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Serum Level of Lactoferrin in Patients with Acne Vulgaris and the Efficacy of Lactoferrin Supplementation

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ABSTRACT

Background: Long-term skin illness known as acne vulgaris (AV) is brought on by clogged hair follicles with oil from the skin and dead skin cells. Acne is caused by a combination of immunologic, inflammatory, and hormonal pathways. A protein found in milk that binds iron is called lactoferrin. Lactoferrin is an iron-binding protein involved in innate defence that has antibacterial and anti-inflammatory properties.

Objective: This study was conducted to evaluate serum level of lactoferrin in patients with acne vulgaris, and the efficacy of oral lactoferrin supplementation in moderate acne vulgaris.

Patients and Methods: In addition to 42 healthy volunteers who were matched by age and gender who served as the control group, the trial comprised 42 patients who had acne vulgaris. The cases group was further split into two equal subgroups: Subgroup (A) consisted of 21 patients who took 100 mg of lactoferrin-enriched tablets twice a day for four weeks as oral lactoferrin supplementation, and subgroup (B) consisted of 21 patients who did not take oral lactoferrin supplementation. The Global Acne Grading System was used to evaluate the severity of the illness.

Results: Comparing acne cases to control subjects, there was a statistically significant increase in serum lactoferrin. Between the two subgroups of the cases group, there was a statistically significant strong positive connection only for inflammatory lesions and not for non-inflammatory lesions when comparing serum lactoferrin to baseline Global Acne Grading System (GAGS). On the GAGS score, noninflammatory lesions, and inflammatory lesions, there was a statistically significant interaction between the treatment arm and time.

Conclusion: Lactoferrin is a potential diagnostic biomarker in acne vulgaris. Utilization of lactoferrin supplementation is an effective supplementary treatment for acne vulgaris.

Keywords: Acne Vulgaris, Lactoferrin, Using Global Acne Grading System.

INTRODUCTION

Seborrhea, the formation of comedones, erythematous papules, and pustules, as well as, less frequently, knobs, deep pustules, or pseudocysts, are all symptoms of acne vulgaris, a chronic inflammatory illness of the pilosebaceous units (PSU) that can occasionally be accompanied with scarring [1].

Its estimated 9% global prevalence is 0.3% of the world's total disease burden. The disease primarily affects teenagers, and its causes include hormonal imbalances, bacterial infections, stress, and incorrect use of skin care products or food [2].

Multifactorial pathophysiology underlies acne [3]. Specifically, the sebaceous glands in hair follicles harbour Cutibacterium acnes (C. acnes), a Gram +ve anaerobe that is resident bacteria on the skin's surface and is essential to the inflammatory lesions associated with acne. An inflammatory response to acne can be brought on by C. acnes growing and reproducing excessively in the hair follicles [4].

One iron-restricting glycoprotein known to help lessen the severity of microbial illness is lactoferrin (LF). It is an iron-restricting protein that exhibits non-iron-subordinate bactericidal action and sequesters iron that is essential for microbial growth [5]. Particularly at mucosal surfaces, it has been implicated in nonspecific host defence against infections and severe inflammations. Bacteriostasis by the sequestration of free iron and bactericidal action through the destabilisation of the cell wall are two examples of LF's antimicrobial properties [6].

In addition, LF has anti-inflammatory properties that include neutralising lipopolysaccharide (LPS), complement stimulation of cytokine production and/or binding, and inhibition of hydroxyl radical generation [6]. Products for the skin and food industry have been fortified with LF, which is obtained from bovine milk [7]. As a result, LF consumption may benefit skin health. However, not much research has been done on how LF supplementation affects acne vulgaris. Accordingly, there was disagreement between studies that demonstrated the negative effects of lactoferrin on Candida acnes [8], and those that demonstrated the increased levels of serum lactoferrin in patients with acne vulgaris [5] when compared to healthy individuals [2].

The aim of this work was for evaluation of serum level of lactoferrin in patients with acne vulgaris, and the oral lactoferrin supplementation efficacy in moderate acne vulgaris.

PATIENTS and METHODS

In order to assess the effectiveness of oral lactoferrin supplementation in treating mild cases of acne vulgaris, a single blinded randomised parallel-group clinical trial and a case control study were carried out. For a year, the study was carried out at the Dermatology, Andrology, and STDs Department's

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outpatient clinic at Mansoura University Hospitals in Mansoura, Egypt.

