Conserved miRNA Signatures Associated with Burn Pit Exposure through Cross-Cohort and Longitudinal Analysis in Military Personnel

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Abstract

Background: The health impacts of open-air burn pit exposure among military personnel remain poorly understood due to limited historical exposure data. This study aims to identify molecular markers, particularly microRNA (miRNA) profiles, associated with burn pit proximity using serum samples from deployed service members.

Methods: Active-duty Army and Air Force personnel from the Millennium Cohort Study were selected based on deployment status and serum availability. Exposed individuals deployed to Joint Base Balad (large burn pit site) were compared with a pre-deployed unexposed time point. In addition, they were also compared to cross-cohort analysis using two control groups: those deployed to Camp Arifjan (incinerator waste disposal) and undeployed personnel. Serum samples (N = 747) underwent RNA extraction and next-generation sequencing for miRNA profiling. Differential analyses are being conducted to compare miRNA expression between exposed post-deployment and control samples, as well as pre- and post-deployment changes within the exposed group based on burn pit proximity. Additionally, inflammatory/immune response proteins were assessed using Olink technology.

Results: Cross-cohort comparisons identified miRNA changes with miR3168, miR-1246 and miR-486-3p were conserved across more than one differential analysis. Proteomic profiling identified exposure-related protein signatures across deployment durations and highlighted dysregulation of airway injury, immune and metabolic pathways. Integrated multi-omic analyses linked miRNA changes with downstream mRNA targets and differentially expressed proteins, forming coherent regulatory networks that implicated PI3K/AKT, NF-κB, and FGF signaling. A causal toxicology network connected these molecular findings to toxicants characteristic of burn-pit emissions.

Conclusions: Together, these results reveal a robust, biologically plausible molecular response to burn-pit exposure and once validated using an independent set can lead to identification of candidate biomarkers and mechanistic pathways underlying deployment-related health effects.

Methods

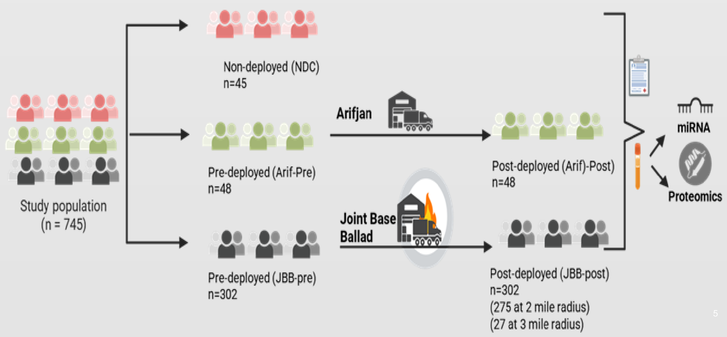


Figure 1- The study population consisted of three groups 1) pre- and post-deployed samples from burn pit exposure site Joint Base Balad (JBB), 2) pre- and post-deployed controls from non-burn pit site Arifjan, and 3) non-deployed controls (NDC). Study plan illustrating the design used to assess molecular effects of burn-pit exposure. Demographic, behavioral, and biological data were collected, and epigenomic (miRNA) and proteomic datasets were analyzed to identify shared signatures. The numbers in the brackets are samples from each of the groups. The figure also outlines the exposure classification (e.g., distance to burn pit site 2 miles or 2-to-3-mile groups).

Associations between circulating miRNA expression and clinical data

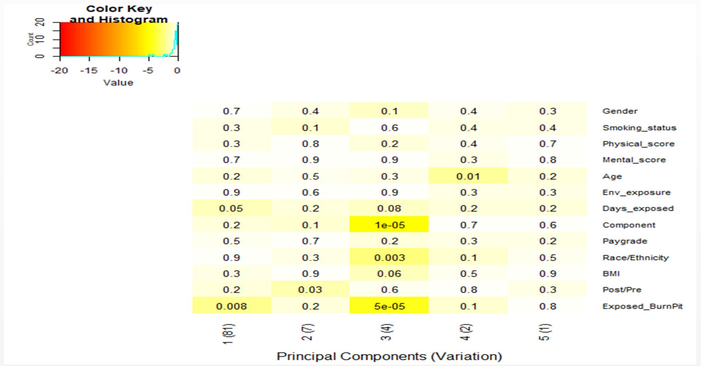


Figure 2- Association of first five components of the data and annotation variables. The p-value is represented in cells by text labels and background color in chromatic scale. The y-axis labels refers to the clinical variables.

Differentially Expressed miRNA

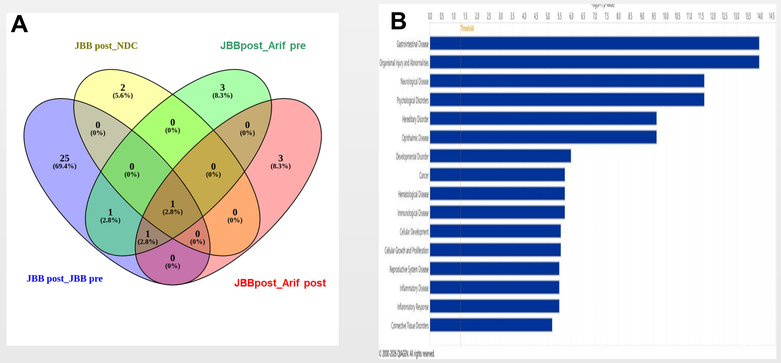


Figure 3 A) Venn diagram of differentially expressed miRNAs identified in analysis groups. Differentially expressed miRNA is identified where pre-and post-burn pit exposure samples were compared. Four groups of comparisons were conducted with A) NDC, B) Arif-pre, C) JBB-pre, and D) Arif-post groups were compared with the JBB-post group, B) Diseases and biofunctions because of differentially expressed miRNA. The y-axis is the negative log of adjusted p-values corrected using the Benjamini-Hochberg procedure.

