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Study on the Profile of Serum Cardiac Biomarkers in Ischemic Heart Disease (IHD).

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ABSTRACT

It is to evaluate the diagnostic ability of the serum cardiac markers in myocardial ischemia by analyzing high sensitive troponin I, N-terminal pro-brain natriuretic peptide, Creatine Kinase, Creatine kinase-MB, Myoglobin, ischemia modified albumin and heart type fatty acid binding protein. Total no of 56 cases and 33 healthy individuals were enrolled in the study and the preliminary blood samples were collected. The subsequent blood samples were collected from the 38 ischemic patients at one hour who had shown positive TMT for ischemia, where other patients does not meet criteria of ischemia. A comparative study for the markers was done between control and cases, pre and post TMT of ischemic cases; finally area under the receiver operator curve was constructed to see the diagnostic efficiency for the markers. NTproBNP, IMA, hscTnI and CK-MB significantly raised in ischemia. NTproBNP (92.98 ± 18.76 and 112.52 ± 21.36) and hscTnI (1.80 ± 0.61 and 2.12 ± 0.60), shown significant elevation in post exercise ($p<0.0001$) compared to pre exercise. Receiver operating curve analysis indicates NTproBNP (AUC, 0.830), hscTnI (AUC 0.86), CK-MB (AUC 0.81), and IMA (AUC, 0.79), better diagnostic efficacy. Marked increase of serum hscTnI, NTproBNP, CK-MB and IMA with more AUC, can be considered as effective diagnostic makers in ischemia.

Keywords: myocardial ischemia, serum cardiac markers, tread mill test, diagnostic ability.

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INTRODUCTION

Coronary artery disease develops when there is damage due to fatty streak formation in which there is a focal increase in the lipoprotein contents in the intima of artery, followed by adhesion, penetration and accumulation of leucocytes forming the plaque. Inflammation in the walls of the coronary arteries leads to narrowed vessels, hence decreased blood flow and oxygen supply to the myocardium, known as ischemia resulting in angina [1]. In the diagnosis of ischemia primarily, ECG is widely used which is highly specific and effective in localizing the region of ischemia, however it is estimated that in one third of patients with AMI is not recognized by the physician or the patient because chest pain is atypical or absent [2]. The ECG can be misleading in 8% and 50% of the patients are arriving at emergency department with non-diagnostic ECG and it is also difficult to identify ischemia that occurs for short period which causes reversible effects by recovering the myocardium[3]. TMT is useful to identify the myocardial ischemia after exercise by observing ECG during exercise. It is said to be positive for myocardial ischemia when the horizontal or down sloping ST-depression of >1mm, 60 or 80ms after the J-point, ST elevation of > 1.0 mm [4]. In addition to ECG and other non-invasive procedures, serum biochemical cardiac markers are more helpful in the diagnosis rather than this high end and other expensive techniques [2]. During myocardial ischemia, there is increase in the serum biomarkers due to cardiac myocyte damage which are released in detectable range but the elevation is not similar at different time periods for each marker because of different release kinetics.

METHODS

This study was approved by Institutional Ethical Committee of Maharajah's Institute of Medical Sciences (MIMS) and informed consent was obtained from all the subjects.

Study Population

Total 56 subjects were enrolled complaining with chest pain and other ischemic symptoms for the first time without prior medication or treatment. These subjects were considered and enrolled in the study based on the criteria or guidelines of myocardial ischemia. To establish the diagnosis, blood samples were drawn from the individuals and on the advice of the cardiologist and physician, they were subjected to TMT-ECG. On confirmation of ischemia on TMT-ECG based diagnostic criteria, a second blood sample was collected from 38 patients within one hour after TMT. The serum samples of 18 subjects collected before TMT, who do not show any abnormality pertaining to IHD i.e., chest pain of non cardiac origin and two patients were unable to perform TMT due to dyspnoea, were discarded and excluded from the study. Finally, out of 56 cases, 38 patients with an age group ranging from 31 to 70 years and 33 age and sex matched healthy individuals comprises control were enrolled for the study.

