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***Research Paper***

# STUDY OF NON TRANSFERRIN BOUND IRON IN PATIENTS OF MYOCARDIAL INFARCTION

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**Abstract**

Heart disease is the leading cause of death for both men and women. Coronary heart disease is the most common type of heart disease. Cardiovascular pathology has multi factorial origin. One of the causes is increased supply of free radicals. Iron being transitional metal carries the potential to generate the reactive oxygen species (ROS) especially when it is not bound to its shielding molecule like transferrin in the circulation which is broadly known as non transferrin bound iron. Iron mediated heart diseases have been explained by several pathways, mainly by tissue cell loss and atherosclerosis through ROS production. ROS known to be produced by NTBI are evident at the site of inflammation and contribute to cell damage. The present study was performed with 50 control subject and 25 myocardial infarction patients. The level of NTBI was measured in both the groups along with other routine biochemical parameters to find out the significant change in non transferrin bound iron level and its correlation with other analytes. We did not find elevated value of serum NTBI by the BPS detectable isoforms of non transferrin bound iron in myocardial infarction patients. Further study is required which do the parallel analysis of samples collected in affixed clinical cases through different methodology.

Key words: Coronary heart disease, reactive oxygen species, non transferrin bound iron, myocardial infarction, Transferrin.

## INTRODUCTION

Heart disease is the leading cause of death for both men and women. Coronary heart disease is the most common type of heart disease, killing more than 385,000 people annually [1]. Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, results from the interruption of blood supply to a part of the heart, causing heart cells to die. Routinely done diagnostic tests are an electrocardiogram (ECG), echocardiography, cardiac MRI and various blood tests. The most often used blood markers are the creatine kinase-MB (CK-MB) fraction and the troponin levels.

Cardiovascular pathology has multi factorial origin. One of the causes is increased supply of free radicals or non radical oxidant which causes number of organ dysfunction. This link can also be observed as higher cardiac complications in thalassemia and other overload conditions where high iron enhances the oxidative stress.

Iron being transitional metal carries the potential to generate the reactive oxygen species (ROS) especially when it is not bound to its shielding molecule like transferrin in the circulation. The free form of iron is found to be the conclusive culprit for many undesirable changes in the body. The toxicity of iron is mostly due to the over production of reactive oxygen species generating the free radicals which cause damaging effect in the liver, heart, pancreas, thyroid and central nervous system mainly through Fenton and Haber-Weiss reaction [2].

Iron mediated heart diseases have been explained by several pathways, mainly by tissue cell loss and atherosclerosis through ROS production. As a well known fact atherosclerosis is one of the major causes of coronary heart disease. Inflammation theory describes atherosclerosis as proliferation of smooth muscle [3]. ROS known to be produced by NTBI are evident at the site of inflammation and contribute to cell damage [4,5]. These reactive species are identified to cause LDL oxidation which induces plaque formation. Various researchers demonstrated that $Fe^{2+}$ could induce platelet aggregation in dose dependent manner [6,7,8,9]. Iron induced oxidative damage has also been linked to increase the loss of cardiomyocytes due to apoptosis [10]. Altered cellular metabolism and / or iron mediated stimulation of cardiac fibroblast that may contribute to increased myocardial fibrosis. In case of reperfusion injury too through ROS iron causes lipid peroxidation, fragility of intracellular lysozyme and release of hydrolytic enzymes and ultimately causing myocardial and endothelial cell necrosis [11,12,13].

