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Glycerol 85% efficacy on atopic skin and its microbiome: A Randomized Controlled Trial with Clinical and Bacteriological Evaluation.

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Glycerol 85% efficacy on atopic skin and its microbiome: A Randomized Controlled Trial with Clinical and Bacteriological Evaluation.

Background: Treating atopic dermatitis (AD) is still a challenge. The staphylococcal skin load is known to aggravate AD. Narrow band ultraviolet B (NB-UVB) and glycerol in low concentration (20-40%) are established therapies for AD. NB-UVB has proven antimicrobial actions, while high concentration glycerol (85-100%) showed similar effects in vitro but hasn't been clinically tested.

Objective: To evaluate the efficacy and tolerability of concentrated glycerol 85% compared to NB-UVB in patients with AD, as assessed by clinical improvement and reduction of staphylococcal colonization of the skin.

Methods: 30 patients with mild to moderate AD were randomized into either nb-uvb or glycerol 85% group. Patients were treated for one month and followed for an additional month. Swabs were taken from the skin and nose to be cultured on mannitol-salt agar for Staphylococci and quantified to determine Colony Forming Units (CFU).

Results: Both groups showed statistically insignificant microbial changes and statistically significant clinical improvement after treatment. The results were comparable between both groups.

Conclusions: Concentrated glycerol 85% is a cheap effective readily accessible alternative for phototherapy in patients with mild-moderate AD who cannot access the facility. Reduction of staphylococcal skin load seems to be involved, but its role is minimal.

Trial registration: Pan African Clinical Trial Registry (www.pactr.org) PACTR201810815694251

Keywords: Atopic dermatitis, glycerol, staphylococcus, ultraviolet therapy, phototherapy, microbiome.

Abbreviations

AD: Atopic dermatitis

BL: Baseline

CFU: Colony forming unit

CONS: Coagulase negative staphylococci

EOT: End-of-therapy

NB-UVB: Narrow-band ultraviolet light

OPC: Out-patient clinic

PACTR: Pan African Clinical Trial Registry

RCT: Randomized controlled trial

S.aureus: Staphylococcus aureus

SCORAD: Scoring atopic dermatitis index

UV: Ultraviolet

Introduction:

Atopic dermatitis (AD) is characterized by intense pruritis that leads to considerable burden on patients' quality of life [1]. The dramatic rise of AD prevalence is well documented [2,3], with prevalence reaching more than 20% of children and 5% of adults in industrialized societies [4,5]. This epidemic was explained by multiple hypotheses such as the hygiene hypothesis, which posits that the more sterile environment of urban settings the more skin microbiota dysbiosis (cutaneous flora imbalance), allowing abundance of pathogenic strains that disrupt the skin barrier and promote allergen sensitization and inflammation [6], partly due to declining exposure to UV radiation in urban settings [7].

Cutaneous flora imbalance in AD is well established. In particular, Staphylococcus aureus (S.aureus) colonisation is found in the vast majority of AD patients, especially in the sweat gland ducts [8]. Some studies found prevalence exceeding 90% of AD patients, while most healthy people do not harbour S.aureus on their skin [9]. After antimicrobial therapies targeting reduction of S.aureus colonisation, clinical symptoms significantly decline along with resettling of diverse microflora [6]. However, the propensity of S.aureus to form protective biofilms and the rapid emergence of antibiotic resistance prompt the search for other antimicrobial agents with less risk of resistance such as phototherapy and non-antibiotic antimicrobial agents [8,10].

Narrow Band ultraviolet B (NB-UVB) is an established treatment for AD [11], that acts as an immunomodulator in such a way that AD pathogenetic loops are suppressed while epidermal antimicrobial functions are enhanced [12,13]. Moreover, it has been shown to suppress superantigen and toxin expression by S.aureus [14]. In vitro and in vivo studies revealed direct UV-induced suppression of S.aureus colony growth and toxin production in a dose dependent manner that paralleled the clinical decline in symptoms and severity [15–18].