Exclusion Criteria

The patients with renal failure, renal transplantation, pregnancy, arrhythmias, aortic dissection, acute heart failure, cardiac contusion, chemotherapy, myocarditis, diabetes mellitus, sepsis, severe neurological disorders, pericarditis, extreme exertion, old AMI cases, non atherosclerotic MI, left ventricular hypertrophy, muscular dystrophy and infectious diseases like HIV, Hepatitis.., were excluded from our study as these conditions lead to changes in some of the cardiac biomarkers. After preliminary screening for HIV and HbsAg, all the samples of controls, and ischemia groups were centrifuged and preserved with necessary precautions at -70^0 C until analysis.

Assay for Cardiac Markers

The parameters analysed were Fasting Blood Sugar (GOD -POD method, normal range: 70-110mg/dL)[5], blood urea (Urease method, normal range: 15-45mg/dL) [6], serum creatinine (Jaffe's method, normal range:0.8-1.4mg/dL) [7], liver function tests (total bilirubin - diazo method, normal range:0.4-1.2mg/dL [8]; serum glutamate oxaloacetae transamianse (SGOT- IFCC kinetic method, normal range: up to 46 IU/L) [9]; serum glutamate pyruvate transamianse (SGPT- IFCC kinetic method, normal range; up to 49 IU/L) [10] ; alkaline phosphatase – IFCC method, normal range: 42-141 U/L [11], were assayed routinely in consideration of exclusion and inclusion criteria. Serum lipid profile (total cholesterol - CHOD-PAP method, normal range: 140-230 mg/dL [12]; triglycerides (TAG - GPO-Trinder method, normal range: 25-160 mg/dL) [13] ; HDL –

phosphotungstic acid method, normal range: 30-65 mg/dL (The values of LDL 60-160 mg/dL and VLDL=TAG/5 were calculated as per Friedewald formula) [14]. Standard controls and samples were assayed routinely and analysed on the Transasia Erba fully automated analyser. Analysis of these parameters in post TMT samples were not carried out as these are not muscle leak enzymes.

Total CK activity was quantified by use of automated analyser assay based on N-acetyl cysteine active (NAC) with a normal limit of 24-170 IU/L [15]. The CK-MB was measured by immunoinhibition kinetic method of Erba kit with a reference range <25 IU/L [16]. Serum Myoglobin was determined by the Accubind Enzyme Immunoassay method with an normal limit ≤96ng/mL supplied by Monobind Inc (Lake Forest, CA, USA) kit for analysis [17]. Serum hscTnI was assayed by using Acculite CLIA microwells supplied by Monobind Inc (Lake Forest, CA, USA) with an upper limit of ≤1.3ng/ml [17]. Serum H-FABP was measured by double antibody sandwich ELISA using the kit of SunRed H-FABP ELISA kit (Shanghai) with minimum detection limit was 0.05ng/ml, serum NTproBNP was measured by double antibody sandwich ELISA by using the kit of SunRed NTproBNP assay (Shanghai) with an assay range of 2 to 360pg/ml. IMA was analysed by colorimetric method based on albumin-cobalt binding assay and standardisation was carried out [18].

Statistical Analysis

Independent sample t-test was used for the comparison of markers between normal and ischemic groups. Paired sample t- test was used for comparing between pre and post ischemic cases by using SPSS version 17.0 software. A value of P≤0.0001 was considered significant. To evaluate the ability of marker levels in ischemic patients, receiver operator characteristic curves (ROC) has been constructed for each marker and the area under the curves (AUC) for all the markers were obtained. Exact 95% confidence interval (CI) for binomial proportions was calculated to determine the diagnostic ability of the markers.

RESULTS

Sex distribution for males in control (n=33) and cases (n=38) 63.6% and 63.2% and the females contributed in control and cases 36.4% and 36.8% respectively, with a mean age in years in control was 50.76 ± 12.23, where as in ischemic cases was 53.16 ± 7.17. Out of the 56 cases enrolled, ischemia was confirmed in 38 subjects and rest of the subjects do not meet the criteria of diagnostic guidelines of ischemia and two cases were unable to perform TMT procedure due to dyspnoea.

Table 1: serum levels of parameters in control group demonstrating range and mean values (n=33).

