Early Diagnosis of Arrhythmia, Angina Pectoris and Acute Myocardial Infarction by using Cardiac Biomarkers and Electrocardiogram (ECG)

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Abstract

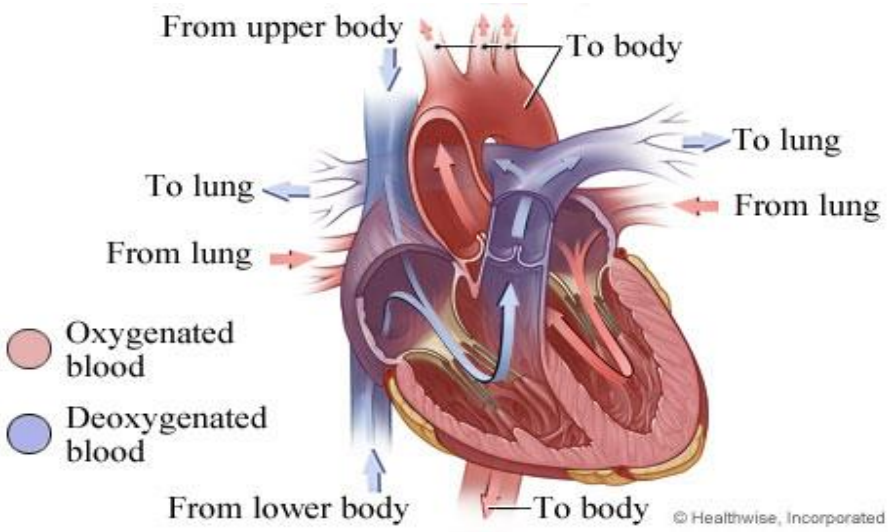
The biochemical analyzer EM 200, biomerieux-mini vidas (protein analyzer) and ECG which are used to identified the early monitoring and screening of angina, arrhythmia and acute myocardial infarction in the suspected patients. chemiluminescence assay, Immunoturbidometric methods are play the important role in rapid determination and quantification of Troponin I, CKMB, CK, from human serum sample and ECG applied to records the electrical impulsesthat are generated from the heart (PR interval period, QRS wave, ST segments and T waves).The biochemical testsare deciding for the reliable measurements and study of acute coronary syndrome, mycocardial fibrosis and chronic heart failure. The use of these tests that covers the elevation of Troponin I, CKMB and CKin the serum sample. Troponin I is an early marker of cardiovascular diseases and ischemic heart diseases. So, biochemical immunoassay and ECG is compulsory to analyses serum sample and visualize the electrical pulses from the cardiac patients. The regular uses of these tests are considered based on the clinical practices.

Key words: *Troponin I, CKMB, CK, PR interval period, QRS wave, ST segments* and *T waves.*

Introduction:

Angina if otherwise called angina pectoris is a sign of a chest pain or abnormal condition of heart rhythms. The insufficient amount blood and deficient amount of oxygen transported in to the cardiac muscle, coronary arteries are blocked due to atherosclerosis condition. There are five different types of angina have been identified, stable angina, unstable angina, variant angina, micro vascular angina and atypical angina. Nitroglycerin is widely used for the angina attack. Nitroglycerins are commercially available in the form of nitosat, nitro quick, nitro lingual, nitrodur, minitran and nitro-bid. Coronary heart disease occurs when the plaque builds up inside the coronary arteries. The plaque is develops in the inner layer of the artery, it is a formation of cholesterol, WBC cells, calcium ions and new materials in the interior walls of arteries. The cramped artery limits the movement of oxygen-abundant blood to various parts of the body.

