Original article

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RISK FACTORS AND CLUSTER ANALYSIS OF ONYCHOMYCOSIS

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Recently, there has been a trend of identifying different disease phenotypes based on clinical parameters. Cluster analysis is a statistical method for categorizing different clinical signs and symptoms based on how closely associated they are.

The aim of this study was to estimate whether cluster analysis could be used to classify distinct clinical phenotypes of onychomycosis and determine risk factors for this infection.

This prospective study evaluated data from the specially designed questionnaire for superficial fungal infections of the skin and adnexa. The questionnaire was composed of three separate groups of questions, including demographic data, symptoms and clinical signs and risk factors. The hierarchical method of cluster analysis, the Ward method with Euclidian distance, was used in statistical analysis.

The applied statistical method separated patients into two clusters based on clinical presentation. The first cluster consisted of patients with onychomycosis of toenails accompanied by pain, complete destruction of the nail plate, involvement of 2/3 of the nail, and nail thickening greater than 2 mm. The second cluster, consisting of patients with onychomycosis of fingernails, was further divided into two subclusters. The first one included patients with lesions of the nail root, inside of the nail, superficial changes, and infected skin around the nail. The other subcluster included nail plate thickening of up to 1 mm, changes of the free edge, involvement of up to 1/3, and brittleness of the nail. The most common risk factors are obesity (50%), positive family anamnesis (32.0%), nail plate trauma (15.0%), and long-term antibiotic therapy.

Phenotyping the infection and considering it alongside the most prevalent risk factors for onychomycosis can significantly improve predictive assessment and diagnosis.

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Key words: *cluster analysis, onychomycosis, risk factors*

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Introduction

Cluster analysis techniques are often used to identify the pattern of clinical signs and symptom expression, facilitating the definition of more precise diagnostic criteria and categories (1, 2). In recent years, the trend of determining different disease phenotypes based on the grouping of most common symptoms and clinical signs in separate clusters has been growing (3) and is practiced in pulmonology (4), cardiology (5), rheumatology (6), and psychiatry (7). Recently, this statistical method was also used in infectology (8, 9).

Considering the fact that fungal infections, both superficial and invasive, are still being treated empirically based on individual predisposition, symptoms, and signs of the infection, figuring out the phenotypes of these diseases would significantly help in diagnosis. According to available data, one of the fungal infections with an extremely high prevalence, treated without prior laboratory confirmation in more than 60% of cases, is onychomycosis (10). Onychomycosis is a fungal nail disease caused by a dermatophyte, non-dermatophyte molds, or yeasts (11). The most common form of onychomycosis is the distal subungual form, which occurs more often on the toenails (12), although a lateral or proximal subungual form, as well as superficial and endonyx onychomycosis, have also been described (13, 14). All of these forms, if left untreated, can result in total dystrophy of the nail (15–17).

Onychomycosis is often chronic, as the causative agents are often difficult to eradicate, with a tendency to relapse and require treatment with systemic antimycotics (18). In most

laboratories, the diagnostic procedure includes a mycological examination of the patient's material (scarifications and swabs from the site of the change) using a conventional methodology, such as microscopic examination and cultivation. The main disadvantage of mycological analyses is the long duration of the cultivation period and the process of causative agent identification (10). Although designing and establishing fast and accurate diagnostic procedures is of great importance in choosing an appropriate course of treatment, molecular techniques, and point of care, immunochromatographic onychomycosis diagnostic tests are not readily available to most laboratories (10). Therefore, in most cases, onychomycosis is treated empirically based on the clinical signs and symptoms (19). Accordingly, the aim of this study was to determine the most common risk factors for the occurrence of this fungal infection, as well as whether cluster analysis can be applied to classify clinical phenotypes of onychomycosis.

Material and Methods

This prospective study was conducted at the Department of Parasitology and Mycology of the Institute of Public Health in Niš. A specially designed questionnaire for superficial fungal infections of the skin and adnexa was used. The questionnaire was composed of three separate groups of questions. The first group of questions included demographic data, the second group focused on symptoms and clinical findings, and the third was focused on risk factors. The attending physician, a specialist in microbiology, conducted the interview, clinical examination, and questionnaire completion. Data from medical records of 100 patients with laboratory-confirmed onychomycosis (significant finding of fungal elements using microscopic examination (mass of fungal conidia/spores and/or hyphae/mycelial fibers) and isolation of yeasts and/or dermatophyte molds) were included in the study.

