EVALUATION OF ISCHEMIA MODIFIED ALBUMIN IN HEPATITIS C POSITIVE HEMODIALYSIS PATIENTS: A PILOT STUDY

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Abstract

Background: Chronic hemodialysis (HD) patients are more prone for infection such as Hepatitis C virus (HCV) infection, which may be associated with increased oxidative stress (OS). Ischemia-modified albumin (IMA) levels rises not only during ischemia but also during chronic OS. Hence the study was aimed to evaluate the levels of IMA and its association with oxidative stress in HCV positive HD patients.

Methods: Twenty two HCV positive [HCV (+)] , 22 HCV negative [HCV (-)] patients with end stage renal disease (ESRD) on maintenance HD and 22 healthy subjects were enrolled in this cross-sectional observational study. Plasma IMA was determined by spectrophotometric Co (II) - albumin binding assay. Plasma MDA was determined by spectrophotometric method.

Results: Both MDA and IMA levels were significantly higher in HCV (+) and HCV (-) HD patients compared to controls (p < 0.001). A significant increase in MDA levels was observed in HCV (+) HD patients when compared with HCV (-) HD patients (p<0.001); however no change in IMA levels were noted between HCV (+) and HCV (-) HD patients. A negative correlation was observed between IMA and MDA levels in both HCV (+) (r= - 0.278, p=0.178) and HCV (-) HD patients (r= - 0.193, p=0.354) which were however not statistically significant.

Conclusion: Plasma IMA and MDA levels were elevated in both groups of HD patients when compared to controls. However, hepatitis C infection does not appear to cause any additional increase of IMA levels in HD patients.

Keywords: Haemodialysis; HCV infection; ischemia-modified albumin; oxidative stress.

1. Introduction:

Chronic hemodialysis (HD) patients are more prone for acquiring infection as HD requires vascular access for prolonged periods. Hepatitis C virus (HCV) infection is prevalent in patients undergoing hemodialysis (HD) with the prevalence of anti-HCV antibody in HD patients ranging between 10% and 55%.1 It has been shown that HCV infection by itself is characterized by an increase in free radical formation manifested by increased hepatic and serum levels of products of lipid peroxidation.2,3 Earlier studies have suggested and proposed Ischemia-modified albumin (IMA) to be a sensitive biomarker of cardiac ischemia. 4, 5 However recently the role and diagnostic significance of IMA in disorders of non-cardiac origin are being explored. 6 IMA is formed as a consequence of modification of albumin by reactive oxygen species (ROS). 7 A decrease in blood flow is believed to trigger ROS formation, which consequently modify the N-terminal portion of albumin leading to IMA formation. 8 The aggravated production of toxic oxygen radicals and related compounds has been noted in HD patients and patient with HCV infection and there is evidence of modification of albumin by both acute and chronic oxidative stress.4, 9 Few studies have evaluated IMA levels in end stage renal disease (ESRD) patients on HD and revealed a significant increase of IMA levels in HD patients compared to controls. 10, 11 To the best of our knowledge we could not find any reports on IMA levels and its relationship with oxidative stress in HCV (+) HD patients. Hence as a preliminary examination the present study was taken up to assess the IMA levels in HCV (+) and HCV (-) HD patients and to study its association with a well known oxidative stress marker, malondialdehyde (MDA).
2. Subjects and Methods:

2.1 Subjects: This cross-sectional study was conducted between January and September 2011, at Sri Venkateswara Institute of Medical Sciences (SVIMS) Tirupati, Andhra Pradesh, India. A total of 44 ESRD patients on HD were included in the study. Among them 22 HD patients were HCV positive [HCV (+)] (tested positive for anti-HCV antibody), and the remaining were HCV negative [HCV (-)]. Control group included 22 healthy subjects who tested negative for anti-HCV antibody, with no history of acute or chronic liver diseases and with normal renal functions. Acute renal failure patients on HD and HD patients with hepatitis B virus (HBV) infection were excluded from the study. The Institutional Human Ethics Committee approved the study protocol and written informed consent was obtained from all the participants enrolled in the study.

2.2 Hemodialysis Unit: The HD unit has two routine HD areas and one isolated area each for HCV (+) and HBV (+) patients. Patients negative for HCV before initiating dialysis were dialyzed in routine dialysis machines and those HCV (+) were dialyzed on dedicated machines in the isolated areas. Patients who seroconverted during HD treatment were shifted to the respective isolated area. All patients were essentially treated with three sessions of routine conventional HD each week (3 to 4 h/session), using standard polysulfone membranes and standard bicarbonate dialysate. The blood flow rate was 200-250 mL/min and the dialysate flow rate was 500 mL/min. All the HD machines were chemically disinfected between each dialysis session. Dialyzers were reused in all patients. Tubings were discarded after dialysis for all patients.

