EFFECT OF ZINC SUPPLEMENT IN THE PROGNOSIS OF BURN PATIENTS IN IRAQ

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SUMMARY. Many studies have reported that zinc plasma levels significantly decrease after a burn, leading to zinc deficiency, and that increased free radical generation and decreased natural antioxidant may negatively affect wound healing and burn outcome in general. Targeting of these changes is considered an important strategy in the treatment of burns in an attempt to improve burn outcome in the clinical setting. Zinc was given orally in a nutritional dose (15 mg elemental zinc) as a zinc sulphate capsule to burn patients in order to improve post-burn zinc deficiency and burn outcome. The study was carried out in 58 burn patients of different age groups, sex, and occupation with different burn size. The patients were allocated to two groups: group A patients (43 in number) were treated with topical povidone-iodine ointment for the first four days post-injury followed by topical silver sulphadiazine cream 1% until discharge in addition to other prescribed drugs according to our burn unit policy; group B patients (15) received the same treatment as group A plus a single daily oral dose of zinc sulphate in a 66 mg capsule, equivalent to 15 mg elemental zinc. In each group, using standard methods, we considered plasma zinc and copper levels, oxidative stress parameters, thyroid, liver, and renal function tests, microbiological factors, mortality rate, healing time, and cost effectiveness. The administration of zinc in dietary doses significantly increased the plasma zinc level in burn patients to around normal control levels and improved the antioxidant status, as represented by elevation of the natural antioxidant level (glutathione), in addition to improving healing time, the incidence of eschar formation, and the mortality rate, compared with the zinc-nonsupplemented group. We conclude that dietary zinc supplementation in zinc-deficient burn patients led to great improvements in their outcome and that zinc deficiency was as an important goal to target during treatment; also, that the use of a combination of topical and systemic antioxidants (povidone-iodine ointment and zinc sulphate, respectively) represented a good strategy for improving results in burn patient treatment.

Introduction

It is well known that burn patients present increased nutritional requirements associated with their resultant hypermetabolic state.1 Among the nutrients required for supplementation are trace elements, which require greater attention because stress alters their level owing to alterations in intestinal absorption, in body losses, in the distribution of body proteins, and in protein concentration.2 The various trace elements have a specific role to play during healing and perform vital functions in the body. They are directly involved in free radical scavenging and are potentially important in burns. Trace element status is altered after major burns, especially during the first week post-injury, when the serum level is severely decreased.1

Burn wound healing is a complicated process that requires several steps, and any alteration at any point may delay the healing process. In cutaneous thermal injury, several factors can increase tissue damage. Among these, the oxygen free radicals are important.3 Oxidative stress contributes to secondary tissue damage and impaired immune functions after burn injury.4 As a consequence of these considerations, the targeting of changes occurring after burn injury (e.g. increase in free radical generation, decrease in natural antioxidants, immune suppression, and decreased levels of trace elements, which collectively may lead to delay in wound healing) is an important strategy in burns treatment in attempts to improve outcomes in the clinical setting. One of the famous trace elements widely used in the clinical setting for this purpose is zinc.

Zinc is an essential trace element in the body, present in all organs, tissues, and body fluids.6 Zinc is needed for more than 300 enzymes used by the body that are responsible for conducting diverse functions such as wound healing, fertility, protein synthesis, cellular reproduction, vision, immunity, free radical protection, DNA replication, and gene transcription. At the cellular level, zinc is critical for cell survival, affecting signal transduction, transcription, and replication.7 Zinc thus plays an essential role in growth, immune function, antioxidant defence, and wound healing.7

Zinc deficiency leads to many pathological signs, e.g. growth failure, neuropathy, diarrhoea, dermatitis, hair loss,
bleeding tendency, hypotension, and hypothermia. Zinc deficiency states are associated with anorexia and alterations of the epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems. Zinc deficiency has been shown to reduce osteoblastic activity, collagen, and proteoglycan synthesis, as well as alkaline phosphatase activity.

In burn patients, many studies have reported that the zinc plasma level is significantly decreased post-injury, and that this is associated with increased urinary zinc excretion. It has been reported that zinc is normally transported in plasma bound to proteins, while post-burn these proteins are broken down owing to an increased catabolic rate and tissue damage. Without proteins to bind it, zinc may be lost in the urine.

Conversely, Voruganti et al. showed that in burn patients zinc concentration in wound exudates exceeded plasma concentration, suggesting that the primary reason for the low plasma zinc level might be due to elevated loss of zinc through wound exudates. In contrast, Agay et al. showed that serum zinc level significantly decreased 6 h after burn injury owing to redistribution to the liver.

