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Comparative study of Intralesional Vitamin-D3 and Autoinoculation in treatment of multiple cutaneous warts

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ABSTRACT-

Background- Persistent and recurrent warts pose a challenge to physicians. Despite the availability of multiple treatment options, warts are resistant to treatment. Intralesional Vitamin D3 and Autoinoculation both are promising treatment modality for multiple warts. **AIM-** To compare the efficacy and safety of intralesional Vitamin-D3 and autoinoculation in treating multiple warts**. Patients and method-** the study included 60 patients divided in two groups (group-A and group-B), each containing 30 patients. Informed consent was taken from each patient. Group-A patients received intralesional Vitamin-D3 0.2-0.5 ml every 3 weeks for maximum of four sessions. Group-B patients were treated by autoinoculation every 4 week for 3 sessions. **RESULTS-** At the end of 3 months 86.6% of patients showed response in group-A, Complete clearance was seen in 76.6% patients. Three months post autoinoculation (group-B), 66.6% patients showed response. Complete clearance was seen in 56.6% post autoinoculation in group-B. **CONCLUSION-** Intralesional Vitamin-D3 had the edge over Autoinoculation in palmoplantar, verruca vulgaris and periungual warts, while autoinoculation had the advantage in the plane variants.

Keywords- Intralesional Vitamin-D3, Autoinoculation, Cutaneous wart

INTRODUCTION

Warts are common epidermal papillomatous growth, caused by various strains of human papilloma virus affecting all age group It can grow anywhere on body, hand and feet are most common site. It is mostly asymptomatic but may be painful, as in palmoplantar wart. Virus remains latent in basal cells and undergo slow replication. It induces hyperplasia and hyperkeratosis in epidermis.[1]

Treatment of warts may be difficult despite of several treatment modalities available. Such cases may be frustrating to the treating physician. Recalcitrant warts are resistant to conventional therapeutic option and may continue to increase in size and number during treatment.[2]

Myriads of treatment modalities are available but none are 100% effective. Topical salicylic acid, topical Trichloroacetic acid, topical imiquimod, topical 5-fluorouracil, cryotherapy, excision, electocautery, laser vaporization, are some primary treatments. But the majority of these available treatment are destructive, require multiple sittings, may cause scarring and chances of recurrence is high. Traditional treatment modalities are limited to local application and do not act systemically. Hence, they are generally considered unsuitable for patients with multiple distant lesions. In multiple warts, especially of palms and soles, destructive procedures are inappropriate and impractical.[3]

Immunotherapy can overcome the limitation of conventional treatment modalities. They resolve lesions which are distant from the site of administration as well as subclinicaly infected virus tissue. Making it a preferable choice for patient with multiple warts, recalcitrant wart, and those with the wart at difficult to treat sites (subungual, periungual, eyelids). Variety of immune stimulants are available, including proinflammatory cytokines like interferons and interleukins-2; vaccines like the mumps, measles, and rubella (MMR) vaccine; Mycobacterium w and bacillus calmette-guerin (BCG) vaccines; intralesional antigens like purified protein derivative; Candida and Trichophyton; immune enhancers like zinc sulfate; and topical contact sensitizers like Diphenylcyclopropenone (DCP), squaric acid dibutyl ester (SADBE).[4]

Immunotherapy acts via recognition by the immune system of antigens produced by virus and wart tissue causing delayed-type hypersensitivity response resulting in the eradication of HPVs.4 Intralesional vitamin D3 is a recent and effective immunotherapy for the treatment of warts. Vitamin D3 acts via regulation of epidermal cell proliferation and differentiation. There is also modulation of cytokine production. Activation of Toll-like receptor also takes place resulting in human macrophages upregulation and expression of VDR and VD1-hydroxylase genes, leading to production of the antimicrobial peptide.[5,6,7]

Autoinoculation therapy is a simple, minimally invasive, inexpensive technique wherein the patient's wart is excised, minced and implanted into a dermal pocket. Exposing HPV antigens to the dermis and the cutaneous vasculature can activate a delayed hypersensitivity response, facilitating clearance of both local and distant warts. Autoinoculation causes activation of a delayed hypersensitivity response to the wart tissue. There is an increased production of Th1 cytokines and viral-specific antibodies.[18]

Hence, this study was undertaken to compare the potential effect of intralesional vitamin D versus autoinoculation in the treatment of multiple extragenital recalcitrant warts.

