Elevated Expression Levels of Lung Complement Anaphylatoxin, Neutrophil Chemoattractant Chemokine IL-8, and RANTES in MERS-CoV-Infected Patients: Predictive Biomarkers for Disease Severity and Mortality

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Background

The Middle East Respiratory Syndrome coronavirus (MERS-CoV) has caused considerable medical and health issues in many countries, particularly in Saudi Arabia. The prevalence of MERS-CoV in the Kingdom of Saudi Arabia (KSA) remains significant. MERS-CoV cases are still being reported in Saudi Arabia, and a high prevalence of MERS-CoV in dromedary camels and direct contact with infected camels have been linked to human infections [1,2,3]. An excessive inflammatory response is a prominent phenotype associated with MERS-CoV infection, which leads to lung immunopathology, disease progression, and poor clinical outcome. MERS-CoV infections are characterized by dysregulation in both the innate and adaptive immune systems [5, 6]. Cross-talk between the complement and coagulation systems plays a crucial role in vascular endothelial damage and thromboinflammation [4]. Over-activation of pulmonary and systemic complement plays a key role in inflammation, endothelial cell damage, thrombus formation, and intravascular coagulation, which results in multiple organ failure and eventually death [5, 6, 7]. This over-stimulation leads to the formation of the complement anaphylatoxins, C3a and C5a. C5a is a chemoattractant for neutrophils, monocytes, eosinophils, and T cells [6]. Following infection, complement anaphylatoxins stimulate phagocytic cells and enhance the production of TNF-α, IL-1β, IL-6, IL-8, granular enzymes, and free radicals. These mediators promote vascular dysfunction, fibrinolysis, and microvascular thrombosis formation [4, 6, 7]. The role of complement in MERS-CoV disease immunopathology and complement-modulating treatment strategies during MERS-CoV infection has received limited attention. Little is known regarding pulmonary complement activation during MERS-CoV infection, the manner in which complement activation affects disease severity or the association of complement response with viral load and mortality. Thus, we performed a comprehensive investigation of the pulmonary complement proteins, IL-8 (CXCL8) and RANTES (CCL5) expression in MERS-CoV-infected patients in addition to viral load determination. We also assessed the correlation between these factors and the fatality rate. To our knowledge, this is the first study demonstrating a relationship between lung complement proteins and complement regulatory factors in MERS-CoV-infected patients

Methods

Patient Selection, Sample Collection, and Preparation and Analysis:

A total of 31 MERS-CoV-positive patients and 15 MERS-CoV non-infected group were enrolled in this study. Lower respiratory samples (bronchoalveolar lavage (BAL) or tracheal aspirate (TA)) were collected. The mean time from the onset of symptoms to hospital arrival, seeking medical attention, and hospital admission was 4.3 days.

MERS-CoV Viral Load Detection:

The MERS-CoV viral loads of the lower respiratory samples were detected by real-time RT-PCR after viral RNA was extracted using QIAamp Mini kit (Qiagen) according to the manufacturer's instructions.

Measurement of Complement Inflammatory Mediators Anaphylatoxins (C3a/C3b and C5a), C1q, C2 and Regulatory Complement Component (factor) Levels:

Pulmonary levels of human complement C3a, C5a, C3b, C1q and C2, as well as

factors P (properdin), I, C4-binding protein (C4-BP), and H, were measured using ELISA assays.

