Ahmad KHAMAYSEH

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THE IMPACT OF ORAL ISOTRETINOIN ON PI3K-AKT-MTOR PATHWAY ACTIVITY AND GENE EXPRESSION IN ACNE VULGARIS PATIENTS

M.Sc. THESIS IN MOLECULAR MEDICINE

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APPROVAL

We certify that we have read the thesis submitted by Ahmad Khamayseh titled **'THE IMPACT OF ORAL ISOTRETINOIN ON PI3K-AKT-MTOR PATHWAY ACTIVITY AND GENE EXPRESSION IN ACNE VULGARIS PATIENTS'** and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Educational Sciences.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

AHMAD KHAMAYSEH

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AHMAD KHAMAYSEH

Abstract

The aim of the present investigation was to examine the impact of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression levels in individuals with acne, both prior to and following oral isotretinoin treatment.

The study's methodology encompassed the procurement of peripheral blood samples both pre- and post-administration of oral isotretinoin. Total RNA was extracted from the collected samples through the process of RNA isolation. Following that, the process of cDNA synthesis was executed through reverse transcription, which involved the conversion of RNA molecules into their corresponding complementary DNA sequences. The utilization of Gradient PCR was implemented to optimize the conditions for the ensuing RT-qPCR analysis.

The expression profiles of *mTOR, AKT, p53,* and *pik3* genes were assessed before and after the administration of oral isotretinoin, in result to the treatment, all examined gene expression levels showed an increase. Correlation analysis using correlation coefficient (r values) and the significance of the relationship (p-values) revealed associations between different gene pairs. Specifically, the expression levels of Akt and p53 genes displayed a significant increase compared to the levels before treatment ($p=0.027$, $p=0.017$, respectively). However, no statistically significant difference was observed in the expression levels of *mTOR* and pik3 genes ($p>0.05$). Furthermore, in the study group, a positive, linear relationship of weak strength was observed between *mTOR* and *pik3* genes ($r=0.486$; $p=0.002$), indicating a positive association. In contrast, a negative, linear relationship of weak strength was found between *mTOR* and *p53* genes ($r=0.486$; $p=0.002$), suggesting a negative association.

In conclusion, the findings indicate that the administration of oral isotretinoin in individuals with acne results in an increase in the expression of the *Akt* and *p53* genes. The results of this study provide evidence for the participation of the *PI3K-Akt-mTOR* pathway and *p53* signalling in the efficacious outcomes of isotretinoin treatment. Additional investigation is required to clarify the fundamental molecular mechanisms and examine the clinical ramifications of these discoveries.

THE IMPACT OF ORAL ISOTRETINOIN ON *PI3K-AKT-MTOR* PATHWAY ACTIVITY AND GENE EXPRESSION IN ACNE VULGARIS PATIENTS

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Summary

The present study aims to examine the impact of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression levels in individuals with acne, both prior to and following oral isotretinoin administration. Isotretinoin (CHEBI:6067) is a synthetic retinoid that is derived from all-trans-retinoic acid by isomerizing the double bond in the α,β-position to the carboxy group to a Z configuration.

The investigation utilized a molecular medicine methodology to examine the fundamental mechanisms of isotretinoin's operation and its influence on the *PI3K-Akt-mTOR* pathway. The study encompassed the collection of samples from individuals with acne who received isotretinoin treatment. The samples were subjected to RNA isolation, cDNA synthesis, and analysis utilizing gradient PCR and RT-qPCR methodologies. The findings of the study demonstrated a noteworthy augmentation in the levels of *Akt* and *p53* gene expression subsequent to the intervention, thereby signifying the regulation of the *PI3K-Akt-mTOR* pathway. Moreover, it was observed that the gene expression levels of *mTOR* and *pik3* were comparatively elevated in patients receiving treatment, although statistical significance was not attained. The aforementioned discoveries enhance our comprehension of the molecular mechanisms implicated in isotretinoin therapy and offer perspectives on plausible therapeutic objectives for the management of acne. This study highlights the significance of conducting additional research in this field to clarify the exact mechanisms and enhance treatment approaches for individuals with acne.

Keywords: isotretinoin, acne, *PI3K-Akt-mTOR* pathway, gene expression, retrospective study.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

1. SR - significant number
2. NIH - National Institutes of Health
3. P. acnes - Propionibacterium acnes
4. IGF-1 - Insulin-like Growth Factor 1
5. AC - Acne
6. PSU - Pilosebaceous units
7. SB - Sebum
8. PO - Pores
9. LS - Lesions
10. SH - Shoulder
11. BK - Back
12. J. - Johnson
13. SG - Sebaceous glands

14. FB - Follicular base

15. INF - Inflammation

16. CY - Cysts

17. ADO - Adolescence

18. PREG - Pregnancy

19. GF - Genetic factors

20. NIA - Non-inflammatory acne

21. IA - Inflammatory acne

22. CA - Cystic acne

23. COM - Comedones

24. BH - Blackheads

25. WH - Whiteheads

26. TM - Topical medications

27. BP - Benzoyl peroxide

28. RET - Retinoids

29. OM - Oral medications

30. AB - Antibiotics

31. OC - Oral contraceptives

32. ISO - Isotretinoin

33. DERMA - Dermatologist

34. ERY - Erythema

35. XER - Xerosis

36. CH - Cutaneous hypersensitivity

37. S. aureus - Staphylococcus aureus

38. PHOT - Photosensitivity

39. TRET - Tretinoin

40. ADAP - Adapalene

41. TAZ - Tazarotene

42. SA - Salicylic acid

43. BHA - Beta-hydroxy acid

44. TC - Tetracycline

45. MC - Minocycline

46. DOXY - Doxycycline

47. HF - Hormonal fluctuations

48. VA - Vitamin A

49. SD - Skin dryness

50. HL - Hair loss

51. µl - Microliter

52. mL - Milliliter

53. µM - Micromola

54. P53 - Tumor protein

55. AH - Androgen hormones

56. EL - Elevated levels

57. CM - Comorbidities

58. EE/NGM - Ethinyl estradiol and norgestimate

59. EE/DRSP - Ethinyl estradiol and drospirenone

60. DSG/EE - Desogestrel and ethinyl estradiol

61. OI - Oral isotretinoin

62. CP - Chemical peels

63. GA - Glycolic acid

64. LA - Lactic acid

65. LT - Light therapy

66. BL - Blue light

67. RL - Red light

68. LM - Lifestyle modifications

69. CE - Comedone extractor

70. PI3K - PI3K-AKT-mTOR pathway

71. DHT - Dihydrotestosterone

72. PCOS - Polycystic ovary syndrome

73. FH - Follicular hyper-keratinization

74. IL-1 - Interleukin-1

75. TNF-alpha - Tumor necrosis factor-alpha

76. TLRs - Toll-like receptors

77. IR - Insulin receptor

78. IGF-1R - Insulin-like Growth Factor 1 receptor

79. PIP2 - Phosphatidylinositol 4

80. PIP3 - Phosphatidylinositol 3,4,5-trisphosphate

81. mRNA - Messenger RNA

82. Rb - Retinoblastoma protein

83. Bcl-2 - B-cell lymphoma 2
84. TRIZOL - A chemical reagent used for RNA extraction
85. DNAse - Deoxyribonuclease
86. RNase - Ribonuclease
87. cDNA - Complementary DNA
88. RT - Reverse transcriptase
89. dNTPs - Deoxyribonucleotide triphosphates
90. abm - Applied Biological Materials Inc.
91. PCR - Polymerase chain reaction
92. qRT-PCR - Quantitative reverse transcription polymerase chain reaction
93. mTOR - Mammalian target of rapamycin
94. AKT1 - Protein kinase B alpha
95. PIK3 - Phosphatidylinositol-4,5-bisphosphate 3-kinase
96. P53 - Tumor protein p53
97. TaqMIx - Taq DNA polymerase mix
98. Fwd primer - Forward Primer
99. Rev primer - Reverse Primer
100. DH2O - Deionized water
101. Fig. - Figure
102. $p > 0.05$ - Not statistically significant difference reached
103. AU - Arbitrary unit
104. p-value - p
105. r - Correlation coefficient

106. Mann-Whitney U test - Mann-Whitney U test
107. Spearman correlation test - Spearman correlation test
108. p53 - Tumor protein p53
109. PI3K-Akt-mTOR pathway - Phosphoinositide 3-kinase-Akt-mTOR pathway

CHAPTER I

Introduction

Acne is a well-known dermatological condition that affects a significant number of individuals annually (SR). Acne occurs when the sebaceous gland becomes hypersensitive to normal circulation level of androgens, Propionbacterium acnes (P. Acnes) and inflammation. Factors that may affect the occurance of acne are medications like steroids as well as a high level of sunlight exposure, usage of pads, endocrine disorders (e.g. polycystic ovarian syndrome) and pregnancy. Moreover, genetic factors may also affect the percentage of branched fatty acids within the sebum, this effect is estimated to be between 50 to 90% (Sutaria, 2023). Furthermore, it has been seen that grade I acne exhibits two types of comedones: the first being open comedones that are due to plugging of pilosebacebous orifice and the second are closed are due to keratin and sebum plugging the pilosebaceous orfice below the skin surface. On the other hand, grade 2 reveals inflammatory lesions that present as small papules (small, red, tender pumps) with erythema.

Acne is primarily distinguished by the manifestation of papules (small, red, tender pumps), blackheads, and whiteheads (NIH, 2023). The manifestation of skin lesions can range from mild, which denotes a minimal presence of such lesions on the skin, to moderate to severe, which indicates that the lesions cover a significant surface area on the skin. Grade 3 kesion may additionally exhibit pustules that occur to be small and inflamed sore lesions, as per the National Institutes of Health (NIH, 2023). Grade 4 lesions progress and the pustules can become coalesce that eventually form nodules and cysts (Sutaria, 2023).

Acne has the potential to manifest on various regions of the body, including the back, thighs, and hands; nevertheless, it is predominantly observed on the facial area. The pharmaceutical market offers a range of treatments for acne, which can be classified into three categories: topical medications, oral medications, and physical therapies. Acne's pathogenesis encompasses various molecular pathways, such as the *PI3K-AKT-mTOR* pathway, the androgen pathway, the P. acnes pathway, and the insulin/Insulin-like Growth Factor 1 (IGF-1) pathway. The present study investigates the impact of

isotretinoin, a potent systemic medication, used for the treatment of severe cases of acne and other skin diseases, *PI3K-AKT-mTOR* pathway in the context of acne disease. Furthermore, the article explores various categories of acne therapies, elucidates the molecular mechanisms underlying acne, and examines the plausible involvement of the *p53* tumour suppressor gene in the development of acne.

Acne is a multifaceted dermatological condition that afflicts a significant proportion of the global population. While the aetiology of acne remains elusive, several factors have been implicated in its pathogenesis. Furthermore, it is noteworthy that the causative factors of acne may vary depending on the anatomical location of the affected area. According to Caldwell (2023), the presence of acne in certain areas of the body may indicate underlying health issues. For instance, acne on the arms may be indicative of a deficiency in vitamin A also known as Retinol (A1) $C_{20}H_{30}O$; 3-dehydroretinol (A2) $C_{20}H_{28}O$) and vitamin D, while acne on the back may be attributed to poor hygiene and excessive sweating. Similarly, acne on the chest may be linked to allergies, and acne on the face may be associated with hormonal fluctuations. The aetiology of acne in the human body is attributed to surplus sebum, follicular hyperkeratinisation, bacterial proliferation, and cutaneous inflammation. The pharmaceutical industry offers numerous treatments for acne, but the effectiveness of these treatments depends on the location and severity of the acne lesion on an individual's skin. There is a range of treatment modalities that can effectively target the underlying mechanisms, including both topical treatments and systemic medications (Caldwell, 2023). Isotretinoin or (CHEBI:6067) is a type of retinoic acid which is an all-trans-retinoic acid that has a double bond which a α,β- to carboxy group and is isomerised to Z configuration, is recognized as one of the most efficacious treatments for severe cystic acne. Nonetheless, it is associated with numerous adverse effects, such as skin dryness, alopecia, and photosensitivity. Consequently, it is prescribed in specific dosages and for a limited duration by specialized dermatologists for patients with such conditions (Clinic, 2023). The modulatory effect of isotretinoin on molecular pathways such as the *PI3K-AKT-mTOR* pathway is a key aspect of its mechanism of action. A comprehensive understanding of the precise impact of isotretinoin on these pathways would provide valuable insights into its therapeutic efficacy for the treatment of acne. Numerous acne treatments are

accessible, yet their effectiveness may vary depending on the severity of the acne lesions. This segment presents a comprehensive survey of the various categories of acne remedies that exist, encompassing topical agents, oral agents, and physical modalities.

