A Randomized, Double Blind, Placebo-Controlled Clinical Trial of the Effects of Oral Zinc Gluconate among Diagnosed Acne Vulgaris patients

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INTRODUCTION

Acne Vulgaris is a common disorder of the pilosebaceous follicles characterized by comedones, papules, pustules, inflamed nodules, canalizing and deep, inflamed sacs usually occurring on areas of the body with hormonally sensitive sebaceous glands, including the face, neck, chest, upper back and upper arms. Moderate to severe acne vulgaris affects around 20% of young people. It persists into the 20s in 64% and into the 30s for 43% of individuals.[1] Lesions last for years, result to permanent scarring, and have serious effects on an individual's psychological health such as depression and social withdrawal. The annual years of healthy life lost per 100,000 people from acne in the Philippines is 50.6 in 2013.[2] This shows that despite the known treatment regimens for acne, Filipino patients are still suffering from the effects of the disease.

Acne has four main pathogenic contributors: follicular hyperkeratinization, increased sebum production, *Propionibacterium acnes* (*P. acnes*) within the follicle, and inflammation.[3] Treatment options for acne vulgaris include benzoyl peroxide, topical and oral retinoids, topical and oral antimicrobials, oral corticosteroids, and physical modalities such as acne surgery, laser and light therapy.[3] Reports show that antibiotic resistance is a growing issue in the treatment regimen of acne vulgaris, making it less and less suitable for long-term treatment,[4] hence other options that can be substitutes or adjuncts to treatment may be useful in this condition. For long–term or maintenance therapy, physicians should consider effectivity, cost, and adverse effects. Several studies have explored the effect of oral zinc on acne vulgaris. Since zinc is more cost-effective and has less adverse effects compared to most antibiotics, this may prove helpful for the Filipino patient in terms of safety and economy for long-term therapy.

At present, there are no local studies using only oral zinc supplementation as an adjunct for the treatment of acne vulgaris. Thus, this study aims to determine the effect of oral zinc gluconate versus placebo on inflammatory lesions of acne vulgaris in the Filipino population. Furthermore, this study may also be able to provide more information for further studies towards non-antimicrobial treatment regimens for acne vulgaris in the Philippines.

Review of Related Literature

Acne Vulgaris is one of the most common dermatologic diagnoses affecting approximately 9.4% of the global population making it the eighth most prevalent disease worldwide.[5] A significant number of adult patients continue to develop new-onset acne beyond the teenage years with prevalence found to be at 50-54% in females and 40-42.5% in males.[6] Moreover, the prevalence of acne did not substantially decrease until after the age of 44.[7] Since acne vulgaris is a chronic and persistent skin condition, safe and effective long-term maintenance therapy is often required. Antibiotic resistance in acne vulgaris can occur in *P. acnes* and other organisms, creating an increase in more pathogenic strains of these bacteria.[4] Given the gravity of the consequences of this trend, it is a challenge to maximize use of non-antimicrobial therapy when treating acne.[4] Other options for treatment of moderate to severe acne vulgaris are already being explored. A study by Stein Gold et al has shown that the fixed combination of adapalene 0.3%/ benzoyl peroxide 2.5% is an effective and safe single-agent topical therapy for moderate and severe inflammatory acne.[8,9]

Oxidative status has been implicated in the pathogenesis of several skin diseases, including acne. A study measured oxidative stress by taking plasma levels of catalase (CAT),

superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA). MDA in acne patients were significantly higher as compared with that of the controls, whereas activities of the antioxidant enzymes SOD and CAT were lower. Hence, this suggests that oxidative stress plays a key role in acne vulgaris.[10]

Zinc is present in all body tissues and has been shown to have antioxidant properties.[11] It is an essential element of more than 200 metalloenzymes, including the antioxidant enzyme, superoxide dismutase, and it has the ability to effectively decrease reactive oxygen species, a key factor in oxidative stress.[12] Zinc also contributes to normal epithelial differentiation and development, protects against UV radiation, enhances wound healing, contributes to proper immune, reproductive and neuropsychiatric functions, and may decrease the relative risk of cancer and cardiovascular disease.[13]

