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Assessment of cbiL gene expression and vitamin B12 levels in acne vulgaris: involvement of both in-vivo and in-vitro studies

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Abstract:

Acne vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous follicles that affects nearly 90% of teenagers; half of them continue to experience symptoms as adults. There have been several reports of monomorphic acneiform eruptions in patients treated with intramuscular cobalamin injections. The eruptions resolved after the cessation of the therapy. Cyanocobalamin, pyridoxine (B_6), and riboflavin (B_2) have been reported to exacerbate existing acne. Moreover, it was reported that in acne patients, the serum level of vitamin B_{12} was significantly decreased after treatment, but no explanation was given. We propose a vitamin B_{12}-mediated bacterial mechanism for acne pathogenesis on the evidence mentioned above. One hundred twenty AV patients were recruited.

Our results proved that vitamin B_{12} was involved in the pathogenesis of AV; in vivo, it proved that AV patients had significantly higher serum levels of the vitamin than controls and showed a positive correlation to the disease duration and severity. In vitro, it proved that vitamin B_{12} supplementation to p. acnes cultures

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significantly raised the porphyrins produced in these cultures when compared to the non-supplemented ones. It also proved that this shift in the metabolism is secondary to inhibition of the cbiL gene of the vitamin B_{12} synthesis in the bacteria; as porphyrins and vitamin B_{12} share the same precursors, vitamin B_{12} does has a role in either elicitation or aggravation of AV.

Keywords: Acne vulgaris, vitamin B_{12}, cbiL gene, inflammation.

Introduction:

Acne vulgaris (AV) is a pilosebaceous follicle-related chronic inflammatory condition. Acne is one of the most common skin illnesses, affecting approximately 90% of teenagers, half of whom continue to have problems as adults. Acne is still present in 1% of males and 5% of women over the age of 40 [1]. Depression, suicidal ideation, anxiety, psychosomatic symptoms, shame, embarrassment, and social inhibition can all result from [2], [3].

Interestingly, different four pathogenic mechanisms have been proposed: increased sebaceous gland activity with seborrhea, abnormal follicular differentiation with increased keratinization, microbial hypercolonization of the follicular canal, and increased inflammation primarily through adaptive immune system activation [4]. This is related to genetic susceptibility, neuroendocrine regulatory mechanisms, and diet, all of which can contribute to this multifactorial process [5], [6].

P. acnes is a Gram-positive bacterium that thrives as a symbiont in the anaerobic environment of the sebaceous unit. The presence of P. acnes in advanced acne lesions, clinical inflammation, and pustules when P. acnes is applied to unaffected skin, and the soothing properties of topical and oral antimicrobials may serve as evidence for the identification of P. acnes [7].

Although P. acnes is generally considered a harmless symbiont, it shares many of the characteristics of a disease-causing organism. One is its ability to make porphyrins, which have become the target of new light-based acne treatments [8]**.** Porphyrins can generate reactive oxygen species in keratinocytes and induce inflammation [9].

Previously, vitamin B_{12} has been observed to cause acne in some people. There have been several reports of monomorphic acne rashes in patients treated with intramuscular cobalamin. The rash subsided after treatment ended. Cyanocobalamin, pyridoxine (B_6), and riboflavin (B_2) have been reported to exacerbate existing acne [10]. In addition, it has been reported that serum vitamin B_{12} levels were significantly reduced after treatment in acne patients [11]. This study was carried out to find out the relation between the serum levels of vitamin B_{12} of acne patients and the severity and duration of the disease and also, to investigate the metabolic effects of vitamin B_{12} on p. acnes to specify a vitamin B_{12}-mediated mechanism for acne pathogenesis to be directed towards a new targeted therapy for the disease.

Patients and methods:

A) patients

120 patients with acne vulgaris (males and females; aged 14-35 years with Mild, moderate, and severe acne, using topical acne treatment; tazarotene, tretinoin, salicylic acid, and benzoyl peroxide) were recruited from the clinic of dermatology at Beni Suef University Hospital between **January 15th, 2023 and February 15th, 2023**. The study was approved by the Faculty of Medicine, Beni-Suef University Research Ethical Committee Under this code (FMBSUREC/12022023/Radi), and patients' consent was obtained.

Control group: it consisted of 100 healthy subjects (males and females; aged 14-35 years) with no obvious signs of energetic disease or curative ailment.

Furthermore, some cases were excluded from this study such as treatment with systemic antibiotics 2 before sampling, pregnant women, treatment with isotretinoin 3 months before sampling, patients suffering from any neurological disorders, and treatment with isotretinoin 3 months before sampling. In addition, age, sampling site, site of acne engrossment, sex, duration of the disease, housing area, and treatment history was recorded.

According to Global Acne Severity Scale (GASS), acne was graded on a scale from 0 to 5 by board-certified dermatologists [12]. Moreover, the control group's skin was verified by board-certified dermatologists and distinct people with no cane lesions on the chest, face, or back.

Specimen collection and processing:

Firstly, the skin was rubbed with 70% ethanol followed by extraction of acne lesions by sterile swab sticks, and was transferred to an anaerobic thioglycolate medium (Merck, Germany).

Blood sampling: 5 ml of blood was obtained by vein perforation from control and patient subjects, and then centrifuged at 4000 revolutions per min (rpm) (Sigma centrifuge 2-16P, USA) for 5 min to get non-hemolysed pure sera. If the investigation was not done immediately, the sera were preserved at -80 ^{o}C till use.

B) Methods and materials

- Each patient's sample was used to make a direct smear.
- Each transfer medium specimen was inoculated in two plates that included Blood agar (Conda, Spain), and one of those was incubated in an aerobic environment at 37°C for 24 hours and the other in anaerobic conditions for one week. Gram stain and specific tests such as catalase, indole, gelatin, and esculin were used to identify P. acnes colonies. If the P. acnes culture was negative after one week, a subculture was grown, and the substance from the Thioglycolate medium was determined again.
- Then the isolated P. acnes strains were deposited in glycerol broth at -70 °C for additional investigations.

The following investigations were carried out:

I) Assessment of the serum levels of vitamin B_{12} of patients and controls:

Serum samples from P. acnes patients and controls were tested for vitamin B_{12} levels using

ELISA according to the manufacturer's instructions **(BioMérieux, France),** and the results were presented for comparison between the two groups.

ELISA constituents:

- Deionized or distilled water.
- Container for Wash Solution.
- Eppendorf tubes for diluting samples.
- Microplate reader with 450 ± 10nm filter.
- Absorbent paper for blotting the microtiter plate.
- Precision single or multi-channel pipettes and disposable tips.