**Zinc’s antioxidant effect**

Zinc’s ability to retard oxidative processes has long been recognized. In general, the mechanism of antioxidation can be divided into acute effects and chronic effects. The chronic effects involve exposure of an organism to zinc on a long-term basis, resulting in induction of some other substance that is the ultimate antioxidant, such as the metallothioneins. Chronic zinc deprivation generally results in increased sensitivity to oxidative stress. The acute effects involve two mechanisms: protection of protein sulphydryl; 2. steric hindrance as a result of binding to other proteins. Protection of protein sulphydryl groups is thought to involve reduction of sulphydryl reactivity through one of three mechanisms: 1. direct binding of zinc to the sulphhydril; 2. steric hindrance as a result of binding to other protein site in close proximity to the sulphhydril group; 3. a conformational change from binding to other site on the protein. Antagonism of redox-active transition metals, such as iron and copper. Protection of protein sulphhydril groups is thought to involve reduction of sulphydryl reactivity through one of three mechanisms: 1. direct binding of zinc to the sulphhydril; 2. steric hindrance as a result of binding to other protein site in close proximity to the sulphhydril group; 3. a conformational change from binding to other site on the protein. Antagonism of redox-active transition metal-catalysed, site-specific reactions has led to the theory that zinc may be capable of reducing cellular injury that might have a component of site-specific oxidative damage, such as post-ischaemic tissue damage. Zinc is capable of reducing post-ischaemic injury to a variety of tissues and organs through a mechanism that may involve the antagonism of copper and iron activity.

In our study zinc was given orally in a nutritional dose (15 mg elemental zinc) as zinc sulphate salt to burn patients to improve their post-burn zinc deficiency. This may improve burn outcome as a result of its antioxidant and immunomodifier effect and its beneficial effect on wound healing.

**Patients and methods**

This study was conducted on 58 patients (27 males and 31 females) of varying age (6-67 yr) (mean, 35.6 ± 19.4, ± SD), with a burn percentage ranging from 15 to 70% estimated according to the rule of nine and a burn degree of first to third. The causes of the burns were direct flame in 45 patients (77.5%) and hot water in 13 patients (22.5%).

The patients admitted to the burn unit in the Department of Surgery in Baquba General Hospital, Diyala, Iraq over a period of six months.

The patients were allocated to two groups:

Group A: 43 patients (20 males and 23 females), treated according to the hospital’s therapeutic policy with topical povidone-iodine ointment 10% (PVP-I) for the first four days of admission followed by topical silver sulphadiazine cream 1% (SSD) until discharge day, in addition to other prescribed drugs determined by burn unit policy.

Group B: 15 patients (7 males and 8 females), treated with the same treatment as Group A, plus a single daily oral dose of a capsule containing 66 mg zinc sulphate, equivalent to 15 mg elemental zinc (which is the recommended dietary allowance). This was taken from the first day of admission until discharge day, in the morning with a cup of water.

In addition, 12 healthy subjects (5 males and 7 females), with the same age range as that of the patients, were selected to serve as control for basic comparison.

**Sample collection and preparation**

Blood samples were collected from all subjects by venipuncture. Ten millillites were taken on admission (zero time) to the burns unit and then every two days of treatment and on discharge day to check changes in the parameters studied. All blood samples were collected in a heparinized plain tube. Erythrocytes were separated by centrifugation at 3000 rpm for 10 min at 4 °C; the plasma obtained was used for biochemical analysis, which included:

1. Determination of plasma zinc and copper

For the measurement of plasma zinc levels, the plasma sample was diluted fivefold with deionized water and aspirated into an atomic absorption spectrophotometer. Calculations were performed by comparison with a standard zinc chloride solution prepared for the purpose. For copper determination, the plasma sample was diluted with an equal volume of deionized water and directly aspirated in-
to the atomic absorption instrument, and the amount of copper was calculated by comparison with a standard copper sulphate solution treated in the same manner.** Results were expressed as µg/dl.

2. Measurement of oxidative stress parameters
   - Measurement of the plasma malondialdehyde (MDA) level: MDA is a by-product of lipid peroxidation and its measurement is based on the reaction of thiobarbituric acid (TBA) with MDA, forming TBA2-MDA adducts, according to the standard method of Stocks and Dornamy.** measurement of the plasma glutathione (GSH) level: GSH contents (measured as total sulphhydryl groups) were measured according to the method of Godin et al..

3. Thyroid function test: in each subject triiodothyronine (T3) and thyroxin (T4) plasma levels were measured by enzyme immunoassay according to the methods of Utiger and Wistoni; respectively; a ready-made kit was used for the purpose (BioCheck, Burlingame).

