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Detection of Transferrin Oxidative Modification In vitro and In vivo by Mass Spectrometry. Hereditary Hemochromatosis is a Model

Hereditary Hemochromatosis (HH) is an inherited recessive autosomal disorder characterized by the accumulation of excess iron. When iron-binding proteins become saturated, concentrations of free or Non-transferrin-bound iron (NTBI) rise, a condition thought to be responsible for the adverse effects associated with HH. To investigate whether disturbing iron homeostasis plays a role in free radical injury in HH, protein carbonyls were found to be 1-7 times higher in patients with HH than in controls, with the greatest increases observed in untreated HH patients with high ferritin and > 90% transferrin saturation with iron. An unpaired t-test revealed a p value of 0.0278 ($p < 0.05$), which is considered statistically significant.
In vitro oxidation of transferrin standards with hydrogen peroxide and excess iron, followed by immobilized trypsin digestion (Poroszyme), high-resolution LC-MS/MS analysis (Q-TOF Ultima, Waters), and MS/MS data processing (PEAKS, Bioinformatics Solution), identified several tryptic peptides containing oxidized Methionine (Met), Tryptophan (Trp), and Histidine (His) residues. Using the same methodology, oxidized residues were subsequently detected in transferrin isolated from plasma samples of patients severely affected by HH. Comparison of MS/MS spectra of in vitro oxidized samples with the most fragment ion peaks in common with oxidized peptide MS/MS spectra from patient samples revealed a strong correlation between the two. These data show that elevated NTBI may be involved in the oxidative modification of transferrin and that such modifications may play a significant role in the pathophysiology of HH.

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Prevalence of Hemolysin (hlyA)-producing Uropathogenic E. coli and Phenolics-mediated Suppression: Experimental and Bioinformatic Evidence

Urinary Tract Infections (UTIs) are common opportunistic diseases, primarily caused by Escherichia coli, which utilizes various virulence factors, including the hlyA gene encoding hemolysin. Phenolic compounds in fruits and vegetables, known for their antimicrobial properties, were examined for their effects on E. coli. This study involved 60 E. coli isolates from Aleppo University Hospital, identified via biochemical and molecular tests. The hemolytic ability was assessed phenotypically, and the hlyA gene was detected using PCR. The impact of pyrogallol and catechol on these isolates was also evaluated. Results showed a 54.6% isolation rate of E. coli, with a higher rate in females (71.7%) than males (28.3%). The 20-40 age group was most affected, comprising 38.4% of cases. Hemolytic activity was observed in 45% of isolates, and the hlyA gene was present in 41.6% of cases. Pyrogallol exhibited a bactericidal effect at high concentrations and mild growth at lower levels, while catechol showed no antibacterial effects. These experimental investigations were validated by docking those polyphenols to the hlyA predicted, validated 3D structure where pyrogallol exhibited stronger binding affinity than catechol (-5.2 vs. -4.8 kcal/mol). The study underscores the significance of the hlyA gene in E. coli virulence and highlights the potential antibacterial properties of phenolic compounds at specific concentrations.
