Prevalence of Squalene Epoxidase Mutation in Terbinafine Resistance: A Systematic Review

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Abstract. Dermatophytosis is a global burden of disease, as it affects around 20–25% of worldwide population. Many antifungal drugs have been developed to treat dermatophytosis that mainly inhibit the ergosterol biosynthesis. Terbinafine as the drug-of-choice has fungicidal effect by inhibiting ergosterol synthesis at the level of squalene epoxidation. Unfortunately, recently many countries reported the emerging terbinafine resistance. However, the exact mechanism of terbinafine resistance is still not clearly understood. Therefore, this paper aimed to provide a systematic literature review that describes the terbinafine resistance via squalene enzyme mutations. We conducted systematic searching to describe the prevalence of squalene enzyme mutation in terbinafine resistance. MEDLINE database was searched with keywords: dermatophytosis, terbinafine, resistance, squalene, mutation. According to our criteria and search protocol, six studies were included that performed squalene enzyme mutation analysis among terbinafine-resistant isolates. Data of those studies were collected to determine a cumulative prevalence of squalene enzyme mutation. According to our systematic review, we found that squalene mutation occurred in 80.39% of terbinafine resistant patients. The consistent finding of squalene enzyme mutation in terbinafine resistance may be seen to provide better strategy in dermatophytosis treatment. Further research may be developed to target another mechanism of anti-mycosis, as well as overcoming the drug resistance to benefit patients with dermatophytosis.

Keywords: dermatophytosis · terbinafine · resistance · squalene · mutation

1 Introduction

Dermatophytosis is currently the most prevalent dermatologic disease. This contagious fungal infection is estimated to affect 20–25% of global population (1). Dermatophytosis ranked fourth highest incidence (around 2.1 billion of cases) when compared to 328 other diseases and injuries worldwide. Eventhough rarely becoming lethal, dermatophytosis causes substantial burden on patients. Skin fungal infection may impact quality of life. Psychosocial life may be negatively affected, for example: lower self-esteem, discomfort or embarrassment and social withdrawal. Inflammation in dermatophytosis causes discomfort that affects ability of daily activities (2). Further, if left untreated dermatophytosis may cause significant morbidity including cellulitis and ulcers (3). In addition,

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dermatophytosis also significantly impact economically. In United States, annual costs of dermatophytosis is around 802 million dollar (4). In regard to morbidity and economic burden of the disease, dermatophytosis is considered to be an important health issue in Dermatology (3).

Dermatophytosis or Tinea is caused by Dermatophytes, filamentous fungus that basically may grow in keratinous materials including cutaneous layer of the skin. In order to maintain their cell membrane integrity and growth, Dermatophytes need ergosterol (5). Therefore, fungicidal actions of many antifungal agents are mediated by blocking ergosterol biosynthesis, such as: allylamines, azoles, morpholines and thiocarbamates (5,6).

Terbinafine is the most widely used anti-mycoses that belongs to allylamines. Terbinafine has antifungal properties to various fungal infections. It has high efficacy to inhibit dermatophytes growth via ergosterol biosynthesis inhibition at the level of squalene epoxidation (5). Terbinafine treatment blocks an enzyme, squalene epoxidase resulting to accumulation of squalene, while the ergosterol becomes deficient. As the result, the membrane cells integrity of the treated fungus is compromised and the growth is finally inhibited.

Recently, recalcitrant case of dermatophytosis is found following terbinafine resistance. The emerging terbinafine resistance is found in many countries, attracting concern by Dermatologists. Terbinafine resistance is defined as recalcitrant case after adequate dose and duration of Terbinafine. Resistance to antifungal treatment can be caused by several underlying factors, including suboptimal dosing, topical steroids misuse or dysregulated host immune response (7). Molecular mechanisms of dermatophytes recalcitrant are attributed to gene mutations of several target enzymes or interaction disruption between drug molecule and fungal enzymes. In terbinafine resistance, a gene mutation that encodes squalene epoxidase (SQLE) was reported as the underlying mechanism. Single amino acid substitution at one of four positions (Leu393, Phe397, Phe415, His440) appeared in Terbinafine resistance of T. rubrum and T. interdigitale. However, the global prevalence of SQLE mutation in terbinafine resistance is not available yet. Therefore, in this study we reviewed literatures that study SQLE mutations in dermatophytic treatment to understand the global prevalence of SQLE mutation. With the increasing reports of Dermatophytes recalcitrant, prevalence of SQLE mutations may be reviewed to understand the molecular mechanism, as well as to robust strategy to solve terbinafine resistance.

2 Methods

We performed a systematic literature searching with the PICO approach, which involved the clinical question: “How is the prevalence of squalene enzyme mutation in Terbinafine resistance?”. After developing this question, we managed to define the following PICO strategy:

P: dermatophytosis patients.
I: squalene enzyme mutation.

Table 1. List of keywords developed from PICO.

P	I	C	O
Fungal infection Mycosis Dermatomycosis Tinea Dermatophytes Tricophyton	Terbinafine Antimycotics Allylamines	–	Terbinafine Antimycotics Resistance Failure Resistant

Table 2. Searching results from PUBMED search engine.

Keywords	**Results**
(fungal infection) OR (skin mycosis) OR (dermatophyt*) OR (tinea) OR (tricophyton)	26,983
((terbinafine) OR (allylamines) OR (antimycotic)) AND ((resistan*) OR (recalcitrant)) OR (failure))	371
((fungal infection) OR (skin mycosis)) OR (dermatophyt*) OR (tinea) OR (tricophyton)) AND ((terbinafine) OR (allylamines) OR (antimycotic)) AND ((resistan*) OR (recalcitrant) OR (failure)) AND (squalene mutation)	38

C: without squalene enzyme mutation.
O: terbinafine resistance.