This research was carried out in accordance with the principles of the Declaration of Helsinki of 1975 and was approved by the Ethics Committees of the Faculty of Medicine, University of Niš (Decision Number: 12-6316-2/1-2016) and Institute of Public Health in Niš (Decision Number 07-4665/2016).

Statistical analysis

Data are presented as arithmetic mean ± standard deviation, frequency, and percentage. Comparison of different variables between groups was performed by the Mann-Whitney test.

The data analysis was conducted using cluster analysis—the agglomerative hierarchical Ward method, which generated a dendrogram with Euclidean distance. The hypothesis was tested with a significance threshold of $p < 0.05$. Data analysis was performed in the R software package (20).

Results

One hundred patients with onychomycosis were included in the study. Mycotic lesions were more common on the toenails (62.0%) compared to the fingernails (38.0%). Fungal nail disease occurs more often in females (79.0%). The age structure of the examined population was uniform, from 17−79 years (Table 1), and the average age was 45.85 ± 19.61 years. Most of them were from the city (86.0%), and half of the studied population was obese.

The most common pathological changes observed in the studied population were nail deformation (74.0%), raised nail (50.0%), involvement of more than two-thirds of the nail (45.0%), complete alteration of the nail (43.0%), brittle nails (43.0%), pain (37.0%) and disruption of the free edge (32.0%). Other symptoms and clinical signs occurred in less than a third of the examined patients (Table 2).

Cluster analysis included the following parameters: involvement of the free edge of the nail, involvement of the nail root, presence of surface changes, changes inside the nail, complete nail involvement, localization of changes, involvement of up to a half and more than a half of the nail, thickening greater than 2 mm, pain, brittleness of the nail and involvement of the skin around the nail.

Based on the cluster analysis, two clusters were identified. The following symptoms and signs are grouped into the first cluster—lesions of the toenails: a complete change of the nail, more than 2/3 of the nail involved, thickening of the nail greater than 2 mm, and the presence of pain. The second cluster involved fingernail lesions further divided into two sub-clusters (Figure 1). The first one included changes to the fingernail root, inside the nail, nail surface change, and involvement of the skin around the nail. The second sub-cluster contained the following characteristics: thickening of up to 1 mm, disruption of the free edge, involvement of up to 1/3 of the nail, and nail brittleness.

We found that the following risk factors are most common in the studied population: obesity (50% of patients with onychomycosis were with body mass index (BMI) ≥ 25 kg/m^2), positive family history (32%) and existing peripheral vascular disease (11%). The prevalence of other risk factors, each accounting for less than 10% of the examined population, is shown in Figure 2.

Table 1. Demographic and clinical characteristics of the studied population

	Total	
	Number	**%**
Gender		
Male	21	21.0
Female	79	79.0
Age	45.85 ± 19.61	17–79
Age categories		
< 19	10	10.0
20–39	29	29.0
40–59	33	33.0
60–79	28	28.0
BMI*	25.08 ± 4.74	15.83–38.22
Nourishment		
Undernourished < 18.5	9	9.0
Normal 18.5–24.9	41	41.0
Obese > 25.0	50	50.0
Place of residence		
The countryside	14	14.0
City	86	86.0

* BMI—body mass index; people with BMI < 18,525 kg/m^2 are considered undernourished; BMI from 18.525 to 24.9 kg/m^2 counts as normal body mass, and obese are with BMI ≥ 25 kg/m^2.

Table 2. Clinical signs and symptoms in the studied population

Clinical signs and symptoms	Total*
Deformation of the nail	74
Raised nail	50
More than 2/3 of the nail involved	45
Nail plate thickening >2 mm	45
Complete nail alteration	43
Brittle nails	43
Pain	37
Disruption of the free edge	32
Less than 1/3 of the nail involved	26
No thickening or less than 1 mm	26
Affected skin around the nail	24
Nail involvement of 1/3 up to 2/3	23
Nail plate thickening 1–2 mm	23
Surface changes	11
Changes inside the nail	11
Alterations of the nail root	7

