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Evaluation of Cardiac Markers in Chronic Renal Failure

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Abstract: *Chronic renal failure (CRF) results from progressive and irreversible destruction of nephrons, regardless of the cause. Patients with CRF are a major cardiac population. The diagnosis of Myocardial Infarction (MI) is difficult in these patients as they exhibit base line changes in electrocardiogram, echocardiogram and atypical cardiac symptoms. They are not fit for exercise tolerance test. Hence the diagnosis is by Biochemical markers.The present study considers the use of serum cardiac markers for the diagnosis of acute coronary syndromes in patients with CRF without ischemic heart disease with focus on CK MB and AST/SGOT.50 patients with CRF admitted into Government General Hospital and Nagarjuna Hospitals, Vijayawada were assessed for CK MB and AST. Of the 50 patients, 30 of them were on chronic maintenance hemodialysis and 20 were on conservative treatment. 15 of the patients on dialysis were studied for serum cardiac markers before and after dialysis. Patients with previous ischemic heart disease and other related diseases with CK MB elevation were excluded.CK MB levels were elevated in CRF patients than those obtained in healthy controls. They were elevated in those undergoing dialysis and also were elevated in 26% of patients post dialysis compared to levels predialysis. CRF patients had lower AST values compared to healthy individuals. They were also lowered in patients on dialysis but were increased post dialysis compared to predialysis values. Both CK MB and AST show variation in CRF patients but not as in MI. So cardiac markers should be interpreted with caution and other methods for cardiac evaluation should be done in these patients. The results were analyzed by applying Chi-Square test, p value calculated and compared among the three groups. P value <0.0001 and 0.01 which is significant.*

Keywords: *Myocardial Infarction, Electrocardiogram, Echocardiagram, Acute coronary syndromes, Hemodialysis.*

I. Introduction

Chronic renal failure results from progressive and irreversible destruction of nephrons, regardless of the cause. This diagnosis implies that the glomerular filtration rate (GFR) is known to have reduced for at least 3 to 6 months. Often there is a gradual decline in GFR occurs over a period of years.

Patients with chronic renal failure (CRF) are at major cardiac risk. Coronary artery disease is highly prevalent in these patients and accounts for much of their observed morbidity and mortality. In patients on dialysis more than one-half of the deaths are of cardiovascular aetiology [1].The diagnosis of Myocardial Infarction (MI) is quite difficult in these patients as they exhibit base line changes in electrocardiogram, echocardiogram and atypical cardiac symptoms. They are not fit for exercises tolerance test. Hence the diagnosis is by biochemical markers.

The sine qua non of the diagnosis of acute coronary syndromes is the elevation of the cardiac enzymes. Aspartate aminotransferase (AST) was the first one to be used, which subsequently stimulated a number of investigations on different compounds. Creatine phosphokinase (CK) and Lactate dehydrogenase (LDH) replaced AST which were later replaced by their isoenzymes. Since then CK MB has become the "gold standard" for the diagnosis of myocardial infarction (MI).

CK MB is mainly found in the heart muscle, but also in tongue, diaphragm, uterus, prostate and skeletal muscle, although in low amounts of only 1% to 3% [2]. After MI the serum CK MB increases within 3-8 hours. It peaks at 9-24 hrs. and returns to normal by 48-72 hrs. An elevated CK MB is usually specific of MI.CK MB, the traditional marker for myocardial injury loses its specificity in the setting of renal failure .In CRF patients, non specific modest elevations of CK MB can cause false positive results at rates of 20% to 30% in the absence of myocardial injury. This elevation has been attributed to increased reexpression of fetal CK MB in myopathic skeletal muscles in renal patients by Chan et al. Injured skeletal muscle undergoing regeneration may produce increased amounts of CK MB to reach the proportion found in myocardium.

AST is one of the group of transaminases and found in the cardiac muscle, liver, skeletal muscle, kidneys, pancreas, spleen, lungs, erythrocytes in decreasing order of concentration. Following MI [3,4] an increased level of AST activity appears in serum. It increases within 4-6hrs after onset of chest pain, peaks after

24-36 hrs and activity falls to within normal range by fourth or fifth day. AST levels are decreased (e.g.: by 20%-50%) in 10%-90% of dialysis patients. The reason is unknown. Many explanations have been advanced, including inhibition of transaminase activity in the serum by uremic toxins. AST values have been shown to increase after dialysis, the increase presumed to be due to (a) Removal of a dialyzable inhibitor (b) Increased release of the enzyme from erythrocytes in the extracorporeal circuit, and/or (c) Ultra filtration induced hemo concentration.