Procedures:

- All samples, reagents, and standards were prepared
- Each well was filled with 50µL of standard or sample. Then directly 50µL of ready detection reagent A was added. Shake and combine. Incubated for one hour at 37ºC.
- Aspirate and rinse three times.
- 100µL of the prepared detection reagent B was added and incubated for 30 minutes at 37ºC.
- Aspirate and rinse three times.
- 90µL of the substrate solution was added and incubated for 15-25 minutes at 37ºC.
- 50µL of the stop solution was added and the absorbance was read at 450 nm immediately.

II) Assessment of porphyrin levels in p. acnes cultures with and without vitamin B_{12} supplementation:

The identified P. acne strains were grown on MRS broth **(Oxoid, USA)** with 10 mg/ml vitamin B_{12}, a dose identical to that employed in prior work with E. coli and without vitamin B12 supplementation, and incubated anaerobically for 48-72 hours [13].

Cultured broth with and without vitamin B_{12} was centrifuged for 5 minutes at 5000rpm, and the supernatant was submitted to the ALMOKHTABER laboratory for porphyrin levels to be measured in both groups, and the data was then recorded for further comparison between the two groups.

III) Detection of Vitamin B_{12} gene expression levels with and without vitamin B_{12} supplementation:

The isolated P. acnes strains were grown on MRS medium (Oxoid, USA) with and without vitamin B_{12} supplementation for one week before being incubated anaerobically. By using real-time polymerase chain reaction (PCR), the isolated colonies were evaluated for the expression of the cbiL gene, which regulates vitamin B_{12} synthesis in P. acnes, according to the instructions for extraction, amplification, and detection of this gene (clinilab, France) [14], [15].

Primer Sequence for cbiL gene:

cbiL 3'-GACCCGGACGGGACC-5'

PCR kit (clinilab, France):

- DNA extraction kit from cell 50 test spin column bioflux (Fermentas, UK).
- Primer salt free 0.2 µmol, 49 bp (Germany).

- DNA ladder 100 bp, 50µg (sibenzyme, Russia).
- Master mix 100 test (larova Germany).
- Agarose 100 g.
- TBE buffer 10x.
- Proteinase K 100 mg.
- PCR tubes sterile BBI 1000/pk (Canada, BBI).

DNA extraction

Methods:

- Lysis buffer was prepared at first: 20mM Tris-HCL, Ph8.0, 2mM EDTA, 1.2%TritonX-100, and lysozyme to 20mg/ml were added immediately before use.
- Bacteria were harvested from overnight broth cultures in 1.5 to 2 ml microcentrifuge tube by centrifugation for 10 min at 5000 xg then the supernatant was discarded.
- The pellet was resuspended in 180 ul of the prepared lysis buffer and then incubated for 30 min at 37°C.
- Proteinase K solution (20 ul Fermantas) and 200 ul of lysis solution were added and then mixed by vortexing or pipetting to obtain a uniform suspension.
- The samples were incubated at 56°C and vortexed occasionally until the cells were completely lysed (about 30min).
- 20 ul of RNase A solution was added, mixed by vortexing then incubated for 10 min at room temperature This treatment efficiently lysed P. acne cells and prevented DNase activity.
- 400 ul of 50% ethanol was added and then mixed by vortexing.
- The prepared lysate was transferred to a gene JET^{TM} Genomic DNA Purification Column that was inserted in a collection tube, that was centrifuged for 1 min at 6000 xg then the flow-through solution was discarded, and the purification column was put in another collection tube.
- 500 ul of Wash buffer I (with ethanol) was added and then the tube was centrifuged for 1 min at 8000 xg. The flow-through solution was discarded, and the purification column was transferred to the collection tube.
- Then 500 ul of Wash buffer II with ethanol was added and also was centrifuged for 3 min at maximum speed (≥12000 xg)
- 200 ul of elution buffer was added to the center of the gene JET^{TM} Genomic DNA Purification Column to elute genomic DNA, then the whole solution was incubated for 2min at room temperature and centrifuged for 1min at 8000 rpm.
- Finally, the purification column was discarded then the purified DNA was stored at -20°C.

Statistical analysis:

Data were analyzed using the SPSS software version 18. The Frequency distribution with its percentage and descriptive statistics with mean ± S.D was calculated (S.D: standard deviation). Chi-square, t-test, and correlations were

done whenever needed. *P* values of less than 0.05 were considered significant.

Results

A total of 120 acne vulgaris patients attending the dermatological clinic at Beni-Suef University Hospital were recruited for the study between **January 15th, 2023, and February 15th, 2023.** The participants were 93 female patients and 27 male patients. The patient's age ranged from 12 to 33 years with a mean of 20.8 years. The disease duration varied from 1 to 132 months, with a mean duration of 34.39 months. Another 100 control people coming to our hospital shared in our study. There were no significant differences between patients and controls regarding age and sex.

P. acnes isolated from acne lesions:

Out of our 120 acne patients, p. acnes strains were isolated from 43 patients (35%); 6 of them were males and 37 were females with ages between 14 and 29 with a mean age of 19.51. Thirty-six of these patients had their acne distributed on the face, while 7 of them distributed on the face, chest, and back. The swabs were taken from the cheeks of 37 patients, the foreheads of 2 patients, the chins of 2 patients, and the backs of 2 patients. Forty-one patients didn't use any treatment before sampling, while 2 patients used topical retinoids for 2 months before sampling; one of them, with very severe acne, mentioned receiving systemic isotretinoin 3 months before sampling but didn't complete her course of treatment.

Classification of acne according to the Global Acne Severity Scale (GASS):

Out of our 43 isolated p. acnes strains and according to the Global Acne Severity Scale; 12 patients had mild acne, 23 patients had moderate acne, 7 patients had severe acne and 1 patient had very severe acne vulgaris as shown in **Table (1) and Figure (1).**

Table 1: Table (4): GEA scaling of acne.

