

# Comparative study of serum zinc concentration in recurrent herpes labialis patients and healthy individuals

**Zahra Ranjbar**

Shiraz University of Medical Sciences

**Maryam Zahed** (✉ [maryamzhd@yahoo.com](mailto:maryamzhd@yahoo.com))

Shiraz University of Medical Sciences <https://orcid.org/0000-0001-5047-4018>

**Mohammad Ali Ranjbar**

Shiraz University of Medical Sciences

**Zahra Shirmardan**

Shiraz University of Medical Sciences

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## Research article

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# Abstract

**Background:** Recurrent herpes labialis (RHL) is one of the most common recurrent infective vesiculoulcerative lesions. Topical and systemic administration of Zinc compounds has been found to have preventive and therapeutic effects. The purpose of this article is to evaluate the serum level of zinc in patients with RHL and healthy individuals.

**Methods:** This cross-sectional study was performed on 43 patients with the history of recurrent herpes labialis and 42 healthy subjects with no history of the lesion. Blood samples were taken and serum zinc level was measured. Chi-Square test was used to compare the qualitative relationships and to compare the quantitative relationships independent T-test was used. To observe the relationship of quantitative factors including serum zinc level, the number of relapses and recovery rates correlation test was taken.

**Results:** The results show that serum zinc level has no significant difference in healthy subjects and patients ( $p > 0.05$ ). Also, zinc level was not related to age and sex factors and frequency of relapse ( $P > 0.05$ ). But surprisingly in the patients group, there was a significant relationship between zinc level and recovery period. As the serum zinc level was lower, the duration of recovery was significantly higher ( $p = 0.009$ ).

**Conclusion:** The results of this study indicate that zinc deficiency is a risk factor for increasing the duration of herpes labialis lesions. Therefore, the evaluation of serum zinc level in subjects with recurrent herpes labialis and subsequent administration of zinc is recommended in such patients.

## Background:

Herpes simplex virus type 1 (HSV-1) a member of the herpes virus family is the etiologic factor of a variety of diseases. It generally affects the upper parts of the human body. The primary infection appears after fluid or skin contact with contaminated individuals. The virus tends to remain in the sensory ganglions or skin epithelium after the initial infection, in most cases. Factors such as sunlight, shock, stress, fever, and hormonal changes can trigger a secondary or recurrent lesion such as herpes labialis.(1, 2)

Herpes labialis appears primarily as a vesicle in the skin area around the oral cavity, lips, nose or the oral mucosa. The vesicle erupts and turns into a painful ulcer which is followed by a crust in the subsequent days. This lesion can be very irritating and disabling due to the severe pain and unappealing appearance. (3, 4)

Common drugs such as acyclovir and valacyclovir and less common medication such as cytarabine and vidarabine are used in the treatment of viral herpes infections. Acyclovir is widely used and it is the selective treatment for herpes labialis infections. Nowadays, new treatments with fewer side effects and less toxicity are warranted for herpes simplex infections. (3, 5–7)

Zinc is an important health-related element that is present in all human body cells. It is an essential feature for the natural operation of cells, tissues and organs of the body. It leads to the enhancement and migration of keratinocytes during wound healing.(8, 9) The local use of zinc promotes the production of epithelial cells and endothelium of the vessels. The use of zinc compounds is found to enhance the local defense system, reducing the inflammation caused by bacteria.(10)

In viral infections zinc has the capacity to stimulate cellular immunity. T-lymphocytes involved in cellular immunity are important in protecting against viral, fungal and protozoic infections, as well as malignant and autoimmune diseases.(11, 12) Zinc appears to increase the number of effector and helper T-cells. Zinc deficiency has been shown to reduce host immunity by reducing circulating T-cells and decreasing the phagocytic activity of other cells, which generally affects cell-mediated immunity.(13)

The topical use of zinc solutions for the treatment and prevention of herpes simplex infections has long been established.(12, 14, 15) Furthermore, saliva zinc deficiency and its role in the recurrence of this viral infection have also been investigated.(16) It is shown that salivary zinc is significantly lower in patients suffering from recurrent herpes labialis compared to healthy individuals. (16) But serum levels have not been investigated previously in these patients.

