Human β-Defensin-3 In Plasma of Egyptian Asthmatic Children

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Abstract: Defensin are antimicrobial peptide components of the innate immune system. According to structural features at the gene and protein levels there are three subfamilies, α-defensin, β-defensin, and θ-defensin; they are produced in a tissue-restricted manner in response to microbial products or pro-inflammatory cytokines. Recent studies have demonstrated that defensin is also able to modulate inflammatory responses, to stimulate adaptive immunity and contribute to tissue repair. Neutrophil defensins, originally identified as broad-spectrum antimicrobial peptides, have been implicated in the regulation of inflammatory and immunological processes. AIM: To estimate plasma level of human β-defensin-3 in asthmatic children and its relation to disease severity. METHODS: The concentrations of Human β-definsin-3 (HβD-3) in the plasma from 26 patients with moderate asthma and 16 normal children were measured by radioimmunoassay. Results: Increased plasma concentrations of HβD-3, was found in patients with moderate asthma compared with control subjects with highly positive correlation with pulmonary score. Conclusion: these findings will help to elucidate the role of HβD-3 in host immune responses and identify the pathophysiological significance of this molecule in bronchial asthma.
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1. Introduction

The integrity of the airway epithelium is an important prerequisite for an efficient host defense system. As has been observed in various inflammatory lung diseases, epithelial injury is followed by a repair process that serves to restore epithelial integrity[1]. During this process, inflammatory cells such as neutrophils are recruited to the site of injury, where they are believed to contribute to host defense, injury, and the repair process itself[2,3]. The airway epithelium forms a continuous barrier against potentially harmful inhaled agents. In response to epithelial injury, it is essential that a repair process is initiated that comprises subsequent epithelial cell migration, proliferation, and differentiation[4]. In inflammatory lung diseases such as asthma and chronic bronchitis, epithelial cell injury is observed[5]. Various studies have shown that activation of the epidermal growth factor (EGF) receptor following ligand binding may play an important role in epithelial repair processes by inducing cell migration, proliferation, and differentiation[6].

Human Defensins (HD) are small, arginine-rich cationic peptides that contain 6 highly conserved cysteine residues, forming a compact looped structure. Human Defensins are divided into α- , ß- and theta defensins families depending on the position of the cysteine residues that participate in disulphide linkages[7](fig.1). They are produced in a tissue-restricted manner in response to microbial products or pro-inflammatory cytokines[8,9]. Defensins released by stimulated neutrophils are members of the α-defensins subfamily and are stored in large amounts in the azurophil granules[10]; these α-defensins are also known as human neutrophil peptides 1-4 [HNP1-4]. Neutrophil defensins, which were originally identified as broad-spectrum antimicrobial peptides, have been implicated in the regulation of inflammatory and immunological processes. HBD-3 protein shows antimicrobial activity against both Gram-negative and Gram-positive bacteria[11].

The aim of the present study was to estimate plasma level of human β-defensin-3 in asthmatic children and its relation to disease severity.

2. Patient and methods:

The present study was conducted on 42 subjects divided into two groups; Asthmatic group 26 children aged from 6 to 13 years with acute moderate asthma and control group 16 healthy children with the same age and sex. Patients were chosen from Allergy and Immunology Outpatient Clinic, Abu-Elreish Children Hospital, Faculty of Medicine, Cairo University. Diagnosis of Asthma among the studied cases were based on careful history, physical examination as well as a documented reversible and variable airflow obstruction as describes in GINA, 2008[12]. Non of the participants had a respiratory tract infection in the last

6 weeks prior to the study. All of the studied children were subjected to full history taking, thorough clinical examination and measurements of β-defensin-3. Assessment of severity of acute attacks upon enrollment to the study was done according to pulmonary score as described by Smith et al, 2002[13] (Table 1). Measuring lung function using spirometry upon enrollment in the study. Legal consents were taken from their parents. Measurements of β-defensine-3 was measured using the enzyme-Linked immunosorbent assay (ELIZA) Kit according to method by Schroder et al, (1999)[14].

Statistical analysis:

Data are expressed as mean ± SD. The data were analyzed by t-test for equality of means, using SPSS software. A probability value of less than 0.05 was considered statistically significant.

3. Results:

Table (2) shows age and sex distribution of studied children, where there are no statistical difference in age and sex distribution between both groups.