The recommended daily allowance (RDA) of zinc is 8 mg elemental zinc for adult females and 11 mg elemental zinc for adult males.[14] The tolerable upper intake level of zinc is 40 mg elemental zinc for both adult males and females.[14] Possible side effects related to oral zinc, usually from zinc sulfate, include nausea, vomiting, abdominal pain and diarrhea.[14,15] According to Sardana et al, the effervescent preparations and zinc gluconate had better results as compared to zinc sulfate and citrate.[16] Zinc gluconate is better tolerated by the gastric mucosa hence causing much less of the known adverse effects of zinc. Zinc gluconate contains 14% elemental zinc, meaning a 100 mg tablet contains 14 mg elemental zinc.[17,18]

Data suggest that zinc deficiency exist both in developed and developing countries, and several factors that may affect serum zinc levels include low intake of highly absorbable zinc in fresh foods, high phytate content of some staple food, pregnancy, lactation, and increased demands of physiological processes such as growth and sexual maturation.[19] Whole blood zinc levels were measured from healthy subjects in relation to sex and age using atomic absorption spectrophotometry. Mean level of zinc obtained in whole human blood from males was 607.0 +/- 105.3 micrograms/100 ml and in females 585.2 +/- 122.9 micrograms/100 ml. The results showed that males had slightly higher zinc levels but the difference was not statistically significant.[20]

A few researches have been done in other countries comparing zinc levels in blood with the severity of acne.[21,22] A study by Saleh done in Iraq measured trace elements in the blood, such as zinc and copper, and correlated it to the severity of acne vulgaris. The results showed that patients with severe acne had significantly lower levels of zinc.[23] As shown in a study done in Iran, zinc levels may be related to the severity and type of acne lesions in patients with acne vulgaris, the relative decrease of serum zinc level in acne patients suggests a role for zinc in the pathogenesis of acne vulgaris.[24] Another study of 173 acne patients published in the British Journal of Dermatology showed that retinol binding protein and serum zinc levels were significantly lower in severe acne.[25] In an unpublished study, a significant relation of whole blood zinc levels with the severity of acne vulgaris was found in Filipinos, with severe acne showing significantly lower zinc levels.[26] Some studies concluded that due to this, it might be helpful to give zinc supplements to acne vulgaris patients.[27,28]

Zinc is still being studied and was found to have multiple uses in medicine and general dermatology as oral and/or topical therapy for conditions such as warts, cutaneous leishmaniasis, leprosy, herpes genitalis, dermatophytosis, bromhidrosis, pityriasis versicolor, acne vulgaris, rosacea, hidradenitis suppurativa, eczema, ulcers, and alopecia areata.[29-31] Oral zinc has been

compared to oxytetracycline in a study by Michaelsson et al, and showed no significant difference in the effect on acne vulgaris.[32] On the other hand, a study by Dreno et al showed that minocycline was superior to oral zinc in the treatment of acne vulgaris[33] and a study by Cunliffe et al showed that although oral zinc was correlated with decreased inflammatory lesions, tetracycline has a superior effect.[34] These studies compared oral zinc with antibiotics, which are already established treatment options for acne vulgaris. There are some instances, however, when patients are already resistant to antibiotics or are reluctant to take antibiotics for long-term. Oral zinc for acne vulgaris may be useful for patients who cannot or do not want to take oral antibiotics in the long-term and for those who already have erythromycin- or clindamycin-resistant strains of *Cutibacterium acnes (Propionibacterium acnes)*.[16] Multiple sources showed that when oral zinc was compared to placebo, there was a significant improvement in inflammation and number of acne lesions.[35-41] Furthermore, adding zinc salts in vitro to culture media of *Cutibacterium acnes (Propionibacterium acnes)* reduced resistance of the bacteria to erythromycin.[41] One of the anti-inflammatory effects of oral zinc in acne was found to be in the inhibition of TLR2 surface expression by keratinocytes.[42] Studies about giving oral zinc gluconate supplements versus placebo in the Philippine setting have not yet been done.

