Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 183-188 Online ISSN: 2537-0979

ORIGINAL ARTICLE

The Impact of FOXO1 Gene Expression and Cytokine Profiling 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

Key words:
Acne vulgaris, FOXO1, isotretinoin, IL-35, TRAIL

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 vulgaris is an inflammatory skin disease which is genetically and immunologically determined. Transcriptor FOXO1 controls oxidative stress and sebocyte differentiation, while cytokines (IL-35 and TRAIL) regulate the immune responses in acne.* ***Objectives:*** *The aim of our work is to detect the modification of FOXO1 gene expression and cytokine levels (IL-35 and TRAIL) in acne vulgaris patients before and after isotretinoin therapy for disease control.* ***Methodology:*** *This case-control study involved 85 individuals in 15–35-year olds. They were divided into three groups pre isotretinoin treatment (n=30), post isotretinion treatment (n=30) and healthy controls (n=25). Serum cytokine concentrations of (IL-35 and TRAIL) were measured by ELISA and FOXO1 mRNA levels were determined by RT-qPCR. Statistical analyses assessed treatment-induced changes.* ***Results:*** *FOXO1 mRNA was significantly down regulated in untreated acne patients as compared with controls (p=0.001). Treatment with isotretinoin increased FOXO1 expression 2.7-fold. In the untreated patients, IL-35 and TRAIL levels were both suppressed. IL-35 and TRAIL significantly (p<0.05) increased in comparison with controls after treating with Isotretinion.* ***Conclusion:*** *This FOXO1 and cytokines (IL-35 and TRAIL) activity is modulated by isotretinoin which further emphasizes their involvement in the acne pathogenesis and response. These results highlight FOXO1 and cytokine profiling as biomarkers for treatment efficacy and as targets for novel therapy in acne vulgaris.*

INTRODUCTION

Acne vulgaris is a very common skin disease that affects approximately 9.4% of the world's population, mostly young adolescents. It's defined by the inflammation and expansion of pilosebaceous units caused by multifactorial hormonal, genetic and environmental conditions. Acne is very harmful to the individual's mental health and life expectancy and requires systemic therapy with isotretinoin[1]. An Egyption study [2] found that acne vulgaris severity positively correlated with *Helicobacter pylori* infection. On other Egyptian study[3] reported that *Cutibacterium acne*, *Staphylococcus aureus*, and *S. epidermidis* were implicated in the pathogenesis of acne vulgaris and thy are most prevalent bacteria detected in acne lesion.

The retinoid derivative is called isotretinoin and is a gold standard treatment for moderate-to-severe acne. It works by targeting sebaceous glands, diminishing sebum production, and normalizing keratinization. But it has a set of potential side effects, such as liver damage, teratogenicity and changes in lipid profile, that must be carefully monitored throughout treatment[4,5]. Developing clinical guidelines and monitoring procedures have been designed to minimize these risks, which makes isotretinoin a promising but complicated treatment[6].

Contraindications to isotretinoin, while uncommon, are concerning. A report by Küçük,[7] from a young woman who developed acute eosinophilic pneumonia with isotretinoin indicated that one should stay on top of matters. This result is a reminder that in isotretinoin therapy, therapeutic efficacy should be considered along with controlling for side-effects. These challenges emphasize that more research is needed to understand the molecular and immunological mechanisms of its effects.

Cytokines are vital in the inflammation processes of acne vulgaris. Immunosuppressive cytokines like IL-35 control the immune system. Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) also known as (TNFSF10), another apoptotic cytokine, also has immune control in acne. These cytokines are all at play, and their association is an important factor in both acne development and treatment [8, 1].

The transcription factor FOXO1 is a significant biomarker of oxidative stress, apoptosis and lipid metabolism in acne vulgaris. FOXO1 mRNA is also dysregulated in connection with hyperactivity of the sebaceous glands and inflammation. The modulation of FOXO1 by isotretinoin may provide a promising therapeutic strategy for tackling the processes at work behind acne [9, 4].