Parameters	Minimum	Maximum	Mean	Std. Deviation
AGE	31	70	50.76	12.23
UREA (mg/dl)	18	44	34.58	6.75
CREATININE(mg/dl)	0.8	1.2	1.03	0.15
FBS(mg/dl)	75	106	92.97	6.61
T.CHOLESTEROL(mg/dl)	136	226	178.86	32.01
TAG(mg/dl)	73	184	151.58	45.61
HDL(mg/dl)	28	56	42.48	4.39
LDL(mg/dl)	54	149	115.21	35.54
VLDL(mg/dl)	15	41	29.55	8.345
TB(mg/dl)	0.8	1.2	0.98	0.14
DB(mg/dl)	0.1	0.4	0.40	0.13
AST(IU/l)	25	42	38.79	10.98
ALT(IU/l)	20	36	36.73	8.79
ALP(IU/l)	25	102	94.55	29.07

The characteristics of the basic parameters are shown in Table 1and 2 for control and ischemia cases respectively. The total cholesterol, TAG, HDL, LDL and VLDL (p<0.0001) were statistically significant, whereas, no significant difference is observed in FBS, urea, creatinine, bilirubin, AST, ALT and ALP in comparison with control. The ischemic patients having the levels of FBS ranging from 88 to 112 mg/dl with a mean of 92.58 ± 8.94 showing a significant difference of FBS from the control. Serum creatinine 1.0 to 1.4 mg/dl with a mean of 1.095 ± 0.18 and blood urea 22 to 48 mg/dl with a mean of 35.63 ± 6.09 do not show significant elevation in

ischemic cases. In case of lipid profile, total serum cholesterol ranging from 157 to 270 mg/dl with a mean of 227.8 ± 32.98, TAG 120 to 249 mg/dl having mean 191.8 ± 39.58, HDL 30 to 48 mg/dl with a mean of 35.66 ±3.98, LDL 93 to 181.87 mg/dl with a mean of 145.32 ± 26.21, VLDL 24 to 50 mg/dl with a mean of 38.47 ± 7.98, were registered in the present study were statistically significant (p<0.0001). The liver function test (LFT) panel, serum TB 1.0 to 1.5 mg/dl with mean value 1.02 ± 0.15, DB 0.2 to 0.4 mg/dl with a mean of 0.39 ± 0.09, AST 15 to 44 U/l with a mean of 38.34 ± 5.35, ALT 20 to 42 U/l with mean 36.32 ± 8.44, ALP 25 to 122 U/l with a mean of 95.45 ± 12.56. LFT do not show any significant change in ischemia cases as compared to control in our study.

Table 2: serum levels of parameters in ischemic group showing the range and mean values (n=38).

Parameters	Minimum	Maximum	Mean	Std. Deviation
Age	39	68	53.16	7.17
UREA(mg/dl)	22	48	35.63	6.09
CREATININE(mg/dl)	1.0	1.4	1.09	0.18
FBS(mg/dl)	88	112	92.58	8.94
T.CHOL(mg/dl)	157	270	227.82	32.98
TAG(mg/dl)	120	249	191.87	39.58
HDL(mg/dl)	30	48	35.66	3.98
LDL(mg/dl)	93	181	145.32	26.21
VLDL(mg/dl)	24	50	38.47	7.98
TB(mg/dl)	1.0	1.5	1.02	0.15
DB(mg/dl)	0.2	0.4	0.39	0.09
AST(IU/l)	15	44	38.34	5.35
ALT(IU/l)	20	42	36.32	8.44
ALP(IU/l)	28	122	95.45	12.56

Table 1 and 2 shows that the analysis of blood samples in ischemia cases and controls for total cholesterol, TAG, HDL, LDL, VLDL the difference were statistically significant(p<0.0001), but there is no significant difference is observed in FBS, blood urea, serum creatinine, total bilirubin, AST, ALT and ALP in comparison with healthy subjects.

Table 3: comparison of serum cardiac parameters between normal and ischemic group

Parameters	normal vs. Ischemic	No. Of cases	Mean	Std. Deviation
IMA (units/ml)	NORMAL	33	64.06	11.66
	ISCHEMIC	38	92.68	13.63
CK(IU/l)	NORMAL	33	121.21	29.90
	ISCHEMIC	38	124.89	48.72
CK-MB(IU/l)	NORMAL	33	18.36	4.32
	ISCHEMIC	38	31.63	13.90
myoglobin(ng/ml)	NORMAL	33	73.86	17.55
	ISCHEMIC	38	75.63	25.19
hscTnI(ng/ml)	NORMAL	33	0.49	0.27
	ISCHEMIC	38	1.80	0.61
NTproBNP(pg/ml)	NORMAL	33	56.11	35.51
	ISCHEMIC	38	92.98	18.76
H-FABP(ng/ml)	NORMAL	33	2.73	1.06
	ISCHEMIC	38	3.32	1.72

Table4: Comparison of serum marker levels in ischemia patients of pre and post TMT stages.

