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Serum Zonulin and Its Role in Rosacea Pathogenesis: A Comprehensive Estimation Study

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ABSTRACT

Introduction: Rosacea is a chronic inflammatory skin disorder distinguished by recurrent episodes of inflammatory papules, persistent erythema, facial flushing, pustules, and telangiectasia. Any disturbance in the gut microbiome could influence the immune system equilibrium in rosacea by releasing zonulin, leading to increased intestinal permeability and the passage of many microbes into the circulation, causing inflammation.

Objectives: We aimed to estimate the serum zonulin levels in cases with rosacea compared with healthy controls. Some fecal bacteria were investigated in an attempt to find a relationship between gut microbiome and rosacea.

Methods: This case-control study was performed on 42 participants aged above 18 years: 21 patients with a clinical diagnosis of rosacea approved by dermoscopy and 21 healthy individuals as controls. The serum zonulin level was estimated by the enzyme-linked immunosorbent assay technique, and some gut microbiomes were investigated using real-time quantitative PCR.

Results: There was a statistically significantly higher serum zonulin level in rosacea cases than in controls. There was a statistically significant elevation in *Bacteroides* and *Lactobacillus* gut microbiomes in rosacea patients compared to controls, while there was no statistically significant increase in *Fusobacterium* microbiome in patients. Zonulin levels did not show a significant correlation with gut microbiome.

Conclusions: Serum zonulin measurement can be used as a discriminating marker between rosacea and healthy controls, due to getting a specific cut-off point in ROC analysis with the highest specificity and sensitivity (100% and 100%, respectively). Gut microbial dysbiosis could play a valuable role in the disease pathogenesis.

Introduction

Rosacea is a chronic inflammatory skin disease that affects 2–10% of the general population. It displays erythema, edema, flushing, telangiectasia, papules, pustules, ocular lesions, and phymatous changes that progress in the centrofacial region alone or in combination [1]. Rosacea is clinically categorized into four subtypes: (1) papulopustular, (2) erythematotelangiectatic, (3) phymatous, and (4) ocular rosacea [2]. In patients with rosacea, there are numerous flare triggers, such as exercise, hot and cold temperatures, ultraviolet radiation, spicy foods, and alcohol [3]. These factors can increase the susceptibility of patients to skin disorders by disrupting immune function or altering the skin's epidermal barrier function [4]. Rosacea appears to be the result of the innate and adaptive immune systems' dysregulation as well as of neurovascular dysfunction, skin barrier dysfunction, microbial dysbiosis, sebaceous gland dysfunction, metabolic dysfunction, and genetic predisposition [5]. The protein zonulin was initially identified as an endogenous bacterial enterotoxin human analogue, zonula occludens toxin (Zot), which is produced by the intestinal bacterium Vibrio cholerae [6]. Zonulin is a critical regulator of tight junctions in intestinal epithelial cells, as it reversibly opens the intercellular tight junctions in the small intestine. It operates through PAR2, which results in the tight junction proteins and the actin filaments, subsequently increasing intestinal permeability [7]. This allows passage of macromolecular proteins, toxins, microorganisms, and inappropriate antigens into systemic circulation across the mucosa, initiating an innate immune response. When the process persists, an adaptive immune response begins, producing pro-inflammatory cytokine. [8]. This process further facilitates the passage of antigens by opening the paracellular pathway, thereby establishing a vicious cycle. The consequence is the breakdown of immune tolerance and chronic inflammation [8]. Luminal zonulin release is induced by either gliadin exposure or microbiome imbalance [9]. The barrier endothelial and epithelial layers function in the intestine can be preserved by inhibiting the zonulin pathway with zonulin antagonists (larazotide acetate), epidermal growth factor receptors (EGFR) inhibitors, or PAR2 modulators [10]. A previous study revealed a significant increase in serum zonulin levels in rosacea patients compared to healthy controls [11]. The link between gut microbiome and rosacea has been reported [12].

The human microbiome is a diverse collection of microorganisms, including bacteria, that are both internal and external to the human body. Intestinal flora is one of the human microbiota ecosystems that has a significant impact on our health [13]. An inflammatory response may be initiated by any quantitative or qualitative alterations to the intestinal microbiomes, which may be followed by autoimmune processes or tissue damage [14]. These modifications, which encompass a variety of dietary components, illnesses, lifestyles, prebiotics, probiotics, antibiotics, and novel biologic medications, have the potential to modify gut microbial communities. These modifications can result in dysbiosis, which can further reduce the gut mucus layer integrity, allow microbes to pass through the intestinal barrier, induce harmful effects by neurotransmitters of the gut microbes or the host, produce toxic products, produce B cell hyperresponsiveness, impair T cell differentiation, and create low IgA secretion levels [15]. In the process of passing through the circulatory system, all these substances transform the state of the skin from healthy to dysbiotic [15].

Objectives

The present study aimed to estimate the serum zonulin level in patients with rosacea compared with healthy controls. In addition, some fecal bacteria were investigated and correlated with the disease severity in an attempt to find a relationship between rosacea and gut microbiome.