2.3 Specimen collection and Laboratory data: Samples of blood were collected from the patients in bottles containing heparin anticoagulant from the arterial end of the dialyzer before the start of the dialysis session. The blood samples were transferred into clean, dry, sterile centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The plasma was separated and stored at -80°C until further analysis. The medical records were reviewed for details regarding history, duration of dialysis, and other biochemical data.

2.4 Biochemical Assays: Ischemia-modified albumin (IMA) was determined by a manual colorimetric assay described by Bar-Or et al known as Co (II)-albumin binding assay. This method consists of adding a known amount of exogenous Co (II) to a plasma sample and measuring unbound Co (II) colorimetrically using dithiothreitol (DTT). The results are given in absorbance units (ABSU). Plasma thiobarbituric acid-reactive substances (TBARS) were estimated and expressed as MDA, as described by the method of Okawa et al.

2.5 Statistical Analysis: Data was expressed as median and its interquartile range as the data is non-normally distributed. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Statistical comparisons of the groups were made using Kruskal-Wallis one-way analysis of variance with posthoc analysis using a Mann-Whitney U test. Spearman rank correlation was used to explore the associations between IMA with MDA. A \( p \) value of less than 0.05 was considered statistically significant. The statistical analysis was performed using statistical software, SPSS version 16.0.

3. Results:
The results are shown in Tables 1, 2 and Figs. 1, 2, 3a, b. Table 1 and 2 shows the demographic, clinical and laboratory data of the controls and patients. There were no statistically significant differences between the groups with respect to age and weight \( (p>0.05) \). Dialysis duration was comparable between HD patients with and without HCV infection \( (p>0.05) \). AST and ALT were significantly increased in HD patients compared to controls, however there was no significant difference between HCV (+) and HCV (-) HD patients.

IMA levels in controls ranged from 0.32 to 0.69 with median 0.43 ABSU, for HCV (-) patients IMA ranged from 0.63 to 0.88 with median 0.83 ABSU and for HCV (+) patients it ranged from 0.50 to 1.14 with median0.78 ABSU. IMA was significantly higher in HCV (+) HD patients \( (0.80 \pm 0.15) \) and HCV (-)ve patients \( (0.80 \pm 0.07) \) compared to controls \( (0.43 \pm 0.08) \) \( (p<0.001) \), however, there was no significant difference in MDA levels between HCV (-) and (+) HD patients \( (p=0.854) \) as shown in Fig. 1. MDA levels in controls ranged from 0.18 to 1.41 with median 0.99 µmol/L, for HCV (-) patients MDA ranged from 2.19 to 4.57 with median 2.89 µmol/L and for HCV (+) patients it ranged from 4.25 to 7.87 with median 5.69 µmol/L. MDA levels were significantly higher in HCV (+) HD patients \( (5.80 \pm 0.91) \) and HCV (-) ve patients \( (3.08 \pm 0.67) \) compared to controls \( (1.00 \pm 0.24) \) \( (p<0.001) \), and the difference in MDA...
levels between HCV (-) and (+) HD patients was also statistically significant (p<0.001) as depicted in Fig.2.
A negative correlation was observed between plasma IMA and MDA levels in HCV (-) HD patients as shown in Fig 3a. Similarly, a negative correlation was observed between plasma IMA and MDA levels in HCV (+) HD patients as shown in Fig 3b. However, the correlations observed between IMA and MDA levels in HD patients without and with HCV infection were statistically not significant (p=0.178; p=0.354 respectively). No significant correlation between IMA and variables such as age, duration of dialysis, hemoglobin, total proteins, albumin, ALT and total bilirubin was found.

