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

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Introduction:

Acne Vulgaris is a disorder of the pilosebaceous unit characterized by formation of comedones, papules, pustules, inflamed nodules, and cysts.[1] Most common predilection sites are those with hormonally sensitive sebaceous glands such as the face, neck, chest, upper back and upper arms.[1] Acne can persist for years and result in disfigurement and permanent scarring. It can cause serious adverse effects on psychosocial development, resulting in emotional problems, withdrawal from society, and depression.[2] Nearly all adolescents experience different severity levels of acne vulgaris, with moderate to severe levels affecting around 20% of this population.[2] In the Philippines, high school students were found to have a mild impairment of quality of life (QOL) due to acne, regardless of severity.[3] Despite the known treatment regimens for acne, people 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.[1, 4] 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.[5] Other options that can be substitutes or adjuncts to treatment may be useful in this condition.

Zinc is an essential element for normal epithelial differentiation and for normal development. It has also been shown to have antioxidant properties.[6] At present, there is paucity of data regarding the role of zinc levels in the development or severity of acne vulgaris. Also, data on favorable effects of dietary factors such as zinc, omega-3 fatty acids, antioxidants, vitamin A, and dietary fiber on acne vulgaris are limited specially in the Philippines.

This study will be conducted to measure the whole blood zinc levels in patients with acne vulgaris and compare it with healthy controls. This aims to determine the relationship between whole blood zinc levels and disease severity in Filipino patients with acne vulgaris in order to bring new insight to guide further studies regarding zinc's role in the pathogenesis and possible further treatment of acne vulgaris in the Philippines.

Review of Related Literature:

Acne Vulgaris is the eighth most prevalent disease in the world, affecting 9.4% of the global population.[7] In adults, acne prevalence ranges from 50-54% in females and 40-42.5% in males.[8,9] Acne is commonly misunderstood to affect primarily teenagers; however, a large proportion of individuals continue to suffer from acne or acquire new onset acne far after their adolescent years.[8] Up to 64% of individuals have acne persisting into their 20s, and 43% have acne persisting into their 30s.[2] Moreover, the prevalence of acne did not substantially decrease until after the age of 44.[9]

Safe and effective long-term maintenance therapy is necessary in order to address the chronic and persistent nature of acne.[5] In the last 3 decades, there has been a significant increase in resistance to antibiotics commonly used to treat acne. This was measured by the increased minimal inhibitory concentration (MIC) against *Propionibacterium acnes* for antibiotics such as erythromycin and tetracycline in patients receiving long-term antibiotic treatment. Increase in MIC was not found in acne patients who were not on antibiotic therapy and in non-acne patients.[5, 10] This is a significant international public health concern because aside from increase in pathogenic *P. acnes*, resistance can occur in other bacteria more dangerous than *P. acnes*.

Given this situation and the complexity of the repercussions of this trend, it is a challenge to maximize the use of non-antimicrobial therapy when treating acne .[5, 11-14]

The development of a variety of skin disorders, including acne, has been linked to oxidative status. Oxidative stress can be measured using plasma levels of catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA). MDA, a marker for oxidative stress, was found to be higher in acne patients compared to controls. TAC together with the antioxidant enzymes, SOD and CAT, were lower for patients with acne. These results suggest that oxidative stress appears to play a major role in the pathogenesis of acne.[15]

Zinc is found in all body tissues. In skin, it is five to six times more abundant in the epidermis than in the dermis. Zinc is an essential element for cell health and proper bodily functions like glandular, reproductive, immune, and neuropsychiatric processes since the conformity, stability and activity of more than 200 metalloenzymes are affected by zinc. Its antioxidant properties have numerous benefits from protection against ultraviolet rays and oxidative damage by decreasing reactive oxygen species (ROS) to improvement of wound healing and prevention of cancer and cardiovascular disease.[6, 16]