II. Aim Of The Study

The present study considers the use of biochemical cardiac markers for the diagnosis of acute coronary syndromes in patients with chronic renal failure without ischemic heart disease, with focus on CK MB and AST/SGOT. Study of the cardiac markers in patients with CRF on hemodialysis, not on dialysis and comparison of the cardiac markers before and after dialysis has been attempted.

III. Materials And Methods

The present study was conducted on 50 patients with chronic renal failure. The patients were admitted into Government General Hospital and Nagarjuna Hospitals, Vijayawada in October 2014. Their ages were ranging from 20-70 years. No specific reference of patients was made with reference to sex. None of these patients had any evidence of ischemic heart disease either by way of history and physical examination or evidenced by E.C.G.

Inclusion criteria:

1. Age 20-70 years
2. Patients with chronic renal failure (CRF).
3. Patients with End Stage Renal Failure (ESRD).

Exclusion criteria:

1. Previous ischemic heart disease.
2. Patients undergoing percutaneous coronary intervention.
3. Patients with trauma.
4. Patients with hypothyroidism.
5. Patients with recent convulsions.
6. Patients with recent cerebrovascular diseases.
7. Patients with muscular dystrophies and dermatomyositis.

Out of the 50 patients with chronic renal failure, 30 of them were on chronic maintenance hemodialysis (maintenance bicarbonate dialysis for 4 hours) three times a week and 20 patients were not on dialysis (on conservative treatment) 15 of the 30 patients on chronic maintenance hemodialysis were studied for serum cardiac damage markers before and after dialysis.

Samples of 25 healthy age and sex matched individuals served as controls. They do not have any evidence of either renal or heart disease. Random blood samples were collected into two clean bottles, one without anticoagulant and the other with coagulant (sodium fluoride and potassium oxalate). Serum was separated taking precautions to avoid hemolysis from the first bottle and the following tests were done.

1. Estimation of serum CK MB by Immunoinhibition method. Principle [5]: The serum sample is incubated with CK MB reagent containing antibody specific to CK-M submit which completely inhibits the CK-M monomer, leaving the activity of CK-B subunits unaffected. Thus, the CK MB activity is the samples is calculated from the CK-B activity not inhibited41, by the following reaction sequence.

$$\text{Creatine phosphate} + \text{ADP} \xrightleftharpoons[\text{Hexokinase}]{\text{CK-B}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightleftharpoons[\text{Glucose-6-phosphate}]{} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6- phosphate} \xrightleftharpoons[\text{Dehydrogenase}]{} \text{6-phosphogluconate} + \text{NADPH} + \text{H+}$$

Prewarm at 37ºC the required amount of working solution. Pipette 1ml of working solution and 0.05ml of serum/plasma. Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at the 300th second and subsequently few more reading

with 30 seconds interval at 340nm. Calculations: Calculate the average change in absorbance per minute Activity of CK-MB in IU/L = Δ Absorbance/min x 6752

2. Estimation of serum Aspartate aminotransferase (AST/SGOT) by International Federation of Clinical Chemistry, Kinetic method [6, 7]

Principle:

$$\alpha\text{- oxoglutarate} + \text{L – aspartate} \xrightarrow{\text{AST}} \text{Glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

$$\text{Sample pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}$$

Assay Procedure: Pipette 1000µl of working reagent and 100µl of sample. Mix well and aspirate. Calculation: The general formula for converting absorbance change into international Units (IU) of activity is: IU/L = (Δ A/min) x T.V. x10/ S.V. x Absorptivity x P Activity of AST = Δ Abs/min x1768

3. Estimation of serum Creatinine by Folin Wu Tungstic method [8]. Principle: Creatinine produces a red color with alkaline picrate solution. The rate of red colored complex formation is directly proportional to the creatinine concentration.

From the second bottle containing anticoagulant blood sample is processed for the tests:

1. Estimation of Blood Sugar by GOD-POD method [6, 10] Principle: The substrate D-Glucose is oxidized by Glucose oxidase to form Gluconic acid and hydrogen peroxide. The hydrogen peroxide so generated oxidizes the chromogen system consisting of 4-Amino Antipyrine and phenolic compound to a red quinonemine dye. The intensity of the color produced is proportional to the glucose concentration and is measured at 505nm.

2. Estimation of Blood Urea by Berthelot method [11, 12] Principle: Urea breaks down urea into Ammonia and Carbon dioxide. In alkaline medium ammonia reacts with Hypochlorite and salicylate to form dicarboxy indophenols a colored compound. The reaction is catalyzed by sodium nitroprusside. The intensity of the color is directly proportional to urea in the serum, which is measured at 578nm.