Parameter	Pre TMT Mean ± SD	Post TMT Mean ± SD	*t*- test	P value
Myoglobin(ng/ml)	75.63 ± 25.19	79.00 ± 24.60	1.40	=0.17
IMA(units/ml)	92.68 ± 13.63	90.76 ± 15.06	1.60	=0.11
CK(IU/L)	124.89 ± 48.72	128.79 ± 46.55	1.66	=0.70
CK-MB(IU/l)	31.63 ± 13.90	31.93 ± 13.35	0.43	=0.66
hscTnI(ng/ml)	1.80 ± 0.61	2.12 ± 0.60	6.82	<0.0001
NTproBNP(pg/ml)	92.98 ±18.76	112.52 ± 21.36	7.91	<0.0001
H-FABP(ng/ml)	3.32 ± 1.72	3.40 ± 1.59	1.30	=0.20

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Table 3. Contains the serum values of confirmed ischemic cases (before conducting of TMT) shows the statistical significance for HscTnI (p<0.0001), NTproBNP (p<0.0001), CK-MB (0.66) and IMA (p<0.11). There is no statistical difference between myoglobin (p=0.17), CK (p=0.70) and H-FABP (p=0.20). In ischemic subjects in comparison with control, NTproBNP with a mean of 56.11 ± 35.51 in control against IHD cases with 92.98 ± 18.76 indicates the significant elevation. The hscTnI showing with a mean of 0.49 ± 0.27 in control and mean of 1.80 ± 0.61 in IHD cases registered a significant raise. IMA with a mean of 64.06 ± 11.66 in control group and a mean of 92.68 ± 13.63 in IHD cases shows significantly raised level. Control group demonstrating the mean serum CK 121.21 ± 29.90 against IHD 124.89 ± 48.72 which is though raised but not significant. Serum CK-MB with a mean of 18.36 ± 4.32 in control in comparison with a mean of 31.63 ± 13.90 in IHD cases showed significant variation. The control group shows serum myoglobin with a mean of 73.86 ± 17.55 and in IHD cases a mean of 75.63 ± 25.19 which is statistically non significant. Serum H-FABP with a mean of 2.73 ± 1.06 in control and 3.32 ± 1.72 in IHD cases showed no statistically significant difference. The comparisons of serum cardiac parameters in pre and post TMT cases of ischemia are in Table 4. NTproBNP (p<0.0001) and hscTnI (p<0.0001) shows significant elevation in post TMT condition indicates the influence of exercise in elevation of markers.

The receiver operator (ROC) for the area under the curve (AUC) was constructed (Table 5 and Figure 1.) for each serum marker to evaluate the diagnostic ability in ischemic patients. The AUC for hscTnI is 0.86 (95% CI 0.70 – 0.98), NTproBNP 0.83 (95% CI 0.74 to 0.96) and CK-MB 0.81 (95% CI 0.74 to 0.92) (p<0.001) showed more area under the curve, when compared to other markers in ischemic subjects.

Table 5: Area under the curve for the parameters of ischemic subjects.

Test result variables	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower bound	Upper bound
NTproBNP	0.83	0.056	0.000	0.74	0.96
IMA	0.79	0.054	0.000	0.77	0.88
CK	0.66	0.067	0.051	0.57	0.78
CK-MB	0.81	0.047	0.000	0.74	0.92
Myoglobin	0.65	0.045	0.000	0.56	0.79
H-FABP	0.57	0.069	0.268	0.46	0.68
hscTnI	0.86	0.046	0.000	0.70	0.98

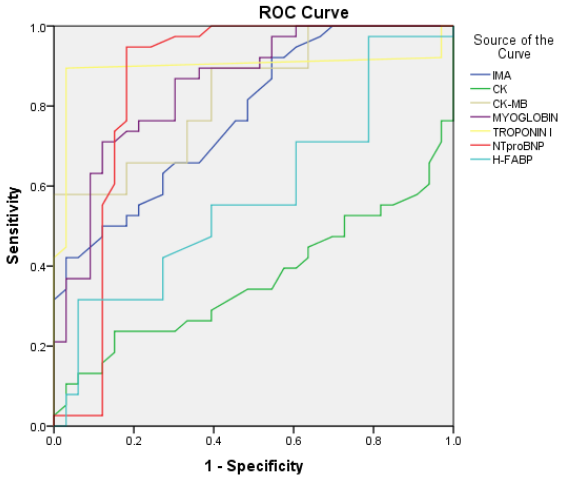

Figure 1: AUC of parameters in ischemic cases.

