Salivary lysozyme levels in patients with primary immunodeficiencies

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ABSTRACT

Background: Lysozyme is a muramidase that acts on the peptideoglycan wall of Gram positive bacteria, causing cell death. It plays part in innate immunity and is present in blood, external fluid, as well in lysossomal granules of the phagocytes. Primary Immunodeficiencies are a diverse group of illnesses that, as a result of abnormalities of the immune system, increase susceptibility to infection. Among the examples of impaired natural immunity are defects in phagocytes and in the complement system. Innate immunity could be important in protecting mucosas against infections in patients with different forms of primary immunodeficiencies. The aim of this study was to investigate lysozyme concentrations in saliva from patients with primary immunodeficiencies.

Methods: Lysozyme levels in saliva samples from 34 patients with primary immunodeficiency (30 children and adolescents between the age of 3-13 years and 4 adults between the age of 20-33) and 60 age-matched healthy controls (49 children and adolescents between the ages of 3-15 and 11 adults between the ages of 22-42) were determined by the lysoplate method.

Results: There was no statistically significant difference between the lysozyme concentrations in the saliva of the immunodeficient subjects and those of the healthy controls.

Conclusion: The results in the present work clearly show that salivary lysozyme levels in primary immunodeficient patients are equivalent to those found in healthy controls, suggesting that this enzyme still represents a remaining (but not a compensatory mechanism), contributing to the protection of there patients against infections.

Key words: Primary immunodeficiency. Lysozyme. Saliva. Compensatory mechanism. Mucosal immunity.

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RESUMEN

Introducción: La lisozima es una muramidasa que actua sobre la pared de peptidoglicano de las bacterias Gram-positivas, provocando la muerte celular. Desempeña un papel en la inmunidad innata y está presente en la sangre, secreciones externas y en los gránulos de los fagocitos. Las inmunodeficiencias primarias constituyen un grupo diverso de enfermedades que, como consecuencia de las anormalidades en el sistema inmune, presentan un aumento de susceptibilidad a las infecciones. Ejemplos de deficiencias de la inmunidad natural son los defectos de los fagocitos y del sistema de complemento. La inmunidad innata puede ser importante para la protección

contra las infecciones de las mucosas en los pacientes con diferentes tipos de inmunodeficiencia primaria. El objetivo de este estudio es investigar la concentración de lisozima en la saliva en pacientes con inmunodeficiencia primaria.

Métodos: Los niveles de lisozima en las muestras de saliva de 34 pacientes con inmunodeficiencia primaria (30 niños y adolecentes con edad entre 3-13 años y 4 adultos con edad entre 20-33 años) y 60 controles sanos de la misma edad (49 niños y adolescentes con edad entre 3-15 años y 11 adultos con edad entre 22-42 años) fueron determinados por el método *lysoplate*.

Resultados: No se observó una diferencia estadística significativa entre las concentraciones de lisozima en la saliva de los individuos inmunodeficientes y de los controles sanos.

Conclusión: Los resultados del presente trabajo claramente indican que los niveles de lisozima en la saliva de los pacientes con inmunodeficiencias primarias son equivalentes a los de los controles sanos, sugiriendo que este enzima representa un mecanismo remanescente, pero no compensador, que contribuye a la protección contra las infecciones en estos pacientes.

Palabras clave: Inmunodeficiencias primarias. Lisozima. Saliva. Mecanismo compensador. Inmunidad de las mucosas.

INTRODUCTION

Defense against microbes is mediated by the early reactions of innate immunity and the later response of adaptative immunity. Adaptative immunity is stimulated by exposure to infectious agents, which make the body capable of recognizing and reacting to a larger number of microbial and nonmicrobial substances. Innate or natural immunity consists of cellular and biochemical defense mechanisms that are in place even before infection and poised to respond rapidly to infection. These mechanisms react only to microbes and not to noninfectious substances, and they respond in essentially the same way to repeated infections. The principal components of innate immunity are physical and chemical barriers, phagocytic cells and NK cells, blood proteins - including members of the complement system and other mediators of inflammation, like lactoferrin and lysozyme.

Lysozyme is a 1-4-β-N acetylmuramidase, which acts on the peptideoglycan layer of Gram positive bacterial wall, causing cell lysis. It's a soluble endoglycosidase present in blood, in external fluids as well in the lysossomal granules of the phagocytes[1,2]. Lysozyme is also present in human milk throughout lactation in a concentration of 100 μg/ml, and unlike other factors, its concentration increases towards the end of lactation. Lysozyme is produced by monocytes, neutrophils, Paneth cells (in the intestinal tract)[3] and salivary glands[4]. In phylogeny, lysozyme is highly conserved, being present right through from plants to mammals[5].