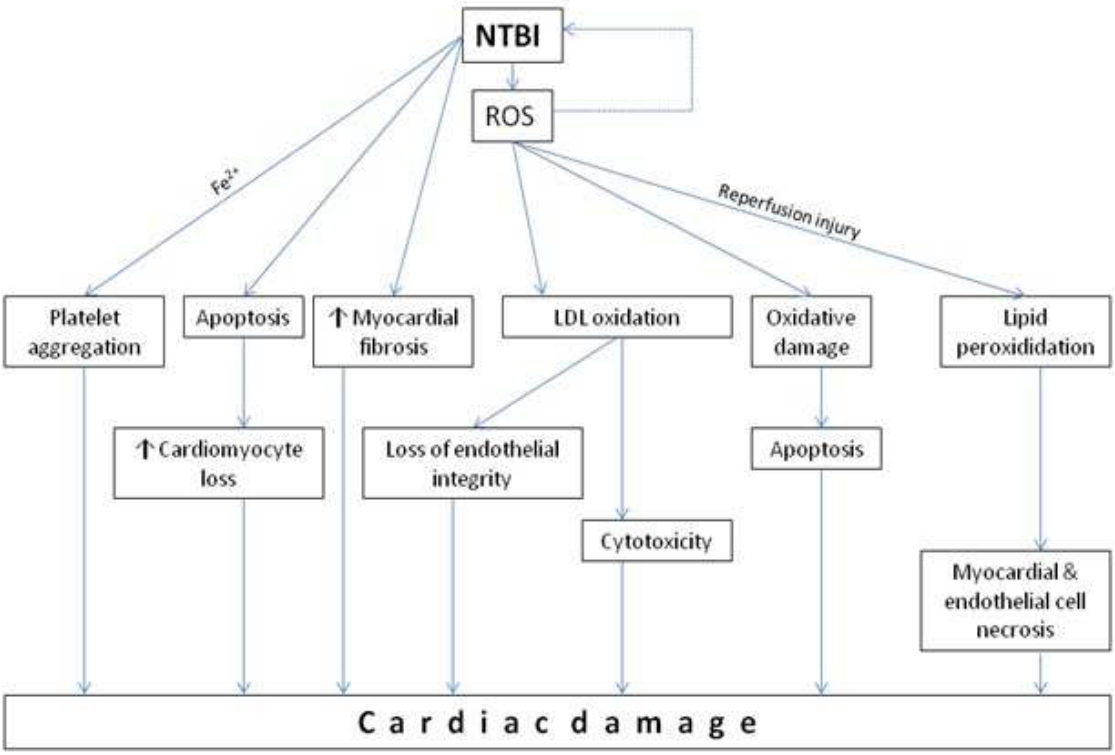



**Figure1: NTBI induced Cardiac damage**

The nature of non transferrin bound iron (NTBI) is unclear at present time. Various names were explored to address probably different isoforms of this molecule according to their biochemical nature by different researchers like free iron, catalytic iron, labile iron, redox active iron etc. They are also addressed according to their reactivity with various chelators like Bathophenanthroline detectable iron, Desferrioxamine- chelatable iron, Bleomycin detectable iron which reveal the heterogeneity of this molecule. The detail explanation of these terms was reviewed in our previous paper [14].

Serum NTBI estimation is a better anayte to be checked for iron toxicity, as compare to stable protein bound iron as it is the main active culprit to cause various detrimental effects through its redox activity.

Non transferrin bound iron (NTBI) is the form of plasma/ serum iron which is devoid of its classical carrier protein transferrin (Tf). Presences of NTBI in the serum have been proposed as a new risk factor for diabetes mellitus (DM), myocardial infarction (MI), renal damage and many other non iron-over load conditions. However due to lack of clarity about the nature of NTBI and unavailability of the gold standard method for its estimation the reports of this analyte are quite controversial.

The present study was performed with 50 control subjects (group I) and 25 myocardial infarction patients (Group II). The level of NTBI was measured in both the groups along with other routine biochemical parameters. The results were further analyzed to find out the significance of serum NTBI analysis in MI patients and to check the correlation of serum NTBI values with the other routinely estimated parameters.

## MATERIALS AND METHODS

### Subject selection

55 healthy volunteers (age and gender matched) as a control (Group I) from general population and 25 patients with myocardial infarction (Group II) were enrolled for the study after taking their written concent.

MI patients with chest pain only were included in the study. All subjects with known acute and chronic illness were excluded in control. Patients having the acute and chronic illness other than the suggested one were also excluded form Group II.

### Sample collection

Blood sample were collected from the cubital vein into plain bulb for S. NTBI and the other parameters except blood sugar estimation in both the groups. Single sample was collected for Group I where as two random samples were collected from MI patients i.e. first at the time of hospitalization and second at 24 hours after admission. Serum was separated within 30 minutes of sample collection by centrifugation. All tests except NTBI were performed within 24 hours after collection. For S.NTBI estimation the samples were stored at -55°C. All of the samples for S.NTBI estimation were thawed at the time of analysis within 3 months of their collection. Haemolyzed samples were discarded.

Blood was collected in fluoride bulb for sugar estimation.

