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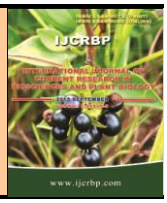

Original Research Article

Effectiveness of Some Serum Constituents as Biochemical Markers in Acute Myocardial Infarction Patients

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Abstract

The aims of the present work were: to compare the characteristics of Acute Myocardial Infarction (AMI) patients with healthy people with respect to a number of blood biochemical parameters, to investigate the effectiveness of each of these parameters as a risk or illness indicator, and to investigate the use of groups of parameters as risk factors and illness markers. The study covered (161) AMI patients and (156) healthy subjects. The two groups contained males and females whose ages ranged between (34 - 87) years. Among the investigated parameters where: the enzymes which include creatine kinase (CK), creatine kinase isoenzyme (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), xanthine oxidase (XO), deoxy ribonuclease (DNase), and the proteins which include troponin (Tn), and C- reactive protein (CRP). The results indicated a significant increase in the level of activity of the investigated enzymes in the serum of AMI patients with ($p < 0.001$).Furthermore, the results showed a significant increase in the concentrations of troponin and C – reactive protein in the serum of AMI patients as compared with healthy subjects by 202 and 15 times respectively. The cutoff values for the different parameters were also estimated through calculating the sensitivity, specificity and efficiency for each of the parameters. The values of the specificity and sensitivity were then used to evaluate the effectiveness of each of them as a risk factor or marker for AMI. For XO, the sensitivity and specificity were both 100% while for Tn and CRP were 100% and 92% respectively for the sensitivity and 0% and 86% for the specificity. To increase the usability and effectiveness of parameters as illness indicators or risk factors, an attempt to group them through discriminant analysis was performed. It was found that the group of age and sex made a very effective risk indicator with a discriminant efficiency of 95.6%. However, the enzymes group showed discriminant efficiencies of 99.6% as illness markers respectively. Following in the effectiveness were the proteins with efficiencies of 96.5%. This proved that grouping the parameters is much more effective as indicated by the higher discriminating efficiencies indicated by the analysis as compared with the single parameter values.

Keywords

Acute myocardial infarction
Creatine kinase
C- reactive protein
Troponin

Introduction

Acute myocardial infarction (AMI) is the most common diagnosis among hospitalized patients in industrialized countries (WHO, 2011). Myocardial infarction (MI) occurs when coronary blood flow to an area of the cardiac muscle is decreased abruptly after a thrombotic occlusion of a coronary artery that was previously narrowed by atherosclerosis leading to depletion of oxygen supplies. The heart tissue becomes inflamed and necrotic at the point of obstruction (Thygesen et al., 2007; Murray et al., 2009). Myocardial cells, deprived of necessary oxygen and nutrients, lose contractility and diminish the pumping ability of the heart (Sue and Kathryn, 2004). Myocardial oxygen reserves are used very quickly after complete cessation of coronary flow (Murray et al., 2009). H^+ and lactic acid accumulates because myocardial tissues have poor buffering capabilities and myocardial cells are very sensitive to low cellular pH. Acidosis may make the myocardium more vulnerable to damaging effects of lysosomal enzymes. This results in the release of certain intracellular enzymes through the damaged cells membrane into the interstitial space (Ganong, 2005). The lymphatic's picks up the enzymes and transports them into the blood stream, where detected by serologic tests.

A significant recent advancement in the diagnosis of heart disease has resulted from the development of quantifying methods for enzymes activity levels as well as certain proteins concentrations in blood serum and their correlation as indicators for myocardial damage. The present work aims at investigating a number of enzyme and proteins as blood biochemical parameters in AMI patients, investigating the effectiveness of each of the parameters as risk or illness indicator, and correlating selected parameters groups as risk factors and illness markers in AMI patients.

Materials and methods

The patients group

The experiment was designed carefully to ensure the random selection of AMI patient's sample that will reflect the actual state of the illness among the citizens of the city of Mosul and its suburbs. Samples were collected from patients admitted to Al-Salam hospital and Ibn-Sina hospital in Mosul. Blood samples were collected through vein puncture. The blood was immediately transferred into a clean, dry polystyrene tube, incubated for10 minutes in a 37^{o}C water bath, and centrifuged at 3000g for 10 minutes to separate the serum. The separated serum is then kept in deep freeze, for further investigation (Tietz, 1999). A total of 161 patients were scanned covering an age ranging from (34-87) years with a mean of (54.6 ± 10.8) year.

