Clinical and Experimental Health Sciences

First Molecular Investigation of VSSC-Linked Permethrin Resistance in Human Scabies in Türkiye

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ABSTRACT

Objective: Scabies, a longstanding public health concern, is a contagious and pruritic skin condition caused by the parasite *Sarcoptes scabiei*, affecting an estimated 300 million individuals worldwide annually. Recent increases in incidence can be attributed to challenges in accurate diagnosis and instances of treatment resistance. Permethrin, an insecticide belonging to the pyrethroid group, is the primary choice for scabies treatment. However, recent treatment failures suggest the emergence of permethrin resistance. Pyrethroids, widely employed as insecticides over the past three decades, have led to resistance development across various organisms. Pyrethroid acaricides like permethrin target the neuronal voltage-sensitive sodium channel (*VSSC*) protein, crucial for action potential generation in excitable cells. Specific mutations in the *VSSC* gene have been associated with pyrethroid resistance. Our objective is to elucidate the correlation between treatment failure and pyrethroid resistance stemming from *VSSC* gene mutations in *Sarcoptes scabiei* mites responsible for scabies cases in the Sivas region, Türkiye.

Methods: In this study, we analyzed 30 scabies cases where initial permethrin treatment proved ineffective. The *VSSC* gene of scabies mites was partially isolated from genomic DNA to identify potential mutations via DNA sequencing.

Results: Results yielded significant insights into the relationship between permethrin resistance and *VSSC* gene mutations. Notably, 43.3% of mites exhibited mutated *VSSC* genes.

Conclusion: This study represents the first investigation into Vssc-associated permethrin resistance in human scabies. The study highlights the importance of detecting genotypic resistance in 43.3% of phenotypically resistant cases.

Keywords: *Sarcoptes scabiei*, Membrane Transport Proteins, Single Nucleotide Polymorphism, Drug Resistance, Scabies.

1. INTRODUCTION

Sarcoptes scabiei is an obligate mammalian ectoparasitic arthropod responsible for causing scabies, a contagious, pruritic skin disease in humans, and mange in other mammals. This arthropod completes its life cycle within the stratum corneum layer of the skin, as it fulfills its oxygen requirement through skin respiration. Consequently, it remains confined to the stratum corneum, predominantly settling in body areas with higher temperatures and thinner stratum corneum layers (1). According to The Global Burden of Diseases, Injuries, and Risk Factors Study conducted in 2015 and 2017, the global prevalence of scabies was reported as 204,151,715 and 175,406,000, respectively (2,3). The annual prevalence of scabies remains approximately 300 million people worldwide, irrespective of race, age, or gender, persisting as a health concern for centuries (4). However, recent years have witnessed an increase in the disease's prevalence due to challenges in accurate diagnosis and the emergence of drug resistance.

The primary treatment for scabies is permethrin, a locally applied drug from the pyrethroid group (5). Pyrethroids, extensively utilized as insecticides worldwide over the past three decades, have led to the development of resistance in numerous organisms. Studies by Mazzatenta et al. (6), Balestri et al. (7), and Meyersburg et al. (8) have all reported decreased sensitivity to permethrin, suggesting treatment failure associated with permethrin resistance (9,10).

This resistance is attributed to mutations in the voltage-sensitive sodium channel (*VSSC*) protein, the target of pyrethroid acaricides like permethrin. Mutations in the *VSSC* gene directly contribute to resistance in various arthropod species (7,11). The voltage-sensitive sodium channel (*VSSC*) is

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a membrane protein composed of four homologous domains and six intermembrane domains. It plays a crucial role in generating action potentials in excitable cells and serves as the target for pyrethroid acaricides such as permethrin.