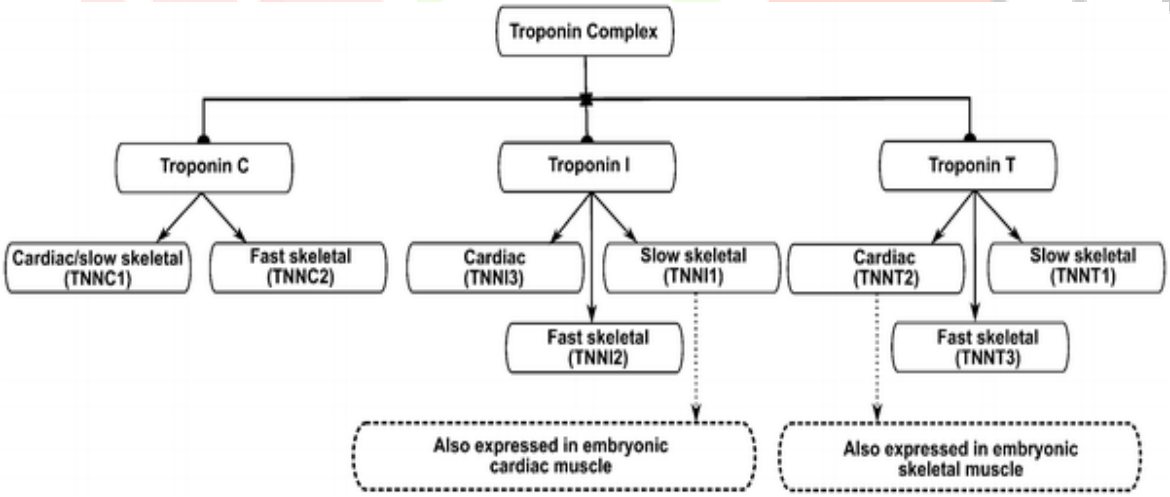
Blood flows in heart



Cardiac Biomarkers:

Troponin I, troponin T, CKMB, CK, ST2 and NT pro BNP biomarker are widely used for heart attack. Biomarkers are assessable substance which indicates the biological or pathological process. Cardiac biomarkers are predominantly used in the diagnosis of acute coronary syndrome, myocardial fibrosis and chronic heart failure. Vertebrate contains three different type of muscle such as smooth muscle, skeletal muscle and cardiac muscle. Cardiac muscle is also an involuntary muscle but it is striated in structure, present only in the heart. Troponin is made up of three subunits, troponin C, troponin I and troponin T.

Structure of Troponin complex:



The two isoforms of cardiac troponin are found in the serum sample, troponinT and troponinI are immunologically different from the amino acid sequences of the myocardial troponins. Cardiac troponins (cTn) are clinically important because they are high effective indicators of myocardial infarction and myocardial fibrosis. Cardiac troponin-I are present in the cardiac muscle with molecular weight of 23.9 kDa. It consists of 209 amino acid residues. Troponin molecules are interacting with the actin and tropomyosin, to form a troponin-tropomyosin complex. Myosin does not bind to actin from the relaxed muscle. When calcium ions interact with troponin C to encourage the contour alterations which begin to dislocation of

troponin I and ultimately tropomyosin moves the binding site for myosin on actin directing to contraction of muscle. Troponin biomarker are gold standard for the diagnosis of acute myocardial infarction and is also elevated in a number of other conditions including heart failure, chronic renal failure, subarachnoid hemorrhage, pulmonary embolus and sepsis.Cardiac markers execute simultaneously with the ultrasonographic device to observe the early modification of cardiovascular system. Molecular genetics techniques identified the new genes called ST2 and its analogous protein was obviously generates the mechanically overburden to cardiac muscle cells. The gene ST2 otherwise referred as T1, IL1RL1, or Fit1 is arranged on chromosome 2q12 as the component of interleukin 1 (IL-1) gene cluster. Four different isoforms are transcriptional product of the gene from the interleukin-1, so two of them are important: IL1RL1-b or ST2L, which is a membrane receptor member of the interleukin-1 receptor family, IL1RL1-a (or) ST2a, which is constricting the soluble receptor that can be identified in serum. The study about ST2 and ST2L mechanisms on cardiovascular system attend to recognize the evaluation of serum plasma levels of ST2 as an important cardiac bio marker in heart failure and ischemic heart diseases. Dyspnoea is the individual symptom of breathlessness and it usually identifies the different pathologic conditions like heart failure, chronic obstructive Molecules, bronchitis, pulmonary disease, asthma, pneumonia, malignancy, pulmonary embolism.

Biomarkers:

S.No	Old Biomarkers	Current Biomarkers
1	Total CK Activity	CK-MB
2	Aspartate Amino Transferase Activity	Troponin
3	Lactate Dehydrogenase Activity	ST2
4	LD1/LD2 Ratio	NT proBNP

Creatine kinase otherwise called creatine phosphokinase (CPK) or phospho-creatine kinase, is an intracellular enzyme found in more amounts in myocardium, skeletal muscle and brain, small amounts in visceral tissues. The cytosolic CK enzymes are a dimeric molecule which is made up of either B subunits(from brain) or M subunits (from muscle). CK-MM, CK-BB and CK-MB isoenzyme gene domain are located on the different chromosomes: B on 14q32 and M on 19q13. CK is a cardiac biomarker, usually recognize a patients with chest pain, acute renal failure, myositides and rhabdomyolysis.CK catalyses the transformation of creatine and consumes ATP to generate Phosphocreatine and Adenosine Diphosphate. This CK enzyme is capable of assuming or producing either of two states and thus ATP can be originating from PCr and ADP. Cells that are consuming ATP rapidly, particularly smooth muscle, skeletal muscle, brain cells, spermatozoa, cells of the retina and hair cells.

