

Original Article

Comparative analysis of salivary zinc level in recurrent herpes labialis

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ABSTRACT

Background: Recurrent Herpes Labialis (RHL) is one of most common infective vesiculoulcerative lesions. according to some studies administration of topical and/or systemic zinc compositions has been effective in treatment and prevention. This article aims to comparison of zinc level in healthy subjects and RHL patients in acute and convalescent phases.

Materials and Methods: This was a retrospective case – control study, carried on 80 individuals (40 normal and 40 RHL patients) mean age=34.5 and 34.4, respectively. Saliva samples were taken in patients in acute phase once and after healing of lesions in convalescent phase (averagely 21 days later) and in normal individuals. Salivary zinc level concentration was measured by flame atomic absorption spectrophotometer by dry digestion method. The results were statistically analyzed with SPSS software by *t*-test ($\alpha=0.05$).

Results: Results showed that salivary zinc level in case group in acute and convalescent phases were 160.8 ng/ml and 205.7 ng/ml respectively and significant differences between them were existed ($P<0.05$). Also significant differences were existed between zinc concentration in healthy subjects and patient groups (in both phases) ($P=.001$ and $.002$ for acute and convalescent phases respectively).

Conclusion: According to the results, zinc level is significantly lower in acute phase than in convalescent phase and significantly lower in both phases compared to healthy individuals, so determination of serum zinc level and prescribing zinc complement in low serum status has both treatmental and preventive effects in RHL patients.

Key Words: Acute phase, convalescent phase, recurrent herpes labialis, zinc level

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INTRODUCTION

Infection with herpes simplex virus (a DNA virus) is the most prevalent infective disease of human after viral respiratory infections.^[1,2]

There are two major types of virus (HSV₁ and HSV₂). Classically, HSV1 causes a majority of oral and pharyngeal infection, meningo-encephalitis and dermatitis above the waist while HSV2 is implicated in most genital infections.^[1] After the initial infection,

which is often an acute stomatitis, the causal microorganism, herpes simplex virus (HSV) type 1, remains dormant in the trigeminal ganglion and may be reactivated by stress, fever, upper respiratory tract infections, ultraviolet light, trauma and immune incompetence, but the condition is variable and unpredictable in most patients.^[3] Recurrent herpes infection of the mouth [Recurrent herpes labialis (RHL)] occurs in patients who have experienced a previous herpes simplex infection.^[1]

Many patients with RHL experience several episodes of recurrence during a year so the disease is an annoyance for the majority of patients and it can be disabling for patients with severe frequent lesions for example immunocompromised individuals.^[1] These factors necessitate treatment of the lesion as they can cause interference with eating and speaking. Acyclovir, the principal antiherpes drug, has been

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shown to be effective in treatment of HSV infections, but may have toxicity or side effects and can lead to viral resistance.^[3]

Consequently, a safer and more effective method to prevent, eliminate or substantially reduce recurrences should be considered. Recently the effectiveness and preventive role of zinc components in many animals and human abnormalities and diseases has been considered.^[3,4] Zinc has several roles in metabolism. There are more than 100 zinc metalloenzymes, including a large number of nicotinamide adenine dinucleotide (NADH) dehydrogenases, RNA and DNA polymerases, and DNA transcription factors as well as alkaline phosphatase, superoxide dismutase and carbonic anhydrase.^[3] Zinc is involved in nucleic acid and protein metabolism and in processes of cell differentiation and replication.^[4] Zinc deficiency can have several effects but clinical assessment of mild zinc deficiency is difficult because many of the signs and symptoms are non-specific. Acrodermatitis enteropathica is a more severe childhood form of zinc deficiency, which manifests with periorificial (oral, anal, genital) and acral dermatitis, diarrhea, behavioral and mental changes, neurological disturbances and secondary bacterial and fungal infections, though it has not been reported to predispose to herpetic lesions.^[3] biochemical signs associated with zinc deficiency include decreased levels of plasma zinc level ($<70 \mu\text{g/dl}$), decreased serum level of alkaline phosphatase, alcohol dehydrogenase in the retina (which accounts for night blindness), and decreased plasma testosterone as well as impaired T-lymphocyte function, decreased collagen synthesis (resulting in poor wound healing), and decreased RNA polymerase activity in several tissues.^[3] The zinc has capacity for the safe control of viruses by cell-mediated immunity stimulation. The T-cell lymphocyte response is the basis of cellular-mediated immunity (CMI). The CMI is important in protection against virus, fungal and protozoan infections, as well as against malignant and autoimmune disease.^[3] Zinc appears to increase number of helper or effector T-cells, or precursors of antibody forming cells or increased suppressor cell activity. Zinc ions stimulate lymphocyte DNA synthesis within a few days and approximately 10-40% of lymphocytes are transformed into lymphoblasts. Additionally zinc-8 hydroxyquinoline unsaturated complexes are stimulatory to lymphocyte mitosis in animals and at least two mechanisms appear to exist for zinc to stimulate lymphocytes in