Methods

This case-control research was carried out on a total of 42 individuals: 21 rosacea cases and 21 healthy sex- and age-matched individuals. They were randomly chosen from the Dermatology Outpatient Clinic in AL Zahraa University Hospital and Damanhour Medical National Institute during the period from February 2021 to December 2022. The Ethics Committee of the Faculty of Medicine for Girls at Al-Azhar University, Egypt, approved this research. IRB (202102652).

The sample size was determined using the Epi-Info statistical application developed by the World Health Organization and the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, in 2002. The study's power was calculated at 84%, with a 5% accepted error and a 95% confidence limit. The annual influx into the hospital ranged from 30 to 50 cases. Accordingly, the minimum estimated sample size constituted 21 cases and 21 healthy controls. Patients of both sexes aged above 18 years with any subtype of rosacea were involved in the research. Exclusion criteria included patients with systemic diseases or autoimmune diseases known to affect levels of zonulin, for example (type 1 diabetes, coeliac disease, rheumatoid arthritis, multiple sclerosis, autoimmune goiter, and inflammatory bowel disease), patients who were taking antibiotics, including tetracycline and probiotics, within six weeks before the study that might have influenced the gut environment, use of antibiotics within four weeks before the study in the control group, patients who were undergoing radiotherapy and chemotherapy, and

patients with dermatological diseases, for example, psoriasis, lichen planus, and atopic dermatitis.

Clinical Assessment

A general and dermatological examination was performed on each patient to rule out the presence of any additional systemic or related dermatologic conditions. We used metric units to measure the body mass index (BMI). The presence of either one of the diagnostic phenotypes, including phymatous changes or centrofacial persistent erythema in a characteristic pattern that could periodically intensify was the basis for a diagnosis of rosacea [16]. In the absence of these features, the diagnosis was established by the presence of any two of the following major features–telangiectasia, flushing/transient erythema, papules, or pustules–and the following ocular manifestations: lid margin telangiectasia, interpalpebral conjunctival injection, spade-shaped infiltrates in the cornea, scleritis, and sclerokeratitis [16, 17], in accordance with the recommendations of the global ROSacea COnsensus (ROSCO) panel [16]. Dermoscopic examination was done by using a DermLite (DL4W) polarized dermatoscope to confirm the diagnosis of rosacea. The diagnostic patterns of rosacea included orange-yellow colored areas, polygonal blood vessels, a pinkish background, superficial white scales, vellus hair, follicular plugs, pustules, perifollicular white coloration, follicular yellow clods, and sebaceous hyperplasia (Figure 1). Some clinical and dermoscopic photos of our patients with different subtypes of rosacea are illustrated in Figure 2.

Laboratory Assessment

Serum zonulin level measurement: 10 ml of blood was collected from all participant groups after 12 hours of fasting and deposited in gel vacuum biochemistry tubes. The serum was separated by centrifugation at 3,000 rpm for 20 minutes after coagulation for 10–20 minutes. The serum that was separated was maintained at –80° C until it was analyzed. A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) reagent (Shanghai Sunred Biological Technology Company) was employed to conduct the zonulin test. The kit's measurement range was 0.25-70ng/ml.

Measurement of the Gut Microbiome: We collected the stool specimen 24 hours prior to the sampling visit. Specimens were transported to the laboratory using cooler bags and stored at –20°C until analysis. Real-time quantitative polymerase chain reaction (qPCR) was conducted using specific primers targeting the following bacterial taxa: Bacteroides (Gram-negative, non-spore-forming bacteria in the phylum Bacteroidetes), Lactobacillales (Gram-positive, non-spore-forming bacteria in the phylum Firmicutes), and Fusobacterium (anaerobic, Gram-negative bacteria in the phylum Fusobacteria). Briefly, DNA was extracted from stool samples using the QIAamp® Fast DNA Stool Mini Kit Protocol according to the manufacturer's instructions. Real-time qPCR experiments were performed with the Step One Plus Applied Biosystems using the FastStart DNA Master SYBR Green kit (Roche Diagnostics, Indianapolis, IN). The thermal cycling conditions used were as follows: an initial DNA denaturation step at 95° C for 10 minutes, followed by 45 cycles of denaturation at 95° C for 10 s, primer annealing at optimal temperature for 20 s, and extension at 72° C for 15 s. Finally, the bacterial concentration from each fecal sample was calculated by comparing the Ct values obtained from the standard curves with Applied Biosystems software. Standard curves were constructed for each experiment using serial tenfold dilutions of bacterial genomic DNA (of known concentration) from pure cultures, corresponding to 1X106 to 1X 107 16S rRNA gene copies/gram of feces. Standard curves were normalized to the copy number of the 16S rRNA gene for each.