METHODS

An open non-randomized comparison study was conducted in the Skin & V.D. Department of Jawaharlal Nehru Medical College Ajmer, a tertiary care centre in Rajasthan from April 2021 to Dec 2021. A total of 60 patients of age 12 and above with multiple verruca vulgaris and palmoplantar warts (>5 in number) attending skin opd. Our exclusion criteria were:

pregnancy and lactation, patients age less than 12 years, immunosuppression due to drugs or disease, diabetes, history of keloids or hypertrophic scars, inability to come for follow-ups and those with mucosal warts.

History of onset and progress of lesions, family history of similar complaints and treatment taken in past were recorded. All patients included were tested for HIV infection and found negative. A routine hemogram, fasting blood glucose, serum urea, creatinine and liver function tests were done. The first session of autoinoculation was then carried out. Patients who were not immunized against tetanus were given intramuscular tetanus toxoid (0.5 ml) at the baseline visit.

Institutional ethics committee approval was taken. Informed consent from each patient was sought prior to commencement of treatment.

A 3-week washout period was allowed if the patients were still on any therapy before coming to us. They were also instructed not to use any other wart therapies during the course of the study. The characteristics of the warts such as type, size, number, presence or absence of side effects, and clinical photographs were recorded at the start of the study and at each follow-up visit.

Based on the previous treatment history and patient consent, patients were allocated to group-A (intralesional vitamin-D3 injection) or group-B (autoinoculation).

Group A - Using a 26-gauge syringe with the bevel facing upward, 0.2 to 0.5 mL Vitamin-D3 solution (600,000 IU; 15 mg/mL) was slowly injected into the base of each wart. A maximum of 5 warts were injected per session. The injections were performed at 3 weekly intervals until complete resolution or for a total of 4 sessions. In addition to routine investigations, the serum calcium levels were monitored during each visit.

Group B- The procedure was performed under strict aseptic precautions in the dermatology operation theatre. A wart of substantial volume was chosen as the donor and anesthetized by 2% lignocaine infiltration. It was then shaved using a number 15 scalpel blade. Hemostasis was achieved with cauterization. The tissue thus obtained was minced on a sterile glass slide with a surgical blade into fine bits (increase surface area of antigen exposure). Volar aspect of the non-dominant forearm was used as the recipient site. After disinfection and local anaesthesia, a dermal pocket was created using 20G sterile needle. Using a 20-gauge needle, a dermal pocket extending up to the subcutis was created over the volar aspect of the left forearm, 5 cm below the antecubital crease with prior infiltration of anesthesia. The minced bits of the donor wart were introduced into this pocket using the tip of the same needle. Both donor and recipient sites were dressed with sterile medicated gauze and adhesive plaster. Systemic and topical antibiotics (oral amoxicillin + clavulanic acid and topical mupirocin) were prescribed for 5 days. Patients were advised not to wet or remove the plaster for 5 days after the procedure. After first inoculation, patients were advised to return after 1 week for change of dressing and to note any adverse effect. All patients were advised to follow up on 4^{th} week of each autoinoculation for three episodes and after three months of third follow up visit. At each visit a different wart was chosen to obtain tissue for inoculation and a new dermal pocket was made because the old pocket healed in 5-7 days. If no lesions were present after the first treatment, the patient was followed-up without further inoculation.

Evaluation of treatment efficacy- Responses to the treatment, including a decrease in number of warts on serial images, were noted at each visit. Response to the treatment was graded as

follows: complete clearance (100% clearance of wart; appearance of normal skin), partial response (50-99% reduction), and no response (<50% reduction in the number of warts or worsening).

