A Biometric Assessment of a Combined Topical Levofloxacin, Retinol, Cloxacillin and Ascorbic acid Against Facial Acne

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Abstract

Acne vulgaris is a common pilosebaceous lesion affects skin over the face and upper chest. It has about 14 million incidence rate that cost 100$ for each case. In this study, biometric outcomes of the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle on different types of acne lesions in human were investigated. Twenty one person were included in two groups; test (N =10) and control (N = 11) with follow up of objective software based biometric analysis parameters including: keratolysis induction, redness reduction and healing of infected lesion. The test formula revealed a significant keratolysis induction as compared with control. Eight out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group, P-Value = 0.005. Similar effects were obtained in redness reduction (redness reduction ratio induced with test formula = 2.5 with confidence interval CI over 0.95) and impetiginization healing at $P < 0.05$. From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions.

Key words: *Facial acne vulgaris, RGB image processing, keratolysis, impetiginization*

Introduction

Acne vulgaris is a common dermatological lesion characterized by progressive popular to nodular skin lesion over the face and sometimes upper chest, back and shoulders [(1,2)]. Studies estimated 14 million presentations to the clinic suffering from acne vulgaris with 85% of cases were between 15-17 years of age[(3-5)] . It is presented in different forms: close, open, and black and white. It affects both sexes with average age incidence[(6)]. Acne vulgaris lesions cost 100$ for each case in average[(7)].

In Iraq and nearby countries prevalence of acne was 13.1% among skin diseases[(8,9)]. Acne vulgaris has multifactorioal causation. Androgen hyperactivity especially dihydrotestesterone[(10,11)] expression of binding factor (binding protein II and proline rich protein I) to Propionibacterium acne, excess inflammatory response to P. acne [(12)]and microcomidos formation with closure of sebaceous duct and accumulation of sebum [(13,14)]. Different cytokines and chemotaxis factors are noticed in excessive amount in acne lesion like IL1, IL12, IL8 and prostaglandins [(15)].Of the most common complications that are associated with this dermatological disease, facial and neck impetiginization and scar.

Factors that predispose to acne complications include hormonal hypersensitivity, hormonal imbalance, bacterial infections, age, weight, cosmetics and skin histopathological typing [(16,17)]. The most commonly used antiacne treatments include keratolytics, antibacterial and peeling agents [(18-20)]. However, a fraction of acne lesions are refractory to treatment that mandate more therapeutic researches.

The objective of this study is that to assess biometric outcomes of the combined topical formula of;

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levofloxacin, disintegrating agent, retinol, hydrocarbon mineral carboxylate, cloxacillin, pH buffering agent, ascorbic acid and vehicle base on different types of acne lesions in human.

Samples, Materials and Method

Study design

Controlled clinical trials of acne vulgaris was designed to exclude:

- Age: 25yr < age < 13yr
- Medications and antiacne intake
- Chronic medical illnesses like HT,DM and hormonal disturbances
- Weight: 70 kg < wt < 50 kg

Prerequisites of medical ethics (according to Geneva and Helsinki declarations) submitted to local ethical committee.

- All of the used drugs and excipients are FDA approved for safety and efficacy
- All of the used drugs were passing phase I (i.e. not used as a first time)
- Benefit is prevailed
- All individuals were informed about study design and expected side effects
- All individuals permission, autonomy and consents have been obtained
- All of the used drugs were through topical route of administration
- All individuals were prior tested for any skin hypersensitivity and side effects.
- All individuals names, private secrets, faces were respected and insured
- All rights were reserved.
- All were free to be subjected to medical treatment or test treatment.

Material

1) **Hydrocarbon cream o/w**

Of multiple esters; Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.05 ml

2) **Active ingredient and vehicle base:**

- Retinol (Egypt, product date and expiry date; PD-ED: 2013-2015)

Package of 200000 IU. The used dose in designing the topical test formula against acne was 0.05%.

- Cloxacillin (Ajanta, India, PD-ED: 2014-2015). The used dose was o.1%
- Levofloxacin (Pharma International, Jordan; PD-ED: 2014-2015). The used dose was 0.1%.
- Ascorbic acid (Merck, PD-ED: 2013-2015). The used dose was 0.1%
- Hydrocarbon base was used as a vehicle of Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.0 mL.

Constituents of the combined antiacne formula;

(Levofloxacin, disintegrating agent, retinol, hydrocarbon mineral acyls, cloxacillin, pH buffering agent, ascorbic acid, vehicle base)

Constituents of the blank controlled formula;

(Hydrocarbon base+ mineral oils+ pH buffer, disintegrating agent)

Pharmaceutical analysis is done to assess formula pH and physical consistency was assessed with PHELECT computerized pH meter electrode (USA). pH was buffered around 6.3.

Methods

Acne sample selection

The lesion to be monitored with biometric method was not randomly selected

Criteria of selection

- More prominent lesion in the face is selected to be monitored.
- Type of lesion was randomized (to include all presenting lesion types for further analysis and concluding the overall effect on all types)

- Monitoring was done in conserved temperature but different ambient lighting because this was processed and normalized by software.