Statistical Analysis

The Statistical Program for Social Science (SPSS version 24.0) was employed to analyze the data. The mean±standard

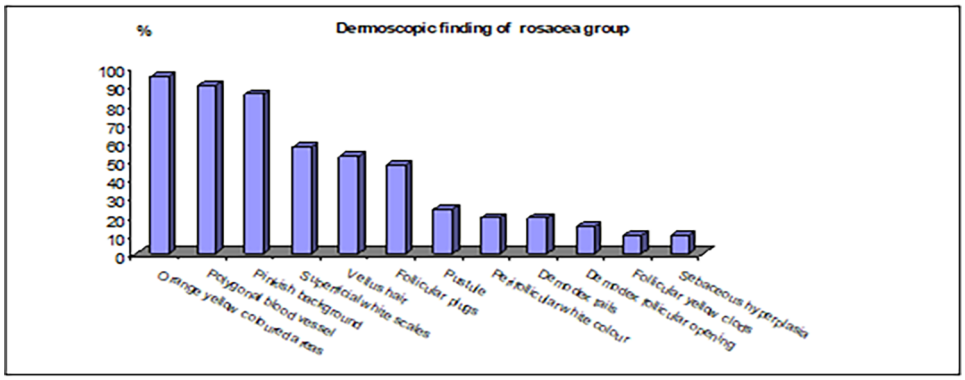

Figure 1. Dermoscopic findings of the rosacea group.

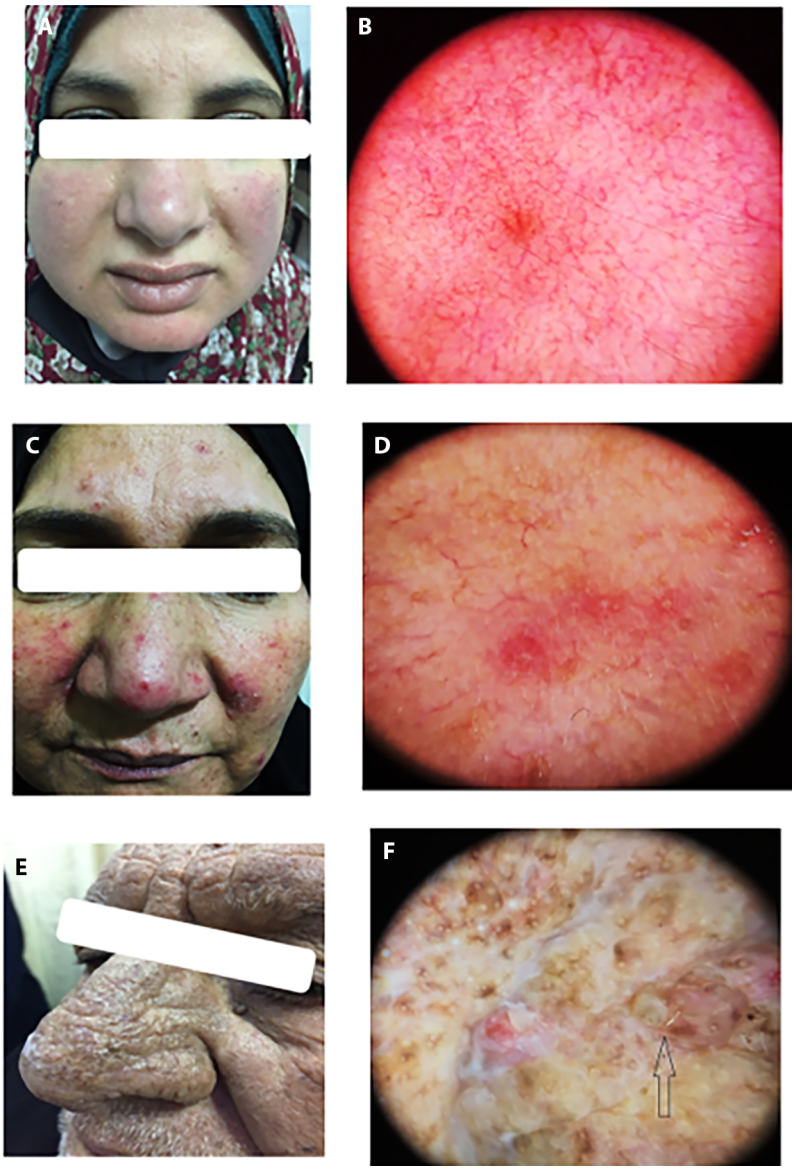

Figure 2. Clinical and dermoscopic photos of patients with different subtypes of rosacea. A. 39-year-old female patient, BMI=27.7 kg/m2 with moderate erythematotelangiectatic rosacea as of one year affecting both cheeks, chin, and nose (telangiectasia score 2, erythema score 2); B. Dermoscopic photo showing polygonal blood vessels with pinkish background. C. 55-year-old female patient, BMI=29 kg/m2 with moderate papulopustular rosacea as of two months affecting both cheeks and nose (inflammatory score 2, telangiectasia score 2) (erythema score is 1); D. Dermoscopic photo showing follicular pustules with orange-colored areas and linear vessels forming incomplete polygons. E: 55-year-old male patient, BMI=29.6 kg/m2 with rhinophyma as of one year; F: Dermoscopic image showing sebaceous hyperplasia characterized by follicular yellow clods (black arrow).

deviation (SD) was utilized to represent the parametric quantitative data. Median and interquartile range (IQR) were utilized to represent the non-parametric data. Frequency and percentage were the metrics employed to represent qualitative data. When comparing two means, an independent-samples t-test of significance was employed. When contrasting more than two means, a one-way analysis of variance (ANOVA) was employed. For multiple comparisons among various variables, the post hoc test was used. To contrast the proportions of two qualitative parameters, the chi-squared ($\chi 2$) test of significance was used. The data was correlated using Pearson's correlation coefficient (r) test.