The control group

The control group was selected carefully from people with no history of heart diseases or others. Total of 156 individuals were sampled following the same procedure mentioned in patients group. The mean age of the group is (45.6 ± 11.6) years and (54.5%) of the sample were males.

Estimation of enzymes activity

The CK activity was determined quantitatively following the enzymatic method described by (Tietz, 1999). The increase in absorbance was proportional to CK activity in the sample when measured. CK-MB activity was determined using the estimation technique depends on the fact that creatine kinase is a dimmer. Its monomeric subunits are designated M (Muscle) and B (Brain). The subunits combine to form three isoenzymes namely CK-BB, CK-MB, CK-MM. The M subunits of CK-MM and CK-MB are inactivated by reaction with an antibody (immunoinhibition), while the B-subunit is kept active and measured enzymatically, (Moss and Henderson, 1999). LDH activity was measured by reduced the absorbance at 340nm (Tietz, 1999). The ALT activity was measured spectrophotometrically using the method of Reitman and Frankel (Anderson and Cockayne, 1993). XO activity was assayed following the method of (Ackermann and Brill, 1974), which depends on the enzymatic oxidation of xanthine. The increase in UV absorption which occurs on depolymerization of DNA when it was incubated with DNase, is used to measure the activity of DNase which was expressed as the rate of change in absorbance, (Udou and Ichikawa, 1980).

Estimation of protein concentration

Troponin concentration was estimated using the VIDAS Troponin I Ultra (TNIU) Assay is an enzyme-linked fluorescent immunoassay (Dimension RxLS® Cardiac Troponin I (CTNI) Assay, biomeneux-USA.com). The estimation follows the procedure of (Chapelle, 1999).

Determination of C - reactive protein CRP in blood serum was assayed by slide agglutination (Latex-Agglutination-Test, Inmesco, GmbH-Germany). The assay presents a rapid test for the qualitative and semi quantitative estimation of CRP in serum (Amos et al., 1977; Minnaard et al., 2013).

Statistical analysis

Means and Standard deviations (SDs) were calculated for continuous variables, and absolute and relative frequencies were measured for discrete variables. Differences between groups were tested by the χ^2 test or Fisher's exact test in the case of discrete variables and by a 2-sample *t* test in the case of continuous variables. Multiple logistic regression was used to test the independent contribution of univariate risk predictors. The complete analyses of the results for each parameter were presented. Within group and among groups comparisons for both the patients and normal subject groups are conducted on the basis of all subjects, sex as well as age.

For subgroup analysis, additional comparisons within the patients group were also conducted on the basis of high blood pressure (HBP), diabetes mellitus (DM), smoking and locality. Statistical analysis was carried out using the commercially available statistical package STATISTICA 5.5 of StatSoft Inc.-1999. For all statistical evaluations, a $p<0.05$ was considered statistically significant (Snedecor and Cochran, 1986).

Results

Comparisons within the control group

The results showed that there is no difference between the males and females ages within the group (45.92 ± 1.31, 45.21 ± 1.31 years). With the parameters (enzymes and proteins), there were no significant differences in relation to sex and age.

Comparisons within the patients group

The specific patient characteristics were shown in Table 1.The distribution is fairly normal and what is worthy to note is the relatively large number of patients in the 30-40 years age group which constituted about 12% of the total sample. Among the patients, (78.3%) were males with an age mean of (53.9 ± 10.9) year, and (21.7%) females with an age mean of (57.3 ± 10.4) year.

Table 1. Some specifics characteristics of the patients group.

