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ORIGINAL ARTICLE

P53 Gene Expression, Antibiogram Profile of *Staphylococcus aureus* and Hormonal Modulation in Acne Patients Treated with Isotretinoin

[1]Inaam A. Abed[*], [1]Mohammed S. Abdul-Razaq, [2]Wisam A. Ameen
[1]Department of Medical Microbiology, College of Medicine, University of Babylon, Iraq
[2]Department of Dermatology, College of Medicine, University of Babylon, Iraq

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Corresponding Author:
Inaam A. Abed.
Department of Medical Microbiology, College of Medicine, University of Babylon, Iraq
Tel.: 07706544991.
med733.inaam.ali@student.uobabylon.edu.iq

ABSTRACT

Background*: Acne is a complex skin disorder caused by hormonal changes, bacterial infections and the genetic background. Staphylococcus aureus, cause inflammatory reactions in acne patients. Sebaceous gland hyperactivity and inflammation are intensified by hormonal regulation (particularly high free testosterone levels). P53 is a key mediator of sebocyte death and inflammation.* ***Objectives****: The aims of the study are to detect the involvement of Staphylococcus aureus, and hormonal dysregulation in acne patients, and to evaluate the p53 gene expression, as a relation between acne patients and healthy controls, and evaluates therapeutic effects of isotretinoin on the p53 gene expression in acne patients.* ***Methodology:*** *S. aureus were detected by the routine bacteriology methods. The hormone tests evaluated free testosterone by ELISA kit. The qRT-PCR of the P53 gene expression was measured both before and after administration of isotretinoin. The statistical significance of the results were carried using t-tests; $p<0.05$, and one-way ANOVA; $p<0.05$.* ***Results****: Staphylococcus aureus was identified in 35 % of acne patients and 15 % of healthy volunteers, where it was especially resistant to antibiotics showed 40% resistance to clindamycin and 35% to fusidic acid. Free testosterone level increased in acne patients (634.69±83.92 ng/dL) compared with healthy control subjects (469.50±66.55 ng/dL, $p=0.001$). The gene expression of P53 were much less in patients who didn't receive isotretinoin treatment.* ***Conclusion****: Free testosterone is high in acne patients and makes sebaceous glands reactiv, while isotretinoin increases p53 signaling in a therapeutically effective way. Future treatments is required to resistant S. aureus.*

INTRODUCTION

Acne vulgaris is one of the most prevalent skin conditions that afflict 9.4 % of the world's population and can be both physically and psychologically detrimental [1]. It has a multifactorial pathogenesis: genes, hormonal abnormalities, microbes, and the environment. And yet even though acne is so widespread, its treatment is still complicated, and treatments are effective and safe today. The acne vulgaris is typically classified in to three grads mild, moderate and severe an egyptian study [2] reported that the studied cases had 20% mild, 30% moderate, and 50% severe degree acne.

Isotretinoin is an indispensable product for moderate- to- severe acne because it reduces the sebaceous gland activity, inflammation and keratinization. But it is not safe. There are now reports of interactions between isotretinoin and side-effects like acute eosinophilic pneumonia and epistaxis, suggesting the importance of vigilant patient care[3-4]. Moreover, due to the potential liver effects and lipid profiles, laboratory tests during treatment are also recommended[5]. Severe complications (keloid formation following skin surgery, for example) illustrate why it's critical to time isotretinoin treatments so as not to be a risk-taker [6].

The microbial environment that has also pathophysiological implications for acne. Bacterium *Staphylococcus aureus* drives inflammation by generating virulence factors, such as toxins and biofilms[7]. The proliferation of antibiotic-resistant *S. aureus* strains makes acne more difficult to treat and requires other microbiome modulating drugs[8-9]. Such methods target pathogens while maintaining healthy skin microbes.

Hormonal dysregulation, specifically excess androgen, is a major cause of acne. Androgens activate sebaceous glands, which in turn generates more sebum to provide a rich lipidic environment for bacteria growth and inflammation[10-11]. This is the reason why acne flares up at puberty, as well as some of the other conditions that are exacerbated by elevated androgens like polycystic ovary syndrome [12].

The p53 tumor suppressor gene is a known candidate for the formation of acne. As a death- and inflammation-controller, p53 keeps sebocytes turning over and stops them building up sludge [13- 14]. The gene expression of P53 is augmented by isotretinoin and other targeted therapies has been shown to be effective in restoring clinical results in acne patients.