* Prevalence percentage of the corresponding clinical sign or symptom

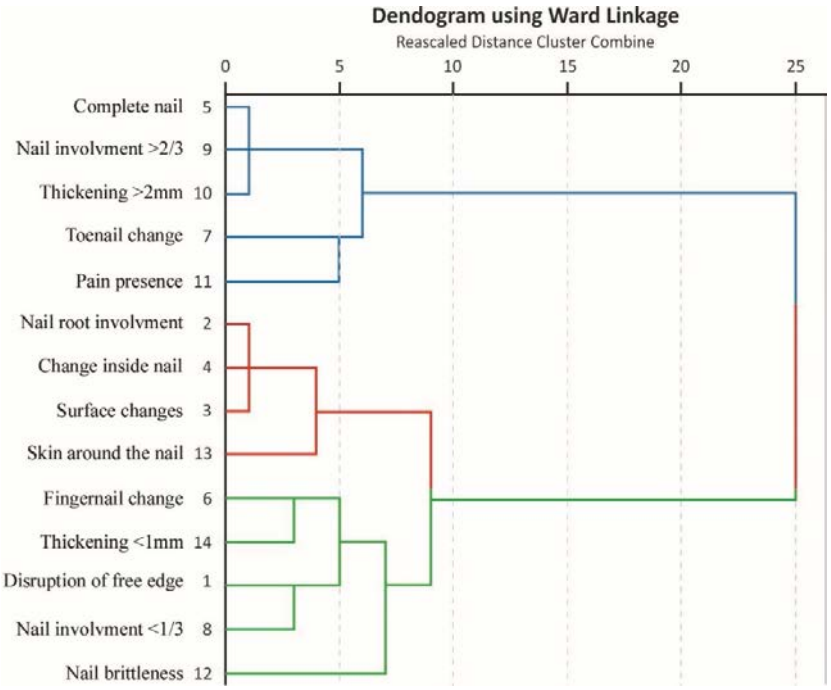

Figure 1. Cluster analysis of symptoms in patients with onychomycosis

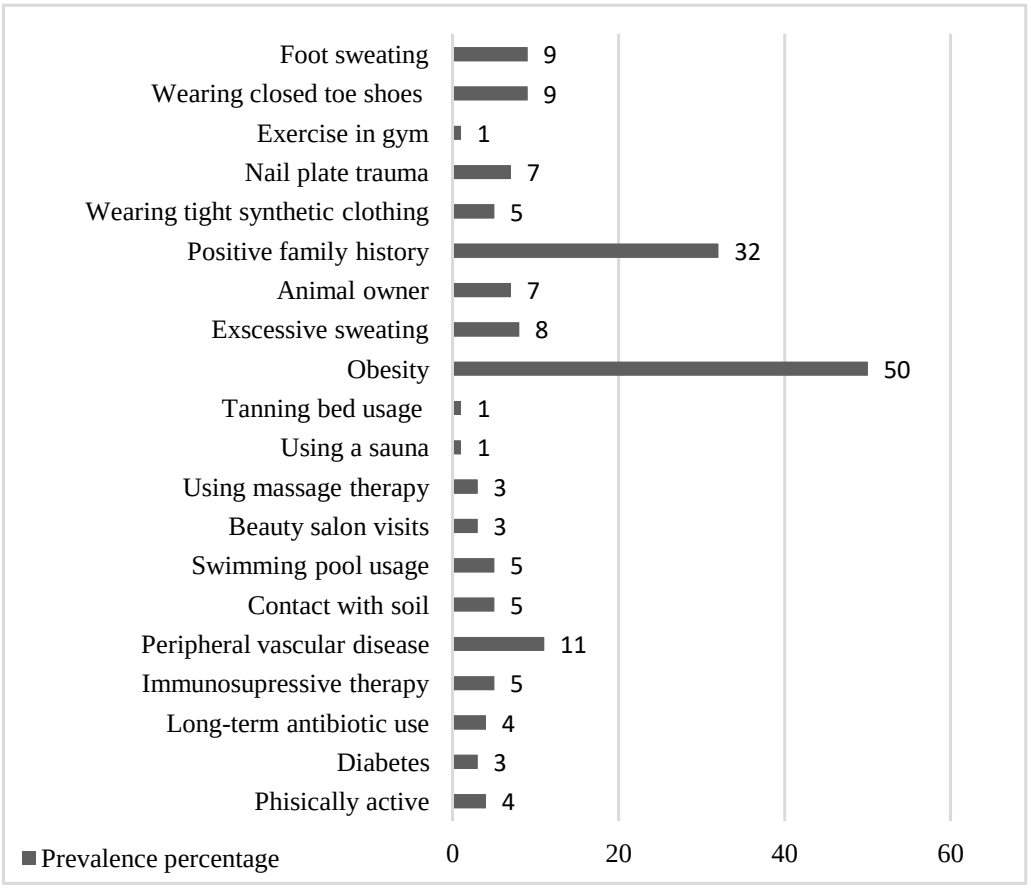

Figure 2. Distribution of risk factors in the examined population

Discussion

All observed signs and symptoms in the studied population are characteristic of onychomycosis (21, 22). However, numerous manifestations of fungal infections can also be found in other diseases (23), so they must be taken cautiously and interpreted in accordance with the complete clinical presentation, predisposing factors, and, in the best case, after a laboratory, mycological examination. Empirical treatment of onychomycosis, the duration of mycological analysis to confirm the fungal infection in the laboratory, as well as the wide range of different symptoms and signs by which onychomycosis manifests, indicate the need to separate these infections into different phenotypes, to be able to set an accurate diagnosis easier.

The cluster analysis can be used to associate clinical manifestations that occur together to achieve this separation of the different phenotypes. For this purpose, the hierarchical cluster analysis method can be used. It begins with one cluster and then gradually combines variables to form the smallest number of clusters with the greatest similarity of variables within clusters and the greatest dissimilarity between them.

Namely, this statistical method is increasingly used in diseases that can present with numerous various symptoms and signs. For example, it is used to classify and group symptoms in chronic diseases, especially in oncology (24, 25). On the other hand, this type of analysis is rare in the case of infectious diseases. So far, this statistical method for determining phenotypes of manifest infection with laboratory evidence has been used in cases of vulvovaginal candidosis and Aspergillus-otomycosis (8, 9). However, this paper represents one of the first studies of cluster analysis of nail fungal infection.

The hierarchical method of cluster analysis in this study shows the first differentiation of toe-onychomycosis and fingernail-onychomycosis as two clusters. Interestingly, the cluster of fingernail onychomycosis also contains two additional subclusters. That is, the analysis of the results indicated the existence of one phenotype of onychomycosis of the toenails and two phenotypes of fingernail onychomycosis.