To determine the best cut-off point for this marker, we calculated its positive predictive value (PPV), sensitivity, specificity, negative predictive value (NPV), and area under the curve (AUC) utilizing a receiver operating

characteristic (ROC) curve. A 95% confidence level was used, and a 5% tolerance for error was allowed. Assuming a significance level of P < 0.05, the results indicated statistical significance.

Results

Both groups were age- and sex-matched. The age differed insignificantly between both groups, ranging from 18 to 58 years (mean ± SD = 39.52 ± 10.73 years for the rosacea group and 36.57 ± 7.03 years for the control group; *P* = 0.298). Rosacea duration ranged from 1 to 72 months (median=12 months). The demographic data of all participants and rosacea patients' clinical characteristics were determined in Table 1.

The patient group had a significantly greater zonulin level compared to the control group (*P*=0.001) (mean ± SD = 33.71 ± 10.77 ng/ml for the rosacea group and 10.59 ± 2.17 ng/ml for the control group). The zonulin ROC curve in rosacea patients at a cut-off point >16.25 ng/ml demonstrated 100% specificity and 100% sensitivity of zonulin (Tables 2–3; Figure 3).

Table 1. Demographic Data of All Participants and Clinical Characteristics of Rosacea Patients.

Variables			Cases (N=21)		Controls (N=21)	Test	P-Value
Age (year)	Range		19 – 58		25 – 50	t: 1.055	0.298
	Mean ± SD		39.52 ± 10.73		36.57±7.03		
BMI	Range		22 – 34.4		22.7 – 33.3	t : 0.078	0.939
	Mean ± SD		27.45 ± 3.29		27.52±3.07		
Sex	Male	N	3		3	X2:0.0	1.0
		%	14.3%		14.3%		
	Female	N	18		18		
		%	85.7%		85.7%		
Occupation	Non worker	N	17		18	X2: 0.171	0.679
		%	81.0%		85.7%		
	Worker	N	4		3		
		%	19.0%		14.3%		
Residence	Urban	N	7		6	X2: 0.111	0.739
		%	33.3%		28.6%		
	Rural	N	14		15		
		%	66.7%		71.4%		
Clinical data			**N**	**%**			
Subtype of rosacea	Erythematotelangiectatic		14	66.7			
	Papulopustular		5	23.8			
	Rhinophyma		2	9.5			
Degree of severity	Mild		4	19.0			
	Moderate		12	57.1			
	Severe		5	23.8			
Bowel habits	Normal		19	90.5			
	Diarrhea		1	4.8			
	Constipation		1	4.8			
Food habits	Not increased		6	28.6			
	Increased with spicy		8	38.1			
	Increased with hot liquid		5	23.8			
	Increased with both		2	9.5			
Duration	Range		1 – 72 m				
	Median		12 m				

Data are presented as mean ± SD, or number (%), t: student test, χ2: Chi-square test, BMI = body mass index; SD = standard deviation.

Table 2. Zonulin Level Comparison in Rosacea Cases and Controls.

		Cases (N=21)	Controls (N=21)	t.	P-Value
Serum zonulin (ng/ml)	Range	17.3 – 49.2	7.5 – 15.2	9.650	0.001*
	Mean±SD	33.71 ± 10.77	10.59 ± 2.17		

Data are presented as mean ± SD. P<0.05 statistically significant. P≥0.05 statistically not significant. Abbreviations: SD: standard deviation; t: Student t test.

Table 3. ROC Analysis of Serum Zonulin Showing AUC, Best Cut-off Point, Sensitivity, and Specificity for Zonulin to Predict Cases vs. Controls.

Cut-off Point	Sensitivity %	Specificity %	PPV	NPV	AUC	P-Value	95% CI	
16.25 (ng/ml)	100	100	100	100	1.000	<0.001	1.000	1.000

Abbreviations: AUC: area under the curve; CI: confidence Interval; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver operating curve.

