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Research Article

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Antioxidant Effects of Oral Zinc Sulfate in Patients with Behçet's Disease

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Abstract: Treatment of Behcet disease (BD) is still considered challenging since no single agent has yet proved to reverse the underlying oxidative stress-induced inflammatory process. Zinc sulfate treatment has been shown to have an effective therapeutic outcome, however adequate exploration of the exact mechanism for its therapeutic benefit is still lacking. This study was carried out to assess and relate the oxidative stress parameters with the clinical manifestation index before and after oral zinc sulfate treatment. Twenty-five Behcet disease (BD) patients were recruited in the study besides an equal number of healthy individuals representing the control group. Patients were treated with 300mg/day oral zinc sulfate in three divided doses for one month. Blood samples were collected from the control group, patient group (before starting treatment),and treatment group (after zinc sulfate treatment) to measure serum oxidative stress parameters as well as antioxidant parameters. A dermatologist who was blind to the treatment calculated the clinical manifestation index (CMI) score and the oral ulcer parameters for each individual in the patient and treatment groups. Serum MDA, copper levels were found significantly higher in the patient group than in the control group. However, both parameters were considerably lower in the treatment group than in the patient group. Serum glutathione (GSH), zinc levels and RBC catalase activity were significantly lower in the patient group than in the control group, but they were substantially higher in the treatment group as compared to the patient group. The CMI score and oral ulcer parameters decreased significantly in the treatment group (after zinc sulfate treatment) compared to the patients' group. The CMI demonstrated a significant positive correlation ($P<0.01$) with the oxidative stress parameters. In conclusion, BD patients were suffering from oxidative stress, the severity of which was directly proportional to the CMI score. The antioxidant activity of oral zinc sulfate treatment is at least one of the mechanisms by which zinc exerts its action in the treatment of BD.

Keywords: Behcet's disease, oxidative stress, zinc, antioxidant, clinical manifestation index

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1. INTRODUCTION

BD is a heterogeneous relapsing-remitting multisystem disease characterized by oral aphthae and ulcers, skin lesions, uveitis, arthritis, and arthralgia. The etiopathogenesis of BD is unknown, but it has been postulated that immune system abnormalities, induced by the microbial pathogen in genetically susceptible individuals are essential in its pathogenesis.[1] Being primarily a mucocutaneous disease, the clinical severity of BD is universally assessed by using the clinical manifestation index (CMI) score. This enables dermatologists to classify BD patients into mild, moderate, or severe cases. A growing body of evidence is supporting the role of oxidative stress in BD via an increased production of ROS which is believed to cause an imbalance between the oxidant and antioxidant components of the skin and the mucous membranes in patients with BD. [2, 3] Despite the presence of a good number of studies supporting the existence of oxidative stress in BD, however, analyzing the correlation between the disease severities with the oxidative stress/ antioxidant blood parameters is still lacking so far. Zinc is a trace element that has shown effectiveness in the treatment of BD [3] as well as demonstrating potent antioxidant and anti-inflammatory actions. [4] This study was carried out to compare the clinical and oxidative stress/ antioxidant parameters of BD patients before and after zinc sulfate treatment. Moreover, this study aimed to elucidate the correlation of the oxidative stress and antioxidant parameters with the clinical parameters of BD patients before and after oral zinc sulfate treatment.

2. MATERIALS AND METHODS

Twenty-five BD patients (13 males and 12 females) whose age range was 21 - 53 years registered at the BD clinic in Baghdad Teaching Hospital were recruited in this study between February and August 2018. The design, nature and duration of the study were explained to each patient who agreed to enroll by signing an informed consent. The study procedure was in line with the declaration of Helsinki promulgated in 1964 as amended in 1996. Human Research Ethical Committee approval was obtained from the Ministry of health before commencing the study. The control group comprised 25 healthy non-smoker volunteers, well-matched with the patient group for their ages and genders. Females recruited in the study had not been taking oral contraceptives for at least 3 months before the blood sample collection. Neither the patients nor the control subjects have been using any multivitamin supplements for at least 2 weeks.