PATIENTS

This study included 84 participants who were classified into 2 groups; **Group (1)** that included (42) patients with acne vulgaris, who were diagnosed clinically as acne vulgaris according to "Acne vulgaris: review and guidelines 2009" [9]. Patients in this group had moderate acne vulgaris and were treated with topical erythromycin *(trade name Aknemycin 2% solution)* twice daily for a month in combination with topical retinoids *(trade name Adapalene 0.1% gel)* once at night for a month and systemic antibiotic therapy Azithromycin (*trade name Azrolid 500 mg tabs)* once daily for 3 days/week for a month. This group also was furtherly divided into two equal subgroups; **subgroup (A)** that Included 21 patients who received oral lactoferrin supplementation at dose 100 mg of lactoferrin enriched tablets twice daily for 4 weeks in addition to the previously mentioned treatment while s**ubgroup (B) i**ncluded 21 patients who received the previously mentioned treatment only without receiving oral lactoferrin supplementation. While **Group (2)** included (42) matched healthy individuals who act as a control.

Patients with moderate acne vulgaris who were between the ages of 13 and 35 were included in the study; they had not had any treatment in the preceding six weeks. However, we did not include individuals who were pregnant or nursing, those with a known or suspected allergy to lactoferrin, those with biliary cirrhosis, cholestasis, severe liver impairment, or those with any systemic diseases that could affect lactoferrin levels, such as anemia or renal impairment.

METHODS

Complete medical histories were taken of all patients; these included personal histories (name, age, sex, employment, and place of residence); current histories (beginning, course, and duration of the disease, as well as factors that precipitated and relieved it); past medical histories (nature, route, dosage, compliance, duration, effect, and side effects); and family histories of acne vulgaris or other dermatomes.

The clinical examination included general examination to exclude any systemic diseases and estimating of BMI. The dermatological examination included local examination for acne lesion and distribution by using Global Acne Grading System (GAGS) [10].

The forehead factor is 2, the right cheek factor is 2, the left cheek factor is 2, the nose factor is 1, the chin factor is 1, and the chest and upper back factor is 3. This system separates the face, back, and chest into six sections. Depending on their severity, each form of lesion is assigned a number (Grade): no lesions = 0, comedones = 1, papules = 2, pustules = 3, and nodules = 4. The most severe lesion multiplied by the area factor yields the score for each area (Local score): Local score = location factor × Grade (0-4). The total of all local scores is the global score.

Assessment of the Disease Severity

The severity of the acne was determined by using the Global Acne Grading System (GAGS). Patients were categorized as having mild acne if their score was less than 18, as moderate acne if their score was between 19 and 30, and as severe acne if their score was greater than 31.

Assessment of Lactoferrin Levels

Sandwich-ELISA was the procedure employed with the ELISA kit. Stripplate wells were used to combine standards or samples with the appropriate antibody. Subsequently, a lactotransferrin (LTF)-specific antibody was applied to every Microelisa stripplate thoroughly and allowed to incubate. To every well, the TMB substrate solution was applied. Only the wells with conjugated away LTF and HRP. The LTF antibody will first appear blue, but once the stop solution is added, it will turn yellow.

Assay Procedure

Following the collection of the blood sample, the sample was centrifuged at 2,000–3,000 rpm for 20 minutes to remove the clot. The sample should then be centrifuged once more after 10–20 minutes of undisturbed room temperature storage.