Statistical analysis- The efficacy of therapy was calculated in terms of percentage of patients showing response. Inferential statistics were applied depending upon nature of data and variables. Chi-Square test applied to find difference in procedure group (vitamin-D3 vs autoinoculation) and result group (complete clearance, partial clearance and no result) by demographic characteristics. Independent sample t-test and one-way ANOVA analysis were performed in assessing any difference in mean age, mean duration of illness and number of warts by procedure group and result group. P values less than 0.05 were considered statistically significant.

RESULTS

Clinico-demographic data of study participants are highlighted in (Table 1). Males outnumbered female in both treatment groups. Mean age of both the groups was comparable. Patients were not below 12 years of age. Both the groups were comparable in terms of clinical profile. Mean duration of illness was approximately 9 months in both the groups. Minimum number of warts were 5 in both the groups.

At the end of 3 months 86.6% of patients showed response in group-A, who received intralesional vitamin-D3 injection. Complete clearance was seen in 76.6%. Three months post autoinoculation (group-B), 66.6% patients showed response. Complete clearance was seen in 56.6% post autoinoculation in group-B. (Table-2)

Subgroup analysis (Table-3) within the both groups showed no statistically significant difference with respect to age or sex and response to therapy. Importantly, the number of warts at baseline did not affect the degree of reduction in warts.

In group-A with intralesional Vitamin-D3 therapy, 92.3% cases of palmoplantar warts show clearance (complete clearance in 84.6%). Among patients having verruca vulgaris, clearance was seen in 83.3% (complete clearance in 75%). Improvement in patients with periungual and filiform warts was 50% and 100% complete clearance rates, respectively. Verruca plana did not shown any response.

With autoinoculation therapy (group-B), best response was seen in cases of palmoplantar warts with clearance seen in 71.4% cases (complete clearance in 64.2%). Among patients having verruca vulgaris, response was seen in 60% (complete clearance in 50%). Response in periungual and filiform warts was 33.3% and 100% complete clearance rates, respectively. Verruca plana shown clearance in 50% cases.

Adverse effects were seen in 9 patients after intralesional Vitamin-D, but all were minor with no life-threatening complications. Most common adverse event was pain at site of injection, seen in 5 patients. Swelling at the site of injection was seen in 3 patients which resolved without any treatment. Dyspigmentation was seen in one patient. No signs of hypervitaminosis D or systemic side effects were seen in any patient.

In 5 patients warts resolved with hypopigmentation, following autoinoculation. Two patients reported secondary infection at site of autoinoculation. Post inflammatory hyperpigmentation at site of autoinoculation was seen in 7 patients.

DISCUSSION

In our study we observed complete clearance in 76.7% patients in intralesional Vitamin-D3 (group-A) and 56.7% patients in autoinoculation (group-B). Partial clearance was observed in 10% patients, in each group.

Several studies have evaluated the efficacy of intralesional vitamin-D3 in the treatment of cutaneous warts. Results in our study in intralesional vitamin-D3 group were comparable to following studies.[5-11] In a study by Singh *et al.*,[8] involving 40 patients, 72.5% cases had complete response. In study by Kavya *et al.*,[6] involving 42 patients with multiple warts, 78.57% patients showed complete response. Raghukumar *et al.*,[7] reported complete clearance in 90% patients. Aktas *et al.*,[5] used intralesional vitamin-D3 for plantar warts in 20 patients and reported complete clearance in 80% of the patients at the end of 8 weeks. In a study by Kumar SS *et al.*,[9] involving 88 patients, 71.5% cases had complete response. Potlapati A *et al.*, [10] and Insha Latif *et al.*, [11] reported clearance in 76.6% and 68.5% respectively.

Shivkumar *et al.*, performed autoinoculation in 60 patients, suffering from verruca vulgaris and palmoplantar warts and total clearance rate was 73.3%. Studies from India reported response to autoinoculation therapy in 65% to 88% of patients with verruca vulgaris within a follow-up period of 3 to 6 months.[13-17] Study by Faleiro et al reported complete clearance in 46% patients, palmoplantar wart and verruca vulgaris showed complete clearance in 82.3% and 28% patients respectively. In our study best response was seen in palmoplantar wart (complete clearance in 64.2% patients) similar to study by Faleiro et al. Complete clearance in verruca vulgaris was showed by 50% patients. 1(50%) patients of verruca plana showed complete clearance.