Statistical analysis: The results were analysed by applying Chi-square test, p value calculated and compared among the three groups.

IV. Results

The present study comprises of 50 cases of chronic renal failure and 25 healthy age matched controls. No specific reference was made to sex. The mean age, S.D. and SEM of control and test group are 40.2±9.4±1.92 versus 43.52±11.78±1.66.

Comparison of Controls and Cases:

50 cases with CRF have been studied out of which 30 patients were on chronic maintenance hemodialysis and 20 were not on dialysis. 15 of the 30 dialysis patients have been studied for serum cardiac markers before and after dialysis.

The mean, S.D., and S.E.M. of creatine kinase MB (CK MB), Aspartate aminotransferase (AST), random blood sugar (RBS), blood urea and serum creatinine of the control group are within the established normal values.

Table 1: Various study parameter values mean, S.D., S.E.M. and p values of Control and Test group

Variables	Mean	S.D.	S.E.M.	P value
CK MB in controls	10.28	5.07	1.03	<0.0001
In cases	22.60	16.96	2.39	Significant
AST in controls	27.16	2.78	0.33	<0.0001
In cases	18.46	2.78	0.39	Significant
RBS in controls	115.00	8.31	8.31	<0.0001
In cases	153.62	64.92	9.18	Significant
Bl. Urea in controls	20.12	3.44	3.44	<0.0001
In cases	132.72	41.68	5.89	Significant
S. Creatinine in controls	0.95	0.12	0.025	<0.0001
In cases	9.21	2.90	0.41	Significant

Table 1 shows the mean, S.D. S.E.M. and p values of various parameters in the control and test group.

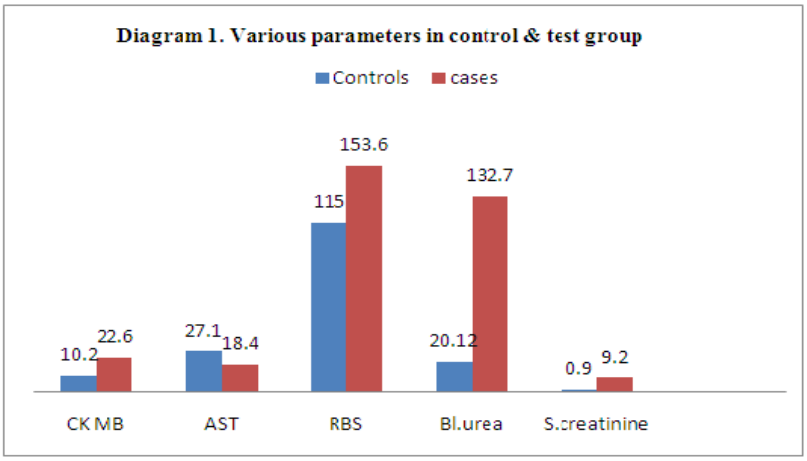

Diagram 1 shows means of the various parameters in cases and controls

Statistical Analysis:

CK MB levels are higher in patients with CRF than in those obtained in healthy individuals (CK MB 22.60±16.90±2.39 versus 10.28±5.07±1.03 [p<0.0001]). It is significantly elevated.

CRF patients have lower AST levels in comparison to healthy individuals (AST 18.46±2.78±0.39 versus 27.16±1.62±0.33 [p<0.0001]). It is also statistically significant.

The other parameters that were taken to support the diagnosis were random blood sugar, blood urea, serum creatinine and serum total cholesterol. RBS is significantly elevated in CRF patients in comparison to healthy individuals (RBS 153.62±64.92±9.18 versus 115.00±8.31±1.66 [p<0.0001] indicating that CRF is more prevalent in diabetic population. Blood urea and serum creatinine levels are also significantly elevated which are diagnostic of CRF (Blood urea 132.72±41.68±5.89 versus 20.12±3.44±0.68 [p<0.0001]) and (serum creatinine 9.21±2.90±0.41 versus 0.95±0.12±0.02 [p<0.0001]).

