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ORIGINAL ARTICLE

Elevated Mammalian Target of Rapamycin (mTOR) Gene Expression in Acne Vulgaris Patients

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Abstract

Background: **Multiple factors contribute to acne vulgaris, a disorder of the pilosebaceous units. Mammalian target of rapamycin (mTOR) pathway dysregulation is associated with many inflammatory conditions.**

Aim: **This study aims was to assess mTOR gene expression as a prognostic molecular marker for acne vulgaris severity or recurrence.**

Patients and methods: **This study was a case-control that consisted of 50 persons with acne vulgaris and 50 age also sex-matched healthy persons. Patients were recruited from the Dermatology Department, Suez Canal University Hospital. mTOR gene expression was conducted using polymerase chain reaction (PCR) at the Oncology Diagnostic and Research Unit, Faculty of Medicine, Suez Canal University.**

Results: **In the patient group, there were 34 (68 %) with mild acne, 15 (30 %) with moderate, and only one (2 %) with severe acne. The mean mTOR expression in the patients' group was statistically significantly greater by fivefold than in the control groups. There was a positive nonsignificant association between mTOR expression and the duration of the disease in Acne vulgaris patients ($r = 0.253$, $P > 0.05$).**

Conclusion: **mTOR is higher expressed in acne patients than in control. mTOR can be employed as a biomarker for acne vulgaris prognosis and response to treatment.**

Keywords: Acne vulgaris, Acne, Gene expression, Mammalian target of rapamycin, Polymerase chain reaction

1. Introduction

Acne vulgaris is an inflammatory condition of the pilosebaceous units that has many causes.[1] There are three main dietary elements that have been linked to acne vulgaris pathogenesis: (i) high glycemic index carbohydrates; (ii) insulinotropic milk and dairy products; and (iii) saturated fats.[2] Growing data suggests that growth hormone, insulin and IGF-1 signaling interact throughout puberty, potentially contributing to the development of acne by affecting adrenal and gonadal androgen metabolism.[3] According to previous studies, high-glycemic-load meals worsen acne, lead to postprandial hyperinsulinemia and raise serum levels of free IGF-1.[4,5] Milk has been found to stimulate the mammalian target of the rapamycin signing pathway, which is an endocrine growth-promoting signal in animals.[6]

Mammalian target of rapamycin (mTOR) controls cellular growth, lipid synthesis, proliferation and protein translation in a nutrient-sensitive manner. It does this through 2 different signaling complexes, mTOR 1 also mTOR 2.[7] Rapamycin-sensitive mTOR 1 encourages cell growth as well as proliferation, which may translate into enhanced lipid production

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in sebaceous glands, which are in charge of seborrhea, and the proliferation of acroinfundibular keratinocytes.[8]

Acne has been suggested to be an obvious mTOR-driven disease of civilization, appearing to develop in metabolic conditions with elevated mTORC1 signaling.[9]

Mammalian target of rapamycin (mTORC1) may regulate the accessibility of amino acids, especially the BCAA leucine, required for its own activation as well can integrate many intra-in addition to extracellular mediators, including growth hormones (insulin, IGF-1) and energy-sensing signals (glucose, AMP/ATP ratio).[10]

Melnik and Schmitz[11] highlighted the antagonistic connection amongst the metabolic transcription factor forkhead box transcription factor O1 (FOXO1) also the nutrient-sensitive kinase mTORC1 by integrating mTORC1 signaling into the complicated picture of acne etiology.[11] So, the purpose of this trial was measurement the mTOR gene expression and how it could be used as a molecular marker for acne severity and complications.

2. Patients and methods

Study design: Case-control research was conducted. The Dermatology Department of Suez Canal University Hospital was the source of the individuals. The Oncology Diagnostic and Research Unit in the Department of Medicine at Suez Canal University ran the molecular PCR tests.

2.1. Study population

The study population counted 50 persons identified with acne vulgaris also 50 age and 6-matched well controls. Inclusion criteria were participants between (15 and 35 years) from both sexes and exclusion criteria were autoimmune diseases, chronic dermatological illnesses, history of any topical and/or systemic anti-acne therapy three months before blood collection, and patients with other hyperandrogenic states, e.g., polycystic ovarian syndrome, hirsutism, severe androgenic alopecia were excluded. Patients were matched to the control group regarding age and sex. Acne was organized in accordance with Global Acne Grading System (GAGS).[12]

2.2. Ethical considerations

Studies Committee, Local Research Committee as well and Research Ethics Committee all gave their stamps of approval to the study's protocol before it could get underway. Participants gave their express consent in writing (Code: RPBR-3).