Sluggishness in standard wells in a Microelisa stripplate totaled ten. 50 pl of standard dilution buffer and 100 µl of standard solution were added to wells 1 and 2 and thoroughly mixed. 100 µl of the solutions from wells 1 and 2 were added to wells 3 and 4, respectively. After that, 50 µl of the standard dilution buffer were added and thoroughly mixed, and 50 µl of the solution were removed from wells 3 and 4. 50 µl of the solutions from wells 3 and 4 were added to wells 5 and 6, respectively. Next, 50 µl of standard dilution buffer were added and well mixed. 50 µl of the solutions from wells 5 and 6 were added to wells 7 and 8, respectively. Next, 50 µl of standard dilution buffer were added and well mixed. 50 µl of the solutions from wells 7 and 8 were added to wells 9 and 10, respectively. After that, 50 µl of the standard dilution Buffer were added and thoroughly mixed, and 50 µl of the solution were removed from wells 9 and 10. Following dilution, the concentrations were 3600 pg/ml, 2400 pg/ml, 1200 pg/ml, 600 pg/ml, and 300 pg/ml in each well, with a total volume of 50 µl. We left a well in the Microelisa stripplate vacant to serve as a blank control. Sample dilution buffer (40 µl) and sample (10 µl) were introduced to sample wells (dilution factor is 5). Samples were placed such they don't come into contact with the well wall. Well combine*)* and was shaken gently. We cultured thirty. We used distilled water to dilute the concentrated washing buffer 30 times for 96T and 20 times for 48T. The closure plate membrane was

carefully peeled off, and then the wash solution was replaced and aspirated. After the wash solution has rested for thirty seconds, it was discarded. Five times over, the washing process was repeated. With the exception of the blank control well, we applied 50 pi HRP-conjugate reagent to each well.

Each well received 50 µl of chromogen solution A and 50 µl of chromogen solution B. The wells were then mixed gently and incubated for 15 minutes at 37°C. To put an end to the process, 50 µl of stop solution were added to each well. The well's colour needed should shift from blue to yellow. At 450 nm, we measured the absorbance OD, using a microtiter plate reader. The blank control well's OD value was set to zero. The assay needed to be completed in 15 minutes following the addition of the stop solution.

Calculation of Results

Plotting the known concentrations of the human LTF standard on the x- and y-axes, respectively, shows the corresponding readings of the OD. Plotting the sample's OD. on the Y-axis allowed for the determination of the concentration of Human LTF in the sample. One can determine the initial concentration by multiplying the dilution factor.

Follow-up

For four weeks, lactoferrin was administered orally to eligible patients in a non-institutionalized setting twice a day. A dermatologist conducted clinical evaluations of facial acne at baseline and at weeks 1, 2, 3, and 4. The dermatologist evaluated inflammatory and non-inflammatory lesions associated to acne at each evaluation.

Ethical consideration

The Institutional Review Board, Faculty of Medicine, Mansoura University, authorized the entire study design (MS.21.05.1498). At every stage of the study, privacy and confidentiality were upheld. Prior to their involvement in the study, all individuals provided written informed consent. Patients were able to leave the study at any moment and wouldn't face any repercussions for doing so. No additional purpose has been or will be assigned to the collected data. The Helsinki Declaration was followed throughout the study's conduct.

Statistical Analysis

Utilising the SPSS application for Windows (version 25), the gathered data were coded, processed, and examined. Using the one-sample Kolmogorov-Smirnov test, the data's normality was initially assessed. Numbers and percentages were used to describe the qualitative data, which were compared by Chi-square test or Fisher exact test. For parametric data, continuous variables were shown as mean ± SD (standard deviation), and for non-parametric data, as median (Min-Max). The two means were compared using the student t-test. In cases where the chance of mistake is less than 5% ($p < 0.05$), the results were deemed significant.

RESULTS

There were no statistically significant differences between acne patients and control subjects in terms of sex, age, BMI, or weight categories. By comparing acne cases to control subjects, there was a statistically significant increase in serum lactoferrin. Two cutoff values with a Youden index (sensitivity + specificity – 1) of 0.928 for each cutoff were found via ROC curve analysis. The 95-percentage confidence interval (CI) for the area under the curve (AUC) was 958-1.000. The Youden index for the two cutoff values was 928. There are two different cutoff values: 995.67, which has 98 percentage sensitivity and 95% specificity, and 1026.2, which has 95 percentage sensitivity and 98 percentage specificity (Table 1 and figure 1).