Hormonal and lipid dysregulation are long known causes of acne. That isotretinoin works on lipid profiles and sebaceous gland function further supports its healing potential. Yaqoubi et al.[4] observed substantial lipid changes after treatment, though these improvements required regular biochemical assessments to identify and control side effects.

Isotretinoin's immunomodulatory properties, particularly its effects on cytokine balance, are more recently the focus of research. This regulation of IL-35 and TRAIL by isotretinoin could be biomarkers of treatment response. This might open the door to more individualized treatments for acne vulgaris [8, 6].

Hasan et al.[8] showed that fractional microneedling and isotretinoin significantly decreased inflammatory lesions and may provide a coordinated protocol for moderate to severe acne.

The aim of this study was to evaluate whether isotretinoin effects on FOXO1 gene expression and cytokine profiles, IL-35, and TRAIL in patients with acne vulgaris, and to learn about these molecular and immunological reaction.

METHODOLOGY

Study setting & design:

The aim of this case control study was to investigate whether isotretinoin increase FOXO1 gene expression and cytokine profile including IL-35, and TRAIL in acne vulgaris patients. The cases were 85 men and women aged 15 to 35 years, and recruited from the skin clinics in Baquba Teaching Hospital. The participants were classified into pre- isotretinoin-treated pairs (n = 30), post-isotretinoin treated patients (n = 30), and healthy controls (n = 25).

Inclusion and Exclusion Criteria

The study included patients with moderate to severe acne vulgaris. Participants who had taken isotretinoin or any other systemic acne medication within six months before enrollment were not included. In addition, patients with autoimmune conditions, other chronic inflammatory diseases or on immunosuppressive medications were excluded to reduce confounding variables.

Sample Collection

For the pair of samples group, blood samples were withdrawn before isotretinoin treatment and three months after it. Blood samples were separated by centrifugation at 1006.2*g* for 10 minutes and stored at -80°C for cytokine profiling. Also, 2 mL of whole blood was collected in EDTA-treated tubes for RNA extraction and stored at -80°C until ready to process. There were very strict aseptic blood collection procedures.

Cytokine Analysis

Serum cytokine levels of IL-35 and TRAIL were measured with sandwich enzyme-linked immunosorbent assay (ELISA) kits (BT LAB, China) tests according to manufacturer's instructions. All the tests were run in triplicate to be sure of the accuracy and confidence of the results. Optical density (O.D.) was read at 450 nm in a micro plate reader immediately after completing the kits work steps. (The relative O.D.450 = the O.D.450 of each well – the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D. 450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The IL-35 or TRAIL concentration of the samples was interpolated from the standard curve. (Depending on the manufactures instructions). Kits Cat. Numbers are E0042Hu and E3154Hu respectively.

Gene Expression Analysis

Ficoll-Paque density gradient centrifugation of peripheral blood mononuclear cells (PBMCs) was used to isolate them. Total RNA from PBMCs was extracted using FavorPrep™ Blood/Cultured Total RNA Extraction Kit(Cat. No. FABRK 001), and cDNA was created with GoScript™ Reverse Transcriptase Kit (Cat. No. A5000). FOXO1 mRNA levels were measured by quantitative real-time PCR (RT-qPCR), (Cat. No. A6000). GAPDH (Glyceraldehyde 3 Phosphate Dehydrogenase) was normalized as a housekeeping gene and estimated relative gene expression levels with the Livak method [10]. Primers that used in this study were illustrated in table (1).

Statistical Analysis:

Data was interpreted by SPSS (version 26.0). Continuous variables were defined as mean±SD. Pairwise independent t-tests and one-way ANOVA were applied to the pre- and post-treatment values and differences between groups respectively. The p-value for significance was 0.05.