4. Discussion:
IMA is relatively a new marker, which has been studied mostly in the context of cardiac ischemia. However it is being revealed that IMA levels do rise in conditions not related to cardiac ischemia such as in diabetes 6, ESRD 13 and HD. 11 Although studies have reported elevated IMA levels in ESRD patients10,11,14 the impact of HCV infection on IMA levels in HD patients has not been explored further. Hence this may be considered as a novel study which has tried to evaluate the influence of HCV infection on plasma IMA levels in HD patients.
In the present study, an increase in IMA levels in both HCV (+) and HCV (-) ESRD patients on HD were observed when compared to controls which is in agreement with the literature 10, 11 The increase of IMA could be attributed to the increase of oxidative stress normally observed in HD patients. Structurally modified albumin cannot bind to cobalt used in the assay, which leads to availability of more free cobalt for reacting with dithiothreitol to develop more color indicating higher IMA levels. The test is hence dependent upon the presence of normal or modified albumin, and also maybe upon the albumin levels. If the albumin levels are decreased as seen in HD, it may lead to more amount of free unbound cobalt and higher IMA levels. This low albumin levels coupled with modifications of the existing albumin due to ROS may explain the increase in the test result in the HD groups when compared to controls.
The oxidative status of subjects was determined by also measuring MDA, a well known and accepted OS marker, which showed a significant increase in HCV (+) HD patients when compared with HCV (-) HD patients and controls. These findings indicate that HCV infection which by itself is known to cause oxidative stress15, 16 is further augmenting this state of oxidative stress in ESRD patients on maintenance HD. It is well established that ESRD by itself is also associated with OS.13 Therefore it appears that HCV infection is adding onto the burden of oxidative stress in HD patients.17 Similarly we anticipated that IMA levels will be elevated in HCV (+) HD patients, as few studies have advocated that there is a modification of albumin in both acute and chronic oxidative stress leading to elevated IMA levels. 18, 19 But on the contrary we did not find any further significant increase in IMA levels in HCV (+) HD patients when compared to HCV (-) HD patients.
Bilirubin is a well known antioxidant and when bound to human albumin scavenges free radical thereby protecting albumin bound fatty acids and most likely the protein itself from oxidation.20 We however found no significant difference in bilirubin and albumin levels between the HD groups, which may partially explain the similar results of IMA in both groups. An invitro study of Erdern et al reported an increase of IMA and MDA levels with phototherapy, suggesting that this may be due to high levels of bilirubin which acts as an antioxidant.21 Elevated bilirubin levels in a state of OS may prove to be beneficial as an antioxidant. Hence there appears to be a plausible underlying relation between bilirubin and IMA levels. And it is well known that HCV infection may not always be associated with high bilirubin or AST levels as was observed in this study.
So far few studies8, 22 reported that IMA is likely to serve as an effective oxidative stress indicator and serum IMA levels have a close relationship with oxidative balance in ESRD patients. IMA was generated in vitro via exposure of albumin to hydroxyl radicals.21 However, in the present study a negative correlation was observed between IMA with MDA levels in both HCV (+) and HCV (-) HD patients, which was however not significant.
A study reported that anaemia, is related to mild hypoxia owing to low hemoglobin levels and this hypoxia is responsible for the alteration in metal albumin binding capacity which may also be a possible contributory factor to increased IMA levels in HD patients.10 In the present study we found a significant decrease of hemoglobin levels in both groups of HD patients when compared to controls, but with no significant
change of hemoglobin levels between the HD patients with and without HCV infection. Probably as the factors proposed to influence IMA levels such as bilirubin, albumin and hemoglobin status are all similar between the HCV (+) and HCV (-) HD patients, we could not demonstrate any significant change in IMA levels in HCV (+) HD when compared to HCV (-) HD patients. The influence of these factors needs to be addressed in further experimental studies supported by clinical designs which may include patients with higher bilirubin levels and studying its association with IMA. Effect of anemia and albumin levels on IMA also needs to be studied further.

It is evident from this study that HCV infection is contributing to the burden of OS in HD patients in the form of a more pronounced elevation of MDA levels. This increase in OS has however not reflected in IMA levels in the present study. Another plausible explanation may be that the oxidative stress encountered in HD has by itself has contributed sufficiently to the modification of the albumin molecule and imposing more OS may not cause any further modifications of albumin. Therefore IMA levels may not truly reflect the severity of OS, but nevertheless it is a definite indicator of OS and for all practical purpose demonstration of OS is by itself sufficient to understand the underlying pathophysiology.

4.1 Limitations of the study: Only one oxidative stress marker was used to portrait oxidative status. Other oxidative stress markers which are recognizably elevated in HD population such as markers of protein, lipids and DNA damage were not measured. This should be considered as a pilot study and the results should be replicatd in larger studies to support the presented results.

Conclusions:
The present study is novel in that it investigated serum IMA levels in ESRD patients on maintenance HD with HCV infection. As a preliminary study, it demonstrated that
1. IMA levels are increased in ESRD patients on maintenance HD When compared to controls. However the levels are similar in both HD patients with and without HCV infection.
2. HCV infection does not appear to cause any additional increase of IMA levels in HD patients.
3. As, there is no change in IMA levels between HCV (+) and HCV (-) HD patients, monitoring IMA concentrations for assessing the severity of oxidative stress may not be suitable, however it can also be considered as an indicator of oxidative stress in these patients.

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Author Contributions:
V. Pradeep Kumar: Collection and assembly of data; Dr.M.M.Suchitra: Conception, design and interpretation of data; A. Madhusudhana Rao: Data Analysis and Drafting of the article; Dr. Aparna R. Bitla: Statistical Analysis; DR. V. Sivakumar: provision of patients; DR. P.V.L.N. Srinivasa Rao: Critical revision of the article for important intellectual content; All authors read and approved the final manuscript.