Glycerol is a humectant commonly used in treatment of AD in low concentrations, usually 20 to 40% [19], owing to its multiple beneficial effects on the skin including stratum corneum hydration, skin barrier repair, anti-irritant effect [20]. It also has proven in vitro antibacterial action in higher concentrations (85-100 %) [21,22]. No in vivo studies to date have investigated the effect of glycerol on S.aureus colonization in AD.

Materials and methods

Setting and design:

This study was designed as a randomized, controlled, parallel-group, single-blinded clinical trial with two interventional arms. A control non-interventional group was also included, with apparently healthy individuals, for interpretation of laboratory data when no standard values were defined (the microbial load). The study was performed at the dermatology outpatient clinic (OPC) of Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University. An initial pilot phase: glycerol 85% was first tested for its tolerability on normal skin of the authors, then on the lesional skin of two adult patients with AD.

The study protocol was approved by the ethical committee of the Faculty of Medicine, Cairo university. This study report follows the CONSORT guidelines for reporting randomized controlled clinical trials [23].

Participants:

Patients of both genders were eligible for the study enrollment if they were diagnosed with mild to moderate AD, defined according to Hanifin and Rajka criteria [24] by the presence of 3 major and 3 minor criteria, and by scoring less than 50 points in SCORAD [25,26]. Patients 6 years old and above were included (younger children are not allowed to be treated by whole cabinet NB-UVB per Kasr-Al-Ainy phototherapy unit regulations).

Exclusion criteria included: i) Severe AD: SCORAD>50 [26], including erythrodermic patients. ii) Patients who cannot attend regular sessions of phototherapy due to time or distance restraints.

Included patients were allowed a clearance phase of one month for systemic therapy and 2 weeks for topical treatment. Patients were randomized into one of two interventional arms (A or B) based on a computer-generated list in blocks of 5 [27]. Patients were treated for four weeks (end-of therapy, EOT), and followed for additional four weeks (End-of-study, EOS) for monitoring of relapses.

15 apparently healthy volunteers were included for comparative assessment of normal microbial skin load.

Interventions:

(1) Group A: Glycerol group (15 patients). Patients applied concentrated glycerol daily on body parts affected with AD lesions for 4 weeks (EOT). An extemporaneous preparation of 85% glycerol, free of additives and preservatives, was provided in 100ml rations.

(2) Group B: Narrow band ultraviolet B (NB-UVB) group (15 patients). Patients received three sessions weekly of NB-UVB till EOT, defined as 12 completed sessions.

Patients' assessment and outcomes definition:

An informed written consent for participation and photography was signed by all included patients or by guardians of those younger than 21 years. All patients were subjected to thorough history taking and examination to confirm the diagnosis and exclude masquerading diagnoses. Photographs of the whole body were taken at day 0 and EOT.

All patients were supplied with rations of pure petroleum jelly, that they applied once at bedtime and following baths to all dry skin. Patients in group B were instructed to clean it

off their skin with a moist towel or a bath prior to their sessions. Patients were advised not to use any other topical or systemic treatments.

Skin swabs were taken from normal controls skin as well as from patients at baseline (lesional and non-lesional skin) then at EOT (from same sites). Nasal swabs were taken from patients at baseline and normal controls, as the nose is a common reservoir of *S.aureus* [28]. Swabs were processed for assessment of skin load of s.aureus and coagulase negative staphylococci (CONS).

For determination of clinical efficacy, the SCORAD score was calculated at baseline (BL) and EOT by one non-blinded and two blinded investigators and the mean was calculated.

The percent change from baseline was determined by the following formula: SCORAD BL − SCORAD EOT SCORAD BL x 100%= % change from baseline. Accordingly, improvement was rated on a 4-point scale as excellent (>75%), good (50-75%), fair (25-50%) and failure of treatment (<25%). Pruritus severity was assessed at day 0 and EOT by the patients on a visual analogue scale of 0 to 10.

Patients were followed up for side effects and flares. Patients were excluded from the study if they developed phototoxic reactions to NB-UVB, irritant contact dermatitis to glycerol or uncontrollable flare of AD. Other adverse events and skin infections were monitored and recorded.

