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Original Article

Comparison of *in vitro* susceptibility of *Sarcoptes scabiei* var.*hominis* to topical 5%permethrin and topical 1% ivermectin

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Abstract

Objective To compare the *in vitro* susceptibility of *Sarcoptes scabiei* var.*hominis* to 5% permethrin and 1% ivermectin.

Methods A randomized, controlled trial was conducted in OPD, Dermatology Department, Military Hospital, Rawalpindi, Pakistan over a period of six months from December 17, 2014 to June 16, 2015.A total of 80 mites from 80 patients were taken. Mites were randomly allocated to two groups: group A (5% permethrin) and group B (1% ivermectin). 5% permethrin and 1% ivermectin were applied in a thin film over a glass slide. Live mites were gently transferred to the glass slide. These were inspected microscopically for leg movements at an hourly interval for 6 hours. Death was declared once all leg movements had ceased. All mites which died within 6 hours were considered as susceptible to drug, while mites having active leg movements even after 6 hours were considered as non-susceptible.

Results There were 35 (84.5%) mites which showed *invitro* susceptibility in the group A (5% permethrin), whereas all the mites from group B (1% ivermectin) were susceptible.

Conclusion The *in vitro* susceptibility was statistically significant (p=0.021) in both the groups. 1% ivermectin was better than 5% permethrin in terms of *invitro* susceptibility.

Key words
Scabies, *Sarcoptes scabiei*, ivermectin, permethrin, *in vitro* susceptibility.

Introduction

Scabies is a common public health problem affecting about 300 million patients in the world yearly.[1] It is important especially in the resource poor countries where it may cause serious complications such as post-streptococcal glomerulonephritis and systemic sepsis. So, treatment should be started early if it is suspected clinically.[2,3]

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Definitive diagnosis can be made on microscopic identification of mites or its products in scrapings from skin lesions. Other tests like burrow ink test, video-dermatoscopy, PCR/ELISA, and specific IgE against major mite components may help.[4,5]

Permethrin, benzyl benzoate, crotamiton, lindane, and ivermectin are the various drugs used for treating scabies.[6] Among these 5% permethrin is the most effective scabicide with fewer side effects and a reported 96.9% efficacy.[7]However, the efficacy of this treatment has diminished, due to the emergence of resistance especially in the developing world.[8]

Ivermectin is the only oral scabicide available with potential antibacterial, antiviral, and anticancer role. It is one of the world's most successful and promising drugs.[9,10] It has worked as a wonder drug in eliminating river blindness and elephantiasis.[11,12]

Oral ivermectin is already being used in the treatment of scabies effectively.[13]Topical ivermectin is also effective.[14] 1% ivermectin lotion in propylene glycol applied topically showed a reported efficacy of 69.3% at one week of application.[15]

Topical treatment of scabies is a burden on the caregivers because it must be applied to the whole skin below the neck. It is cumbersome and messy, and interferes with daily activities of patients. The presentation of scabies patients with recurrent infestations indicates the possibility of emerging drug resistance or poor compliance to topical permethrin, which is the most commonly prescribed treatment for scabies.[16,17] Newer treatment options should be studied, aiming for a better treatment modality.

Our study was designed to compare the susceptibility of *Sarcoptes scabiei* mite to 5% permethrin and 1% ivermectin. Considering all the available research on ivermectin done previously, we planned this *in vitro* assay to explore this drug further. The two drugs are compared by observing the *in vitro* susceptibility of mites to either of two. By making a comparison, we will be able to choose a better treatment modality for use in future.

Methods

This randomized, controlled trial was conducted in dermatology outpatients department, Military Hospital, Rawalpindi, Pakistan over six months i.e. December 17, 2014 to June 16, 2015.

Samplesize was calculated by using WHO sample size calculator, taking level of significance as 5%, power of test as 90%, anticipated population proportion P1 as 96.9%[7] and anticipated population proportion P2 as 69.3%.[15]There were 40 mites in each group making a total of 80 mites.

All patients of any gender between age limit of 14 years and 65 years, with a clinical diagnosis of scabies were explored for live mites. Diagnosis was confirmed by extruding live mites from the lesions of scabies and later on confirmed by light microscopy. Only previously treated patients of scabies (treated within last two months) were excluded.