4. Liver function test: alkaline phosphatase activity was measured colorimetrically according to the method of Kind et al., utilizing a ready-made kit for the purpose (BioCheck, Burlingame).

5. Renal function test
   - Blood urea: the determination of serum urea level was performed using the urease-modified Berthelot reaction, utilizing a ready-made kit for the purpose;
   - Serum creatinine: serum creatinine was evaluated utilizing a ready-made kit for the purpose (BioMerieux, France). Also, the activities of glutamate-pyruvate transaminase (SGPT) and glutamate-oxaloacetate transaminase (SGOT) enzymes were evaluated colorimetrically according to the method of Reitman and Wistom utilizing a ready-made kit for the purpose (RANDOX, UK).

6. Wound swabs for microbiological examination and characterization of the invading micro-organism, if present, were taken on admission and at two-day intervals during follow-up until discharge.

7. Mortality rate, healing time (reported as the time required for complete healing of the burn wound without any sign of infection), and cost of treatment were calculated on a per course of treatment basis.

8. Statistical analysis
   - Results were expressed as mean ± SD;
   - The Student t-test was used to examine the degree of significance, and a p value of less than 0.05 was considered significant.

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**Results**

As shown in Table I and Fig. 1, the plasma zinc levels in burn patients were significantly reduced (p < 0.05) compared with healthy control subjects; the lowest plasma zinc value was at zero time (time of resuscitation) in both group A and group B compared with control. In group A, plasma zinc levels increased nonsignificantly after two days, four days, and at discharge time, while in group B (the zinc-supplemented group) plasma zinc levels significantly increased (p < 0.05) four days after initiation of treatment and at discharge time, reaching a highest value that was slightly more than that of control (Fig. 1).

In contrast, plasma copper level showed nonsignificant changes in burn patients compared with control in both groups. The Cu/Zn ratio showed a 183% increase in group A burn patients at zero time, an increment that became 56% at discharge time compared with the control value, while in group B (the zinc-supplemented group) the elevation in the Cu/Zn ratio dropped from 98% at zero time to 2% at discharge time, which was the normal ratio Fig. 2.

Table I also shows the effects of treatment in group A and group B on plasma MDA levels; the end product of lipid peroxidation in group A MDA plasma levels was significantly higher (p < 0.05) than control values at zero time.

**Table I - Effects of treatment in group A and group B on plasma zinc and copper levels and oxidative stress parameters in burn patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Duration</th>
<th>Plasma Zn level (µg/dl)</th>
<th>Plasma Cu level (µg/dl)</th>
<th>Cu/Zn ratio</th>
<th>Plasma MDA (µmol)</th>
<th>Plasma GSH (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>- -</td>
<td>110 ± 5</td>
<td>172 ± 3</td>
<td>1.563</td>
<td>0.06 ± 0.02</td>
<td>0.126 ± 0.018</td>
</tr>
<tr>
<td>Group A</td>
<td>43</td>
<td>Zero time</td>
<td>44 ± 10</td>
<td>195 ± 24</td>
<td>4.431</td>
<td>0.21 ± 0.01</td>
<td>0.045 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 days</td>
<td>67 ± 26</td>
<td>171 ± 31</td>
<td>2.552</td>
<td>0.06 ± 0.02</td>
<td>0.074 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 days</td>
<td>75 ± 13</td>
<td>187 ± 21</td>
<td>2.493</td>
<td>0.055 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discharge day</td>
<td>81 ± 11</td>
<td>198 ± 18</td>
<td>2.444</td>
<td>0.05 ± 0.01</td>
<td>0.082 ± 0.02</td>
</tr>
<tr>
<td>Group B</td>
<td>15</td>
<td>Zero time</td>
<td>58 ± 16</td>
<td>180 ± 24</td>
<td>3.103</td>
<td>0.25 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 days</td>
<td>88 ± 13</td>
<td>192 ± 18</td>
<td>2.181</td>
<td>0.059 ± 0.03</td>
<td>0.092 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 days</td>
<td>106 ± 27</td>
<td>183 ± 54</td>
<td>1.726</td>
<td>0.051 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discharge day</td>
<td>119 ± 17</td>
<td>191 ± 33</td>
<td>1.605</td>
<td>0.048 ± 0.01</td>
<td>0.15 ± 0.04</td>
</tr>
</tbody>
</table>

Group A: burn patients treated with topical povidone-iodine ointment for the first 4 days after admission followed by topical silver sulphadiazine cream in addition to other prescribed drugs.