Various topical treatments, including benzoyl peroxide, retinoid, and antibiotics, are available in the form of gels, creams, lotions, and face washes to address specific aspects of acne pathogenesis. These treatments aim to eliminate bacteria that may contribute to the development of acne and unclog pores. (Danby, 2015). The application of benzoyl peroxide and antibiotics is intended to mitigate inflammation and eradicate bacteria, specifically Staphylococcus aureus, with the ultimate goal of diminishing the occurrence of acne. Retinoid, which are derived from vitamin A, have been found to target cell turnover and decrease the formation of comedones, thereby promoting youthful skin for an extended duration (Danby, 2015). Although these therapies have demonstrated significant efficacy in treating mild acne, they should be employed in conjunction with other acne treatments, which will be subsequently elaborated upon, to address severe acne and attain optimal outcomes. Pharmaceuticals administered orally, such as antibiotics, hormonal agents, and isotretinoin, are utilized to target systemic factors. Antibiotics play a crucial role in the eradication of bacteria, including a gram (+) anaerobic bacterium Propionibacterium acnes, which is a significant contributor to skin ailments like acne vulgaris (McDowell 2016). In addition, the utilization of hormonal agents has been found to be efficacious in the management of acne by reducing the synthesis of androgens, which are responsible for the excessive secretion of sebum, leading to the obstruction of hair follicles. Finally, it can be asserted that Isotretinoin is the most effective oral medication for treating acne due to its ability to permanently reduce acne lesions by completely inhibiting the production of oil by the sebaceous glands. According to dermatological clinics, physical therapies such as light therapy, microdermabrasion, and chemical peels are considered alternative methods to address acne (Nestor, 2016). These therapies are typically not the primary treatment option and are instead utilized when other medications have proven to be ineffective. Acne pathogenesis is mediated by various molecular pathways, including the androgen pathway, the Propionibacterium acnes pathway, the insulin/Insulin-like Growth Factor pathway, and the *PI3K-AKT-mTOR* pathway. The androgen pathway encompasses

androgens, including testosterone, which are synthesized in the gonads. This pathway facilitates the generation of sebum from the sebaceous glands, thereby exerting a direct impact on the development of acne. The pathway of Propionibacterium acnes primarily pertains to the excessive proliferation of Propionibacterium, which relies on obstructed follicles for its growth, thereby facilitating the development of acne lesions (McDowell, 2016). The Insulin/Insulin-like Growth Factor pathway has been observed to exert an influence on the development and differentiation of skin cells, as well as the production of excess sebum. This pathway is believed to stimulate the ovaries and adrenal glands to produce androgens, which in turn facilitate the development of acne (Ray, 2022). The *PI3K-AKT-mTOR* pathway is primarily concerned with regulating cell growth, survival, and metabolism in the context of acne. Any disruption to this pathway may result in an overproduction of sebum, abnormal keratinization of follicles, and inflammation, ultimately leading to the development of acne (Ruan, 2020).

Isotretinoin exerts an impact on the *PI3K-AKT-mTOR* pathways by means of downregulating the said pathway. The aforementioned phenomenon can be attributed to the revelation that there is a decline in the activity of sebaceous glands, a proliferation of keratinocytes, and a reduction in inflammation of both the skin tissue and acne lesions. Isotretinoin is an effective treatment for acne due to its modulation of *the PI3K-AKT-mTOR* pathway, which targets various pathogenic factors associated with the disease. This mechanism of action leads to sustained remission and amelioration of acne symptoms.

The *P53* gene, which is widely recognized as a tumour suppressor gene that regulates cellular proliferation and inhibits oncogenic activity, has been implicated in the pathogenesis of acne. The implication of *p53* in the regulation of sebocyte differentiation, apoptosis, and inflammation has been reported in the literature. Consequently, an aberration in the regulation of *p53* has the potential to impact the escalation of sebaceous gland activity and inflammation, which are commonly observed in acne.

Problem Statement

Isotretinoin is a successful pharmaceutical when it comes to the treatment of acne, a common dermatological condition that affects a significant portion of the population. Despite its clinical success, the molecular mechanism underlying the therapeutic effects of Isotretinoin on acne pathogenesis remains not completely understood. Specifically, the involvement of the *PI3K-Akt-mTOR* pathway in the development of acne and the impact of Isotretinoin treatment on gene expression levels as well as activity remain largely unexplored. Having said this, it has come to light that there is a hindrance in the comprehensive understanding of Isotretinoin's mode of action and its potential implications for targeted interventions. Therefore, this study aims to investigate the effect of Isotretinoin on the *PI3K-Akt-mTOR* pathway, assess potential changes in gene expression levels before and after oral Isotretinoin treatment, and shed light on the molecular mechanisms involved in the therapeutic effects of Isotretinoin in acne.

Purpose of the Study

The purpose of this study is to investigate the effect of Isotretinoin on the *PI3K-Akt-mTOR* pathway while assessing the changes in gene expression levels before and after oral isotretinoin treatment. Through the exploration of the different molecular mechanisms of the effect of isotretinoin in acne, this research aims to understand the influence of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression.

Research Questions / Hypotheses

Oral isotretinoin treatment in patients with acne vulgaris leads to significant alterations in the expression levels of genes within the *PI3K-Akt-mTOR* pathway, suggesting that the therapeutic effects of isotretinoin are mediated, at least in part, through modulation of this pathway. Therefore, this urges the question: what is the effect of oral isotretinoin treatment on the expression levels of genes within the *PI3K-Akt-mTOR* pathway in patients with acne vulgaris?

Significance of the Study

The research holds importance for multiple reasons, as it contributes to the comprehension of the therapeutic mechanism of isotretinoin in the management of acne. Additionally, the results of this investigation may pinpoint distinct genes within the *PI3K-Akt-mTOR* pathway that demonstrate varying levels of expression subsequent to isotretinoin administration. This is of significance as these genes may hold promise as a prospective avenue for the management of acne and the optimization of therapeutic interventions, thereby facilitating a more individualized treatment strategy and ultimately an enhanced clinical outcome.

Limitations

The present investigation has encountered a constraint pertaining to the limited sample size, which consisted solely of 44 specimens. The restricted number of participants in this study could have an impact on the applicability of the results and potentially introduce inconsistencies and partiality. Increasing the sample size would have augmented the statistical power and bolstered the validity of the research by affording a more comprehensive representation of the population.

One of the constraints inherent in the design of this study is its observational nature, which precludes randomization or intervention. Hence, the researchers possess restricted authority over the variables that could potentially impact the research findings. This constraint poses a limitation as it elevates the likelihood of confounding factors, including but not limited to the sequence of events and the variability among participants.

The present research design may give rise to potential biases, such as selection bias. This is due to the fact that all participants have been recruited from a single clinic, thereby excluding individuals with acne who are taking isotretinoin from the broader population. As a result, this approach may compromise randomization and limit the generalizability of the findings to the entire population.

Observational studies have the capability to discern associations between two variables, but they are incapable of establishing a causal relationship in the natural world. The

challenge of establishing causality is largely attributed to the significant impact of confounding bias, which impedes the identification of variables that may affect the observed associations and the determination of the actual causal relationship.

In general, this study exhibits certain constraints in its research design, such as the absence of command over variables that could potentially impact results, thereby amplifying the confounding factors and posing challenges in establishing causality. The research design may give rise to potential bias, as clinical trials are susceptible such as selection bias as it is confined to one hospital and a limited set of group in one area. Nonetheless, it is crucial to acknowledge that this research approach can yield significant benefits, such as investigating correlations, formulating conjectures, and examining enduring consequences.

CHAPTER II

Literature Review

Acne Disease

Acne is a widely recognized dermatological condition that arises from obstructed pilosebaceous units located beneath the skin's surface. According to Acne 2023, sebum plays a crucial role in safeguarding the skin from dehydration, whereas the accumulation of deceased skin cells obstructs the pores, culminating in the emergence of lesions, popularly referred to as acne or pimples. Acne predominantly manifests on the facial region; however, it may also manifest on the shoulder and back areas.

The pathogenesis of acne is attributed to the hypersecretion of sebum by the sebaceous glands situated at the follicular base. The surplus production of sebum can obstruct the pores, leading to a favourable milieu for bacterial proliferation (Johnson, 2023). Propionibacterium acnes (P. acnes) is a type of bacteria that is typically found on the skin and has the potential to induce inflammation and the development of pimples and cysts (Bojar, 2004).

The occurrence of acne has been attributed to hormonal fluctuations, which may arise during periods of adolescence or pregnancy. These fluctuations have been observed to stimulate the production of oil and excess sebum, thereby elevating the activity of specific hormones. Additionally, the development of acne may be influenced by genetic factors. Individuals with a family history of acne are more susceptible to developing this dermatological condition.

Acne can be categorized into various types, such as non-inflammatory acne, inflammatory acne, and cystic acne (Saturia,2020). Comedonal acne, commonly referred to as non-inflammatory acne, is characterized by the appearance of blackheads and whiteheads. Inflammatory acne, comprising pustules, papules, and nodules, exhibits distinctive features, including the manifestation of erythematous and edematous lesions. Cystic acne is a variant of inflammatory acne that is characterized by the presence of sizable and distressing cysts.

The standard approach for managing acne involves a combination of pharmacological agents administered topically and orally. The application of topical medications, such as

benzoyl peroxide and retinoids, has been found to be effective in mitigating inflammation and inhibiting the development of fresh acne lesions (staff, 2020). Acne can be treated with oral medications, including antibiotics and oral contraceptives. In instances of heightened severity, a dermatologist may suggest the administration of isotretinoin, an orally ingested medication typically reserved for more severe cases of acne, which has been shown to provide a sustained remission of the condition.

Apart from medical interventions, modifications in lifestyle can also aid in the management of acne. The modifications entail adhering to a nutritious dietary regimen, refraining from consuming oily or greasy edibles, and abstaining from the application of oily or heavy topical preparations. It is imperative to refrain from manipulating acne lesions, as this behaviour may result in the formation of permanent scars and the dissemination of pathogenic microorganisms.

To sum up, acne is a prevalent and intricate dermatological ailment that affects a significant proportion of individuals and results from a confluence of factors, including hormonal fluctuations, heredity, and the proliferation of specific bacteria. The condition is characterized by the occurrence of comedones, namely pimples, blackheads, and whiteheads, in various regions of the body (Plewig & Kligman, 2000). The treatment regimen for this condition involves a combination of topical agents, oral medications, and specific modifications to one's lifestyle and dietary habits (Plewig & Kligman, 2000). Nonetheless, it is crucial to obtain assistance from a qualified expert if the ailment is severe or long-lasting, as it may result in scarring and self-confidence concerns.

Types of Acne Treatments

Topical Medications:

Topical medications are commonly employed to alleviate pain or address various issues such as allergies, bacterial infections, and acne by being applied to a specific area of the body. Topical formulations such as creams, ointments, lotions, gels, and powders are utilized to attain a therapeutic outcome (Russell, 2000). Topical medications are commonly used as a primary treatment for individuals experiencing blackheads,

pimples, and whiteheads. Among the available options, a gel face wash is a popular and easily accessible product. Its versatility allows for concurrent use with other treatments, resulting in an expedited resolution of acne symptoms. The treatment regimen for acne encompasses a range of ingredients, including salicylic acid, retinoids, benzyl peroxide, and antibiotics. Each of these constituents is tailored to address a particular aspect of the condition and is administered in accordance with the specific requirements of the patient. The primary objective of these products is to eliminate blockages in the pores, eradicate any bacterial presence on the skin, and mitigate the occurrence of redness and inflammation. Retinoids, which are primarily recognized as a derivative of vitamin A, facilitate the treatment of acne by promoting cellular regeneration, thereby reducing the occurrence of comedones and also promoting a more youthful skin complexion. Topical antibiotics are a crucial component in the reduction and treatment of bacterial growth on the skin, including pathogens such as Staphylococcus aureus. Some may contend that they are the sole means of achieving this objective (Heather L. Brannon, 2022). Topical medications are highly sought-after in the field of dermatology due to their numerous advantages. These medications offer targeted and localized applications while also exhibiting minimal side effects. Notwithstanding, topical medications may entail adverse effects such as erythema, xerosis, or cutaneous hypersensitivity, and in some cases, individuals may experience allergic responses. This paper aims to provide a comprehensive analysis of the various types of topical medications utilized in the treatment of acne. Additionally, it will examine the efficacy of these medications in addressing the symptoms of acne (Russell, 2000).

Benzoyl peroxide

Benzoyl peroxide (MB) is a topical pharmaceutical agent that is frequently employed for the management of acne. The mechanism of action involves the reduction of the population of P. acnes bacteria on the skin, which is the causative agent of acne (Kleinman, 1963). The reduction of sebum, an oily substance produced by the sebaceous glands, is a mechanism by which pores are unclogged, thereby influencing the development of acne. Multiple concentrations of Benzoyl peroxide are available for the

treatment of acne. It is reported that strengths of 2.5%, 5%, and 10% are commonly used and available in different formulations, including gels, creams, lotions, and facial cleansers (Klienman, 2020). Benzoyl peroxide is widely acknowledged as a safe and efficacious therapeutic agent for managing mild to moderate cutaneous acne. It can be utilized either as a monotherapy or in conjunction with other acne therapeutics such as retinoids or antibiotics. It is advisable to initiate the utilization of benzoyl peroxide at reduced concentrations, such as 2.5% and 5%, and subsequently elevate the concentration in a gradual manner as the skin adapts. Utilizing sun protection is crucial when utilizing benzoyl peroxide due to its potential to augment the skin's photosensitivity.