We observed no correlation between age or sex and response to intralesional Vitamin-D3 or autoinoculation. We found that response differed with the type of warts. Best response was seen in palmoplantar wart in both the groups, but intralesional Vitamin-D3 showed higher clearance (complete clearance in 84.6% patients) as compared to autoinoculation (complete clearance in 64.2% patients). Similarly in verruca vulgaris and periungual wart higher clearance was seen in intralesional Vitamin-D3 therapy as compared to post autoinoculation. Filiform wart shown similar response in both the groups. But verruca plana showed 0% response in intralesional Vitamin-D3 group.

Adverse effect was seen in 9 patients after intralesional Vitamin-D3 therapy and 14 patients post autoinoculation. Dyspigmentation was more in autoinoculation, wart resolved with hypopigmentation in 5 patients post autoinoculation as compared to only 1 patient showed dyspigmentation post intralesional Vitamin-D3 therapy. Swelling and pain reported in 3 and 5 patients respectively in Intralesional Vitamin-D3 group. Secondary infection and post inflammatory hyperpigmentation at site of autoinoculation reported in 2 and 7 patients respectively.

CONCLUSION

In summary, one can say it was evident that Intralesional Vitamin-D3 had the edge over Autoinoculation in palmoplantar, verruca vulgaris and periungual warts, while

autoinoculation had the advantage in the plane variants. Filiform wart showed similar response in both the groups. Intralesional Vitamin-D3 is a simple, well tolerated treatment method with minimal side effects, that is easy to administer in outpatient clinics. No correlation between age or sex and response to intralesional Vitamin-D3 or autoinoculation. We found that response differed with the type of warts.

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DECLARATIONS

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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Table 1: Clinico-demographic profile.

Variables	**Total (n=60)**	**Vitamin D (n_2=30)**	**Autoinoculation (n_1=30)**	**P value**
Age(years) Mean±S.D.	27.57±7.674	26.30±7.548	28.83±7.715	0.204
Gender (Male)	38(63.3%)	18(60.0%)	20(66.7%)	0.592
Duration of Illness(months) Mean±S.D.	9.28±6.140	9.37±6.462	9.20±5.910	0.917
<3	7(11.7%)	3(10.0%)	4(13.3%)	
3-6	17(28.3%)	10(33.3%)	7(23.3%)	
6-12	22(36.7%)	9(30.0%)	13(43.3%)	
>12	14(23.3%)	8(26.7%)	6(20.0%)	
Type of Warts				
Palmo-plantar	27(45.0%)	13(43.3%)	14(46.7%)	0.935
Verruca Vulgaris	22(36.7%)	12(40.0%	10(33.3%)	
Periungual	5(8.3%)	2(6.7%)	3(10%)	
Verruca Plana	3(5%)	1(3.3%)	2(6.7%)	
Filiform	3(5%)	2(6.7%)	1(3.3%)	
Number of Warts Mean±S.D.	9.33±4.565	9.50±4.584	9.17±4.617	0.780
<10	37(61.7%)	18(60.0%)	19(63.3%)	
11-20	21(35.0%)	11(36.7%)	10(33.3%)	
>20	2(3.3%)	1(3.3%)	1(3.3%)	
Site				
Face	4(6.7%)	3(10.0%)	1(3.3%)	0.687
Hand	29(48.3%)	12(40.0%)	17(56.7%)	
Hand Feet	6(10.0%)	3(10.0%)	3(10.0%)	
Hand Leg	5(8.3%)	2(6.7%)	3(10.0%)	
Feet	14(23.3%)	9(30.0%)	5(16.7%)	
Trunk	2(3.3%)	1(3.3%)	1(3.3%)	

Table 2: Response in group-A and group-B following therapy.