Primary outcomes were defined as: i) clinical effectiveness as assessed by reduction of SCORAD and/or of pruritus by at least 50% at EOT (SCORAD50 and ITCH50 respectively); ii) reduction of staphylococcal skin load of treated patients at EOT, as assessed quantitatively by log Colony Forming Units (CFU); iii) impact of staphylococcal colonization and initial severity on treatment response.

Secondary outcomes were defined as reduction of flares of the disease that need other topical or systemic therapy during follow-up period.

Laboratory methods:

The location of non-lesional skin chosen for swabbing was always an unaffected armpit. The lesion chosen was always the itchiest one determined by the patient. At EOT the swabs were taken from the same sites as BL. The wet swab method of harvesting bacteria from skin and nose was used [29] with some modifications. Mannitol-Salt Agar (Oxoid LLC, UK) was used as a selective culture medium. The plates were incubated at 37°C for 24 hours aerobically. After incubation, S. aureus and CONS colonies were identified and enumerated manually to give a total viable count expressed as colony forming units per cm^2 skin (CFU/ cm^2) according to the formula: *CFU/cm^2 = number of colonies X 1/dilution*.

Colony forming units per cm^2 of skin sampled were then transformed into log^{10} values according to the following formula, to account for zero values and normalize the data [30,31]: *Log transformed CFU/cm^2 = log (CFU/ cm^2 + 1).*

Statistical methods:

Sample size was calculated using G*Power 3.1.9.2 statistical software version 2.7.2 for MS Windows. Based on reported data about NB-UVB effect on SCORAD at 12 weeks [32] and data reported about the effect of glycerol on SCORAD at 2 weeks [33], the effect size was considered to be extremely large using Cohen's criteria [34]. With a two tailed alpha error probability set at 0.05, the projected sample size needed with the more conservative effect size (6.53) is approximately N = 6 in each group. 15 patients per cohort were recruited to additionally allow for subgroup analysis of microbiological outcomes.

Data were statistically described in terms of mean ± standard deviation or frequencies (counts) and percentages when appropriate. Comparison of numerical variables within each group was done using Wilcoxon signed-rank test, and comparison between the study groups

was done using Mann Whitney *U* test . For comparing categorical variables, McNemar's test was performed on paired data and Fisher Exact test on independent samples. Two-sided *p* values less than 0.05 was considered statistically significant. Data for analysis of treatment success were analyzed on intention-to-treat basis.

Results

Figure 1 demonstrates the patients' flow diagram. 18 males (40%) and 27 females (60%) with mild-moderate AD were recruited and randomized to both interventional arms. Their ages ranged from 6 to 37 years with a mean age 12.1±7.3 years. Only 25/30 patients completed the study.

Baseline characteristics of total group of patients (n=30) compared to healthy controls (n=15) showed that patients and controls were matching homogenously as regards age and gender. However, patients' cutaneous carriage of S.aureus was markedly higher (p=0. 002), with most of that difference attributable to S.aureus on AD patients' lesions, as their healthy skin counts were similar to those of healthy controls. On the other hand, the healthy skin counts of CONS were significantly higher in patients than controls (p=0.001). The odds ratio of skin S.aureus colonisation in cases vs controls was 11.23 (95% CI: 2.13 to 59.26, p= 0.004), denoting marked prevalence of S.aureus skin carriage in AD patients compared to normal population. In the patients group (n=30), the proportion of *S.aureus* in lesional skin was prevalent, while in non-lesional skin *CONS* was prevalent.

As shown in **table 1**, comparative baseline characteristics of patients in both interventional groups were homogenous as regards clinical and laboratory parameters.

Longitudinal analysis (before-after therapy comparison) showed that both glycerol and NB-UVB achieved a significant reduction of clinical scores (SCORAD and itch score) after 4 weeks in comparison to baseline (p<0.001 and p=0.003 respectively). On the other

hand, both lesional and non-lesional S. aureus log CFU/cm^2 did not show significant changes after therapy in any group, neither did lesional and non-lesional CONS count.