Primary immunodeficiency disorders are a diverse group of illnesses that, as a result of abnormalities in the immune system, increases susceptibility to infection[6]. However, little is known about mucosal innate soluble factors that contribut to the host defense of these patients. Synergism between lysozyme, IgA and the complement system has been reported to occur in microorganism lysis, but the biological role of lysozyme is not yet fully known[7]. In the present work, we investigated the lysozyme concentrations in saliva from patients with primary immunodeficiencies to verify if lysozyme might act as a compensatory mechanism for the mucosal protection of these patients.

MATERIAL AND METHODS

Thirty saliva samples were collected from children and adolescents aged between 3 and 13 years and 4 from adults aged between 20 and 33 years at Instituto da Criança of Hospital das Clínicas and Escola Paulista de Medicina, São Paulo, Brazil.

The deficiency diagnosis was: X-linked Agammaglobulinemia (8 cases), Ataxia Telangiectasia Syndrome (5 cases), Common Variable Immunodeficiency (6 cases), Polysaccharide Antibody Deficiency –which represent normal serum levels of immunoglobulins and IgG subclasses, good antibody response to proteic antigens and severely impaired antibody response to polysaccharide antigens– (4 cases), Hyper-IgM Syndrome (2 cases), Chronic Granulomatous Disease (2 cases), IgG2 Deficiency and Down Syndrome (1 case), Silver-Russel Syndrome with Common Variable Immunodeficiency (1 case), Chédiak Higashi Syndrome (1 case), Selective IgA Deficiency (1 case), Hyper IgE Syndrome (1 case), and Agammaglobulinemia with partial deletion of chromosome 2 (1 case).

Saliva samples were also collected from 49 healthy 3-15 year-old children and adolescents and from 11 of 20-42 year-old adults who were used as controls. The lysoplate method was utilied to determine the lysozyme concentrations in saliva samples. This test is based on the agar plate diffusion

method first described by Osserman & Lawlor (1966)[7]. To prepare the agar plate, a 10 mg amount of *Micrococcus lysodeikticus* (Sigma, M 3770, USA) was suspended in 10 ml of phosphate buffer (PB), and was added to a 10 ml solution of 2 % agarose, also diluted in PB, pH 6.2. Wells were made in the agarose to place the standard curve and saliva samples. For the construction of the standard curve, egg lysozyme (Sigma, L-6876,USA) was diluted to 1 mg/ml in PB pH 6.2 and the fresh saliva samples were centrifuged at 23.15 g for 10 min, the pellet was discarded and the supernatant was utilized. To quantify the lysozyme content of saliva, 5 μg/ml of each saliva sample was transferred to each well. The plates were incubated 18 h at 24 °C and cleared zone ring diameters of lysis were measured. A standard curve was constructed by relating the egg lysozyme concentration and the cleared zone ring diameter of lysis. The values were plotted on the graph to determine the lysozyme concentration of the saliva samples. Graphpad Prism program was utilized to calculate these results.

Statistical analysis was carried out using variance analysis with three factors. It was performed with 95 % confidence limits and a probability value $p < 0,05$ would be considered as significant.

RESULTS

No difference was found between the lysozyme levels in saliva of the immunodeficient patients and lysozyme concentrations in the healthy controls (table I), regardless of age, sex and types of primary immunodeficiency (fig. 1). Variance analysis revealed that comparison among the groups failed to reach significance (age, $p = 0.136$; sex, $p = 0.205$; group, $p = 0.663$; age versus sex, $p = 0.508$; age versus group, $p = 0.941$ and sex versus group, $p = 0.592$).

DISCUSSION

Innate immunity is increasingly recognized as crucial for the resistance to infection. Lysozyme could represent a contributory factor to mucosal immunity and to protection against Gram-positive bacterium in patients with severely impaired immune response[8].

In this paper, it is demonstrated that salivary lysozyme levels in patients with several primary immunodeficiencies are equivalent to those of healthy age-matched controls, including one patient with Chédiak-Higashi Syndrome, who was previously described as having low activity of this enzyme inside the macrophage[9]. Similar results have been reported by Kirstila et al (1994), who demonstrated that in 15 patients with Common Variable Immunodeficiency (CVI), lysozyme and other innate immunity substances such as lactoferrin, salivary peroxidase, myeloperoxidase, hypothiocyanite, thiocyanate and agglutinins are equivalent to those found in healthy controls. Those authors suggest that some of these agents do, in fact, seem to be produced in higher amounts to compensate the low antibody titers. It is possible that the unspecific factors form a "backup system" in patients with CVI, in accordance with findings in human infants who are still physiologically immature with respect to immunoglobulins but not as regards to lysozyme, total peroxidase activity or hypothiocyanite[10]. It was also described that concentration of salivary lysozyme in HIV patients with oropharyngeal candidiasis was higher than the HIV