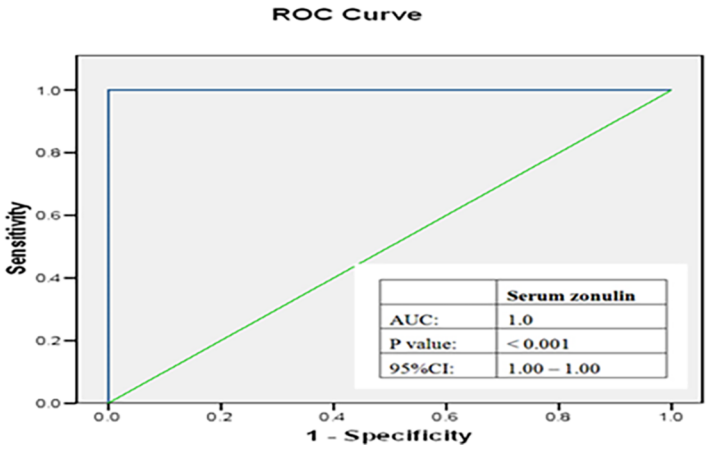

Figure 3. ROC curve for serum zonulin to predict cases versus controls.

Zonulin levels did not show a significant correlation with the age of the patient, BMI, duration of disease, or gut microbiomes (Bacteroides, Lactobacillus, and Fusobacterium), nor a significant relation with sex, occupation, residency, subtypes of rosacea, degree of severity, or food habits in the rosacea group. The rosacea group had a significant increase in Bacteroides and Lactobacillus gut microbiomes compared to controls (mean ± SD =8.49± 0.58 for Bacteroides in the rosacea group and 8.14 ± 0.46 for the control group, *P*=0.041), (mean ± SD =11.21 ± 1.20 for Lactobacillus in the rosacea group and 9.89 ± 0.52 for the control group, *P*=0.001). In contrast, Fusobacterium did not show a significant difference between rosacea group and controls (*P*= 0.842) (Table 4).

Bacteroides and Fusobacterium gut microbiomes showed statistically significant relations with subtypes of rosacea, while Lactobacillus showed no significant relation with subtypes of rosacea. The strongest significant relation was found between papulopustular and rhinophyma subtypes regarding Bacteroides and Fusobacterium (*P*=0.007 and *P*=0.004, respectively). A less significant relation was detected between erythematotelangiectatic and rhinophyma subtypes regarding Bacteroides (*P*=0.022), with a less significant relation between erythematotelangiectatic and papulopustular subtypes regarding Fusobacterium (*P*=0.015) (Tables 5–6).

Lactobacillus gut microbiome showed a significant relation with degree of severity, with the highest significant relation found in patients with mild and moderate disease (*P*=0.014) (Table 7), while there was no statistically significant relation between Fusobacterium or Bacteroides gut microbiomes and degree of severity in rosacea patients.

Discussion

This study detected a statistically significant higher serum zonulin level in rosacea cases than in controls. Our finding confirms the results of a previous study done by Yüksel and Ülfer [11], who revealed a significant elevation in serum zonulin levels in rosacea patients compared to healthy controls.

Table 4. Comparison Between Rosacea Cases and Controls Regarding Gut Microbiomes (Bacteroid, Lactobacillus, and Fusobacterium).

Variables		Cases (N=21)	Controls (N=21)	t.	P-value
Bacteroide	Range	7.2 – 9.5	7.3 – 8.9	2.108	**0.041***
	Mean ± SD	8.49 ± 0.58	8.14 ± 0.46		
Lactobacillus	Range	9.1 – 12.9	9.08 – 10.8	4.645	**0.001***
	Mean ± SD	11.21 ± 1.20	9.89 ± 0.52		
Fusobacterium	Range	5.1 – 8.02	5.1 – 7.9	0.200	0.842
	Mean ± SD	6.41 ± 1.00	6.34 ± 1.08		

Data are presented as mean ± SD. P<0.05 statistically significant. P≥0.05 statistically not significant. Abbreviations: SD: standard deviation; t: Student t test.

Table 5. Relationship Between Bacteroid Gut Microbiome and Subtypes of Rosacea in the Case Group.

Bacteroide	Subtype of rosacea		
	Erythematotelangiectatic	Papulopustular	Rhinophyma
Range	7.5 – 9.06	8.01 – 9.5	7.2 – 7.9
Mean ± SD	8.50 ± 0.42	8.82 ± 0.71	7.55 ± 0.49
F. test	4.617		
P-Value	**0.024***		
Erythematotelangiectatic and papulopustular	Erythematotelangiectatic and rhinophyma	Papulopustular and rhinophyma	
0.232	**0.022***	**0.007***	

Data are presented as mean ± SD. P<0.05 statistically significant. P≥0.05 statistically not significant. Abbreviations: F: ANOVA test; SD: standard deviation.

Table 6. Relationship Between Fusobacterium Gut Microbiome and Subtypes of Rosacea in the Rosacea Group.

Fusobacterium	Subtype of Rosacea		
	Erythematotelangiectatic	Papulopustular	Rhinophyma
Range	5.1 – 7.9	5.2 – 5.8	7.3 – 8.02
Mean ± SD	6.57 ± 0.93	5.44 ± 0.23	7.66 ± 0.51
F. test	6.254		
P-value	0.009*		
Erythematotelangiectatic and papulopustular	Erythematotelangiectatic and rhinophyma	Papulopustular and rhinophyma	
0.015*	0.093	**0.004***	

Data are presented as mean ± SD. P<0.05 statistically significant. P≥0.05 statistically not significant. Abbreviations: F: ANOVA test; SD: standard deviation.

