

CLINICAL RESEARCH

## Red Blood Cell Forms in Acne and their Complex Treatment with Application of Light-Emitting Diode Influences

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### Abstract

The efficiency of the laser light-emitting diode (LED) that is used in the complex treatment of acne was studied with light microscopy, scanning electron microscopy, and planimetry. It was found that the light emission of the photon matrix by A.Korobov–B.Korobov “Barva-Fleks/BIR” ( $\lambda_1=470\text{nm}$  and  $\lambda_2=940\text{nm}$ ) combined with the drug treatment are an effective means for the treatment of acne.

**Keywords:** *acne, LEDs, morphology, red blood cells.*

### Introduction

Acne is one of the most common skin diseases, which affects up to 80% of people aged 12–25 years and 40% of those over 25 years [1,2]. The main feature of the pathology is that even after favorable recovery it leaves quite deep scars on the visible surface of skin, in particular on the face, which sometimes occur as keloid growths. For this reason, preventing skin from scarring is an important point in the treatment of acne.

At present, perhaps the majority of dermatological pathologies are either treated with one or another form of phototherapy or diagnosed with aid of photodiagnostics, which include laser and other types of light emission [1, 3-10]. The capacities of phototherapy hold particular interest in the treatment of acne. The use of laser therapy, particularly of He-Ne laser-HNL, in the treatment of acne has a history of more than a quarter of century. Recent years were marked by implementation of new methods of laser therapy, in combination with drugs and other methods of physiotherapy in a complex treatment of acne and prevention of scarring [1,3,6-14].

The laser light-emitting diode (LED) emission has also been used increasingly during recent years [9,11,12]. Dermatoses and infectious skin lesions are associated not

only with significant structural changes in the skin, but also with marked changes in the ratio of discocytes, normal red blood cells (RBCs), and their pathological forms as well. Such imbalance is notable in peripheral blood, obtained from fingers, and particularly, in blood obtained from the areas of skin lesion [14]. Shifts in the balance of discocytes and pathological forms of RBCs (PF-RBC), along with changes in the capillary wall, cause a marked disturbance of microcirculation [12,14].

However, the changes in the form of RBCs in acne, which represents one of the most common skin pathologies, especially during adolescence and young adulthood have never been studied, and LED emission has not yet been included in the diagnostic algorithm of acne. This omission has defined the purpose of the current study – to study the changes in the ratios of discocytes and PF-RBCs in acne and the influence of complex treatment with LED phototherapy.

### Material and Methods

The study included 84 patients aged from 18 to 30 years. The control group constituted 20 healthy, age-matched, randomly selected persons.

The combined effects of blue LED and infrared emissions of photon matrices Barwa-Fleks/BIR (Ukraine), along with traditional treatment of acne, were studied in the mode of closest approach to the surface of affected skin. The matrix was covered with a case of thin polyethylene to prevent contamination of the surfaces of LEDs. The radiation power

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density (determined by formula  $P_s = P/S$ ) comprised 1.25 J/cm<sup>2</sup> [3,8] The matrix contains 12 blue ( $\lambda=470$  nm) and 12 infrared ( $\lambda=940$  nm) light-diode emitters [12,14].

Medicamentous treatment includes antibiotics, vitamins, enterosorbents, and resorptional means. Duration of treatment was 14–17 days. Seventy-four patients received daily a 10-minute irradiation of acne zones with Barwa-Fleks/BIR (for 8–10 days) in a complex therapy with medicamentous treatment.

Blood of all patients, obtained from finger and areas with acne, was studied before and after treatment. Blood for light microscopy was studied by the “express –method of thick drop (EMTD). The technique of EMTD was developed in the laboratory of pathologic anatomy of RSCS named after acad. V. Vakhidov. It is patented in the patent offices of the Republic of Uzbekistan as “Method for determining the forms of RBC” № ICI 6 and 61 B 10/00, along with PC software created for this purpose – “Express diagnosis of RBC forms” № ED-5-05. To perform this technique, the pad of the ring finger or acne plaques is punctured with a scarificator and 2–3 drops of received blood are placed in 2ml fixating 2.5% solution of glutaraldehyde prepared on a phosphate buffer (pH 7.4). A drop of fixed, unstained blood is then placed on a slide and covered with a coverslip. This forms a layer of “thick film,” which can be studied under a light microscope. The proposed technique allows saving the natural state of RBC to a certain extent and having the RBC approximately the same as those in the vascular lumen. This, in turn, facilitates more adequate assessment of the functional morphology of RBC. This method is applicable to the qualitative study of RBC forms, as well as to the morphometric study of ratios of normal and pathological forms.

