Identification of Saliva Biomarkers for Asthma by Proteomic Analysis

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ABSTRACT

Poor and inconsistent diagnosis of asthma is one main reason for the burgeoning cases of death from asthma. On top of that, the nature of current diagnosis methods being invasive and unpleasant limits their applicability. Hence the identification of biomarkers to non-invasively and conveniently detect asthma will facilitate interventions to prevent disease progression. Whole saliva may be analysed for the diagnosis of asthma, since it can be readily collected and contains identifiable serum constituents. The purpose of this study was to characterize the human salivary proteome in asthmatics of different severity to identify potential biomarkers. By using proteomic approaches such as 2D gel electrophoresis and Bioplex. We found 11 potential candidate markers whose levels were significantly different. These 11 proteins include Cystatin S, Cystatin SA, Cystatin SN, Cystatin SA-III precursor, Cystatin D precursor, Calgranulin B, Zn-Alpha2-glycoprotein, Prolactin-Induced Protein, Alpha Amylase, IL-1 beta and IL-8. These techniques identified candidates that may prove to be of diagnostic significance. This is followed by investigation of individual samples via Dotblotting where we have discovered proteins (Calgranulin B, Lactoferrin and Complement 4) with diagnostic potential, all having AUC of 0.8 and above. Further large scale and quantitative work have to be done to conclusively conclude their diagnostic utility for clinical use.

INTRODUCTION

The world-wide estimate of people with asthma is three hundred million. Still, global burden is projected to increase by another 100 million cases by 2025. Poor diagnosis of asthma has been put forward as a potential reason for delays in obtaining treatment, which may contribute to increasing severity and deaths from asthma. In view of the shortcoming of current diagnosis methods, we propose the use of saliva. Therefore, in this study, I have two main objectives. Firstly, to identify potential candidate and diagnostic markers for asthma in whole saliva (defined as an ROC of >0.7 at p<0.05). The second objective of this project is to understand whether is there a difference in the whole saliva protein profile for asthma at different levels of control. Hence throughout the analysis, patients were stratified according to their level of control following the Global Initiative for Asthma (GINA) guidelines. Finally, all findings in this project can also add to the knowledge on the mechanism of inflammation in asthma and saliva as a biological fluid.

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MATERIALS AND METHODS

Unstimulated saliva was collected from National University Hospital. Population was stratified along the GINA guidelines. Experimental technique such as Bioplex was used to screen a total of 12 cytokines. Besides the cytokine base technique, other general techniques were employed. 2D-gel electrophoresis-Mass Spectrometry was performed to plot out the gel protein profile whole saliva between asthmatics and non-asthmatics, where a comparative analysis will be done. Dotblotting was performed to analyze the levels of these proteins in individual samples. Multiple complementary techniques were performed in hope to identify candidates that may prove to be of diagnostic and therapeutic significance. Mann-Whitney U test and ROC were used to evaluate markers.

RESULTS

Bioplex

IL-2, IL-12(p70), IFN-gamma, GM-CSF, TNF-alpha, IL-6 and IL-13 were detected at low levels or not detected. IL-1 beta and IL-8 may be a potential candidate cytokine biomarker to differentiate asthma cases according to its severity.

2D Gel Electrophoresis

The Mann-Whitney analysis revealed a significant difference in protein markers. We identified nine potential candidate markers (Down-regulated in A&E asthmatics- Alpha Amylase; Up-regulated in A&E asthmatics- Cystatin S, Cystatin SA, Cystatin SN, Cystatin SA-III precursor, Cystatin D precursor, Calgranulin B, Zn-alpha 2-glycoprotein and Prolactin-Induced Protein.

Dotblotting

Calgranulin B and Lactoferrin were significantly greater in amount (intensity) in A&E asthmatics. Their AUC was 0.833 and 0.801 respectively. On the contrary C4 was significantly lower in A&E having an AUC of 0.826. All differences were statistically significant with p<0.05.

DISCUSSION

Multiple approaches have been proven to be able to justify the accuracy between techniques and aims to be non-exhaustible in identifying more markers. Some markers were already discovered by other scientists; such as alpha amylase. It was believed that asthmatics having high level of stress would activate Hypothalamus-Pituitary-Adrenal axis, in doing so, it lowers sympathetic activity resulting in lowered salivary alpha amylase levels.

The other markers were first found in this study. All forms of cystatins were up-regulated in A&E asthmatics’ saliva. Cystatins is involved in the protection of cells from inappropriate proteolysis by regulating cysteine proteinases. During asthma inflammation, apoptosis would trigger the release of cysteine proteinases. In response, the body will produce more cystatins in hope to prevent excessive protein degradation. IL1-beta and IL-8 were suggested to have a role in the development of exaggerated obstruction in asthmatic airways. They behave as pro-inflammatory mediators in asthma. According to literatures, I hypothesised that Calgranulin B
and Lactoferrin may work together in bringing about airway inflammation. Calgranulin B being up-regulated, when released into the extracellular space would activate the expression of cells such as monocytes, neutrophils and macrophages. Supporting Calgranulin B’s function, Lactoferrin is released into the blood where it modulates the migration, maturation and function of these immune cells. From this we can see that asthma disease mechanism is complex and there may be many processes yet to be discovered. Nevertheless, this study has allowed us to identify markers capable of diagnosing acute exacerbation using saliva.

REFERENCES