DISCUSSION

The ischemic patients demonstrating the serum levels of parameters rose significantly for lipid profile (p<0.0001) and there is no significant difference for FBS, urea, creatinine and liver functions test parameters. Our Study showed normal fasting glucose level in ischemic patients as diabetes mellitus is one of the exclusion criteria. However, hyperglycemia is a common associated phenomenon in ischemia and worsening the angina

has been reported by Ishihara M et al, 2003[19], Lickerman A et al, 1997 [20]. Serum creatinine and blood urea did not show significant elevation in ischemic cases and our results are similar to the study of Robert ostfeld et al 2010, reported that, urea and creatinine has no diagnostic influence on ischemia, and the elevation in some cases may be due to renal impairment and have no influence on ischemia [21]. In case of lipid profile, the serum values of lipids, is one of the risk factor of ischemia, increased in chronic stable IHD but there is no change with HDL when compared to control group, which is cardiac protective [22,23]. HDL reduces the oxidized lipid particles in LDL and turns them as less atherogenic and low HDL increases the risk of CAD [24]. The oxidised LDL has got sticky nature and infiltrates into intimal region of arterial wall and taken up by macrophages to cause foam cell formation followed by atherosclerosis. The LFT panel, do not show any significant change in ischemia cases as compared to control in our study. Some studies observed slight rise in liver enzymes especially AST and ALT in IHD due to fatty infiltration in the liver cell associated with obesity [25].

Comparison of serum levels of cardiac parameters of ischemic subjects (pre-TMT) with control group, ischemic cases in the present series had their serum levels of cardiac parameters raised significantly. NTproBNP, hscTnI, IMA and CK-MB were significantly elevated in ischemic group in comparison with control ($p<0.0001$). The increase of NTproBNP in serum samples of ischemic patients attributed to ventricular stretch of myocardium in ischemic patients. Studies show that IMA is a potential marker of recognising unstable angina rather than ECG but lacks specificity [26]. In one study, IMA is an ideal marker with high sensitivity in myocardial ischemia which can be detectable in the reversible early phase of acute coronary syndrome (ACS) where other markers are absent [27]. CK-MB showed significant variation ($p<0.0001$) and CK and myoglobin demonstrated a slight raise in comparison with healthy group but not significant. Our study correlates with study observed no variation of serum myoglobin ($p=0.73$) in angina and in healthy individuals [28]. H-FABP showed no statistical significant difference and our study do not correlate with the findings reported that, a significant rise of H-FABP in IHD and considered it as a sensitive marker in ischemia [29]. The hscTnI in IHD cases registered a significant raise and explains that it has diagnostic role in detecting stable angina and multiple vessel disease and our observations are in concurrence with the study by Eryol NK et al 2002[30]. To summarize the data in comparison between control and ischemic cases, it infer that NTproBNP, hscTnI, IMA and CK-MB showing significant rise in their serum levels.

In comparing the pre TMT samples with post TMT, the ischemia cases had their post TMT serum levels of cardiac parameters NTproBNP ($p<0.0001$) and hscTnI ($p<0.0001$) were raised significantly when compared to pre TMT of ischemic cases. Our study claims that, post exercise stress test increases the serum NTproBNP and cardiac troponins and in agreement with other studies [31]. The assessment of NTproBNP along with stress test improves the prediction of CAD, Suggesting that exercise-induced ischemia or its associated regional wall stretch abnormalities triggers the release of NT-pro-BNP before and immediately after exercise can distinguish patients with and without ischemia [32, 33]. Cardiac troponin (cTn) is sensitive and specific for the detection of myocardial damage but may not rise during reversible myocardial ischemia. Heavy exercises like stress test may severely lead to myocardial damage. The study of post-stress test troponin readings, in patients with stable angina pectoris as well as in patients with negative stress test result, may be of great help in detecting the patients with ischemia [34]. Ischemia Modified Albumin (IMA) has recently been shown to be a sensitive and early biochemical marker of ischemia. In our study we did not observe any significant difference between pre and post states of TMT and not correlating with the study of Z.P Koc et al 2012 [35], who reported a significant increase in post-TMT IMA levels as compared with pre-TMT IMA levels. Previous reports of post-exercise elevations in serum concentrations of CK-MB could result in cardiac injury [36]. Our study found that there is modest elevation of CK-MB in post TMT conditions of IHD cases however not showing significant variation as compared to pre TMT condition. In contrast to our study, Alon T Marmor [37] stated that, there was elevation of total CK after exercise but there is no rise in case of CK-MB in myocardial ischemia induced by treadmill exercise. Studies reported that there is no rise of CK, CK-MB and troponin followed by 20 minutes exercise session which does not result in myocardial necrosis [38].