All the patients suffering from CRF were divided into two groups

- Those on dialysis
- Those not on dialysis

Comparison of CRF patients on dialysis and patients not on dialysis:

Table 2: Mean, S.D., S.E.M. and p values of CK MB &AST in patients on dialysis and those not on dialysis

Variables	Mean	S.D.	S.E.M.	p value
CK MB				
Dialysis patients	25.90	18.33	3.35	>0.07
Patients not on dialysis	17.60	13.36	3.07	Not significant
AST				
Dialysis patients	17.4	3.01	0.55	<0.01
Patients not on dialysis	19.25	1.74	0.39	Significant

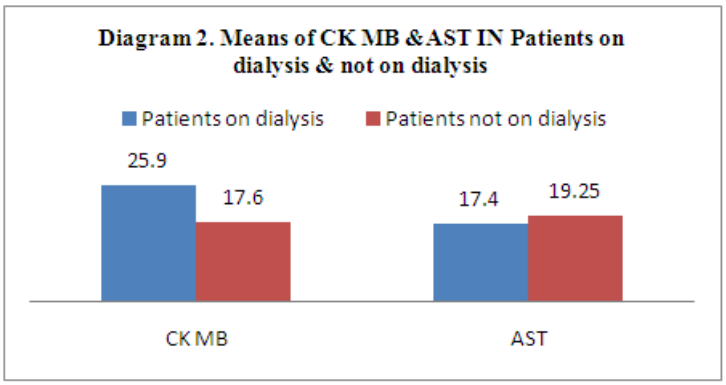

Diagram 2 shows the means of the cardiac enzymes in patients on dialysis and patients not on dialysis

Statistical Analysis:

CK MB levels are apparently elevated in patients on dialysis but are not statistically significant. (25.90±18.33±3.35 vs. 17.60±13.36±3.07 [p>0.07]).

AST levels are lower in patients on dialysis than in those obtained in patients not on dialysis(17.4±3.01±0.55 versus 19.25±1.74±0.39 [p<0.01]). It is of statistical significance.

Comparison of predialysis and post dialysis samples:

Table 3: Mean, S.D., S.E.M. and p values of CK MB &AST in predialysis and post dialysis

Variables	Mean	S.D.	S.E.M.	p value
CK MB				
Predialysis	35.53	19.97	5.33	<0.50
Post dialysis	30.93	18.85	5.04	Not significant
AST				
Predialysis	17.80	3.09	0.82	<0.01
Post dialysis	20.80	2.48	0.66	Significant

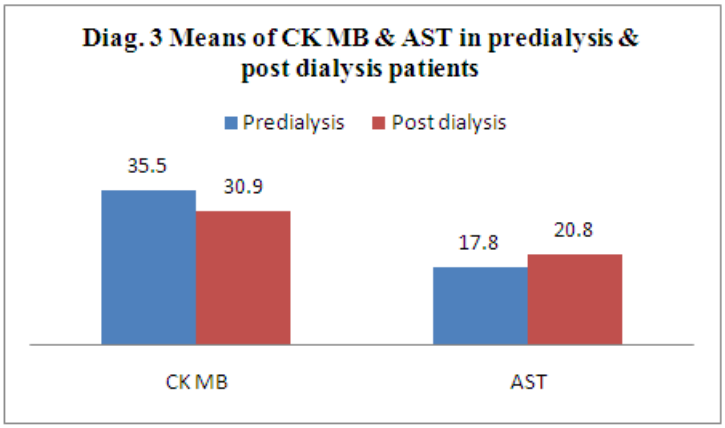

Diagram 3 shows the means of these cardiac enzymes in the predialysis and post dialysis samples.

Statistical Analysis:

CK MB levels are reduced post dialysis as compared to the levels predialysis 30.93±18.85±5.04 versus 35.53±19.97±5.33 [p<0.50]. It is not statistically significant.

Post dialysis AST values are greater than predialysis AST levels (20.80±2.48±0.66 versus 17.80±3.09±0.82 [p<0.01]). It is statistically significant.

V. Discussion

The present study included 50 cases of CRF out of which 30 cases were of patients on dialysis and 20 were of patients not on dialysis. 25 healthy individuals served as controls.

As per the results obtained it is found that patients with CRF frequently have elevated levels of CK MB compared to healthy controls. The AST levels are significantly decreased in dialysis patients in comparison to patients not on dialysis. The comparison between predialysis and post dialysis samples in the dialysis group show significant elevations of AST level post dialysis.

The results obtained in the present study can be discussed under the following categories.

1. **CK MB levels in CRF Patients:** CK MB levels are significantly elevated in CRF patients in comparison to healthy controls (22.60±16.96±2.39 versus 10.28±5.07±1.03 [p<0.0001]).

