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PLATELET CONTAMINATION AFFECTS MITOCHONDRIAL DNA (mtDNA) LEVELS SIGNIFICANTLY IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM HIV PATIENTS

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OBJECTIVES: MtDNA copies/cell in peripheral blood mononuclear cells (PBMCs) have been suggested as a potential marker for HIV lipodystrophy. Isolation of PBMCs coincides with platelet contamination. Since platelets contain mtDNA and no nuclear DNA, this could skew results that would make inter- and intrastudy comparisons difficult. In this study, mtDNA copies/cell were quantitated in PBMCs from HIV+ and HIV- patients with and without platelet contamination. Platelet removal was verified by flow cytometry.

METHODS: PBMCs were isolated from blood of three HIV seronegative and three seropositive patients using a standard Ficoll gradient protocol. Cells from each individual were assayed fresh or viably frozen. Cells were labelled with CD61 platelet-specific magnetic microbeads for chromatographic removal of the platelets using Miltenyi auto MACS magnetic column separation. The elute (platelet and PBMCs) and flow through (PBMCs) fractions were evaluated by flow cytometry using CD61 PE (platelet-specific) and CD45 FITC (PBMC-specific) labeling. MtDNA copies/cell were quantitated by real-time PCR pre- and post-chromatography on all cell fractions.

RESULTS: Platelet contamination varied significantly in Ficollisolated PBMCs. Seronegative and seropositive PBMCs had an average 20% platelet contamination, ranging from 3–33%. Chromatography reduced platelet contamination to 5% in the seronegative and 10% in the seropositive PBMCs. MtDNA copies/cell were decreased in the chromatographically isolated frozen HIV+ PBMCs (209 + 64) compared with the HIV- PBMCs (357 + 40), $P=0.028$.

CONCLUSION: MtDNA quantification from PBMCs isolated using Ficoll separation may be affected by platelet contamination and this can be as much as 20%. Platelet removal by magnetic chromatographic separation can minimize the variability caused by contamination.

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