Principle of a biomerieux Minividas Chemiluminescence Immunoassay:

The Solid Phase Receptacle acts a stable state aspiration device for the test. Reactants for the test are prepare-to-use and pre allocate in the covered reagent strips. Most of the experiment steps are done

automatically by the mini vidas immunological analyzer. In and out of cycled mechanisms of SPR test processed regularly. Free ingredients are removed through cleaning buffer and the last reaction step is 4-Methyl-umbelliferyl phosphate cycled in and out of the SPR. Then, hydrolysis of this substrate into a luminance product by immobilized enzyme, the 4-Methyl-umbelliferone luminance is detected at 450 nm. The power of the luminance based on the absorption of alkaline phosphatase present on the SPR that alter the substrate. At the end, results are automatically measured by the analyzer. For some tests, two detection steps are performed successively. For antigen detection, the SPR is generally coated on the interior with capture antibody or sometimes with byproducts of the analyte. For antibody detection, the SPR is coated with an encapsulated antigen or antibody administered to the antigen. Depending on the test, the conjugate can be byproducts of the analyte or an antibody labeled with alkaline phosphatase. For more particular details, mention to the assay package inserts.

Materials and Methods:

Quantification of Troponin-I Ultra Mini VIDAS:

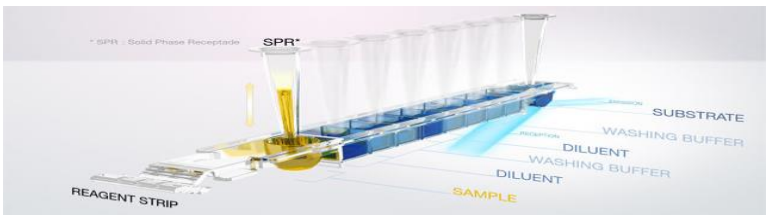
The serum sample collected under the good laboratory practices. The samples were tested quickly after collection. The sample is stable for 3 to 5 days at 2°C to 4°C. If the sample unable to process within 24 hours, freeze until the test can be done. Permit the sample to reach room temperature before testing. The sample was collected by using micropipette and added for 3 to 5drops (100-150 µl) of sample into the 1stwell of the strip and read the result.

Biomerieux Minividas Chemiluminescence Immunoassay kit:

The biomerieux minividas chemiluminescence Immunoassay kit contains everything required to carry out a specific assay, single or dual reagent strips, SPRs (Solid Phase Receptacle), standard/calibrator(s), controls, diluents.

Single Reagent Strip:

Single reagent strip is made of polypropylene and contains ten wells. The sample is added in the 1stwell. Conjugate, diluents, and wash buffer are already coated in the 2nd well in to 8th well. The luminance of the substrate is measured finally (10th well). Tags assure that the SRP is properly placed in the guided channel.



SPR:

The polystyrene device (SPR) is able to grabbing the soluble proteins, viruses and bacteria. It's labeled with a color-coded, bar-coded dot drill in the middle. SPR vial is not re-usable. The SPR is the

stable state for the immunological response.SPR interior walls are coated with antibody or antigen that captures a target analyte. The target analyte from the sample binds to the SPRs interior coating (antibody or antigen) to form a (sandwich) or antigen -antibody-anti-antibody labeled complex. The stimulant change the hydrolysis state of the molecule into a luminous end product .The SPR is used to pipette samples and reagents and perform the following operations sampling, incubation, mixing, washing. The beveled tip of the SPR enables it to pierce the protective seal that covers the wells in a reagent strip. The reagent strip tray then moves in and out to allow liquids to be transferred from one well to another.

Loading reagent strips and SPR.

Lift the cover of the reagent strip section, hold the strip by its handle and insert it into a test position, slide the strip into the section channel, open the SPR block door, place the SPR in the SPR block position directly over the reagent strip, Repeat these steps for other strips and SPRs to be loaded, Close the SPR block door and the reagent strip tray cover.