Patients fulfilling the inclusion criteria were selected through non-probability sampling, after taking formal approval and permission from Hospital Ethical Committee, Military Hospital, Rawalpindi and an informed consent from the patients.

Only adult mites were subjected to study. The mites were identified on clinical examination of the patients as pale white coloured papules at the end of the burrows and were extracted manually with the help of a sterilized common pin. A relevant history and physical examination of all the patients was recorded. All extracted mites were allocated to two groups randomly by using the random numbers table.

Topical permethrin was 5%w/w of permethrin cream (brand name Lotrix® by GlaxoSmithKline laboratories). Topical 1% ivermectin was 1%w/w of ivermectin (brand name Mectis® by Genome pharmaceuticals, Pakistan) in propylene glycol. Since, topical ivermectin is not available commercially; to standardize the preparation it was prepared by a single chemist.

Live mites had active leg movements under microscope. These were transferred gently to glass sides with a thin film of either drug and maintained at room temperature. Mites were inspected under the microscope for leg movements at one hourly interval for 6 hours. Death was declared once all leg movements had ceased. All data were recorded on a proforma.

Data were analysed by using SPSS version 22. The quantitative variables like age and duration of illness were calculated by taking mean and standard deviation. The qualitative variables like gender and outcome variables like *in vitro* susceptibility of mites (yes/no) were calculated by taking frequency and percentages and compared by Chi-square test. A *p* value of 0.05 or less was considered as significant. The confounding variables like age; duration of illness and gender were controlled by stratification. Poststratification Chi square test was applied, keeping *p* value 0.05 or less as significant.

Results

Total 80 patients were included in our study meeting the inclusion criteria. Mean age (years) in both the groups was 37.10±14.70 and 44.82±15.16, respectively, as shown in **Table 1**. There were 28 (70.0%) and 30 (75.0%) male patients, respectively in each of the groups whereas there were 12 (30.0%) and 10 (25.0%) female patients, respectively in the groups. Mean duration of illness was 12.73±4.85 days and 11.45±4.63 days, respectively.

There were 35 (84.5%) mites which showed *in vitro* susceptibility in the group A (which was given 5% permethrin), whereas all the mites in group B (which was given 1% ivermectin) were susceptible (**Table 2**), (*p*-value 0.021) i.e. 1% ivermectin was better than 5% permethrin against *Sarcoptes scabiei* in terms of *invitro* susceptibility.

In the age group ranging from 14-40 years, there were 24 (32.0%) patients in which *in vitro* susceptibility was observed whereas in age group 41-65 years, there were 51 (68.0%) patients in which *in vitro* susceptibility was observed (**Table 3**), *p*value 0.029.

Effect modifier like duration of illness was also compared with *in vitro* susceptibility (death of mite confirmed at 6 hours). Mean duration of illness was 12.12±4.82 days in those patients in which *in vitro* susceptibility was observed. Independent sample t-test was used to compare duration of illness with *in vitro* susceptibility (death of mite confirmed at 6 hours) which was statistically not significant (*p*value 0.781), as shown in **Table 4**.

There were 56 (74.7 %) male patients in which *in vitro* susceptibility was observed whereas there were 19 (25.3%) female patients in which *in vitro*susceptibility was observed, *p* value 0.093, as shown in **Table 5**.

Kaplan-Meier survival analysis was used to compare 1% ivermectin with 5% permethrin in terms of average time of death of mites. Mean time of death of mites in ivermectin group was 2.35 hours whereas in permethrin group was 3.59 hours as is shown in **Table 6**.

The graph also depicts the survival rate (average time of death) of mites. The green line shows the 1% ivermectin whereas blue line shows 5% permethrin group.The graph shows that 1% ivermectin group required less average time for the death of mites as compared to 5% permethrin as shown in **Figure 1**.

Table 1 Demographic and clinical characteristics of study population (n=80)

	Group A (5% permethrin) N=40	*Group B (1% ivermectin) N=40*
Mean age (years)	37.10±14.70	44.82±15.16
Sex		
Male	28 (70%)	30 (75%)
Female	12 (30%)	10 (25%)
Duration of illness (days)	12.73±4.85	11.45±4.63

Table 2 Comparison of *in vitro* susceptibility of mites at 6 hours (n=80).