It is noteworthy that the initial use of benzoyl peroxide may result in dryness, peeling, and irritation. Therefore, it is recommended to apply a thin layer of the product once or twice a day, as per the guidance of a healthcare professional (Clinic, 2022). It is possible for some people to experience an allergic reaction to the medication, which may show up as symptoms like erythema, pruritus, or dermatitis. In the event of such an occurrence, it is advisable to cease usage and seek the guidance of a healthcare practitioner, if deemed necessary.

Retinoids

Retinoids are a category of topical pharmaceuticals that are derived from vitamin A and are frequently employed in the management of acne. It is applied to the face once every day at night, topical retinoids like adapalene, isotretinoin and tretinoin may help in the treatment of mild and moderately severe acne. Such retinoids are in first line treatment for comedonal and inflammatory acne but may take some time before seeing any actual improvement. They may reduce the expression of scarring on the skin caused by acne, as well as treat hyperpigmentation that may have occurred due to inflammatory dermatoses, this works by the inhibition of melanosome transfer and facilitate melanin dispersal. According to Zasada and Budzisz (2019), the mechanism of action of these agents involves the facilitation of exfoliation of the epidermis, clearance of obstructed pores, and mitigation of inflammatory processes. Retinoid possess the added benefit of

stimulating cellular regeneration and collagen synthesis, thereby enhancing the general aesthetic quality of the skin. It has been identified that tretinoin, adapalene, and tazarotene as retinoids that are commonly employed in the treatment of acne (Ema, 2021). Retinol is stored in the liver and is then transported to the tissues by a specific transporter protein, known as retinol binding proteins (RBP), which also holds a binding site for the all-trans retinol molecule (Blaner, 1989). Moving on, transthyretin plays a major role as a physiological transporter of thyroxine and retinol-binding protein, while RBP and retinoids may be stabilizers of Transthyretin. Cellular retinol binding proteins (CRBP) has the affinity for retinol, while cellular retinoic acid binding proteins (CRABP) has the affinity for retinoic acid. CRBP is able to bind to cytosolic CRBP I and II receptors, when CRABP binds to CRABP I and prevents nuclear uptake of retinoic acid in the cytoplasm. On the other hand, CRABP II has different nuclear receptors, tissues and physiological actions. According to Zasada and Budzisz (2019), the mechanism of action of retinoinds involves the binding of these compounds to particular receptors located in the skin, which in turn modulate the expression of genes that play a role in the proliferation and differentiation of skin cells. This aids in the reduction of sebum production, thereby facilitating the unclogging of pores. In addition, retinoids exhibit anti-inflammatory characteristics that aid in the reduction of erythema and edema linked with acne. Retinoids are deemed efficacious for mild to moderate acne and can be utilized either as monotherapy or in conjunction with other acne therapeutics, such as benzoyl peroxide or antibiotics. It is crucial to employ sun protection measures when using retinoids due to their potential to heighten the skin's photosensitivity (DeJohn, 2022).

Salicylic acid

Salicylic acid is a topical medication that is frequently employed for the treatment of acne. According to Frothingham (2022), beta-hydroxy acid (BHA) serves to facilitate the exfoliation of dead skin cells, mitigate inflammation, and alleviate pore blockages. Salicylic acid is commercially available in a range of concentrations, typically ranging from 0.5% to 2%, and is offered in diverse formulations, including gels, creams, lotions,

and facial cleansers (Frothingham, 2022). Analogous to the aforementioned, salicylic acid may elicit certain adverse effects such as erythema, pruritus, and xerosis. Nevertheless, despite their ubiquity, if employed with expert guidance, they can be an effective remedy for acne.

Oral Medications:

Oral medication is typically reserved for moderate to severe acne cases, as it is a more potent treatment option utilized when topical medications have proven to be inadequate. Typically, oral medications are classified into two primary categories, namely antibiotics and hormonal agents. Antibiotics function by reducing the activity of bacteria that contribute to the development of acne vulgaris, with Propionibacterium acnes being the primary causative agent (Heather L. Brannon, 2022). Additionally, antibiotics inhibit the formation of fresh acne lesions. Several antibiotics that fall under this category are tetracycline, minocycline, and doxycycline. Hormonal agents are a commonly utilized oral medication in the treatment of acne. Oral contraceptives, in particular, have been found to be efficacious in managing acne in females experiencing hormonal fluctuations. The therapeutic approach involves the regulation of hormonal levels by mitigating sebum production and the incidence of acne eruptions. Notwithstanding, it is worth noting that the predominant form of oral medication utilized for the treatment of severe cystic acne is isotretinoin, which is a derivative of vitamin A and exerts a permanent reduction in acne. Oral medications are considered highly advantageous due to their ability to effectively reduce the severity of cystic acne. One limitation associated with these medications is the occurrence of skin dryness and hair loss, as well as the challenge of maintaining consistency to achieve optimal results. Additional information will be elaborated upon in subsequent texts.

Oral Contraceptives

Oral contraceptives, commonly referred to as birth control pills, are an oral medication that has been found to be effective in treating acne among women. Their mechanism of

action involves the regulation of androgen hormones, which have been implicated in the pathogenesis of acne. The production of sebum in the skin is attributed to androgen hormones. Elevated levels of these hormones can cause excessive sebum production and obstructed pores, ultimately leading to the development of acne (Cobb, 2018). The mechanism of action involves the inhibition of ovulation and the regulation of the menstrual cycle, thereby mitigating the hormonal fluctuations that may exacerbate acne (Cobb, 2018).

The utilization of combination pills, which comprise both estrogen and progestin, is a prevalent approach for managing acne. However, progestin-only pills are a viable alternative for women who are contraindicated for estrogen administration (Cooper, 2022). Several examples of oral contraceptives include Ethinyl estradiol and norgestimate, Ethinyl estradiol and drospirenone, and Desogestrel and ethinyl estradiol, (Cooper, 2022).

The use of oral contraceptives is contraindicated for certain subpopulations of women with acne, namely those who have a smoking habit, a medical history of thromboembolic events, or comorbidities such as hypertension or diabetes. It is imperative to comprehend that the efficacy of oral contraceptives in treating acne is not immediate and may take a few months to manifest.

Oral Isotretinoin

Isotretinoin is an orally administered medication that is commonly employed in the treatment of various forms of acne, such as severe or cystic acne (Layton, 2009). According to Layton (2009), this substance is a derivative of vitamin A that functions by diminishing the secretion of sebum by the sebaceous glands, resulting in unobstructed pores and decreased inflammation. Additionally, it possesses the ability to decrease the population of Propionibacterium acnes, which is a bacterium that plays a role in the development of acne.

Isotretinoin is commonly prescribed for severe or cystic acne due to its classification as a potent medication with associated adverse effects. Typically, a regimen lasting

between four and six months is prescribed, with dosages being adjusted in accordance with the patient's weight, the severity of their acne, and their individual response to the medication.

Isotretinoin has a molecular formula of $C_{20}H_{28}O_2$, it's IUPAC name is (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl) nona-2,4,6,8-tetraenoic acid. Cis-retinoic acid is a yellow-orange to orange crystalline powder; orange-brown chunky solid (NTP, 1992). Isotretinoin's prescribed dose is 0.5 to 1 milligram (mg) per kilogram (kg) of body weight per day, and is usually taken for 3 to 6 months, however some may undergo a second round according to the recurrence of acne. A counter indication of this treatment are people who suffer from vitamin A sensitivity, retinol sensitivity and tretinoin sensitivity. Moreover, an important counter indication to this treatment is pregnancies, it is not prescribed for women during pregnancy periods as it can have a teratogenic effect on the fetus, causing physiological abnormalities and developmental abnormalities.

The administration of isotretinoin may result in adverse effects such as cutaneous, labial, and ocular dryness. However, these effects can be effectively mitigated through the application of moisturizing agents and ointments. The administration of isotretinoin may result in a transient exacerbation of acne during the initial weeks of treatment, commonly referred to as the "initial breakout" phase. The administration of isotretinoin is contraindicated in pregnant women due to its teratogenic potential. Hence, it is mandatory for women in their reproductive years who are prescribed isotretinoin to employ dual contraceptive measures and undergo periodic pregnancy screenings.

Physical Therapies

Physical medications offer an alternative approach to treating acne, typically involving non-invasive methods that target the development of scars and residue on acne-prone skin while simultaneously reducing the severity of breakouts. Chemical peels are a physical treatment modality for acne that involves the application of chemical agents,

such as glycolic acid or lactic acid, to the skin. The primary objective of this procedure is to mitigate pore size and sebum production while also promoting skin exfoliation and rejuvenation. In addition, physical medications such as light therapy and surgery are employed as treatment modalities. Light therapy employs specific wavelengths to target acne-causing bacteria and facilitate wound healing. Surgical intervention is considered a final resort in the management of acne following unsuccessful attempts with other modalities. This procedure entails the excision of individual acne cysts and pimples using a scalpel. The significance of lifestyle modifications cannot be overstated, as they have a direct influence on both physical and skin health. A healthy diet and lifestyle are crucial factors that are frequently undervalued. Adequate hydration and the avoidance of foods that promote acne can potentially mitigate the development of acne and sebum secretion. The subsequent texts will provide further elaboration on the various categories of physical therapy.

Chemical Peels

Chemical peels are a form of physical intervention that may be employed for the management of acne. The process involves the application of a chemical solution onto the skin, which results in the exfoliation of the uppermost layer of skin cells (Castillo & Keri, 2018). Consequently, this process aids in the elimination of obstructions within the pores, the mitigation of inflammatory responses, and the stimulation of the proliferation of novel dermal cells. Chemical peels are employed in diverse potencies, and the selection and potency of the peel are contingent upon the individual's skin type and the gravity of their acne condition (Castillo & Keri, 2018).

According to Beautysimply1 (2022), there are three primary categories of chemical peels that are utilized for the management of acne. The initial category of peels is commonly referred to as superficial or light peels. These peels employ alpha-hydroxy acids or beta-hydroxy acids as a mild exfoliant for the skin. Subsequently, medium peels employ trichloroacetic acid and/or glycolic acid to achieve deeper penetration into the skin and eliminate impaired cells. Finally, deep chemical peels employ phenol to penetrate the deepest layers of the skin and yield more significant outcomes. Chemical

peels can be utilized either as a monotherapy or in conjunction with other acne therapies, such as topical or oral medications, as previously delineated. These treatments aid in reducing the visibility of acne scars and enhancing the skin's texture and tone. Chemical peels have certain drawbacks, such as inducing skin redness, dryness, and flakiness, as well as heightening the skin's susceptibility to solar radiation. After undergoing a chemical peel, it is crucial to prioritize sun avoidance and the application of sunscreen.

Light Therapy

Phototherapy, also referred to as light therapy, is a physical therapy modality that has been employed in the management of acne (Light Therapy, 2023). The application of diverse light modalities is utilized to specifically target the bacteria accountable for the onset of acne, diminish inflammation, and facilitate the process of wound healing, as described in the literature on light therapy. Numerous modalities of phototherapy are available for employment in the management of acne. Various forms of phototherapy include blue light, red light, and a hybrid of blue and red light.

Blue light therapy employs a distinct wavelength of blue light to specifically target the P. acnes bacteria, which is accountable for the onset of acne (Dai, 2012). Blue light has the ability to permeate the skin and eliminate bacteria, thereby contributing to the reduction of acne outbreaks. Conversely, red light therapy employs a precise wavelength of red light to mitigate inflammation and foster skin rejuvenation. The utilization of blue and red light in combination is employed to effectively address both bacterial growth and skin inflammation. Phototherapy can be utilized as a monotherapy or in conjunction with other therapeutic modalities for acne management, such as topical or systemic pharmacological agents. The treatment is widely acknowledged as a safe and efficacious remedy for acne of mild to moderate severity. The administration of light therapy may result in certain adverse effects, such as transient mild discomfort or erythema; however, these effects are predominantly of a temporary nature.

Phototherapy can be administered in a clinical or salon environment, while certain devices are also accessible for personal use at home. In addition, it is advisable to seek

guidance from a skincare expert prior to engaging in any light therapy and to adhere to the appropriate post-treatment guidelines.

Lifestyle Changes

The implementation of alterations in one's lifestyle has been demonstrated to be a highly efficacious method for the management and mitigation of acne. According to Hickey (2023), certain modifications in one's lifestyle may aid in the prevention and management of acne. Adhering to a nutritious dietary regimen, characterized by the consumption of ample amounts of fruits, vegetables, whole grains, and lean protein, has been shown to facilitate the cultivation of healthy skin. Similarly, refraining from consuming foods that have a high sugar content can aid in mitigating inflammation and averting the occurrence of skin breakouts.