Patient details	Total	Male	Female
	Mean ± SD	Mean ± SD	Mean ± SD
Gender	161 (100%)	126(78.3%)	35(21.7%)
Age (year)	54.6 ± 10.8	53.9 ± 10.9	57.3 ± 10.4
HBP (%)	41.6% ± 0.49	32.5% ± 0.47	74.2% ± 0.44
DM (%)	29.8% ± 0.495	22.2% ± 0.41	57.1% ± 0.5
Smoke amount (pack/day)	1.59 ± 0.78	1.64 ± 0.8	±0.4 1.18
* Data is represented as Mean ± SD or No (%)			

Comparisons between enzyme activities in sera of patients with AMI and control subjects

Table 2 indicates that there is a significant increase, (with $p<0.001$) in serum CK activity of AMI patients in comparison with that of the control group, which is in accordance of the results of (Keller et al., 2010). This increase could be attributed to the presence of the enzyme in a much higher concentration inside the cell where its destruction as result of myocardial necrosis causes the release of the enzyme into the systemic circulation. The high level may be associated with strong change in blood pressure; high plasma catecholamine levels after myocardial cell damage. The results showed no significant difference between CK activity of male and female patients. A significant increase in the activity of CK-MB in the patients serum with $p<0.001$ is also shown in Table 2. The high concentration of CK-MB inpatients is in accordance of other reported (Nasrollah and Naser, 2014). This may be due to the presences of this specific cardiac isoenzyme in high concentrations in myocardial cells, hence the necrosis of such cells cause its release into the blood stream. CK-MB is then a very specific indicator of cardiac injury. In patients with AMI, LDH leaks from injured or necrotic heart muscle into the blood stream.

The results of Table 2 showed significant increase in the LDH activity in sera of patients with AMI when compared with healthy subjects with ($p<0.001$), which is

in agreement with previous observations by (Khalil et al., 2013). Serum LDH increase starts in relatively late times after the onset on chest pain, peaks on the second day and decline gradually thereafter. However, it stays elevated till the fourth day (Nasrollah and Naser, 2014). The leakage of the enzyme from even a small mass of damaged tissue can increase the observed serum level of LDH to a significant extent, this explanation confirm the observation of high LDH activity in AMI patients. The results did not show any significant effect of sex within the patients group on the activity of LDH. The results in Table 2 also showed a significant increase in AST activity, AST is the next to appear after CK which is the first heart enzyme to appear in the blood stream after heart attacks (Bishop et al., 2005), the findings are in agreement with the study of Ajlan and Baqir (2011) at (p<0.001), this elevation is due to myocardial cell destruction after the onset of AMI which cause the release of the enzyme into the blood stream and it is consider as a marker for the disease. Other workers explain the increasing level of AST as a result of inflammatory conditions (Ajlan and Baqir, 2011).

Table 2. Comparisons between enzymes activities in sera of patients with AMI and control subjects.

Parameter	Gender	Mean ± SD		*P*
		Control	Patients	
CK (U/l)	Male	105.5 ± 10.1	169.9 ± 312.1	***
	Female	108.8 ± 11.9	964.9 ± 392.7	***
	Total	106.8 ± 7.7	1516.2 ± 257.7	***
CK-MB (U/l)	Male	5.6 ± 0.54	46.5 ± 7.8	***
	Female	6.4 ± 0.38	67.6 ± 35.7	MS
	Total	5.8 ± 0.37	51.5 ± 10.2	***
LDH (U/l)	Male	95.6 ± 8.9	677.7 ± 92.9	***
	Female	98.9 ± 9.7	649.6 ± 126.7	***
	Total	96.9 ± 6.6	670.9 ± 76.5	***
AST (U/l)	Male	8.8 ± 0.72	19.1 ± 1.3	***
	Female	7.5 ± 0.5	18.3 ± 2.8	***
	Total	8.3 ± 0.5	18.9 ± 1.1	***
XO (U/l)	Male	6.5 ± 0.7	120.6 ± 36.1	***
	Female	7.6 ± 0.5	47.8 ± 14.8	***
	Total	6.9 ± 0.48	104.0 ± 28.7	***
DNase (U/l)	Male	22.2 ± 1.5	735.3 ± 456.6	*
	Female	25.1 ± 1.3	978.6 ± 832.0	*
	Total	23.3 ± 1.1	808.3 ± 380.6	***

* Significant difference (p≤0.05); *** Significant difference (p<0.001); MS Marginal Significant difference.

