

Mechanism of gallstones formation in women during menopause (EPR study)

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The worldwide incidence of gallbladder disease is highly variable. Identification of metabolic alterations like cholesterol metabolism or free radicals may provide insight into the formation of gallstones and provide a basis for prognostic markers. The aim of the study was to identify the pathogenesis and prognostic value of metabolic disorders in gallstone formation in menopausal women.

Methods: Menopause-aged women with (group I, 58 patients) and without gallstone disease (group II, 25 patients) were investigated. In each group blood lipid metabolism and blood redox parameters (free Mn^{2+} -ions) and antioxidant system activity (ceruloplasmin/ Fe^{3+} -transferrin) were studied using micro enzymatic method and Electron Paramagnetic Resonance (EPR) spectroscopy. Surgically removed gall bladder stones were studied by routine laboratory methods (cholesterol and bilirubin content) and EPR spectroscopy. In model experiments *in vitro* purified bilirubin was irradiated with visible light in different conditions (in presence and without oxygen).

Results: Intensive signal of oxidized bilirubin free radical was detected in the EPR spectra of gallstones. Reduced activity of blood antioxidant ceruloplasmin/ Fe^{3+} -transferrin system and increased content of prooxidants Mn^{2+} -ions were detected in menopausal women blood with gallstone disease. *In vitro* experiments demonstrated, that prolonged exposure of bilirubin to visible light in the presence of oxygen induces formation of the bilirubin free radicals (EPR signal $g = 2.003$ $\Delta H = 1.0$ mT); irradiation of bilirubin in vacuum was not associated with generation of free radicals. Correlation analysis revealed statistically significant correlation between EPR signal intensity of bilirubin free radical in gallstones, activity of ceruloplasmin/ Fe^{3+} -transferrin antioxidant system and content of free Mn^{2+} ions in patients' blood ($r = -0.5725$, $p = 0.0324$; $r = 0.7805$, $p = 0.0010$, respectively). The tight correlation between marker of oxidative stress, Mn^{2+} ions EPR signal intensity and HDL-, LDL-cholesterol content in blood ($r = 0.910629$, $p = 0.0324$; $r = 0.029477$, $p = 0.0010$, respectively) was also revealed.

Conclusion: The results of the present study demonstrated the pathophysiological significance of alterations of blood redox-homeostasis during menopause in the gallstone formation. The risk of bilirubin oxidation, which plays a crucial role in the gallstones formation, increases with intensification of oxidative stress. Crystallization of cholesterol in polymeric network of oxidized bilirubin may contribute to gallstone formation.