

Original Article**Investigation of the Role of Zn/Cu Index and its Correlation with Physiological Activity of SOD 1 and GRx in Males with Acne Vulgaris****Naji, H. H¹, AL-Azawi, R. S. A², Ibrahim, N. J¹, Kzar, H. H^{1*}**

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Abstract

It has been approved that the normal physiology of skin can be adversely affected by acne vulgaris (AV). This disorder leads to impairment of stratum corneum hydration and causes trans epidermal water loss. The normal physiology of the males' skin is different from the normal physiology of females' skin. Therefore, in case of any skin disorder, choosing the best strategy and treatment should be investigated seriously in each gender. Therefore the current study was designed to investigate the effect of two important trace elements (i.e., zinc [Zn] and copper [Cu]) on skin health and the correlation of Zn/Cu index with the physiological activity of antioxidant enzymes superoxide dismutase (SOD1) and glutaredoxins (Grx) in males with AV. In total, 100 samples were obtained from 60 males (in the age range of 17-20 years) with a definite diagnosis of AV (AVM group) and 40 males (in the age range of 18-20 years) with normal skin as the control group (CON group). The blood samples were obtained from each participant. The blood samples were centrifuged for the measurements of Zn, Cu, Zn/Cu index, SOD1, and GRx, and serum samples were preserved at -20°C until use. Moreover, Zn, Cu, and Zn/Cu index were determined using spectrophotometric kits. The enzyme-linked immunosorbent assay, as the preferred method, was performed for SOD1 and GPx measurements. On the other hand, in this study, body mass index (BMI) and age were considered to have a possible association with the incidence of acne in males. The recorded data showed that there were no significant differences between the AVM group and controls in terms of BMI. The recorded data showed that Zn (AVM:151±10.7; CON:189±9.7) and Cu (AVM:55±5.2; CON: 77±4.8) concentration was significantly reduced ($P<0.05$) in the AVM group, compared to controls. On the other hand, the Zn/Cu index was significantly lower in the AVM group (1.05±0.19), compared to the control group (1.78±0.08). The results of the SOD1 and GRx assay showed that the AVM group suffered from a significant reduction in the SOD1 and GRx concentration, compared to the group of control. Overall, it can be concluded that the improvement of the antioxidant enzyme activity and supplementation of trace elements may significantly reduce the incidence of AV in males.

Keywords: Acne vulgaris, Copper, GRx, SOD1, Zinc

1. Introduction

Acne vulgaris (AV) is defined as any alteration in the normal physiology of the activity of the hair follicle, hair shaft, and sebaceous gland which causes non-inflammatory and inflammatory lesions, such as open and closed comedones, papules, pustules, and nodules,

respectively. The AV causes varying degrees of damage and scarring in the skin. It is worth mentioning that 85% of AV cases are observed during adolescence and are mostly related to the lifetime of an individual (1).

It is well documented that AV is more common in males, compared to females. On the other hand, the

incidence of AV in women is highest from the age 20 and 29 years (2). Acne usually appears in adolescence and persists through the early thirties in males. It is well documented that one's lifestyle is strongly associated with the incidence of acne, and urban populations are more susceptible to acne than rural populations (3).

The incidence rate of severe acne with a high scarring index is about 20%. On the other hand, the race of individuals is considered to be the most important factor in AV incidence. In a study conducted by Juhl Bergholdt (4), it was revealed that Asians and Africans tend to develop severe acne, compared to the white populations, and that darker skin has greater potential to develop hyperpigmentation in general.

Moreover, acne in neonates often resolves spontaneously (5). During puberty, under the influence of androgens, sebum secretion is increased as 5-alpha reductase converts testosterone to more potent DHT, which binds to specific receptors in the sebaceous glands. Sebum production and secretion are increased during puberty. This procedure leads to more hyperproliferation of the follicular epidermis and retention of sebum (6).

Psoriasis, atopic eczema, and acne are skin disorders that interfere with one's social and occupational functioning and lead to an impairment of life quality (7). Skin disorders may have several social and psychological morbidities which may be ignored or not properly addressed. Consequently, the adverse effects of these complications are usually underestimated by healthcare professionals (8). Acne vulgaris is also known as juvenile acne since it has a high incidence rate among adolescents in males and females (9).

It is well documented that several skin and hair disorders occur due to the insufficient amount of some trace elements and antioxidant enzymes (10). Therefore, this study aimed to investigate the effect of some levels of trace elements, such as Zinc (Zn) and Copper (Cu), Zn/Cu index, and the correlated physiological activity of antioxidant enzymes, SOD1 and GRx, in males with AV.