It should also be emphasized that using this method, the objective morphometric evaluation of deformability of RBCs can be obtained within 10–15 minutes with simple light microscopy. This makes it possible to monitor the structural and functional status of RBCs and other blood cells to assess the severity of a pathological process and the adequacy of the treatment. Previously conducted comparative studies of RBCs, counting peripheral blood specimens with SEM and EMTD, have shown reliability of the last [14].

Counting and determination of the ratio of RBCs was performed with magnification power 10x40 and with samples containing no less than 1000 RBC for each stage and series of study. The results were subsequently statistically analyzed. All values are presented as mean  $\pm$  standard error of the mean. The significance of differences between means was evaluated by a student t-test for unpaired data and by a two-way analysis of variance (ANOVA) followed by Duncan’s multiple range test.

The microscopes used for this purpose were Biolam-12 and Axioscop 40-ZEISS. Light-optical micrographs were obtained on Axioscop 40-ZEISS with an attached digital camera and saved and stored on a PC with Microsoft programs.

For scanning electron microscopy, tissues and RBCs were fixed in a 2.5% glutaraldehyde solution in a phosphate buffer (pH-7.4) and were post-fixed in osmium tetroxide in a phosphate buffer; samples were dehydrated in alcohol of ascending concentration and acetone, and dried by transition

through the critical point of nitrous oxide in the apparatus HCP-2; then they were gold-shadowed in “IB-2” and studied in SEM Hitachi-S405. Samples were then mounted on aluminum bases with electro conductive glue. Micrographs were obtained from the microscope screen with a Canon digital camera and further processed and stored with Microsoft application programs. Samples were then studied and photographed in SEM Hitachi S-405A with a Canon digital camera from the monitor screen of the microscope.

Morphometric counting of normal and PF-RBC ratios were carried out, using at least 1000 RBCs in each case.

RBCs of peripheral blood were studied with the aid of SEM and EMFD after medical treatment without ILIB application and with ILIB application. Blood was also taken from practically healthy volunteers aged from 20 to 30 years (n=8). Blood sampling was conducted in October-November and March-April in order to minimize the influences of too hot and too cold temperatures.

## Results and Discussion

Acne leads to a substantial increase in the proportion of PF-RBCs. The increase of PF-RBCs is notable in peripheral blood obtained from the finger and, particularly, in the blood taken from areas of acne. This leads to the conclusion that the share of PF-RBC accounts for nearly half of all RBCs (Fig. 1-3; 5-7; Table 1). Particularly, a significant increase occurs in the proportion of stomatocytes and RBCs with crest and this is more than three times higher than the control group. The total share of PF-RBC in the blood taken from areas of lesions comprises more than one-third of all RBCs.

**Table 1**

**Ratio of discocytes and pathological forms of RBC in acne before and after treatment with LED matrix Barva-Flex/BIR**

| Form of RBC        | Control Group (1) | Blood from finger before treatment (2) | Blood from acne zones before treatment (3) | Blood from acne zones, drug therapy alone (4) | Blood from acne zones, treatment with Barva-Flex/BIR (5) |
|--------------------|-------------------|--|--|---|--|
| Discocytes         | 89 $\pm$ 1.7%     | 65 $\pm$ 1.4%*                         | 54 $\pm$ 1.6%*                             | 70 $\pm$ 1.6%**                               | 83 $\pm$ 1.0%**  |
| Echinocytes        | 4 $\pm$ 0.6%      | 6 $\pm$ 1%*                            | 7 $\pm$ 1%*                                | 5 $\pm$ 1%                                    | 4 $\pm$ 2%**   |
| Stomatocytes       | 3 $\pm$ 0.04%     | 14 $\pm$ 0.2%*                         | 19 $\pm$ 0.9%*                             | 12 $\pm$ 0.2%                                 | 5 $\pm$ 0.1%*  |
| RBC with crest     | 3 $\pm$ 0.01%     | 13 $\pm$ 0.3%*                         | 18 $\pm$ 0.4%*                             | 12 $\pm$ 0.2%**                               | 6 $\pm$ 0.2%**   |
| Irreversible forms | 1 $\pm$ 0.07 %    | 2 $\pm$ 0.2%*                          | 2 $\pm$ 0.2%*                              | 1 $\pm$ 0.2%                                  | 2 $\pm$ 0.1%   |

\*  $P < 0.05$  vs control group ; \*\*  $P < 0.05$  vs (3)

Conventional drug therapy increases the share of discocytes and decreases the share of PF-RBC. Thereby, the overall share of stomatocytes and RBCs with crest decreases from one-third to one-fourth of all RBCs.

