# Validation of a liquid chromatography tandem mass spectrometry method to determine whole blood oxidized and reduced forms of glutathione for an assessment of degree of oxidative stress

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Reactive oxygen species (ROS) has a considerable degree of reactivity and can cause potential biological damage in human. As a possible marker of oxidative stress, a lot of studies have measured whole blood or erythrocyte reduced glutathione (GSH), oxidized glutathione (GSSG) and/or GSH/GSSG ratios. However, because of large differences in GSH and, particularly in GSSG levels between different studies, accurate revision of the methods used and analytical validation of newly modified method is needed. We modified recently reported measurement methods for GSH and GSSG and evaluated its analytic performance. In addition we evaluated clinical utility of GSH/GSSG ratio by testing the increase of GSH/GSSG ratio according to the amount of oxidative stress induced by iron in mouse model.

To prevent artifactual GSH auto-oxidation, we pretreated sample with N-ethylmaleimide (NEM) immediately after sample collection. After derivatization with NEM, protein precipitation using sulfosalicylic acid (SSA) and chromatographic isolation and quantitation of the anlaytes by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was conducted. GSH-NEM and GSSG were detected using a positive electrospray ionization in the multiple reaction monitoring (MRM) pairs of m/z 433-> 304 and m/z 613-> 355, respectively. This method was validated for precision, linearity, limit of quantification, limit of detection, and ion-suppression. Twenty mice were randomized to four treatment groups: a) control (300 μL of normal saline intra peritoneal injection (IP) per day, n = 5), (b) 5 mg of iron dextran IP per day, (n = 5), (c) 10 mg of iron dextran IP per day (n = 5), or (d) 15 mg of iron dextran IP per day (n = 5). Whole blood GSH and GSSG concentrations were determined from mice after 4 weeks of treatments. The mean GSH/GSSG ratios were 163.1, 31.0, 27.9 and 12.8 for control, 5 mg injection group, 10 mg injection group and 15 mg

injection group, respectively, showing decrease of GSH/GSSG ratios according to the amount of oxidative stress induced (Figure 1). The assay linear range was 100 μmol/L to 5000 μmol/Lfor GSH-NEM and 0.1 μmol/L to 50.0 μmol/L for GSSG. Intra-assay CVs (n = 5) were 2.00-4.92 % for GSH-NEM and 5.87-7.84 % for GSSG. Inter-assay CVs (n = 5) were 2.72-4.82 % for GSH-NEM and 5.99-8.53 % for GSSG. No ion suppression was observed at the retention time for GSH-NEM and GSSG. Our methods for GSH and GSSG using LC-MS/MS was reliable and GSH/GSSG ratio could provide an assessment of degree of oxidative stress.

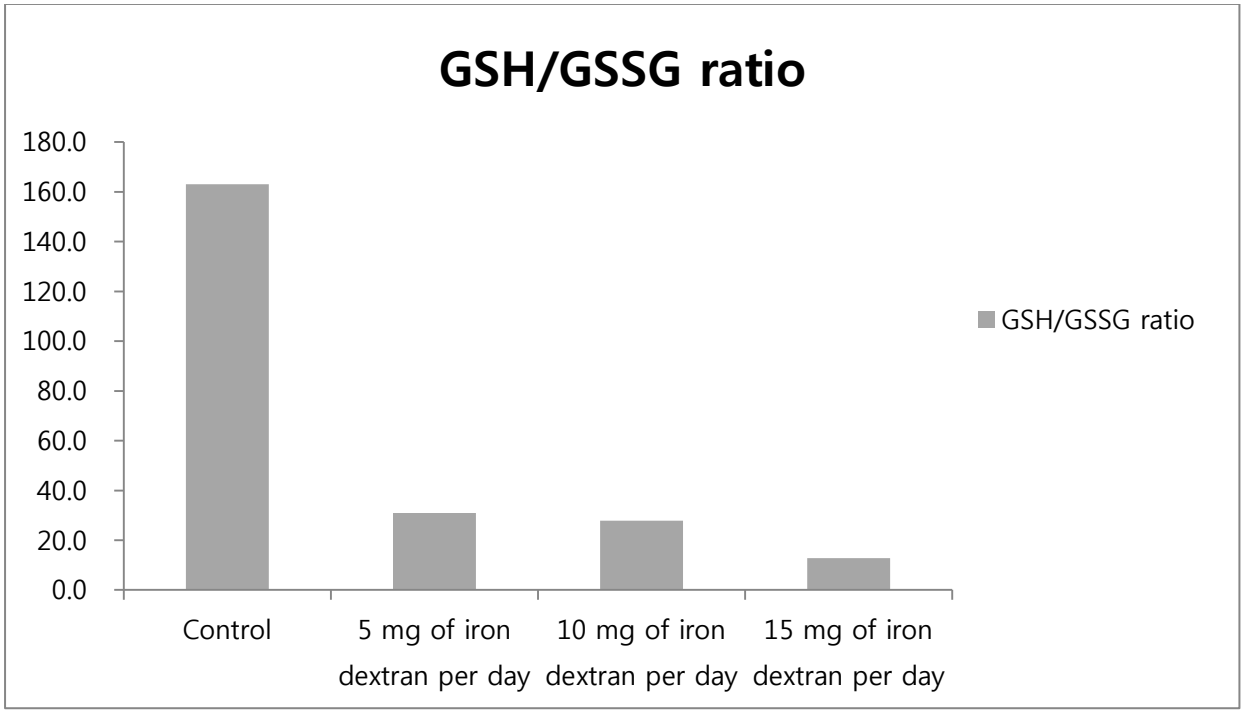


Figure 1. GSH/GSSG ratios according to the amount of oxidative stress induced