2. Materials and Methods

2.1. Study Design

SOD1, GRx, Zn, Cu, and Cu/Zn index were considered as markers of the antioxidant capacity. These markers were measured in 100 male participants who were divided into Group 1 and Group 2. Group 1 (AVM group) consisted of 60 males (in the age range of 17-20 years) with a definite diagnosis of AV, and Group 2 (CON group) included 40 males (in the age range of 18-20 years) with normal skin. The blood samples were obtained from each participant and centrifuged for the measurements of Zn, Cu, Zn/Cu index, SOD1, and GRx. Serum samples were preserved at -20°C until use. Spectrophotometric kits were used to determine Zn, Cu, and Zn/Cu index. The enzyme-linked immunosorbent assay (ELISA) was performed, as the preferred method, to measure SOD1 and GPx. On the other hand, in this study, body mass index (BMI) and age were considered two probable factors associated with the incidence of acne in males.

2.2. Determination of Zn, Cu, and Zn/Cu Index

Analysis of Cu, Zn, and Cu/Zn index were measured using flame atomic absorption spectrometry (Thermo Jarrel Ash, Germany), according to the method used by Kirgbright (11). Serum samples were diluted by deionized water. Different concentrations of trace elements were prepared for calibration of standard graphs. Absorbances were read at 324.7 nm and 213.9 nm for Cu and Zn, respectively. For accuracy, the standard solutions were run for every 10-test sample. Serum samples were run in triplicate, and individual values were averaged.

2.3. Determination of SOD1 and GRx

SOD1 and GRx were assayed using the ELISA technique (Sandwich-ELISA). The provided Micro-ELISA strip plate has been pre-coated with an antibody specific to SOD1 and GRx. Standards and samples were added to the appropriate Micro-ELISA strip plate wells and combined to the specific antibody to SOD1 and GRx.

2.4. Statistical Analysis

All Zn, Cu, Zn/Cu, SOD1, and GRx values were reported as mean±SD. Statistical significance was assessed using Student's t-test. *P*-values less than 0.05 (*P*<0.05) were considered statistically significant.

3. Results and Discussion

The recorded data showed that AVM and control group had no significant differences in terms of BMI. However, it was revealed that BMI and age could not be considered factors associated with the incidence of AV (Table 1).

The standard curve of SOD1 and GRx enzymes activity is depicted in figure 1, as determined using ELISA.

Table 2 tabulates the results of the serum samples analysis. It is revealed that the Zn (AVM: 151±10.7; CON:189±9.7) and Cu (AVM: 55±5.2; CON:77±4.8) concentrations were significantly lower in the AVM group, compared to the control group. On the other hand, the Zn/Cu index was dramatically lower in the AVM group (1.05±0.19), compared to the control group (1.78±0.08).

The results of the SOD1 and GRx assay showed that the AVM group suffered from a significant reduction in the SOD1 and GRx concentration, compared to the control group (Table 3). Figure 2 represents the correlation between the Zn/Cu index with levels of SOD1 and GRx (IU) in the AVM group.

Table 1. Different gross parameters as probable factors in the incidence of acne vulgaris

Parameters	AVM group(N=60)	CON group (N=40)	<i>P</i> -value
Age (years)	17-20	18-20	0.127
BMI (Kg/m ²)	23.87 ^a	24.75 ^a	0.081
Duration (years)	1-5	-	-

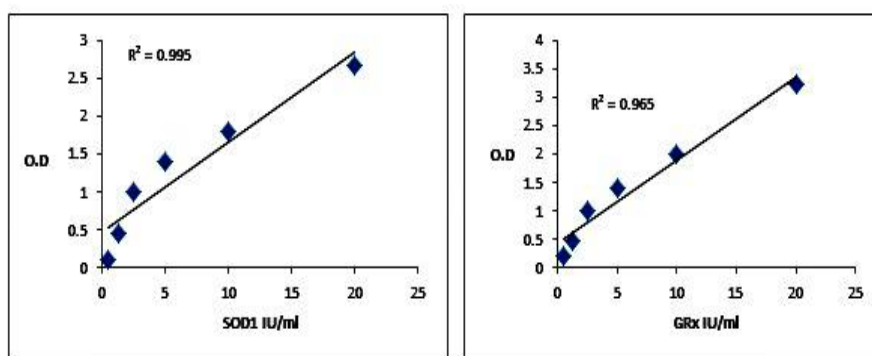


Figure 1. Standard curve of SOD1 and GRx

Table 2. Differences between Zn, Cu, and Zn/Cu index levels in AVM and CON groups