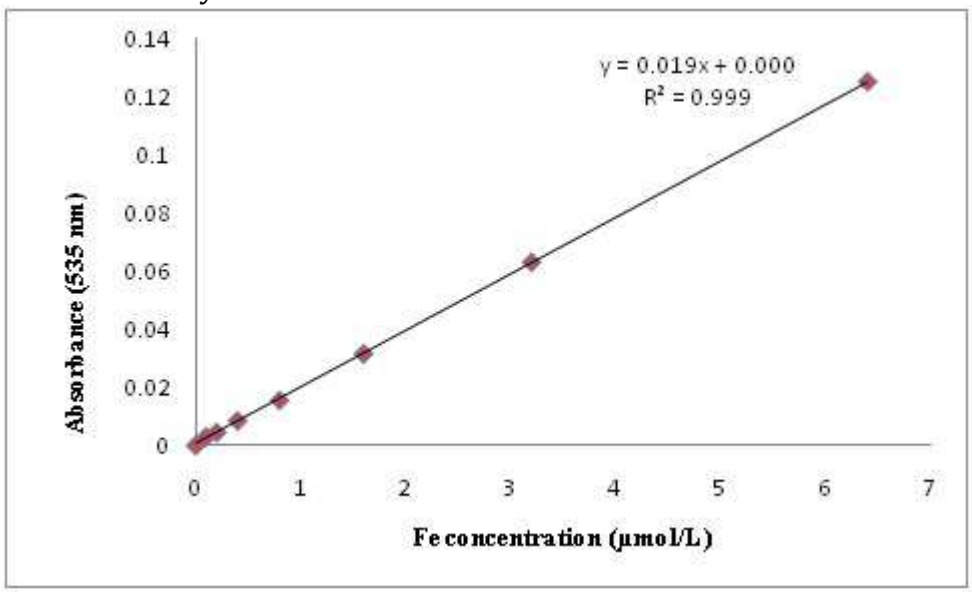
### Instrumentation and methodology

Laboratory investigations were carried using ERBA-XL-300 fully auto analyzer and Statfax-3300 semi auto analyzer. Estimation of S.NTBI was carried out by bathophantroline disulphonate (BPS) based chromogenic method suggested by [15] using double beam UV spectrophotometer after preparing the standard graph with high linearity.

All statistical analysis was performed using SPSS 12.0.

### Standard curve

Standard curve was constructed as indicated in the reference method using various concentration of ferrous ammonium sulfate [15] which shows excellent linearity and was further used in the analysis.




## RESULTS

Table-1 shows the level of serum NTBI in different groups studied. The values have been expressed as their mean ± SD and their p value.

**Table 1: Serum NTBI values in different groups**

| Group | | S. NTBI (µmol/L) | p value |
|---|---|---|---|
| I | | 0.02 ± 0.06 | -- |
| II | Day 1 | 0.05 ± 0.08 | 0.104 |
| | Day 2 | 0.08 ± 0.14 | 0.083 |

Table 2 shows the % clinically significant S.NTBI positivity by considering 0.3 µmol/L as the cut off value.

**Table 2: Clinically significant serum NTBI positivity in different groups' subjects (n=80)**

| Group | | Number of significantly positive sample | % significant positivity |
|---|---|---|---|
| I | | 0 | 0.00 |
| II | Day 1 | 0 | 0.00 |
| | Day 2 | 1 | 5.88 |

Table 3 shows the p value of different analytes by comparing the mean values of these analytes between the group I and group II.