Result	**Total (n=60)**	**Vitamin D (n_2=30)**	**Autoinoculation (n_1=30)**	**P value**
Complete Clearance	40(66.7%)	23(76.7%)	17(56.7%)	0.213
Partial Clearance	6(10.0%)	3(10%)	3(10%)	
No Result	14(23.3%)	4(13.3%)	10(33.3%)	

Table 3: Subgroup analysis within each group.

Type of warts	Complete clearance N (%)	Partial clearance N (%)	No response N (%)	Total (%)
Vitamin D Group				
Palmo-plantar	11(84.6%)	1(7.7%)	1(7.7%)	13(100%)
Verruca Vulgaris	9(75.0%)	1(8.3%)	2(16.6%)	12(100%)
Periungual	1(50.0%)	1(50.0%)	0(0.0%)	2(100%)
Verruca Plana	0(0.0%)	0(0.0%)	1(100%)	1(100%)
Filiform	2(100%)	0(0.0%)	0(0.0%)	2(100%)
Autoinoculation Group				
Palmo-plantar	9(64.2%)	1(7.1%)	4(28.5%)	14(100%)
Verruca Vulgaris	5(50%)	1(10.0%)	4(40%)	10(100%)
Periungual	1(33.3%)	1(33.3%)	1(33.3%)	3(100%)
Verruca Plana	1(50.0%)	0(0.0%)	1(50.0%)	2(100%)
Filiform	1(100.0%)	0(0.0%)	0(0.0%)	1(100%)

FIGURE LEGENDS

FIGURE 1- (a) Patient 1 with multiple verruca vulgaris and (b) showing complete clearance after Intralesional Vitamin-D3.

FIGURE 2- (a) Patient 2 with multiple plantar warts before treatment and (b) showing complete clearance after Intralesional Vitamin-D3.

FIGURE 3- (a) Patient 3 with multiple verruca vulgaris pre autoinoculation and (b) complete clearance post auto inoculation.

FIGURE 4- (a) Patient 4 with multiple verruca vulgaris pre autoinoculation and (b) complete clearance post auto inoculation.