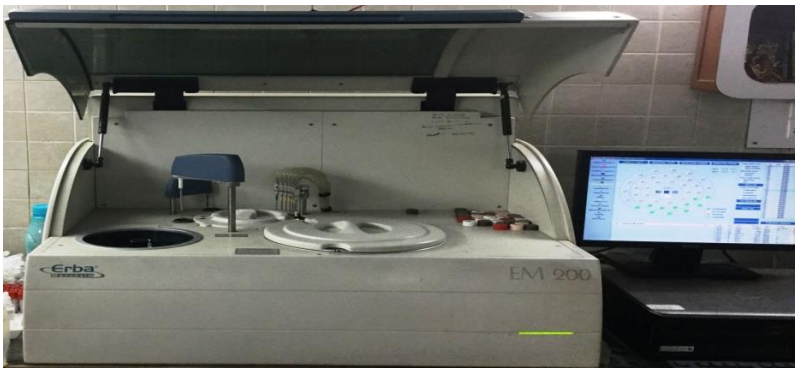
Minividas protein Analyzer System:

Quantification of CKMB and CK - EM 200:

EM 200 is an automated clinical Biochemistry analyzer. It has computerized pipetting of reagent and sample by a mechanical arm which assure the proper liquid handling of minimum 2µl and maximum 300µl with an accumulative volume of 0.1µl. Aspirating probe have liquid level sensor to check the existence of adequate liquid from the container. Vertical stumbling block detection sensor secures the probe thereby expanding its durability. It has three level cleaning systems. It is useful for immunoturbidometric assays. EM 200 has free independent cleaning station to assure proper result. EM 200 has Levey-Jennings chart depend on the quality control to satisfy the quality of reagent and instruments. Waste from curette is collected in a special container. Cleaning done by distilled water and ERBA XL Wash. After cleaning curettes are send to drying section .Provides convenience for sample loading in primary tubes of 500 µl, 2 ml, 5 ml, 7 ml, 10 ml, and sample cups. All the thirty-nine positions can be allocated for Blank/Sample/Calibrator/Control/STAT Samples. An optional QR BARCODE Scanner makes the operation easy and user friendly, thereby developing operator's efficiency. Consist of 50 cooled positions for system pack bottles, flexible options of 50 ml, 20 ml, bottles and 5 ml adapter. Linear, Non-linear, multi-point and K factor type of calibration makes EM 200 system reliable. For immunoturbidometric assays up-to 10

standards can be used. Calibration system provides accurate information about the curve, date and time of calibration.

TransAsiaEM200-BiochemistryAutoanalyser:



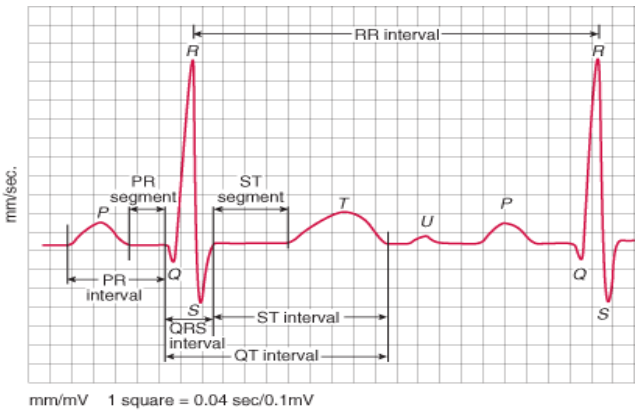
Electrocardiogram:

The electrocardiogram is a rapid, painless, safe and non-expensive test that is mainly used to detect the atrial fibrillation and chest pain.Electrocardiography is study the recording of the electrical impulses that are generated in the heart. These impulses initiate the contraction of cardiac muscles. The word vector is recognizable to characterize these electrical impulses in a graphical method to exhibit the intensity and the guidance of the electrical impulse is 60 to 100 times performs per minute. Arrhythmia (also referred to as dysrhythmia) is an abnormal or irregular heart rhythm, which can cause the heart to pump less effectively. Atrial arrhythmia is an arrhythmia caused by a dysfunction of the sinus. The conductive system of the heart is consists of five specialized tissues are Sino atrial node (SA node), atrioventricular node (AV node), bundle of his, left bundle branch (LBB) and right bundle branch (RBB), Purkinje fibers.ECG machine thatrecords electrical impulses coming from heart through 12 small electrode patches (six limb leads I, II, III, aVR, aVL ,aVF)and (six chest leads V1toV6) connected to the skin of your chest, arms and legs.