Maintaining adequate hydration levels through the consumption of water can facilitate the elimination of harmful substances from the body and promote the maintenance of healthy skin (Mayer, 2022). It is advisable to consume a minimum of eight glasses of water per day. Furthermore, it has been demonstrated that the management of stress can result in an increase in sebum production, potentially resulting in the development of acne. The implementation of diverse stress management techniques has been found to be effective in mitigating the frequency and intensity of acne. Engaging in activities such as meditation or exercise has been identified as beneficial for reducing stress levels (Mayer, 2022). The avoidance of specific products, such as heavy makeup and certain skin care products, may prevent the obstruction of pores, ultimately reducing the likelihood of experiencing breakouts.

The process of exfoliation can aid in the removal of deceased skin cells and the unclogging of pores (Mayer, 2022). However, it is crucial to exercise caution when exfoliating, as excessive exfoliation may result in irritation and, consequently, an increase in the occurrence of breakouts. Finally, obtaining sufficient sleep is a crucial element not only for general well-being but also for preserving a healthy and well-hydrated appearance of the skin. Insufficient sleep can trigger the production of stress

hormones in the body, thereby increasing the likelihood of experiencing breakouts, as previously noted. The recommended duration of sleep per night is 7 to 8 hours (Hogan, 2023).

Surgery

In the realm of acne treatment, surgery is generally considered a final recourse and is reserved for instances of severe acne that are unresponsive to primary treatment modalities (DocDoc, 2020). Various surgical procedures can be employed for the management of acne. One of the primary methods for treating acne is through a procedure known as acne excision, which entails the surgical removal of individual acne cysts or nodules using a scalpel. Commonly employed for instances of severe cystic acne. Additionally, a common method for addressing acne is through a procedure known as acne extraction, which involves the use of a comedone extractor to remove blackheads and whiteheads from the skin. This treatment modality is commonly employed for acne cases that range from mild to moderate in severity.

Additionally, acne surgery involves the amalgamation of acne excision and acne extraction techniques. Cysts and comedones are both present in cases of acne, which calls for the use of this treatment option. Subsequently, laser therapy is employed to specifically target the bacteria accountable for the onset of acne, diminish inflammation, and facilitate the process of wound healing. This treatment modality is indicated for individuals with moderate-to-severe acne. Finally, subcision is a medical intervention that entails the fragmentation of fibrous tissue with the aim of enhancing the aesthetic quality of acne scars. This treatment modality is commonly employed for managing acne scars that range from moderate to severe in intensity (DocDoc, 2020). It is noteworthy that surgical procedures can give rise to unfavourable outcomes such as erythema, edema, and cicatrisation, and their appropriateness may not be universal.

Prior to undergoing any surgical intervention for acne treatment, it is advisable to seek consultation with a qualified dermatologist or plastic surgeon.

Molecular Pathways Involved in Acne Disease.

Multiple different pathways play a role in the development of acne on the skin, Androgen pathway, the Propionibacterium acnes pathway, the Insulin/Insulin-like Growth Factor pathway, and the *PI3K-AKT-mTOR* pathway, are examples of different pathways that contribute in the growth of acne lesions (Taylor, 2011).

To begin, the first pathway that will be covered is the androgen pathway that includes androgens like testosterone, that exert the production of sebum from the sebaceous glands that lead to clogged pores and oily skin. An excess of the production of sebum contributes to the development of acne. The Propionibacterium acnes pathway is interesting because unlike the others, it revolves around the overgrowth of bacteria called Propionibacterium acnes on the skin. Such bacteria depend greatly on clogged hair follicles to grow while also producing some substances which specialise in the trigger of an immune response, contributing in the formation of inflamed acne lesions (Taylor, 2011). Another pathway is the Insulin/Insulin-like Growth Factor pathway, hence the name, it contains insulin that promote the production of sebum and skin cells. Insulin and insulin-like growth factors may sway the sebaceous glands, which lead to an excessive increase of sebum production. Furthermore, they may also influence the growth and differentiation of skin cells, contributing to the development of acne. Last but not least, the *PI3K-AKT-mTOR* pathway is an important factor in the development of acne because it is involved in the regulation of various cellular processes, like cell growth and survival (Taylor, 2011). Dysregulation of this pathway may lead to an increased sebum production, abnormal follicular keratinization, and inflammation, all of which play a role in acne development.

Androgen Pathway

Androgen Pathway is a type of pathway that is involved in the development of acne, specifically in the management of sebum production. Androgens are hormones that form from testosterone and its more potent derivative, dihydrotestosterone (DHT). Such hormones affect the pathogenesis of acne as well as sebum production and sebaceous gland activity (Naamneh, 2022). Sebaceous glands are responsible for the production of

sebum, and present on the glands are androgen receptors. This is true when these androgens bind to their receptors on the gland cells, they begin to initiate the secretion of sebum. Because sebum is more or less the only substance that is oily, it is able to lubricate the skin that in turn, contributes to acne formation in excessive forms.

Many factors can lead to skin oiliness. One of them is an increase of androgen levels. Such fluctuations can be because of hormones occurring in puberty or during the menstrual cycle (Wakelin, 2012). However, more specific fluctuations can occur because of medical disorders such as polycystic ovary syndrome (PCOS). The higher the androgen levels are the more the sebaceous glands produce sebum, consequently leading to oilier skin (Naamneh, 2022).

Sebum composition contains triglycerides, fatty acids, and wax esters. Any alterations in these secretion levels can contribute to the progression of acne. This is true because any increased levels or excessive levels of sebum are able to clog the hair follicles and trap the shredded dead skin cells, which in turn, can cause whiteheads and blackheads on the skin.

The importance of follicular hyper-keratinization is it occurs during microcomedone and subsequent acne lesions. This happens when the cells of the follicle become cohesive and is not able to shed normally. However, androgens help in the regulation of keratinocyte proliferation and differentiation. Therefore, any changes like an increase of androgen levels can consequently lead to abnormal follicular keratinization in this is worrying as it defeats the lining of the hair follicles, causing them to become sticky. The sticky cells are able to clog the follicles and lead to the formation of acne. The importance of the excessive presence of sebum, together with blocked follicles, creates an environment that encourages the growth of P. acnes, a bacterium that is associated with acne. P. acnes is very crucial because it leads to the discharge of pro-inflammatory molecules. These include chemokines and cytokines that merely lead to the trigger of an inflammatory response. This response will also contribute to the development of skin lesions and acne lesions like nodules, pustules, and papules.

Propionibacterium Acnes (P. acnes) Pathway:

The Propionibacterium acnes (P. acnes) pathway has been identified as a molecular pathway that is linked to the onset of acne. Propionibacterium acnes is a microorganism that typically inhabits the integumentary system, with a specific predilection for the sebaceous follicles (Leheste, 2017). The presence of P. acnes on the skin is a common occurrence, owing to its status as a member of the skin microbiota. However, certain factors can contribute to its proliferation and consequent involvement in the pathogenesis of acne. The colonization of sebaceous follicles by P. acne, in conjunction with the presence of sebum, creates an optimal environment for the proliferation of P. acne colonies. This is due to the provision of nutrients that facilitate their growth and sustenance. In addition, the impact of P. acne on sebum production via sebaceous glands facilitates the conversion of triglycerides into fatty acids, thereby promoting a conducive milieu (Leheste, 2017).

The release of P. acne and its associated substances, such as proteases and lipases, can elicit an inflammatory response that triggers the activation of immune cells, such as macrophages and neutrophils. These immune cells subsequently produce a variety of inflammatory molecules, including cytokines and chemokines, as part of their activation response. Toll-like receptors (TLRs) are also implicated in the innate immune response. The activation of TLRs is attributed to the impact of P. acne on the synthesis of cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF-alpha) (Leheste, 2017).

The inflammation of acne lesions triggers the immune response, which causes the release of inflammatory molecules that affect the development of acne lesions. The aforementioned lesions are characterized as nodules, pustules, and papules. In addition, P. acne exerts a crucial impact on follicular hyperkeratinisation, whereby the bacterium facilitates the liberation of keratinocytes that contribute to the pathogenesis of acne lesions.

The Insulin/Insulin-like Growth Factor 1 (IGF-1) Pathway

The pathway involving Insulin/Insulin-like Growth Factor 1 (IGF-1) plays a pivotal role in the development of acne. The aforementioned pathway encompasses hormones such as Insulin and IGF-1, which regulate various cellular processes that facilitate the onset of acne. The pathogenesis of acne can be attributed to the dysregulation of this particular pathway (Field, 2008).

The mechanism of action of Insulin and IGF-1 Receptors involves the binding process to particular receptors located on the surface of target cells. The insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) are present on both sebaceous glands and hair follicles.

Insulin and IGF-1 facilitate the activation of sebaceous gland function and lipogenesis, which contribute to the development of sebum production. Activation of the insulin receptor (IR) and the insulin-like growth factor 1 receptor (IGF-1R) results in upregulation of gene expression involved in sebum synthesis. Possible academic rewrite: The molecular factors involved in this process comprise SREBP-1, a transcription factor that binds to sterol regulatory elements, and PPAR, a nuclear receptor that regulates lipid metabolism and adipogenesis. The escalation of insulin and IGF-1 concentrations is associated with a rise in sebum production, which consequently exerts a direct influence on the onset and progression of acne. The impact of insulin and IGF-1 on androgen metabolism can significantly affect the metabolic rate by inducing an elevation in androgen levels. As a result, excessive production of androgens and testosterone in the ovaries can trigger an increase in the activity of the sebaceous glands, culminating in the development of acne (Field, 2008).

The follicular hyper-keratinization of insulin and IGF-1 may affect the growth and differentiation of keratinocytes, which are responsible for lining the follicular hairs. Furthermore, elevated levels of insulin and IGF-1 may result in an atypical proliferation of keratinocytes (Field, 2008). This accumulation of keratinocytes on the skin's surface, along with dead skin cells and sebum, can obstruct hair follicles and give rise to comedones.

The production of pro-inflammatory cytokines, namely interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-alpha), may arise due to the inflammatory response to insulin and IGF-1. The manifestation of an inflammatory skin ailment characterized by erythema and edema of acneiform lesions

***PI3K-Akt-mTOR* Pathway**

The *PI3K-Akt-mTOR* pathway is a crucial signalling pathway that has a significant impact on the regulation of cellular growth, proliferation, metabolism, and viability (Porta, 2014). The activation of the pathway occurs via the binding of growth factors, such as insulin growth factor 1 (IGF-1) and epidermal growth factor (EGF), to their respective receptors located on the cell surface (Porta et al., 2014). Upon binding, PI3K is activated, leading to the phosphorylation of the lipid phosphatidylinositol 4,5-bisphosphate (PIP2) and the subsequent production of phosphatidylinositol 3,4,5-trisphosphate (PIP3). Upon activation, PIP3 facilitates the recruitment of *Akt*, a serine/threonine kinase that modulates several downstream targets.

The mammalian target of rapamycin (*mTOR*) is a kinase that plays a vital role in regulating protein synthesis, cell growth, and proliferation and is considered to be one of the primary downstream targets of *Akt* (Porta, 2014). Upon activation by *Akt*, *mTOR* gives rise to two discrete complexes, namely *mTORC1* and *mTORC2*. The *mTORC1* pathway plays a crucial role in promoting cellular growth and proliferation by regulating the process of mRNA translation into proteins and stimulating the synthesis of lipids and nucleotides while integrating signals from growth factors and hormones. The activation of *mTORC1* may consequent into the phosphorylation of downstream targets involved in protein translation, ribosome biogenesis, and autophagy and lipid synthesis. In contrast, *mTORC2* governs the cytoskeleton and cellular viability as well as metabolism, it also plays a major role in the haemostasis of cells and contributes to the regulation of cell size, proliferation and survival. It is able to respond to growth factors and insulin signalling, while controlling the phosphorylation of specific kinases such as *Akt*. The *PI3K-Akt-mTOR* pathway has been implicated in the pathogenesis of various ailments, including but not limited to cancer, diabetes, and neurological disorders. The occurrence

of mutations in genes that encode components of the pathway, including *PIK3CA* (which encodes the p110α subunit of *PI3K*) and PTEN (which encodes a phosphatase that dephosphorylates PIP3), is frequently observed in various cancer types. These mutations can result in hyperactivation of the pathway (Grüninger, 2022). The hyperactivation of the *PI3K-Akt-mTOR* pathway has been observed to facilitate cellular survival and proliferation, hinder apoptosis, and escalate angiogenesis and metastasis, thereby fostering the advancement and expansion of tumorous growth.