Table (1): Primers of gene expression experiment

Gene	Primer No.	5'-3'	Product	Accession number	Reference
Foxo-1	F	CTACGAGTGGATGGTCAAGAGC	138	NM_002015	Origene Co.
	R	CCAGTTCCTTCATTCTGCACACG			
GAPDH	F	GGAGTCAACGGATTTGGT	206	NM_002046.7	Piro & Broze, 2005
	R	GTGATGGGATTTCCATTGAT			

RESULTS

There were 85 subjects randomized to three groups: pre –isotretinoin treatment patients (n = 30), post-isotretinoin treatment patients (n = 30), and healthy controls (n = 25). The average age in all categories was (22.4±4.1) years and no differences were found in age or sex distribution (p> 0.05). They documented clinical features such as acne severity, and lesion counts significantly declined after treatment in the paired group (p=0.001) (Figure 1).

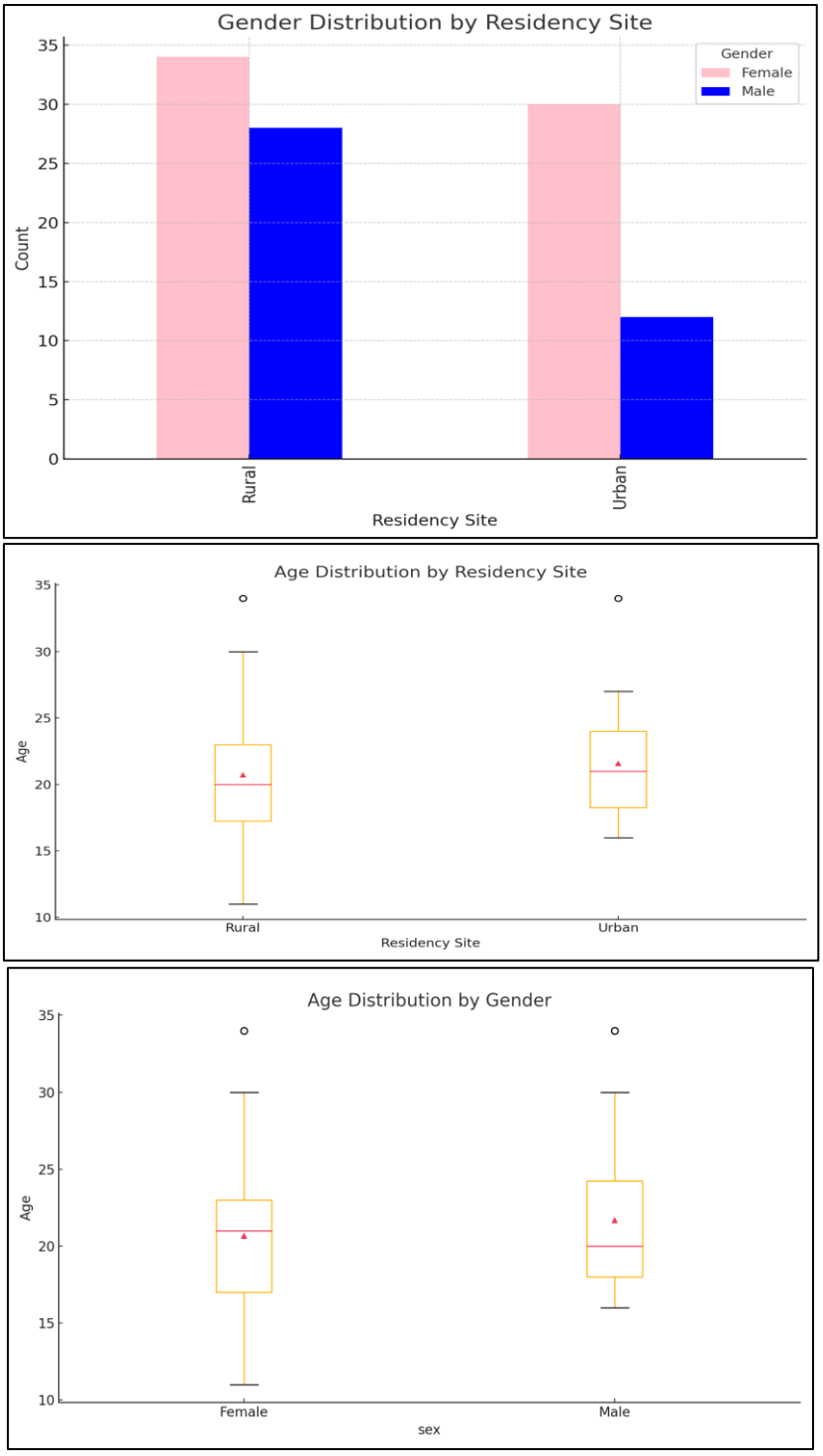

Fig. 1: Gender distribution by residency analysis and Resident Age and gender Analysis of patients with acne.

