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Cysteinylation of albumin leads to reduced antioxidant activity in non-alcoholic fatty liver disease patients

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Backgrounds & Aim: 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 function. Present study aim is to develop a noninvasive diagnostic marker for Non-Alcoholic Fatty Liver Disease (NAFLD) by studying the differential modification pattern of albumin and antioxidant activity in NAFLD patients.

Patients & Methods: 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 was compared between NAFLD and controls. In vitro ROS generation and antioxidant activity was measured by Mean Fluorescence Intensity (MFI) of Dihydrorhodamine (DHR) by flow cytometry in presence of purified albumin of controls and NALFD patients.

Results: Three most prominent isoforms of albumin were observed in the de-convoluted 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 subjects in perfect agreement 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 patients. Cysteinylated isoform of albumin (cys-Alb) was significantly higher in NAFLD patients than controls [100% v/s 52% ($p<0.01$)]. Although NAFLD showed 100% relative abundance of cys-Alb isoform further fatty liver and NASH patients differ on the basis of unmodified albumin isoforms [82% vs. 60% ($p<0.05$)] suggesting varied oxidative stress. Circular dichroism (CD) spectrum showed clear structural alterations in purified albumin from NAFLD patients as compared to purified albumin from controls. Further, purified 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%, $p<0.001$) suggest reduced antioxidant activity of albumin in NAFLD patients. Three most prominent isoforms of albumin were observed in de-convoluted 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. Intact peak with 100% relative abundance in healthy subjects in perfect agreement with calculated theoretical mass (66,438 Da, 542 aa). In contrast, the relative abundance of modified form with addition of +119Da (cysteinylation) of albumin was predominant (100%) in NAFLD patients. Cysteinylated isoform of albumin (cys-Alb) was significantly higher in NAFLD patients than controls [100% v/s 52%, $p<0.01$].

Conclusion: Our results clearly showed that sustained oxidative stress and reduced antioxidant activity is reflected by high levels of cysteinylated albumin in NAFLD patients and might be useful plasma marker for oxidative damage in NAFLD/NASH.

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