

Plasma neutrophil elastase in children with recurrent aphthous stomatitis

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Abstract

The pathogenesis of recurrent aphthous stomatitis (RAS) is poorly understood. The neutrophil elastase is implicated in tissue damage occurring in the course of several chronic inflammatory conditions. This strong proteolytic enzyme may be also a sensitive indicator of inflammation.

EDTA-treated blood was obtained from 12 patients with the acute stage of minor RAS (aged from 1.5 to 16 years, 7 girls and 5 boys) and 15 healthy children. The plasma concentration of neutrophil elastase in complex with alpha₁-proteinase inhibitor (NE- α_1 PI) was measured by ELISA methods.

All RAS patients had significantly ($p < 0.00001$) higher levels of NE- α_1 PI (av. 377.96 ± 306.52 ; median: $226.19 \mu\text{g/L}$) compared to the control group (av. 55.90 ± 20.31 ; median: $57.53 \mu\text{g/L}$). Our data suggest that NE- α_1 PI may be a useful indicator of neutrophils activation in RAS patients and may be also involved in pathogenesis of this disease. These observations could have implications for development of new therapeutic strategies in the future.

Key words: children, recurrent aphthous stomatitis, neutrophil elastase

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Abbreviations: RAS – recurrent aphthous stomatitis, N – neutrophils, NE – neutrophil elastase, NE- α_1 PI – neutrophil elastase complexed with α_1 -proteinase inhibitor, ELISA – enzyme-linked immunosorbent assay

and chronic response and neutrophils activation in different diseases.

The aim of the study was to determine the concentration of plasma NE- α_1 PI in children in the acute period of RAS.

Introduction

Recurrent aphthous stomatitis (RAS) is a common and very painful ulcerative condition of the mouth. The disease has a chronic course with periodic exacerbations. Pathogenesis of RAS is not fully understood. According to some authors immunological and neutrophil abnormalities may be involved in the etiology and pathophysiology of RAS [1]. RAS may be a big clinical and therapeutic problem. It may be the only sign of the onset stage of many systemic diseases. Several indicators have been evaluated in the diagnosis of inflammatory stage, and include various leukocyte indices and acute-phase proteins. The examination of plasma levels of NE- α_1 PI can be used as a novel and sensitive indicator of the acute

Material and methods

Subjects

For this study the blood of 12 children with the acute stage of minor form RAS was obtained. The subject: 7 girls (58%) and 5 boys (42%) at the age of 1.5 to 16 years (median: 12 years) were patients of the Clinic of Immunology, Medical University of Wrocław. None of these patients had a history of any additional acute or chronic disorders. Medications for patients included only topical nonsteroidal anti-inflammatory drugs. Healthy children ($n=15$), aged from 1.5-12.5 years (median: 6 years), without chronic illness in the anamnesis were studied as a control group.

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Assesment of elastase complexed with α_1 -proteinase inhibitor

Plasma NE- α_1 PI was measured by means of reagents manufactured by Merck, Germany. The assessment was carried out by ELISA method.

Statistical analysis

The obtained results were subjected to statistical analysis using the nonparametric Wald-Wolfowitz test. The level of statistical significance was assumed to be $p < 0.05$.

Results

As shown in Figure 1, NE- α_1 PI was significantly increased ($p < 0.00001$), in plasma of all RAS patients compared to control group. In 10 of ill children NE- α_1 PI concentration was above 150 $\mu\text{g/L}$, and in 5 cases above 200 $\mu\text{g/L}$ (RAS: interquartile range (25-75%): 155.06-638.48 $\mu\text{g/L}$; control: interquartile range (25-75%); 38.08-68.18 $\mu\text{g/L}$).