animal models.^[3] Many studies suggest that systemic and/or topical zinc administration appeared to reduce both the number of episodes and the time of recovery of herpes labialis.^[3,5-9]

According to the relative ease with which saliva can be collected compared with the relative invasive method of getting blood samples and noting that saliva is a potential indicator of concentration of many blood components, and along with the relationship between salivary zinc concentrations and dietary intakes reported in the literature^[10-12] while there is not still any documented research that has measured the zinc level in patients with RHL and comparison of it with healthy subjects. The aim of this study was to measure salivary zinc concentration in acute and convalescent phases of RHL in patients with history of at least three episodes per year in comparison with healthy individuals.

MATERIALS AND METHODS

This case-control retrospective study was done between three groups. The first group (study group) consisted of 40 individuals (22 female and 18 male, mean age=34.4, std. deviation=14.52) who had clinically documented herpes labialis at the examination time of ≤ 48 hours duration and history of at least 3 episodes of recurrence each year without any history of immunological disease. The excluding criteria was: having any history of disease that could affect immune function of the body and having history of RHL less than three times a year.

The second group are of the same individuals in group 1 but 21 days later, at the convalescent phase of disease.

The third group (control group) consisted of 40 healthy subjects (24 female and 16 male, mean age=34.5, std. deviation=13.85) with no history of any systemic and immunological disease with immune competency, that are match in sex and age and nutritional habits (for example times of eating milk and fish meals in week) with study group. All subjects were informed of the purpose and informed consent was obtained from all the individuals and local ethical committee approval that was in accordance with the last update of Helsinki declaration was obtained.

Saliva collection

10 cc of whole unstimulated saliva was collected in sterile test tubes from each person between 9.00 and 12.00 am. All subjects were requested to avoid eating

and drinking 2 hours before sampling. Saliva was collected using spitting method (saliva ejection for 5 minutes was collected), then each tube was frozen at -18°C until sending to biochemistry laboratory. Another salivary collection was done only in patient group 21 days later in convalescent phase after which lesions had completely healed. There after 120 saliva samples were sent to biochemistry lab at one time to avoid the problems of several setting up of the unit. Samples were analyzed using the dry digestion method by flame atomic absorption spectrophotometer according to following step:

- Transporting the saliva samples to porcelain circulates and dry in 105°C for 24 hours.
- Heating the samples in 450 cc in electrical furnace 14 hours.
- Dissolving the burnings in 0.5 ml of nitric acid (7 molar) and diluted up to 10 ml with pure water.
- Measurement of zinc level by direct aspiration into spectrophotometer using the hollow multielemental cathodic lamp.

Reliability of analyze was tested with comparison with cow-liver standard test of international institute of standard and technology. Finally results were analyzed in each 3 groups by SPSS statistically software using paired *t*-test ($P < 0.05$).