Pyrethroids disrupt the function of the *VSSC* protein by slowing the channel's activation and inactivation kinetics, leading to prolonged channel opening. This prolonged opening results in paralysis, ultimately causing the death of the organism. Studies have demonstrated that mutations in various positions of the *VSSC* gene, which encodes the channel protein, are directly associated with the development of resistance in many arthropod species. Pasay et al. investigated *Sarcoptes scabiei* var. *canis* mites that had undergone long-term permethrin therapy and exhibited resistance to treatment in vitro, showing reduced sensitivity to permethrin. Specifically, they identified a G → A mutation at position 1535 of the *Sarcoptes scabiei* var. *canis VSSC* gene, leading to a glycine to aspartic acid transition. This mutation has been implicated in conferring resistance to permethrin (11–13).

Despite the absence of reported scabies outbreaks in Türkiye, a notable increase in the number of cases has been observed since 2017. A study conducted across nine different provinces of Türkiye revealed a staggering 7-fold increase in cases from 2017 to 2018, followed by a 30-fold increase from 2018 to 2019. Furthermore, while no instances of resistance were noted until 2018, subsequent analyses indicated 20 cases (13.3%) and 87 cases (13.14%) of topical resistance in 2018 and 2019, respectively (14,15). Another study conducted in Erzurum province highlighted a nearly twofold increase in scabies cases observed in 2019 compared to those reported in the first quarter of 2020 (16). Additional findings from *Özçelik›s* study revealed that 57.6% of patients had undergone multiple treatments due to various factors including improper and inadequate drug application, reinfestation, or drug resistance. These data underscore the escalating burden of scabies in Türkiye and emphasize the importance of addressing challenges such as treatment efficacy, adherence, and resistance to effectively manage the disease (17).

Scabies has become increasingly prevalent in both the Sivas region and Türkiye overall in recent years, with permethrin proving ineffective in most cases. This study aims to investigate the presence of *VSSC* gene mutations associated with the *kdr* resistance phenotype, indicative of pyrethroid resistance, in *Sarcoptes scabiei* var *hominis* mites isolated from permethrin-treated scabies cases that failed treatment in the Sivas region, Türkiye.

2. METHODS

2.1. Ethics

This study was approved by the Sivas Cumhuriyet University Ethics Committee (decision no: 2020-12/04, Date 03/12/2020). The sample size for our study was determined based on practical considerations, including the prevalence of resistance observed in preliminary data related to the availability of cases during the study period. Before the sample collection, informed consent was obtained for the confidentiality of the information and its use in the study. When α (alpha) = 0.05, β (beta) = 0.20 and 1-β = 0.80 were taken, it was decided to include 30 individuals in the study and the strength of the test was found to be p = 0.80060..

2.2. Collecting the Sarcoptes scabiei samples

Sarcoptes scabiei samples were collected from volunteer patients diagnosed with scabies at the Sivas Cumhuriyet University, Faculty of Medicine, Department of Dermatology and Venereal Diseases. The study comprised 30 volunteer patients aged between 18 and 65 years. During sample collection, the skin was scraped multiple times along the tunnels without causing bleeding, using a scalpel and needle. In cases where typical tunnels were not visible, scraping samples were obtained from papules or 4-5 suspicious lesions. Mineralized oil was applied directly to the lesion or onto the scalpel to collect the *Sarcoptes scabiei* and its products in the scraping samples. Mites were positively identified in all skin scrapings. Skin scraping samples were collected both before the initial treatment and after the second treatment.

Subsequently, the mites were separated from the skin tissues under a stereo microscope and collected individually using a needle-tipped loop. DNA isolation was performed using multiple mites. Sarcoptes scabiei mite bodies were confirmed by examining the obtained material under a dry objective, and positive samples were stored at – 20°C for further analysis.

2.3. Genomic DNA Isolation

The genomic DNA (gDNA) samples were isolated utilizing the GeneJET Genomic DNA Purification Kit (Thermo ScientificTM/ catalog no K0721), following the manufacturer's protocol without any modifications. Subsequently, the quality of the isolated gDNA was assessed through agarose gel electrophoresis.