Data suggest that zinc deficiency exist both in developed and developing countries, and several factors may affect the serum zinc levels, which include low intake of highly absorbable zinc in fresh foods, and high phytate content of some staple food, pregnancy, lactation, increased demands of physiological processes such as growth and sexual maturation.[17] 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.[18] Zinc deficiency starts at 66 micrograms/dL for non-pregnant females and 70 micrograms/dL for males greater than 9 years old.[19]

The Agency for Toxic Substances and Disease Registry (ATSDR) states that inductively coupled plasma–atomic emission spectroscopy (ICP-AES) is used for zinc determinations in blood and tissue samples. This method is approved by the National Institute for Occupational Safety and Health (NIOSH). Samples are prepared by ashing or mineralization using acid digestion with nitric acid (HNO_3), perchloric acid ($HClO_4$), or sulfuric acid (H_2SO_4) in order to oxidize the organic sample and leave the inorganic component for analysis.[20] Atomic emission spectrometry (AES) is a technique commonly utilized for analyzing elements. This method records the electromagnetic radiation released by free atoms of the elements in the sample to be analyzed and uses the electromagnetic emission lines to determine the element. The prepared sample, once in the spectrometer, evaporates and atomizes in argon plasma flame, where temperatures can reach up to 10,000 K. The high temperature causes the excitation of atoms to higher electronic states. Atoms returning to the ground electron state emit electromagnetic radiation, which is measured by the spectrometer to determine the element.[21]

A few researches have been done in other countries comparing zinc levels in blood with the severity of acne. 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.[22] A study done in Iran suggested that zinc levels may be related to the severity and type of acne lesions in patients with acne vulgaris, with moderate and severe acne patients having lower serum zinc levels than mild acne

and control patients.[23] 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.[24] The relative decrease of serum zinc levels in acne patients suggests a role for zinc in the pathogenesis of acne vulgaris. Studies about serum or whole blood zinc levels and acne severity in the Philippine setting have not yet been done.

The Global Acne Grading System (GAGS) is an acne severity scale that includes six locations on the face, chest and upper back. A factor is provided for each of the six locations based on size (forehead = 2, right cheek = 2, left cheek = 2, nose = 1, chin = 1, chest and upper back = 3). Each of the six areas are graded on a scale of 0-4 based on the most severe lesion present (no lesion = 0, comedones = 1, papules = 2, pustules = 3, nodules = 4). The local score per location is obtained by multiplying the factor with the corresponding score for the most severe lesion. The sum of the local scores from all locations will result to the global score, with zero as the lowest and 44 as the highest possible global score. Acne severity is rated as mild (1-18), moderate (19-30), severe (31-38), and very severe (≥39) with corresponding cut-off global scores.[25]

Significance of the Study:

While a number of studies have been conducted comparing serum zinc levels with acne vulgaris, no previous studies have been done in the Philippine setting that assesses the relationship of whole blood zinc levels with the severity of acne. Given that acne vulgaris is one of the most common dermatologic diagnoses in the Philippine population, and the increasing resistance to antibiotics commonly used to treat acne, studies about other treatment modalities for acne vulgaris that do not have resistance factors are timely and significant in the country.

For long–term or maintenance therapy, physicians should consider effectivity, cost, and adverse effects. By determining the relationship of whole blood zinc levels to acne severity among Filipinos 18-25 years old, this study can be used as evidence for giving zinc supplements to Filipino people diagnosed with acne vulgaris if a significant relationship is found. 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. 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.

Research Question:

Is there a relationship between whole blood zinc levels and the severity of acne vulgaris in Filipino patients 18-25 years old versus normal controls?

Objectives:

GENERAL

- To study the relationship between whole blood zinc levels and the severity of acne vulgaris in Filipino patients 18-25 years old and normal controls

SPECIFIC

- To describe the clinical profile of Filipino patients 18-25 years old with acne vulgaris

- To determine the whole blood zinc levels of acne patients compared to controls
- To determine the relationship of the clinical severity of acne vulgaris to whole blood zinc levels

Research Hypotheses:

Null Hypothesis: There is no relationship between whole blood zinc levels and the severity of acne vulgaris.