As compared with serum control group, XO activity in patients showed significant increase, (with p<0.001) as shown in Table 2 which confirms the findings of (Michael et al., 2015). The higher activity of XO in sera of AMI patients is due to the function of the enzyme which catalyzes the breakdown of nucleotides of dead myocardial cells causing DNA destructive to form uric acid, which contributes to the antioxidant capacity of blood (Griguerc et al., 2006). Our results show no significant effect of both sex (male and female) on the activity of XO.

The results in Table 2 showed that the activity of DNase is significantly higher in patients with AMI as compared to the control group (with p<0.001), the increase amount is in agreement with the work of Takeshita et al. (2004). Within the patients group, no significant effect of sex on DNase activity had been found. DNase activity in serum was found to be transiently increased in patients with AMI within 2 h after the onset of chest pain. The abrupt increase in serum DNase activity that accompany the onset of AMI could serve as a novel biochemical diagnostic marker for AMI in the very early phase after AMI onset, which is in line with the previous observation by Arakawa et al. (2005). Such early diagnosis of AMI allows more appropriate and earlier therapy, such as thrombolytic agent (e.g. streptokinase) to be administrated to the patients.

Concentration of some proteins in serum of AMI patients and control

The results in Table 3 showed that troponin (Tn) concentration significantly increase in the sera of control subjects in comparison of sera of AMI patients (with $p<0.001$), the results are in agreement with other studies (Sharma et al., 2004). Within the patients group, there was no significant effect of sex on the concentration of troponin as indicated in the Table 3. The elevation of troponin concentration is due to its various important characteristics such as cardio-specificity, unique location in the cardiac tissue matrix, early release as a result of cardiomyocyte injury and longer diagnostic window, ranging from 4 h to 8-10 days. Hence it could prove to be a superior emergency room biochemical marker (Jackson and Makan, 2004). Moreover, it's very low level in normal serum, gives it high cardiac specificity and in light of the results of various studies, Tn has been establish as a reliable biochemical marker, "gold standard" for AMI (Sharma et al., 2004). Most intracellular Tn is bound to the myofibrils in the cardiac myocyte; however a small percentage exists in a cytosolic pool, (3-4% of Tn), (Jacob, 2002). The importance of this pool is as the source of cytosolic Tn released 4-6 hrs after myocardial injury, continuing breakdown of the myofibrillary complex in damaged myocyes result in the prolonged elevation of the concentration of troponin in blood. So this conform the use of this parameter for early and late phase injury diagnosis (Sharma et al., 2004).

As indicated in Table 3, the serum level of CRP was significantly higher in AMI patients (with $p<0.001$) when compared with control groups, and is in agreement with the studies of Faraj et al. (2012). The results highlight the important role of this novel inflammatory marker in the clinical setting of AMI, which result from plaque rupture and thrombus formation (Khalil et al., 2013). The present findings support those of Al-Barwari and Al-Barwari (2011), who suggested that CRP levels increase within 6 h after the onset of AMI. This might be due to the fact that CRP not only reflects on underlying inflammatory process in the atherosclerotic plaque lesion but also directly participates in the promotion of atherosclerotic processes and endothelial cell inflammation (Minnaard et al., 2013). No significant differences were found between males and females in respect with mean values of serum Troponin and CRP within the patients group.

Table 3. Concentration of Troponin, CRP in serum of AMI patient and control subjects

Parameter	Sex	Mean ± SD		*P*
		Control	Patients	
Troponin (µg/L)	Male	0.1 ± 0.00	29.1 ± 3.6	***
	Female	0.18 ± 0.08	26.2 ± 5.1	***
	Total	0.14 ± 0.04	28.4 ± 3.0	***
CRP (mg/L)	Male	3.0 ± 1.3	56.0 ± 10.3	***
	Female	8.0 ± 2.0	61.3 ± 25.1	MS
	Total	3.8 ± 1.2	57.2 ± 9.6	***
*** Highly significant difference ($p<0.001$).				

Diagnostic characteristics of parameters

A measured parameter can only be useful if it is successfully put in a frame work that enables its use as either a "risk factor", to indicate the probability level of developing acute myocardial infarction (AMI) or a "marker", to indicate the presence and severity of AMI in inpatients.

To assess the effectiveness of a parameter as a diagnostic tool, researchers have developed the concepts of sensitivity, specificity and efficiency of tests (Apple et al., 1999).To start with, the results of any test can be classified in any of the categories listed in Table 4.