The *PI3K-Akt-mTOR* pathway has garnered significant attention as a potential target for the development of novel cancer therapies, owing to its pivotal role in regulating cellular growth and survival (Grüninger, 2022). Various pharmaceutical agents that selectively target constituents of the aforementioned pathway, including but not limited to inhibitors of *PI3K, Akt, and mTOR*, are presently undergoing clinical evaluation as potential therapeutic modalities for diverse malignancies. Nevertheless, the intricacy of this pathway and the possibility of unintended impacts make it challenging to specifically aim for it (Porta, 2014). Hence, there is a requirement for additional investigation to enhance comprehension and advance the pathway with efficacious and focused therapeutic interventions.

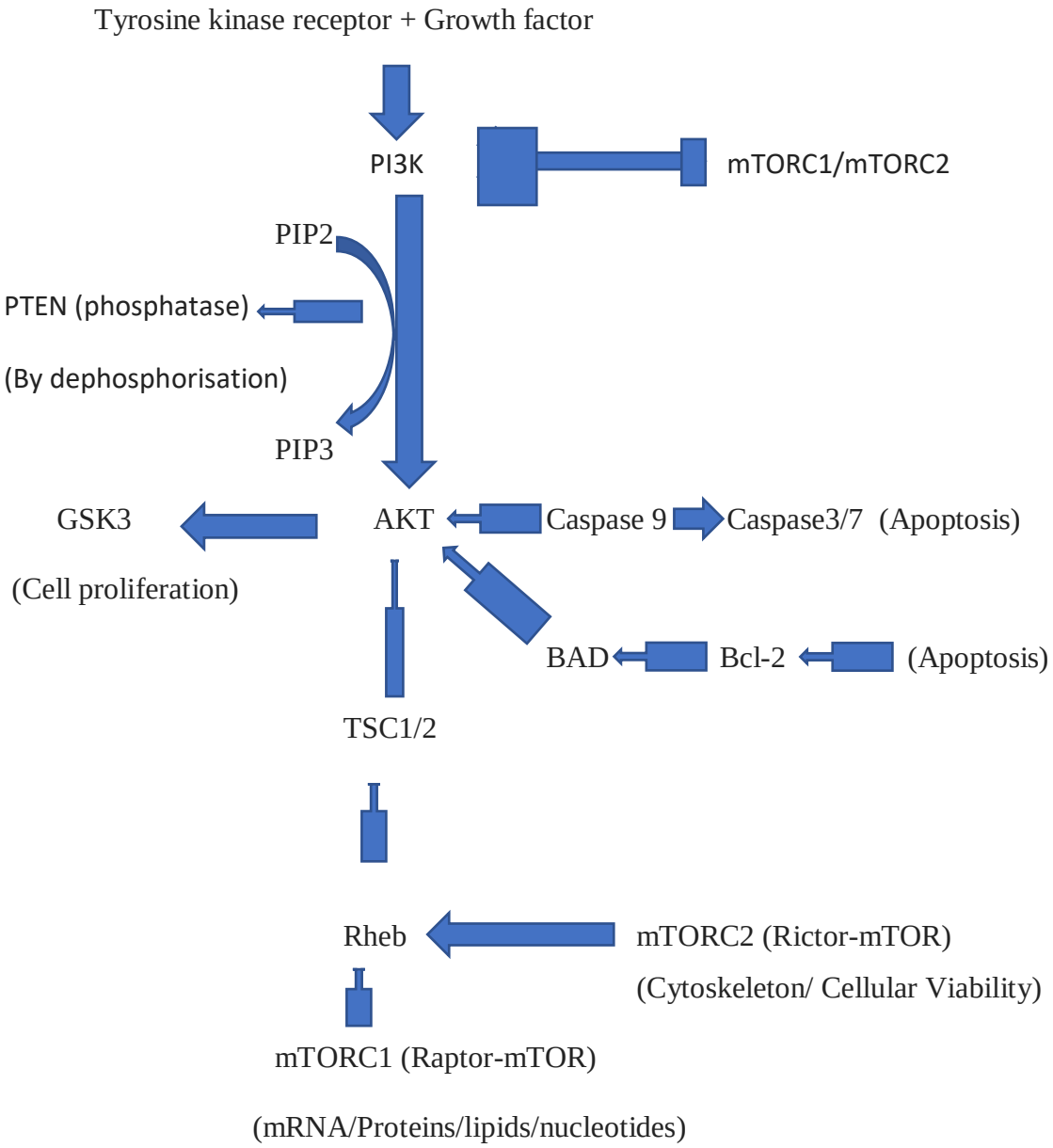

Figure 1 *PI3K/AKT/mTOR Signalling in Eukaryotes*

P53 and Acne Disease

P53 is a gene responsible for suppressing tumours, playing a crucial role in regulating cell proliferation and preventing the development of cancer (Medicine, 2020). The production of the *p53* protein, which is a transcription factor that regulates the expression of target genes by binding to particular DNA sequences, is facilitated by this gene. The activation of *P53* is triggered by various indicators of anomalous cells, such as DNA damage, cellular stress, and other related factors. Upon activation, *p53* facilitates the deceleration and cessation of cellular proliferation through the induction of apoptosis, cell cycle arrest, and DNA repair. Additionally, it has been reported that *p53* plays a crucial role in regulating cellular differentiation and maintaining the integrity of the genome (Molchadsky, 2010).

Mutations in the *P53* gene are frequently observed in various types of cancer and are commonly known as "the guardian of the genome" (Tontonoz, 2019). Various forms of mutations can manifest, such as missense mutations, frameshift mutations, and insertions or deletions. Damage to the DNA turns on kinases called ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related). Subsequently, these kinases proceed to phosphorylate and subsequently activate the p53 protein, which functions as a suppressor of tumorigenesis. Upon activation, the p53 protein coordinates a sequence of processes that encompass the halting of the cell cycle and the initiation of DNA repair mechanisms. The p53 protein initiates the upregulation of p21, a protein that exerts inhibitory effects on cyclin-dependent kinases (CDKs), which are accountable for the progression of the cell cycle. The observed inhibition leads to a cessation of the cellular cycle during the G1 phase, thereby creating a window for DNA repair mechanisms to address the incurred damage. In the event that the DNA damage surpasses the capacity for repair, p53 has the ability to induce apoptosis through the activation of particular genes associated with programmed cell death. The ATM protein is activated in response to DNA damage. The occurrence of non-functional or dominant-negative *p53* proteins can result from these mutations, thereby impeding their ability to bind to DNA and regulate gene expression. Consequently, neoplastic cells can circumvent the typical cellular pathways of growth control and acquire the ability to achieve immortality. The p53 protein is able to interact with the retinoblastoma protein (Rb) at the molecular

level. This means that it can help control different cellular processes. Protein-protein interactions allow the p53 protein to interact with Rb in a specific way. This leads to the formation of a complex that plays a key role in controlling the progression of the cell cycle and keeping the stability of the genome. Different protein domains and binding motifs help the p53 and Rb proteins work together. This makes it possible for a stable complex to form between the two proteins. During this interaction, the p53 protein has an effect on the Rb protein. This affects the Rb protein's activity and the signalling pathways that control the cell cycle and stop tumours from forming. The interaction between p53 and Rb shows how complex the molecular mechanisms are that make up cellular processes. It also shows how important these mechanisms are for maintaining cellular homeostasis and stopping abnormal cell growth. The inhibition of cell division is a crucial function of a key regulator of the cell cycle (Hardwick, 2013). The protein *P53* is known to engage in interactions with the Bcl-2 protein family, which play a crucial role in the regulation of apoptosis, as per the findings of Hardwick and Soane (2013). It is known that the p53 protein has a big effect on how angiogenesis is controlled. Angiogenesis is the physiological process that causes new blood vessels to grow out of and change into existing ones. The role of p53 in the process of angiogenesis is complex and involves both promoting and inhibiting the formation of new blood vessels. In certain situations, p53 can stop angiogenesis from happening by directly repressing the expression of pro-angiogenic factors like vascular endothelial growth factor (VEGF). This makes it harder for new blood vessels to form. Furthermore, the p53 protein has the ability to indirectly regulate angiogenesis through its control over the expression of various factors that have an impact on the processes involved in angiogenesis. In contrast, it has been observed that p53 can facilitate angiogenesis in specific circumstances. It can cause the body to make more anti-angiogenic factors, like thrombospondin-1, which can stop the process of angiogenesis by making it harder for endothelial cells to do their jobs. Also, p53 can affect the production of other regulatory molecules that play a role in the process of angiogenesis. These molecules include both inhibitors and activators of angiogenic growth. The important role of p53 in angiogenesis shows how important it is in controlling this complicated process, which has effects in body health, such as tumor growth, wound healing, and heart problems.

Related Research

In the previous years, high end research has been conducted in order to further understand the molecular mechanisms underlying the therapeutic effects of isotretinoin and its impact on the *PI3K-Akt-mTOR* pathway in the context of acne vulgaris. One relevant study published in the National Library of medicine in 2022, called 'Isotretinoin treatment upregulates the expression of *p53* in the skin and sebaceous glands of patients with acne vulgaris', explored the effect of isotretinoin on *p53* expression. It has been found that isotretinoin treatment led to an increase in *p53* expression in both the skin and sebaceous glands of acne vulgaris patients. This finding shows a potential role of *p53* in the therapeutic mechanism of isotretinoin, as *p53* is a main regulator of apoptosis processes that have been proven to be important in the treatment of acne vulgaris.

Furthermore, a study named 'The *PI3K-Akt-mTOR* and Associated Signalling Pathways as Molecular Drivers of Immune-Mediated Inflammatory Skin Diseases: Update on Therapeutic Strategy Using Natural and Synthetic Compounds', published in 2023, offers a broader perspective on the *PI3K-Akt-mTOR* pathway in immune-mediated inflammatory skin diseases. This review article emphasizes the pivotal role of the *PI3K-Akt-mTOR* pathway in driving inflammatory responses in various skin disorders, including acne vulgaris. It discusses the intricate network of signalling pathways involved while highlighting the therapeutic potential of natural and synthetic compounds that target these pathways. Although this article does not specifically focus on isotretinoin, it covers the importance of the *PI3K-Akt-mTOR* pathway in skin diseases and provides valuable insights into further therapeutic strategies.

Moreover, an intriguing study titled "The increase of *mTOR* expression is consistent with *FoxO1* decrease at gene level in acne but not in psoriasis" explored the expression of *mTOR* and *FoxO1* in acne vulgaris and psoriasis. An upregulation was observed in the *mTOR* expression in acne vulgaris patients, accompanied by a decrease in FoxO1 expression at the gene level. This finding suggests that dysregulation of the *mTOR* pathway may have a role in the pathogenesis of acne vulgaris. While not directly investigating the effects of isotretinoin, this study provides important insights into the molecular alterations associated with acne vulgaris and highlights the relevance of the *mTOR* pathway in the context of this skin condition.

Through the incorporation of these studies into the broader context of this thesis, it is has been helpful to be able to establish the significance of investigating the impact of oral isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression in acne vulgaris patients. The evidence from these articles suggests that isotretinoin treatment may adjust the *p53* expression, highlighting a potential mechanism underlying its therapeutic effects. In addition, the above studies were able to emphasize the major role of the *PI3K-Akt-mTOR* pathway in skin diseases while also offering a potential therapeutic strategy targeting this certain pathway. Altogether, the above findings contribute to the growing body of research on isotretinoin and the molecular mechanisms involved in acne vulgaris, supporting the importance of further investigating the impact of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression in acne vulgaris patients.

CHAPTER III

Methodology

Sample Collection

The samples were obtained from Near East Hospital of Dermatology, after it was approved by the Near East University Scientific Research Ethical Committee (project no. NEU/2022/107-1625). The samples obtained were peripheral blood samples from patients that used oral Isotretinoin to treat acne disease and sorted into EDTA tubes. A total of 44 samples, which included 22 before and 22, 15 of whom are female and 7 of whom are male, for a span of approximately 4 months. The participant's ages ranged between 18 to 35 years and they did not have any underlying chronic diseases while on this treatment. This research was conducted with informed consent from all patient participants through written forms and signatures.