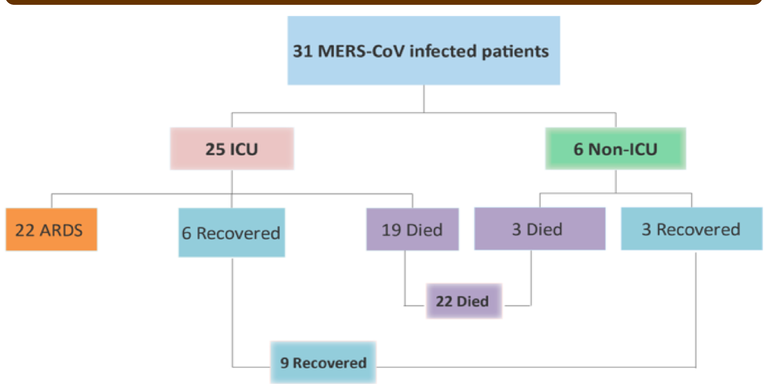
Measurement of Pulmonary Pro-Inflammatory Cytokine and Chemokine Profiles using ELISArray:

The concentrations of major human pro-inflammatory cytokines and chemokines were measured in the respiratory samples of 30 MERS-CoV-infected patients and 18 MERS-CoV non-infected individuals using the multi-analyte ELISArray (Qiagen, Germantown, MD, USA) following the manufacturer's protocol.

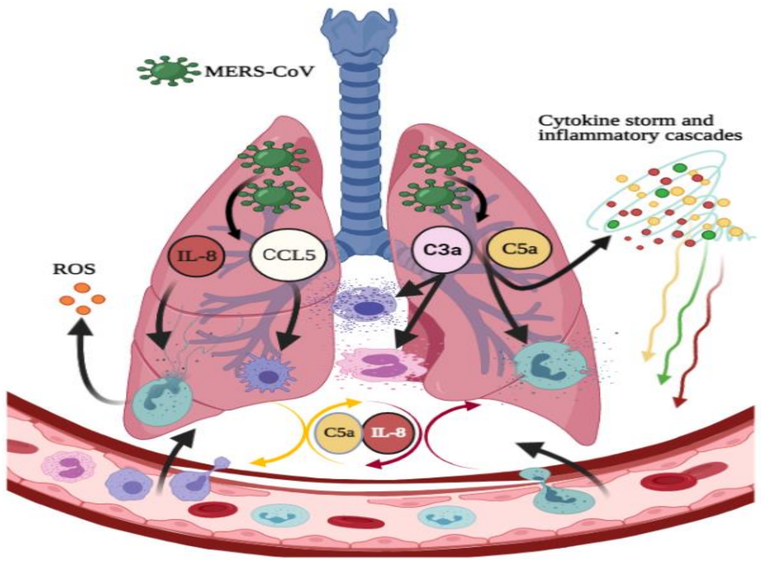
Protein–protein Interaction (PPI) Network Construction and Identification of Hub Proteins:

To further explore the potential interplay among the differentially expressed proteins (C3a, C5a, factor P, IL-8, and RANTES) in the lung of MERS-CoV infected patients with the potential interactors. Protein–protein interaction networks were constructed using bioinformatics resources of the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING version 11.0).