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Figure-1

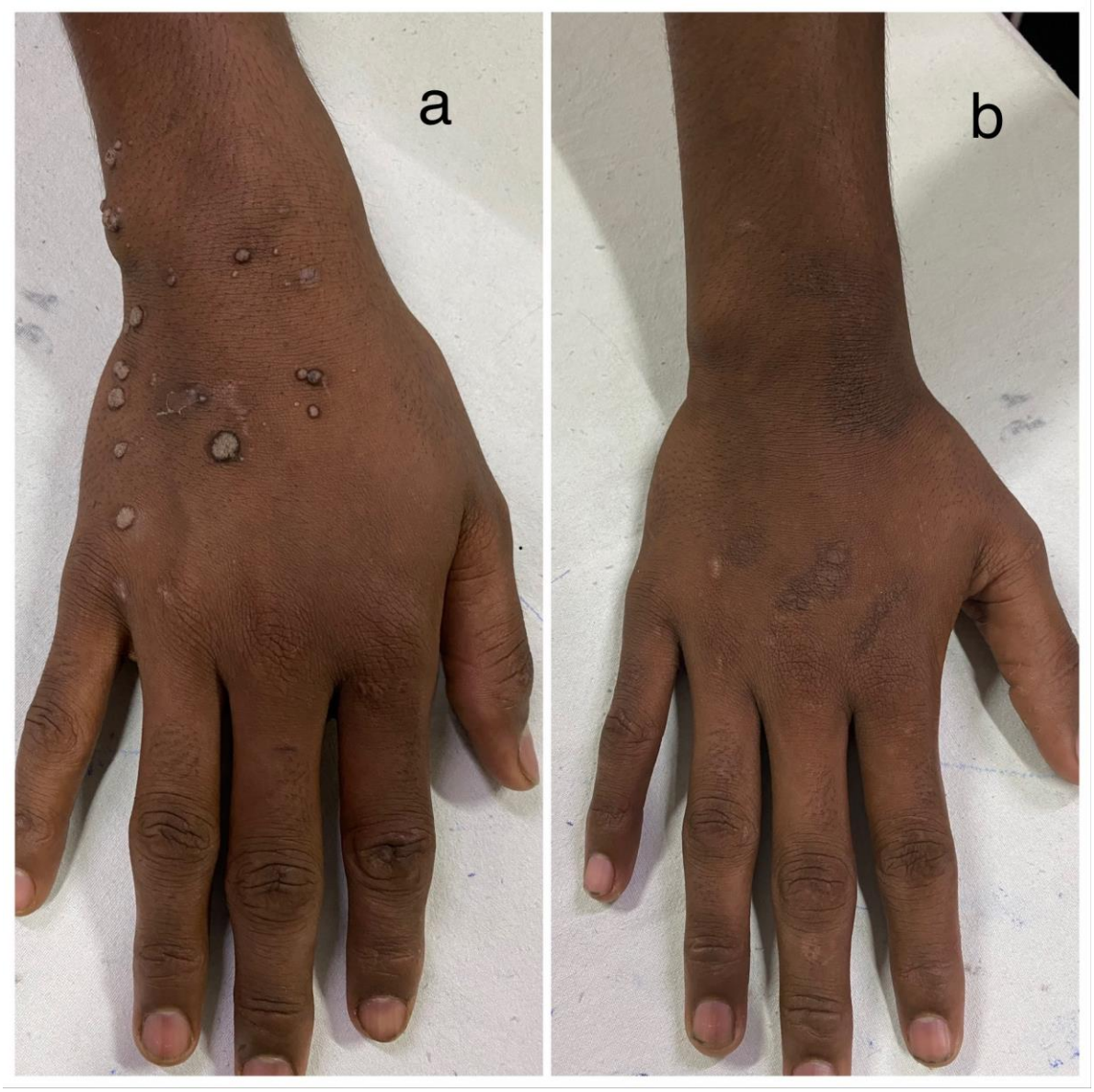

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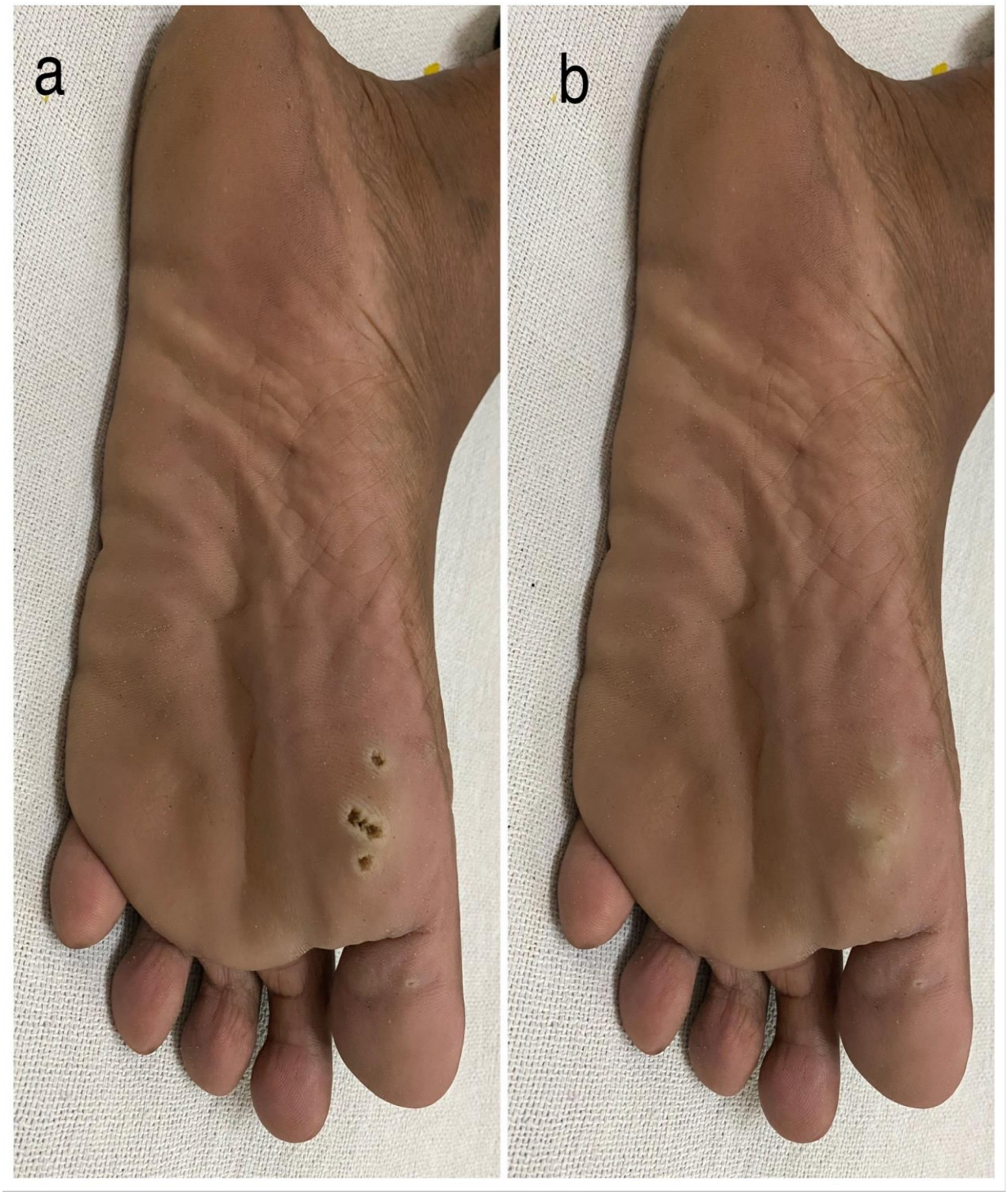
Figure-2