Alternative Hypothesis: There is a relationship between whole blood zinc levels and the severity of acne vulgaris.

METHODOLOGY

Study Design

A cross-sectional comparative study design will be utilized for this study which will be conducted from November 2017 to March 2018.

Study Setting

The study will be conducted at the Dermatology Out-patient Clinic of the East Avenue Medical Center (EAMC). The hospital has adequate staff and infrastructures to provide for the blood extraction of this study to be conducted properly. Laboratory services of Hi-Precision Diagnostics will be used for measuring whole blood zinc levels for this study.

Study Population

The participants of the study will include patients clinically diagnosed with acne vulgaris, aged 18-25 years of age.

Inclusion Criteria for Cases

- Filipino patients, aged 18-25 years old
- Diagnosis of Acne Vulgaris
- Able to read and write in English or Tagalog
- Seen at the Dermatology Out-Patient Department or Skin Center of East Avenue Medical Center

Exclusion Criteria for Cases

- Patients with other chronic dermatoses or systemic disease
- Taking oral supplements or medications for the past 3 months
- Patients who are pregnant or lactating

Inclusion Criteria for Controls

- Filipino patients, aged 18-25 years old
- Able to read and write in English or Tagalog
- Seen at the Dermatology Out-Patient Department or Skin Center of East Avenue Medical Center

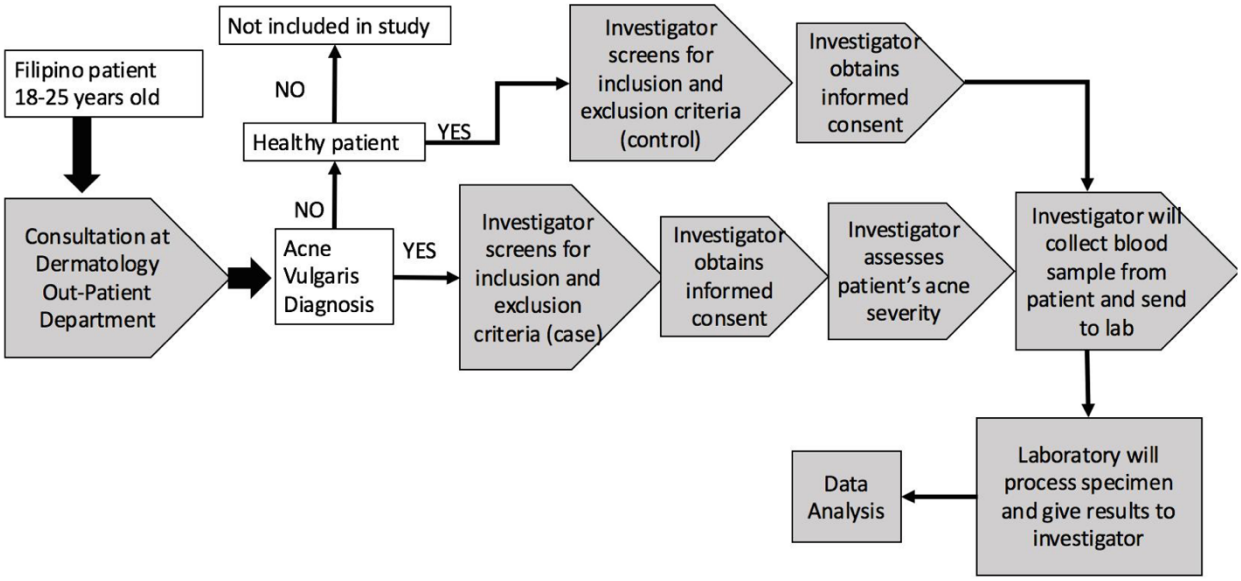
Exclusion Criteria for Controls

- Patients with Acne Vulgaris or other chronic dermatoses or systemic disease
- Taking oral supplements or medications for the past 3 months
- Patients who are pregnant or lactating