**Table 3: Various biochemical parameters of group II (n= 25)**

| Biochemical parameter | Day 1 | | Day 2 | |
|---|---|---|---|---|
| | Mean ± SD | p value | Mean ± SD | p value |
| RBS (mg/dl) | 143.64 ± 58.63 | 0.013 | 119.33 ± 31.14 | 0.316 |
| S. Urea (mg/dl) | 52.72 ± 77.75 | 0.148 | 36.61 ± 17.53 | 0.105 |
| S. Creatinine (mg/dl) | 1.64 ± 1.94 | 0.048 | 1.14 ± 0.54 | 0.026 |
| S. Total cholesterol (mg/dl) | 147.12 ± 31.73 | 0.007 | 128.77 ± 35.57 | 0.000 |
| S. Triglyceride (mg/dl) | 138.08 ± 86.66 | 0.527 | 142.50 ± 126.43 | 0.795 |
| S. HDL cholesterol (mg/dl) | 38.48 ± 13.05 | 0.017 | 38.44 ± 12.76 | 0.034 |
| S. LDL cholesterol (mg/dl) | 63.76 ± 23.77 | 0.000 | 53.27 ± 19.11 | 0.000 |
| S. VLDL cholesterol (mg/dl) | 27.4 ± 17.31 | 0.099 | 30.38 ± 25.04 | 0.478 |
| S. CPK (U/L) | 1371.76 ± 1470.11 | 0.000 | 580.72 ± 438.80 | 0.000 |
| S. CPK - MB (U/L) | 59.28 ± 66.42 | 0.001 | 25.05 ±16.56 | 0.003 |
| S. LDH (IU/L) | 1229.76 ± 941.74 | -- | 996.50 ± 625.27 | -- |
| S. AST (IU/L) | 254.96 ± 482.66 | 0.024 | 127.94 ± 207.62 | 0.046 |
| S. ALT (IU/L) | 162.04 ± 383.99 | 0.083 | 94.22 ± 189.57 | 0.131 |
| S. Albumin (gm/dl) | 4.05 ± 0.35 | 0.589 | 3.75 ± 0.47 | 0.017 |

**Table 4: Pearson correlation coefficients (r) between NTBI and other clinical variables among group I and group II (n=80)**

| Biochemical parameter | Group I | Group II | |
|---|---|---|---|
| | | Day 1 | Day 2 |
| RBS/FBS (mg/dl) | 0.06 | -0.03 | -0.22 |
| S. Urea (mg/dl) | 0.01 | -0.18 | -0.10 |
| S. Uric acid (mg/dl) | 0.08 | ND | ND |
| S. Creatinine (mg/dl) | -0.03 | -0.18 | -0.06 |
| S. Total cholesterol (mg/dl) | -0.07 | -0.01 | -0.16 |
| S. Triglyceride (mg/dl) | -0.01 | -0.11 | -0.22 |
| S. HDL cholesterol (mg/dl) | -0.19 | 0.21 | -0.24 |
| S. LDL cholesterol (mg/dl) | -0.05 | -0.11 | -0.10 |
| S. VLDL cholesterol (mg/dl) | 0.07 | -0.11 | -0.12 |
| S. CPK (U/L) | 0.11 | -0.13 | -0.11 |
| S. CPK–MB (U/L) | 0.10 | -0.10 | 0.10 |
| S. LDH (IU/L) | ND | -0.28 | -0.07 |
| S. AST (IU/L) | -0.06 | -0.19 | 0.36 |
| S. ALT (IU/L) | -0.01 | -0.20 | 0.33 |
| S. Total protein | 0.17 | ND | ND |
| S. Albumin (gm/dl) | 0.24 | 0.23 | 0.16 |

## DISCUSSION

Typical symptoms of acute myocardial infarction include sudden chest pain, shortness of breath, nausea, vomiting, palpitations, sweating,and anxiety [16]. Women may experience fewer typical symptoms than men, most commonly shortness of breath, weakness, a feeling of indigestion, and fatigue [17]. A sizeable proportion of myocardial infarctions (22–64%) are "silent", that is without chest pain or other symptoms [18].

Important risk factors are previous cardiovascular disease, older age, tobacco smoking, high blood levels of low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) cholesterol, diabetes, high blood pressure, lack of physical activity and

obesity, chronic kidney disease, excessive alcohol consumption, the abuse of illicit drugs (such ascocaine and amphetamines), and chronic high stress levels [19, 20, 21]

The role of iron in coronary heart disease (CHD) was proposed by Sullivan in 1981 as an explanation for the sex difference in risk [22]. Now an impressive body of evidences indicates that free radicals and non-radical oxidants might cause a number of cardiovascular dysfunctions. Both direct damage to cellular components and/or oxidation of extracellular biomolecules, e.g. LDL might be involved in the etiology of cardiovascular diseases. The key molecules in this process seem to be iron and copper ions which catalyse formation of the highly reactive hydroxyl radical [23]. In acute myocardial infarction LPI is suggested to be elevated when large fluxes in iron occur [24, 25].

Several studies have successfully demonstrated the correlation between the iron intake, body iron stores and cardiovascular risk in the general population [26, 27, 28, 29]. Numerous pathways have been indicated to explain iron mediated heart diseases which are mainly by tissue cell loss and atherosclerosis through ROS production.

However many other studies refuse the probable correlation of iron with cardiovascular risk, which could be [30, 31, 32, 33, 34, 35].