2.1 Inclusion Criteria

Patients who fulfilled the international study group (ISG) criteria [5] for the diagnosis of BD were included. The ISG criteria require the presence of recurrent oral aphthae plus any two of the following: recurrent genital aphthae, eye lesions, skin lesions and/or a positive Pathergy test.

2.2 Exclusion Criteria

Patients with severe manifestations were excluded from the trial such as severe neurological, cardiovascular, or eye involvement (Posterior or bilateral uveitis), as well as patients whose clinical manifestation index (CMI) score, was more than 15 and pregnant or lactating females. Patients on other drug therapy were asked to stop their treatment 2 weeks prior to their enrollment in the trial. The CMI was calculated for each patient on each visit. A Detailed history of each patient's disease was obtained. The first presenting feature of the BD was recorded as well as the duration and the frequency of the occurrence of symptoms.

2.3 Blood collection

Blood samples were taken from both patient and control groups. 10 ml of venous blood was withdrawn. Then each blood sample was divided into 2 separate tubes. 7 ml was inserted into a plain tube for the serum to be separated by centrifugation at 3000g for 10 minutes. Then the serum was stored at -20°C and kept for further analysis of serum MDA, GSH, zinc and copper levels. The remaining 3 ml of blood was subjected to 3000g centrifugation for 5 min. to obtain packed RBCs, then the samples were stored at -20°C and kept for later estimation of RBC catalase enzyme activity.

2.4 Oxidative stress parameters analysis

Serum MDA was estimated by the method of Buege and Aust. [6] Serum thiol concentration was determined by the modified method of Elleman. [7] Serum GSH was expressed as µmol/dl. CAT enzyme activity in blood hemolysate was done according to the method described by Aebi. [8] A colorimetric measurement of serum copper level was done via a kit purchased from Randox Co. [9] while serum zinc level was determined through a colorimetric test using a kit purchased from Geisse Diagnostics Co. (Roma, Italy). [10]

2.5 *Treatment*

After blood sample collection, BD patients (patient group) were instructed to take 100mg (one capsule) of zinc sulfate three times daily with or immediately after the meal with a full glass of water. The patients were warned not to ingest milk products, antacid-containing tablets, or any drug that may interfere with zinc absorption while taking the treatment. A month later, all treated patients (treatment group) were re-evaluated for BD manifestations, especially oral and genital ulcers. The number, size, and frequency of these ulcers were recorded. Patients were re-examined by an ophthalmologist, rheumatologist and other specialists whenever clinically indicated. Measurement of serum MDA, GSH, copper, zinc, and RBC catalase activity was repeated. Pathergy test was done and the CMI was scored for each patient. Patients were asked to report any side effects from the treatment. [11]

3. STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. A probability P-value of ≤ 0.05 was considered statistically significant. Data were statistically analyzed using SPSS (Statistical Package for Social Sciences) version 20 computer software. Student t-test was used to compare the clinical parameters of the patient group with the treatment group. One-way ANOVA with LSD test (f-test) was used to compare the oxidative stress parameters among the 3 groups (control, patients, and treatment groups). Pearson correlation analysis was used to test the relation between clinical and oxidative stress parameters for the patient group.

4. RESULTS

Significantly higher antioxidant parameter levels (GSH, RBC CAT, and zinc) were found in the control and treatment groups as compared to the patient's group. On the other hand, significantly lower oxidative stress parameters (MDA and s. copper) were detected in the control and treatment groups compared to the patients' group, Table 1.

Table 1: Compares the oxidative stress parameters among the three study groups

		Oxidative stress parameters ± SEM				
		s.MDA µmol/l	**s.GSH** µmol/l	**RBC CAT** mU/gHb	**s.copper** µg/dl	**s.zinc** µg/dl
Study group	**Control**	1.48±0.04**	1.52±0.01**	1.03± 0.03**	81.95±0.39**	88.22±0.34**
	Patient	2.36±0.05	0.71±0.02	0.66±0.02	87.87±0.19	77.67±0.38
	Treatment	1.74±0.01**	1.34±0.02 **	1.15±0.02**	*84.87±0.28	95.79±3.64**

** = P <0.05 compared to the patient group. **= P <0.01 compared to the patient group.*

Statistical analysis of CMI score mean values and the values of oral ulcer parameters for the patients and the treatment group (before and after zinc sulfate treatment) is explained in Table 2.

