Abstract

Iron is an essential trace element which has vital role in the various metabolic processes. The deficiency of this trace element is widespread in Indian population and so its supplementation is also common. Unobserved iron supplementation and various metabolic abnormalities like thalassemia, hemochromatosis etc. are known to cause iron overload. In our body iron is associated with various proteins; most of it is present in association with apotransferrin in circulation. In normal condition the capacity apotransferrin to bind plasma iron is very high and so it do not get saturated which prevent the existence of iron free from its traditional carrier. The circulatory iron which is not bound to apotransferrin is known as Non Transferrin Bound Iron (NTBI). NTBI has been reported as heterogeneous molecule. Different forms of NTBI have been observed in diverse pathological conditions depending on the type of pathological condition and the type of iron overload, its severity as well as its duration.

The thesis mainly focuses on two important aspects, the first is to set up the simple, rapid and user friendly modification in spectrophotometric method of NTBI, which can be opted in Indian laboratories and hospital set ups. The second is to estimate the NTBI in assorted clinical conditions and to find out the probable correlation of NTBI with the other analytes studied in the specific clinical conditions if any. Amongst various methodologies available we had selected spectrophotometric method due to easy availability of spectrophotometer as compared to the others.

The major obstacle in the spectrophotometric estimation of NTBI was the fluctuating non-specific absorbance. Such hitch was solved with the use of ultracentrifugation and ultrafiltration by various researches. We had experimented filtration with PVDF durapore syringe filter, vortex mixing, low speed centrifugation as well as high speed centrifugation at various stage of the experimental protocol. We found that high speed centrifugation with in-between sample pre-incubation had greatly reduced the non specific background noise in NTBI estimating bathophenanthroline (BPS) based spectrophotometric method. The standard graph shows that the detection limit of the present method was 0.1 µmol/L. We compared the values of controls observed in the present study with the values reported by others, the level of fluctuating absorbance was also been considered before accepting the protocol for clinical evaluation.

The modified protocol was used to estimate the serum NTBI values in control subjects and patients of cardiac complications, diabetes mellitus, hemodialysis, β thalassemia major and Chronic Lymphoid Leukemia (CLL). The results showed that the mean serum NTBI value for healthy controls was 0.02 ± 0.06 µmol/L. The NTBI values we observed in patients of diabetes mellitus, hemodialysis and β thalassemia major were significantly higher (p<0.05) than the respective control values. The NTBI positivity for control subjects and patients of cardiac complications, diabetes mellitus, hemodialysis, β thalassemia major and CLL were 14.5 %, 42.8 %, 42.0 %, 39.6 %, 90.0 % and 30.0 % respectively and by considering 0.3 µmol/L NTBI as cutoff the significant positivity were 0 %, 2.7 %, 22.0 %, 18.9 %, 60.0 % and 0.0% respectively.