We used PUBMED search engine to identify relevant literatures on MEDLINE database. We used keywords: fungal infection, terbinafine, and resistance. According to these keywords, the results were as follows (Table 1):

From a total of 42 literatures found from the database, we selected studies based on our eligibility criteria: the study must be conducted within the last five years, studying the prevalence of squalene enzyme mutation from isolates of terbinafine resistant after antifungal susceptibility testing. According to our criteria, a total of 6 studies were included in our review.

3 Result and Discussion

Terbinafine is an antimycotic agent that inhibits squalene enzyme in the ergosterol biosynthesis of dermatophytes. Following terbinafine therapy, membrane cells of the fungus is disrupted, resulting to cellular death. Terbinafine has been used for two decades as the drug-of-choice to treat dermatophytosis and onychomycosis as it demonstrated reliable efficacy against Tricophyton rubrum, Tricophyton mentagrophytes, Epidermophyton floccosum, Tinea pedis, Tinea cruris and Tinea unguium (8). However, recent reports found increasing number of dermatophytosis with terbinafine resistance. Terbinafine resistance or failure may be caused by poor patient compliance, drug interaction or local

host immune response. Wide spread use of topical drug containing steroid, antibiotics and antifungal also enhances the global epidemic of anti-mycosis resistance. In addition, since 2017 terbinafine resistance was reported to be associated with squalene enzyme mutation (9). Therefore, this paper collected studies from the last five years to get a cumulative prevalence of squalene enzyme mutation in terbinafine resistance.

Geographic distribution of squalene enzyme mutation included in this paper was from Europe (Switzerland, Denmark, Germany, Poland and Belgium) and Asia (India). The cumulative prevalence of squalene enzyme mutation was surprisingly very high. We found that 80.28% of isolates with terbinafine resistance had squalene enzyme mutation (Table 2). This high prevalence indicates that enzyme gene mutation was a critical underlying mechanism of terbinafine resistance. Moreover, we also found that the point mutation of the squalene enzyme gene was mostly located at the position of 393 and 397 as substation mutation (Table 3).

Terbinafine resistance is defined clinically as failure of treatment after 6 months therapy (also considered as chronic infection) or patient who relapse 4 weeks after completing standard treatment protocol of Terbinafine (9). Resistant patients usually have typical lesion that is large, sometimes coalescing lesion with severe itching. Confirmation of drug resistance follows in vitro evaluation with antifungal susceptibility testing (AFST). Therefore, in this paper we only included studies that performed AFST for terbinafine, rather than studies that only collected isolates from clinically resistant patients. Minimal inhibitory concentration (MIC50) is used to indicate responsiveness to terbinafine, by which 50% of fungal growth was inhibited. Terbinafine resistance is defined from AFST with MIC50 $\geq$ 1 mg/L. Mean of MIC50 of terbinafine-responsive dermatophytosis patients was 0.153 mg/L (8). We found that the MIC50 of terbinafine resistance patients was extremely higher (13.53 mg/L) (Table 3), more than 100-folds compared to terbinafine-responsive dermatophytes. Thus, squalene mutation might significantly affect dermatophytes responsiveness to terbinafine (Table 4).

Table 3. Prevalence and country distribution of squalene enzyme mutation in Terbinafine resistant isolates**.**

Author	Year	Country	Number of terbinafine resistant	Squalene enzyme mutation (n, %)	
				Yes	No
Yamada et al. (10)	2017	Switzerland	17	17 (100%)	0 (0%)
Singh et al. (11)	2018	India	20	16 (80%)	4 (20%)
Saunte et al. (12)	2019	Denmark	12	9 (75%)	3 (25%)
Nenoff et al. (13)	2020	Germany	13	10 (76.92%)	3 (23.08%)
Lagowski et al. (14)	2020	Poland	4	4 (100%)	0 (0%)
Sacheli et al. (15)	2020	Belgium	5	1 (20%)	4 (80%)
Cumulative prevalence			71	57 (80.28%)	14 (19.72%)

Table 4. Mean MIC50 value and point mutations of squalene enzyme.

Author	Year	Methods	Squalene enzyme mutations	MIC50 (mg/L)
Yamada et al.	2017	PCR	L393F; F397L; F415L; H440Y	3.2
Singh et al.	2018	Gene sequencing	F397L; L393F	32.0
Saunte et al.	2019	PCR, gene sequencing	F397L, L393F, L393S, F415S, H440Y	8.0
Nenoff et al.	2020	PCR	F397L; A448T	2.0
Lagowski et al.	2020	PCR	L393F	32.0
Sacheli et al.	2020	PCR, gene sequencing	F397L	4.0
Mean MIC50 (mg/L)				13.53

4 Conclusions

With the increasing number of terbinafine resistance patients, it is important to understand the underlying mechanism. Squalene epoxidase mutation has been proposed as one of important etiology of the drug resistance. In this paper, we collected the current evidence of squalene enzyme mutation and found that the mutation was highly prevalent: 80.28% among terbinafine resistance dermatophytosis. The most common point mutation of the squalene epoxidase gene was found as substitution mutation at L393F and F397L. As the result, the minimum inhibitory concentration (MIC50) was strikingly higher. Our results may be used as an evidence of Terbinafine resistance mechanism. More importantly, our findings can be used to inspire further research to solve the terbinafine resistance that may benefit dermatophytosis patients.

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