In view of the fact that serum zinc concentration is currently the best and most widely used biomarker of zinc status in populations,(17) we decided to check this marker in herpes simplex patients to further evaluate the effect of this element in the prevention and treatment of this recurrent debilitating lesion.

## **Methods:**

This cross-sectional study was performed on 85 subjects including 43 patients with the history of recurrent herpes labialis and 42 healthy individuals with no history of the disease who were referred to the Oral and Maxillofacial department of Shiraz dental school in the year 2018. All subjects were over the age of 18. The cases were asked to visit an oral and maxillofacial specialist in less than 48 hours after the onset of a new lesion to be precisely diagnosed as recurrent herpetic lesions. They were included in the study if they experienced recurrent lesions more than three times a year. A control group of healthy subjects who were free of any history of similar lesions throughout their life were randomly selected. They were similar in age and gender compared to the case group. Exclusion criteria for both groups included pregnancy, a history of systemic and immunological disease that affects the activity of the immune system and the use of drugs or any supplements.

At the beginning of the study, an informed consent form was obtained from the subjects. Also, a form containing biographical information including age, sex, history of any certain disease, medications and supplements, as well as the rate of relapse of the lesions in a year and the duration of the recovery period of the last lesion experienced was obtained. The patients were referred to the Nursing Station for blood sampling. All blood samples were taken in the morning and were carried out fast to the laboratory of Hafez hospital in Shiraz. Measuring serum zinc level was performed according to standards. The results of the serum zinc level measurements were announced by the laboratory to the patients and our

colleagues. The study was approved by the ethical committee of Shiraz University of Medical Sciences. (IR.SUMS.REC.1397.449)

The results were statistically analyzed using SPSS software version 18. Chi-Square test was used to compare the qualitative factors. To compare the quantitative relationships independent T-test was used. To observe the relationship between quantitative factors including: serum zinc level, number of relapses, and lesion improvement a Correlation test was taken.

## Results:

Of the participants in the study, 67 were women (78%) and 18 were men (22%). The mean age of individuals having herpes lesions and healthy subjects was 37.28 and 36.17, respectively. There was no statistically significant difference between the two groups in terms of gender and sex. (Tables 1 and 2)

For comparison of serum zinc level, independent t-test was used. There was no significant difference in serum zinc level in the case and control group ( $p = 0.313$ ). (Table 3)

Moreover, zinc level was not significantly different in regard to age of the patients, gender and the number of recurrences in a year. ( $p = 0.953$ ,  $p = 0.336$ ,  $p = 0.123$ ). However, a significant relationship between the amount of zinc and the duration of the last recovery period was seen. The higher the serum zinc level, the less the duration of the period ( $p = 0.009$ ). (Table 4)

Table 1  
Number and percentage of gender difference in case and control groups

	Gender		P value* (Student T-test)
	Men (Number Percentage)	Women (Number Percentage)	
Case	10 23.3%	33 76.7%	0.635
Control	8 19%	34 81%	

\* P value < 0.05 is considered significant.

Table 2  
The mean age of groups

	<b>Mean age</b>	<b>Std.Deviation</b>	<b>Sig.(2-tailed)</b>
Case	37.28	9.192	0.636
Control	36.17	12.237	
* P value < 0.05 is considered significant.			

Table 3  
Average of Serum zinc level in case and control groups

	<b>Serum zinc average</b>	<b>Std.Deviation</b>	<b>Sig.(2-tailed)</b>
Case	74.74	14.017	0.313
Control	71.86	12.139	
* P value < 0.05 is considered significant.			

Table 4  
The relationship between serum zinc level and relapse rate in case group

<b>N = 43</b>	<b>Pearson correlation</b>	<b>Sig.(2-tailed)</b>
Duration of lesions	-0.391	0.009
Recurrence rate	-0.009	0.953

\* P value < 0.05 is considered significant.