RNA ISOLATION

The process of extraction was carried out utilizing the TRIZOL reagent (manufactured by Hibrizol, Hibrigen, Istanbul, Turkey). The initial step in RNA extraction involves the addition of 500 microliters of TRIZOL reagent, which serves to chemically disrupt the cellular membrane. Subsequently, introduce 100µl of chloroform to the mixture and agitate using a vortex for a duration of 15 seconds. Allow the solution to remain at ambient temperature for approximately two to three minutes. Additionally, subject the specimen to centrifugation at a force of 12,000 times the acceleration due to gravity for a duration of 15 minutes at a temperature of 3 degrees Celsius. Following the process of centrifugation, three distinct phases were observed, namely the organic phase, interphase, and aqueous phase, each exhibiting unique characteristics. The proteins and lipids are contained within the organic phase, while the interphase is comprised of DNA, and the RNA is held within the aqueous phase. Subsequently, carefully transfer the upper aqueous solution to a new tube, taking care not to disrupt the interphase. A volume of 250 µl of Isopropyl alcohol was utilized for the precipitation of RNA that was present in the aqueous phase, followed by an incubation period of 10 minutes within a temperature range of 15 to 30 degrees. Following the completion of the incubation

process, the samples underwent centrifugation at a temperature of 3 degrees Celsius for a duration of 10 minutes at a force of 12,000 xg. Occasionally, the RNA that has been precipitated may not be discernible to the naked eye prior to centrifugation and instead may coalesce into a pellet with a gel-like consistency at either the bottom or the periphery of the tube. The supernatant should be discarded, and a 1:1 ratio of 75% ethanol to trizol reagent should be added for the purpose of initial homogenization. The resulting solution should be vortexed and centrifuged at 7,500 xg for 5 minutes at a temperature range of 2 to 8 degrees Celsius. Repetition of the aforementioned washing procedure is crucial for attaining a product of high purity. In order to obtain RNA of high purity, it is necessary to eliminate the ethanol and subject the sample to a period of desiccation with dry air, lasting between 5 and 10 minutes. The final stage involves the elution of RNA through the addition of 20 microliters of distilled water that is free from DNase and RNase.

COMPLEMENTARY DNA (cDNA) SYNTHESIS

The cDNA synthesis process involves the utilization of the ABM One Script Plus cDNA synthesis kit, which is commercially available from the ABM business located in Richmond, Canada. The package comprises One Script Plus reverse transcriptase, One Script Plus RT reaction buffer, Oligo(dT) primer, dNTPs mix, and anchored oligo(dT) primer, which were stored within a temperature range of -15 to -25 ℃. In the experimental procedure, 10µl of RNA, 1µl of oligo(dt) primers, 1µl of reverse transcriptase enzyme, 1µl of deoxyribonucleotides triphosphate (dNTPs), 4µl of buffer solution, and 3µl of nuclease-free water were employed for each sample. Following the addition of all the necessary components into the reaction tubes, conventional PCR was employed to facilitate the synthesis of cDNA from RNA via a reverse transcription mechanism. The reaction tubes were subsequently incubated at a temperature of 55°C for 15 minutes.

Primer Optimization for Gradient PCR

During the optimization process, a stock of oligomers for each of the three genes was prepared for the primer. The stock primer specifies a precise quantity of deionized water, which was subsequently diluted to a 10 µM working solution by adding 10 µl of stock primer to 90 µl of deionized water. The primers that were used in gradient PCR were *mTOR, AKT1, PIK3* and *P53*. Gradient PCR was performed using the Bio-Systems 96-well thermal cycler PCR to distinguish the various optimal temperature conditions for qRTPCR. The gradient polymerase chain reaction (PCR) was conducted for each of the four genes specified above at a temperature range of 56 °C to 61 °C. Table 1 displays the results of the calculations performed on six samples and one negative control.

Table 1 *The Calculation for gradient PCR mixture.*

Component	**1X**	**7X**
TaqMIx	12.5µl	87.5µl
Forward Primer	1.25µl	8.75µl
Reverse Primer	1.25µl	8.75µl
DH2O	2µl	14µl

During the experimental protocol, a total of 20µl was transferred into the Eppendorf PCR tubes, consisting of 17µl obtained from the final mixture and an additional 3µl of cDNA. The aggregate amount pertained to six specimens and one negative control. The experimental procedure was replicated for all four genes, utilizing primers that were specific to each respective gene. The experiment presented in Table 1 utilized a range of annealing temperatures spanning from 56 °C to 61 °C for the gradient PCR.

Table 2 *Optimum Conditions used for gradient PCR.*

	Stage	Temperature	Time	Time
	Initial denaturation	95℃	5 mins	1 cycle
	Denaturation	95℃	15 sec	35 cycles
steps	Annealing	56℃ - 61℃	30 sec	
	Extension	72℃	45 sec	
	Termination	72℃	5 mins	1 cycle

Quantitative-PCR (RT-qPCR)

The PCR thermal cycler and RT-qPCR reactions were conducted under sterile conditions. The task was accomplished utilizing a laminar flow hood of category II. The regents and plasticware underwent sterilization prior to their use in this procedure. The table below displays 44 samples of the RT-qPCR master mix.

Table 3 *Calculation for RT-qPCR mixture for mTOR, AKT1, PIK3 and P53.*

Component	1X	Mixture Volume for 45X
SYBR GREEN	10 µl	450 µl
Forward Primers	1 µl	45 µl
Reverse Primers	1 µl	45 µl
DH20	6 µl	270 µl

Table 4 *The optimum condition for qRT-PCR at different stages was recorded and presented in*

	Stage	**Temperature**	**Time**	**Time**
	Initial denaturation	95℃	2 mins	1 cycle
	Denaturation	95℃	30 seconds	30 cycles
steps	Annealing	57℃	30 seconds	
	Extension	72℃	45 seconds	
	Termination	72℃	10 mins	1 cycle

Statistical Analysis

The statistical analysis was conducted using SPSS software (Statistical Package for the Social Sciences 25.0, SPSS Inc., Chicago, IL, USA). The data was presented in the form of a mean ± standard error (SE). The gene expression data was acquired in the form of Cycle Threshold (CI) values, where C1 represents the cycle number at which logarithmic PC plots intersect a calculated threshold line. The 2ΔΔCT method was employed to compare the expression of each gene between depots. In this method, the ΔΔCT value is calculated by subtracting the C1 value of the target gene from the CI value of the housekeeping gene. The study conducted a comparison between normally distributed continuous variables and abnormally distributed continuous variables using the Student's t-test and the Mann-Whitney U test, respectively. A p-value of less than 0.05 will be considered statistically significant.

CHAPTER IV

Findings and Discussion

The expression profiles of *mTOR, AKT, p53* and *pik3* genes among in study group were evaluated (Figure 1). All studied gene expression levels were found to increase after treatment in this clinical trial. We observed that the expression levels of *Akt* (2.3-fold) and *p53* (≈2.5-fold) genes were significantly increased in patients who got treatment compared to the before treatment (***p=0.027, p=0.017,*** respectively). Although, the expression levels of the *mTOR* and *pik3* genes were found to have been slightly high in patients who getting treatment compared to the before treatment, however there was no statistically significant differences were reached (p>0.05, respectively).

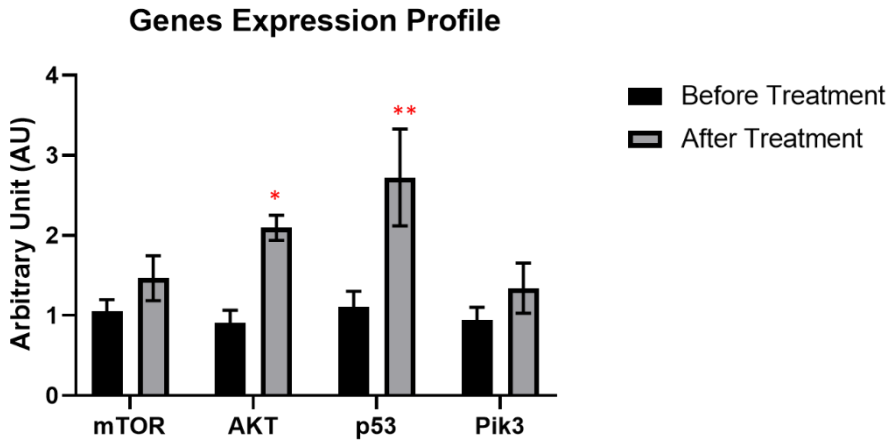

Figure 2 *gene expression profile*

Figure 1: Relative expression levels of *mTOR, AKT, p53* and *Pik3* in study group.

The values are calculated using Mann–Whitney U test. p-value ≤ 0.05 is considered statistically significant. **** p=0.027*** and *****p=0.017***

Correlational studies are conducted with the purpose of examining potential associations between variables. Before conducting an analysis on the association between two

quantitative variables, specifically the expression levels of two distinct genes, it is customary to construct a visual representation known as a scatterplot. This graphical depiction encompasses both variables and facilitates the examination of their relationship.

A scatterplot is a graphical representation that illustrates the association between two quantitative variables, specifically the expression levels of two distinct genes, which have been measured for a common set of individuals. The horizontal axis represents the values of one variable, while the vertical axis represents the values of the other variable. Every data point on the graph represents an individual entity.

The correlation coefficient, denoted as r, quantifies the degree of linear association between two variables of a quantitative nature.

Correlation analysis involves the estimation of a sample correlation coefficient, denoted as r, which is specifically known as the Pearson product-moment correlation coefficient. The sample correlation coefficient, denoted as r, is a statistical measure that falls within the range of -1 to +1. It serves to quantify both the direction and magnitude of the linear relationship between two variables. The relationship between two variables can exhibit positive correlation, where higher levels of one variable are linked to higher levels of the other, or negative correlation, where higher levels of one variable are associated with lower levels of the other(Mindrila & Balentyne, 2013) .

Table 5. *The listed criteria below allows for the measure of strength, (comparing absolute r value) between the relationships of two variables.*

Absolute value of r	Strength of Relationship
$r \leq 0.3$	None or very weak
$0.3 \leq r \leq 0.5$	Weak
$0.5 \leq r \leq 0.7$	Moderate
$r \geq 0.7$	Strong

To Calculate a Pearson correlation coefficient, an assumption is required that the relationship between two variables is linear; Extreme values r=1 and -1 only occur in the case of a perfect linear relationship.

When examining the relationships between variables, multiple factors are taken into consideration. The correlation coefficient, which quantifies the extent of the association between the variables, evaluates the strength of the relationship. Additionally, the correlation coefficient's sign determines whether associations are positive or negative when determining the direction of the relationship. Furthermore, an analysis is conducted to assess the nature of the relationship, with particular emphasis on linearity, as the presence of a linear relationship is a necessary condition for calculating the Pearson correlation coefficient. The p-value, which determines the likelihood that the observed relationship resulted from random chance, serves to determine the statistical

significance of the relationship. These four factors offer a comprehensive comprehension of the nature and importance of relationships among variables.

Correlation coefficients are associated with a p-value, which represents the probability of observing a relationship between two variables that is equal to zero, indicating the null hypothesis of no relationship. Low p-values, which imply a low probability of the absence of a relationship between the variables, indicate significant correlations. In the present investigation, p-values that are less than or equal to 0.05 are regarded as indicators of statistical significance for the observed relationship.

The correlation analyses were done between expression levels of studied genes in this study by the Spearman correlation test (Figure 2 and Figure 3). The results showed that the *mTor* gene expression was positively correlated with *pik3* gene expression in patients who were getting treatment (*r=0.486,* ***p=0.002***) (Figure 2). Nonetheless, the expression of *mTOR* gene was negatively correlated with *p53* expression in patients who got treatment (*r=-0.349,* ***p=0.045****)* (Figure 3).

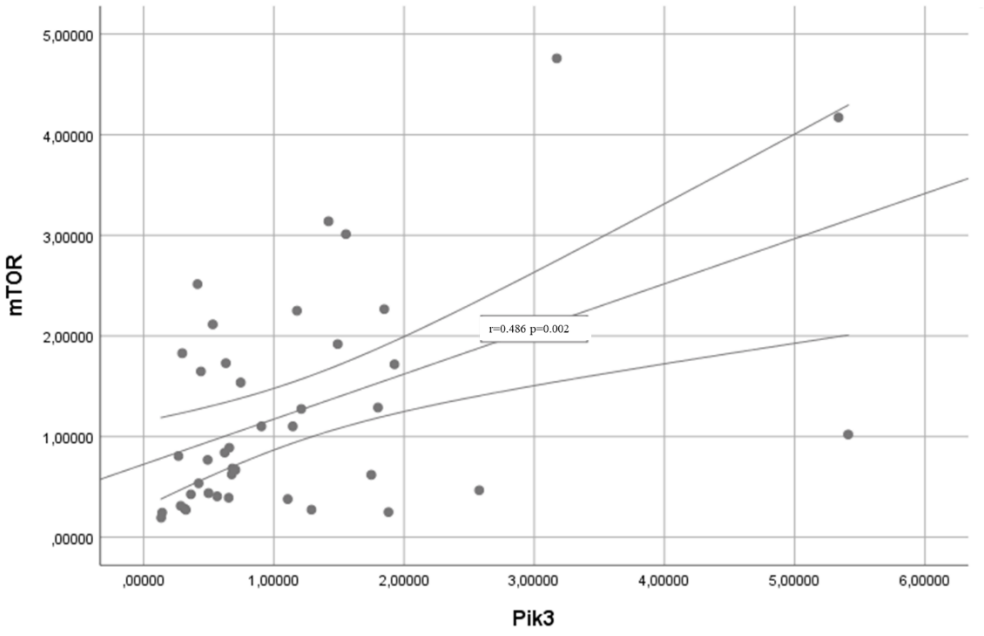

Figure 3 *The correlation analysis between relative expression levels of mTOR, and pik3 in study group.*