Group B: burn patients treated as in group A with the addition of single daily oral dose of 66 mg zinc sulphate (15 mg elemental zinc).

Results represent mean ± standard deviation.

Results with non-identical superscripts were considered significantly different (p < 0.05).

Zero time represents time of resuscitation.
time and decreased significantly after two days, four days, and at discharge time, when it was close to control values; the same finding occurred in group B. The other parameter that expresses oxidative stress is the plasma GSH level, which significantly decreased in burn patients compared with control, significantly increasing after two days, four days, and at discharge time in group A. In group B GSH levels increased significantly ($p < 0.05$) after two days and four days compared with pre-treatment values and increased significantly ($p < 0.05$) at discharge time compared with the four-day value in group B (Fig. 3). The GSH level correlated positively with the plasma zinc level ($r = 0.97$, $p < 0.05$).

Table II clearly shows that in the groups treated there were no significant changes with regard to thyroid hormones $T_3$ and $T_4$, blood urea, and serum creatinine, which
The results presented Table I clearly show that plasma zinc levels were significantly reduced post-burn compared with control, while plasma copper levels remain unchanged. Pochon showed that plasma zinc and copper decreased post-burn, presenting data that are compatible with our data regarding plasma zinc levels but incompatible regarding plasma copper levels, while Agay et al. showed there was an increase in plasma copper levels post-burn, which is also incompatible with our result.

Topical treatment with PVP-I for the first four days post-burn, followed by topical SSD cream in addition to other prescribed drugs according to our burn unit policy, resulted in an increase in plasma zinc levels but non-significantly led to a slight improvement in the Cu/Zn ratio, while in group B, in which zinc sulphate was administered orally to burn patients in a nutritional dose in addition to the same treatment as group A, Table I shows a significant elevation in the plasma zinc level four days after start of treatment, without any effects on the plasma copper level - there was an improvement in the Cu/Zn ratio, which returned to normal at discharge time, a result compatible is with that obtained by Li et al. Voruganti et al. showed in burn patients that zinc concentrations in wound exudates exceeded plasma concentrations, suggesting that the primary reason for low plasma zinc levels was the elevated loss of zinc through wound exudates. Al-Kaisy and Sahib found that the use of topical PVP-I in treated groups for the first four days decreased oozing and fluid loss from the skin of burn patients and also decreased fluid requirements, meaning decreased wound exudates and zinc loss, which may explain the elevation in plasma zinc levels in both groups. It has been shown that after use of topical PVP-I ointment for burned skin the first event is the release of iodine, leaving the PVP polymer alone; the second event is the uptake of water by the polymer from the wound skin, leading to swelling of the polymer and cross-linking when exposed to more water, causing the formation of a layer that is impermeable to water and prevents fluid losses from burned skin. The released iodine, which acts as a disinfectant, has the ability to oxidize sulphhydryl groups and other molecules; it reacts with sites of unsaturation in lipids and converts to iodide. At this stage the action of iodide is completely different - it now acts as an electron donor in the presence of $H_2O_2$ and peroxidase, and the remaining iodine readily iodinates tyrosine. Its action changes from antibiotic to antioxidant, which has a very important role in decreasing microvascular permeability and again fluid loss by antagonizing the reactive oxygen species (ROS) responsible for this process. ROS is widely generated in burns - the important pathway is through the xanthine oxidase enzyme, which leads to formation of $H_2O_2$.

This may also explain the reduction in MDA levels and the elevation in GSH levels in group A, indicating an improvement in oxidative stress parameters.

Zinc supplementation in group B also acted as an antioxidant with various mechanisms, which also led to de-

### Table III - Effects of treatment in group A and group B on the incidence of invading micro-organisms isolated from burn patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Duration</th>
<th>Number of negative swabs</th>
<th>Number of positive swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>43</td>
<td>Zero time</td>
<td>13 (30%)</td>
<td>30 (70%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 days</td>
<td>5 (12%)</td>
<td>38 (88%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 days</td>
<td>37 (86%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discharge</td>
<td>37 (86%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Group B</td>
<td>15</td>
<td>Zero time</td>
<td>5 (33%)</td>
<td>10 (67%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 days</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 days</td>
<td>10 (67%)</td>
<td>5 (33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discharge</td>
<td>12 (80%)</td>
<td>3 (20%)</td>
</tr>
</tbody>
</table>

**Table III** shows that the incidence of invading bacteria isolated from burn patients expressed as a percentage of the total number decreased with time in both groups. While Table IV shows that healing time decreased non-significantly ($p < 0.05$) in group B compared with group A and that the incidence of eschar formation decreased from 2.3% in group A to zero in group B, the most important result in this study is the reduction in the mortality rate from 7% in group A to zero in group B.