The inflammatory score is a grading system that measures inflammatory lesions only. It includes locations such as the face, chest and back. For each location, papules, pustules and nodules are counted. A factor based on the lesion (papule=3, pustule=4, nodule=5) is used to be multiplied to a grade equivalent to the number of lesions. The subtotal scores are added with a maximum possible total score of 144.[35]

The Global Acne Grading System (GAGS) is an acne severity scale that includes six locations on the face, chest, and back. A factor based on surface area and distribution of pilosebaceous units is provided for each of the six locations, and grades are based on the type of lesions per area (1 for ≥ one comedone, 2 for ≥ one papule,3 for ≥ one pustule, and 4 for ≥ one nodule). The factor and the grade are multiplied to get a local score. The local scores are then added with a maximum possible global score of 44. Acne severity is rated as mild, moderate, severe, and very severe with corresponding cut-off scores.[43,44]

Research Question

Does oral zinc gluconate supplementation improve inflammatory acne as measured by the inflammatory score and GAGS score in diagnosed acne vulgaris patients?

Objectives

GENERAL

- To assess the efficacy of oral zinc gluconate in the improvement of disease activity in diagnosed acne vulgaris patients.

SPECIFIC

- To determine the demographic and clinical profile of Acne Vulgaris patients
- To determine the disease activity measured by the inflammatory score and GAGS score of acne vulgaris patients on initial consult, at 4 weeks, and at 8 weeks.
- To determine if there is a significant difference in disease activity as measured by the inflammatory score and GAGS score among acne vulgaris patients given placebo and oral zinc gluconate.

METHODOLOGY

Study Design

A randomized, double blind, placebo-controlled clinical trial was utilized for this study which was conducted from December 2018 to July 2019.

Ethical Considerations

The protocol will be submitted for ethical review and approval by the Technical Review Board and Institutional Ethics and Review Board of the East Avenue Medical Center. Informed consent will be obtained from the participants prior to participation in the study. All data collection will be done in a setting that ensures patient safety and privacy. Patient will be assured of the confidential nature of the patient-provider interactions. Active listening, open-ended questions and clarifications will be used.

The investigators will perform physical examination of the patient to assess the severity classification, and any photographs that will be taken of the patient or the skin lesions will be subject to informed consent. No closed-circuit television or recording devices will be used in the rooms for interview and physical examination.

The Informed Consent Process:

Informed consent will be obtained from the participants prior to participation in the study. At the onset, patient will be assured of the confidential nature of the patient-provider interaction. The investigator will explain that to ensure confidentiality in data collection, the investigators will assign a code to replace patient identifiers (e.g. name). Only authorized members of the research team will be allowed to access the result. All pertinent records will be stored in a locked cabinet. The records identifying the subject will be kept confidential and will not be made publicly available. In obtaining and documenting informed consent, the investigator adheres to Good Clinical Practice guidelines and to sound ethical principles. During the procurement of the informed consent, the investigator will go through the informed consent form which will include the following: that the patient will enter into a research study, the purpose of the study, the procedure to be followed, the possible risks involved (skin irritation from the topical medications and nausea from the oral zinc) and the expected benefits (patient will be provided with free medications). The subject will also be provided contact details of whom to speak to if she or he has any questions or any concerns regarding the study. The investigator will not coerce or unduly influence a subject to participate or to continue to participate in a trial without their consent. It will be emphasized that whether the patient chooses to participate or not participate in the study, he or she will still receive appropriate treatment. It will also be made clear that no compensation will be given for participating in the study. None of the oral and written information concerning the trial will contain any language that causes the subject to waive or to appear to waive any legal rights or that releases or appears to release the investigator for liability from negligence. Active listening, open-ended questions and clarifications will be used. The investigator will provide the subject with ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions will be answered to the satisfaction of the subject. By signing the consent form, the subject attests that the information in the consent form and any other written information was accurately explained and apparently understood by the

subject and that informed consent was freely given. At any time during the process, the subject can opt to refuse from participating in the study.

Study Setting

The study will be conducted at the Out-patient Clinic and the Skin Center of the East Avenue Medical Center (EAMC) Department of Dermatology. The hospital had adequate staff and infrastructures to provide for the requirements of this study to be conducted properly.