The values are calculated using Spearman correlation test. p-value ≤ 0.05 is considered statistically significant. *r=0.486 and* ***p=0.002***

Based on the criteria listed in Table 1, the value of r in this case indicated that there was a positive, linear relationship of weak strength between *mTor* gene expression and *pik3* gene expression in patients who were getting treatment (*r=0.486, p=0.002*)

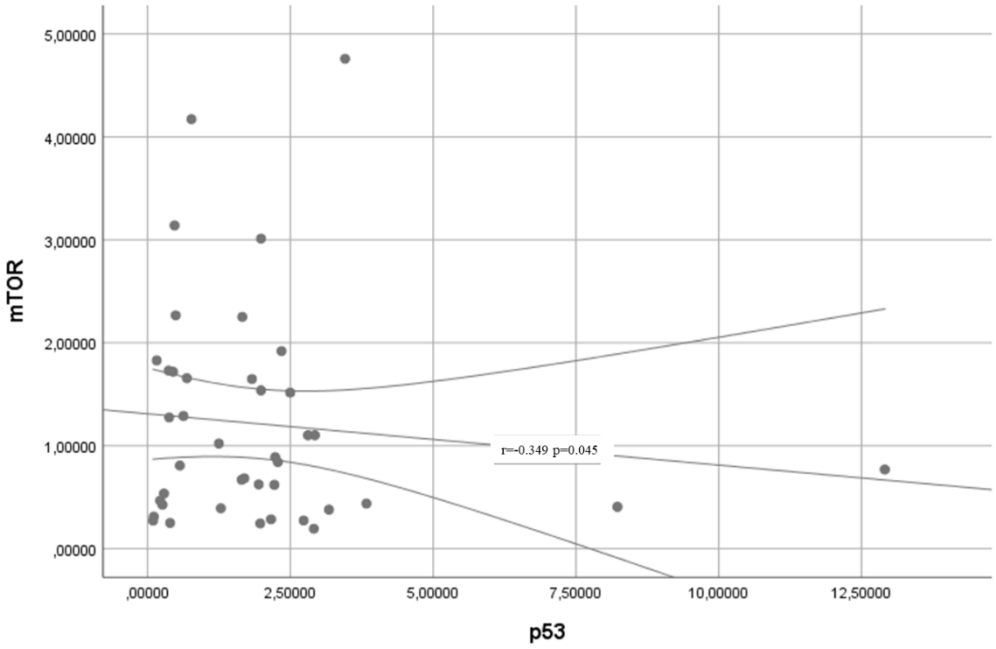

Figure 4: *The correlation analysis between relative expression levels of mTOR, and p53 in study group.*

The values are calculated using Spearman correlation test. p-value ≤ 0.05 is considered statistically significant. *r=-0.349 and* ***p=0.045***

Based on the criteria listed in Table 1, the value of r in this case indicated that there was a negative, linear relationship of weak strength between mTor gene expression and p53 gene expression in patients who were getting treatment (r=-0.349 and p=0.045.)

The presence of outliners in both figures 2 and 3 has the potential to significantly impact the observed correlations. In the context of small populations, outliers can have a

significant impact on the observed relationships. It is evident that the value of r will vary if the outliner is excluded in either figure 2 or figure 3. In general, larger populations, or sample sizes, tend to mitigate the impact of extreme observations. The Pearson assumptions can offer support for associations, specifically in describing a linear relationship. However, it is important to note that these assumptions do not establish causations. While this information is necessary, it is not enough on its own. Additional analyses should be undertaken to gain a more comprehensive understanding of the interaction between genes involved in cell proliferation and death as well as the associated pathways.

CHAPTER V

Discussion

Acne is a prevalent dermatological condition that impacts a significant proportion of the global population. The occurrence is attributed to the obstruction of pilosebaceous units situated beneath the dermis. This phenomenon arises as a result of excessive sebum secretion by the sebaceous glands, which creates a conducive environment for bacterial proliferation and consequent obstruction of the skin pores. Propionibacterium acnes (P. acnes) is the bacterial strain commonly associated with acne. This bacterium is known to induce skin inflammation and promote the development of pimples and cysts. In addition, there are certain factors that can contribute to the augmentation of sebum and oils within the skin, ultimately resulting in the obstruction of hair follicles. These factors may encompass hormonal fluctuations, which can transpire during the adolescent phase or gestation. The occurrence of acne is often associated with hormonal fluctuations of estrogen, progesterone, and testosterone. As a result, individuals who are most commonly affected by this skin condition include teenagers, menstruating women, and post-menopausal women.

Additionally, genetics has been identified as a contributing factor to the development of acne, as individuals with a familial history of the condition are more likely to experience it in the future. It is noteworthy to acknowledge that acne can be categorized into various types based on whether it is non-inflammatory or inflammatory.

Non-inflammatory acne vulgaris comprises whiteheads and blackheads, that runs as a grade 1 type of acne, meaning it is painless and does not get inflamed. On the other hand, inflammatory acne vulgaris encompasses pustules, papules, and nodules, these types can range from grade 2 acne up to grade 4, meaning they can become very painful and have the capability of becoming very red and swollen. Severe cystic acne represents a more severe manifestation of inflammatory acne. The condition is distinguished by the presence of sizable, erythematous, and distressing cysts, which are aesthetically unappealing. The efficacy of acne treatments is contingent upon the specific type of acne that is present and the contextual factors that surround it.

Topical medications, such as gels and creams containing benzyl peroxide and retinoids, are effective in mitigating inflammation and inhibiting the development of fresh acne lesions. Additional categories of pharmaceuticals include oral medications like antibiotics and oral contraceptives, as well as the crucial oral Isotretinoin. In instances of severe cystic acne, a dermatologist may prescribe a treatment that offers sustained remission of the condition. Finally, physical interventions such as lifestyle modifications can be employed to address acne. Empirical evidence suggests that the consumption of dairy products is positively correlated with the incidence of acne. Additionally, the consumption of oily or greasy foods and the use of heavy skin care products have been found to be associated with an increased likelihood of developing acne.

The present investigation sought to examine the management of the *mTOR, AKT, p53,* and *pi3k* gene expression levels in the clinical study. The study's findings demonstrated intriguing results regarding the expression of genes and the administration of isotretinoin. The matter at hand shall be expounded upon within the framework of pertinent literature. Consistent with prior research, our findings indicate an increase in the expression levels of *Akt* and *p53* genes subsequent to the intervention. Our study revealed a similar outcome to the research article titled "Isotretinoin treatment upregulates the expression of p53 in the skin and sebaceous glands of patients with acne vulgaris", published in 2022 where an upregulation of *p53* gene expression was observed because after the use of isotretinoin inducing transcriptomic regulation, thus a major increase of p53 protein and expression was observed in the skin. The significance of the upregulation of *p53* gene expression lies in its implications for cellular responses. This may encompass DNA repair, apoptosis, and regulation of the cell cycle. The anti-proliferative and apoptotic effects of isotretinoin may be attributed to its impact on the p53 activation pathway.

In accordance to our research outcomes, the levels of *mTOR* and *pik3* gene expression did not exhibit a statistically significant variation subsequent to the administration of isotretinoin. The present discovery contradicts the results of a previous investigation titled, "The PI3K-Akt-mTOR and Associated Signalling Pathways as Molecular Drivers of Immune-Mediated Inflammatory Skin Diseases: Update on Therapeutic Strategy Using Natural and Synthetic Compounds". Thus, this study revealed a decrease in the

PI3K-Akt-mTOR pathways in individuals with acne who received isotretinoin treatment. The observed inconsistencies could potentially be attributed to a variety of factors, including but not limited to the length of the acne therapy, the methodology employed in the study, or the characteristics of the patient under the clinical trial investigation.

Additionally, it is noteworthy that the observed increase in *Akt* and *p53* gene expression levels in our investigation implies a plausible implication of the *PI3K-Akt-mTOR* pathway and *p53* signalling in the mode of action of isotretinoin. Elevated *Akt* kinase activity suggests an upregulation of *Akt* gene expression, which may subsequently modulate diverse downstream signalling pathways implicated in cellular growth, survival, and proliferation. Furthermore, the observed increase in *p53* gene expression may suggest the initiation of *p53*-mediated pathways, resulting in cellular responses such as cell cycle arrest and apoptosis. The observed variations between our investigation and prior research highlight the intricacy of the *PI3K-Akt-mTOR* pathway and its control by isotretinoin. It is imperative to acknowledge the constraints of our investigation, notably the restricted sample size that could have potentially impacted the statistical potency of detecting noteworthy variations in gene expression levels. Moreover, the limited number of participants in the study may not accurately reflect the characteristics of the entire population, thus necessitating the need to account for potential confounding variables. Subsequent investigations could endeavor to elucidate the exact molecular mechanisms that underlie the gene expression alterations observed in response to isotretinoin therapy. The potential therapeutic effects of isotretinoin on gene expression can be further understood through longitudinal studies with a larger sample size, which may involve the inclusion of additional molecular markers and an exploration of relevant signalling pathways.

The present investigation has demonstrated an increase in *Akt* and *p53* gene expression subsequent to the administration of isotretinoin, indicating a plausible association with the remedial properties of isotretinoin. Therefore, it can be inferred that the upregulation of these genes may be implicated in the therapeutic mechanism of isotretinoin. Moreover, it is necessary to conduct additional research to elucidate the exact interplay between isotretinoin, the *PI3K-Akt-mTOR* pathway, and other molecular pathways. Such

investigations may enhance our comprehension of the molecular mechanisms underlying the effects of isotretinoin in various diseases and contexts.

CHAPTER VI

Conclusion and Recommendations

To sum up, the present investigation examined the impact of isotretinoin on the expression levels of *mTOR, AKT, p53,* and *PIk3* genes in the clinical study. By scrutinizing the outcomes and the ensuing discourse, a number of significant variables were revealed. Initially, an increase in the expression levels of *Akt* and *p53* genes was noted subsequent to the administration of isotretinoin. The results indicate that isotretinoin could have stimulated both the *PI3K-Akt-mTOR* pathway and the *p53* pathway, which is significant as it could conceivably enhance its therapeutic benefits. Furthermore, these pathways are recognized to comparatively modulate cellular processes such as cell proliferation, cell viability, and programmed cell death, which are crucial in the management of various ailments such as cancer and acne vulgaris (Fei, 2021). However, no significant difference was observed in the expression levels of *mTOR* and the *PI3K* genes after taking the isotretinoin treatment. This discovery posits that the regulation of the aforementioned genes is subject to influences beyond isotretinoin. Further investigation is required to elucidate the underlying mechanisms and potential clinical implications of the intricate interplay within the *PI3K-Akt-mTOR* pathway. Although the study has certain limitations, such as a relatively small sample size of 44 participants, it cannot be considered entirely objective as it may not be representative of the entire population. On the other hand, the results presented may provide significant insights into the molecular effects of isotretinoin treatment, because an increase in the expression of *Akt* and *p53* genes serves as significant evidence for the implication of these genes in the domain of therapy and their reaction to isotretinoin (Bojar, 2004). Subsequent research endeavors pertaining to the subject matter ought to concentrate on expounding upon the exact molecular mechanisms that underlie the noted alteration in gene expression. Additionally, further inquiry into the involvement of *PI3K-Akt-mTOR* pathways in the context of isotretinoin treatment is warranted. Conducting longitudinal studies to continuously examine, with a larger sample size and controlled interventions such as setting up a randomised group from multiple hospital locations and compare them to one another may help yield more reliable outcomes and facilitate a more comprehensive exploration of the effects of isotretinoin on associated

gene expression and pathways. The study contributes to the expanding pool of information regarding the molecular impacts of isotretinoin while also illustrating the involvement of *the PI3K-Akt-mTOR* pathway and the *p53* signalling pathway. By comprehending the mechanisms of isotretinoin's action, it is feasible to enhance its utilization in the clinical setting and refine approaches to manage diverse ailments and follow ups particularly acne vulgaris, which is the most pertinent condition under investigation.

Recommendations

Recommendations According to Findings

To sum up, the present investigation examined the impact of isotretinoin on the expression levels of *mTOR, AKT, p53,* and *PIk3* genes in the clinical trial. By scrutinizing the outcomes and the ensuing discourse, a number of significant variables were revealed. Initially, an increase in the expression levels of *Akt* and *p53* genes was noted subsequent to the administration of isotretinoin. The results indicate that isotretinoin could have stimulated both the *PI3K-Akt-mTOR* pathway and the *p53* pathway, which is significant as it could conceivably enhance its therapeutic benefits. Furthermore, these pathways are recognized to comparatively modulate cellular processes such as cell proliferation, cell viability, and programmed cell death, which are crucial in the management of various ailments such as cancer and acne vulgaris. However, no significant difference was observed in the expression levels of *mTOR* and the *PI3K* genes in the subsequent treatment.