Complex therapy with drugs and Barva-Flex/BIR brings the ratio of discocytes and PF-RBCs to the normal level, where the share of discocytes reaches 83% (Fig.4,8-10; Table 1).

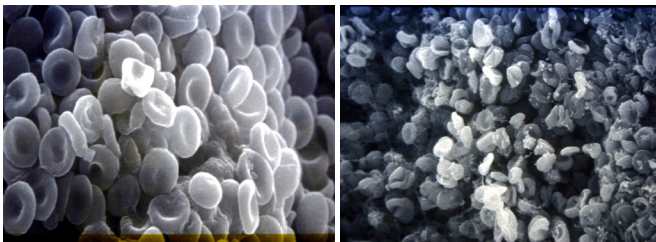
PF-RBCs, such as stomatocytes, echinocytes and RBCs

with crest is a normal finding in peripheral blood. However, their total share normally does not exceed 11%–12%.

RBC is one of the most differentiated cells in mammals and in the human body; it is also devoid of all organelles and nuclei. This determines their sensitivity to changes in the various blood parameters, which take place in various pathological conditions. Shifts in the proportion of discocytes, normal RBCs in the form of biconcave disks and pathological forms are determined primarily by changes in the properties of the plasma membrane [14]. Growth of pathological forms of RBCs disrupts microcirculation since they lose the ability to transform and subsequently restore initial shape – a feature necessary for passing narrow capillaries [14].

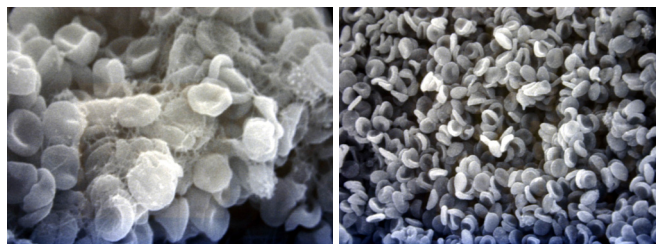
The identified shifts in the ratio discocytes and PF-RBCs in acne are especially marked, mainly in the blood drawn from the skin where acne is localized. The changes, occurring the same way in blood from a finger, confirm the systemic nature of the disturbance in acne, where RBCs have a leading role.

The application of LED matrix Barwa-Flex/BIR in the treatment of acne leads to a significant increase in the proportion of discocytes and a reduction of the share of PF-RBC. Such a trend is observed in the blood from acne areas as well as in peripheral blood from fingers (Fig.1; Table 1).



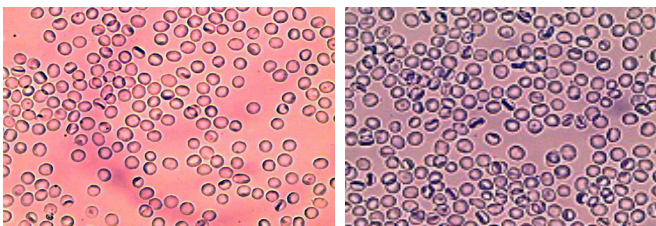
**Figure 1.** Erythrocytes in norm, domination of discocytes. SEM x 2000

**Figure 2.** Erythrocytes from zone of acne, domination of pathologic forms. SEMx 1000



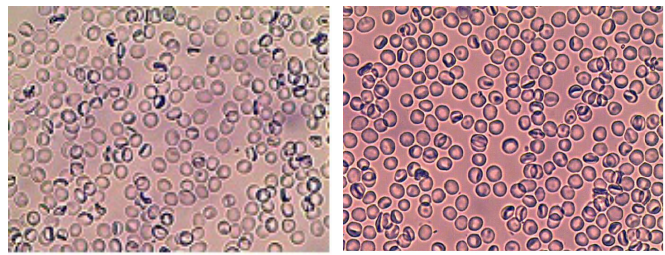
**Figure 3.** Erythrocytes from finger, domination of pathologic forms. SEMx2000

**Figure 4.** Erythrocytes from finger after complex treatment with "Barwa-Flex/BIR". Domination of discocytes. SEMx 1000



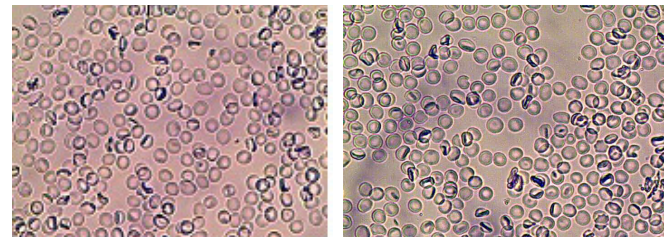
**Figure 5.** Blood from finger. Norm (control): domination of discocytes. EMTD 10x40.