This study detect the hormonal dysregulation, investigate the characteristics of the microbiota on the acne skin, and molecular study of gene-expression of P53 in patients' blood, and detection of P53 level in patients' serum.

METHODOLOGY

Study setting & design:

This study is a case-control work, done in Diyala Province, Iraq; from 1 May to 1 December 2024, to investigate the effects of isotretinoin on p53 gene expression and p53 level; detect the hormonal dysregulation in acne patients; and investigate the bacterial characteristics in acne skin areas. The research was conducted in Baquba Teaching Hospital, Dermatology Consultation Clinic and the lab analysis was conducted in College of Medicine, University of Babylon. The Ethical approval was obtained from the General Health Directorate of Diyala Province and the College of Medicine, Babylon University. All participants gave a written informed consent following discussion of study objectives and protocols. The volunteers were divided into 3 groups: acne vulgaris patients before isotretinoin (30 samples); post-isotretinoin treatment (30 samples); and 25 healthy volunteers without any history of acne as the control. The participants were 85 (15 to 35years old) men and women.

Inclusion and Exclusion Criteria

For inclusion, the patients had to have been diagnosed with acne vulgaris and be between the age limits. Exclusion criteria: pregnancy, lactation, systemic or autoimmune disorders, other dermatological conditions, and chronic infection.

Sample Collection

Bacteriological study

The face of patients were wiped with 70% ethanol and the samples were taken using sterile cotton swabs. The acne pustules were ruptured by sterile hypodermic needle. Collected samples were cultivated on Blood, MacConkey, Chocolate, and Nutrient agars. The plates were incubated at 37°C under aerobic and microaerophlic condition for 24 hrs. Biochemical tests were done by the Vitec system.

Blood samples collection

Venous blood samples were drawn aseptically, 3.5 mL of blood were withdrawn into gel tubes, centrifuged and the serum was aliquoted into five Eppendorf tubes for storage at -80°C. Here, 2 mL of blood was collected in EDTA-treated tubes for RNA extraction and stored at -80°C until used.

Enzyme-Linked Immuno-Sorbent Assay test:

Concentrations of p53 protein were determined using sandwich ELISA kit the Cat. No. is (E1711Hu), and the concentration of Free testosterone hormone were determinated using competative ELISA kit (BT LAB, China), the Cat. No. is (EA0034Hu). The tests were done according to manufacturer's instructions. Serum samples were analyzed with a BioTek ELISA microplate reader (Germany), optical density was 450 nm. A Plot of standard curves was done to determine protein concentrations, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).

Real-time quantitative PCR

Total RNA from whole blood was extracted with FavorPrep™ Blood Total RNA Extraction Kit (FAVORGENE, Korea) (Cat. No. FABRK 001). RNA amount and purity were verified spectrophotometrically. The complementary DNA (cDNA) was constructed with GoScript™ Reverse Transcriptase Kit. (Promega, USA), (Cat. No. A5000). RT-qPCR with GoTaq® qPCR Master Mix (Promega, USA), (Cat. No. A6000) was performed using Mx3005P Stratagene Real-Time (Agilent, USA). Boosting took place in 20 µL reaction volume and the following temperature profile was employed: 95°C for 5 minutes for hot-start activation, then 40 cycles of 95°C for 10 seconds, 60°C 30 seconds, and 72°C 30 seconds. The primers for p53 and for the reference gene GAPDH (Glyceraldehyde 3 Phosphate Dehydrogenase) used in this study included in (Table (1)). The fold changes were calculated by Ct method (Gene expression ratio (reference / target) = $2^{-\Delta\Delta CT}$).

Table 1: The primers for p53 and for GAPDH

Gene	Primer name	5'-3'	Product	Accession number	Reference
P53	F	CCTCAGCATCTTATCCGAGTGG	128 bp	NM_000546.6	Origene Co.
	R	TGGATGGTGGTACAGTCAGAGC			
GAPDH	F	GGAGTCAACGGATTTGGT	206	NM_002046.7	Piro&Broze, 2005
	R	GTGATGGGATTTCCATTGAT			

Data analysis:

Data was analyzed with SPSS Prism version 26. All quantitative data were presented as means standard deviation. Group comparisons were carried out using t-tests; $p<0.05$, and one-way ANOVA, significance threshold was p less than 0.05.