### Discussion

represent the renal function tests, or in the liver enzyme activities of SGOT and SGPT, except for alkaline phosphatase which showed a significant increase ($p < 0.05$) in group B (the zinc-supplemented group) after two days and four days the start of treatment, compared with pre-treatment values, and a significant increase at discharge time compared with values on day 4 day after the start of treatment (Fig. 4). It was also found that there was a positive correlation ($r = 0.98, p < 0.05$) between plasma zinc levels and serum alkaline phosphatase.

**Table III** showed that the incidence of invading bacteria isolated from burn patients expressed as a percentage of the total number decreased with time in both groups.
creased ROS and decreased microvascular permeability and fluid loss. At the same time one of the antioxidant effects of zinc is the protection of protein sulphydryl groups, considered to be a substrate for iodine to convert it to iodide and act as an antioxidant (Fig. 5).

In group B zinc acted as an antioxidant and reduced MDA levels significantly after two days, four days, and at discharge time, while GSH increased significantly after two days and four days, compared with pre-treatment, and at discharge time, while GSH increased significantly after two days; the incidence percentage decreased after four days; the incidence percentage decreased after four days and at discharge time to a nonsignificant value.

Table III presents the incidence of wound infection in the treated groups. These show the same incidence profile, as represented by a high percentage at zero time and after two days; the incidence percentage decreased after four days and at discharge time to a nonsignificant value.

Table IV shows that healing time in group B decreased nonsignificantly compared with group A, in which zinc sulphate was not administered. It was found that zinc deficiency increased the time necessary for wound closure and decreased wound strength. The role of zinc in wound healing has been investigated since the 1950s but the mechanism by which zinc affects the healing process is still not clear.

Wound healing is a complicated process that involves various cell types, structural proteins, cytokines, and ROS. In general, it presents three major stages: inflammation, proliferation, and remodelling. Inflammation is considered to be a critical stage for establishing an environment that facilitates the subsequent stages of the healing process. The initial event during this stage is triggered by ROS, and antioxidant levels have been shown to be depleted in healing cutaneous wounds, and it has also been shown that the inflammatory stage is regulated by the nuclear factor NFkB, a critical transcription factor that regulates the expression of inflammatory mediators. Hence NFkB plays a pivotal role during the inflammatory stage: NFkB activation is thought to be a critical event in early wound healing. NFkB is normally maintained in an inactive form in the cytoplasm, bound to the inhibitory protein IkB; during activation IkB is phosphorylated and degraded, facilitating the translocation of NFkB to the nucleus, where it regulates the expression of immune and inflammatory genes. It has been found that zinc deficiency reduces NFkB-binding activity in vivo and impairs its translocation, and that NFkB specifically requires zinc for optimal DNA binding. Yunsook et al. have therefore suggested that zinc deficiency may delay wound healing as a result of impaired NFkB-binding activity and that high doses of zinc have the same effect on wound healing; they believe that dietary zinc has an optimal effect on the healing process, which may explain the results presented in Table IV.

Table IV also shows that the treatment in group B (the zinc-supplemented group) reduced the incidence of eschar formation from 2.3% in group A to zero. Mauviel showed that zinc was an important co-factor for metalloproteinases, which are likely to play a significant role in the tissue remodelling that occurs after burn injury.

Finally, the results in Table IV show that the mortality rate fell from 7% in group A to zero in group B. As the primary cause of death in burns is the development of post-burn multiple organ failure, and as one of the main factors involved in the genesis and development of multiple organ failure is ROS, the use of a topical antioxidant like PVP-I...
and systemic antioxidants like oral zinc sulphate may have a great benefit in the reduction of mortality rates. The results presented in Table IV can be explained on this basis.

**Conclusion**

This study showed that burn patients had significantly lower plasma zinc levels than control and that zinc supplementation significantly increased plasma zinc levels and improved the Cu/Zn ratio in burn patients. The study also showed that zinc supplementation significantly improved the antioxidant status of burn patients, as represented by a significant elevation of the GSH level (the natural antioxidant); this improvement led to a decrease in healing time, in the incidence of eschar formation, and in the mortality rate (to zero) in burn patients, compared with the non-zinc-supplemented group, thus indicating the great benefit of using a combination of topical PVP-I and a systemic (zinc sulphate) antioxidant to improve burn outcome. The importance of regarding oxidative stress as an important goal for therapeutic targeting in burn treatment protocols is also considered.

**BIBLIOGRAPHY**