Comparison of both patients group at EOT (**table 2**) revealed that both reached a statistically comparable reduction of SCORAD as well as itch scores after 4 weeks. Clinical effectiveness as defined by achievement of SCORAD50 and ITCH50 at the EOT was comparable between both groups **(table 3**). Reduction of S.aureus skin load at EOT was comparably achieved by both therapies, both in lesional and non-lesional skin, as determined by the change in log CFU/cm^2 (**table 2**). Interestingly, while both therapies achieved skin S.aureus eradication in average 40% of colonized patients (p=1), incidental colonization was noted in 1/5 (20%) of the non-colonized patients after glycerol therapy, and in 4/6 (66%) patients after NBUVB therapy (p=0.206).

While lesional CONS load showed a small comparable tendency to increase in both groups, non-lesional CONS load showed a comparable tendency to decrease in both groups at the EOT (**table 2**).

Achievement of treatment success clinically did not significantly depend on the severity of initial presentation (**table 3**), neither did it depend on S.aureus eradication in any interventional group (**table 4**). However, a significant positive correlation was found between the SCORAD index and both lesional and non-lesional S.aureus count (log CFU/cm2) after NBUVB, only including colonised cases [(p= 0.024, rho= 0.857) and (p <0.001, rho=1) respectively]. This clinical-microbial correlation was not mirrored in the glycerol group.

Grading of objective clinical response, as measured by SCORAD, into excellent, good, fair and no response showed both groups achieving comparable results (p=0.426).

Assessment of secondary outcomes revealed that in the 4 weeks after cessation of therapy until EOS, the majority of both groups' patients required systemic or topical

treatment for a flare or exacerbation (66.7% of glycerol group and 53.8% of NB-UVB group, p=0.688).

Few minor adverse events were recorded in both groups: dryness, recurrent abscesses, flare, folliculitis, mild irritation. Noteworthy in the glycerol group, 2 cases with features of hand eczema developed severe hand irritation, one of whom the extent of irritation required discontinuing treatment. In the NB-UVB group, one patient discontinued sessions due to severe phototoxic reaction at the minimal erythema dose.

Discussion

The recent era of skin microbiome research has revolutionized the therapy and understanding of inflammatory skin diseases [35]. Skin microbiota are now recognized as a treatment-specific biomarker in AD, where staphylococcal abundance is consistently documented [36], with proved temporal relationship between S.aureus density and clinical severity of AD [30]. Replicating previous reports [37–39], the baseline S.aureus lesional skin load in our patients group was indeed higher than normal controls, suggesting the need for anti-microbial therapy.

In response to previous in-vitro reports about the anti-microbial effects of glycerol in high concentrations [21,22], the current study aimed to test for the in-vivo anti-microbial effect of concentrated glycerol 85% along its clinical efficacy in AD, in comparison to a well-documented line of therapy in AD with an antimicrobial efficacy, namely NB-UVB [15–18].

Our results show that both therapies are comparable as regards clinical and microbiological efficacy in mild-moderate AD after four weeks of treatment. The clinical effectiveness was demonstrated by comparable treatment success, as defined by a 50% reduction of SCORAD and itch scores at the EOT. The microbiological effectiveness was demonstrated by the comparable percent reduction of lesional and non-lesional skin S.aureus colony counts in both groups at the EOT. The number of patients showing successful skin S.aureus eradication was also comparable in both groups after therapy.

Both glycerol 85% and NB-UVB seem to have modest effect on Staphylococcal skin colonization as shown by the small percent reduction of log CFU/cm^2 in each group, and by the non-significant change after therapy in comparison to baseline. Unlike NB-UVB where the clinical improvement (SCORAD) correlated well with the reduction of S.aureus skin load (both lesional and non-lesional), glycerol clinical effectiveness did not correlate significantly with its anti-microbial action, so other mechanisms in AD may be implicated.

Few randomized trials assessed the clinical efficacy of glycerol for AD, despite its use being widely supported by guidelines [40]. Although matching with the results of a previous 3-week study of 20% glycerol in adults with AD [41], the 48% reduction of SCORAD achieved by concentrated glycerol in our study is lower than most previous reports of 15-20% glycerol application which showed an average 60% reduction of variable clinical scores of AD [42–44]. This could be easily attributed to the drying effect of concentrated glycerol, an effect that we tried to palliate by emollient application to patients in both study arms.