Myoglobin was not found in detectable range in ischemic patients when compared with control and there is no statistical difference in pre and post TMT conditions. Baadsqaard et al 1984, reported that there is no change in the myoglobin in IHD before and after exercise induced angina pectoris and our results were concurrence with the study, that there is slight difference in serum of myoglobin in pre and post exercise states but not statistically significant in ischemic patients [39]. H-FABP shows no variations in our study and we accord with the study demonstrated that there is no significant elevation of H-FABP in ischemia as there is no

significant changes in the serum levels of ischemic patients in pre TMT and post TMT states[29]. To summarize the data in comparison between pre and post TMT serum levels of parameters, NTproBNP and hscTnI registered significant elevation in post TMT conditions (P<0.001) as compared to pre TMT, rather than other markers. From our study, it is clear that, the levels of markers in the serum shows significant variation, especially for hscTnI, NTproBNP, CK-MB and IMA. These markers may be released in ischemia due to transient damage of myocardium.

The receiver operator characteristic curves (ROC) for each marker demonstrated and more area for hscTnI, NTproBNP and CK-MB when compared to other markers in ischemic subjects. Our results were correlated with the study of Haaf P et al, reported, AUC for NTproBNP 0.79 (95% CI, 0.75-0.83), improves the diagnosis of suspected cases [40]. Milenko J. Tanasijevic et al, have found that the area under the receiver operating characteristic (ROC) curve was 0.71, 0.70 and 0.71 for myoglobin, cTnI and CK-MB [41], but in our study it was observed that, CK, H-FABP and myoglobin shows smaller AUC indicates less diagnostic significance and hscTnI showed more diagnostic efficacy with 0.86 AUC (p<0.0001) in identifying the ischemic condition. AUC demonstrated for IMA is 0.79, highly sensitive but poor specific for the presence of ischemia and results were similar to the study of Shu-ming, shown AUC for suspected ischemia cases was 0.75 [42].

CONCLUSION

In ischemia cases, the markers hscTnI, NTproBNP and CK-MB showed significant diagnostic ability in detection of ischemia. IMA also registered significant diagnostic efficiency. In view of multi marker strategy, as the release kinetics of the markers were different hence it is evident from our study that the combinations of hscTnI, NTproBNP and CK-MB increases the diagnostic ability ischemia. The assessment of these markers helpful in understanding the disease stage in IHD or predict the future trends to prevent the progression of IHD into full fledged myocardial infarction.

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REFERENCES

[1] Guyton and Hall. Text book of medical physiology. Elsevier publications, 10th edition, 2004, pp. 128 - 132.
[2] Richard W. Troughton, Mark Richards. B-Type Natriuretic Peptides and Echocardiographic Measures of Cardiac Structure and Function. J Am Coll Cardiol Img 2009;2(2):216-225.
[3] Robbert J Winter, Jeroen G. Lijmer, Rudolph W. Koster, Frans J. Hoek, Gerard T. Sanders. Diagnostic accuracy of myoglobin concentration for the early diagnosis of acute myocardial infarction. Ann Emerg Med 2000; 35:113-120.
[4] Gibbons RJ, Balady GJ, ricker T, et al. ACC/AHA 2002 guideline update for exercise testing: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1997 Exercise Testing Guidelines). Circulation 2002; 106(14): 1883-92.
[5] Trinder, P.Ann. Clin.Biochem 1969. 6(24).
[6] Take, H, and Schubert, G.E. Klin, Wochschr. 1965; 19(43): 174.
[7] Bowers, L..D (1980) Clin Chem. 26;551.
[8] Pearlman, P.C. & Lee, R.T.Clin.Chem. (1974), 20:447.
[9] Thefeld Wetal (1974) : Dtsch Med Wschr 99 : 343
[10] Bergmmeyer, H.U.et al., (1980) Clin. Chem. Acta: 105: 147.
[11] Szasz, G., Rauternberg, H.W. (1971). Z. Kinderheilk., 111,233-239.
[12] Allain C.C., Poon L.S., Chan C.S.G., Richmond W. And Fu P., Clin. Chem 1974; 20(470).
[13] McGowan MW, et al. Clin Chem 1983;29;538.
[14] Burstein M., Scholnic H.R., Morfin R. (1970). J. Lipid Res.,
[15] Tietz N (ED), Fundamentals of clinical chemistry W.B. Saunders Co., Philadelphia PA, 1976