In vitro susceptibility (death of mite confirmed at 6 hours)	*Group A (5% permethrin) N=40*	*Group B (1% ivermectin) N=40*	*P value*
Yes	35 (87.5%)	40 (100%)	0.021
No	5 (12.5%)	0 (0%)	

Table 3 Effect of age group stratification on *in vitro* susceptibilityat 6 hours (n=80).

Age (years)	*In vitro susceptibility (death of mite confirmed at 6 hours)*		*P value*
	Yes (n=75)	*No (n=5)*	
14-40	24 (32%)	4 (80%)	0.029
41-65	51 (68%)	1 (20%)	

Table 4 Effect of duration of illness on *in vitro* susceptibility at 6 hours (n=80).

In vitro susceptibility (death of mite confirmed at 6 hours)	*N*	*Duration of illness (days)*	*P value*
Yes	77	12.12±4.82	0.781
No	3	11.33±3.05	

Table 5 Effect of gender on *in vitro* susceptibility at 6 hours (n=80).

Sex	*In vitro susceptibility (death of mite confirmed at 6 hours)*		*P value*
	Yes (n=75)	*No (n=5)*	
Male	56 (74.7%)	2 (40%)	0.093
Female	19 (25.3%)	3 (60%)	

Table 6 Comparison of mean time to death of mites

	Mean time to death (hours)			
	Estimate	Standardized error	*95% confidence interval*	
			Lower bound	*Upper bound*
5% Permethrin	3.593	0.158	3.282	3.903
1 % Ivermectin	2.350	0.092	2.170	2.530
Overall	2.946	0.114	2.723	3.169

Discussion

Scabies is a global health problem. Many treatment options are available. Among them 5% permethrin is the most effective scabicide with fewer side effects. Efficacy of topical permethrin is challenged by drug resistance due to specific mutations in the neuronal voltage-sensitive sodium channel gene in the mite.These neuronal voltage-sensitive sodium channels are necessary for the generation of action potentials in excitable cells and are the target of permethrin.[18,19]

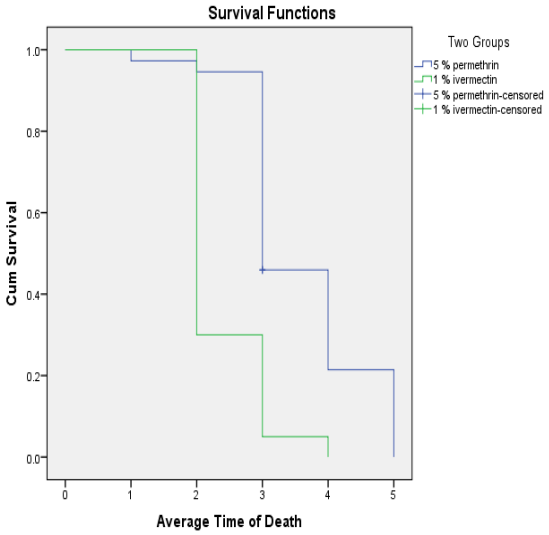

Figure 1 Kaplan–Meier Curve: Comparison of 5 % permethrin and 1% ivermectin in terms of average time of death of mites.

Ivermectin binds selectively with high affinity to glutamate-gated chloride ion channels in muscle and nerve cells of the parasite causing an increase in the permeability of the cell membrane to chloride ions resulting in hyperpolarization of the cell, leading to paralysis and death of the parasite. It also acts as an agonist of the neurotransmitter gamma-amino butyric acid (GABA), thereby disrupting GABA-mediated central nervous system neurosynaptic transmission.[20]

Oral ivermectin is being used increasingly because it is easy to administer. There is no significant difference regarding safety of use between permethrin and oral ivermectin. It also proved effective for the scabies prophylaxis. It has been used in nursing homes and prisons for both prophylaxis, as well as, outbreaks.It can be useful in patients for whom topical treatment is potentially irritant and less well-tolerated.[21,22,23]But it is not approved for use in children who weigh less than 15 kg, or in pregnant or lactating women, due to potential systemic adverse reactions, such as hepatic dysfunction.[24,25]

Ivermectin is known to have limited ovicidal action as compared to permethrin. Since the eggs on the skin at the time of treatment will hatch later. So, a single dose may not effectively eradicate scabies.[26,27] Therefore two doses of ivermectin separated by 7 to 14 days, is usually advised. A cure rate of 56 % after a single dose and 78% after two doses a week apart has been reported.[13]

