Chapter 6

Summary

and

Conclusion
6.1 Distribution of subjects:

A total number of 513 subjects were included in the study.

Out of these, samples of 290 apparently healthy subjects were included in part 1 of the study, where in various modifications were experimented in NTBI estimating BPS based spectrophotometric method and the best one was selected for its operation for part 2 of the study.

223 subjects were actually undergone for the clinical evaluation i.e. part 2 of the study. Such subjects were comprised of normal subjects, cardiac patients, type 1 and type 2 diabetes mellitus patients, kidney patients undergoing hemodialysis, β thalassemia major patients and patients suffering from CLL.

There were 43, 15, 31, 47, 17 and 5 males in group no. I, II, III, IV, V, VI respectively. Numbers of females were 12, 10, 19, 16, 3 and 5 for group no. I, II, III, IV, V and VI respectively.

Number of subjects with < 50 years of age were 31, 5, 15, 29, 20 and 5 respectively for group no. I, II, III, IV, V, VI respectively.

Number of subjects with ≥ 50 years of age were 24, 20, 35, 34, 0 and 5 for group no. I, II, III, IV, V, VI respectively.

6.1.1 Distribution of subjects on the basis of dietary habits:

Out of total 223 subjects studied for part 2, 22, 10, 21, 32, 0 and 2 subjects were vegetarians among group no. I, II, III, IV, V, VI respectively. Number of non vegetarians (subjects taking veg. + non veg.) were 33, 15, 29, 31, 20 and 8 for group no. I, II, III, IV, V, VI respectively.

6.1.2 Distribution of subjects on the basis of alcohol consumption:

Out of total 223 subjects studied for part 2, number of alcoholic subjects were 7, 6, 7, 13, 20 and 3 in group no. I, II, III, IV, V, VI respectively. Number of non alcoholics were 48, 19, 43, 50, 0, 7 for group no. I, II, III, IV, V, VI respectively.

6.2 Blueprint of the methodology:

High and fluctuating background noise can interfere at a great extent while measuring the absorption on spectrophotometer if care is not taken to minimize it especially in present method as the intensity of the endpoint color is very low.
SUMMARY AND CONCLUSION

Filtration with PVDF durapore syringe filter as well as vortex mixing at various stage of the processing failed to minimize non specific and fluctuating absorbance.

High speed centrifugation with in between sample pre-incubation had greatly reduced the non specific background noise in NTBI estimating BPS based colorimetric method.

By using the simple instrument like high speed centrifuge, incubator and spectrophotometer, which are virtually present in almost all medium scale laboratories, NTBI can be effectively measured.

The reliability of the method greatly depends on the sensitivity of the instrument, to improve the sensitivity, we had increased the path length, and while doing so, the sample volume demand had been increased which is unfavorable in clinical practice.

To minimize the sample volume the assay need to be automated before its wide scale applicability or else the test has to be done in microtitre plate with reduced sample volume and the absorbance has to be taken with the sensitive ELISA plate reader.

6.3 Clinical evaluation of various biochemical parameters:

6.3.1 Serum NTBI level in various groups studied:

Mean serum NTBI values were 0.02 ± 0.06 µmol/L, 0.05 ± 0.08 µmol/L, 0.08 ± 0.14 µmol/L, 0.031 ± 0.64 µmol/L, 0.20 ± 0.32 µmol/L, 0.25 ± 0.76 µmol/L, 0.61 ± 0.54 µmol/L and 0.05 ± 0.08 µmol/L for group I, group II: day 1, group II: day 2, group III, group IV: pre-HD, group IV: post-HD, group V and group VI respectively.

The mean serum NTBI value of the control group was comparable with the others reported in the literature.

The mean serum NTBI value for group I, i.e. of control subject was the lowest, where as for group V i.e. of β thalassemia major patient was highest (p < 0.001). The mean serum NTBI values for diabetics and the hemodialysis patients’ pre-hemodialysis and post-hemodialysis samples were significantly higher than the control subjects (p < 0.005, < 0.001 and < 0.05 respectively).
However for cardiac patients and hematological malignancy patient, the mean serum NTBI were not significantly higher than control group. The mean serum NTBI values significantly differ neither between day 1 and day 2 samples of cardiac patients nor in pre and post HD sample of subjects of kidney disease undergoing dialysis.

The positivity of serum NTBI we found was 14.5 %, 40.0 %, 28.0 %, 42.0 %, 39.6 %, 33.3 %, 90.0 % and 30.0 % for group no. I; II: day 1, II: day2; III; IV: pre-hemodialysis, IV: post-hemodialysis; V and VI respectively. % positivity was lowest i.e. 14.5 % for control group and highest i.e. 90.0 % for β thalassemia major patients.

Mean serum NTBI value in subjects < 50 years of age was 0.19 ± 0.38 µmol/L and 0.21 ± 0.56 µmol/L for subjects ≥ 50 years of age. No significant difference was observed in serum NTBI values of subjects of < 50 years and ≥ 50 years (p =0.773).