The results are quite controversial, which could be due to selection of improper indicators or inappropriate techniques to estimate the respective indicator. Many researchers explained the toxic role a binding of iron due to its redox activity. However in normal physiological conditions most of the circulatory iron is in association with apotransferrin which not only transport the iron but also hide its toxic effects. Due to unclear nature of NTBI, gold standard method is not available at present for its estimation. However, various researchers apply different analytical approaches which target one or the other characteristics of NTBI to estimate it [36].

Basically NTBI or its sub fractions get estimated by indirect method using anti-tumor antibiotic bleomycin, chelation of NTBI with chelator followed by its separation and estimation using various analytical techniques and direct estimation of NTBI with iron sensitive fluorescent probe. Most of these methods are complicated costly and demanding specialized requirement of instruments and accessories. Furthermore there is no comparative data available which indicate that these methods estimate the identical analyte. This could be the reason why if we see the level of NTBI or its subfraction reported by different researchers in various clinical conditions they are non identical.

We had estimated the S.NTBI by a chromogenic method using a chromogenic chelator bathophenanthroline in patients suffering from control group and the group of patients suffering from myocardial infarction.

In the present study we have taken up 80 subjects, out of which 55 normal subjects and 25 patients of myocardial infarction were included.

The mean S.NTBI level we found in normal subjects was 0.02 ± 0.06 µmol/L. Many researchers reported absence of NTBI or its sub-fraction in normal subjects by diverse methodology [37,38]. However with the advantage of more sensitive methods low levels of NTBI have been reported in normal subjects too by various researchers. Jakeman et al (2001) reported ≤ 0.1 µmol/L NTBI in normal subjects with graphite furnace atomic absorption spectrometry [39]. 0.154 ± 0.328 µmol/L and 0.038 ± 0.07 µmol/L NTBI and LPI have been reported respectively by Caroline et al (2005) with a fluorescent based method [40]. Other workers reported 0.5 ± 0.2 µmol/L free iron in healthy control with the use of BPS based spectrophotometric method [41,37]. With HPLC based method Lee et al (2006) found 0.04 ± 0.13 µmol/L NTBI in control subjects [42].

With the use of bleomycin based colorimetric method, Lele et al (2009) reported 0.1± 0.06 µmol/L catalytic iron in control group [43]. As suggested in the reference method 0.03 µmol/L of NTBI has been considered as the cut off and any value higher than this had been considered as significantly positive.

In the present study we have included a total of 25 myocardial infarction subjects. The level of NTBI was measured twice as explained in materials and method section. The mean S. NTBI value we found for the first day and second day specimens were 0.05 ± 0.08 µmol/L and 0.08 ± 0.13µmol/L respectively. In none of the cases, the serum NTBI was significantly higher than that of the control subjects (p=0.104 and 0.083 respectively). At the same time no significant difference in serum NTBI value was observed in-between day 1 and day 2. The mean serum NTBI we found for day 1 as well as day 2 was considerably lower than the values reported by Lele et al (2009) for catalytic iron in acute MI patients[43]. With the use of bleomycin based methodology, Lele et al (2009) reported rapid elevation of catalytic iron in MI patients peculiarly before the characteristic elevation of routinely used parameter troponin I [43]. However Daphne et al (2006) found no excess risk of CHD or MI in population with the higher NTBI value as compare to the low NTBI bearing population [44]. The key cause of this difference could be the difference in the methodology. As a specific isoform which is elevated in a certain clinical condition and is determined by a specific analytical method may or may not be determined by the other method, as they target different characteristic of NTBI isoforms. The mean values of all the analytes studied for control group were within the normal range. The overall high value of serum triglyceride could be due to random sample collection without overnight fasting (data not shown). As expected the mean value of serum AST, serum CK, CK-MB and serum LDH were significantly higher than the values of the control group for day 1 as well as day 2 samples. We find neither perfect positive nor perfect negative correlation in between serum NTBI with any of the analytes included in this study.

## CONCLUSION

In MI patients the level of known cardiac markers like serum AST, serum CK, CK-MB and serum LDH were elevated and crossed the normal range for day 1 as well as day 2 samples. We did not find elevated value of serum NTBI by the BPS detectable isoforms of NTBI in MI patients. There was no significant difference in the serum NTBI values in the 2 samples collected at 24 hour interval. Further study is required which do the parallel analysis of samples collected in different clinical cases through different methodology.

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