Table (3) shows data of the clinical examination of children and results of pulmonary score, where the mean value of pulmonary score was 4.6 ±1.1 (moderate asthma).

Table (4) and Figure (2) show the concentration of β-defensin-3 in plasma where the mean± (SD) plasma concentration of β-defensin-3 in patients with acute moderate asthma (1.8808±0.62102 ng/ml) was significantly different from that in normal control subjects (0.4825±0.22347 ng/ml). The plasma concentration of β-defensin-3 was 3.9 folds higher in patients with acute moderate asthma than in normal subjects which was highly significant ($p < 0.01$).

Correlation between plasma concentration of β-defensin-3 in asthmatic patients and their pulmonary score showed a highly positive correlation ($r = 0.0685$) (figure 3).

Table (1) Classification of acute asthma attacks according to pulmonary score for children > 6 years

Pulmonary score of each sign	Respiratory rate	Accessory muscle use	Wheezing
0	< 20	no apparent increase	none
1	21 - 35	mild increase	End of expiration with stethoscope
2	36 - 50	increased	entire expiration with stethoscope
3	> 50	maximal increase	inspiration and expiration with stethoscope

*A total pulmonary score (3 – 7) denote moderate acute attack of bronchial asthma.

Table (2) Data for age and sex of studied children

Groups	Number	Mean age ±SD (years)	Sex		P- value
			Male	Female	
Asthmatic	26	9.47 ± 1.634	13 (50%)	13 (50%)	1.00
Control	16	9.95 ± 2.743	10 (62.5%)	6 (37.5%)	1.00
Total	42	9.71 ± 2.188	23 (54.3%)	19 (45.2%)	-

Table (3) Data of the clinical examination of children

Group	Respiratory rate/ m.			Accessory muscle use			Wheezes				HR	Pulm Score
	<20	21-35	>35	no use	mild inc.	Mod inc.	N.	Term. exp.	Entir. exp.	Inspi. & exp.	mean ± SD	mean ± SD
Asthm.	0	14	12	0	12	14	0	2	11	13	85.7± 3.8	4.6 ± 1.1
Control	7	9	0	0	0	0	0	0	0	0	84.1 ± 4.6	–

Table (4) Concentration of β-defensin-3 in plasma of studied children

Groups	Number	Mean	St.deviation	St. Error of mean
Asthmatic	26	1.8808	0.62102	0.12173
Control	16	0.4825	0.22347	0.05587

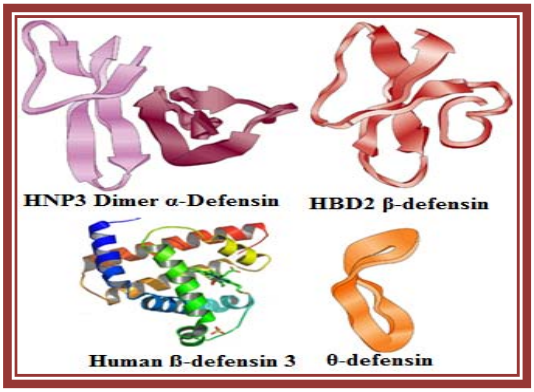

Fig.(1) Human defensins

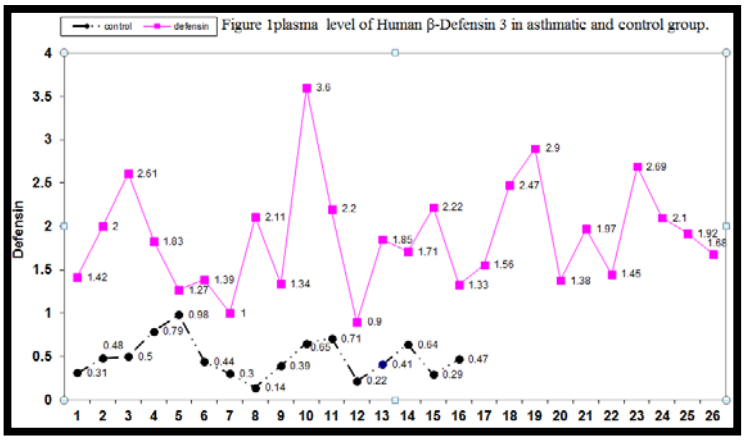

Figure (2) Plasma concentration level of Human β-defensin-3 in asthmatic and control groups.