Study Population

The subjects of the study included patients clinically diagnosed with acne vulgaris, aged 18-27 years of age.

Inclusion Criteria

- Filipino patients, aged 18-27 years old
- New patients diagnosed with Acne Vulgaris and a Global Acne Grading System score of at least 19
- Able to read and write in English or Tagalog
- Seen at the Out-Patient Department of East Avenue Medical Center Department of Dermatology

Exclusion Criteria

- Patients with other chronic dermatoses or systemic disease
- Taking oral supplements or medications within the past 4 weeks
- Patients who are pregnant or lactating

Study Procedure

1. In the Dermatology Out-Patient Department of East Avenue Medical Center, new patients who were diagnosed to have Acne Vulgaris, will be screened if they meet the inclusion/exclusion criteria.
2. Informed consent will be obtained for those who are qualified.
3. Patients will be given proper treatment whether they agree to participate or not. Patients who agree to participate will fill out an information sheet and three resident physicians will assess the patient's Global Acne Grading System (GAGS) and acne inflammatory score on initial visit, after 1 month, and after 2 months.
4. Patients will be given the bottle of zinc gluconate or placebo to be taken for 2 months, with patient education and a daily monitoring sheet. Details of blinding and randomization detailed below. Patients will be asked to follow up every 2 weeks where assessment and pill count will be done. Participants will be advised to continue intake if there are any unconsumed capsules within the 60-day period.
5. Data collected will be tabulated by the investigators and data analysis will be done.

Definition of Variables

Treatment arm

In this study, patients will be randomly assigned to receive either placebo or oral zinc gluconate supplementation. The assignment to a treatment arm was performed randomly.

Improvement in the disease activity

This will be assessed by determining the difference in the GAGS and inflammatory scores of a patient at the start and at the end of the clinical trial. The GAGS and inflammatory scores will be measured by the same investigators at the start and at the end of the trial. Secondary measures include the examiner's assessment and patient's self-assessment scores at the end of the trial.

Randomization

Allocation to either Group A (Zinc Gluconate) or Group B (Placebo) will be done using a computer-generated block randomization scheme (www.randomizer.org). The randomization will be done by a resident physician who was not involved in the trial. The randomization codes will be kept in a locked cabinet and will only be broken at the end of the study. Therefore, the assignments will be concealed from both participants and the investigators. The investigator, also blinded, will be the one to dispense the medications to the patients.

Treatment allocation and blinding

The Solgar Zinc Gluconate 50mg will be repackaged by the TARC Pharmacy Laboratory of the University of Santo Tomas into the clear size 0 capsules. The same laboratory will prepare the placebo capsules and package the cornstarch placebo into the same clear size 0 capsules. This repackaging is necessary to ensure that the physicians and the participants will be blinded during the study. The capsules will be packaged in uniform airtight containers with 60 capsules per container. A third person not directly involved in care and assessment of the subjects, who also did the randomization, will be tasked to encode the capsules into groups A and B. Bottles will be labelled with the corresponding patient number only. Both the physician in charge and the patients will be unaware of which capsules contain Zinc Gluconate or placebo. During the study, patients will be asked to draw a number from 1 to 32, and he/she will get the corresponding bottle. The code will then be revealed during the data analysis portion to allow the statistician to segregate the results into the proper groups.

Intervention

Patients will be randomly assigned to 60 days of oral Zinc Gluconate capsules (one capsule of 200mg daily) versus the placebo capsules. The participants will be given a container filled with 60 capsules. They will be instructed to take 1 capsule once a day 20 minutes before the morning meal in their residence. The starch placebos will be identical to zinc supplements in size, color, and shape hence both patients and investigators will be unable to tell whether participant will be on zinc supplementation or placebo.

All participants will receive the same adapalene 0.3% + benzoyl peroxide 2.5% preparation to be applied two times a day on affected areas. Patient education on Acne Vulgaris, Zinc sources, and basic skin care will be performed on all patients. Instructions will be given to avoid intake of other medication or supplementation during the 60-day trial.