Global acne severity scale	Frequency	Percent
2: mild	12	27.9
3: Moderate	23	53.5
4: Severe	7	16.3
5: Very Severe	1	2.3
Total	43	100.0

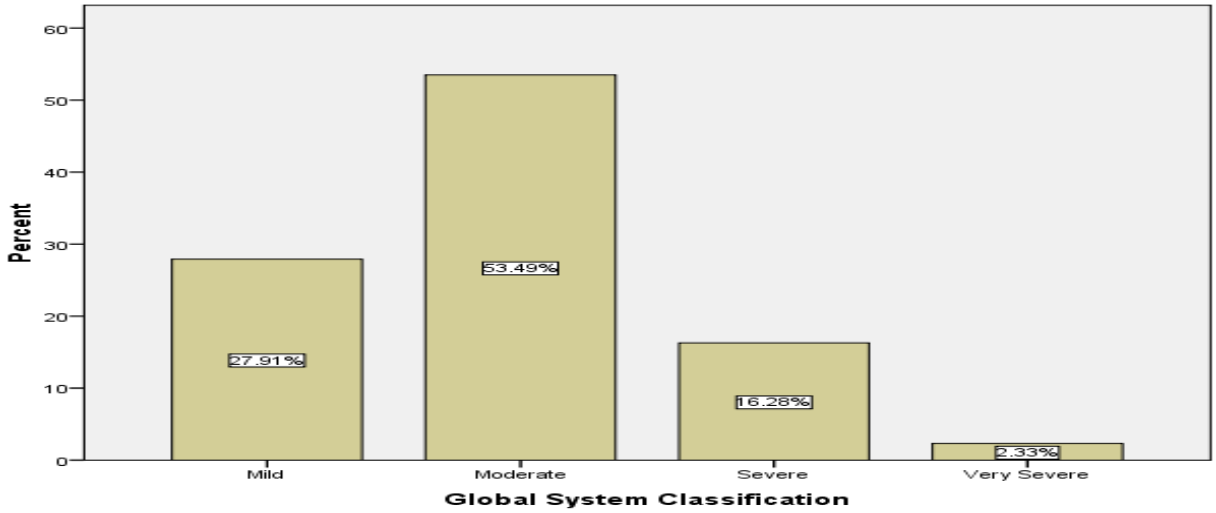

Figure 1: GEA scaling of acne.

Serum levels of vitamin B_{12} of p. acne patients:

The serum levels of vitamin B_{12} in P. acne patients ranged from 283pg/ml to 2000 pg/ml with a mean value of 1098 as shown in **Table (2) and Figure (2).**

Table 2: The serum levels of vitamin B_{12} of patients and normal control.

Parameters	Patients	Control
No.	40	100
Mean	1098.0	543.38
Median	977.0	496.5
Mode	2000.0	423.0
Std. Deviation	598.9	158.44
Range	1762.0	787.0
Minimum	238.0	231
Maximum	2000.0	1018

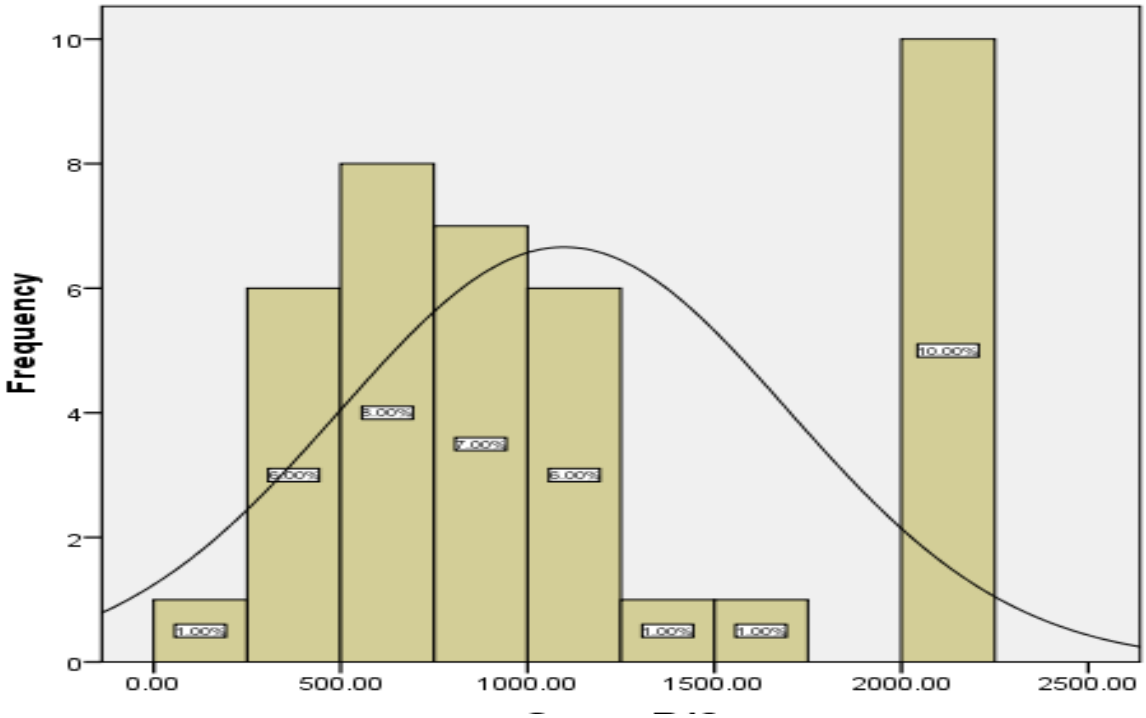

Figure 2: The serum levels of vitamin B_{12} of patients.

Serum levels of vitamin B_{12} of control:

The serum levels of vitamin B_{12} of controls ranged from 231pg/ml to 1018 pg/ml with a mean value of 543.38 as shown in **Table (2) and Figure (3).**