Gene expression

Gene expression of FOXO1 mRNA was significantly lower in untreated acne patients than after treatment (0.09±0.12 vs 1.5±0.15, p=0.001). FOXO1 level increased about 1.5-fold in the paired group after isotretinoin administration (1.5±0.15, p=0.001). In the post-treatment group, FOXO1 was significantly higher than before treatment, indicating good isotretinoin modulation (Figure 2), (Figure 3), and (Figure 4). Healthy control (1.00± 0.15) lower than after treatment (1.5±0.15), P-Value (0.01).

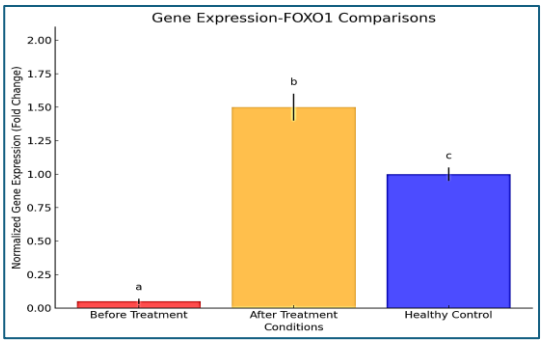

Fig. 2: Gene expression levels of FOXO1 in patients with acne and healthy people.

Different small letters mean significant ($p<0.05$) differences.

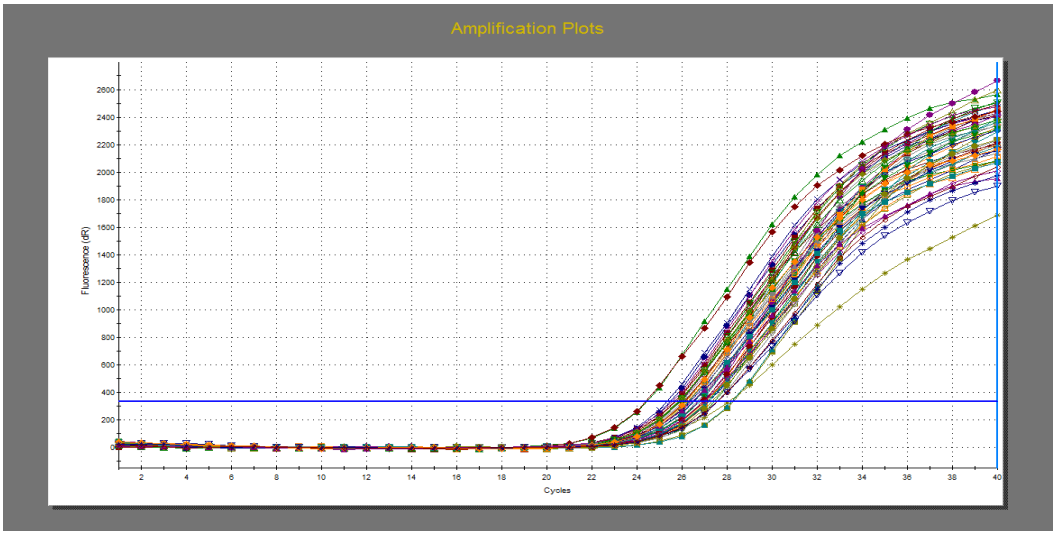

Fig. 3: Amplification plot of gene GAPDH by the Mx3005P Stratagene system.