Checking of Compliance

Participants will be given a Daily Monitoring Sheet where they can note if they have taken

the capsule for the day, as well as side effects or other remarks. The number of remaining capsules will be checked every 2 weeks during follow-up. Patient will be required to bring medicine bottle on follow-up for the pill count.

Final evaluation

After 60 days of either placebo or zinc gluconate, the participant will be instructed to follow up for re-assessment. The GAGS and acne inflammatory score will be re-evaluated. The investigator will perform an examiner's assessment score and the patient will also give a self-assessment score.

Outcome measures

The primary outcome measure will be the change in inflammatory scores, GAGS scores of the zinc and placebo groups from initial consult to the end of the trial. The secondary outcome measures include the examiner's assessment score and the patient's self-assessment score.

Stopping Guidelines

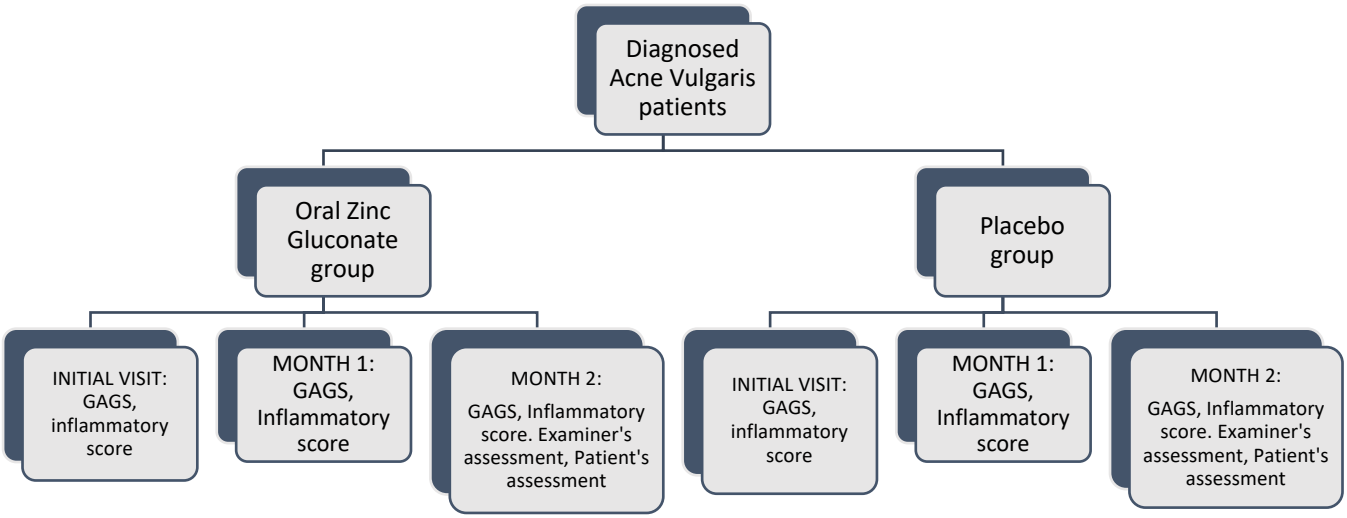
Criteria for stopping the intervention include anaphylactic reaction or allergies to the medications, and severe gastrointestinal symptoms. Patients with severe adverse reactions will be treated accordingly to relieve symptoms. They will be considered as "withdrawals" of the study, and still receive proper treatment for acne vulgaris.

Dropouts and Withdrawals

Withdrawals are defined as patients who discontinued the study for known reasons (did not comply with instructions, had severe adverse reactions). Dropouts are defined as patients who cannot be contacted, had no more follow up, and whose outcomes are unknown by the end of the study period.

Study Framework

Figure 1. Study Framework



Sample Size Computation

The sample size is computed using G*Power software. The effect size used was based on "Low Doses of Zinc Gluconate for Inflammatory Acne" by Dreno, P et al. (1989). According to this, the inflammatory score was statistically lower in the Zinc group than in the Placebo group at 2nd month of observation with p-value less than 0.02. The computed effect size was 0.6708327, this was used given that there was no effect size convention available in G*Power for repeated measures between factors (ANOVA, F-test). The computed sample size of 12 (6 per group) was estimated using 0.05 α-level of significance at 80% actual power of analysis. Twenty percent of the computed sample size was added to adjust for the dropout rate. Thus, the minimum required sample size is 15. The sample sizes for power of analysis are shown in Table I.