**Figure 6.** Predominance of pathologic forms of erythrocyte. EMTD 10x40. Blood from finger.



**Figure 7.** Predominance of pathologic forms of erythrocyte. EMTD 10x40. Blood from finger.

**Figure 8.** Decrease of pathologic forms of erythrocytes after traditional treatment. Blood from zone of acne. EMTD 10x40.



**Figure 9.** Decrease of pathologic forms of erythrocytes after traditional treatment. Blood from finger. EMTD 10x40.

**Figure 10.** Considerable decrease of pathologic forms of erythrocytes after complex treatment with "Barwa-Flex/BIR". Blood from zone of acne. EMTD 10x40.

## Conclusion

The pathologic process in acne involves a shift in the ratios of discocytes and PF-RBCs, both in acne areas and in peripheral blood, which implies that acne is a systemic pathology of the organism, causing, apparently, impairment of microcirculation. LED phototherapy is an effective treatment for acne, stimulating restoration of ratios of RBCs.

Comparison of the findings of changes in the forms of RBCs, obtained by scanning electron microscopy and EMTD, demonstrates their comparability. EMTD can serve as a method for assessment of the severity of the pathological process and the effectiveness of provided treatment.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Abergel P. Laser treatment of acne scars. *Laser Surg Med* 1992; Suppl 4:70.
2. Cordain L, Lindeberg S, Hurtado M, Hill K, Eaton SB, Brand-Miller J. Acne vulgaris: a disease of Western civilization. *Arch Dermatol* 2002; 138(12):1584-90.
3. Farago K. Low power laser in dermatology. *Laser Therapy* 1994; 6. N 1: 43 -52.
4. Geynits AV, Moskvina CV, Azizov GA. Intravenous laser irradiation of blood. Moscow: "Triad"; 2006. [Monograph in Russian].
5. Hou Y-H, Hou Y-Q, Fang X. Effective therapy of low level laser in 810 nm wavelength on severe acne. Proc. 7<sup>th</sup> Internat Congr of WALT, Sun City, South Africa, October 2008, p.117.

6. Moskvina SV, Buylin VA. *Basics of laser therapy*. M: Medicine; 2006. .p 256 [Manual in Russian]
  7. Petrishchev NN, Papayan GV, Arabian ER, Romanova OL, Dmitrieva NYu, Kang U. Fluorescence diagnosis and photodynamic therapy of acne. Scientific and Practical Conference "Recent advances in laser medicine and their application in medical practice". Moscow, 2006:155-6. [Article in Russian].
  8. Seaton E, Charakida A, Mouser PE, Grace I, Clement RM, Chu AC. Pulsed-dye laser treatment for inflammatory acne vulgaris: randomized controlled trial. *Lancet* 2003; 362(9393):1347-52.
  9. Simunovic Z, Trobonjaca T. Low level laser therapy of acne and scars applied as monotherapy and complimentary treatment modality to tetracycline: a multi centre clinical study on 80 patients with control group. *Lasers in Surg Med*. Suppl 12, 2000:7-11.
  10. Swanson JK. Antibiotic Resistance of Propionibacterium in acne vulgaris. *Dermatol Nurs* 2003; 15(4):359-62.
  11. Tuner J, Hode L. *The New Laser Therapy Handbook*. Prima books: Stockholm; 2010.
  12. Baybekov IM, Ibragimov AF, Khashimov FF. Application of LED radiation in the treatment of dermatoses and acne. Materials of XXXIX International scientific and practical conference "The use of lasers in medicine and biology". Kharkiv; 2013:17. [Article in Russian].
  13. Korobov AM. *Phototherapeutical devices by Korobov, "Barva" series*. In: Korobov AM, Korobov VA, Lesnaya TA, editors. Kharkiv; 2010:176. [Monograph in Russian].
  14. Baybekov IM, Mavlyan-Khodjaev RSh, Erstekis AG, Moskvina SV. RBC in norm, pathology and under laser influences. Tver: "Triad";-2008:256. [Article in Russian].
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