[16] Bremmer F W cardiac disease and hypertension in "clinical Chemistry theory, analysis and correlation." Kalpan L and Pesce A (ED) CV Mosby company, 1987.

[17] Apple Fred S. Christenson RH, Valdes RJ, Andriak AB, Duh Show-Hong, Feng Y J, Saeed AJ, Johnson Nancy J, Koplen Brenda, Mascotti K, and Wu Alan J. "Simultaneous Rapid Measurement of Whole Blood Myoglobin, creatine Kinase MB, and cardiac Troponin I by the Triage Cardiac Panel for Detection of MI'. clin chem. 1999; 45: 199-205.

[18] Sudeshna Behera, Manaswani Mangaraj, Prakash Chandra Mohapatro. Diagnostic efficicacy of ischemia modified albumin and its correlation with lipid profile, oxidative stress in acute myocardial infarction patients on admission. Asian pac J Trop Dis 2012;2(1):62-65.

[19] Ishihara M, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Nishioka K, Umemura T, Nakamura S, Yoshida M. Effect of acute hyperglycemia on the ischemic preconditioning effect of prodromal angina pectoris in patients with a first anterior wall acute myocardial infarction. Am J Cardiol 2003;92(3):288-91.

[20] Lickerman A, Grover-McKay M, Dellsperger KC. Hyperglycemia-induced angina pectoris in a patient with diabetes mellitus. Clin Cardiol 1997;20(8):736-7.

[21] Robert Ostfeld, Michael Spinelli, Disha Mookherjee, Dvorah Holtzman, Abu Shoyeb, Michael Schaefer, Thomas Kawano, Sanjay Doddamani, Daniel Spevack, Yunling Du. The Association of Blood Urea Nitrogen Levels and Coronary Artery Disease. Einstein J Biol Med 2010; 25 (1):3-7.

[22] Yadav Arvind S, Bhagwat Vinod. Lipid Profile Pattern in Anginal Syndrome Patients from Marathwada Region of Maharashtra State. Journal of Medical Education & Research, Vol. 2, No.2, July- Dec' 2012.

[23] Sreedhar K, Banumathy Srikant, Laxmikant Joshi, Usha G. Lipid Profile in Non-Diabetic Stroke – A Study of 100 Cases. J Assoc Physicians India 2010; 58:547-51.

[24] Anette Varbo, Marianne Benn, Borge G. Nordestgaard. Remnant cholesterol as a cause of ischemic heart disease: Evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. Pharmacol Therapeut 2014; 141:358–367.

[25] Saverio Stranges, Maurizio Trevisan, Joan M. DORN, Jacek Dmochowski, and Richard p. Donahue. Body Fat Distribution, Liver Enzymes, and Risk of Hypertension. Hypertension 2005; 46(5): 1186-1193.

[26] Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of "Ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J. 2004 ;21(1):29-34.

[27] Shaoqing Ju, Juan Ni, Jianyou Su, Min Pan, Jianhua Zhu. Ischemia-Modified Albumin is Increased in Patients With Unstable Angina: A new Potential Diagnostic Biomarker of This Acute Coronary Syndrome?. Lab Med 2008; 39 (11):668-670.

[28] Bastagli L, Guardigli G, Ruffini M, Spagnolo N, Fontana F, Puddu G, Ventura C, Patrono D, Capelli M, Bernardi P. Changes in plasma myoglobin levels in ischemic heart disease. G Clin Med 1989;70(12):733-5, 739-42.