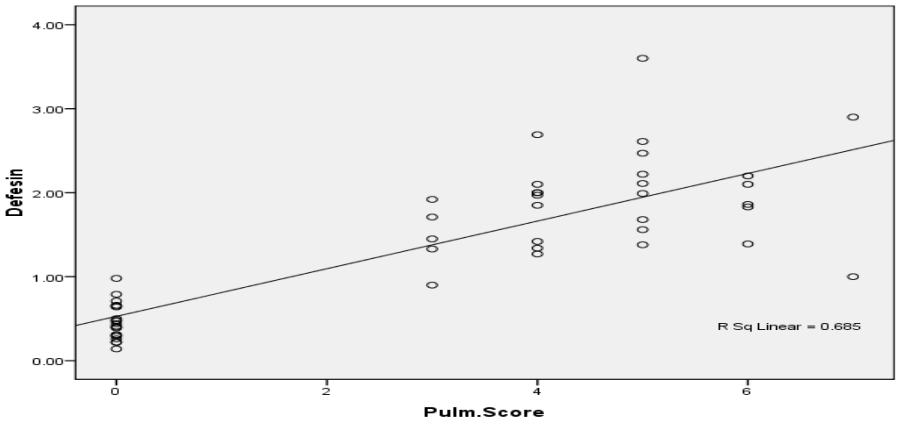

Fig. (3) Correlation between β-defensin-3 in plasma of asthmatic children and their pulmonary score

4. Discussion:

Bronchial asthma is a worldwide problem with 300 million affected individuals. Over the years, the percentage of children suffering from asthma increased significantly. Significant effort needs to be invested in the area of host immune response and the pathophysiology of bronchial asthma[15].

Acute and chronic bronchial inflammations are thought to be central to the pathogenesis of several lung disorders such as asthma. The specific nature of the inflammatory response is determined by the recruitment and activation of immune cells in lungs. These activated cells produce cytokines, oxidants, and many other mediators which are involved in inflammation. Different stimuli, such as allergens and infections, have been shown to induce bronchial inflammation[16].

Host defense against infection involves a multitude of factors and cells that together form the elements of innate and acquired immunity. A substantial number of studies have been focused on defensins, a family of low molecular weight, multifunctional cationic peptides that interact and disrupt microbial membranes and have been identified in plants, animals and humans[17].

In our study, there is highly significant increase in β-defensin-3 in plasma of children with acute moderate asthma when compared to control. The plasma concentration of β-defensin-3 was 3.9 folds higher in patients with acute moderate asthma.

To our knowledge, this is the first time to estimate β-defensin-3 in plasma of asthmatic children. Vega et al, (2011)[16], found that neutrophils from allergic patients release α-defensins via an allergic-dependent mechanism and concluded that α-defensins may contribute to inflammatory process in bronchial asthma.

It has been demonstrated that infections may cause exacerbation of chronic airway disease such as asthma[18,19]. Studies involving patients with allergic rhinitis have demonstrated increase responsiveness to allergens in individuals with common cold symptoms[20,21]. These exacerbation may be in part due to the release of defensins by neutrophils both nonspecifically and specifically with increased responsiveness to allergens.

Jamil el al, (2002)[22], founded that neutrophil defensins enhance proliferation of cells from the airway epithelial cell lines A549 and NCI-H292. Also Ishimoto et al, (2006)[23], found that serum concentration of human plasma β-defensin-3 in patients with bacterial pneumonia increased during the acute phase and normalizing after treatment with antibiotics. Although the mechanism of biosynthesis and release of endogenous human β-defensin-3 peptide are not well understand the release of human β-defensin-3 peptide in the bronchial and bronchiolar epithelia and the change in serum human β-defensin-3 concentrations during antibiotic therapy for bronchial pneumonia suggest that serum human β-defensin-3 might be secreted by bronchial and bronchiolar cells in bacterial pneumonia.

In our results, we found also a highly positive correlation between plasma concentration of human β-defensin-3 in asthmatic children and their pulmonary score indicating that human β-defensin-3 increases with asthma severity.

Conclusion:

Neutrophils from asthmatic patients release β-defensin-3 via an allergen-dependent mechanism which may contribute to the inflammatory processes and its severity in asthma, suggest that defensins may be a marker of neutrophil activity in bronchial asthma.

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