## Discussion:

The results of the present study show that although serum zinc level of recurrent herpes labialis patients was lower than healthy individuals, but this comparison was not significant. What is interesting is that the duration of lesions was significantly higher in patients with low serum zinc levels. And also the recurrence rate in the past year was lower in patients with higher zinc levels.

Why this element may have an effect on reducing the recovery time of this lesion or reducing the recurrence of herpes disease may be due to its various effects in the human body. Zinc is an essential element for the natural function of cells, tissues and organs of the body. As a factor, it leads to the enhancement and migration of keratinocytes during wound healing. Moreover, Zinc has many roles in metabolism. Zinc is involved in the metabolism of nucleic acids and proteins and in the processes of cell differentiation and proliferation. Zinc ions stimulate lymphocyte DNA synthesis in a few days, and about

10–40% of lymphocytes become lymphoblast. Additionally, unsaturated zinc-8 hydroxyquinoline complexes in animals stimulate lymphocyte synthesis.(18, 19)

Moreover, in relation to host immunity, Zinc has the capacity to safely control viruses by stimulating cellular immunity. T-Lymphocyte response is a cellular immune based immunity (CMI). CMI is important in protecting against viral, fungal and protozoic infections, as well as against malignant and autoimmune diseases. Zinc appears to increase the number of effector and helper T-cells, either the precursors of the antibody-forming cells or the increased activity of suppressive cells.(9, 13, 20) In a study conducted by Barman et al., Zinc deficiency has been shown to reduce host immunity by reducing circulating T cells and decreasing the phagocyte activity of other cells, which generally affects cell-mediated immunity.(21)

Although zinc deficiency may have many effects, but clinical evaluation of this deficiency is not easy, because the signs and symptoms are non-specific. Signs associated with zinc deficiency include: decreased plasma zinc levels ( $< 70 \mu\text{g} / \text{dL}$ ), decreased serum alkaline phosphatase, retinal alcohol in the retina (leading to night blindness) and decreased plasma testosterone levels, T- lymphocyte dysfunction, decreased collagen synthesis (resulting in poor wound healing), and decreased RNA polymerase activity in different tissues. (8, 17)

Recently, the role of zinc compounds in the prevention and control of many diseases and abnormalities of humans and animals has been considered. The use of zinc compounds in order to strengthen the local defense system, reduce the inflammation of the bacteria and produce epithelial cells and endothelium vessels in the repair of foot injuries has been proven. Many studies indicate that administration of systemic or topical zinc helps reduce the recovery rate of RHL.(10–12, 14, 19)

In a study conducted by Khozeimeh et al., in 2012, salivary zinc was compared between people with recurrent herpes labialis in both conditions of disease and recovery (21 days after the improvement of lesions of Herpes) and healthy people. The result showed that zinc level was significantly different, in disease state compared to recovery stage and recovery compared to healthy subjects. In this study, there was no significant correlation between zinc levels and age of patients, sex, recurrence rate and duration of healing.(16) However, in our study, serum zinc levels in subjects with the history of herpetic lesions were less than healthy individuals, but this difference was not significant. One of the reasons for this difference is that saliva is probably not an exact criterion for measuring the amount of zinc in the body. As it is shown in a study conducted by Freeland-Graves and colleagues, comparing whole saliva and salivary sediment after the administration of a low zinc diet (3.2 mg / day)in 12 women for 22 days. The zinc level of saliva and salivary sediment (centrifuged saliva) were measured before and after the start of the study. The results were that zinc saliva levels remained constant throughout the study, while zinc levels in salivary sediment significantly increased 22 days after the administration. As a result, complete saliva is by no means an acceptable benchmark for the evaluation of zinc intake.(22)