Comparative Study:

Name of the study	% ↑ in CK MB levels
Green T.R., Golper T.R. et al [15]	88% ↑ in a longitudinal study over a 3 yr period
Mclaurin et al [16]	30% ↑ in CK MB
Golam K. Alam & Davi B. Lieb [17]	30% ↑ in CK MB
Iliou MC et al [18]	7% ↑ in CK MB
Present study	26% ↑ in CK MB (>25U/L at 37ºC)

The 26% rise in CK MB in the present study is consistent with the data of Mclaurin et al. and Golam K. Alam and David B. Lieb. The possible mechanism of elevation could be:

- Increased reexpression of fetal CK MB in myopathic skeletal muscles in renal patients.[13]
- Elevation as a result of skeletal muscle damage [14].
- Chronic dialysis is associated with abnormal protein metabolism and muscle wasting which is a source of ↑ CK MB in these patients.

2. CK MB levels in dialysis patients: CK MB levels are elevated in dialysis patients as compared to patients not on dialysis but is not statistically significant (25.90±18.38±3.35 vs. 17.6±13.31±3.05 [p>0.07]). CK MB levels in dialysis patients in comparison to patients not on dialysis has not been addressed to date. In the present study an attempt was made to compare dialysis patients with those not on dialysis. The elevation shows no statistical significance, so the possible cause may be the above same reason.

3. CK MB levels in predialysis and post dialysis samples: Post dialysis CK MB is reduced in comparison to predialysis levels (Median 50 vs. 39 30.93±18.85±.04 vs. 35.53±19.97±5.33 [p>0.50]). It is statistically not significant. M Gurbilek et al[25] found in their study that predialysis versus post dialysis was not statistically significant (Median 69 vs. 54). Studies carried out by Tarakcioglu show that it was statistically significant (14.8±0.9 vs.13.1±0.9 U/L)[26]. The present study is consistent with that of M. Gurbilek et al.

4. Aspartate aminotransferase levels in CRF: Aspartate aminotransferase levels are lower in patients with CRF than those obtained in healthy individuals. Studies carried out by Fabrizi F. et al. state that there is significant decrease in AST levels in CRF patients when compared to healthy individuals and is statistically significant (18.46±2.78 vs. 17.6±8[p<0.0001]). The reason is not known, many explanations have been put forward including inhibition of transaminase activity in serum by uremic toxins.

5. AST levels in patients on dialysis: AST levels in patients on dialysis were lower than patients with CRF not on dialysis (17.6±13.31 vs. 19.25±1.74 [p<0.01]). A Study by Fabrizi F. et al [19] was made on AST levels in predialysis patients with CRF compared to those in healthy persons and dialysis patients. They state that AST activity in patients with CRF not requiring dialysis has not been adequately addressed to date. The reason for the decrease was not explained. The levels were of statistical significance (16.6±11.6 vs. 17.9±8 IU/L [p<0.01]).

6. AST levels in predialysis and post dialysis samples: Post dialysis AST levels are increased in comparison to predialysis AST levels (20.80±2.48±0.66 vs. 17.80±3.09±0.82 [p<0.01]).It is statistically significant. Comparison of AST levels in predialysis and post dialysis samples has not been addressed to date. Here in this study an attempt has been made to compare serum AST levels in predialysis and post dialysis samples. The increase in AST levels after dialysis could be due to

- Removal of a dialyzable inhibitor
- Increased release of the enzyme from erythrocytes in the extra corporeal circuit and/or
- Ultra filtration induced hemoconcentration [5].

Other parameters in CRF: Random Blood sugar is significantly increased in CRF patients compared to controls. Diabetes mellitus is the cause of 35% of new cases of ESRD annually [15].Blood urea and serum creatinine are also statistically elevated supporting the diagnosis of CRF. increased but not of statistical significance. The high burden of cardiovascular disease in chronic kidney disease (CKD) reflects a combination of traditional and nontraditional risk factors [9]. Patients with CKD have a higher prevalence of traditional risk factors compared to the general population (e.g.: diabetes, hypertension, older age, smoking history and abnormal lipid profiles) [25,26,18]. The sample population that was analysed is small and analysis of a bigger size of population is required for accurate results.

VI. Conclusion

Patients with CRF are a major cardiac risk population [15]. However these biochemical markers of cardiac can be falsely elevated in patients with End Stage Renal Disease (ESRD). Chronic dialysis patients without acute ischemic heart disease frequently had increased creatine kinase and CK MB levels. Serum aminotransferase values are decreased [e.g. by 20% - 50%] in 10% -90% of dialysis patients. They are elevated post dialysis compared to predialysis values. Serum markers of myocardial damage in dialysis patients should be interpreted with caution.

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