These findings suggest that the significant increase in serum Zonulin observed in rosacea patients indicates enhanced intestinal permeability, which may contribute to a "leaky gut" and potentially play a role in the pathophysiology of the disease, indicating that enhanced intestinal permeability could be responsible for a leaky gut in rosacea patients, hence might have a role in the disease pathophysiology [7]. Based on our ROC curve results, serum zonulin level at a cut-off of >16.25 ng/mL could be a reliable predicting marker for patients with rosacea. However, we did not find an association between serum zonulin level and the degree of disease severity. This could be explained by the small sample size. The present study indicated a significant elevation in Bacteroides and Lactobacillus gut microbiomes in rosacea patients

Table 7. Relationship Between Lactobacillus Gut Microbiome and Degree of Severity in the Rosacea Group.

Lactobacillus	Degree of Severity		
	Mild	Moderate	Severe
Range	12.4 – 12.8	9.1 – 12.9	10.1 – 12.3
Mean ± SD	12.58 ± 0.17	10.95 ± 1.21	10.76 ± 0.91
F. test	4.277		
P-Value	0.030*		
Mild and Moderate	**Mild and Severe**	**Moderate and Severe**	
0.014*	0.018*	0.742	

Data are presented as mean ± SD. P<0.05 statistically significant. P≥0.05 statistically not significant. Abbreviations: F: ANOVA test; SD: standard deviation.

compared to the control group, while there was no significant increase in Fusobacterium gut microbiomes in rosacea patients. In line with our findings, Chen et al. [18] found abundant relative Fusobacterium and Bacteroides. However, they found a reduced abundance of Lactobacillus gut microbiota in rosacea patients compared to the controls. Similar to our finding, Nam et al. [19] have detected an increased abundance of Lactobacillus gut microbiota in rosacea patients compared to the controls. However, they detected changes in other microbiomes not investigated by our study.

The disagreement with the Chen et al. [18] study about Fusobacterium and Lactobacillus could be attributed to the differences in demographic and clinical characteristics of the included sample, as well as the difference in sample size and geographical distribution between our and their studies. These findings of changed gut microbiome levels in rosacea patients explain their importance in the pathogenesis of the disease. In a healthy state, a diverse and extensive enteric microbiome is responsible for preventing the passage of noxious substances across the gut mucosal surface. However, a disturbance in the mucosal surface, which may be caused by a difference in the enteric microbiome or intrinsic composition due to inflammatory or autoimmune diseases, may permit harmful substances to enter the bloodstream, leading to adverse effects at peripheral sites [20]. Also, the intestinal microbial population appears to have an immunomodulatory effect on non-enteric systems, including the skin; however, the precise mechanism for this effect is still not fully understood [19, 21]. In this study, we detected a significant relation between Bacteroides and Fusobacterium gut microbiomes with subtypes of rosacea. Bacteroid microbiome showed the highest significant relation between papulopustular and rhinophyma subtypes and a less significant relation between erythematotelangiectatic and rhinophyma subtypes. Fusobacterium microbiome showed the highest significant relation between papulopustular and rhinophyma subtypes and a less significant relation between erythematotelangiectatic and papulopustular subtypes. Regarding Bacteroides and Fusobacterium, these findings may be attributed to papulopustular rosacea, as antibiotic (oral metronidazole) treatment could improve both rosacea symptoms and inflammatory enteritis, suggesting a bacterial pathogen may be involved [22].

Additionally, rosacea is reported to be associated with gastrointestinal comorbidities, and certain studies have linked this coexistence to alterations in the gut microbiome [23]. It has been mentioned by Ellis et al. [24] that alterations in the gut microbiome could lead to cutaneous manifestations through the brain-gut-skin axis, which explains the presence of rhinophyma. They postulated that negative emotional states, for example, anxiety and depression, disrupt the gastrointestinal system, resulting in altered gut flora and increased intestinal permeability in the intestine. It leads to systemic inflammation that can disrupt cutaneous homeostasis by activating T cells and disrupting immunosuppressive cytokines [24]. Besides, cutaneous microbiomes promote efficient immune functions, acting as a potential contributor to the development of rosacea [25]. Rhinophyma is considered a more pronounced inflammatory rosacea with sebaceous gland hypertrophy and excessive fibrous tissue overgrowth of the skin [26]. It is mediated by the fibrogenic cytokine TGF-β and the TGF-β receptor, which is upregulated in rhinophyma by fibroblast activation.