Toenail phenotype includes the presence of the infection on the toes, with complete alteration or involvement of two-thirds of the toenail, thickening of the nail greater than 2 mm, and the presence of pain. Fingernail phenotype encompassed two different sub-phenotypes of the fungal infection. The first one was manifested by the changes in the nail root and endonyx (lesions within the nail plate, surface changes, and involvement of the skin around the nail). In addition, there was a second subcluster within the fingernail onychomycosis group, which included signs such as nail thickening of up to 1 mm, alterations on the distal part of the nail plate, i.e., on the free edge, brittleness, and involvement of up to one-third of the nail.

Analyses of the identified phenotypes indicate a clear grouping of signs of more extensive infection and pain with mycotic lesions of the toenails. Contrary to that, in the case of onychomycosis of the fingernails, signs of the infection are more discrete.

One of the techniques used to determine cluster diversity is the visual observation of graphic displays or dendrograms. Although the dendrogram clearly clustered signs and symptoms into three phenotypes, the possible overlap between variables, particularly between neighbouring clusters, should be highlighted. For the first cluster, the involvement of the nail root can additionally be considered. For the second cluster, the pain presence can be considered. Consecutively, for the third cluster, the presence of skin changes around the nail might be assumed.

Even though the dendrogram construction is one of the critical steps in this type of statistical analysis since every step in the formation of clusters can be visually observed, its interpretation requires caution (21). The major drawback of applying this type of analysis is that different cluster analysis techniques can produce different results. Therefore, it is recommended that cluster analysis should be followed by principal component analysis. In this two-way process, the validity and reliability of the data can be verified with greater confidence (22).

As for risk factors, obesity, positive family history and trauma to the nail plate were most often noted among the examinees. Other authors usually associate these factors with onychomycosis (22, 26). Additionally, long-term use of antibiotics was found in 11%, and diabetes mellitus in less than 10% of our respondents (27). The sporadic presence of these risk factors among those examined can be explained by the severity of the underlying diseases, leading to onychomycosis being neglected and not confirmed by laboratory tests. Phenotyping the infection and considering it together with the risk factors most commonly present in onychomycosis can significantly aid in predictive assessment and diagnosis.