To our knowledge, no previous studies assessed the in vivo effect of high concentration glycerol on cutaneous microbiota. However, a few trials assessed the role of emollients in general in restoring microbial harmony with conflicting results [44–47]. None of the studies reported a correlation between change in bacterial counts after decrease in disease severity in response to emollients. Unfortunately, marked heterogeneity in the studies made inference of conclusions difficult.

Four previous studies reported the effect of phototherapy on cutaneous microbiota of AD patients with variable results [15–18]. None of the studies reported a correlation between decrease in bacterial counts after phototherapy and decrease in disease severity. Dotterud and coworkers [15] reported similar results to our study, with a non significant decrease in lesional S.aureus counts after 4 weeks of UVB treatment, which were followed by an increase after 2 weeks follow up. It is known that UVB radiation induces pyrimidine dimers on the DNA chain and that such damage may be involved in the reduction of proliferating microorganisms as well as reduction of their protein synthesis needed for superantigen production and biofilm formation [17]. Upregulation of keratinocyte synthesis of antimicrobial peptides is also involved [12,48], although a dose-dependent suppressive effect was demonstrated in vitro proving a direct mechanism [14,18,49].

Interestingly, the commensal CONS, the most important species of which is S.epidermidis, increased in lesions of both groups -albeit non-significant statistically- after treatment. This finding suggests that under therapy, normal commensals amensalistically antagonize the effect of S.aureus or displace its overgrowth, an observation supported by previous reports in AD [50–52]. Amensalism is defined as an association between organisms of two different species in which one is inhibited or destroyed while the other remains unaffected [50].

To the best of our knowledge, this is the first report comparing concentrated glycerol to NB-UVB in AD management through objective clinical and microbiological outcomes. We demonstrate that the efficacy of 85% glycerol is comparable to NB-UVB in the treatment of mild-moderate AD for 4 weeks as regards SCORAD and itch reduction, while demonstrating adequate safety. Therefore, glycerol is a viable cheap, safe and accessible alternative to NBUVB especially in mild cases of AD. However, high concentrations of glycerol should not be applied to eroded skin. Continued application of glycerol may be needed to maintain efficacy.

Both glycerol 85% and NB-UVB have modest effect on *Staphylococcal* skin colonization. Unlike NB-UVB, glycerol doesn't significantly affect bacterial counts so other mechanisms of action may be implicated.

This study was limited by its short duration (4 weeks may have not been sufficient for all cases to reach success), and the use of conventional selective culturing and colony counting methods (limiting the panel of species examined and the accuracy of quantification).

Further research focusing on molecular analysis of dysbiosis in AD patients and its response to different concentrations of glycerol is warranted. The combined effect of NB-UVB and glycerol is particularly appealing since glycerol is a known optical clearing agent that purportedly increases UVB penetration and effects at lower radiation doses, making them

a potentially synergistic pair [53–55]. Microbiological assessments of *S.aureus* colonisation of phototherapy unit surfaces, the risk of patient contamination and the efficacy of sterilization measures are to be taken into consideration.

Compliance with ethical standards

Ethical approval status: reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Cairo University. Approval number: N-54-2018. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983.

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Tables legend

Table 1: Baseline characteristics of both glycerol and NB-UVB groups.

Table 2: Comparative results of glycerol and NB-UVB groups after 4 weeks (end-of-therapy).

Table 3: Treatment success outcomes according to initial severity.

Table 4: Treatment success outcomes according to Staphylococcus aureus eradication.

Figures Legend

Figure 1: Patients' flow diagram.

Table 1: Baseline characteristics of both glycerol and NB-UVB groups.