Table 4. Possible outcomes of a test.

Test result	Outcomes of a test	Health condition	
		With AMI	No AMI
	Positive	True positive	False positive
	Negative	False negative	True negative

Sensitivity of a test defines its capability to diagnose a patient and is given by Dardir et al. (1998):

$$S_n = \{N_{TP}/ (N_{TP} + N_{TN})\} \times 100$$

Where: N_{TP} is the number of successfully diagnosed patients and N_{TN} is the number of successfully identified healthy persons in any sample. Furthermore, the

specificity is a measure of the capability of the test to isolate healthy persons, it is defined as:

$$S_p = \{N_{TN}/ (N_{TN} + N_{FP})\} \times 100$$

Where: N_{FP} is the number of healthy persons with positive test in the sample. Finally, the efficiency of any test is defined as:

$$E_f = \{N_{TP} + N_{TN}) N_S\} \times 100$$

Where: N_S is the total sample size.

Successful implementation of the above measures to any marker requires an accurate estimation of the "cutoff value" or "reference interval" of the marker (Pezzilli et al., 2000). It is defined as "the usual value of a healthy population". Cutoff values can either be identified as the 95th or 99th percentiles of the control group values depending on the accuracy of the test. It could also be chosen to optimize the sensitivity and specificity according to the receiver operator characteristics, ROC, method (Bishop et al., 2005).

The enzymes group

Table 5 shows the sensitivities, specificities, and efficiencies for CK, CK-MB, LDH, ALT, XO and DNase at three cutoff values, those are: the 99th and the 95th percentiles of the control group values, as well as the mean value which was added for comparisons. In general, the mean value does not seem suitable as a reference value. This is obvious from the low efficiency values for all parameters. Furthermore, there is a little difference between using the 99th and 95th percentiles as cutoff values except for the CK-MB case where the 95th percentile gave a better sensitivity and efficiency.

The results also indicated that the above mentioned markers have higher specificities and sensitivities. Among the markers, XO showed the best characteristics as an identifier for AMI with S_n = 100%, S_P = 91% and E_f = 95% at the 99th percentile followed by AST with S_n = 90.6%, S_P = 96% and E_f = 92% at the same cutoff level. The rest will follow as shown in Table 5.

Table 5. Clinical sensitivities and specificities for enzymes related to AMI patients.

Parameter		Criterion		
		99%	95%	Mean
CK (U/l)	Cutoff	166.1	166.1	106.8
	$S_{n\,(\%)}$	85	85	63
	$S_{P\,(\%)}$	91	91	44
	$E_{ff\,(\%)}$	88	88	66
CK-MB (U/l)	Cutoff	10.7	7.85	5.88
	$S_{n\,(\%)}$	76	88	72
	$S_{P\,(\%)}$	96	83	46
	$E_{ff\,(\%)}$	84	86	7
LDH (U/l)	Cutoff	180	175	96.85
	$S_{n\,(\%)}$	85	85	97
	$S_{P\,(\%)}$	97	94	67
	$E_{ff\,(\%)}$	89	88	87
AST (U/l)	Cutoff	11.86	11.26	8.33
	$S_{n\,(\%)}$	90.6	90.6	96.8
	$S_{P\,(\%)}$	96	93	60
	$E_{ff\,(\%)}$	92	91	85
XO (U/l)	Cutoff	15.3	11.6	6.94
	$S_{n\,(\%)}$	100	100	100
	$S_{P\,(\%)}$	91	75	55
	$E_{ff\,(\%)}$	95	85	72
DNase (U/l)	Cutoff	27.54	27.53	23.3
	$S_{n\,(\%)}$	70	70	70
	$S_{P\,(\%)}$	76	76	32
	$E_{ff\,(\%)}$	74	74	43