Conclusion

Even though statistical methods are relatively complex, identifying different clusters of onychomycosis's clinical characteristics would significantly contribute to a better understanding of the disease and an adequate assessment of the patients. Our study showed that cluster analysis could be useful in identifying phenotypes of symptoms and signs of nail fungal infections. However, further research is needed to estimate an association between clusters of symptoms and signs and type of causative agent of fungal nail infection, as well as a comparison between phenotypes of onychomycosis with phenotypes of other nail diseases.

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References

1. Everitt B, Landau S, Leese M. Cluster Analysis, 4th edition. London: Edward Arnold Publishers Ltd; 2001.
2. Hartigan JA. Clustering. Annu Rev Biophys Bioeng 1973;2(1):81-102. [CrossRef] [PubMed]
3. Naeem A, Rehman M, Anjum M, Asif M. Development of an efficient hierarchical clustering analysis using an agglomerative clustering algorithm. Curr Sci 2019;117(6):1045. [CrossRef]
4. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008;178(3):218-24. [CrossRef] [PubMed]
5. Katz DH, Deo RC, Aguilar FG, Selvaraj S, Martinez EE, Beussink-Nelson L, et al. Phenomapping for the identification of hypertensive patients with the myocardial substrate for heart failure with preserved ejection fraction. J Cardiovasc Transl Res 2017;10(3):275-84. [CrossRef] [PubMed]
6. Zhu H, Wu C, Jiang N, Wang Y, Zhao J, Xu D, et al. identification of 6 dermatomyositis subgroups using principal component analysis-based cluster analysis. Int J Rheum Dis 2019;22(8):1383-92. [CrossRef] [PubMed]
7. Gonzalez R, Suppes T, Zeitzer J, McClung C, Tamminga C, Tohen M, et al. The association between mood state and chronobiological characteristics in bipolar I disorder: a naturalistic, variable cluster analysis-based study. Int J Bipolar Disord 2018;6(1):5. [CrossRef] [PubMed]
8. Bojanović M, Ignjatović A, Stalević M, Arsić-Arsenijević V, Ranđelović M, Gerginić V, et al. Clinical Presentations, Cluster Analysis and Laboratory-Based Investigation of Aspergillus Otomycosis—A Single Center Experience. J Fungi (Basel) 2022; 8(3):315. [CrossRef] [PubMed]
9. Ignjatović A, Arsić-Arsenijević V, Golubović M, Đenić S, Momčilović S, Trajković A, et al. Recurrent Vulvovaginal Candidosis and Cluster Analysis of Clinical Signs and Symptoms: A Laboratory-Based Investigation. J Fungi (Basel) 2020; 6(3):113. [CrossRef] [PubMed]
10. Otašević S, Momčilović S, Stojanović N, Skvarč M, Rajković K, Arsić-Arsenijević V. Non-culture based assays for the detection of fungal pathogens. J Mycol Med 2018;28(2):236-48. [CrossRef] [PubMed]
11. Trofa D, Gácser A, Nosanchuk JD. Candida parapsilosis, an emerging fungal pathogen. Clin Microbiol Rev 2008;21(4):606-25. [CrossRef] [PubMed]
12. Elewski BE, Rich P, Tosti A, Pariser DM, Scher R, Daniel RC, et al. Onchomycosis: an overview. J Drugs Dermatol 2013;12(7):s96-s103. [PubMed]
13. Hay RJ, Baran R. Onychomycosis: a proposed revision of the clinical classification. J Am Acad Dermatol 2011;65(6):1219-27. [CrossRef] [PubMed]
14. de Berker D. Fungal nail disease. N Engl J Med 2009;360(20):2108-16. [CrossRef] [PubMed]
15. Tasić S, Stojanović S, Poljački M. Etiopathogenesis, clinical picture and diagnosis of onychomycoses. Med Pregl 2001;54(1-2):45-51. [PubMed]
16. Otašević S, Barac A, Pekmezović M, Tasic S, Ignjatović A, Momčilović S, et al. The prevalence of Candida onychomycosis in Southeastern Serbia from 2011 to 2015. Mycoses 2016;59(3):167-72. [CrossRef] [PubMed]
17. Siu WJJ, Tatsumi Y, Senda H, Pillai R, Nakamura T, Sone D, et al. Comparison of in vitro antifungal activities of efinaconazole and currently available antifungal agents against a variety of pathogenic fungi associated with onychomycosis. Antimicrob Agents Chemother 2013;57(4):1610-6. [CrossRef] [PubMed]
18. Gupta A, Ryder J, Johnson A. Cumulative meta-analysis of systemic antifungal agents for the treatment of onychomycosis. Br J Dermatol 2004;150(3):537-44. [CrossRef] [PubMed]
19. Otašević S, Momčilović S, Golubović M, Ignjatović A, Rančić N, Đorđević M, et al. Species distribution and epidemiological characteristics of superficial fungal infections in Southeastern Serbia. Mycoses 2019; 62(5):458-65. [CrossRef] [PubMed]
20. Core Team R. R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna; 2013.
21. Hurt MA, Weedon D. Weedon D. Weedon's Skin Pathology, 3rd ed. London: Churchill Livingstone Elsevier; 2010. [CrossRef] [PubMed]
22. Dubljanin E, Džamić A, Vujčić I, Grujičić SŠ, Arsenijević VA, Mitrović S, et al. Epidemiology of onychomycosis in Serbia: a laboratory-based survey and risk factor identification. Mycoses 2017;60(1):25-32. [CrossRef] [PubMed]
23. Tully AS, Trayes KP, Studdiford JS. Evaluation of nail abnormalities. Am Fam Physician 2012;85(8):779-87. [PubMed]
24. Khan F, Ahmad N, Biswas FN. Cluster analysis of symptoms of Bangladeshi women with breast cancer. Indian J Palliat Care 2018;24(4):397-401. [CrossRef] [PubMed]

