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Cysteinylated plasma albumin leads reduce antioxidant activity in nonalcoholic fatty liver disease (NAFLD)

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Oxidative stress is postulated to play an important role in liver disease progression. The degree of oxidized cysteine (Cys) 34 in human serum albumin (HSA) is correlated with oxidative stress related to pathological conditions and modulates its physiological functions. We analyzed purified plasma albumin from 46 biopsy-proven NAFLD patients and 21 matched healthy blood donors. The albumin modifications were analyzed by liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometer (ESI-TOF/MS). Relative % abundance of unmodified (intact) and modified isoforms of albumin were compared between NAFLD and controls. *In vitro* ROS (reactive oxygen species) generation and antioxidant activity was measured by mean fluorescence (MFI) of dihydrorhodamine (DHR) by flow cytometry in presence of purified albumin of controls and NALFD patients. Three most prominent isoforms of albumin were observed in the deconvoluted ESI spectrum with molecular masses of 66,438±2.8, 66,559±4.8 and 66,603±6 Da in controls and NAFLD patients represents intact, cysteinylated and glycated isoforms of albumin respectively. Unmodified albumin was the predominant peak with 100% relative abundance in healthy with calculated theoretical mass (66,438 Da, 542 aa). In contrast, the relative abundance of modified form with addition of +119 Da (cysteinylation) of albumin was predominant (100%) in NAFLD. Cysteinylated isoform of albumin (cys-Alb) was significantly higher in NAFLD patients than controls (100% v/s 52% - $p<0.01$). Circular dichroism (CD) spectrum showed clear structural alterations in purified albumin from NAFLD patients as compared to controls. Further, albumin antioxidant activity was measured by removal of ROS productions *in vitro*. Significant differences were observed in mean fluorescence intensity of DHR in presence of purified albumin from controls and patients (51.5±5.8% vs 60.3±13.8%, $p<0.001$) showed reduced antioxidant activity of albumin in NAFLD patients. Our results clearly showed that sustained oxidative stress and reduced antioxidant activity is reflected by high levels of cysteinylated albumin in NAFLD patients.

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