

## **Serum Exosomes and miRNAs in Male and Female Current, Former and Non-Smokers**

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**Significance:** Despite recent surge in exosome research, the effect of smoking on serum exosomes remains unclear. Exosomes are present in almost all body fluids including serum. We have evaluated the effect of smoking on serum exosome and its miRNA content in current, former, and non-smokers.

**Methods:** We isolated exosomes from a total of 24 human serum samples (8 current, 8 former, and 8 non-smokers with 4 males/4 females in each group). Nanoparticle tracking analysis was performed with ZetaView. Zeta potential, an indicator of exosome stability, was measured at 23°C. Extracted exosomal RNA was profiled using NanoString Human miRNA assay. miRNA data was exported and normalized. After removing miRNAs with background level expression, we used the LIMMA package in R to identify differentially expressed (DE) miRNAs.

**Results:** The mean age of the study subjects was 55.4, 45.3, and 46.1 years for current, former, and non-smokers, respectively. Exosome diameters were similar across all groups. Significant differences were observed in the size distribution across the spectrum (50-350nm), the amount of the 50-150nm particles, and the zeta potential between the different sex and smoking groups ( $p < 0.05$ ). The transmission electron microscope images indicate that most exosomes were within 50-150nm, consistent with our ZetaView data. We identified 156, 228, and 182 miRNAs in current, former, and non-smokers. With a fold change  $> 1.5$  and  $p < 0.05$ , we identified 12 DE miRNAs in current vs. non-smokers, 13 miRNAs in former vs. non-smokers, 5 miRNAs in former vs. current smokers. Several of them have been associated with cigarette smoking previously.

**Conclusions:** Our study has identified the specific differences in serum exosome and its miRNA contents related to smoking exposure in males and females for the first time. Further investigation is needed due to the relatively small sample size.