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## Simultaneous quantitative and qualitative analysis of human chorionic gonadotropin in urine

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Quantitative analysis for protein remains as a challenge. This is because protein is a macromolecule and most of the proteins undergo post translational modification such as glycosylation and phosphorylation. These modifications cause variable detectability of the proteins when analyzed using mass spectrometer. Although, quantitative analysis of proteins is commonly carried out using bioassay, due to limitation in the sensitivity of bioassays, proteins which are present in the minute quantities in biological fluids cannot be determined accurately. Furthermore, interferences from the matrix will also suppress the sensitivity of bioassays. We have developed a method to simultaneously identify and quantify hCG (human chorionic gonadotropin); this hormone is misused by male athlete to enhance their performances in sport by inducing the secretion of endogenous testosterone. The hormone can be found in trace quantity in the urine of doped male athletes. Besides sensitivity, the use of bioassay for quantification of hCG is being challenged as antibodies for hCG cross react with LH (luteinizing hormone) that resembles hCG. Sample preparation plays a critical role in getting good analysis data. We used immune affinity clean up and enrichment self-packed column to isolate hCG in urine. The isolated hormone was then subjected to reduction, alkylation and digestion with trypsin enzyme. A tryptic peptide that is unique to hCG in its amino acid sequence was selected to be the quantifying marker. The marker peptide detected in the full scan MS was isolated and excited to collision induced dissociation (CID) in the MS/MS scan. The MS/MS data generated gave unique identification for hCG; furthermore, three fragment ions of the marker peptide were used for quantification of hCG. Using such an approach, we eliminated the background interferences and thus increase the sensitivity of the method.

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## Core-fucosylated glycopeptides in hepatocellular carcinoma

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Aberrant core-fucosylation (CF) is often associated with the development of hepatocellular carcinoma (HCC). A mass spectrometry-based methodology has been developed to study changes of site-specific CF of target candidate proteins as well as of non-target serum proteins among cirrhosis and HCC samples. The methods involve trypsin digestion, enrichment of CF peptides, followed by glycan truncation while retaining the innermost N-acetylglucosamine (GlcNAc) and/or core-fucose bound to the peptide. Ceruloplasmin, one of the target proteins was found to be up-regulated in the early stage alcohol-related HCC serum samples compared with alcohol-related cirrhosis samples but not in HBV or HCV-related samples. Four CF sites (sites 138, 358, 397 and 762) were present in ceruloplasmin, among which the core-fucosylation level of sites 138 and 397 were more susceptible to change in disease states. Most interestingly, the CF level of 3 sites of ceruloplasmin increased significantly in alcohol-related HCC samples compared to alcohol-related cirrhosis samples, with the highest AUC (area under the curve) value of 0.838 at site 138. Large scale mass spectrometry-based screening quantified 1300 CF peptides where 20 CF peptides were differentially expressed in alcohol-related HCC samples compared with alcohol-related cirrhosis samples and 26 CF peptides changed in HCV-related HCC samples compared to HCV-related cirrhosis samples. Among these, we found three CF peptides from fibronectin upregulated in alcohol-related HCC samples compared with alcohol-related cirrhosis samples with an AUC value of 0.89 at site 1007 with a specificity of 85.7% at a sensitivity of 92.9% (generated with 10-fold cross-validation). When combined with the AFP index, the AUC value reached to 0.92 with a specificity of 92.9% at a sensitivity of 100%, it significantly improved compared to that with AFP alone (LR test $p < 0.001$).

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