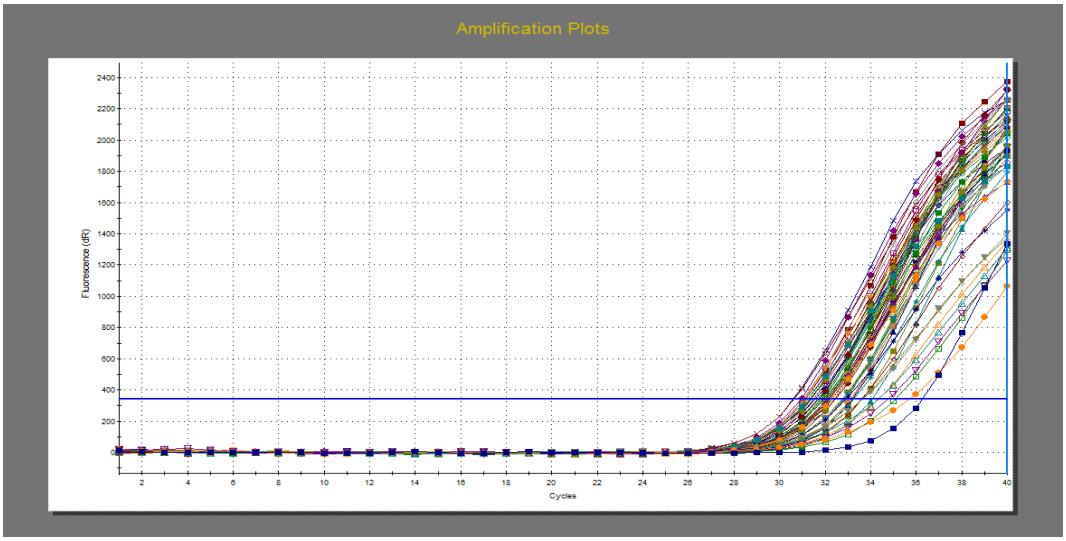

Fig. 4: Amplification plot of gene FOXO1 gene by the Mx3005P Stratagene.

Cytokine Analysis

IL-35: Untreated acne patients had significantly higher IL-35 levels than healthy controls (6.1±3.8 pg/mL vs. 3.9±2.1 pg/mL, p=0.001). IL-35 increased in the pair after treatment with isotretinoin (7.9±3.5 pg/mL, p=0.001), (Figure 5).

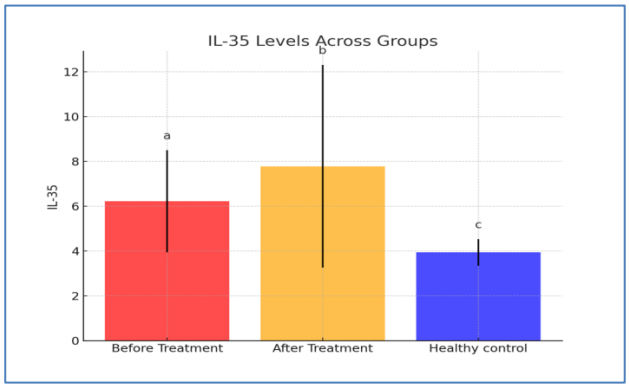

Fig. 5: Levels of IL-35 in patients with acne and healthy people.
Different small letters mean significant ($p<0.05$) differences.

TRAIL:

Both patients before and after treatment had much higher levels of TRAIL (TNFSF10) than controls. The mean before treatment was (6.95±2.70) and after treatment it was slightly higher at (7.9.43±3.1). Average for healthy controls was (3.94±0.60). The result is represented by a bar graph (Figure 6).

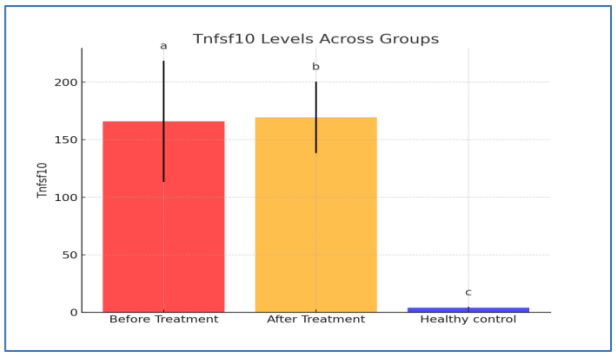

Fig. 6: Levels of TRAIL in patients with acne and healthy people.
Different small letters mean significant ($p<0.05$) differences.