Network Analysis

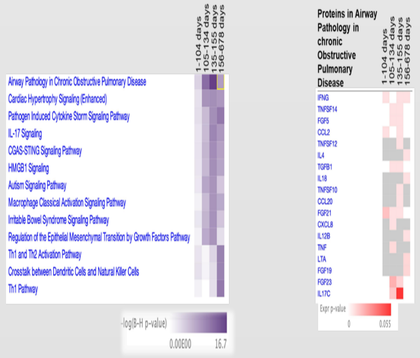
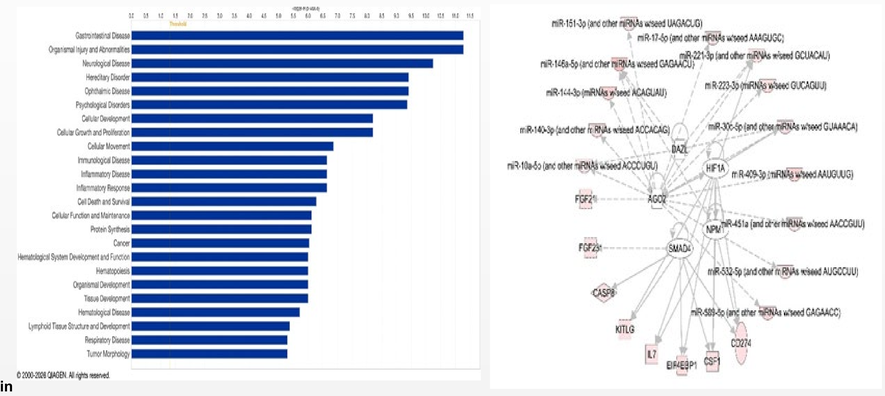


Figure 4- The top 13 canonical pathways derived from IPA gene ontology algorithms for burn pit associated differentially expressed proteins. These pathways emerged following IPA "core analysis". The heatmap indicates the minimum significance level scored as −log (B-H p value) from Fisher's exact test. B) Heatmap of proteins involved in the top identified canonical pathway as observed at each of the time points.

Integrated miRNA–Protein Diseases and Biofunctions



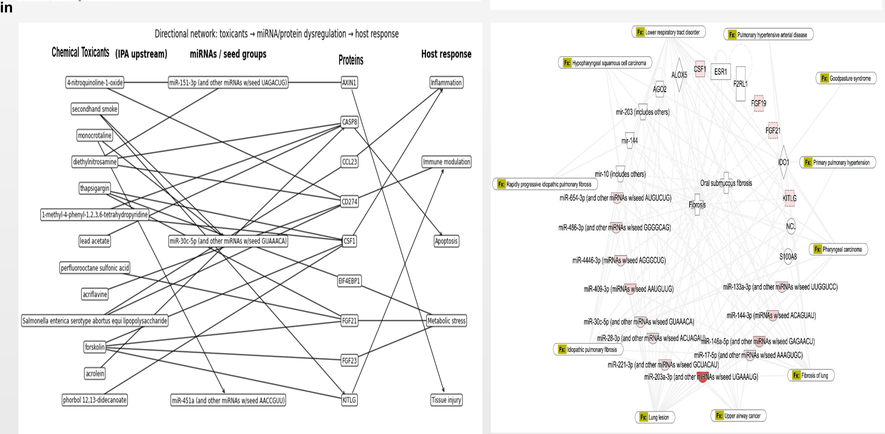


Figure 5 A) Integrated miRNA–Protein Diseases and Biofunctions Associated with burn pit exposure. The y-axis is adjusted p-values corrected using the Benjamini-Hochberg procedure and displayed that have–log (B-H p-value greater than 5.0). B) Integrated Upstream Translational/transcriptional Regulator–miRNA–Protein Analysis: The connections with miRNA, protein and top five upstream regulators are marked as hyphened line. The outer circle represents the molecules from our data connected to upstream regulators (inner molecules). C) Causal network linking different classes of chemical toxicants to shared proteins and miRNA. D) Integrative miRNA-protein analysis- Data linked to the top-scoring network after combining miRNA and protein differentially expressed molecules (pink highlighted) and their association with respiratory disease pathways.

Future Directions

- Characterized molecular responses, in U.S. military personnel, including non-deployed controls and cohorts deployed with or without burn pit exposure.
- Pathway analysis of differentially expressed miRNAs in burn pit-exposed personnel revealed dysregulation in oxidative stress, airway injury, and inflammatory processes
- Our results demonstrate a robust, biologically plausible molecular response to burn pit exposure, establishing candidate biomarkers and mechanistic pathways for deployment-related health effects.

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