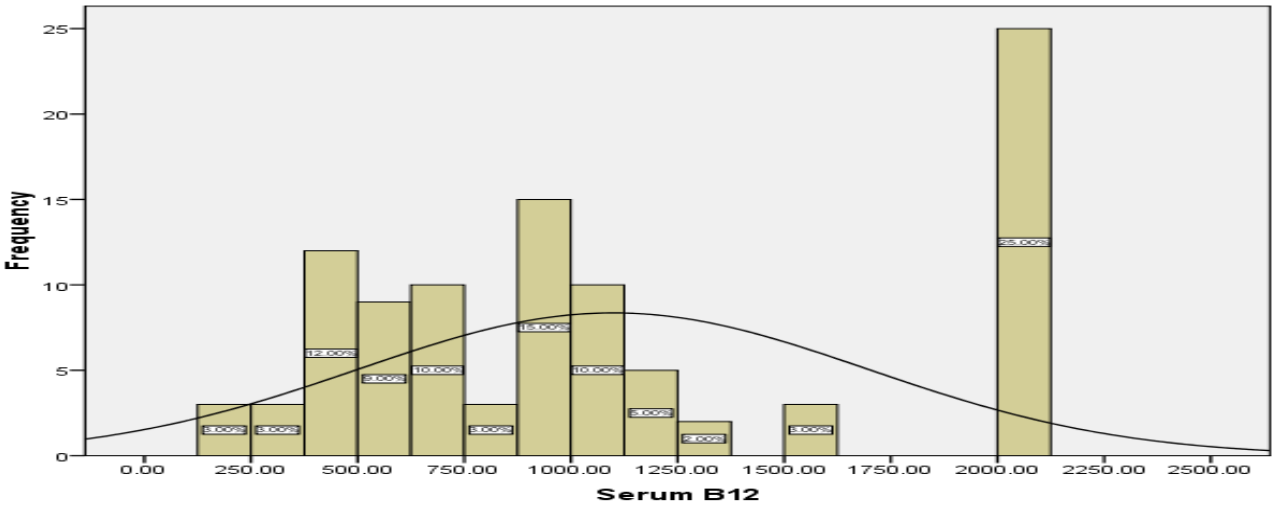

Figure 3: The serum levels of vitamin B_{12} of controls.

Comparison between cases and controls regarding the serum level of vitamin B_{12}:

As shown in **Table (3) and Figure (4);** p. acne patients had higher serum levels of vitamin B_{12} than control people; the mean value is 1098 in patients, while that of controls is 543 (p <0.001).

Table 3: Comparison between cases and controls regarding the serum levels of vitamin B_{12}.

Serum B12	Mean	Std. Deviation	Minimum	Maximum	P value
Cases	1098.0	598.9	238.0	2000.0	<0.001*
Controls	543.4	158.4	231.0	1018.0	

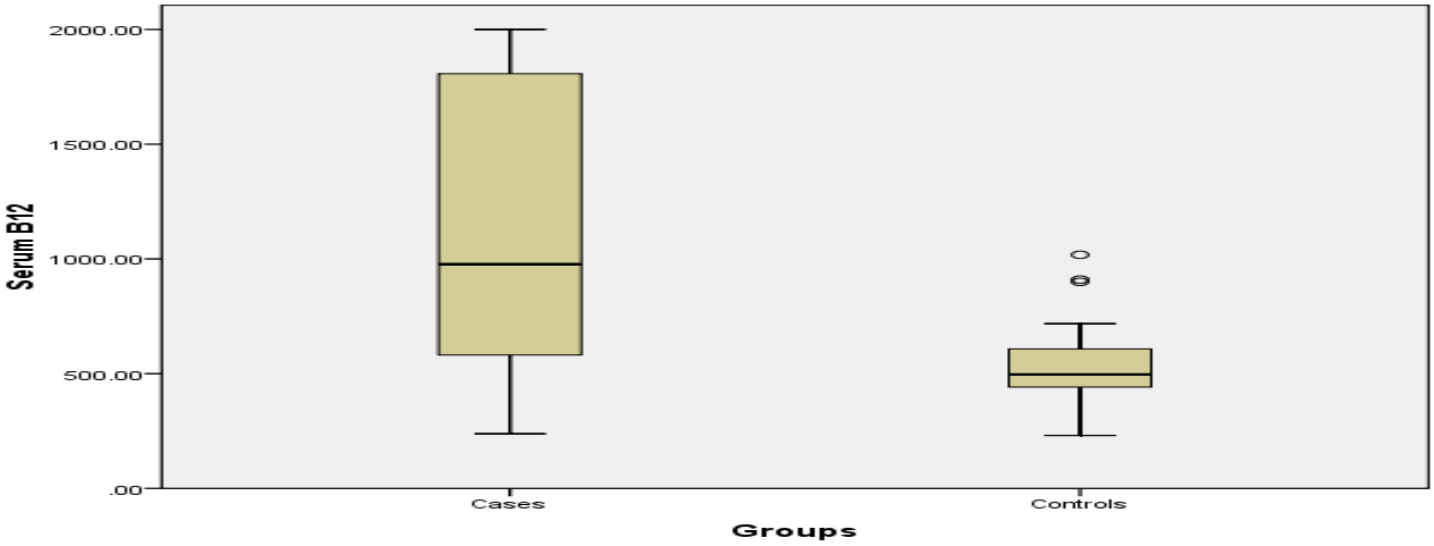

Figure 4: Comparison between cases and controls regarding the serum levels of vitamin B_{12}.

The porphyrin levels measured in p. acnes cultures without vitamin B_{12} supplementation:

The porphyrin levels measured in p. acnes cultures not supplemented with vitamin B_{12} ranged from 0.0012µg/ml to 0.0076 µg/ml with the mean value of 0.0044 as shown in **Table (4) and Figure (5).**

Table 4: The porphyrin levels in p. acnes cultures with and without vitamin B_{12} supplementation

Parameters	Vitamin B12 supplementation	Without vitamin B12 supplementation
No.	43	43
Mean	0.0357	0.0044
Median	0.031	0.0057
Mode	0.02	0.01
Std. Deviation	0.0158	0.00216
Range	0.054	0.01
Minimum	0.012	0.0012
Maximum	0.066	0.0076

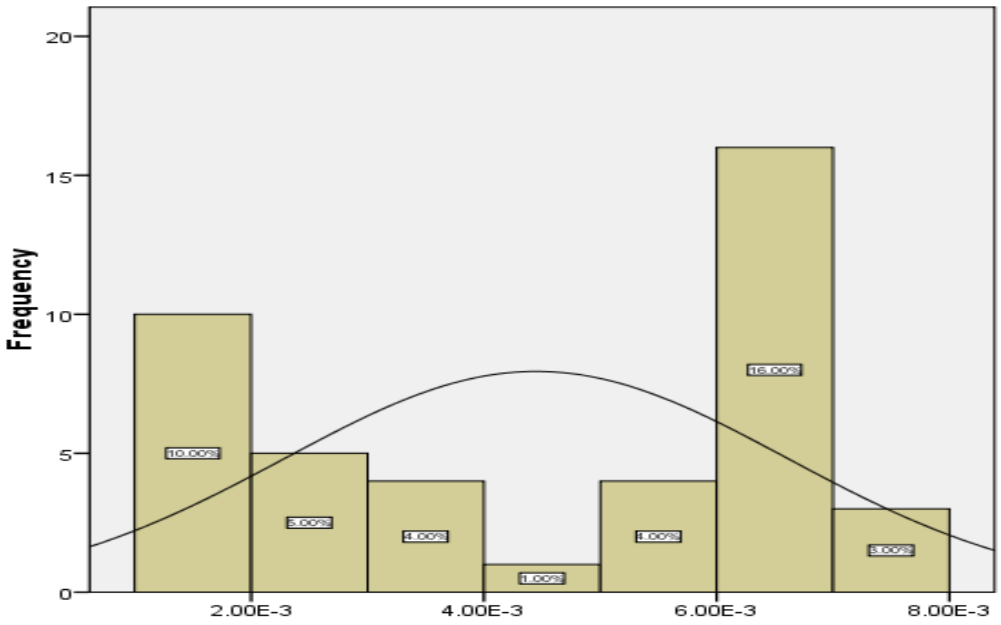

Figure 5: The porphyrin levels in p. acnes cultures without vitamin B_{12} supplementation

