INVESTIGATION OF POTENTIAL BIOMARKERS FOR LYMPHANGIOLEIOMYOMATOSIS (LAM) IN BLOOD

A thesis submitted in partial fulfilment of the degree of Master of Science (MSc) by Research

by

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Ayesha Javed declare that this thesis is submitted in fulfilment of the requirements for the award of Master of Science by Research, in the School of Life Sciences and Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research was supported by Australian Government Research Training Scheme and the LAM Australia Research Alliance.

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Date: 21-01-2019

Acknowledgements

I would first like to thank my thesis supervisor Dr Najah Nassif of the School of Life Sciences and Faculty of Science at the University of Technology Sydney. She was always available to answer any query pertaining to my research project and was very supportive. She consistently steered me in the right direction whenever she thought I needed it.

I would also like to thank the experts who taught me various techniques, procedures and provided guidance: Associate Professor Sarah Lal and Professor Fatima Shad, without their passionate participation and input I would not have been able to accomplish my goals in this project.

I would also like to acknowledge Lisa Windon for her support in completing tasks, providing encouragement and guidance and I am gratefully indebted to her for her very valuable suggestions.

Finally, I would like to express very profound gratitude to my parents and to my husband Naveed Ahmad for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

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ABSTRACT

LAM is a rare multisystem disease primarily involving lungs. There is an average lag time of 4 years from the onset of symptoms till the diagnosis is made. Current methods of diagnosis are invasive and associated with a lot of limitations. Clinically, interstitial lung disease (ILD) resembles LAM and there are no methods available that are less invasive, and yield quicker results, in order to differentiate the two. The aims of this study are to identify potential biomarkers for LAM diagnosis and also determine its clinical severity just by using a simple blood test.

This pilot study was conducted to investigate and validate the differential expression of 10 proteins in the blood of patients suffering from lymphangioleiomyomatosis (LAM) as compared to interstitial lung disease (ILD) (idiopathic pulmonary fibrosis) and normal healthy subjects in order to determine if any of the proteins could serve as a potential biomarker for the early diagnosis of LAM. The 10 proteins selected for study were lipopolysaccharide binding protein (LBP), Fetuin-B, apolipoprotein A1 (APO A1), apolipoprotein A4 (APO A4), heparin cofactor 2, phospholipid transfer protein (PLTP), plasma serine protease inhibitor 5 (SERPIN A5 gene product), N-acetylmuramoyl-L-alanine amidase (PGLYRP2), sex hormone binding globulin (SHBG) and Talin 1. These proteins were selected on the basis of a proteomics study (LC/MS) which showed altered expression levels (either increased or decreased) of these proteins in sera from LAM patients, as compared to normal controls.

Validation of differential expression of the selected protein targets was carried out using either ELISA or western analysis using sera from LAM patients (n=10), ILD patients (n=10) and normal age matched individuals (n=10). Blood samples were obtained from and a blood biobank

established. The expression of each of the 10 candidate proteins was validated in all three sample groups and data obtained was analysed to establish the statistical significance of any detected protein level alterations.

The validation experiments carried out showed that Fetuin-B was significantly decreased in LAM patient sera as compared to ILD patients ($p=0.023$). Heparin cofactor 2 was significantly decreased in LAM patients as compared to normal healthy individuals ($p=0.030$). PGLYRP2 was significantly reduced in LAM as compared to ILD patients ($p=0.00047$) and normal healthy human subjects ($p=0.0061$). Further, clinical severity of disease (ILD and LAM) was also correlated with the protein level differences observed. Correlation analysis showed that, in LAM, levels of heparin cofactor 2, PGLYRP2 and Fetuin-B decrease with increasing severity of the disease. In ILD, levels of APO A4 were shown to be decreased with increasing disease severity in ILD, while plasma serine protease inhibitor levels were increased with the increasing disease severity.

This study data identified some novel potential biomarkers for diagnosing LAM by differentiating it from other similar diseases and normal individuals. These include Fetuin-B, Heparin cofactor 2 and PGLYRP2. Additionally, some potential protein biomarkers of severity were also identified for LAM and ILD. These potential biomarkers may, in future be used for diagnostic purposes in LAM and also for determining stage/severity of LAM and ILD.