ABSTRACT

Present study is concerned with the development of enzyme linked immunosorbant assay (ELISA) for myeloperoxidase (MPO). Coating antibody for MPO (APO36 code: BM240-N4C7 SCIPAC USA) was selected at 1µg/ml in borate buffer pH 8.0 for two nights at room temperature (RT) in humid chamber. 50 µl per well of 1% ByCo A (hydrolysed gelatin) was used in borate buffer for two hours at room temperature in humid chamber to block the non specific binding of antigen. Stability of coating antibody (APO36 code: BM240-N4C7 SCIPAC USA) was done using 1% ByCo A and 2% mannitol in distilled water for 1 hour at RT. Detecting antibody (APO35 # 189-176-4) was biotinylated with Sulfo-NHS-LC-Biotin and was used at 1:20K with streptavidin-HRP conjugate at 1:25K to bind with detecting antibody. 0.1% Triton X-100 was used for removal of unbound reagents during the assay procedure. To recognize the bound HRP conjugate 3, 3' 5, 5' tetramethyl benzidine (TMB) was used at room temperature for 20 minutes. To stop the enzymatic activity 1M sulphuric acid was selected. Assay was validated by recovery and dilution experiment. In recovery experiments samples show high % recovery i.e., 101.9%, 102.3%, 93.6%, 99.43%, 102.1 and 92.4%. Dilution experiment showed that samples when diluted serially at 1:2 and 1:4 dilution showing that assay is sensitive enough as all requires.

Second part of the present study was clinical application of developed assay. Using newly developed ELISA assay serum samples with 50 µl of sample volume were found most appropriate. 82 normal controls were included with the mean age 45.89± among males and 50.2± among females. The MPO levels among males were 18.75± ng/ml and among females it was 13.70± ng/ml. it was further found that in both groups normal controls with BMI > 25 had higher levels of MPO. In the disease population 15 patients were included, sample were collected from Coronary Care Unit from the Department of Cardiology, Shaikh Zayed Medical Complex. The results of patients were