This discovery posits that the regulation of the aforementioned genes is subject to influences beyond isotretinoin. Further investigation is required to elucidate the underlying mechanisms and potential clinical implications of the intricate interplay within the *PI3K-akt-mTOR* pathway. Although the study has certain limitations, such as a relatively small sample size of 44 participants, it cannot be considered entirely objective as it may not be representative of the entire population. The results presented may provide significant insights into the molecular effects of isotretinoin treatment. The

observed increase in the expression of *Akt* and *p53* genes serves as significant evidence for the implication of these genes in the domain of therapy and their reaction to isotretinoin. Subsequent research endeavours pertaining to the subject matter ought to concentrate on expounding upon the exact molecular mechanisms that underlie the noted alteration in gene expression.

Additionally, further inquiry into the involvement of *PI3K-Akt-mTOR* pathways in the context of isotretinoin treatment is warranted. Conducting longitudinal studies with a larger sample size and controlled interventions can yield more reliable outcomes and facilitate a more comprehensive exploration of the effects of isotretinoin on associated gene expression and pathways. The study contributes to the expanding pool of information regarding the molecular impacts of isotretinoin while also illustrating the involvement of the *PI3K-Akt-mTOR* pathway and the *p53* signalling pathway. By comprehending the mechanisms of isotretinoin's action, it is feasible to enhance its utilization in the clinical setting and refine approaches to manage diverse ailments, particularly acne vulgaris, which is the most pertinent condition under investigation.

Recommendations for Further Research

Drawing upon the outcomes and constraints of this dissertation, a number of suggestions can be put forward for future investigations aimed at broadening the scope and comprehension of the subject matter. Initial steps should involve conducting prospective studies with larger sample sizes and control groups to establish a more robust basis for exploring the impact of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression levels. It is recommended that these studies incorporate extended follow-up periods to ascertain the durability of the observed alterations and appraise the treatment response across time. Furthermore, there is a requirement for additional mechanistic investigations to explicate the fundamental molecular mechanisms through which isotretinoin impacts the *PI3K-Akt-mTOR* pathway and gene expression. In order to elucidate the particular pathways and signalling molecules implicated, in vitro investigations utilizing different cell lines in literature or animal models may be utilized. This will aid in the elucidation of complex molecular interactions and offer valuable

insights into potential therapeutic targets along the pathway. In addition, it is recommended that forthcoming studies prioritize the enhancement of treatment approaches by tailoring them to individual genetic profiles and the expression levels of particular genes that are linked to treatment response. The implementation of a personalized approach has the potential to optimize treatment outcomes while simultaneously reducing the occurrence of adverse effects.

The identification of biomarkers or genetic signatures that can anticipate patient response to isotretinoin treatment would facilitate the categorization of patients and the selection of the most suitable treatment alternatives to enhance efficacy. Conducting longitudinal studies is imperative for evaluating the enduring impacts of isotretinoin on the *PI3K-Akt-mTOR* pathway and gene expression that extend beyond the duration of treatment. The examination of the prolonged impacts and possible hazards linked with extended isotretinoin treatment will yield significant insights for medical practice and the handling of patients. The investigation of combination therapies that involve isotretinoin and other targeted agents that modulate the *PI3K-Akt-mTOR* pathway may yield advantageous outcomes. The utilization of combinatorial strategies has the potential to augment the effectiveness of treatments, mitigate drug resistance, and decrease the occurrence of unfavourable outcomes.

Conducting research on the synergistic impacts of combined treatment regimens in both preclinical and clinical settings would be beneficial in enhancing treatment approaches. Finally, it is imperative to explore approaches aimed at alleviating possible negative consequences linked to the administration of isotretinoin. The identification of strategies to effectively manage adverse effects, such as dryness, mood alterations, or impacts on other signalling pathways, can enhance patient compliance and overall satisfaction with treatment. The implementation of supportive care interventions and monitoring protocols can effectively mitigate the negative impact of adverse effects on the quality of life of patients.

To summarize, the above-mentioned suggestions for additional investigation intend to overcome the constraints of this dissertation and offer significant guidance for forthcoming research endeavours concerning comprehending the impacts of isotretinoin

on the *PI3K-Akt-mTOR* pathway and genetic expression levels. The execution of these investigations will make a valuable contribution to the progression of knowledge in the domain of molecular medicine and establish a path for the development of more efficient and tailored therapies for acne.

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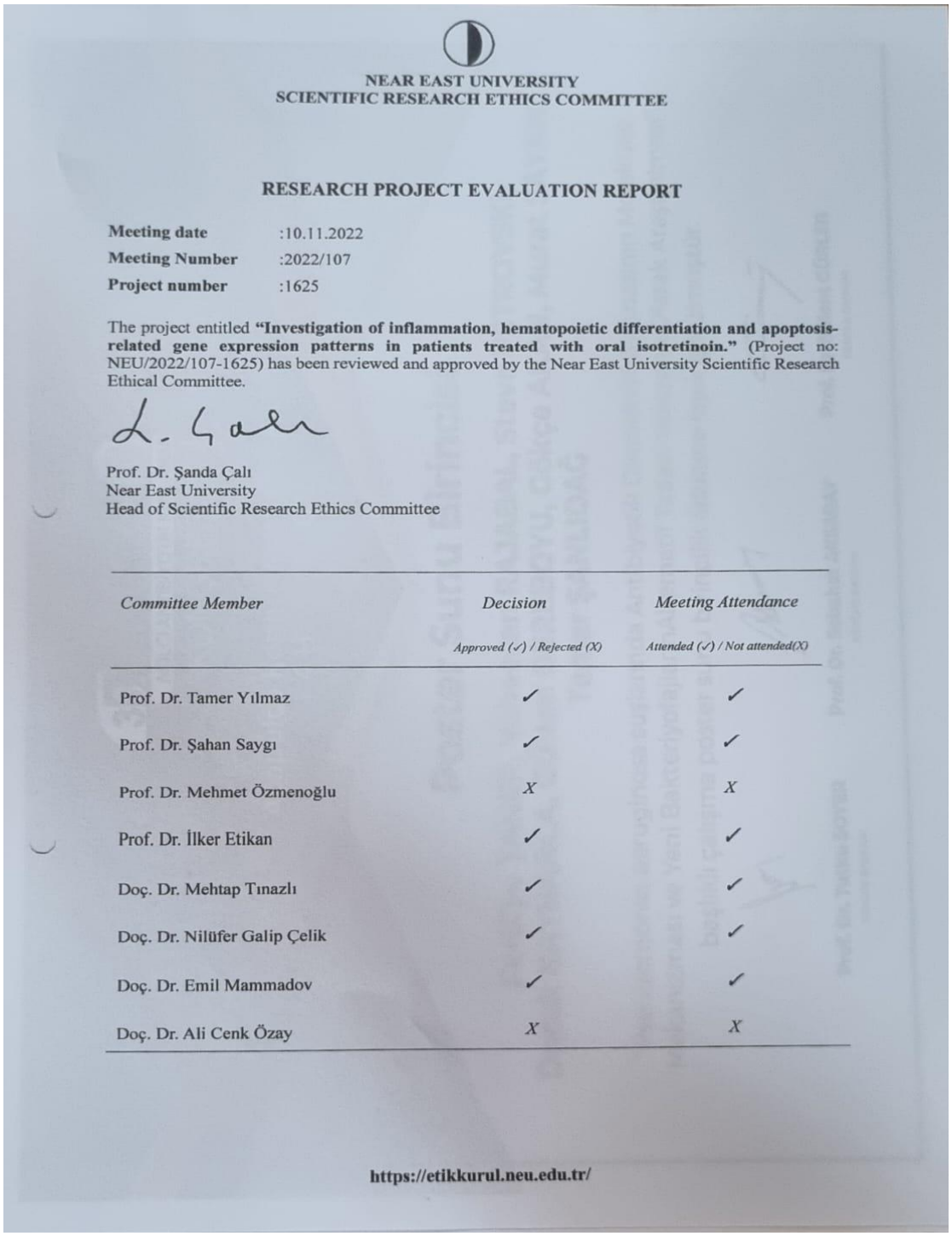
Appendices

Appendix 1



NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :10.11.2022
Meeting Number :2022/107
Project number :1625

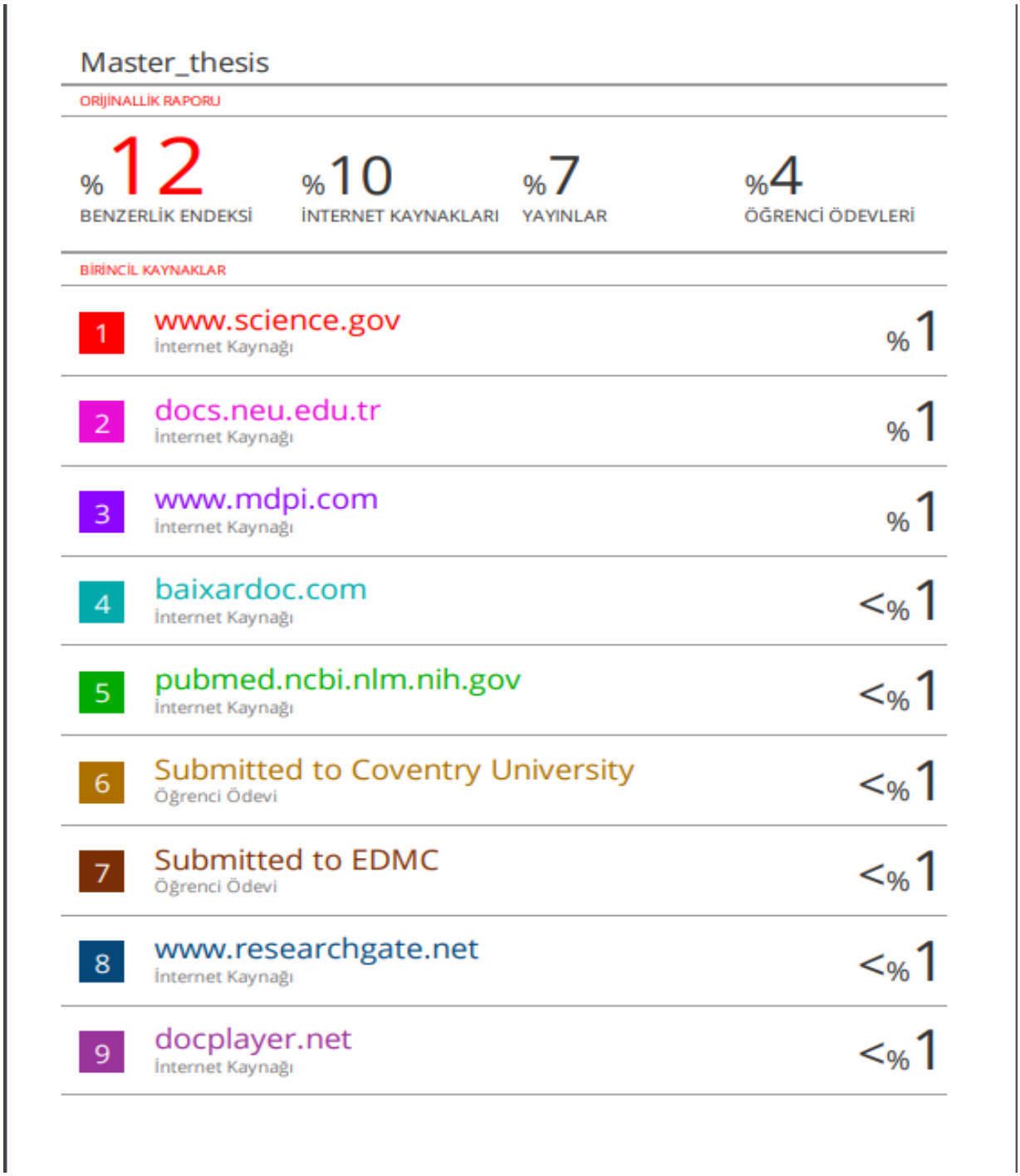
The project entitled **"Investigation of inflammation, hematopoietic differentiation and apoptosis-related gene expression patterns in patients treated with oral isotretinoin."** (Project no: NEU/2022/107-1625) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Mehmet Özmenoğlu	X	X
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	X	X

https://etikkurul.neu.edu.tr/

Appendix 2



Master_thesis

ORİJİNALLİK RAPORU

%12	%10	%7	%4
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.science.gov İnternet Kaynağı	%1
2	docs.neu.edu.tr İnternet Kaynağı	%1
3	www.mdpi.com İnternet Kaynağı	%1
4	baixardoc.com İnternet Kaynağı	<%1
5	pubmed.ncbi.nlm.nih.gov İnternet Kaynağı	<%1
6	Submitted to Coventry University Öğrenci Ödevi	<%1
7	Submitted to EDMC Öğrenci Ödevi	<%1
8	www.researchgate.net İnternet Kaynağı	<%1
9	docplayer.net İnternet Kaynağı	<%1