RESULTS

Bacteriological Findings:

It was found that *S. aureus* is more predominant in patients with acne vulgaris than in controls. 35% of patients with acne were positive for *S. aureus* but only 15% of the control group were positive. The 45% of acne patients were positive for *S. epidermidis* and 34% of healthy control were positive for *S. epidermidis*, (Figure 1).

The antibiotic resistance data points to major differences between *Staphylococcus aureus* and *Staphylococcus epidermidis*, as shown in (Figure 2).

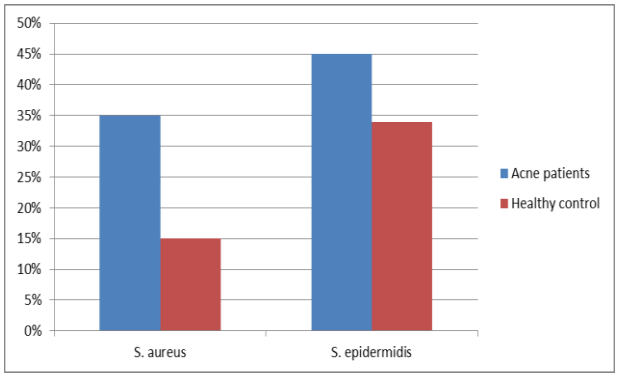

Fig. 1: Distribution of bacterial types by groups.

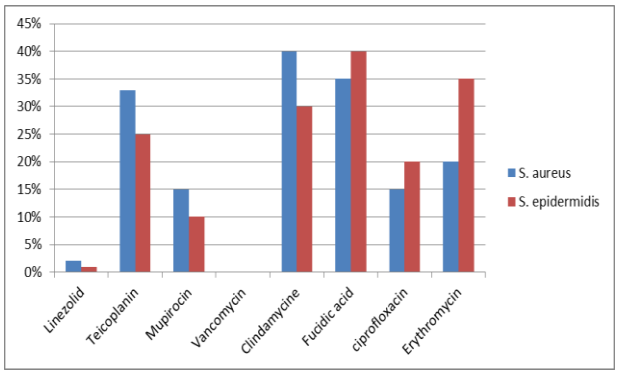

Fig. 2: Antibiotic resistance of *S. aureus* vs. *Staph. epidermidis*.

Linezolid (30 mg), Teicoplanin(30 mg), Mupirocin (200 mg), Vancomycin (30 mg), Clindamycin(2 mg), Fucidi acid(10 mg), Ciprofloxacin(5 mg), Erythromycin (5 mg).

2% of *Staphylococcus aureus* were resistant to linezolid; and *Staphylococcus epidermidis* resistance level was only 1%. Teicoplanin, resistance in *S. aureus* was (33%) and *S. epidermidis* was (25%). Mupirocin resistance in *Staphylococcus aureus* was 15%, while resistance in *Staphylococcus epidermidis* was less than 10%. *S. aureus* and *S. epidermidis* have no resistance to vancomycin. Clindamycine resistance in *Staphylococcus aureus* is 40% and for *Staphylococcus epidermidis* it's about 30%. Fusidic acid resistant in *S. aureus* was more than 35%, and in *staphylococcus epidermidis* was about 40%. Ciprofloxacin resistance in *Staphylococcus aureus* 15%, and 20% for *Staphylococcus epidermidis*. Erythromycin resistance among *S. aureus* was 20%, and 35% among *S. epidermidis*.

ELISA Results:

Detection of Hormone: Free testosterone was significantly higher in the acne patients (634.69±83.92 ng/dL) than in healthy controls (469.50±66.55 ng/dL), P-Value (0.004), (Figure 3).

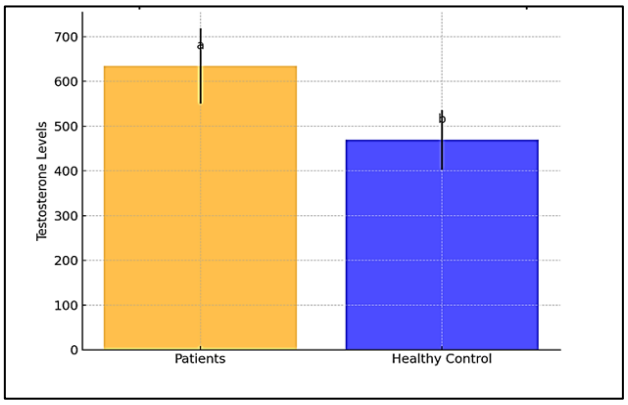

Fig. 3: Comparison of free testosterone levels in acne patients and healthy peoples.
Different small letters mean significant ($p<0.05$) differences.