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### Table 1: Demographic characteristics of the study groups

| Variables                  | Controls median (Range) | HCV (-) HD patients median (Range) | HCV (+) HD patients median (Range) |
|----------------------------|-------------------------|-----------------------------------|-----------------------------------|
| Number of Patients         | 22                      | 22                                | 22                                |
| Age (Years)                | 49 (30-70)              | 54.5 (22-64)‡                    | 47 (32-65)†‡§                    |
| Gender                     |                         |                                   |                                   |
| Male (%)                   | 11 (50.0)               | 16 (72)                          | 17 (77)                           |
| Female (%)                 | 11 (50.0)               | 06 (27)                          | 05 (23)                           |
| Weight (Kg)                | 55 (40-63)              | 61.2 (40-74)‡                    | 59.5 (32-85)‡§                    |
| SBP (mm Hg)                | 127 (120-135)           | 150 (100-190)***†               | 150 (110-190)***§                 |
| DBP (mm Hg)                | 80 (70-83)              | 90 (70-120)‡²                   | 90 (70-110)‡³                     |
| Duration of dialysis (months) | -----                  | 39 (26-47)‡                     | 44 (32-51)³                      |

Data represented as median (25th -75th Percentiles); HCV-hepatitis C virus; HD- hemodialysis; SBP-systolic blood pressure; DBP-diastolic blood pressure. Statistically significant (compared to controls * p value 0.05 to 0.01; ** p value 0.01 to 0.001; *** p value <0.001, ‡ not significant); (compared to HCV (-) HD patients † p value 0.05 to 0.01; †† p value 0.01 to 0.001; ††† p value <0.001, § not significant)
Table 2: Biochemical parameters in the study groups

| Variables         | Healthy Controls median (Range) | HCV (-) HD patients median (Range) | HCV (+) HD patients median (Range) |
|-------------------|----------------------------------|------------------------------------|-----------------------------------|
| Hemoglobin (g/dL) | 12.1 (10.9-15.2) ***             | 10.0 (6.0-14.5) ***                | 10.0 (6.6-16.4) ***§             |
| Glucose (mg/dL)   | 93 (56-170) ***                  | 97 (72-147) **§                   | 105 (54-189) ***§                |
| Urea (mg/dL)      | 23 (16-49) ***                   | 8.53 (2.43-13.5) ***              | 7.34 (4.58-14.60) ***§           |
| Creatinine (mg/dL)| 0.90 (0.20-1.30) **             | 138 (72-204) **                   | 137 (102-182) **§                |
| Cholesterol (mg/dL)| 99.5 (89-193) **              | 92 (60-223) **                    | 112 (74-234) **§                 |
| Triglycerides (mg/dL)| 82.5 (60-140) **         | 8.53 (2.43-13.5) ***              | 8.53 (2.43-13.5) ***             |
| HDL-C (mg/dL)     | 41.5 (30-54) *                   | 6.6 (6.0-7.4) **                  | 6.5 (5.8-7.5) **§                |
| Total Proteins (g/dL)| 7.8 (6.4-8.0) **             | 3.9 (3.4-6.0) **                  | 3.8 (3.5-5.1) **§                |
| Albumin (g/dL)    | 4.9 (4.2-6.0) **                 | 45.5 (29-66) **                   | 49 (28-79) **‡                   |
| ASL (IU/L)        | 21 (11-49) **                    | 36.0 (30-56) **                   | 44 (20-59) **‡                   |
| Total Bilirubin (mg/dL)| 0.55 (0.30-0.90) *   | 0.90 (0.50-1.40) *                | 0.80 (0.60-1.40) *§              |

Statistically significant (compared to controls * p value 0.05 to 0.01; ** p value 0.01 to 0.001; *** p value <0.001, § not significant); (compared to HCV (-) HD patients † p value 0.05 to 0.01; †† p value 0.01 to 0.001; ††† p value <0.001, § not significant); HD, hemodialysis; HCV, hepatitis C virus; HDL-C, high density lipoprotein cholesterol; ALT, alanine aminotransferase; ASL, aspartate aminotransferase.

Fig. 1. Plasma levels of Ischemia Modified Albumin (IMA) in healthy controls and in hemodialysis patients with and without HCV infection.
Fig. 2. Plasma levels of Malondialdehyde (MDA) in healthy controls and in hemodialysis patients with and without HCV infection

Fig. 3.a. Correlation between plasma IMA and MDA in HCV negative HD patients
Fig. 3.b. Correlation between plasma IMA and MDA in HCV positive HD patients