2.3. Study procedures

All participants underwent the following procedures: (1) Detailed medical report; (2) Measurements for weight, height, BMI and waist circumference (WC); (3) General also systemic examination; (4) Sampling for 3 mL of blood that was taken from the peripheral veins and put on ethylenediaminetetraacetic acid (EDTA) vacutainers. The specimens were centrifuged for 15 min at 4000 rpm for separating the plasma to be used for molecular analysis. Within 24 h, the samples were processed.

2.4. Molecular biology technique for mTOR expression analysis

RNA was isolated from the patient and control plasm using the RNeasy Mini Kit for total RNA extraction (QIAGEN, USA, Catalog no.74904). B) The RNA was kept at (−80). C) until the required gene expression was assessed. The nanodrop1000 UV spectrophotometer was employed to find out the total RNA concentration. cDNA was synthesized using two hundred ng of total RNA according to the PrimeScript Reverse transcriptase kit's supplied procedure (TaKaRa, Shiga, Japan, Catalog no. RR014A). Step one real-time polymerase chain reaction (PCR) instrument (Applied Biosystems, Foster City, CA, USA) was utilized to evaluate the relative expression of the mTOR gene in a 25 μl reaction volume comprising 12.5 μl SYBR Green real-time PCR master mix (Toyobo, Osaka, Japan), 1 μL of 15 pmol from each primer which was shown in Table 1 and 250 ng of cDNA. After 5 min of denaturing at 95°, PCR amplification was carried out for forty cycles of fifteen seconds at 95°, 1 min at 62°

Table 1. Polymerase chain reaction primers used for quantification of Mammalian target of rapamycin and GAPDH.

Gene	Primer Sequence	Amplicon size (Base pairs)
mTOR	5′-AGCATCGGATGCTTAGGAGTGG-3′ 5′-CAGCCAGTCATCTTTGGAGACC-3′	146
GAPDH	5′-GTCTCCTCTGACTTCAACAGCG-3′ 5′-ACCACCCTGTTGCTGTAGCCAA-3′	235

and 1 min at 72°. The CT values of the mTOR gene's mRNA expression levels were standardized with the average expression of Glyceraldehyde 3-Phosphate dehydrogenase (GAPDH) according to Ref.[13]

2.5. Statistical analysis

The information was loaded into the IBM SPSS software program version 25 (IBM Corporation, Armonk, New York). Numbers besides % were performed to describe qualitative information. The Kolmogorov-Smirnov test was applied to check the distribution's normality. Range (min and max), mean, standard deviation, median and interquartile range were used to characterize quantitative data (IQR). The significance of the acquired outcomes was identified at the five percent level. Used tests were: (1) χ^2 test: For categorical parameters, for comparison amongst changed groups, (2) Student *t*-test: For normally distributed quantitative parameters, to evaluate among 2 investigated groups, (3) Mann Whitney test: For abnormally distributed quantitative parameters, to compare amongst 2 examined groups, (4) Spearman coefficient: To associate amongst 2 distributed normally quantitative variables.

3. Results

The descriptive analysis of the studied cases showed that; Mild acne was observed in 34 (68 %), moderate in 15 (30 %) and only 1 (2 %) severe case of the total 50 patients. Regarding the family history of acne, 11 (22 %) patients had a positive family history, while the other 39 (78 %) patients were negative. In addition, comedonal or inflammatory cases were 7 (14 %) of patients while 43 (86 %) were mixed (Tables 2 and 3).

The mean mTOR expression in the patients' group was 6.62 ± 5.51 μm, it was markedly elevated than controls with a highly significant alteration amongst the two groups ($P > 0.05$) (Fig. 1). There was a positive nonsignificant connection among mTOR expression and duration of acne vulgaris (Fig. 2) ($r = 0.253$, $P > 0.05$). ($r = 0.231$, $P = 0.127$).

4. Discussion

According to this study, the mean mTOR expression in the patients' group was 6.62 vs. 5.51 μm, which was significantly more than the control group's mean of 1 μm with a significant variance among the two groups. These results were consistent with those of Monfrecola et al.[7] who discovered that mTOR gene expression was elevated by 17.96–20.77-fold (mean values) in non-lesioned and lesioned skin from acne sufferers, respectively, in contrast to skin obtained from volunteers in good health.