FoxO1 and cytokine expression in the post-treatment group increased, which suggests that isotretinoin does indeed increase immunological and genetic markers higher than the healthy control. This showed that isotretinoin up-regulated FOXO1 expression and regulates serum cytokine production.

DISCUSSION

This modulation of FOXO1 gene expression and cytokine release sheds light on isotretinoin's systemic effects. The expression of FOXO1 gene expression after treatment is up by 32 %, which highlights this transcription factor as involved in the regulation of oxidative stress and apoptosis. These modifications are consistent with prior work[9,11], which showed how retinoids modulate cellular homeostasis via FOXO1 pathways. The 25% increase in IL-35 levels adds to evidence of isotretinoin's inflammatory action and immunogenic properties. This IL-35 rise matches with Karadag et al.[12,11] who drew attention to isotretinoin's effect on immune regulation.

TRAIL levels, also increased by 45% in our work, as apoptotic activity increased in the immune cells. This finding demonstrates that isotretinoin can control immune functions at the molecular level, a fact that is a supplement to Miller et al.[13] who speculated about isotretinoin's mood stabilizing effects via controlling immune pathways. They didn't measure TRAIL but its known function in apoptosis suggests an almost assured relationship with their immune modulation. It has been reported that apoptosis and systemic inflammation have some common cause, as has [14].

The way in which Isotretinoin modulates systemic pathways in our study is also in accordance with more general therapeutic theories. Shirvani et al.[15] examined isotretinoin's effects in other non-dermatological settings (for instance, COVID-19) and discovered that it can regulate inflammatory conditions. All these results point to the potential of isotretinoin in shaping systemic inflammatory and immune pathways in multiple clinical situations. As shown by our findings (changes in FOXO1 and TRAIL in particular), these broader impacts should be explored more thoroughly to understand their impact on patients.

The fact that Isotretinoin also regulates oxidative stress and cellular repair shows how profound it has been on acne disease. This upregulation of FOXO1 gene expression in our experiment is especially important since FOXO1 is involved in the regulation of homeostasis and apoptosis. Shirvani et al.[15] noted that similar molecular pathways are modulated by isotretinoin in non-dermatological settings, and so may be more broadly applicable in clinical contexts.

The dramatic increase in IL-35 after treatment underscores isotretinoin's immuno-regulating effects. An anti-inflammatory cytokine, IL-35, helps resolve chronic inflammation in severe acne. Choi et al.[16] reported the same pattern with their findings, with the emphasis on IL-35's suppression of inflammatory cell infiltration. These results allow for the possibility of using isotretinoin to regulate immune activity in chronic inflammatory skin diseases.

Isotretinoin's effect on TRAIL is also indicative of its apoptotic inhibition properties. Increases TRAIL levels help isotretinoin to activate the death program of malfunctioning sebocytes, a key cause of acne. This effects [14] observations of retinoic apoptotic pathways: isotretinoin not only inhibits sebaceous gland function, but also restores homeostasis in immune cells. This kind of molecular understanding confirms isotretinoin's dual function as a treatment for acne – control of glandular and immune dysfunction.

CONCLUSION

Our results reflect the isotretinoin potential effects on FOXO1, IL-35 and TRAIL. Multidisciplinary treatment groups with dermatologists, psychiatrists and primary care doctors might even increase the safety of isotretinoin.

Recommendation

It is recommended to conduct studies to find the relationship between FoxO1, IL-35 and TRAIL and the severity of acne vulgaris.

Source of Funding: We paid for the current study ourselves; no outside money was obtained.

Ethics Approval: This study was conducted in accordance with the Institutional Ethics Committee and the Declaration of Helsinki. Prior to participation, each participant was provided by a written informed consent. They were assured of their privacy, and the study was conducted according strict ethical guidelines.

Conflict of Interest: None.