Mean serum NTBI value in males was 0.22 ± 0.51 µmol/L and 0.16 ± 0.44 µmol/L for females. No significant difference was observed in serum NTBI values in between male and female (p = 0.361).

Mean serum NTBI value in vegetarians was 0.21 ± 0.57 µmol/L and 0.20 ± 0.42 µmol/L for non-vegetarians. No significant difference was observed serum NTBI value in vegetarians and non-vegetarians (p = 0.900).

Mean serum NTBI value in alcoholics was 0.24 ± 0.47 µmol/L and 0.19 ± 0.49 µmol/L for non alcoholics. No significant difference was observed in these two categories (p = 0.502).

0.3 µmol/L of serum NTBI value was considered as the cut off value and all the samples giving values higher than that were considered as clinically significantly positive samples. The cut off value was determined by the values of serum NTBI found in control subjects and the cut off suggested by other researchers.

Overall positivity and significant positivity of serum NTBI for subjects < 50 years were 48.50 %, 17.16 % and the same for ≥ 50 years were 36.87 %, 14.37 % respectively.

Overall positivity and significant positivity of serum NTBI for male were 43.33 %, 17.61 % and the same for female were 27.38 % and 10.71 % respectively. The % positivity and significant positivity were considerably high in case of male as compare to female.
Overall positivity and significant positivity of serum NTBI for vegetarian were 38.40 %, 15.20 % and the same for non-vegetarian were 39.05 %, 15.97 % respectively. Significant difference was observed neither in % positivity nor in % significant positivity in between vegetarians and non vegetarians.

Overall positivity and significant positivity of serum NTBI for alcoholics were 49.01 %, 19.60 % and the same for non alcoholics were 36.62 %, 14.81 % respectively.

The significant positivity was 0.00 %, 0.00 %, 5.88 %, 22.00 %, 23.80 %, 13.47 %, 60.00 % and 0.00 % for controls; cardiac patients: day 1, day 2; diabetic patients; hemodialysis patients: pre-HD, post-HD; β thalassemia major patients and patients suffering from hematological malignancies respectively.

The mean serum NTBI value in β thalassemia major patients was highest amongst all the groups studied but far lower than the values reported by others. Such low serum NTBI values could be due to limited chelatability of BPS in the absence of the extra mobilizer explored in most of the other methods.

6.3.2 Other significant parameters studied in various groups:

Various biochemical parameters also studied in normal subjects, diabetes patients, cardiac patients, in end stage renal disease patient undergoing hemodialysis and β thalassemia major patients.

In diabetes patients, the mean plasma sugar level was highest amongst all the groups studied. The mean plasma values in diabetic patient group were higher than the suggested normal range.

The mean level of serum CPK, serum CPK-MB, serum LDH, serum AST and serum ALT were higher in cardiac patients than the normal subjects and suggested normal value. The mean value of serum CPK and serum CPK-MB were higher in samples collected on day 1, i.e. on the day of hospitalization as compared to the sample collected on day 2. Serum AST and serum ALT also followed the same pattern.

The mean level of serum urea, serum creatinine and serum uric acid were high in patients undergoing hemodialysis especially in pre dialysis samples however the levels were decreased after dialysis.
The mean level of serum iron, TIBC and % Tf saturation were normal even though frequent blood transfusion in β thalassemia patients.

No significant difference in other serum parameters were observed between normal controls and the clinical cases studied.

6.3.3 Correlation of serum NTBI values with the other biochemical parameters in different groups:

By comparing the serum NTBI values with the other parameters studied, no perfect correlation was observed for any of the analyte in any groups’ subjects. However in diabetic patients’ group, serum total cholesterol; in hemodialysis patients’ group in pre HD samples serum urea, serum albumin and serum globulin; in post HD sample serum uric acid and in β thalassemia major patients’ serum iron found to be correlated strongly with serum NTBI values.

6.4 Conclusion:

BPS based colorimetric method is a simple, rapid, economical and feasibly adopted method for NTBI estimation in a medium scale Indian laboratories. To make the method more user friendly, the microwell version should be practiced.

The BPS detectable NTBI isoforms are significantly elevated in β thalassemia patients, diabetes patients and hemodialysis patients especially before dialysis.

No significant difference was observed in mean serum NTBI values in between male Vs female; patients < 50 years of age Vs patients ≥ 50 years of age; vegetarians Vs non vegetarians and alcoholics Vs non-alcoholics.

Further evaluation along with other relevant parameters is required to establish the significance of serum NTBI estimation in conditions associated with oxidative stress and in iron overload patients receiving chelation therapy.

The key need in this area is to estimate serum NTBI with two or more different methods simultaneously from the identical samples of different clinical conditions. This will register the significance of the specific isoform in definite clinical condition, which is measured by a particular method.