Table (1): Comparisons of acne cases vs. control subjects

Characteristic	Acne group	Control group	p-value
Sex			
Male	10 (23.8%)	13 (31%)	0.463
Female	32 (76.2%)	29 (69%)	
Body weight			
Ideal	32 (76.2%)	32 (76.2%)	1.000
Overweight	8 (19%)	7 (16.7%)	
Obese	2 (4.8%)	3 (7.1%)	
Age (years)	18.9 ± 2.1	19.1 ± 2.4	0.667
BMI (kg/m^2)	23.7 (22-24.8)	23.4 (22.4-24.8)	0.918
Serum lactoferrin (pg/ml)	2447.9 ± 1169.7	740.8 ± 165.9	**<0.001**
Serum lactoferrin (pg/ml)			**<0.001**
<995.67	1 (2.4%)	40 (95.2%)	
≥995.67	41 (97.6%)	2 (4.8%)	
Serum lactoferrin (pg/ml)			**<0.001**
<1026.2	2 (4.8%)	41 (97.6%)	
≥1026.2	40 (95.2%)	1 (2.4%)	

Quantitative data are presented as mean ± standard deviation if parametric or as median (range) if non-parametric data.

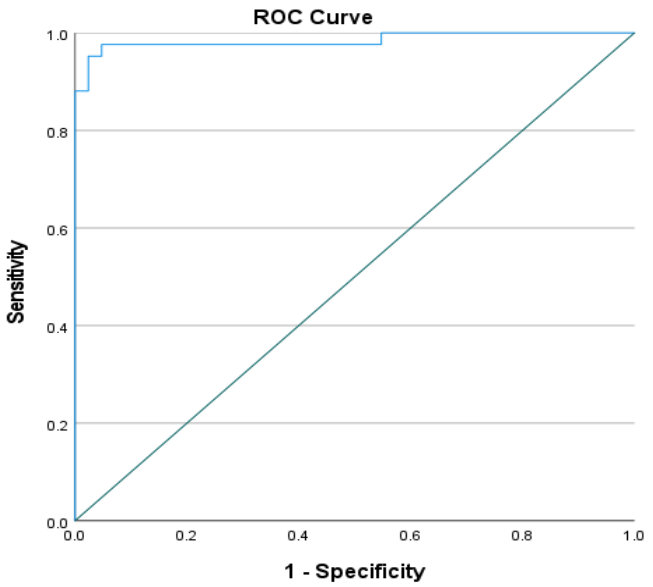

Figure (1): Serum lactoferrin ROC curve

This study involved 21 acne patients who received lactoferrin (group A) and 21 acne patients who didn't receive lactoferrin (group B). Table (2) showed no statistically significant differences in sex, age, BMI as well as weight categories, acne duration, and serum lactoferrin between the two treatment arms. Table **(3)** showed a statistically significant strong positive correlation between serum lactoferrin vs. baseline GAGS and inflammatory lesions but not non-inflammatory lesions.

Table (2): Comparisons of the two treatment groups

Characteristic	Group A	Group B	p-value
Sex			1.000
Male	5 (23.8%)	5 (23.8%)	
Female	16 (76.2%)	16 (76.2%)	
Body weight			**0.844
Ideal	17 (81%)	15 (71.4%)	
Overweight	3 (14.3%)	5 (23.8%)	
Obese	1 (4.8%)	1 (4.8%)	
Acne duration (years)	4.1 ± 1.9	3.7 ± 2	$0.476
Age (years)	19.2 ± 2.3	18.6 ± 2	$0.313
BMI (kg/m^2)	23 (22-24.6)	23.9 (21.9-25.6)	$$0.435
Serum lactoferrin (pg/ml)	1649 (1266-300	2753 (1845-3547	$$0.134

Quantitative data are presented as mean ± standard deviation if parametric or as median (range) if non-parametric data.

Table (3): Correlations of serum lactoferrin with baseline GAGS, inflammatory and non-inflammatory lesions

Parameter	All cases		Group A		Group B	
	r_s	p-value	r_s	p-value	r_s	p-value
Total GAGS score	0.865	**<0.001**	0.901	**<0.001**	0.831	**<0.001**
Non-inflammatory lesions	-0.105	0.507	-0.222	0.333	0.072	0.757
Inflammatory lesions	0.796	**<0.001**	0.843	**<0.001**	0.768	**<0.001**

r_s = Spearman's correlation coefficient.

A statistically significant interaction between treatment arm and time on GAGS score was seen in table (4). On noninflammatory lesions, table (5) demonstrated a statistically significant interaction between treatment arm and time. A statistically significant interaction between treatment arm and time on inflammatory lesions was observed in table (6).