Study Procedure

1. The study will be submitted to the Technical Review Board and Institution Ethics and Review Board for approval.
2. In the Dermatology Out-Patient Department of East Avenue Medical Center, patients seeking consult for their skin lesions which were diagnosed to be Acne Vulgaris will be screened if they meet the inclusion/exclusion criteria.
3. Informed consent will be obtained for those who are qualified.
4. Patients will be given proper treatment whether they agree to participate or not. For the patients who agree to participate, the investigator will assess the patient's acne severity using the Global Acne Grading Scale (GAGS).
5. Twelve milliliters of blood will be extracted from the patient with proper aseptic technique and waste disposal.
6. Blood sample will be properly stored in three EDTA tubes, labelled, and transported to the laboratory for determination of whole blood zinc levels through ashing acid digestion-inductively coupled plasma (ICP) using Shimadzu ICPS-7510, an inductively coupled plasma emission spectrometer.
    a. Blood samples are viable and stable for 5 days at 15-25 degrees Celsius.
    b. A memorandum of agreement between the principal investigator and Hi-Precision Diagnostics, Inc. has been made. Hi-Precision Diagnostics will be providing the materials for extraction, picking-up, and transporting the blood sample using a special insulated medical specimen bag (Versapak Pathology and Specimen bag) with ice packs and thermometer to help maintain the ideal temperature of 15-25 degrees Celsius.
    c. Schedule of specimen pick-up is from 8:00 am- 8:00 pm and may be done on an as-needed basis. The blood specimens will be stored in the refrigerator immediately after extraction.
7. Results will be obtained by the investigator via the online results portal of the laboratory.
8. Data collected will be tabulated and data analysis will be done.

Study Framework/Process



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.

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. The investigator will do physical examination of the patient to assess the severity classification, but no photographs will be taken of the patient or the skin lesions. 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 participant 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 (minimal chances of bleeding, bruising, infection, faintness or brief dizziness) and the expected benefits (patient will be able to know

his/her blood zinc levels). The participant 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 patient 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 participant 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 participant 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 participant. By signing the consent form, the participant attests that the information in the consent form and any other written information was accurately explained and apparently understood by the participant and that informed consent was freely given. At any time during the process, the participant can opt to refuse from participating in the study.

STATISTICAL ANALYSIS PLAN

Justification of Sample Size:

PASS 2008 was used for the computation of minimum sample size. Parameters for the computation was obtained from previously published studies. For the one-way ANOVA, a minimum of 40 patients (10 for each acne severity: mild, moderate and severe acne and 10 for controls) achieves 95% power to detect differences among the means versus the alternative of equal means using an F test with a significance level of 0.05. The size of the variation in the means is represented by their standard deviation which is 13.30 mg/dL and the common standard deviation within a group is assumed to be 20.00 mg/dL.[22]

Plan of Data Analysis:

Data will be encoded by the researcher in MS Excel. The excel file will be converted into Stata file for further data processing and analysis. Stata SE version 12 will be used for both descriptive and inferential statistics. Quantitative variables will be presented as mean or median while qualitative variables will be presented as percentages.

In order to compare the whole blood zinc levels between cases and controls, independent T-test will be used. ANOVA will be used to compare the whole blood zinc levels by acne severity. Multiple linear regression analysis will then be performed in order to determine the association of whole blood zinc levels with acne severity after controlling for the effects of significant confounding variables. All p values ≤ 0.05 will be considered as significant.

CONCLUSION

The correlation of whole blood zinc levels with severity of acne vulgaris in Filipino patients may help clinicians recommend more detailed dietary advice and add non-antibiotic supplements to their current treatment of acne vulgaris. The results of the study can serve as a guide for

further studies related to whole blood zinc and its role in the pathogenesis of acne vulgaris in Filipino patients.

Key Words: Whole Blood Zinc, Acne Vulgaris, Filipino

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