P53 Protein Levels in patients serum samples using ELISA method:

Before treatment with isotreatenion (541.11 ± 235.90). After treatment with isotretinion (567.31 ± 128.25). healthy control (3.94 ± 0.60).In the case of healthy control, p53 protein was significantly lower than treated acne patients in (p=0.001), and lower than untreated acne patients (p=0.01). The p53 protein level is increased after treatment with isotretinion than before treatment with isotretinion (P-Value = 0.001). (Figure 4).

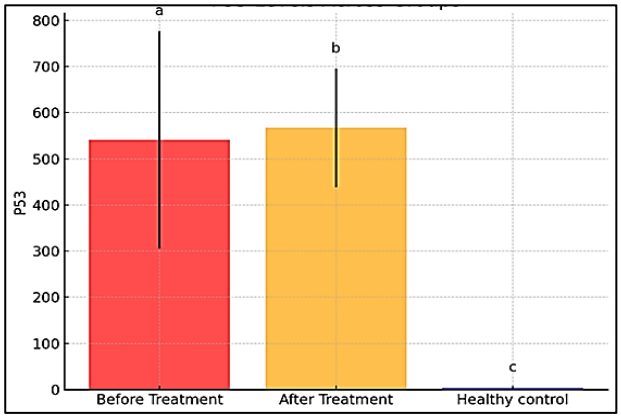

Fig. 4: Comparison of P53 protein in acne patients' serum samples before and after isotretinion treatment and healthy peoples.
Different small letters mean significant ($p<0.05$) differences.

Gene Expression Analysis of P53 mRNA in acne patients' blood samples using RT-qPCR analysis:

Significantly less p53 mRNA expression was detected in the untreated acne patients (0.05 ± 0.03) than healthy controls (0.90 ± 0.10), (p=0.00015). After Isotretinoin treatment acne patients greatly increased p53 mRNA expression (1.50 ± 0.15) compared to untreatment acne patients (p=0.00002), and compared to healthy control (p=0.005), (Figure 5).

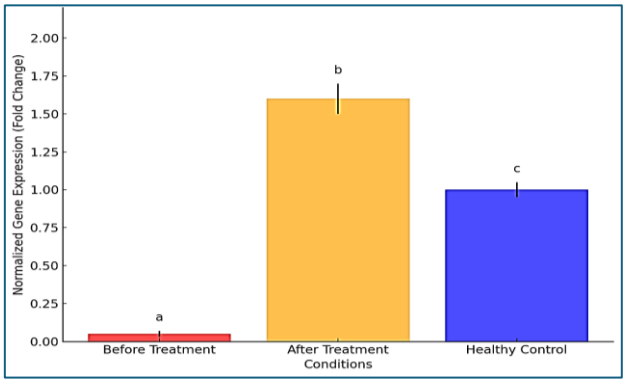

Fig. 5: Comparison of P53 (mRNA) Gene expression in acne patients' blood samples before and after treatment with isotretinion and healthy peoples.
Different small letters mean significant ($p<0.05$) differences.