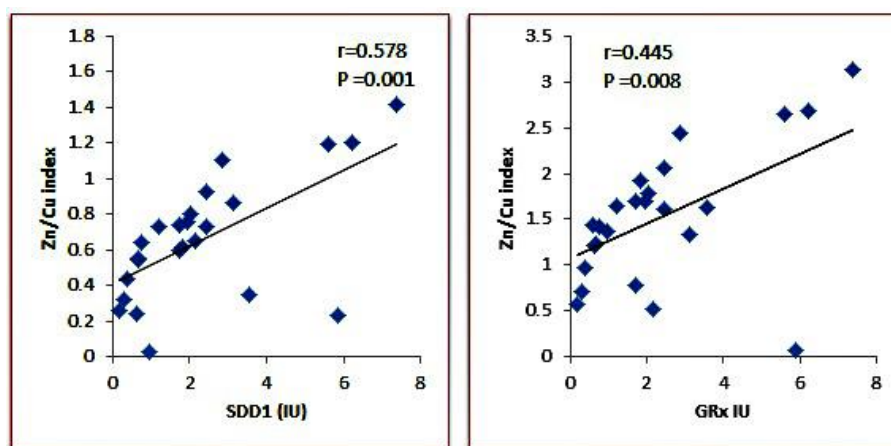
Trace Elements (µg/dl)	AVM group (N= 60) Mean ± SD	CON group (N= 40) Mean ± SD	<i>P</i> -value
Zn	151±10.7 ^a	189±9.7 ^b	0.05
Cu	55±5.2 ^a	77±4.8 ^b	0.05
Zn/Cu	1.05±0.19 ^a	1.78±0.08 ^b	0.021

^{a,b} Different superscripts in a row represent significant differences

Table 3. Levels of SOD1 and GRx in AVM and CON groups

Parameters	AVM group(N= 60)	CON group (N= 40)	P-value
SOD1 (IU/l)	14.41 ± 4.9 ^a	19.54 ± 4.7 ^b	0.05
GRx (IU/l)	1.34 ± 0.21 ^a	2.98 ± 0.01 ^b	0.05

^{a,b} Different superscripts in a row represent significant differences

**Figure 2.** Correlation between SOD1 and GRx levels with Zn/Cu index in ANM group

A previous study conducted by Bashir et al. revealed that up to 95% of males and 83% of females were suffering from AV until they reach their twenties (12). Moreover, it was revealed that AV became severe in about 10% of AV cases (13).

It is well documented that the possible etiological factors for AV progression include 1) hormones, 2) use of cosmetics and/or drugs, and 3) chronic stress (14). Genetic factors also play a significant role. Goulden, Clark (15) showed that the majority (67%) of patients had a first-degree family history of post-adolescent acne (15). In our study, out of 454 adult participants examined 322 (70.9%) participants, including 50 males (72.5%) and 272 females (70.6%), declared a family history of acne ($P=0.872$) (15). The results of this study suggested positive correlations between the Zn/Cu index and levels of SOD1 and GRx in the AVM group ($P<0.005$). Even though genetics, hormones, and infection have been proven to play a role in the pathogenesis of AV, there is very little evidence on whether such factors as dietary restriction, facial hygiene, and sunlight exposure influence acne and its

management positively or negatively. Although still unproven and controversial, the perception that diet is a cause or aggravating factor of acne is a strongly held belief among adolescents and participants with acne worldwide (16-18). This study showed a strong correlation between levels of Zn and Cu (mg/dL) as important trace elements with the incidence of AV in males. Based on the evidence, AV is a common skin disease affecting approximately 9.4% of the world's population with the highest prevalence in adolescents. It affects over 90% of males and 80% of females in all ethnic groups (19-21) and has been the presenting complaint in 90% of people aged 13 to 25 in the Iraqi population. This study suggested that the mean age of AV incidence in males was 17 years. The number of adults with acne, including people over 25 years, is lower than those under 25 years and not included in this study. Previous studies investigating the potential link between lifestyle and AV have shown controversial results (19). Moreover, the literature review suggested that the prevalence of AV varies with several genetic and environmental factors. A study conducted by

Ballanger, Baudry (22) found a strong association of causation of AV with both genetic and environmental elements.

Overall, it can be concluded that the improvement of the antioxidant enzyme activity and trace elements supplementation can reduce the incidence of AV significantly in males.

Authors' Contribution

Study concept and design: H. H. K

Acquisition of data: H. H. N.

Analysis and interpretation of data: H. H. K.

Drafting of the manuscript: H. H. K.

Critical revision of the manuscript for important intellectual content: N. J. I.

Statistical analysis: R. S. A.

Administrative, technical, and material support: H. H. K.

Ethics

The study design was approved by the ethics committee of Al-Qasim Green University, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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