Power of Analysis	Sample Size	Group 1	Group 2
99%	24	12	12
95%	18	9	9
90%	16	8	8
80%	12	6	6

Table I. Sample sizes for power of analysis

Statistical analysis plan

For the profiling of patients, quantitative variables (e.g. age, inflammatory scores, and GAGS scores) will be summarized using the mean and standard deviation or median and range (Min-max) if the data appears scattered. Meanwhile, qualitative variables (e.g. sex, examiner's assessment, patient's self-assessment) will be described as frequencies and percentages. The point and 95% confidence interval estimate of the mean inflammatory scores and GAGS scores

of the two treatment groups will be measured at baseline, at one month, and at the end of the trial. Lastly, independent two-sample t-test will be used to compare the two treatment groups in terms of: 1) the mean change in the inflammatory scores from baseline to end of the trial; 2) the mean change in the GAGS scores from baseline to end of the trial. Alternatively, Mann-Whitney U-Test will be used as an alternative if the data will be dispersed. Repeated measures ANOVA will be used to compare the inflammatory score and GAGS score of acne vulgaris patients on initial consult, at 4 weeks, and at 8 weeks. Friedman test will be used as a non-parametric alternative.

A 5% level of significance will be used in this hypothesis testing. SPSS version 25.0 will be used for all output and the necessary descriptive and inferential statistics in this study.

DUMMY TABLES

Table 2. Demographic profile of patients

	Zinc Gluconate (n=)		**Placebo (n=)**	
	Mean	**SD**	**Mean**	**SD**
Age in years				
	Frequency	**Percentage**	**Frequency**	**Percentage**
Sex				
Male				
Female				

Table 3. Mean Inflammatory Scores

Mean Inflammatory Scores				
Period	**Zinc Gluconate (n=)**		**Placebo (n=)**	
	Mean	**SD**	**Mean**	**SD**
Initial				
Month 1				
Month 2				

Table 4. Mean GAGS Scores

Mean GAGS Scores				
Period	**Zinc Gluconate (n=)**		**Placebo (n=)**	
	Mean	**SD**	**Mean**	**SD**
Initial				
Month 1				
Month 2				

Table 5. Comparison of two treatment groups in terms of mean change in the inflammatory scores and GAGS scores from baseline to end of the trial

Mean change of outcomes from baseline to end of the trial					
Outcomes	**Zinc Gluconate (n=)**		**Placebo (n=)**		**p value**
	Mean	**SD**	**Mean**	**SD**	

Inflammatory Score			
GAGS score			

Table 6. Frequency of each category for examiner's assessment

Examiner's Assessment of Improvement of Acne				
Assessment	**Zinc Gluconate (n=)**		**Placebo (n=)**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
No Improvement				
Slight Improvement (<50%)				
Marked Improvement (≥50%)				

Table 7. Frequency of each category for patient's self-assessment

Patient's Self-Assessment of Improvement of Acne				
Assessment	**Zinc Gluconate (n=)**		**Placebo (n=)**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
No Improvement				
Slight Improvement (<50%)				
Marked Improvement (≥50%)				

