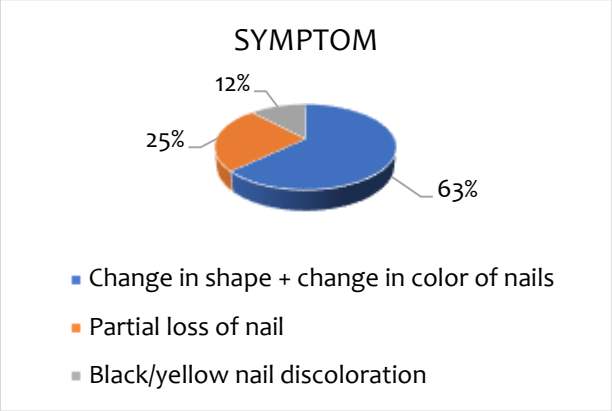

Figure 6. Characteristics of nails scraping based on symptom patterns

The clinical symptoms found in all samples were thickened fingernails or toenails, wavy surfaces, blackened or yellowed colors, and even partially broken nails. This means the samples most likely had onychomycosis, a fungal infection of the nails, often caused by dermatophytes, but can also be due to yeasts or non-dermatophyte molds. However, similar symptoms can also appear in psoriasis, lichen planus, or trauma, so diagnosis usually requires laboratory confirmation using KOH test, culture, or PCR. This study use culture to confirm the diagnosis as mention previously.

Table 1. Results of Chi-square test between fungal type and risk factors of farmer's nail in study area

Risk Factor	Value P	Interpretation
Type of fungus	0.21	No significance correlation
Age	0.3	No significance correlation
Environment	0.05	Constant
Hygiene pattern	0.001	Strong significance correlation
The disease history	0.44	No significance correlation

Based on the SPSS software test results for onychomycosis-causing fungal type (*Aspergillus* sp.) and several intrinsic and extrinsic risk factors on farmers' nails (Table 1), the p-value is 0.21, indicating no significance. The age factor has a p-value of 0.3, also indicating no significance. The environmental factor remains constant because it only includes one variable, rice fields. The hygiene pattern shows a p-value of 0.001, indicating strong significance, while the disease history has a p-value of 0.44, indicating no significance.

DISCUSSION

Onychomycosis is a fungal infection that gradually affects the nails and can involve the surrounding skin, causing dystrophic changes in the infected nails.[7,8] Farmers' nails suspected of Onychomycosis, the shape of the fingernails or toenails is thickened, the surface is wavy, the colour is black or yellow, and there are even nails that are partially broken when scraped. In this study, 11 samples were identified as Aspergillus sp, which is classified as a non-dermatophyte fungus. It is in accordance with research conducted by 18 people who studied onychomycosis. This study used samples of farmers' toenails and fingernails, while [20] used samples of chicken sellers' fingernails. It proves that Aspergillus sp. are filamentous fungi, which are prevalent in the environment [21,22] Aspergillus sp is a type of fungus found in the samples after undergoing

laboratory examination. Aspergillus sp. is the largest genus with 250 species. This fungus belongs to a non-dermatophyte fungus that can invade the nails.

The diagnosis of onychomycosis is based on clinical symptoms such as nail discolouration, hyperkeratosis or subungual debris, onycholysis, and onychauxis. The diagnosis is confirmed through microscopic examination using Potassium Hydroxide (KOH) solution and fungal culture on Sabouraud Dextrose Agar (SDA) media.[9] Macroscopic morphological evaluation is performed after fungal colonies have grown on SDA media, where Aspergillus sp. colonies begin to appear on the 3rd to 4th day after planting. The colonies initially appear yellowish-white, then change to greenish-yellow, and in some cases appear greenish-black with a folded cotton-like texture. Figure 2 shows the similarity of macroscopic morphology that can be used as a basis for identifying the type of fungus, which is then confirmed by the results of microscopic examination using KOH. Furthermore, Figure 3 shows that the microscopic morphology of the two samples has similarities, such as a biseriate structure with several phyllids and round conidia with a pale green colour before staining. The staining used in this study was a KOH solution, so the resulting microscopic image was less clear when compared to staining using Lactophenol Cotton Blue, which generally provides better visual contrast.

The risk factors supporting the occurrence of Onychomycosis in this study were found to be age group and medical history as intrinsic risk factors, while environment and hygiene patterns were identified as extrinsic risk factors. The elderly age group was most commonly found in these cases, influenced by the declining immune system in the elderly, which reduces the immune response to pathogenic infections and poses a greater risk. Medical history found in positive samples included diabetes and/or hypertension. Diabetes is classified as a systemic disease that leads to immune suppression, thus increasing the risk of infection. Hypertension is often associated with abnormalities in blood vessels that can reduce perfusion in the lower extremities, particularly in the nails, leading to inadequate oxygenation and, consequently, metabolic nutrient deficiencies in these areas. It can hinder nail growth and elevate the risk of infection.[9,23]

In both men and women, susceptibility to nail fungus infection tends to be influenced by additional factors according to their habits. Men are more likely to engage in strenuous activities or heavy labour, which may lead to trauma and serve as an entry point for fungal infections. Women, on the other hand, are influenced by the diversity of footwear choices, which can potentially injure the nails. However, in this study, it was found that there were more female samples than male samples.[9]

Environment and hygiene patterns are risk factors for Onychomycosis. A humid environment can provide an ideal breeding ground for fungi, such as in rice fields. Farmers who spend prolonged periods in humid environments are at risk of being infected by pathogenic fungi that cause Onychomycosis. Hygiene patterns also play a significant role in these cases. The discovery of poor hygiene patterns in the samples increases the risk of fungi growing on extremities that are frequently exposed to pathogens.

CONCLUSION

Based on this research, it has been concluded that Aspergillus sp. is a type of fungus that acts as a pathogen in Onychomycosis, and intrinsic risk factors (age and medical history) and extrinsic risk factors (environment and hygiene patterns) influence Onychomycosis in Pacciro Village, Ajangale District, Bone Regency.

ETHICAL CONSIDERATIONS

The study has received approval from the ethical committee of Faculty of Medicine and Health Scieces Universitas Muhammadiyah Makassar with ethical approval number: 20230926100

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CONFLICT OF INTEREST

None

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