- Selection of blank treated and test treated was randomized

- There was no person with any application of either test or blank to insure ethical requisites. On the other hand, no person was with standard medical treatment.

Methods of observation and analysis

Places of application were therapeutic research lab/ Kufa College of medicine for assessment of students.

Private lab for assessment of the volunteers out of the college as well.

Ambient conditioning

Lighting was objectively evaluated to be controlled

Ambient temperature has averaged 22-25 ºC

A prior imaging of the lesion was taken then after 4 day of the treatment another image was acquired for MIP.

MIP was a mathwork 2013a image processing blockset design by Dr Hussein AbdulKadhim for processing and analyzing RGB and lesion pattern for:

1- RGB shift

2- Lesion dimension

3- Pattern of lesion response (keratolysis, impetiginization, dimentions and bevel).

Figure 1: Mathwork 2013a image processing and analysis used for analysis of the outcomes by the combined Microsoft thermograph camera.

Treatment Mode

The entire face was messaged with the test combination and left overnight then morning washing. Another application was done for 1hr duration to be washed prior to attending time.

Biometric Analysis Method

The biometric set composed of a Microsoft combined thermographic tissue camera with MIP for objective image analysis.

Analyzing histogram vector RGB and lesion in form

of red pixelate shift was objectively monitored and data were analyzed statistically with spline interpolation and risk reduction at 0.95 C.I. Discrete data was analyzed with chi square test at $P < 0.05$. Statistical software packages were Matlab 2013 statistical toolbox and Minitab 2014 statistics.

Results

1- **Findings and analysis of keratolytic activity of the test combined topical formula.**

Chi-Square Test: test; control

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	test	control	Total
1	8	2	10
	4.76	5.24	
	2.202	2.002	
2	2	9	11
	5.24	5.76	
	2.002	1.820	
Total	10	11	21

Chi-Sq = 8.025; DF = 1; P-Value = 0.005
1 cells with expected counts less than 5.

Figure 2: Number of individuals who showed keratolysis in response to the applied test formula as compared to those used a blank cream base after 14 days of treatment. So that 8 out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group. P-Value = 0.005.

2- **RGB analysis findings for acne lesions**

Color model interpolation and the mean red value estimation with redness reduction ratio.

In control group:

MIP red value was reduced from 240-200 = 40

In test group:

MIP red value was reduced from 250-150 = 100

So the redness reduction ratio of test formula = 2.5 at C.I. 0.95.

Figure 3: Mean values of red bins by which the MIP had shifted after 14 days of treatment. This was a direct indicator of acne reactivity in response to treatment.

3- Lesion impetiginization response

Chi-Square Test: test; control

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	test	control	Total
1	1	5	6
	3.25	2.75	
	1.558	1.841	
2	12	6	18
	9.75	8.25	
	0.519	0.614	
Total	13	11	24

Chi-Sq = 4.531; DF = 1; P-Value = 0.033
2 cells with expected counts less than 5.

Discussion

A trial of assessment of topical antiacne formula has a significant consideration since it can be reasonably safe alternate to systemic administration of drugs for prolonged period.

The reason behind selecting combined active ingredients in designing the test formula was to induce synergism since P. acne, S. aureus and S. pyogen are rapidly emerging resistance against the commonly used antimicrobials[21,22] and to potentiate peeling with retinol and keratolysis with ascorbic acid[23,24]. This principle causes augmentation of antiacne effect.

However this study concerned a limited number of population and needs for further confirmation in larger samples. Overall clinical and biometric outcomes are best to be included in further studies.

The clinical evaluation of keratolytic activity of the combined formula showed highly significant induced keratolysis (Chi-Sq = 8.025; DF = 1; P-Value = 0.005) in comparison with blank treated group. That was a clinical sign of improvement since keratlysis can convert closed comedos to opened type. Moreover, keratolysis insure more antiacne drug absorption fraction since it causes thinning of the corneocytes portioning. Different studies showed the importance of the use of keratolytics in treatment of acne[25].

Redness is a major indicator of inflammation. It could be assessed objectively by RGB shift analysis. Redness reduction ratio was 2.5 at C.I. 0.95.

Test formula revealed a significant remission of impetiginization in comparison with blank treatment (Chi-Sq = 4.531; DF = 1; P-Value = 0.033).

That effect may be attributed to the synergistic activity of levofloxacin and cloxacillin. Some studies on assessment of comedolytic effects of ciprofloxacin and ampicillin revealed significant influence of these drugs on improving acne[26-29].

Conclusion

From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions. Larger sample size is necessary for further confirmation of the antiacne activity of the test formula. We recommend that other congeners of the used antimicrobials and keratolytics are to be included. And, for future studies, other comparative studies between antiacne drugs alone and in combination to determine the synergistic ratio.

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