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**Measurement of Mitochondrial Respiratory Chain Complexes Enzyme Activities in Human Fibroblast using Enzyme-linked Immunosorbent Assay**

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**ABSTRACT**

The mitochondrial respiratory chain (MRC) plays crucial roles in cellular energy production through four enzymatic complexes, CI to CIV. Deficiencies of Complex I and IV are the most common causes of mitochondrial oxidative phosphorylation (OXPHOS) disorders. Since our current kinetic enzyme assay for analysing activities of these complexes is very tedious and less precise, we aimed to evaluate an alternative method using enzyme-linked immunosorbent assay (ELISA) to measure activities of CI and CIV. Wild-type strain human fibroblast was used for linearity and precision studies. 15 post-mortem’s skin samples taken from infants suspected of having IEM were cultured and the fibroblasts were analysed for CI and CIV activities using both ELISA and the current kinetic enzyme assay. The well of the ELISA plates were pre-coated with specific antibodies which will immobilised the complexes. Substrates were added and the colorimetrical oxidation of NADH to NAD+ at 450nm and reduced cytochrome c at 550nm were captured by Eon™ spectrophotometer. The measurements of activities were expressed as rate (mOD/min) per mg of cell lysate. Data were analysed using SPSS software to determine their correlation. Linearity of CI and CIV activities using ELISA was up to 0.25 mg/mL of fibroblast lysate. The mean activities of CI and CIV were 37.86±4.03; and 74.85±8.83 (mOD/min/mg) (n=15), respectively. Precision study of both complexes using ELISA showed coefficient of variation (CV) less than 15% compared to 25% in kinetic assay. Enzyme activities in both methods showed a normal distribution (p<0.05). Pearson's correlation coefficients between ELISA and kinetic enzyme assays was moderate with r = 0.577 and 0.553 for CI and CIV, respectively. ELISA assay of CI and CIV are more precise and comparable to current kinetic enzyme assay and can be used as an alternative method to measure MRC complexes activities.