REFERENCES

1. Bhate K, Williams H. Epidemiology of acne vulgaris. *British Journal of Dermatology*. 2013;168(3):474-485.
2. Acne in the Philippines. *HealthGrove*. 2017. Accessed February 20. http://global-disease-burden.healthgrove.com/l/84612/Acne-in-Philippines
3. Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K. *Fitzpatrick's Dermatology in General Medicine*. McGraw Hill; 2012.
4. Humphrey S. Antibiotic resistance in acne treatment. *Skin Therapy Letter*. 2012;17(9):1-3.
5. Tan J, Bhate K. A global perspective on the epidemiology of acne. *British Journal of Dermatology*. 2015;172(Supp 1):3-12.
6. Collier CN, Harper JC, Cafardi JA, et al. The prevalence of acne in adults 20 years and older. *Journal of the American Academy of Dermatology*. 2008;58:56-59.
7. Goulden V, Stables G, Cunliffe W. Prevalence of facial acne in adults. *Journal of the American Academy of Dermatology*. 1999;41(4):577-580.
8. Thiboutot DM, Dreno B. Practical management of acne for clinicians: An international consensus from the Global Alliance to Improve Outcomes in Acne. *Journal of the Americal Academy of Dermatology*. 2018;(78):S1-23.
9. Stein Gold L, Weiss J, Rueda MJ, Liu H, Tanghetti E. Moderate and Severe Inflammatory Acne Vulgaris Effectively Treated with Single-Agent Therapy by a New Fixed-Dose Combination Adapalene 0.3 %/Benzoyl Peroxide 2.5 % Gel: A Randomized, Double-Blind, Parallel-Group, Controlled Study. *American Journal of Clinical Dermatology*. 2016;17(3):293-303.

10. Al-Shobaili H. Oxidants and anti-oxidants status in acne vulgaris patients with varying severity. *Annals of clinical and laboratory science*. 2014:202-207.
11. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. *Free Radic Biol Med*. 1990;8(3):281-91. doi:10.1016/0891-5849(90)90076-u
12. Prasad AS. Zinc is an Antioxidant and Anti-Inflammatory Agent: Its Role in Human Health. *Frontiers in nutrition*. 2014;
13. Rostan MD EF, DeBuys MD HV, Madey PhD DL. Evidence supporting zinc as an important antioxidant for skin. *International Journal of Dermatology*. 2002;
14. NIH Zinc Fact Sheet. Accessed March. https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/.
15. Drug Bank Zinc Gluconate. Accessed April. https://www.drugbank.ca/drugs/DB11248.
16. Sardana MD K, Chugh MD S, Garg MD VK. The role of zinc in acne and prevention of resistance: Have we missed the "base" effect? *International Journal of Dermatology*. January 2014:125-127.
17. Examine.com. Accessed May 8. https://examine.com/supplements/zinc/.
18. Zinc for Testosterone. Accessed July. https://supplementsinreview.com/testosterone/zinc-testosterone/.
19. Marcos JM, Perlas LA, Trio PZ, et al. Zinc Status of Filipinos by Serum Zinc Level. *Philippine Journal of Science*. 2015;144(2):139-148.
20. Buxaderas S, Farré-Rovira R. Whole blood and serum zinc levels in relation to sex and age. *Revista Espanola De Fisiologia*. 1985;41(4):463-470.
21. Amer M, Bahgat MR, Tosson Z, Abdel Mowla MY, Amer K. Serum zinc in acne vulgaris. *Int J Dermatol*. Oct 1982;21(8):481-4. doi:10.1111/j.1365-4362.1982.tb03188.x
22. Arora PN, Dhillon KS, Rajan SR, Sayal SK, Das AL. Serum Zinc Levels in Cutaneous Disorders. *Med J Armed Forces India*. Oct 2002;58(4):304-6. doi:10.1016/S0377-1237(02)80083-1
23. Saleh BO, Anbar ZN, Majid AY. *Serum Trace Elements (Zinc, Copper and Magnesium) Status in Iraqi Patients with Acne Vulgaris:(Case- Controlled Study)*. Iraqi Journal of Pharmaceutical Sciences; 2011.
24. Mogaddam MR, Ardabili NS, Maleki N. Correlation between the Severity and Type of Acne Lesions with Serum Zinc Levels in Patients with Acne Vulgaris. *BioMed research international*. July 2014:1-6.
25. Michaëlsson G, Vahlquist A, Juhlin L. Serum Zinc and Retinol-Binding protein in Acne. *British Journal of Dermatology*. 1977;96(3):283-286.
26. Lim MC, Encarnacion MNF, Lavadia MAM. *Relationship of Whole Blood Zinc Levels to Acne Severity Among Filipinos 18-25 years old: A Cross-sectional Comparative Study in a Tertiary Government Hospital*. 2018.
27. Ozuguz P, Kacar SD, Ekiz O, Takci Z, Balta I, Kalkan G. Evaluation of serum vitamins A and E and zinc levels according to the severity of acne vulgaris. *Cutaneous and Ocular Toxicology*. 2013;
28. Kaymak Y, Adisen E, Erhan M, Celik B, Gurer MA. Zinc Levels in Patients with Acne Vulgaris. *Journal of the Turkish Academy of Dermatology*. 2007;1(3)
29. Gupta M, Mahajan VK, Mehta KS, Chauhan PS. Zinc Therapy in Dermatology: A Review. *Dermatology research and practice*. 2014;