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Figyure-3

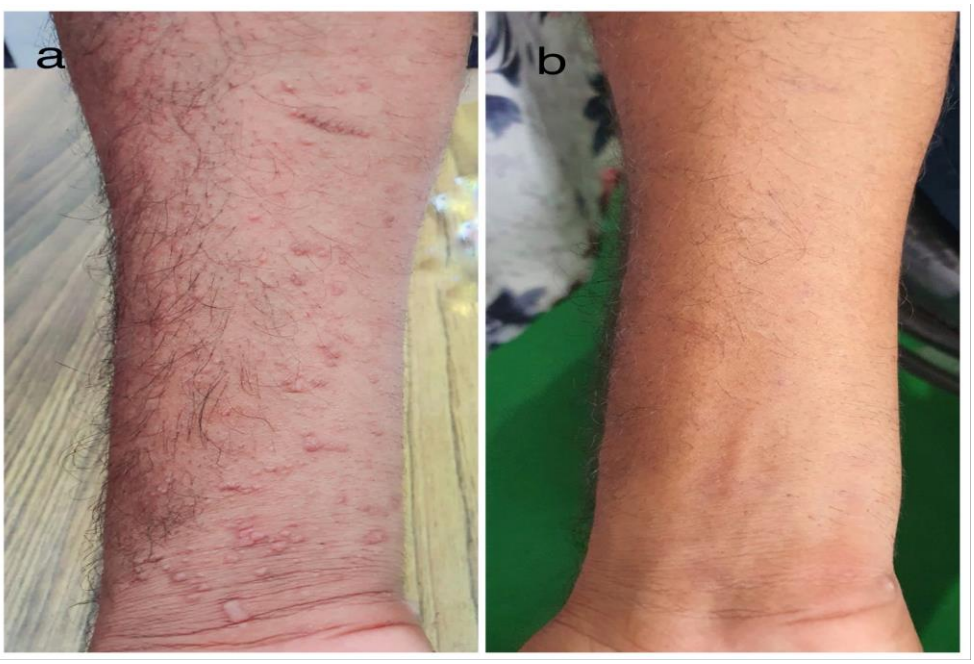

Figure-4