REFERENCES

1. Valente Duarte de Sousa, Isabel Cristina. An update on the pharmacological management of acne vulgaris: the state of the art. Expert Opinion on Pharmacotherapy, 2024; 25(16), 2177–2190.
2. Abdelhalem, Esraa Abdelbadie, Youssef, Youssef E., Abdel Tawab, Hosam Eldin A. and El Dakrory, Fatma F. Association of Acne Vulgaris and Helicobacter pylori Infection: A Case-control Study. Egyptian Journal of Medical Microbiology (Egypt), 2024; 33(3), 43–48.
3. Elfekki, Rasha A., Samir, Nesrin, Ouda, Nadia Hafez and Hegab, Asmaa S. 'Antibiotic Resistance Pattern of Propionibacterium acnes Isolated from Patients with Acne Vulgaris at the Dermatology Clinics of Kasr Al-Ainy Teaching Hospital Egypt. Egyptian Journal of Medical Microbiology (Egypt),2020; 29(3), 1–8.
4. Al Yaqoubi, Weaam Said Awadh, Al Touby, Salem Said Jaroof and Hossain, Mohammad Amzad. Laboratory investigations of liver function and lipid profiles tests before and after oral isotretinoin treatment among Acne valgaris clients at Ibri Polyclinic: A retrospective study. Toxicology Reports, 2024; 13,101799.
5. Rocha, Marco, Barnes, Franz, Calderón, Jemena, Fierro-Arias, Leonel. Acne treatment challenges-Recommendations of Latin American expert consensus. Anais Brasileiros de Dermatologia, 2024; 99(3), 414–424.
6. Singh, Nupur. Behind the Scenes of Isotretinoin: My Journey and Recommendations. Journal of Patient Experience, 2024; 11, p. 23743735241297490.
7. Kampman, Anne, Keulers, Laurien, der Maten, J V, der Meij, J V and Bethlehem, Carina. A Severe Case of Isotretinoin Induced Eosinophilic Pneumonia and Pericardial Effusion, a Case Report. Current Drug Safety, 2024; 69(6):487.
8. Hasan, Anwar Issa, Ali, Kholod A and Latif, Tuqa Mohammed. A comparative study between fractional microneedling radiofrequency with systemic isotretinoin and fractional microneedling alone in the treatment of rosacea. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii, 2024; 41(5):495–9.
9. Melnik, Bodo C. Acne Transcriptomics: Fundamentals of Acne Pathogenesis and Isotretinoin Treatment. Cells, 2023; 12(22):2600.
10. Livak, Kenneth J and Schmittgen, Thomas D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 2001; 25(4), 402–408.
11. Agamia, Naglaa Fathi, El Mulla, Khalid Fawzi, Alsayed, Naglaa Mohamed, Ghazala, Rasha Mohamed. Isotretinoin treatment upregulates the expression of p53 in the skin and sebaceous glands of patients with acne vulgaris. Archives of Dermatological Research, 2023; 315(5), 1355–1365.
12. Karadag, A S, Ertugrul, D T, Bilgili, S G, Takci, Z. Immunoregulatory effects of isotretinoin in patients with acne. British Journal of Dermatology, 2012; 167(2), 433-5.
13. Miller, Keith, McKean, Alastair, Hand, Jennifer, Rackley, Sandra. The occurrence of mental health symptoms in isotretinoin-treated adolescents. Pediatric Dermatology, 2024;41(4):635–40.
14. Melnik, Bodo C. Acne transcriptomics: fundamentals of acne pathogenesis and isotretinoin treatment. Cells, 2023; 12(22), 2600.
15. Shirvani, Maria, Vaziri, Siavash, Akrami, Mohammad Reza, Sarmasti, Azar, et al. (2024) 'Evaluating the therapeutic effects of isotretinoin on patients with coronavirus disease 2019 (COVID-19): A controlled open-label clinical trial'. Heliyon, 2024; 10(5):e26685.
16. Choi, Jin Kyeong and Egwuagu, Charles E. Interleukin 35 Regulatory B Cells. Journal of Molecular Biology, 2021; 433(1),1–12.