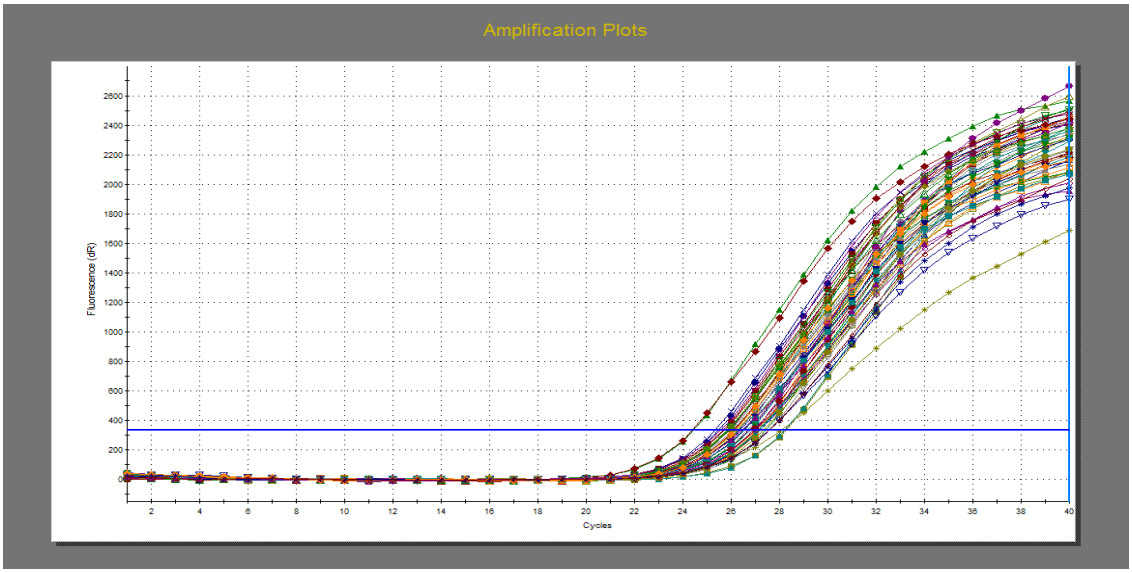

Fig. 6: Amplification plot of gene GAPDH by the Mx3005P Stratagene system.

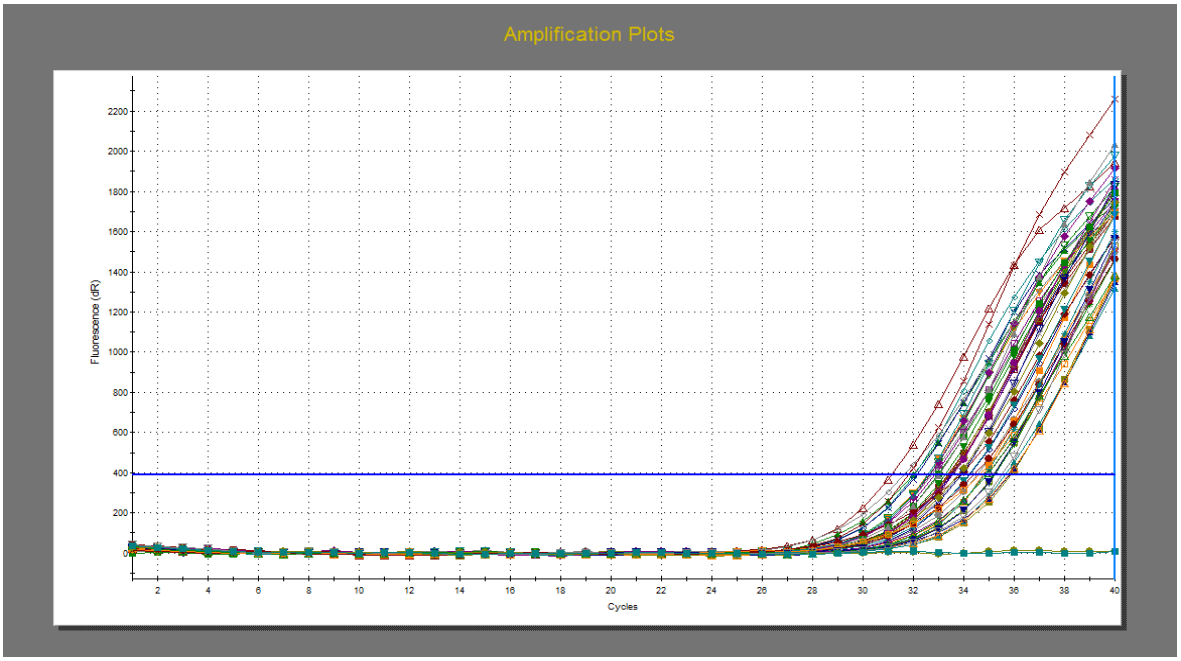

Fig 7: Amplification plot of P53 gene by the Mx3005P Stratagene system.

DISCUSSION

Our results highlight acne vulgaris' multifactorial character: bacterial colonization, hormonal dysregulation and p53 gene expression are all factors involved. These data show that isotretinoin is effective in reducing these causative factors and this support, as well as add to, the existing research.