Furthermore, a higher number of fibroblasts correlates with increased mast cell number, which is fibrosis-driven [27]. Moreover, Demodex folliculorum has been implicated in triggering rosacea due to inflammation within pilosebaceous follicles [28], which PCR can detect because it is a very sensitive tool for the diagnosis of Demodex in patients with rosacea. In a case-control study, Trave et al. [29] found that PCR is a sensitive method for identifying the presence of Demodex on the faces and scalps of patients with papulopustular rosacea. Taken all together, these cutaneous mechanisms enhance the effect of gut microbiomes. This study found a

significant relation between the Lactobacillus gut microbiome and the degree of severity. Lactobacillus had the highest significant relation in patients with mild and moderate disease. Some intestinal bacterial species, such as Lactobacillus, are reported to confer a health advantage to the host [30].

Limitations

Study limitations include the small sample size and the lack of confirmation of diagnosis of rosacea with the gold standard histopathology, as it could have given a better assessment of rosacea than dermoscopy. Also, body mass index (BMI)-matched healthy individuals limited the evaluation of obesity in relation to rosacea.

Conclusion

Concerning the descriptive results of our study, the serum zonulin shows a significant elevation in rosacea patients compared to healthy controls. Accordingly, high sensitivity and specificity of serum zonulin measurement can be used as a discriminating marker between rosacea and control due to getting a specific cut-off point in ROC analysis, especially in dark-skinned people, who might be underdiagnosed. The findings of changed gut microbiome levels in rosacea patients explain their importance in the pathogenesis of the disease. Further studies with a larger sample size of rosacea patients should assess the serum zonulin level and correlate it with subtypes of rosacea and the degree of severity. More research should also explore the gut microbiome's role in rosacea pathogenesis by studying more subtypes of the microbiome. Wider studies are recommended to correlate serum zonulin levels with various types of gut microbiomes, because bacterial zonulin system induction is not completely explained. Based on the relation between zonulin and intestinal dysbiosis, zonulin receptor antagonists need to be investigated as a future new target in rosacea treatment. The role of gluten restriction on rosacea severity and zonulin level needs to be investigated. In addition, the role of probiotics and prebiotics on zonulin levels needs to be inspected.

References

1. Daou H, Paradiso M, Hennessy K, Seminario-Vidal, L. Rosacea and the Microbiome: A Systematic Review. *Dermatol Ther (Heidelb)*. 2021;11:1-12. DOI: 10.1007/s13555-020-00460-1. PMID: 33170492.
2. Rodrigues-Braz D, Zhao M, Yesilirmak N, Aractingi S, Behar-Cohen F, Bourges JL. Cutaneous and ocular rosacea: Common and specific physiopathogenic mechanisms and study models. *Mol Vis*. 2021;27:323-53. PMID: 34035646; PMCID: PMC8131178.
3. Buddenkotte J, Steinhoff M. Recent advances in understanding and managing rosacea. *F1000Res*. 2018;7:F1000 Faculty Rev-1885. DOI: 10.12688/f1000research.16537.1. PMID: 30631431
4. Park K, Ikushiro H, Seo HS, et al. ER stress stimulates production of the key antimicrobial peptide, cathelicidin, by forming a previously unidentified intracellular S1P signaling complex. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;113(10):E1334-42.DOI: 10.1073/pnas.1504555113. PMID: 26903652.
5. Chen C, Wang P, Zhang L, et al. Exploring the Pathogenesis and Mechanism-Targeted Treatments of Rosacea: Previous Understanding and Updates. *Biomedicines*. 2023;11(8):2153. DOI: 10.3390/biomedicines11082153. PMID: 37626650.
6. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. J *Cell Sci. 2000*;113 Pt 24:4435-40. DOI: 10.1242/jcs.113.24.4435. PMID: 11082037.
7. Serek P, Oleksy-Wawrzyniak M. The Effect of Bacterial Infections, Probiotics and Zonulin on Intestinal Barrier Integrity. *Int J Mol Sci*. 2021; 22(21):11359. DOI: 10.3390/ijms222111359. PMID: 34768787.
8. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers*. 2016; 4(4):e1251384. DOI: 10.1080/21688370.2016.1251384. PMID: 28123927.
9. Wood Heickman LK, DeBoer MD, Fasano A. Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity. *Diabetes Metab Res Rev*. 2020; 36(5):e3309. DOI: 10.1002/dmrr.3309. PMID: 32162764.
10. Veres-Székely A, Szász C, Pap D, Szebeni B, Bokrossy P, Vannay Á. Zonulin as a Potential Therapeutic Target in Microbiota-Gut-Brain Axis Disorders: Encouraging Results and Emerging Questions. *Int J Mol Sci*. 2023;24(8):7548. DOI: 10.3390/ijms24087548. PMID: 37108711.
11. Yüksel M, Ülfer G. Measurement of the serum zonulin levels in patients with acne rosacea. *J Dermatolog Treat*. 2022; 33(1) 389–392. DOI: 10.1080/09546634.2020.1757015
12. Wang FY, Chi CC. Association of rosacea with inflammatory bowel disease: A MOOSE-compliant meta-analysis. *Medicine (Baltimore)*. 2019; 98(41):e16448. DOI: 10.1097/MD.0000000000016448. PMID: 31593075
13. Muszer M, Noszczyńska M, Kasperkiewicz K, Skurnik M. Human Microbiome: When a Friend Becomes an Enemy. *Arch Immunol Ther Exp (Warsz)*. 2015; 63(4):287-98. DOI: 10.1007/s00005-015-0332-3. PMID: 25682593
14. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients*. 2012; 4(8):1095-119. DOI: 10.3390/nu4081095. PMID: 23016134.
15. Mahmud MR, Akter S, Tamanna SK, et al. Impact of gut microbiome on skin health: gut-skin axis observed through the lenses of therapeutics and skin diseases. *Gut Microbes*. 2022;14(1):2096995. DOI: 10.1080/19490976.2022.2096995. PMID: 35866234.
16. Schaller M, Almeida LMC, Bewley A, et al. Recommendations for rosacea diagnosis, classification and management: update from the global ROSacea COnsensus 2019 panel. *Br J Dermatol*. 2020;182 (5):1269-1276. DOI: 10.1111/bjd.18420. PMID: 31392722.