The porphyrin levels measured in p. acnes cultures with vitamin B_{12} supplementation: After supplementing the p. acnes cultures with vitamin B_{12} at a rate of 10mg/ml, the porphyrin levels measured raised significantly to reach a maximum of 0.066µg/ml with the mean value of 0.0357 as shown in **Table (4) and Figure (6).**

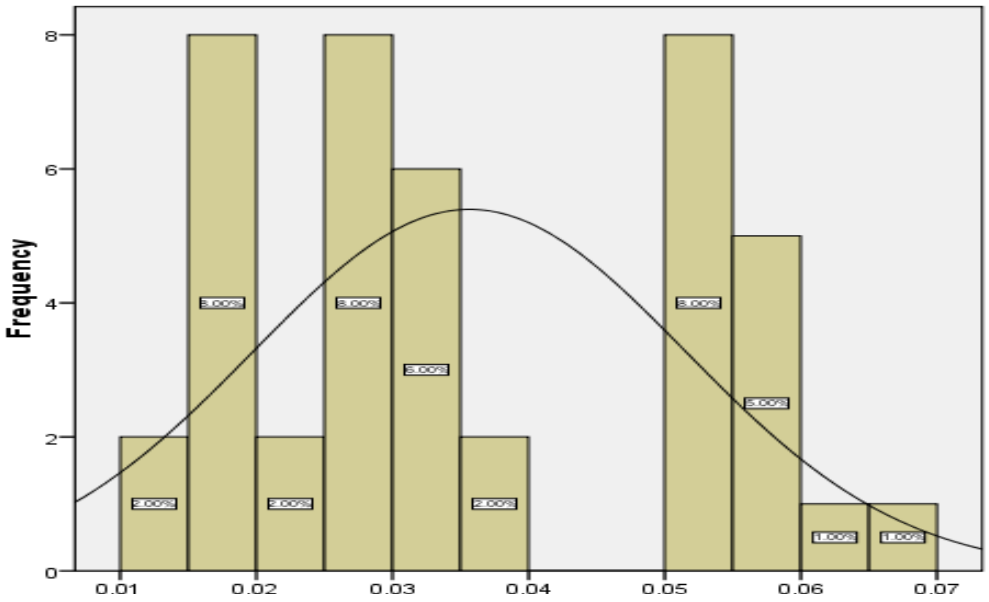

Figure 6: The porphyrin levels in p. acnes cultures with vitamin B_{12} supplementation

Comparison between the porphyrin levels measured in p. acnes cultures with and without vitamin B_{12} supplementation:

As shown in **Table (5) and Figure (7),** the porphyrin levels measured increased significantly in cultures supplemented with vitamin B_{12} at a rate of 10mg/ml, when compared to the non-supplemented cultures, so that the mean value was raised from 0.0044 before supplementation to reach 0.0357 after supplementation (p<0.001).

Table 5: Comparison between the porphyrin levels in p. acnes cultures with and without vitamin B_{12} supplementation.

Parameters	Mean	Std. Deviation	P value
Porphyrin in non-supplemented cultures.	0.0044	0.00216	<0.001*
Porphyrin in supplemented cultures.	0.0357	0.01589	

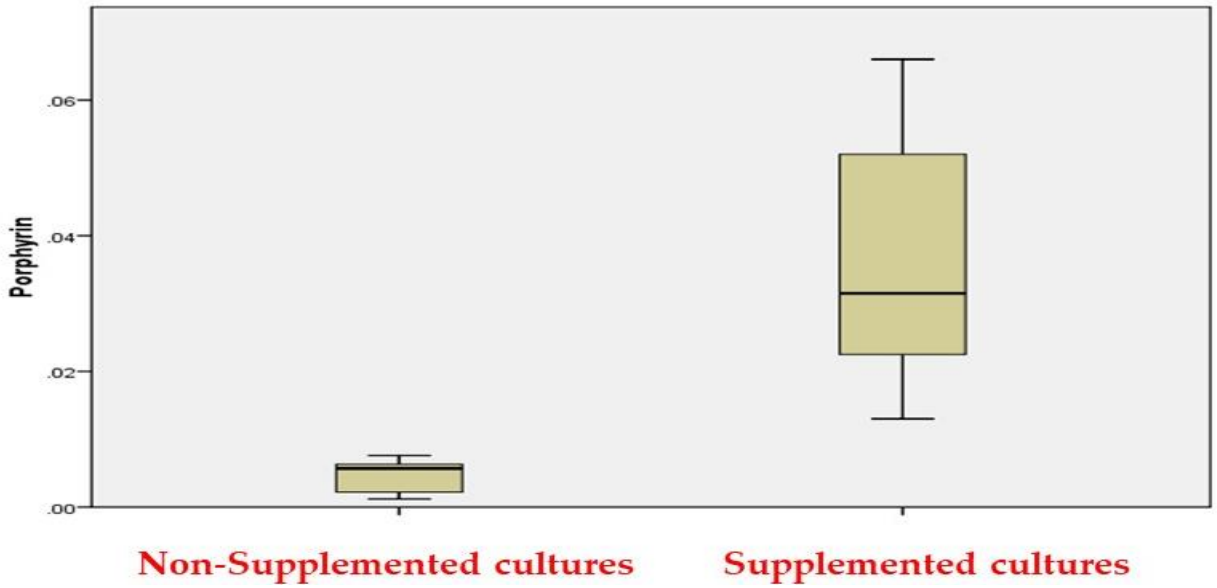

Figure 7: Comparison between the porphyrin levels in p. acnes cultures with and without vitamin B_{12} supplementation.