Table 2: depicts a t-test comparison between the clinical parameters of the patient group and the treatment group.

	group	mean	SD	SEM
CMI Score	**Patient**	8.32	2.56	0.51
	Treatment	2.84**	1.72	0.34
Oral ulcer Number	**Patient**	3.60	1.15	0.23
	Treatment	1.00**	0.76	0.15
Oral ulcer size (mm)	**Patient**	4.20	0.96	0.19
	Treatment	1.48**	1.12	0.22
Oral ulcer duration (days)	**Patient**	8.72	2.61	0.52
	Treatment	3.32**	2.23	0.45

*** = P < 0.01 compared to patient group. CMI = clinical manifestation index.*

The correlation between the clinical parameters (CMI score, oral ulcer parameters) and the oxidative stress parameters (serum MDA, GSH, copper, zinc ,and RBC CAT activity) is shown in Table 3.

Table 3: Depicts correlation analysis between clinical and oxidative stress / antioxidant parameters.

Correlation (r)	s. MDA	s. GTH	RBC catalase	s. copper	s. zinc
CMI Score	0.92**	-0.57*	-0.93**	0.91**	-0.87**
Oral ulcer Number	0.91**	-0.29	-0.89**	0.88**	-0.81**
Oral ulcer size (mm)	0.87**	-0.73**	-0.50*	0.84**	-0.83**
Oral ulcer duration (days)	0.87**	-0.59*	-0.86**	0.87**	-0.81**

*r = correlation coefficient. * = P < 0.05. ** = P < 0.01.*

Less than 30% of cases complained of gastric upset and mild anorexia, and this in most of the cases was due to improper timing of drug intake by the patient or due to taking the drug on an empty stomach. Lethargy was experienced by 3% of patients only that was somehow comforting to the patient rather than annoying.

5. DISCUSSION

The role of reactive oxygen species (ROS) produced by activated neutrophils during the inflammatory response in BD is a well-established fact. [12] The elevated serum MDA level in BD patients in the current study as compared to the control group was in agreement with results reported by previous investigations. [13-16] Other studies reported that RBC and plasma MDA levels as well were significantly higher in the patient's group than those of the healthy control group. [16, 17] This reflects the extent of the oxidative stress in BD patients that eventually leads to oxidative damage in their skin and mucous membranes. The significant reduction in Serum GSH level in patients with BD as compared to that of the healthy control group in the current study is consistent with findings reported by prior researchers. [17-19] This finding signifies the exhaustion of glutathione stores as a result of the severe oxidative stress in BD patients. It is worth mentioning that GSH functions as a direct antioxidant, scavenging a variety of radical species, as well as acting as a component of the glutathione peroxidase antioxidant enzyme. Erythrocytes are highly susceptible cells to lipid peroxidation due to the excessive and continuous leakage of peroxy radicals into the bloodstream in diseases affected by oxidative stress. This, together with the nature of erythrocytes' cell membrane being rich in lipids, makes them more prone to oxidative damage against which CAT enzyme is the predominant H_2O_2 scavenger. [17] Owing to the chronicity of the disease, GSH stores will eventually be depleted and CAT antioxidant activity will be exhausted. This may explain the reason behind the reduction in both serum GSH level and erythrocyte CAT activity in patients with BD.Consistent with the results stated by Kökçam and co-workers, CAT enzyme activity in the RBCs in the present study was found to be significantly decreased in BD patient group as compared to the healthy control group. [19] The significantly higher serum copper concentration in the patient's group as compared to the