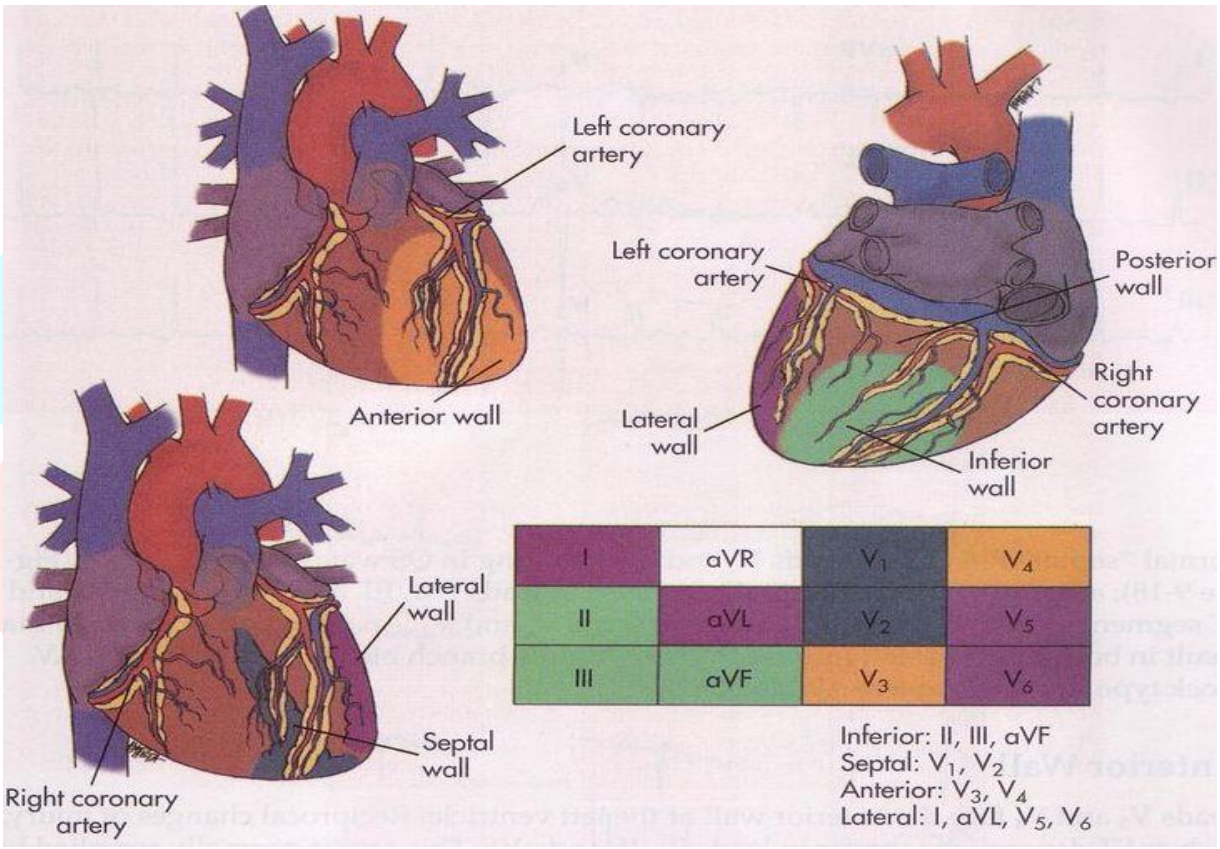
ECG changes during myocardial infarction:

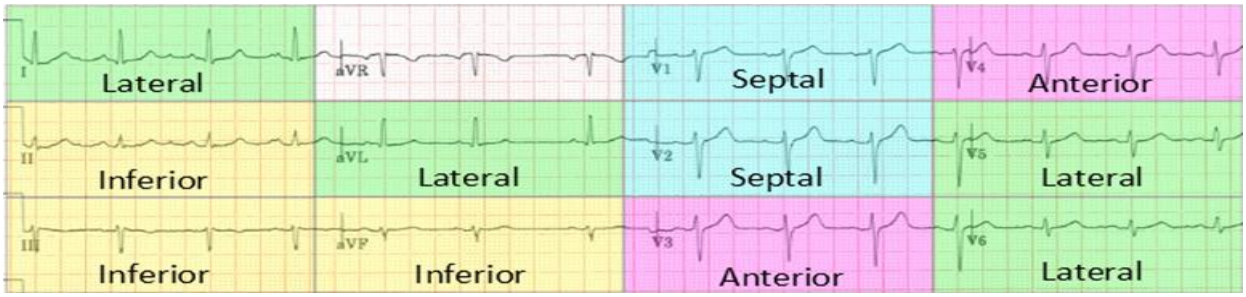
Wall Affected	Leads Showing ST Elevation	Leads Showing Reciprocal ST Depression	Suspected Artery
Septal	V1, V2	None	Left Anterior Descending - LAD
Anterior	V3, V4	None	Left Anterior Descending - LAD
Anteroseptal	V1, V2, V3, V4	None	Left Anterior Descending - LAD
Anterolateral	V3, V4,V5,V6,I,aVL	II, III, aVF	Left Anterior Descending – LAD, Circumflex – LCX
Extensive Anterior (Can be called Anteroseptal w/Lateral Extension)	V1, V2, V3, V4,V5,V6,I,aVL	II, III, aVF	Left Main Coronary Artery – LCA
Inferior	II,III,aVF	I, aVL	Right Coronary Artery –RCA, or Circumflex - LCX
Lateral	I,aVL,V5,V6	II, III, aVF	Circumflex - LCX
Posterior (Often associated w/Inferior or Lateral but also can be isolated)	V7,V8,V9	V1, V2, V3, V4	Posterior Descending – PDA, (branch of RCA or Circumflex – LCX)
Right Ventricular (Usually associated w/Inferior)	II,III,aVF,V1,V4R	I, aVL	Right Coronary Artery - RCA

ECG waves:



Normal ECG Morphology : Rhythum strip:





The P wave outcome from atria contraction, P wave is normally about 1 box broad or 1 box long. P wave that exceeds these condition indicate atria hypertrophy that is enlargement. The PR interval is measured from the start of the P wave to the start of Q wave. It represents the duration of atria depolarization. Regular duration is from 0.12 to 0.20 seconds, about 3 to 5 boxes wide. If the PR interval is greater than 0.20 seconds, then an AV block might be present. The QRS complex is measured from the start

of Q wave to the end of S wave. It represents the duration of ventricle depolarization. Regular duration is from 0.08 – 0.12 seconds, about 2 to 3 box wide. If duration is longer, it might indicate presence of bundle branch blocks. The Q T/QT_c is measured from the start of the Q wave to the end of T wave. QT intermission distance show the period of activation and improvement of the ventricular muscle. This time period varies backwards with the heart rate. The regular QT_c is generally 0.41 seconds or an authentic measurement, it is corrected with the heart rate with the following formula to get QT_c.QT_c = QT + 1.75 (HR – 60).The ST segment is measured from end of S wave, J point, to the start of T wave. This segment is important in identifying pathology such as myocardial infarctions (elevations) and ischemia (depressions).

Pwave,QRS complex and T waves:

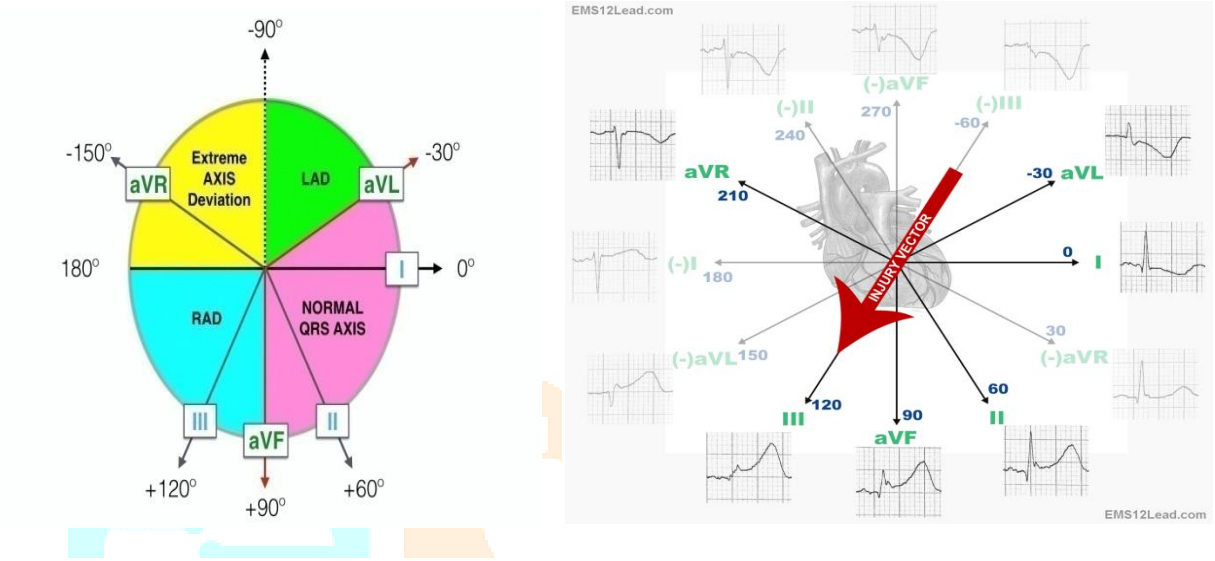
Analysis contents	Normal values	Abnormal values
Heart rate	60-100 ms	less than 60 ms and greater than 100 ms
Heart rhythum	Regular	Irregular
P wave	Sinus p waves	Non sinus p waves
PR interval period	0.12-0.20 sec	Less than 0.12sec and greater than 0.20sec
QRS wave	Normal QRS waves	QRS duration augmentation, pathological Q waves
ST segments	Normal ST segments	Elevation & depression of ST segments
T waves	Normal T waves	Tip, fiat or inverted T waves
Others		U-Waves

Determining Axis:

The axis of the Electro cardiogram is the considerable orientation of the overall electrical activity of the heart. It can be normal (axis lies between –30° and +100°), an axis of –30° or more negative is left axis deviation, or LAD (leftward), and one that is +100° or more positive is right axis deviation, or RAD (rightward). The QRS axis is the maximum significance to determine. So, the P wave or T wave axis can also be studied. To determine the QRS axis, the limb leads (not the precordial leads) need to be observed. The clarification of the standard leads and their correlation to the cardiac axis is given below. Record the

lead I is at zero degrees, lead II is at +60 degrees, and lead III is at +120 degrees. Lead aVL (L for left arm) is at -30 degrees and lead aVF (F for foot) is at +90 degrees. The negative of lead aVR (R for right arm) is at +30 degrees; the positive of lead aVR is actually at -150 degrees. Although memorizing the above picture is necessary to calculating the correct axis, some alternative routes to rapidly identify the axis are outlined below.

Determining Axis:



The normal QRS axis must be between -30 and +90 degrees. Left axis deviation is explained as the major QRS vector, lowering between -30 and -90 degrees. Right axis deviation occurs with the QRS axis and is between +90 and +180 degrees. Indeterminate axis is between +/- 180 and -90 degrees. This is summarized in the image below. The rapid, non-specific method to evaluate the QRS axis is to observe the major direction of the QRS complex positive or negative in leads I and aVF. The QRS complex is upstanding (positive) in the couple of lead I and lead aVF, subsequently the axis is normal. The QRS is upstanding in lead I (positive) and downward in lead aVF (negative), then the axis is between 0 and -90 degrees. The left axis deviation is determined as between -30 and - 90, this scenario is not always technically left axis deviation. This text, the QRS axis could free fall between 0 and -30, which is within normal limits. To moreover differentiate the normal from left axis deviation in this position, look at lead II. The lead II is downhill (negative), then the axis is more towards -120, and left axis deviation is present. If the QRS complex in lead II is upstanding (positive), then the axis is more towards +60 degrees, and the QRS axis is normal. The QRS is mostly negative in lead I and positive in lead aVF, then the axis is rightward (right axis deviation). The QRS is downward (negative) in lead I and downward (negative) in lead aVF, then the axis is indeterminate or northwestern axis.

Results and Discussions:

Quantification of Troponin-I Ultra Mini VIDAS.

Thechemiluminescence Immunoassay of troponin-I detected by using biomerieux-mini VIDAS protein analyzer instrument and collect the recorded sample data results observed as acute coronary

syndrome, myocardial fibrosis and chronic heart failure. (Negative value is ‹ 0.11 ug/L and Positive value is ›0.11 ug/L).