Staphylococcus aureus was detected in 35% of acne patients. However, *Staph. epidermidis* was recorded at 45%. No detection to *Streptococcus Pyogenes* was seen, The results confirm *S. aureus'* role in acne flare-ups. Our data is coordinate with the egyptian study[15] which found that *S. epidermidis* was predominant (61%) followed by *S. aureus* in (28%) samples collected from acne lesion. The report of Oulès et al. [16] who described how isotretinoin treated acne, reduced the bacterial burden and broke down the biofilms that support *S. aureus*. *Staphylococcus aureus* showed the higher resistance to Clindamycine (40%) and *Staphylococcus epidermidis* showed higher resistance to Fusidic acid (40%), that because the widespread usage of these antibiotics in the dermatology. These results call for non-antibiotic interventions as Almuzaini et al[4] have also noted in their research an isotretinoin induced microbial regulation.

High levels of free testosterone were detected in acne patients (634.69±83.92 ng/dL), a well-established correlate of hyperactivity of the sebaceous glands. Levels are significantly low in healthy control (515.32±72.41 ng/dL). Del Rosso and Kircik [11] demonstrated that isotretinoin induces suppression of androgens. That kind of hormonal regulation is important for ending the cycle of excessive sebum production and bacterial invading.

The reduction of p53 gene expression and protein levels in acne patients before treatment revealed the role of this gene in acne pathology. P53 levels are low before treatment, which means compromised regulatory function in acne patients. This result aligns with Agamia et al.[13], who notice decrease in p53 activity in acne patients prior to isotretinoin therapy. Low p53 levels lead to defective sebocyte death and overproduction of sebum, both of which lead to acne.

In the analysis, p53 mRNA expression was up (1.6) fold and protein levels (2.2) fold post-treatment, suggesting that isotretinoin restored apoptotic and inflammatory equilibrium. Melnik [18] also noted that isotretinoin boosts p53, leading to sebocyte death and fewer inflammation. The reason why there were differences between ELISA and gene expression could be that not all mRNA of P53 translated to protein.

The healthy control group shows p53 gene expression approximately (1.0) fold which is normal activity for individuals without acne. That's compatible with Abdelhamed et al.[17], who reported p53 levels in healthy patients were unchanged and untroubled by the pathological processes found in acne patients. The fact that the p53 levels in the treated population remained relatively high when compared with the controls was a testament to the active regulatory effect of isotretinoin therapy.

On the other hand, isotretinoin comes with a significant side effects. Küçük [3] described a novel case of acute eosinophilic pneumonia caused by isotretinoin, and Almuzaini et al. [4] reported a 12% rate of patients

with epistaxis. These results call for surveillance and tailored treatment. Furthermore, Yaqoubi et al. [5] emphasized the effects of isotretinoin on liver function and lipid metabolism and emphasized the importance of monitoring biochemical changes regularly throughout therapy.

The condition is a disorder of hormones, which causes acne vulgaris and its manifestations by causing an overproduction of sebum and inflammation caused by the overproduction of androgens. These high levels of free testosterone echoed what Del Rosso and Kircik[11] reported: that isotretinoin works to shut down androgen production, which in turn suppresses excessive activity of the sebaceous gland. This hormonal control of sebum also re-routes to the root of blocked pores and inflammation, making it an all-around solution to acne[19].

Beyond hormone activity, isotretinoin's effects on genetic circuitry have been given renewed vigor. That p53 gene expression and protein levels have been raised after treatment points to the drug's ability to restore apoptotic and inflammatory equilibrium. This coordinate with Melnik's[18] report that isotretinoin activated p53 to trigger sebocyte death and inhibit the production of inflammatory cytokines. Such molecular processes illustrate how isotretinoin addresses acne's two drivers, hormonal and genetic.

CONCLOSION

The research proves that isotretinoin works to diminish bacterial infections, restore hormonal balance, and increase p53 expression.

Recommendations

Future research should focus on optimizing isotretinoin treatments, and on how to tackle looming problems such as antibiotic resistance and treatment resistance.

Source of Funding:
The authors provided all funding for the current investigation; no outside funding was obtained.

Ethical Approval:
This research was done under the Declaration of Helsinki and the institutional ethics committee. Everyone in the study gave a written informed consent before joining. They promised privacy and the research had strict ethical procedures all along.

Conflict of Interest: None.

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