Correlation between the serum level of vitamin B_{12} of acne patients and the disease duration:

As shown in **Table (6);** there is a positive correlation between the serum levels of vitamin B_{12} of patients and the disease duration in those patients (p<0.013).

Table 6: The serum levels of vitamin B_{12} of p. acne patients in relation to the disease duration

Parameters		Serum B_{12}
Disease duration	Pearson Correlation	0.387
	P value	0.013*
	N	40

Correlation between the serum level of vitamin B_{12} of acne patients and the porphyrin levels in p. acnes cultures:

As shown in **Table (7)**; the p-value is 0.157 between the serum vitamin B_{12} level of acne patients and the porphyrin levels measured in p. acnes cultures of the same patients.

Table 7: The relation between the serum level of vitamin B_{12} of patients and the porphyrins measured in p. acnes cultures of patients.

Parameters		Serum B12
Porphyrin levels in P. acnes cultures.	Pearson Correlation	0.228
	P value	0.157
	N	40

Association between Global Acne Severity Scale (GASS) and the serum B_{12} levels of acne patients:

As shown in **Table (8)** and according to the GASS of acne, the mean value of serum B_{12} levels of acne patients was found to be higher in the severe acne group than in the moderate and mild acne groups.

Table 8: The Global Acne Severity Scale in relation to the serum levels of vitamin B_{12} of patients.

Global Classification		N	Mean	Std. Deviation	P value
Serum B_{12}	Mild	11	1015.4	639.6	0.858
	Moderate	21	1125.2	600.8	
	Severe	7	1198.0	630.8	
	Very Severe	1	738.0		

Association between the sex of patients and each of the serum B_{12} levels of patients and the porphyrin levels measured in p. acnes cultures:

The mean value of the serum vitamin B_{12} levels of males was 1309.2 and that of females was 1060.8. The mean value of the porphyrin levels in p. acnes cultures in males was 0.0054 and that of females was 0.0043 **(Table 9).**

Table 9: Association between the sex of patients and each of the serum B_{12} levels of patients and the porphyrin levels measured in p. acnes cultures.

Parameters	Sex	N	Mean	Std. Deviation	P value
Serum B12	Male Female	6 34	1309.2 1060.8	787.8 565.9	0.356
Porphyrins in p. acnes cultures	Male Female	6 37	0.0054 0.0043	0.00207 0.00216	0.258

Comparison between the cbiL gene expression in p. acnes cultures with and without vitamin B_{12} supplementation:

Using the RT-PCR, it was found that the cbiL gene expression levels were downregulated on day 10 with vitamin B_{12} supplementation at a rate of 10mg/ml in 28 out of 42 p. acnes isolated strains from acne patients (66.7%), while 14 strains showed no changes in their gene expression levels (33.3%). At the same time, on day 10 the gene expression levels were not changed in the same P. acnes strains not supplemented with vitamin B_{12}.

Discussion

Acne vulgaris (AV) is a chronic skin disease that affects a large proportion of the world's population. Its growth is influenced by both external and internal factors [11].

Several reports of monomorphic acneiform eruptions in patients treated with intramuscular cobalamin injections were initially reported. The eruption went away after the therapy was stopped.

Acne has been reported to be exacerbated by cyanocobalamin, pyridoxine (B_6), and riboflavin (B_2) [16]; however, the underlying mechanism of this clinical observation is unknown. Lareb published an article in 2008 about acneiform dermatitis in ten patients treated with vitamin B_{12}. Up

until 2010, Lareb had received 35 reports of vitamin B12 being linked to acne or acneiform dermatitis. The SmPC Cyanokit® reports pustular rash, primarily on the face and neck. Except for four patients, all reports concerned women. Almost all of the patients were premenopausal. Two patients (I and D) were under the age of 16.

Here, in our research, we propose a vitamin B_{12}-mediated bacterial mechanism for acne pathogenesis and its relation to the disease severity and duration.

Out of our 120 acne lesions examined, p. acnes strains were isolated from 43 lesions (35%), this was consistent with another study by **Dhillon and Varshney, 2013** where p. acnes were isolated from 32% of acne lesions, but much lower than study by **Mendoza et al., 2013** where p. acnes were isolated from 75% of acne lesions [17]. Then the serum levels of vitamin B_{12} measured in the same patients with isolated p. acnes strains were compared with 100 control people selected with the same age and sex as patients, then the in vitro impact of vitamin B_{12} supplementation on the porphyrin levels produced in cultures was investigated, and the cbiL gene expression levels responsible for the vitamin B_{12} biosynthesis pathway in p. acnes were compared with and without vitamin B_{12} supplementation.

As regards the serum levels of vitamin B_{12}, our results showed that acne patients had significantly high serum levels of the vitamin (1098 +/- 598.8) when compared to the control group (543.38 +/- 158.44).

Going with our results, **Karadag et al., 2011** showed that the serum level of vitamin B_{12} in 60 acne patients decreased significantly after 4 months of isotretinoin treatment at a dose of 0.5-0.75 mg/kg [18]. However, the mechanism of vitamin B_{12} as a contributing factor to the disease was not illustrated in their study.

Another study, by **Göklap et al., 2014**, compared the serum vitamin B_{12} levels of 120 acne patients before and after 6 months of isotretinoin treatment and observed that pre-treatment vitamin B12 levels were statistically significantly higher ($P = 0.002$), and post-treatment vitamin B12 and folic acid levels were statistically significantly lower ($P = 0.05$).

Another study by **Arora et al., 2012** evaluated the serum levels of vitamin B_{12} and the homocysteine levels of 60 female patients with severe acne vulgaris. Their results showed that the homocysteine levels were significantly high in patients when compared to controls ($p < 0.0001$), while the serum levels of vitamin B_{12} of acne patients were 487 +/-24 pg/mL, and that of the controls were 498 +/-26 pg/mL, that difference in serum levels of vitamin B_{12} in patients when compared to controls was not significant statistically; the serum B_{12} levels were even towards the lower limits of reference value in both groups, they explained their low results as both groups were on a vegetarian diet. Another explanation that we suggested for their results is that their research included only patients with very severe acne lesions who must have been using treatment for the

condition especially that isotretinoin and systemic antibiotics were not in the exclusion criteria of the research [19].