Research on rats by Ruan et al.[14] found that acne etiology is connected to increased mTORC1 activity and mTORC1 works as a nutrient/energy/redox sensor to govern protein & lipid synthesis. People who eat a high-sugar, high-protein diet for an extended period of time are more prone to get acne. Mammalian Target of Rapamycin 1 regulates lipid production in the sebaceous gland via modulating the expression of SREBP-1 (Sterol-regulatory element binding proteins.[15] Through the mTORC1 signaling pathway, changes in amino acid in addition energy metabolism in the body can impact acne.[16]

Table 3. Distribution of the studied patients according to the nature of the disease.

Age of disease onset (years)	Min	Max
Range	13	25
Mean ± SD	17.74 ± 2.28	
Disease duration (years)	**Min**	**Max**
Range	0	13
Mean ± SD	5.80 ± 4.10	
Acne severity	*N* (%)	
Mild	34 (68 %)	
Moderate	15 (30 %)	
Severe	1 (2 %)	
Family history	*N* (%)	
Negative	39 (78 %)	
Positive	11 (22 %)	
Features	*N* (%)	
Comedonal only	7 (14 %)	
Comedonal + inflammation	43 (86 %)	

SD, standard deviation, *P* greater than 0.05 = nonsignificant; *t*, student *t*-test; χ^2, Chi-square.

Table 2. General characteristics of studied groups.

Sex	Patients No. (%)		Controls No. (%)		Test for Significance	
					χ^2	*P*
Males	10 (20.0)		11 (22.0)		0.102	0.749
Females	40 (80.0)		39 (78.0)		0.164	0.870
Total	50 (100)		50 (100)			
Age (y)	**Min**	**Max**	**Min**	**Max**	**t**	**P**
Range	16	35	18	26		
Mean ± SD	23.54 ± 5.60		22.40 ± 2.46		1.319	0.192

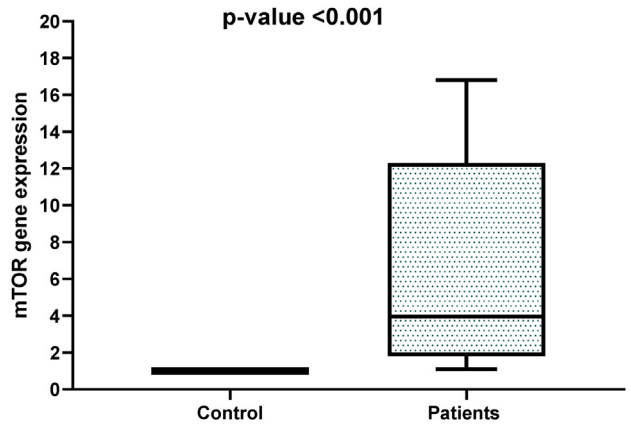

Fig. 1. Comparison between the two studied groups according to Mammalian target of rapamycin expression. Mann-Whitney test was used to calculate the P-value. P-value less than 0.05 is considered significant.

In acne, mTOR expression is important for sebum fatty acid synthesis, sebogenesis & monosaturated fatty acid processing.[8] mTORC1 activation has been reported in inflammatory IL-17-driven dermatoses such as psoriasis, acne vulgaris also hidradenitis suppurative (HS).[7,10] Enlarged mTOR core protein expression has been detected in both lesional and non-lesional skin of hidradenitis suppurative individuals, showing that mTORC1 signaling is exacerbated in hidradenitis suppurative.[17]

In this study, the severity of acne vulgaris was observed in 34 (68 %) of the patients, moderate in 15 (30 %) and only one severe case (2 %) of the total 50 patients with no statistically significant association amongst mTOR expression and the severity of acne vulgaris (*P* more than 0.05). These findings were comparable with those of Monfrecola et al.[7] who discovered that mTOR progressively rose from healthy volunteers to acne sufferers, with a statistically significant connection (P 0.05). However, they discovered that the increase in mTOR was not directly linked to acne severity or body mass index, which was similar to our findings, but mTOR expression may indirectly affect acne severity because it correlated with the severity of HS, which was linked to higher BMI.[18]

Significantly, increased levels of mTOR expression have been seen in cases with inflammatory lesions, particularly migrating furunculoid lesions.[7] Melnik et al. and Monfrecola et al. disagreed with us, claiming that mTOR gene expression statistically connects with disease severity and as in acne vulgaris, with BMI.[7,11] Because eruption patterns besides risk factors fluctuate contingent on BMI, low and high BMI patients may constitute two clinically distinct subgroups. As a result, isotretinoin may have significant anti-inflammatory benefits in people with a higher BMI.[19] In this connection, it should be noted that metformin, another mTORC1 inhibitor, improved acne, most likely by a similar mechanism of action to isotretinoin.[20]