2.4. Partial isolation of VSSC gene

The conventional PCR method was used to amplify a 144 bp region of the *VSSC* gene containing the mutation. Gene-specific primers, VSSC_F_2 (5'-GAGCAGCCAGAGAAAGAAGTCAA-3') and VSSC_R_2 (5'-AGATCCGCCGGCTTTCTTT-3'), were utilized for this purpose. Each reaction was prepared in a total volume of 50 µL, consisting of 10 µL of 5X Phusion HF buffer, 0.5 µL of Phusion DNA Polymerase (2 U/µL) (Thermo Scientific™, Phusion™ High-Fidelity DNA Polymerase, catalog no. F530S), 10 mM dNTP mix, primers (0.5 µM each), and 5 µL of gDNA. The PCR conditions were set as follows: an initial denaturation at 96°C for 30 seconds; 27 cycles of 96°C for 15 seconds (denaturation), 58°C for 15 seconds (primer annealing), and 72°C for 30 seconds (extension); followed by a final extension

at 72°C for 5 minutes. PCR products were analyzed by agarose gel electrophoresis to confirm the expected band size and subsequently purified using the GeneJET PCR Purification Kit (Thermo Scientific™, catalog no. K0701), following the manufacturer's protocol without modifications.

2.5. DNA Sequencing

The purified PCR products were directly submitted for DNA sequencing without being transferred into a plasmid vector. The analysis was conducted at the Central Research Laboratory of Ankara Yıldırım Beyazıt University using gene-specific primers.

2.6. Multiple Sequence Analysis

The DNA sequencing results obtained from the forward and reverse directions of the *VSSC* gene, sampled from 30 individuals, were analyzed by aligning them using MEGAX software (18). All results were compared to the wild type *VSSC* mRNA (NCBI ID: DQ077149.2) to identify any mutations (19). Subsequently, the sequences obtained from multiple sequence alignment were translated into protein sequences and subjected to multiple sequence alignment again to identify amino acid mutations.

3. RESULTS

In the study, a point mutation resulting in a glycine to aspartic acid transition (GGC → GAC) in the *Sarcoptes scabiei VSSC* gene was identified in 13 out of 30 patients. All raw data and multiple sequence alignment of the DNA sequences are provided in the supplementary files (S1, S2 and S3). The multiple alignment of the protein sequences is illustrated in Figure I.

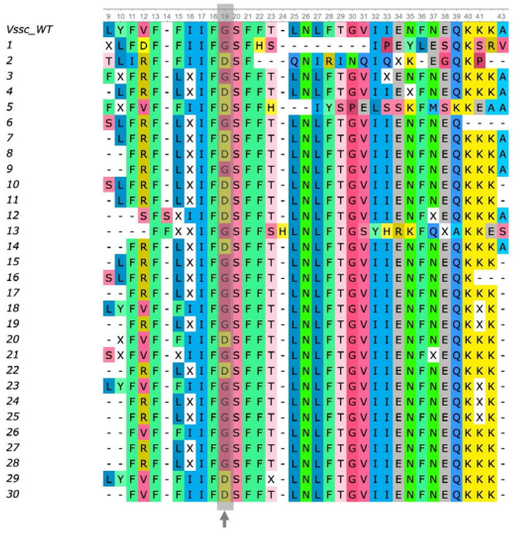

Figure 1. *The multiple sequence alignment of sequencing results with the reference gene (VSSC_WT). (A) Forward and (B) reverse orientation results of the DNA sequencing results.*

The patients who come to clinic who do not respond to the treatment are followed up by our clinic. All volunteers (30 cases) consisted of permethrin resistant scabies patients who did not respond to second or third permethrin treatment. Permethrin treatment was terminated in our patients (13 cases) who did not respond to the first three permethrin treatments and were treated with pomades containing sulfur and benzyl benzoate. 17 out of 30 cases, lesions were decreased after the second treatment, and a significant decrease in the number of parasites was observed in the skin scraping samples. Complete recovery was noted after the fourth drug application. However, 13 out of 30 cases, showed no response to permethrin treatment only. Therefore, we can associate the presence of mutation with the treatment response. However, we can consider patients who do not have a mutation and who do not respond to permethrin as not using the drug correctly or using it inadequately in the first treatment.