30. Bae YS, Hill ND, Bibi Y, Dreiher J, Cohen AD. Innovative Uses for Zinc in Dermatology. *Dermatologic Clinics*. 2010;28(3):587-597.
31. Nitzan YB, Cohen AD. Zinc in skin pathology and care. *Journal of Dermatological Treatment*. 2006;17:205-210.
32. Michaelsson G, Juhlin L, Junghall KL. A double-blind study of the effect of zinc and oxytetracycline in acne vulgaris. *British Journal of Dermatology*. 1977;97:561-566.
33. Dreno B, Moyse D, Alirezai M, et al. Multicenter Randomized Comparative Double-Blind Controlled Clinical Trial of the Safety and Efficacy of Zinc Gluconate versus Minocycline Hydrochloride in the Treatment of Inflammatory Acne Vulgaris. *Dermatology*. 2001;203:135-140.
34. Cunliffe WJ, Burke B, Dodman B, Gould DJ. A double Blind trial of a zinc sulphate/citrate complex and tetracycline in the treatment of acne vulgaris. *British Journal of Dermatology*. 1979;101:321-325.
35. Dreno B, Amblard P, Agache P, Sirot S, Litoux P. Low Doses of Zinc Gluconate for Inflammatory Acne. *Acta Dermato-Venerealogica*. 1989;69:541-543.
36. Hillstrom L, Pettersson L, Hellbe L, Kjellin A, Leczinsky C-G, Nordwall C. Comparison of oral treatment with zinc sulfate and placebo in acne vulgaris. *British Journal of Dermatology*. 1977;97:681-684.
37. Goransson K, Liden S, Odsell L. Oral zinc in acne vulgaris: a clinical and methodological study. *Acta Dermato-Venereologica*. 1978;58(5):443-448.
38. Verma K, Saini A, Dhamija S. Oral zinc sulphate therapy in acne vulgaris: a double-blind trial. *Acta Dermato-Venereologica*. 1980;60(4):337-340.
39. Sardana K, Garg VK. An observational study of methionine-bound zinc with antioxidants for mild to moderate acne vulgaris. *Dermatologic Therapy*. 2010;23:411-418.
40. Vahlquist A, Michaelsson G, Juhlin L. Acne treatment with oral zinc and vitamin A: effects on the serum levels of zinc and retinol binding protein (RBP). *Acta Dermato-Venereologica*. 1979;58(5):437-442.
41. Dreno B, Foulc P, Reynaud A, Moyse D, Habert H, Richet H. Effect of zinc gluconate on propionibacterium acnes resistance to erythromycin in patients with inflammatory acne: in vitro and in vivo study. *European Journal of Dermatology*. 2005;15(3):152-155.
42. Jarrousse V, Castex-Rizzi N, Khammari A, Charveron M, Dreno B. Zinc salts inhibit in vitro Toll-like receptor 2 surface expression by keratinocytes. *European Journal of Dermatology*. 2007;17(6):492-496.
43. Adityan B, Kumari R, Thappa DM. Scoring Systems in Acne Vulgaris. *Indian Journal of Dermatology, Venereology and Leprology*. 2009;75(75):323-326.
44. Zohra F, Sultana T, Islam S, Nasreen T. Evaluation of Severity in Patients of Acne Vulgaris by Global Acne Grading System in Bangladesh. *Clinical Pathology and Research Journal*. 2017;1(1):1-5.