Table (4): GAGS scores over time in the two treatment arms

Time	Group A		Group B		Group*Time interaction		
	M	SD	M	SD	F	p-value	Partial η^2
Basal	23.3	3.06	24	2.8	7.669	**<0.001**	0.161
One week	21.2	3.02	22.9	3.5			
Two weeks	17.6	4.3	21.2	3.3			
Three weeks	13.7	4.2	17.7	2.6			
Four weeks	11.3	3.7	17	3.1			

Table (5): Non-inflammatory lesions over time in the two treatment arms

Time	Group A		Group B		Group*Time interaction		
	M	SD	M	SD	F	p-value	Partial η^2
Basal	6.71	1.42	7.19	0.68	9.202	**<0.001**	0.187
One week	6.38	1.46	7.10	0.62			
Two weeks	6.05	1.53	7.05	0.67			
Three weeks	5.67	1.68	7.00	0.63			
Four weeks	5.33	1.68	7.00	0.63			

Table (6): Inflammatory lesions over time in the two treatment arms

Time	Group A		Group B		Group*Time interaction		
	M	SD	M	SD	F	p-value	Partial η^2
Basal	16.62	3.47	16.81	2.93	4.843	**0.004**	0.108
One week	14.86	3.35	15.76	3.53			
Two weeks	11.38	4.44	14.29	3.24			
Three weeks	8.14	4.04	10.71	2.57			
Four weeks	5.95	3.35	9.95	3.03			

Comparing GAGS scores, noninflammatory lesions, and inflammatory lesions between the two groups (treatments) at each time point allowed for a simple main effect analysis of the groups. According to table (7), there was no statistically significant difference in GAGS scores between the two groups at either the basal level or one week. At the other three time periods, there was a significant difference in the scores at two, three, and four weeks. At the base level, there was no statistically significant difference between the two groups' noninflammatory lesions. At 1, 2, 3, and 4 weeks, the scores differed significantly. The scores differed significantly at all 4 other time periods. At basal level and 1 week, there was no statistically significant difference in the number of inflammatory lesions between the two groups. At two, three, and four weeks, the p-values indicated a significant difference in the scores at the three other time periods.

Table (7): Simple main effect of group (treatment) on GAGS scores noninflammatory lesions, and inflammatory lesions

Time	F	p-value	Partial η^2
GAGS scores			
Basal	0.542	0.466	0.013
1 week	2.558	0.118	0.060
2 weeks	9.653	**0.003**	0.194
3 weeks	13.811	**<0.001**	0.257
4 weeks	29.114	**<0.001**	0.421
Noninflammatory lesions			
Basal	1.923	0.137	0.046
1 week	4.221	**0.046**	0.095
2 weeks	7.513	**0.009**	0.158
3 weeks	11.546	**0.002**	0.224
4 weeks	18.041	**<0.001**	0.311
Inflammatory Lesions			
Basal	0.037	0.849	0.001
1 week	0.725	0.400	0.018
2 weeks	5.855	**0.020**	0.128
3 weeks	6.052	**0.018**	0.131
4 weeks	16.474	**<0.001**	0.292

The test of significance is general Linear model (univariate) with GAGS score, noninflammatory lesions and inflammatory lesions at a certain time point as the dependent variable and treatment (group) as the fixed factor.

DISCUSSION

The chronic, self-limiting inflammatory disease known as acne vulgaris affects the pilosebaceous unit. On the face, neck, trunk, or proximal upper extremities, it is a common cutaneous condition characterised by the persistent or repeated development of papules, pustules, or nodules [11].

It is the most prevalent skin condition, and while it often appears during puberty and gets worse during adolescence, epidemiological research indicates that it can occur at any age. 85% of young people globally are impacted by it [12]. Acne is caused by a combination of immunologic, inflammatory, and hormonal pathways [13].

A large protein called lactoferrin is secreted into a number of bodily fluids, including sweat, and it is also a part of neutrophil granules [14]. It was therefore assumed that acne vulgaris would induce its existence. Due to its high rate of microbial death, anti-inflammatory qualities, and safety for human usage, lactoferrin may be a promising target for antimicrobial medication development [15, 16].

To the best of our knowledge, no research has been done to identify the part lactoferrin plays in the etiology of acne vulgaris. Therefore, the purpose of this study was to assess the blood level of lactoferrin in acne vulgaris patients as well as the effectiveness of oral lactoferrin supplementation in patients with moderate acne vulgaris.