17. Kang CN, Shah M, Tan J. Rosacea: An Update in Diagnosis, Classification and Management. *Skin Therapy Lett*. 2021;26 (4):1-8. PMID: 34347259.
18. Chen YJ, Lee WH, Ho HJ, Tseng CH, Wu CY. An altered fecal microbial profiling in rosacea patients compared to matched controls. *J Formos Med Assoc*. 2021; 120(1 Pt 1):256-264. DOI: 10.1016/j.jfma.2020.04.034. PMID: 32446756.
19. Nam JH, Yun Y, Kim HS, et al. Rosacea and its association with enteral microbiota in Korean females. *Exp Dermatol*. 2018;27(1):37-42. DOI: 10.1111/exd.13398. PMID: 28636759.
20. O'Neill CA, Monteleone G, McLaughlin JT, Paus R. The gut-skin axis in health and disease: A paradigm with therapeutic implications. *Bioessays*. 2016; 38(11):1167-1176. DOI: 10.1002 /bies.201600008. PMID: 27554239.
21. 21 Searle T, Ali FR, Carolides S, Al-Niaimi F. Rosacea and the gastrointestinal system. *Australas J Dermatol*. 2020; 61(4): 307-311. DOI: 10.1111/ajd.13401.PMID: 32761824.
22. Koçak M, Yağli S, Vahapoğlu G, Ekşioğlu M. Permethrin 5% cream versus metronidazole 0.75% gel for the treatment of papulopustular rosacea. A randomized double-blind placebo-controlled study. *Dermatology*. 2002; 205(3):265-70. DOI: 10.1159/0000 65849. PMID: 12399675.
23. Han J, Liu T, Zhang M, Wang A. The relationship between inflammatory bowel disease and rosacea over the lifespan: A meta-analysis. *Clin Res Hepatol Gastroenterol*. 2019; 43(4): 497-502. DOI: 10.1016/j.clinre.2018.09.009. PMID: 30389397.
24. Ellis SR, Nguyen M, Vaughn AR, et al. The Skin and Gut Microbiome and Its Role in Common Dermatologic Conditions. *Microorganisms*. 2019; 7(11):550. DOI: 10.3390/microorganisms 7110550. PMID: 31717915.
25. Holmes AD. Potential role of microorganisms in the pathogenesis of rosacea. *J Am Acad Dermatol*. 2013; 69(6):1025-32. DOI: 10.1016/j.jaad.2013.08.006. PMID: 24011460.
26. Rainer BM, Kang S, Chien AL. Rosacea: Epidemiology, pathogenesis, and treatment. *Dermatoendocrinol*. 2017; 9(1):e1361574. DOI: 10.1080/19381980.2017.1361574. PMID: 29484096.
27. Steinhoff M, Schauber J, Leyden JJ. New insights into rosacea pathophysiology: a review of recent findings. *J Am Acad Dermatol*. 2013; 69(6 Suppl 1):S15-26. DOI: 10.1016/j.jaad .2013.04.045. PMID: 24229632.
28. Chen W, Plewig G. Are Demodex mites principal, conspirator, accomplice, witness or bystander in the cause of rosacea? *Am J Clin Dermatol*. 2015; 16(2):67-72. DOI: 10.1007/s40257-015-0115-y. PMID: 25666117.
29. Trave I, Salvi I, Canepa P, Parodi A, Cozzani E. Detection of demodex mites in papulopustular rosacea using microscopic examination and polymerase chain reaction: a comparative case-control study. *Arch Dermatol Res*. 2024;316(7):485. DOI: 10.1007 /s00403-024-03228-1. PMID: 39042262.
30. Zhu W, Hamblin MR, Wen X. Role of the skin microbiota and intestinal microbiome in rosacea. *Front Microbiol*. 2023;14:1108661. DOI: 10.3389/fmicb.2023.1108661. PMID: 36846769.