25. Miaskowski C. Future Directions in Symptom Cluster Research. Semin Oncol Nurs 2016;32(4):405-15. [CrossRef] [PubMed]
26. Gazes MI, Zeichner J. Onychomycosis in close quarter living review of the literature. Mycoses 2013;56(6):610-3. [CrossRef] [PubMed]
27. Gupta AK, Versteeg SG, Shear NH. Onychomycosis in the 21st century: an update on diagnosis, epidemiology, and treatment. J Cutan Med Surg 2017;21(6):525-39. [CrossRef] [PubMed]

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FAKTORI RIZIKA ZA POJAVU ONIHOMIKOZA I KLASTER ANALIZA

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Definisanje različitih fenotipova bolesti na osnovu kliničkih parametara predstavlja trend u istraživanjima sprovedenim poslednjih godina. Klaster analiza je statistička metoda za kategorizaciju različitih kliničkih znakova i simptoma na osnovu stepena njihove povezanosti.

Ovaj rad je za cilj imao da ispita mogućnost primene klaster analize za klasifikaciju različitih kliničkih fenotipova onihomikoze i određivanje faktora rizika za nastanak ove infekcije.

U ovoj prospektivnoj studiji korišćeni su podaci dobijeni posebno dizajniranim upitnikom u vezi sa površinskim gljivičnim infekcijama kože i adneksa. Upitnik se sastojao od tri grupe pitanja, koje su obuhvatale demografske podatke, simptome i kliničke znake, kao i faktore rizika. U statističkoj obradi podataka korišćena je hijerarhijska metoda klaster analize, Vordova metoda sa euklidskom distancom.

Primenjenom statističkom metodom bolesnici su podeljeni u dva klastera. Prvi klaster činili su bolesnici sa onihomikozom noktiju na stopalima, praćenom bolom, potpunim uništenjem nokatne ploče, zahvaćenošću 2/3 nokta i zadebljanjem nokta većim od 2 mm. Drugi klaster koji su činili bolesnici sa onihomikozom noktiju na šakama, dalje je podeljen na dva potklastera. Prvi je uključivao bolesnike sa lezijama korena nokta, unutrašnjosti nokta, površinskim promenama i zahvaćenom kožom oko nokta. Drugi potklaster obuhvatao je bolesnike kod kojih su uočeni zadebljanje nokatne ploče do 1 mm, promene slobodne ivice, zahvaćenost do 1/3 nokta i lomljivost nokta. Utvrđeno je da su najčešći faktori rizika bili gojaznost (50%), pozitivna porodična anamneza (32,0%), trauma nokatne ploče (15,0%) i dugotrajna terapija antibioticima (11,0%).

Fenotipizacija infekcije i njeno razmatranje uz najzastupljenije faktore rizika za onihomikozu mogu u velikoj meri poboljšati procenu i dijagnozu bolesti.

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***Ključne reči**: klaster analiza, onihomikoza, faktori rizika*