The protein group

Table 6 shows the results of this group. The troponin gave 100% sensitivity due to the fact that the control group contained only healthy persons and the troponin is very specific to the cardiac muscle. Members of this group are both very good markers for diagnosing AMI. Specificity for troponin = zero, because the cutoff value of (0.1 µg/l) is used which represent the threshold of the assay (ELFA assay) using Minividas analyzer. Troponin cutoff values are very assay dependent, other researchers reported that the cutoff value of troponin according to assay had been used was < 0.8 µg/l for AXSYM assay and < 0.15 µg/l for Dade Behring stratus assay (Apple et al., 1999) and the sensitivity and specificity were (91.8% and 92.4%) respectively (Apple et al., 1999).CRP showed S_n = 92% and S_P = 76% and E_{ff} = 88% at a cutoff concentration ≥ 10.75 mg/l. Previous observation found a cutoff value for CRP (12.8 mg/l), S_n=60.9%, S_P=89.1% and E_{ff} =77.6% (Pezzilli et al., 2000).The high sensitivity and specificity for this test does confirm the use of them as future diagnostic markers for AMI.

The effectiveness of groups of parameters as diagnostic tools

In the proceeding sections, the characteristics of the individual parameters were analyzed and discussed. The differences between patients and healthy subjects were outlined and the use of the different parameters as risk factors or illness markers was discussed as well. To increase the power of diagnosis, the trend now is to use two or more parameters in a group form to diagnose or indicate illness (Pezzilli et al., 2000). This task can be achieved through performing discriminant analysis which uses present patients and healthy subjects' data to extract discriminant functions that can be used to assign subjects to any of the two groups based on the outcome of such functions after feeding them with the required test values.

The analysis starts from the biochemical groups with the addition of age and sex to each of the groups. A backwards stepwise technique is used to eliminate ineffective parameters whose p>0.05. The outcome of the analysis is a function of the form:

$$\mathbf{f = a_o + \Sigma\, a_i x_i\ ,\ i = 1, n}$$

Where a's are constants, the x's are the studied parameters and f is the discriminating variable. Based on the value of f, (f < 0 or f > 0), the examined subject is assigned to group one or two, (healthy or sick, low risk or high risk). f = 0 marks the equal probability line for the subject to belong to any of the groups. The final outcome is the discrimination efficiency (ηd) which marks the correctness extent of the function in assigning subjects.

The enzymes group discrimination function

The enzymes group members: LDH, CK, CK-MB, AST, XO and DNase plus the age and sex are analyzed. The variables age, sex and LDH were removed by the program and the final model, (discrimination function), is given as:

$$\mathbf{f_e = -\ 63.1883 + 2.9466 \times AST + 0.01294 \times CK + 0.1499 \times CK\text{-}MB + 0.06344 \times XO + 0.03189 \times DNase}$$

Where fe is the enzymes discrimination function, (discrimination variable). It acts as an illness marker and discriminates between subjects with AMI (fe > 0) and subjects with no AMI (fe < 0). The discrimination efficiency of the obtained function is 99.6% which again is higher than the individual efficiencies of the constituents each by itself.

Table 6. Clinical sensitivities and specificities for Tn and C-reactive protein

Parameter		Criterion		
		99%	95%	mean
Troponin (µg/l)	Cutoff	0.1	0.1	0.14
	$S_{n\,(\%)}$	100	100	86
	$S_{P\,(\%)}$	0	0	90
	$E_{ff\,(\%)}$	81	81	87
CRP (mg/l)	Cutoff	11.75	10.75	3.88
	$S_{n\,(\%)}$	92	92	80
	$S_{P\,(\%)}$	86	76	59
	$E_{ff\,(\%)}$	84	88	88

The proteins group discrimination function

The model included age, gender, troponin and CRP. It is given by:

$$f_p = -13.78 + 0.0793 \times age + 1.1152 \times sex + 0.4557 \times trop \times 0.0831 \times CRP$$

Where: f_p is the discrimination function for proteins group. The discrimination function in this case is an "illness marker" since these substances are AMI specific especially the troponin where the ratio of patients to control concentrations means of Tn and CRP are (203.5 and 14.74) respectively. The discrimination efficiency of the above function is (96.5%) which is higher than those for Tn and CRP individually as reported in Table 6. The P value for the function is very small ($p<10^{-6}$)

Conclusion

There is a significant increase in Tn and CRP concentration in serum of AMI patients, in addition to the significantly increased activity of related enzymes (CK, CK-MB, LDH, AST, XO, and DNase). The grouping of parameters gave tools for using as a risk factor and /or diagnostic markers with efficiency over individual parameters.

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