Regarding the correlation between the serum levels of vitamin B_{12} and disease duration and severity, our results showed a positive correlation between the serum levels of the vitamin and the disease duration (p = 0.013). However, as regards disease severity, our results showed that patients with severe acne lesions had relatively higher serum vitamin B_{12} levels (mean 1198) than those with moderate acne lesions (mean 1125.2) and those with mild acne lesions (mean 1015.4), surprisingly the mean value of the very severe acne lesion was the lowest when compared to the others (mean 738), but this can be explained as it only included one patient who was found on our history that she previously received systemic isotretinoin for 2 months and stopped it 3 months before sampling and since then she used a topical retinoid cream instead; however further studies should be focused on that observation with an equal number of patients at each grade of the disease to get non-doubtful results.

Porphyrin biosynthesis is inversely related to vitamin B_{12} biosynthesis in propionibacteria. According to the p. acnes metabolic pathway, L-glutamate is a precursor for both the vitamin B12 and porphyrin biosynthesis pathways [20].

Hence, vitamin B_{12} is the regulator of its biosynthesis pathway; as studies showed that high vitamin B_{12} levels repressed the expression of cob/cbi operons in the vitamin B_{12} biosynthesis pathway[21]**.** So, based on those facts our theory supposes that high serum levels of vitamin B_{12} in acne patients down-regulate vitamin B_{12} synthesis in p. acnes and hence shunt the metabolic flow of p. acnes towards the porphyrin biosynthesis pathway which is known to cause inflammation in acne lesions [9].

Our results did verify that hypothesis. As seen in our results the mean value of the porphyrin levels measured in p. acnes cultures without vitamin B_{12} supplementation (0.0044) raised significantly with the vitamin B_{12} supplementation to become (0.0357) (p<0.001).

There was no statistically significant difference between males and females regarding the serum levels of vitamin B_{12} of patients or the porphyrin levels measured in P. acnes cultures.

In line with our findings, a study by **Borelli et al., 2006** compared the porphyrin levels in p. acnes of 16 untreated patients and another 16 and 17 patients receiving isotretinoin and minocycline respectively, their results showed that the porphyrin levels significantly decreased after 2 months of treatment (***p***<0.05) [8].

Meyer et al., 2015 found that analysis of cheek skin in the nasal area revealed significantly higher fluorescence (500-800 nm) in image-based and spectroscopic analysis from acne subjects, indicating a higher presence of the bacterial metabolite porphyrin in those with AV [22].

Finally, we verified that this increase in the porphyrin levels with vitamin B_{12} supplementation was due to the downregulation of the vitamin biosynthesis pathway in p. acnes and not due to any

other factors by testing the inhibition of one of the genes responsible for the vitamin B_{12} biosynthesis pathway in p. acnes, the cbiL gene [14]**,** with vitamin B_{12} supplementation to the cultures using RT-PCR. Our results showed that in 28 out of 42 isolated p. acnes strains from 42 acne patients, the gene was downregulated on day 10 after vitamin B_{12} supplementation to the culture (66.7 %), while the 14 other isolated strains showed no change in their gene expressions (33.3%). Verifying that this shunt in the metabolic biosynthesis pathway of p. acne is secondary to inhibition of the vitamin B_{12} biosynthesis pathway in the bacteria, we re-examined the gene expression in the non-supplemented p. acnes cultures after 10 days and found out that it remained not inhibited. Complementing the mentioned above; we explained the 33.3% of the non-inhibited gene expression that our research was conducted on only one of the three genes responsible for the vitamin B_{12} biosynthesis pathway in p. acnes.

Supporting our results, a study by **Kang et al., 2015,** showed that the porphyrin level increased in p. acnes cultures supplemented with vitamin B_{12} measured on day 8 after the supplementation ($p < 0.0043$)[23]. It also showed down-regulation of the genes responsible for vitamin B_{12} biosynthesis pathway in p. acnes proven by qRT PCR technique, however, their study was conducted upon 33 p. acnes isolates; 20 of them were p. acnes isolates from healthy individuals not suffering from acne vulgaris moreover 10 of those healthy individuals were receiving intramuscular injections of vitamin B_{12} (1000 μg/ml) for the general well-being, so it was an in vitro study about the effect of vitamin B_{12} on the metabolic activities of p. acne without its correlation with the actual serum level of the vitamin in real patients and disease severity or duration.

At last, we know that lots of doctors prescribe multivitamins for the general well-being of their patients, many individuals also take multivitamins for good performance with not indeed need for them, thinking that vitamins are good for their skin and intellectual functions and have no harm or worsening effects on their looks, however, based on this study, we sincerely hope for that attitude to be dumped. We need to conduct awareness campaigns to our population about that issue and how vitamin B_{12} injections had led to acne elicitation in a subset of individuals and also prevent using the vitamin supplementation unless they show the manifestations of its deficiency.

Also, as dermatologists, maybe we should advise our acne patients to consume a more plant-based diet with less consumption of animal products that are rich in vitamin B_{12}, eggs, milk, poultry, and beef.

Conclusion

Our study concluded that vitamin B_{12} is involved in the pathogenesis of acne vulgaris; as in vivo, it proved that acne vulgaris patients had significantly higher serum levels of vitamin B_{12} than controls and showed a positive correlation to the disease duration and severity. In vitro, it proved that vitamin B_{12} supplementation to p. acnes cul-

tures significantly raised the porphyrins produced in these cultures when compared to the non-supplemented ones. It also proved that this shift in the metabolism is secondary to inhibition of the cbiL gene of the vitamin B_{12} synthesis in the bacteria as porphyrins and vitamin B_{12} share the same precursors.

Limitation

- The study included an examination of 43 p. acne patients with various grades of acne vulgaris of non-equal numbers at each grade grouping.
- Only one gene in the vitamin B_{12} synthesis pathway was examined.

Ethical committee:

Data was confidential and anonymous. Socio-demographic questions were for identifying the characteristics not identity. Respondents were aware of the steps of the study and were allowed to express their opinion and comments. Informed consent was signed by the concerned officials. The patients themselves were not obliged to participate. **Under this code (FMB-SUREC/12022023/Radi)**

Conflict of interest:

None

Fund:

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Authors contributions:

A.A.S and A.A.M performed the experiments. A.A.S, A.A.M, G.S.F, A.E, and N.A.R designed the experiments, analyzed data, and wrote the manuscript.

References

[1] A. L. Dawson and R. P. Dellavalle, "Acne vulgaris," *BMJ*, vol. 346, no. 7907, 2013, doi: 10.1136/BMJ.F2634.