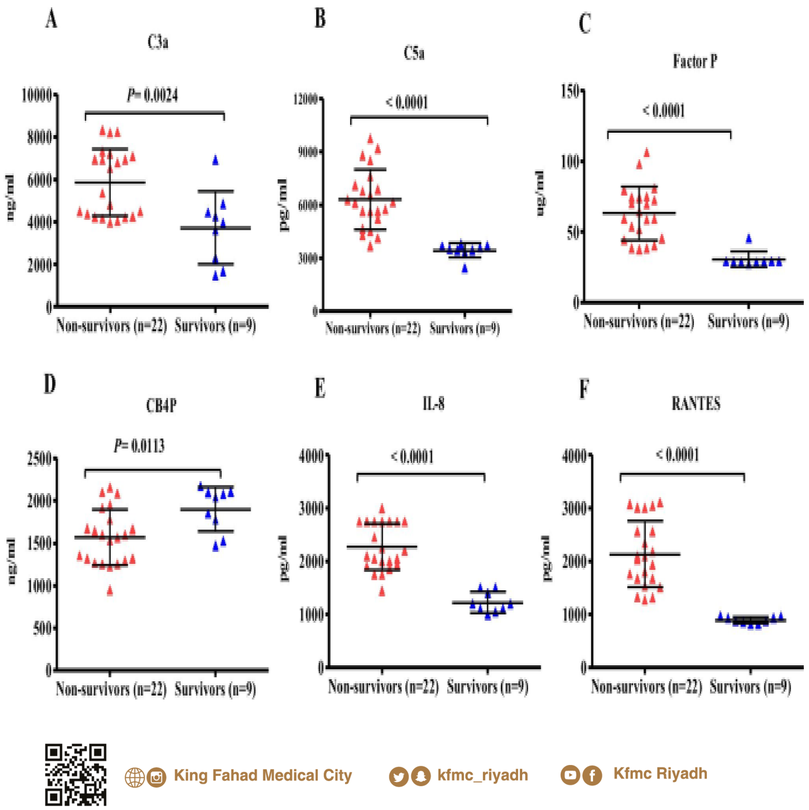
Results



Results



Non-surviving MERS-CoV-infected Patients Exhibit High Levels of Complement Anaphylatoxins (C3a and C5a), Factor P, IL-8, and RANTES



Protein–protein Interaction (PPI) Network Analysis and Hub Proteins

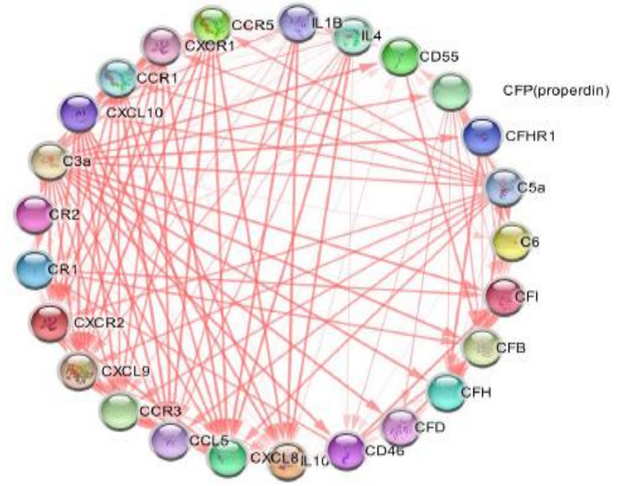


Fig. 4 Actions of C3a, C5a, factor P, IL-8 and RANTES protein-protein interaction network using Cytoscape software. PPI network contains 25 nodes, 149 edges, average node degree 11.9 and the PPI enrichment p-value is < 1.0e-16. A node represents a protein, and a line represents an interaction. The line thickness represents the degree of confidence prediction of the interaction.

Conclusion

We conclude that the high levels of complement anaphylatoxins, C5a and C3a; positive regulatory complement protein (factor P); IL-8; and CCL_5 in the lower respiratory tracts of MERS-CoV-infected patients are associated with immunopathology, higher fatality rates, more severe disease, and ARDS development. High levels of complement mediators, disease severity, and increased mortality appear to be linked to the degree of complement activation against MERS-CoV. Furthermore, the levels of C3a, C5a, IL-8, and CCL_5 in the lung may be useful biomarkers to predict a more severe MERS-CoV infection and mortality. Targeting any of these mediators may offer an effective treatment for MERS-CoV infection. As such, future large studies characterizing components of the complement system at different stages of MERS-CoV infection may offer an effective immunotherapeutic strategy.

Translational Potential

A deeper understanding of the role of the C3a, C5a, IL-8, and CCL5 in the lung may be useful biomarkers to predict a more severe MERS-CoV infection and mortality. Targeting any of these mediators may offer an effective treatment for MERS-CoV infection and may prove instrumental of more efficacious immunotherapeutic approaches. Our findings also explain the events of complement activation and its causal relation to the lung tissue damage during MERS-CoV infection. Additionally, the hub proteins identified in our PPI network may become targets for follow-up investigation.

References

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