It is noteworthy that in several studies it has been determined that serum zinc is the most widely used biomarker for assessing the status of zinc in the population. Serum zinc concentration indicates recent consumption of zinc or regular zinc intake, which means that in populations with low zinc diet, the serum

zinc level is lower, indicating high zinc deficiency risk. These studies showed that the best time to measure serum zinc was whilst fasting in morning.(17, 23)

In another article, published by Femiano et al. in 2005, they examined the effect of zinc on RHL. In this paper, 20 patients (including 12 women and 8 men) with an average age of 26.6 with a history of RHL more than 6 times per year and a recovery period of more than 8–14 days were treated with zinc sulfate 22.5 mg twice daily for 4 months. Patients were followed for 12 months. The study concluded that herpes ulcers decreased by less than 4 times a year and the recovery period were reduced to less than 7 days at each relapse. As a result, this study showed that systemic zinc sulfate reduced the relapse rate and the period of RHL. This study also found that people with lower serum zinc had a longer recovery period, as well as a higher rate of relapse in a year. However, our study did not address the treatment of such individuals.(24)

In a study conducted by Brody et al. they used a zinc sulfate solution on the site of herpes lesions on the skin and the oral mucosa (used on the skin at a concentration of 0.05 – 0.025% and on the mucus at a concentration of 0.01–0.025%). This study also showed that treatment with this solution on the skin prevents post-herpetic erythema multiform in addition to its herpes preventive role.(25)

In a laboratory study conducted by Max Arens et al., they investigated the effects of different concentrations of different zinc salts (zinc acetate, zinc lactate, zinc sulfate, zinc gluconate) on isolated HSV virus in culture medium. The results showed that isolated HSV, in laboratory conditions, is effectively inactivated by treatment with Zinc salts, and the degree of inactivation of the virus depends on the type of HSV, zinc concentration and treatment length.(26)

In a study published by Antoine and colleagues in 2016, ZOTEN (zinc oxide tetrapod nanoparticle) was used as an intravaginal vaccine in female rats. The ability to inhibit HSV-2 by ZOTEN reduced clinical symptoms and decreased rat death. ZOTEN inhibited recurrent infection with increasing T cell-mediated and antibody-mediated response. Overall, the effect of the vaginal microbial vaccine against primary and secondary vaginal herpes virus infection was determined. (27)

In the case of other viral disease and body minerals, in a study conducted by Okwara et al. 51 adult patients with HIV viral infection and 48 healthy people were selected. Selenium, zinc and magnesium were measured. All the minerals were lower in HIV-infected individuals compared to healthy subjects. The difference in the type of viral disease and the difference in sample size may be due to the differences in the results of the current study with this study.(28)

It is worthy to note that due to the fact that herpes is a common and self-limiting disease, usually referral to a dermatologist or dentist is rare, therefore patient selection was somehow difficult. Also encouraging the patients for blood sampling was not easy. Further studies with larger sample sizes are suggested. It is also suggested that other viral diseases, should be checked in regard to zinc level. It is reasonable to compare saliva and serum simultaneously. Moreover comparison of plasma zinc levels both disease and recovery state can be beneficial.

## Conclusion:

The results of this study indicate that zinc deficiency can be a risk factor for increasing the duration of recovery period of herpes labialis. Therefore, the evaluation of serum zinc level in individuals with the history of recurrent herpes labialis and subsequent administration of zinc might be beneficial. Further studies are warranted to evaluate this issue profoundly.

## Declarations

*Ethics approval and consent to participate:* This study was approved by the ethical committee of Shiraz University of Medical Sciences. (IR.SUMS.REC.1397.449) All patients signed an informed consent form.

*Consent for publication:* Not applicable.

*Availability of data and materials:* The data that support the findings of this study are available upon reasonable request from the corresponding author. But restrictions apply to the public availability of these data, because of patient confidentiality.

*Competing interests:* The authors declare that they have no competing interests.

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*Authors' contributions:* MZ, ZR and MAR designed this work; ZSH was responsible for patient selection and blood acquisition; MZ, ZR and ZSH have drafted the work; all authors have approved the submitted version.

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