The current study included 42 individuals with acne vulgaris and 42 healthy individuals served as the control group. In the acne vulgaris and control groups, the proportion of female participants was 76.2% and 69%, respectively. There was no statistically significant difference seen between the two study groups (p = 0.463).

This was in line with the findings of **Tayel *et al.*** [17], who demonstrated that there was a gender difference in the study and that women reported acne at a higher rate than men (30.30 vs. 39.13%, p = 0.009). Similarly, women had a greater prevalence of clinically verified acne (28.64%) compared to men (20.20%, p = 0.006).

Our findings contradicted those of **Al-Kubaisy *et al.*** [18] cross-sectional study at the Syrian International University for Science and Technology, which involved the selection of a sample of 500 students. The study revealed that the prevalence of acne in males was higher than in females (42.9% versus 23.6%, P <0.0001), possibly as a result of increased sebum production.

This does not imply that females are more likely than males to get acne, but rather that girls seek therapy because they are more conscious of the way their faces look [19]. Women were more likely to seek medical

attention, felt more ashamed, and worried more about the condition than men did. In addition to being more emotional and sensitive about their looks and the possible impact of the illness on their marital status, women are more conscious of the beauty of their skin [20, 21].

The acne vulgaris group in the current study had a mean lactoferrin level of 2447.9 ± 1169.7 pg/ml, which was statistically substantially higher than the control group's level of 740.8 ± 165.9 pg/ml. This was in line with research by **Sharara *et al.*** [22] in which 40 AV patients, divided into 20 mild and 20 severe instances, had their serum LF measured using an enzyme-linked immunosorbent test compared to 20 healthy controls. The study's findings demonstrated a significantly increased serum LF level in all AV patients compared to healthy controls. Furthermore, serum LF levels were considerably higher in each subgroup when compared to healthy controls (P≤0.001) and significantly reduced in mild acne cases (142.75±28.90 ng/ml) compared to severe instances (P≤0.001).

The current findings also agreed with those of **Alkady *et al.*** [5], who separated the 90 participants into Group A, which consisted of 70 patients who had skin break out vulgaris based on a clinical analysis. 20 distinctly solid participants made up Gathering B (control gathering). They demonstrated that the subjects' serum levels of lactoferrin were measured. The lactoferrin level was 233.25±160.93 in the cases group and 93.75±26.99 in the control group. The study patients' serum lactoferrin levels were found to be considerably greater than those of the control group (P<0.001).

In the present investigation, the optimal threshold for lactoferrin levels to distinguish cases of acne vulgaris from the control group was around 995.67 pg/ml. Excellent specificity (95 percentage) and sensitivity (98 percentage) characterise this value. p < 0.001 indicated a high statistical significance of the area under the curve, which was 0.984. Serum lactoferrin vs. baseline GAGS exhibits a statistically significant strong positive connection with inflammatory lesions, but not with non-inflammatory lesions.

Serum LF levels above 175 ng/ml in the **Sharara *et al.*** [22] trial demonstrated a 95% sensitivity and specificity in differentiating between mild and severe acne cases using the ROC curve**.** Additionally, **Alkady and associates**[5] found a significant correlation between the severity of acne and lactoferrin; at a cutoff value of lactoferrin >174.2, this correlation has good predictive discrimination between mild and severe acne, with an AUC of 0.979.

As a first-line defence protein, lactoferrin is thought to protect against a wide range of microbial infections and regulate the production of proinflammatory cytokines. Lactoferrin broad antibacterial and moderating actions reduce skin irritation in this way [23]**.** At the sites of aggravation, LF can lessen the harmful effects of reactive oxygen species produced by leukocytes [24-26]. Biological actions of lactoferrin include antiviral, antibacterial, anti-inflammatory, and anti-cancer properties [27].

Lactoferrin is a commonly used component in food items, medications, and cosmetics because it is safe and effective [28]**.** Through its involvement in the regulation of the expression of genes linked to adipocyte proliferation and differentiation as well as proteins associated to lipid metabolism, lactoferrin has been shown in previous research to effectively regulate the development and metabolism of adipocytes. These results lead to the hypothesis that lactoferrin could be a useful target for controlling adipocyte dysfunction or managing metabolic diseases [29, 30].