RESULTS

Study subjects consisted of 18 men and 22 women (mean age=34.4 years, std. deviation=14.52) and healthy subjects consisted of 16 men and 24 women (mean age 34.5 years with std. deviation=13.85)

There were 120 salivary samples that 40 were from healthy persons and 40 from patients at acute phase and 40 from same patients in convalescent phase. Acute phase is the time that patient has lesions with symptoms such itching, burning, crusting and ...

Convalescent phase is 21 days after beginning of lesion after lesions completely healed but viral shedding in saliva may be continued.

Duration of lesions in study group was at least 4 up to 15 days and mean was 9.2 days (std. deviation=2.84).

Number of recurrence each year was at least 3 up to 10 and most prevalence was three times of recurrence and mean was 4.8 (std. deviation=1.65)

Results of salivary zinc measurements in three groups are shown in [Table 1]. According to Table 1 there is significant difference between salivary zinc levels

Table 1: Mean salivary zinc concentration (ng/ml) in patients in acute and convalescent phases and safe group

Study groups	N	Salivary zinc concentration (ng/ml)	STD. deviation
Patients in acute phase	40	160.8	136.06
Patients in convalescent phase	40	205.7	149.03
Safe group	40	317.5	161.83

in acute and convalescent phases of disease in patient group ($P < 0.05$) and between both of phases and healthy group too ($P < 0.05$).

Analyzes of the relation between salivary zinc levels in acute and convalescent phases and other questioned parameters (time of recurrence per year, duration of disease, age and sex, revealed no significant differences between zinc level and any of this parameters. ($P > 0.05$ in all comparisons).

DISCUSSION

Herpes labialis is one of the most prevalent and clinically obvious viral disease and recurrence is a common characteristic and many patients experience frequent, large, painful or disfiguring lesions so the disease is bothering for patients and interfere with their social activity and can produce many psychological problems.^[1]

Studies have suggested several mechanisms for reactivation of latent HSV, including low serum IgA,^[13] decreased cell-mediated immunity^[14] and depression of antibody-dependent cell cytotoxicity (ADCC).^[15] Although antiviral agents, including Acyclovir, may be effective against recurrent herpes labialis, they may have many side effects and can promote viral resistance.^[1,3] On the other hand many studies had emphasized the effects of zinc components in treatment of RHL.^[3,5-9]

According to many investigations, zinc is associated with taste and smelling power and wound healing. It is essential to the integrity of the immune system as it plays a role in stabilization of cell membranes.^[3,4] the zinc has potential in safe control of viruses by cell mediated immunity stimulation,^[3] thus its deficiency may associated with many abnormalities. The purpose of this study was determination and comparison of salivary zinc level in patients with RHL in both acute and convalescent phase of disease and between the two phases of disease and healthy subjects.

According to the results of this study there is a significant differences between the mean value of salivary zinc level in acute phase of disease (160.8) and convalescent phase (205.7) ($P=0.001$) [Table 1]. Significant increase of zinc level during convalescent phase of RHL may reveal the reparative role of zinc in mucosal lesions. Some biochemical signs associated with zinc deficiency include decreased collagen synthesis (resulting in poor wound healing) and decreased RNA polymerase activity in several tissues.^[3]

Also this finding can confirm previous researches finding about the effects of topical and systemic compositions of zinc in shortening the duration of disease and fastening the healing process of lesions. There was significant differences between mean concentration of salivary zinc levels in patients in both phases (160.8 and 205.7, respectively) and healthy individuals (317.5), demonstrated that zinc level in healthy subjects is about two times more than its level in acute phase and 1.5 times more than its level in convalescent phase.

These results emphasized the important role of zinc as a preventive and reparative agent in mucocutaneous lesions. Thus in cases of zinc deficiency, administration of zinc may have significant therapeutic and prophylactic effects in many diseases such as RAS,^[16-23] RHL,^[3,5-9] periodontal diseases as indicated in many studies.^[3-9,23]

CONCLUSIONS

The results of this study suggest that zinc deficiency is a potential risk factor for recurrent herpes labialis. So prescribing zinc complement if zinc level is low in patients with RHL has both therapeutic and preventive effects and is recommended.

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