Table I

Salivary lysozyme concentrations (μg/ml) in immunodeficient patient and healthy control groups

	Children/adolescents		Adults	
	ID Mean ± SD (n)	N Mean ± SD (n)	ID Mean ± SD (n)	N Mean ± SD (n)
Male	27.2 ± 9.4 (20)	26.9 ± 11.6 (32)	35.0 ± 12.25 (4)	34.0 ± 15.0 (3)
Female	26.6 ± 4.0 (10)	23.4 ± 12.5 (17)	*	25.0 ± 7.0 (7)

*Saliva sample from immunodeficient females weren't available.
ID: immunodeficients; N: healthy controls; SD: standard deviation; n: number of samples tested.

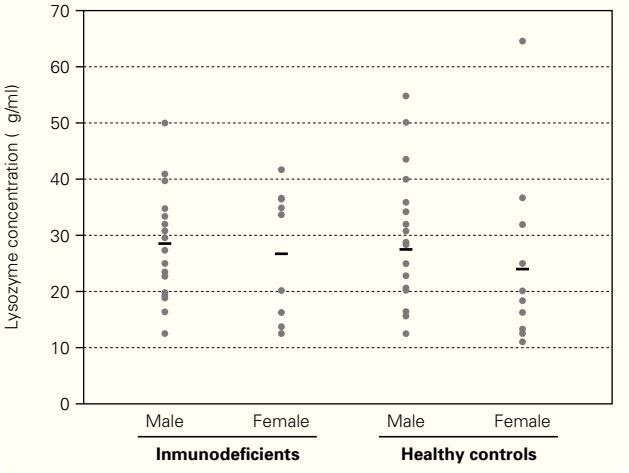

Figure 1.—Salivary lysozyme concentrations in male and female immunodeficient patients and in age-matched male and female healthy controls – indicates mean values.

ones without oropharyngeal candidiasis or the healthy control group[11].

The results of the present work clearly show that salivary lysozyme levels in primary immunodeficient patients are equivalent to those found in healthy controls, suggesting that this enzyme still represents a remaining (but not compensatory) mechanism, contributing to the protection of these patients against infections.

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REFERENCES

1. Ribeiro MA. Determinação dos níveis de lisozima, IgM total e proteína C-Reativa em líquido cefalorraquidiano como parâmetros auxiliares no diagnóstico diferencial das meningites. Tese de mestrado-ICB-USP, Brazil 1990. Department of Immunology: Universidade de São Paulo, Brazil; 1990.
2. Jenzano JW, Hogan SL, Lundbland RL. Factors Influencing Measurement of Human Salivary Lysozyme and Turbidimetric Assays. J Clin Microbiol. 1986;24:963-7.
3. Rubio CA. Colorectal adenomas produce lysozyme. Rev Anticancer Res. 2003;23:5165-71.
4. Miyzaki T, Fujiki T, Inoue Y, Takano K. Imunoelectron microscopis identification of lysozyme- expressing cells in human labial salivary glands. Arch Histol Cytol. 1998;61:199-214.
5. Jollés P. A possible physiological function of lysozyme. Biomédicine. 1976;25:275-6.
6. Abbas AK, Lichtmah AH, eds. Congenital and Acquired Immunodeficiencies. Cellular and Molecular Immunology. 5th ed. Philadelphia: W.B. Saunders Company; 2003. p. 453-76.
7. Porto MHO, Ribeiro MA, Carvanha-Neto M, Franco Jr JG, Zugaib M, Carneiro-Sampaio MMS. Amniotic fluid lysozyme activity in fetal distress. Brazilian J Med Biol Res. 1990:23:403-8.
8. Osserman EF, Lawlor DP. Serum and urinary lysozime (muramidase) in monocytic and monomyelocytic leukemia. J Exp Med. 1966;124:921-52.
9. Goldsby RA, Kindt TL, Osborne BA, eds. Aids e outras imunodeficiências. Kuby Imunologia. 4th ed. Rio de Janeiro: Revinter; 2002. p. 467-96.
10. Conley ME, Stiehm ER. Immmunodeficiency disorders: general considerations. In: Stiehm ER, ed. Immunology Disorders in Infants e Children. 4th ed. Philadelphia: W.B. Saunders Company; 1996. p. 202-52.
11. Kirstila V, Tenovuo J, Ruuskanen O, Nikoskelainen J, Irjala K, Vilja P. Salivary defense factors and oral health in patients with common variable immunodeficiency. Clin Immunol. 1994;14:229-36.
12. Bard E, Laibe S, Clair S, Biichle S, Millon L, Drobacheff C, et al. Nonspecific secretory immunity in HIV-infected patients with oral candidiasis. Acquir Immune Defic Syndr. 2002;31:276-84.