[2] Y. Kubota, Y. Shirahige, K. Nakai, J. Katsuura, T. Moriue, and K. Yoneda, "Community-based epidemiological study of psychosocial effects of acne in Japanese adolescents," *J. Dermatol.*, vol. 37, no. 7, pp. 617–622, 2010, doi: 10.1111/J.1346-8138.2010.00855.X.

[3] U. Gieler, T. Gieler, and J. P. Kupfer, "Acne and quality of life – impact and management," *J. Eur. Acad. Dermatology Venereol.*, vol. 29, no. S4, pp. 12–14, 2015, doi: 10.1111/JDV.13191.

[4] J. J. Lai, P. Chang, K. P. Lai, L. Chen, and C. Chang, "The role of androgen and androgen receptor in skin-related disorders," *Arch. Dermatological Res. 2012 3047*, vol. 304, no. 7, pp. 499–510, 2012, doi: 10.1007/S00403-012-1265-X.

[5] C. C. Zouboulis *et al.*, "What is the pathogenesis of acne?" *Exp. Dermatol.*, vol. 14, no. 2, pp. 143–143, 2005, doi: 10.1111/J.0906-6705.2005.0285A.X.

[6] I. Kurokawa *et al.*, "New developments in our understanding of acne pathogenesis and treatment," *Exp. Dermatol.*, vol. 18, no. 10, pp. 821–832, 2009, doi: 10.1111/j.1600-0625.2009.00890.x.

[7] E. A. Tanghetti, "The role of inflammation in the pathology of acne," *J. Clin. Aesthet. Dermatol.*, vol. 6, no. 9, pp. 27–35, 2013.

[8] C. Borelli *et al.*, "In vivo porphyrin production by P. acnes in untreated acne patients and its modulation by acne treatment," *Acta Derm. Venereol.*, vol. 86, no. 4, pp. 316–319, 2006, doi: 10.2340/00015555-0088.

[9] M. Schaller *et al.*, "Induction of a chemoattractive proinflammatory cytokine response after stimulation of keratinocytes with Propionibacterium acnes and coproporphyrin III," *Br. J. Dermatol.*, vol. 153, no. 1, pp. 66–71, 2005, doi: 10.1111/J.1365-2133.2005.06530.X.

[10] A. Zamil, D. H. Perez-Sanchez, A. Katta, D. H. Zamil, A. Perez-Sanchez, and R. Katta, "Acne related to dietary supplements," *Dermatol. Online J.*, vol. 26, no. 8, pp. 2–3, 2020, DOI: 10.5070/D3268049797.

[11] A. Podgórska, A. Puścion-Jakubik, R. Markiewicz-żukowska, K. J. Gromkowska-Kępka, and K. Socha, "Acne vulgaris and intake of selected dietary nutrients—a summary of information," *Healthc.*, vol. 9, no. 6, p. 668, 2021, doi: 10.3390/healthcare9060668.

[12] B. Dréno *et al.*, "Development and evaluation of a Global Acne Severity Scale (GEA Scale) suitable for France and Europe," *J. Eur. Acad. Dermatology Venereol.*, vol. 25, no. 1, pp. 43–48, 2011, doi: 10.1111/J.1468-3083.2010.03685.X.

[13] M. D. Lundrigan and R. J. Kadner, "Altered cobalamin metabolism in Escherichia coli btuR mutants affects btuB gene regulation," *J. Bacteriol.*, vol. 171, no. 1, pp. 154–161, 1989, doi: 10.1128/JB.171.1.154-161.1989.

[14] M. J. Warren, E. Raux, H. L. Schubert, and J. C. Escalante-Semerena, "The biosynthesis of adenosylcobalamin (vitamin B12)," *Nat. Prod. Rep.*, vol. 19, no. 4, pp. 390–412, 2002, doi: 10.1039/B108967F.

[15] A. A. Shoun, R. Abozahra, K. Baraka, M. Mehrez, and S. M. Abdelhamid, "Identifying Different Mutation Sites Leading to Resistance to the Direct-Acting Antiviral (DAA) Sofosbuvir in Hepatitis C Virus Patients from Egypt," *Microorganisms*, vol. 10, no. 4, p. 679, 2022, doi: 10.3390/microorganisms10040679.

[16] M. J. Warren, E. Raux, H. L. Schubert, and J. C. Escalante-Semerena, "The biosynthesis of adenosylcobalamin (vitamin B12)," *Nat. Prod. Rep.*, vol. 19, no. 4, pp. 390–412, 2002, doi: 10.1039/B108967F.

[17] N. Mendoza, P. O. Hernandez, S. K. Tyring, K. A. Haitz, and A. Motta, "Antimicrobial susceptibility of Propionibacterium acnes isolates from acne patients in Colombia," *Int. J. Dermatol.*, vol. 52, no. 6, pp. 688–692, 2013, doi: 10.1111/J.1365-4632.2011.05403.X.

[18] A. S. Karadag, E. Tutal, D. T. Ertugrul, and K. O. Akin, "Effect of isotretinoin treatment on plasma holotranscobalamin, vitamin B12, folic acid, and homocysteine levels: non-controlled study," *Int. J. Dermatol.*, vol. 50, no. 12, pp. 1564–1569, 2011, doi: 10.1111/J.1365-4632.2011.05027.X.

[19] M. K. Arora, S. Seth, and S. Dayal, "Homocysteine, folic acid and vitamin B12 levels in females with severe acne vulgaris," *Clin.*

Chem. Lab. Med., vol. 50, no. 11, pp. 2061–2063, 2012, DOI: 10.1515/CCLM-2012-0228/MACHINEREADABLECITATION/RIS.

[20] E. Barnard *et al.*, "Porphyrin Production and Regulation in Cutaneous Propionibacteria," *mSphere*, vol. 5, no. 1, 2020, doi: 10.1128/msphere.00793-19.

[21] A. G. Vitreschak, D. A. Rodionov, A. A. Mironov, and M. S. Gelfand, "Regulation of the vitamin B12 metabolism and transport in bacteria by a conserved RNA structural element," *RNA*, vol. 9, no. 9, pp. 1084–1097, 2003, doi: 10.1261/RNA.5710303.

[22] K. Meyer *et al.*, "Evaluation of Seasonal Changes in Facial Skin With and Without Acne.," *J. Drugs Dermatol.*, vol. 14, no. 6, pp. 593–601, 2015.

[23] D. Kang, B. Shi, M. C. Erfe, N. Craft, and H. Li, "Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis," *Sci. Transl. Med.*, vol. 7, no. 293, p. 293ra103, 2015, doi: 10.1126/scitranslmed.aab2009.