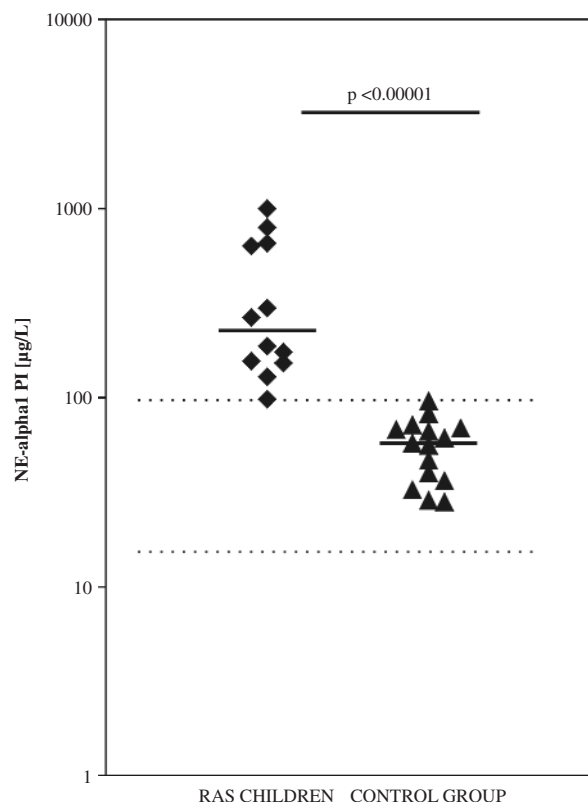


Fig. 1. Plasma levels of NE- α_1 PI in children with RAS and control group. The solid short lines designate median values in each group. The dashed lines indicate 95% confidence intervals determined for control group

Discussion

Many of studies indicate different causes of RAS e.g. haematologic and nutritional deficiencies, food allergies, immune disorders, stress. There is also evidence indicating for certain abnormalities of neutrophils in RAS concerning both their count and function. Neutrophils (N) not only provide a principal means in defense against bacterial or fungal infection, but also have been implicated in the pathogenesis of non-infectious inflammatory diseases. Neutrophilic inflammation is a characteristic feature e.g. in acute and chronic lung diseases, Sweet's syndrome [2, 3].

The granules of N contain the strong proteolytic enzyme – elastase (NE). During degranulation NE is released into intracellular space where together with oxygen radical and other enzymes, such as cathepsin G, myeloperoxidase, lactoferrin, inactivates foreign phagocytized substances. After excessive stimulation N release enzymes and oxidizing substances outside the cell. Free NE perpetuates the cycle of inflammation by promoting the generation of chemoattractants particularly interleukin-8 and leukotriene B4, which recruit more N into the tissue. In the extracellular environment free NE is rapidly bound and inactivated mainly by α_1 -proteinase inhibitor (α_1 -PI). Degranulation of large numbers of N causes the release of so large amounts of NE that it can't be neutralized by local inhibitors. The extent of tissue injury depends on the balance between the enzymes and its inhibitor. NE can destroy e.g. elastin, collagen, immunoglobulins, clotting factors and proteinase inhibitors [2]. NE may degrade cadherins – components of endothelial basement membrane [4] and contribute to microvascular injury, which facilitates the migration of not only the cells taking part in the inflammation but also some harmful factors. The value of free NE and NE- α_1 PI may be useful as an indicator of local and systemic inflammation. The concentration of the NE- α_1 PI correlates with the released free elastase. Schroeder et al. [5] demonstrated in the biopsy specimens neutrophil infiltrates in the ulcers margins. The function of N performed by Sisti et al. [1] show that RAS patients had significantly lower spontaneous migration and ingestion values in the acute period. Also ingestion ability of salivary N in patients with acute RAS was reduced [6]. These disorders may contribute to the recurrence of the aphths. Another study demonstrated that in patients with minor RAS no significant differences in chemotaxis and spontaneous migration of N in blood were found [7]. On the other hand the increased reactive oxygen intermediates and increased expression of CD11b on resting and fMLP-stimulated N in RAS patient [8], and increased adherence of stimulated N were demonstrated [9]. The excessive activation of N and action of NE, as shown in the present study, may initiate immunopathological changes that lead to RAS.

Conclusions

Our data suggest that the stimulation status of N in inflamed tissue in RAS patients can be estimated by measuring the plasma concentration of NE- α_1 PI. These observations could have implications for development of new therapeutic strategies in the future.

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