In the present trial, a nonsignificant positive connection between mTOR expression and both the duration of acne vulgaris patients was observed. This is comparable with the results of Monfrecola and colleagues who found that mTOR gene expression elevated and activated in acne cases equated to healthy controls, indicating the importance of mTORC1 signaling in acne etiology.[7] Increased protein/lipid synthesis, cell proliferation

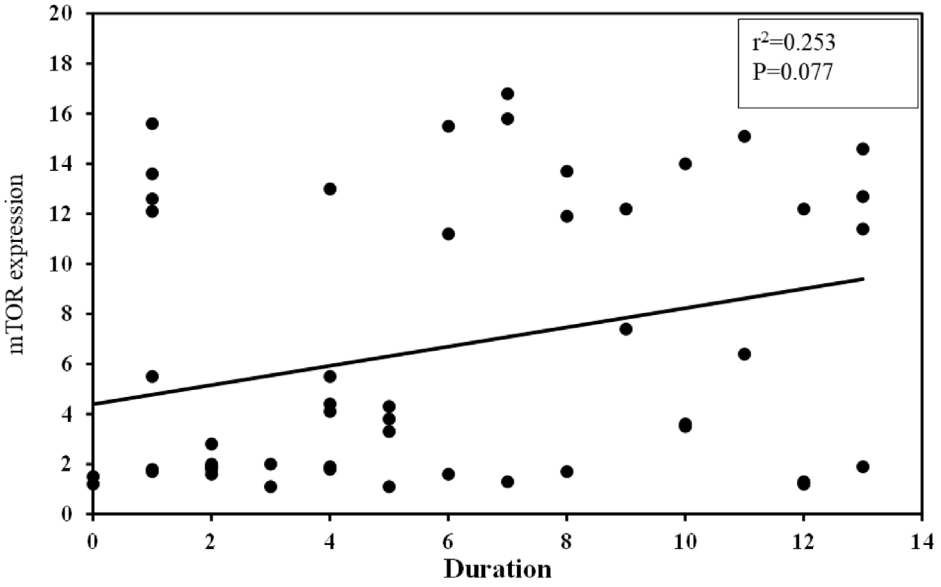

Fig. 2. Correlation between Mammalian target of rapamycin expression and acne duration.

as well as inflammation occur in sebocytes when the PI3K/Akt/mTOR pathway is activated by insulin.[21,22]

In several research, a link between milk consumption and increased BMI was observed.[23] In addition, acne risk was lowered in Italian adolescents and young adults with minor body mass index.[24] A lower body mass index has been linked to a decreased acne prevalence.[25]

In another study, milk protein consumption raised body mass index and C-peptide plasma levels in overweight Danish adolescents.[26]

The branched-chain amino acid (BCAA) leucine is essential for mTORC1 activation among all amino acids. Milk has the most leucine of any animal protein, making it ideal for optimizing mTORC1 activation for postnatal development.[27,28] Morris et al.[29] highlighted the association between raised plasma BCAA profiles, higher BMI & insulin resistance.

Thus, high GL and milk intake result in increased mTORC1 signaling, which both play important roles in the etiology of food-aggravated acne. Exaggerated mTORC1 signaling generated by the Western diet activates the kinase S6K1, which adversely regulates insulin signaling at the level of insulin receptor substrate-1 (IRS-1) phosphorylation.[8] Over-stimulation of Mammalian Target of Rapamycin 1-S6K1 signaling by nutrients causes insulin resistance, a feature of heavy milk intake, acne also diseases linked with metabolic disorder.[30]

Evidence suggests that components in dairy, such as estrogens and androgens and IGF-1, may affect the skin's pilosebaceous unit. Furthermore, skim milk and the ingredients elaborated in its processing may contribute to comedogenicity and acne formation. High-fiber diets have been demonstrated in certain trials to promote skin health.[31]

The visual indications of acne, according to Clatici et al.[32] can be a signal of elevated mTORC1 activity and a probable predictor of future metabolic disorders such as overweight and insulin resistance. They came to the conclusion that lowering 'over-stimulated mTORC1 signaling by food may have favorable benefits not just on acne, but it may also prevent the development of more severe, chronic illnesses'. They proposed that dietary intervention would entail lowering overall calorie consumption by reducing dairy protein, sugar also foods high in leucine.

4.1. Conclusion

According to the findings of this study, mTOR is much greater in acne vulgaris cases than in normal well participants. As a result, mTOR can be employed as a biomarker for acne prognosis.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article.

Conflict of interest

The authors declared that there were NO conflicts of Interest.

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