The study to evaluate the efficacy of lactoferrin supplementation included 42 patients with moderate acne vulgaris taking topical erythromycin twice daily for a month in combination with topical retinoids once at night for a month and systemic antibiotic therapy (Azithromycin) once daily for 3 days/week for a month. This group was furtherly divided into two equal subgroups: 21 acne patients who received lactoferrin supplementation and 21 acne patients who didn't receive lactoferrin supplementation. The two treatment arms showed no statistically significant differences in sex, age, BMI as well as weight categories, acne duration, and serum lactoferrin.

In the current study, GAGS at 2 weeks, GAGS at 3 weeks and GAGS at 4 weeks in the acne vulgaris group who received lactoferrin supplementation were statistically significantly lower as compared to the acne vulgaris group with no lactoferrin supplementation with p-value of <0.001. Also, the percent of reduction of GAGS at 2 weeks, at 3 weeks and at 4 weeks in the acne vulgaris group who received lactoferrin supplementation were statistically significantly higher as compared to the acne vulgaris group with no lactoferrin supplementation. The noninflammatory lesions (comedones) were statistically insignificantly different between the two groups at basal level (p = 0.137). The noninflammatory lesions (comedones) were significantly improved in the acne vulgaris group with lactoferrin supplementation at all 4 other time periods with p-values of 0.046, 0.009, 0.002, and <0.001 at 1, 2, 3, and 4 weeks, respectively than the acne vulgaris group with no lactoferrin supplementation. The inflammatory lesions were statistically insignificantly different between the two groups at basal level (p=0.849) and 1week (p=0.400). The inflammatory lesions were significantly improved in the acne vulgaris group with lactoferrin supplementation at all 3 other time periods with p-values of 0.020, 0.018, and <0.001 at 2, 3, and 4 weeks, respectively than the acne vulgaris group with no lactoferrin supplementation.

The current findings are consistent with those of **Mueller *et al.*** [8], who gave chewable tablets containing bovine lactoferrin twice a day for eight weeks to 43 teenagers and young adults with acne vulgaris. The increase in acne lesion counts above baseline was the

main effectiveness goal. By the time the trial ended, there had been a mean decrease from baseline in the number of inflammatory lesions (20.2%), non-inflammatory lesions (23.5%), and total lesions (22.5%). At the end of the research, 76.9% of participants had fewer lesions overall.

Furthermore, this was consistent with the findings of **Chan *et al.*** [31] double-blind, placebo-controlled experiment, in which 168 participants between the ages of 13 and 40 were randomized to either a placebo or a lactoferrin capsule formulation with zinc and vitamin E twice a day for three months. A decrease in the quantity of acne lesions in comparison to the placebo was the main outcome measure. In comparison to the placebo group (n = 82), they found that the lactoferrin group exhibited a substantial median percent reduction in total lesions as early as 2 weeks, with the largest reduction happening at week 10. At week 10, there was also the greatest decrease in inflammatory lesions and comedones (32.5%, $P < 0.0001$).

107 participants with mild to moderate acne were treated with oral lactoferrin and a 0.15 percent retinol cream gel for eight weeks as part of the **Fabbrocini *et al.*** [32] trial. No other topical or systemic treatment was permitted throughout the study. A quality of life questionnaire and the Global Acne Grading System (GAGS) were used to assess the severity of the acne and the effectiveness of the treatment. The treatment's acceptability and tolerability were also noted. The majority of patients exhibited a successful therapeutic response, as evidenced by a 51% reduction in their GAGS global score. There were no patient withdrawals from the research due to adverse effects, and in 87.8% of the sample, tolerability was good or very acceptable.

Although the data are valuable, there are certain limitations to the study. This included the limited sample size and the use of only one acne severity scale, which may have reduced the power of the results. Estimating a desirable amount of lactoferrin that improves acne vulgaris may also be aided by measuring the serum level of lactoferrin following treatment.

CONCLUSION

We came to the conclusion that patients with acne vulgaris are primarily middle-aged and adolescent patients, with the skin condition being common in both age groups. One possible biomarker for diagnosis in acne vulgaris is lactoferrin. Acne vulgaris can be effectively treated with lactoferrin supplements.

Conflict of interest: No conflicts of interest are disclosed by the investigators.

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