healthy control group was in agreement with that reported by older research studies. [20, 21-23] Copper ions were found to be involved in both the generation and the defense against ROS in cells. The best-known antioxidant role of copper is that of being the major cofactor in the CuZn–superoxide dismutase enzyme, which catalyzes the dismutation of superoxide. The generation of superoxide and hydrogen peroxide is due to the interaction of intracellular copper ions with thiols such as GSH and oxygen, which are abundant intracellularly. [23, 24] Intracellular Cu^{+} may further interact with hydroperoxides to generate hydroxyl or alkoxyl radicals in Fenton-type reactions, especially in conditions where cellular hydroperoxide concentrations are well above basal levels [25], similar to what happens in BD cases. Moreover, a rise in serum copper as an inflammatory marker may play a significant role in initiating the inflammatory process in BD patients. [2 6]In the present work, serum zinc in patients with BD was significantly lower than that of the control group. Low serum zinc was also reported by other researchers during the last decade. [3] This result is in contrast with a relatively recent study outcome which reported an insignificant difference between serum zinc levels in BD patients compared to the control group. [22]This depletion of serum zinc in BD patients is believed to be linked to the increased demand and therefore overconsumption of zinc in areas with oxidative stress due to its robust antioxidant activity, ulcer healing effect which is the hallmark in BD patients. Moreover, zinc is continuously required by the body to maintain the immune system integrity via its immunomodulatory function. Additionally, it has been proven that zinc decreases NF-κB activation and its targets, especially TNF-α and IL-1β, as well as upregulating A20 and PPAR-α genes expression which code for the two zinc finger proteins with anti-inflammatory properties. [27] The significant favorable shift of the oxidative stress parameters a month after oral zinc sulfate treatment to BD patients clearly indicates the substantial replenishment of antioxidant stores (increased serum GSH and RBC CAT activity) and the halt of the lipid peroxidation manifested by decreased serum MDA. The significant decline in serum copper level after one month of zinc sulfate treatment may be ascribed to two main reasons. Firstly, the reduced inflammatory events in BD patients, since copper is a marker for inflammation. Secondly, the fact that zinc possesses an antagonistic relation with copper; i.e. serum copper levels have been found to be high in individuals with low serum zinc levels, and hypocupremia has been observed in patients treated with high doses of zinc supplements. [25] These results point to the fact that zinc has compensated for the deficiency in the antioxidant reserve and assisted to prevent free radical-induced lipid peroxidation. The significantly increased serum zinc level in the treatment group as compared to the patient group can be chiefly accredited to the dose of zinc sulfate therapy rather than the reduction in oxidative stress status of the patients. This long-term antioxidant effect of zinc element is thought to be an essential mechanism that took part in achieving the improvement in the clinical as well as the oxidative stress parameters of BD patients after one month of oral treatment with zinc sulfate. The presence of a significant positive correlation of serum MDA and copper levels with the CMI score, oral ulcer size, number, and duration confirms the importance of the continuous reinforcement of endogenous antioxidant mechanisms to reduce the severity of the disease. When there is a worsening of clinical features, there was a concomitant increase in serum MDA level (Free radical damage marker) and a decrease in serum GSH level and erythrocyte CAT activity (endogenous antioxidants), and a decrease in serum zinc level. These results provide additional supporting evidence to the theory of oxidative stress in the pathogenesis of BD. Several points serve to rank zinc as a competing option in the treatment of BD. First, it is a natural supplement possessing a wide margin of safety enabling zinc to be used during pregnancy and lactation. In contrast to other drugs with serious and teratogenic side effects like thalidomide, methotrexate, and colchicine. Secondly, it is readily available and an inexpensive over-the-counter supplement. Thirdly, it has proven to have comparable efficacy to that of dapsone in ameliorating BD manifestations. [28]

6. CONCLUSION

It can be confirmed that BD patients suffer from oxidative stress; the intensity of which is directly proportional to the severity of clinical manifestations index score of BD. The antioxidant activity of zinc supplements is at least one of the mechanisms by which zinc is exerting its therapeutic action in the treatment of BD. This study was conducted during the early stage of the covid-19 pandemic in 2020. Therefore, the main limitation of this study was the failure to recruit an adequate number of patients during the short time of the study duration. Moreover, for the same reason, a significant number of patients failed to follow up for the second visit on time (after one month of zinc sulfate treatment).

7. ACKNOWLEDGMENT

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8. AUTHORS CONTRIBUTIONS STATEMENT

The study was designed by Assoc. Prof. Dr. Marwan Azzubaidi. The co-investigator from the Dermatology department was Prof. Dr. Khalifa Sharquie. Oxidative stress and antioxidants activities assessment was done by Prof. Dr. Rafid Najm. Statistical analyses were conducted by Assoc. Prof. Dr. Uday Younis Hussein Abdullah. All authors almost equally contributed to finalizing the article sections.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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