After being taken orally, ivermectin is absorbed into the blood and reaches skin via sebaceous glands. The effective concentration in the stratum corneum ranged from 40-80ng/g in patients with scabies who responded to oral ivermectin, which is therefore regarded to be the effective concentration.[28]

Based on the above principle, a new treatment named “whole-body bathing method” is suggested.[29] Patients may be bathed in a fluid containing more than the effective concentration for treatment of scabies by making an effective solution containing about 80ng/mL ivermectin. This may be done by putting only 12mg of ivermectin into a bathtub full of water, approximately 150L. This bathing fluid can be used repeatedly within a group, such as a family.

This method was tested on rats and was found useful. When the rats were bathed in the fluid containing 100ng/mL of ivermectin, the concentration in the skin was more than 400ng/g wet weight. It rapidly appeared in the skin within 5 minutes of bathing and the concentration was maintained for 8hours after the end of bathing irrespective of the bathing time. It was not detected in the plasma. But as the skin permeability and absorption of ivermectin may be different between rats and humans, further investigations should be done to evaluate the feasibility and the optimal bathing conditions in humans.[29]

In vitro study by Walton*et al*.[30] showed better efficacy for ivermectin. 5% permethrin had the slowest killing time, with 35% of mites still alive after 3 hours, and 4% still alive after 18-22 hours of constant exposure. In contrast, no mites were alive after 3 hours exposure to 100-8000ng/g of ivermectin.

Goldust*et al*.[14] compared topical 1% ivermectin at a dose of 400 microgram/kg with permethrin 2.5% cream. Two applications of ivermectin were as effective as two applications of permethrin at the 2-week follow-up, 63.1% versus 65.8%. After repeating the treatment, ivermectin was as effective as permethrin at the 4-week follow-up, 84.2% versus 89.5%, respectively.

Chhaiya*et al.*[15] compared the efficacy and safety of topical permethrin 5%, oral ivermectin 200 micrograms/kg, and topical ivermectin 1% lotion in propylene glycol in the treatment of uncomplicated scabies. Patients were randomly allocated to 3 groups. First group received permethrin 5% cream as single application; second group received tablet ivermectin 200 micrograms/kg as single dose; and third group received ivermectin 1% lotion as single application. The patients were followed up weekly for 4 weeks. If there were no signs of clinical cure, the same intervention was repeated at each follow-up. At the end of first week, cure rate was 74.8% in permethrin group, 30% in oral ivermectin group, and 69.3% in topical ivermectin group. At the end of second week, cure rate was 99%, 63%, and 100%, respectively. At the end of third week, 100% cure rate was observed in permethrin and topical ivermectin group while 99% in oral ivermectin group. The study concluded that topical permethrin and topical ivermectin were equally effective against scabies while oral ivermectin was significantly less effective at 2 weeks.[15]

In our study 35 (84.5%) mites showed *in vitro* susceptibility in the group A (which was given 5% permethrin), whereas all the mites in group B (which was given 1% ivermectin) were susceptible. The *in vitro* susceptibility was statistically significant (*p*value 0.021) in both the groups and showed that 1 % ivermectin was better than 5% permethrin against *Sarcoptes scabiei* in terms of *in vitro* susceptibility. All mites in the ivermectin group died within 4 hours with a mean death time of 2.35 hours. The mean death time in the permethrin group was 3.5 hours; which is longer as compared to 2.35 hours for the ivermectin group. Therefore, ivermectin not only showed 100% susceptibility of mites but also a shorter mean death time.

Conclusion

1% ivermectin is more effective than 5% permethrin, against *Sarcoptes scabiei* var.*hominis* in terms of *in vitro* susceptibility.Mean killing time of ivermectin is shorter than permethrin making it a more effective antiscabies drug. As 12.5% of mites in the permethrin group were not susceptible, it is therefore recommended that further *in vitro* susceptibility assays should be performed to see any emerging pattern of resistance in local population.

Our study suggests that topical ivermectin is a good alternative for the treatment of scabies. With a shorter death time, it might need a shorter contact time and thus patients might comply better with it. Further *invitro* and *in vivo* trials are needed with larger sample sizes to evaluate this treatment option in detail.

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