[29] Kanda, K, Sugama, K, Sakuma, J, Kawakami, Y & Suzuki. 'Evaluation of serum leaking enzymes and investigation into new biomarkers for exercise-induced muscle damage.' Exerc Immunol Rev 2014; 20: 39-54.

[30] Eryol NK, Başar E, Ozdogru I, Çiçek Y, Abaci A, Oguzhan A, Topsakal R, Cetin S. Should troponin-T be assessed during exercise stress testing in patients with stable angina pectoris? Anadolu Kardiyol Derg. 2002; 2(2):132-7.

[31] Michael Weber, Veselin Mitrovic, Christian Hamm. B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide – Diagnostic role in stable coronary artery disease. Exp Clin Cardiol 2006; 11(2): 99–101.

[32] Robert S. Foote, Justin D. Pearlman, Alan H. Siegel, Kiang-Teck J. Yeo. Detection of Exercise-Induced Ischemia by Changes in B-Type Natriuretic Peptides. J Am Coll Cardiol 2004; 44(10):1980-87.

[33] Konig D, Neubauer O, Nics L, Kern N, Berg A, Bisse E, Wagner KH. Biomarkers of exercise-induced myocardial stress in relation to inflammatory and oxidative stress. Exerc Immunol Rev. 2007; 13:15-36.

[34] Robert Shave, Aaron Baggish, Keith George, Malissa Wood, Jurgen Scharhag, Gregory Whyte, David Gaze, Paul D. Thompson. Exercise-Induced Cardiac Troponin Elevation Evidence, Mechanisms, and Implications. J Am Coll Cardiol 2010; 56, (3): 169-176

[35] Koc ZP, Erkilic M, Basarıcı I, Deger N, Ozdem S, Saka O. Ischemia modified albumin levels cannot predict stress induced ischemia shown by myocardial perfusion scintigraphy. Esp Med Nucl Imagen Mol. 2012; 31(4):202-6.

[36] Martin Juneau, Nathalie Roy, Anil Nigam, Jean-Claude Tardif, Lucie Larivee. Exercise above the ischemic threshold and serum markers of myocardial injury. Can J Cardiol. 2009; 25(10): e338–e341.

[37] Alon T Marmor, Roberto Klein, Michael Plich. Elevated CK-MB lsoenzyme after Exercise Stress Test and Atrial Pacing in Patients with lschemic Heart Disease. Chest1988; 94 (6):1216-20.

[38] Devi D. Munjal , Joyce A. McFadden , Patricia A. Matix ,Kim D. Coffman,Stephen M. Cattaneo. Changes in serum myoglobin, total creatine kinase, lactate dehydrogenase and creatine kinase MB levels in runners. Clini Biochem 1983; 16(3): 195–199.

[39] Baadsgaard O, Schmidt JF. Myoglobin concentration, Creatine kinase, and creatine kinase sub-unit B activity in serum after myocardial ischemia. Scand J Clin Lab Invest. 1984; 44(8):679-82.

[40] Haaf P, Balmelli C, Reichlin T, Twerenbold R, Reiter M, Meissner J, Schaub N, Stelzig C, Freese M, Paniz P, Meune C, Drexler B, Freidank H, Winkler K, Hochholzer W, Mueller C. N-terminal pro B-type natriuretic peptide in the early evaluation of suspected acute myocardial infarction. Am J Med. 2011; 124(8):731-9.

[41] Milenko J. Tanasijevic, Christopher P. Cannon, Elliott M. Antman, Donald R. Wybenga, George A. Fischer,Christine Grudzien, C. Michael Gibson, James W. Winkelman, Eugene Braunwald. Myoglobin, Creatine-Kinase-MB and Cardiac Troponin-I 60-Minute Ratios Predict Infarct-related Artery Patency After Thrombolysis for Acute Myocardial Infarction- Results from the Thrombolysis in Myocardial Infarction Study (TIMI) 10B. J Am Coll Cardiol 1999;34(3):739-747.

[42] Shu-ming Pan, Chao-yang Tong, Qing Lin, Chen-Ling Yao, Jie Zhao, Zhi Deng. Ischemia-modified albumin measured with ultra filtration assay in early diagnosis of acute coronary syndrome. World J Emerg Med 2010; 1 (1):37-40.