	Glycerol group (n=15)	**NB-UVB group (n=15)**	***P* value**
Age (years) **Mean ± SD**	9.9±4.1	13.7±8.7	0.226
Gender **Females** **Males**	 7 (46.7%) 8 (53.3%)	 11 (73.3%) 4 (26.7%)	 0.264
SCORAD **Mean ± SD**	34.42±10.95	37.24±9.06	0. 595
Itch score **Mean ± SD**	6.7±2.7	7±1.9	0. 883
Skin *S.aureus* colonisation **no** **yes**	 5 (33.3%) 10 (66.6%)	 6 (40%) 9 (60%)	 1
Nasal *S.aureus* colonisation **no** **yes**	 8 (53.3%) 7 (46.7%)	 5 (33.3%) 10 (66.7%)	 0. 461
Lesional skin *S.aureus* colonisation **no** **yes**	 6 (40%) 9 (60%)	 6 (40%) 9 (60%)	 1
Non-lesional skin *S.aureus* colonisation **no** **yes**	 10 (66.7%) 5 (33.33%)	 12 (80%) 3 (20%)	 0. 682
Lesional *S.aureus* (log CFU/cm^2)	1.6±1.6	1.6±1.6	1
Non-lesional *S.aureus* (log CFU/cm^2)	0.4±0.7	0.4±0.8	0.632

CFU: Colony forming units, S.aureus: staphylococcus aureus. *P* value < 0.05 is significant.

Table 2: Comparative results of glycerol and NB-UVB groups after 4 weeks (end-of-therapy).

	Glycerol group (n=12)	**NB-UVB Group (n=13)**	***P* value**
% Change in SCORAD: Mean ± SD (95% CI)	-48.6	-50.8	0. 479
% Change in itch score: Mean ± SD (95% CI)	-53.6	-55.7	0. 934
Change in non-lesional *S.aureus* (log CFU/cm^2) Mean ± SD (95% CI)	-0.14±0.64 (-1.49 to 1.04)	-0.01±0.97 (-1.77 to 1.18)	0. 623
Change in lesional *S.aureus* (log CFU/cm^2) Mean ± SD (95% CI)	-0.50±2.12 (-3.85 to 2.30)	-0.03±2.15 (-1.79 to 1.79)	0. 564
Change in non-lesional CONS (log CFU/cm^2) Mean ± SD (95% CI)	-0.02±1.34 (-1.20 to 1.09)	-0.14±1.22 (-1.33 to 1.00)	0.935
Change in lesional CONS (log CFU/cm^2) Mean ± SD (95% CI)	+0.55±1.39 (-0.33 to 1.43)	+0.53±1.70 (-2.17 to 4.15)	0.971
Treatment success (intention to treat analysis)	**Glycerol group (n=15)**	**NBUVB group (n=15)**	***P* value**
SCORAD50			
Success	4 (26%)	7 (46.7%)	0.449
Failure	11 (73.3%)	8 (53.3%)	
ITCH50			
Success	7 (46.7%)	8 (53.3%)	1
Failure	8 (53.3%)	7 (46.7%)	

CFU: Colony forming units, CONS=coagulase negative Staphylococci, SCORAD: score for severity of atopic dermatitis, S.aureus: Staph aureus.

Table 3: Treatment success outcomes according to initial severity.

	Glycerol group (n=15)	***P* value**	**NBUVB group (n=15)**	***P* value**
Baseline SCORAD Mean±SD				
SCORAD50 success	25.1±12.1	0.104	7 (46.7%)	0.382
SCORAD50 failure	39.5±9.2		8 (53.3%)	
Baseline itch score Mean±SD				
ITCH50 success	35.5±8.1	0.694	37.0±11.3	0.673
ITCH50 failure	6.75±2.12		7.20±1.79	

Based on intention-to-treat analysis

Table 4: Treatment success outcomes according to Staphylococcus aureus eradication.

(n=25)	SCORAD50		ITCH50		Needed systemic or topical treatment in follow-up until EOS	
	no	yes	no	yes	no	yes
No eradication	10 (71.4%)	7 (63.6%)	6 (60%)	11 (73.3%)	8 (47.1%)	9 (52.9%)
Eradication	4 (28.6%)	4 (36.4%)	4 (40%)	4 (26.7%)	2 (25%)	6 (75%)
P value	1		0.667		0.402