4. DISCUSSION

Scabies presents a significant global public health challenge, affecting individuals of all ages, races, genders, and socio-economic backgrounds, often leading to severe itching and diminishing quality of life. Particularly in recent years, there has been a notable increase in scabies cases worldwide. Permethrin application (5% topical) is typically recommended as the first-line treatment according to current guidelines for scabies management. However, despite the utilization of various treatment schedules, a decline in the efficacy of permethrin has been observed (6). Furthermore, two complementary studies have indicated decreased treatment effectiveness even with more aggressive treatment approaches suggested by guidelines and diverse treatment regimens (7,8).

Several potential reasons have been proposed for the failure of permethrin treatment. These include inadequate exposure time or amount of application, misuse of permethrin, failure to maintain short nails (which may harbor mites), insufficient treatment of hyperkeratosis, omission of permethrin application on the heads of children, failure to reapply permethrin after hand washing, reinfection from contact with infected individuals or contaminated items such as clothing, sheets, and towels, and the development of resistance to permethrin. Additionally, treatment failure may occur due to the inability to treat all family members simultaneously, particularly in communal living conditions (20).

Although not conclusively proven, it is hypothesized that permethrin resistance may result from various mechanisms, including the formation of mutations. Increased transcription rates of genes encoding glutathione-S-transferase, cytochrome p450, and monooxygenase in *Sarcoptes scabiei* mites have been suggested as potential mechanisms contributing to permethrin resistance (9,11). Pasay et al. identified a single point mutation at position 1535 of the *VSSC* gene in *Sarcoptes scabiei* mites resistant to permethrin (11).

This study identified a G→A single point mutation in the *VSSC* gene, resulting in a glycine → aspartic acid change in 13 out of 30 patients. This mutation causes a structural alteration in the protein, preventing permethrin binding and thereby conferring resistance (21). While a knockdown resistance (*kdr*) mutation associated with permethrin resistance has been detected in *Sarcoptes scabiei* var. *canis* mites and lice, it was not found in *Sarcoptes scabiei* var. *hominis* mites (22). Another study reported permethrin's effectiveness against *Sarcoptes scabiei* mites, attributing treatment failure to patient non-adherence (23).

In cases of permethrin resistance, sulfur ointments have proven to be an effective and safe alternative. Another viable option is the use of topical crotamiton (5% or 10%) applied for three to five consecutive days. Benzyl benzoate (25% for adults, 10% for children) applied topically for three consecutive days is also considered an effective alternative treatment. These options provide clinicians with multiple strategies to address resistance while tailoring treatment to individual patient needs (24).

Ivermectin is another treatment option for scabies, with potential mechanisms of action including genetic alterations and changes in the structure of the glutamate chloride channel and p-glycoprotein membrane transport protein. Ivermectin tablets (3 mg) are approved for the systemic treatment of scabies. The recommended dosage is 200 µg/kg of body weight for patients weighing 15 kg or more (24,25). Although recent meta-analyses suggest the efficacy of permethrin treatment, clinical observations indicate its diminishing effectiveness compared to previous years. Prospective genetic analyses are warranted to corroborate these findings (20).

5. CONCLUSION

Scabies has become increasingly prevalent in recent years, posing challenges in treatment efficacy. Our study reveals the presence of mutations previously documented in the literature in patient samples. Specifically, mutations were observed in 13 out of 30 samples collected. Significantly, our study highlights the importance of detecting genotypic resistance in 43.3% of phenotypically resistant cases, representing the first report from the Sivas region, Türkiye. Given the ineffectiveness of traditional scabies treatments in these cases, exploring alternative treatment options and developing novel therapeutic agents are imperative.

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Conflicts of interest
The authors declare that they have no conflict of interest.

Ethics Committee Approval: *This study was approved by Ethics Committee of Sivas Cumhuriyet University Clinic Ethics Committee (Approval date 03/12/2020; Number: 2020-12/04)*

Author Contributions:
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