Quantification of CKMB and CK - EM 200:

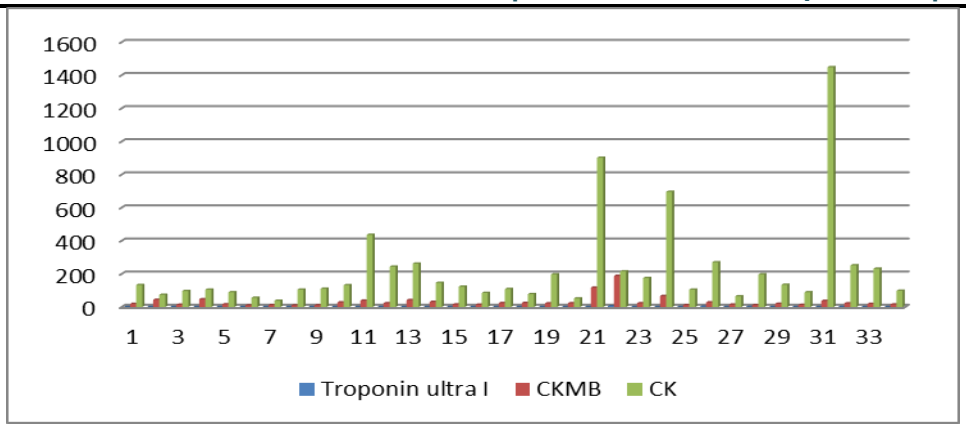
The CKMB and CK detected by using EM200biochemical analyzer instrument and collect the recorded sample data results observed as acute coronary syndrome, myocardial fibrosis and chronic heart failure. (CKMB negative value is›25ug/L and CK value negative is (Men: upto170u/L, Women: up to 145 u/l)).

Table 1: Quantification of troponinn I, CKMB and CK.

S.No.	Age	Gender	Troponin ultra I (‹ 0.11ug/L /›0.11 ug/L)	CKMB (›25u/L)	CK(men:upto170u/L, women:up to 145 u/l)
1	53	Male	0.01	19	133
2	64	Male	0.01	44	74
3	65	Female	0.01	14	97
4	53	Male	0.01	47	105
5	40	Female	0.01	17	89
6	65	Male	0.01	10	56
7	65	Female	0.01	12	38
8	31	Male	0.01	10	105
9	31	Male	0.01	11	111
10	62	Female	0.01	28	132
11	41	Female	0.01	39	436
12	80	Female	0.09	23	244
13	81	Female	0.08	42	262
14	60	Male	0.01	31	167
15	45	Male	0.01	16	123

16	62	Female	0.01	14	85
17	40	Male	0.01	24	109
18	37	Male	0.01	25	78
19	37	Female	0.06	22	125
20	73	Female	0.01	23	52
21	68	Male	0.53	117	902
22	71	Male	5.06	188	215
23	24	Male	0.01	23	164
24	63	Male	0.01	66.5	696
25	47	Male	0.35	12	105
26	65	Male	2.98	28	270
27	49	Male	0.01	15	65
28	63	Male	1.28	12	197
29	73	Male	0.01	20	134
30	73	Female	0.01	13	89
31	66	Male	0.01	37	1449
32	73	Female	1.14	22	252
33	52	Male	0.01	19	232
34	53	Male	0.01	16	98

Figure 1:luminescence Immunoassay of Troponin-I using biomerieux-mini VIDAS and ckmb ,ck by EM200



Electrocardiogram:

The electrical impulses were generated, recorded the graphical data, measured the value of heart rate, monitored the heart rhythms, calculated the PR rate, QRSD rate, QT rate, QTC rate, and determining the P axis, QRS axis, Taxis value were tabulated .The pericarditis or acute myocardial infarction, heart attack, left atrial enlargement at lead(s) II and v4-abnormal biphasic p wave and abnormal progression at lead(s) v5 and v6 observed the results from the patient.

Age	Gender	Heart Rate(60-100bpm)	Heart Rhythum	PR Rate(120-200 ms)	QRSD Rate	QT Rate(390-400 ms)	QTC Rate(<350-450>)	P Axis	QRS(69-103ms) Axis	T Axis
68	Male	57	Sinus	184	85	438	427	33	68	49
71	Male	61	Sinus	181	90	425	428	68	66	35
47	Male	75	Sinus	194	84	381	426	72	67	62
65	Male	69	Sinus	189	84	403	432	79	77	46
63	Male	90	Sinus	107	92	354	433	71	79	50
73	Female	84	Sinus	105	88	346	420	68	76	48

Fig: 1 Borderline ST elevation (V2-V6) -pericarditis or acute myocardial infraction

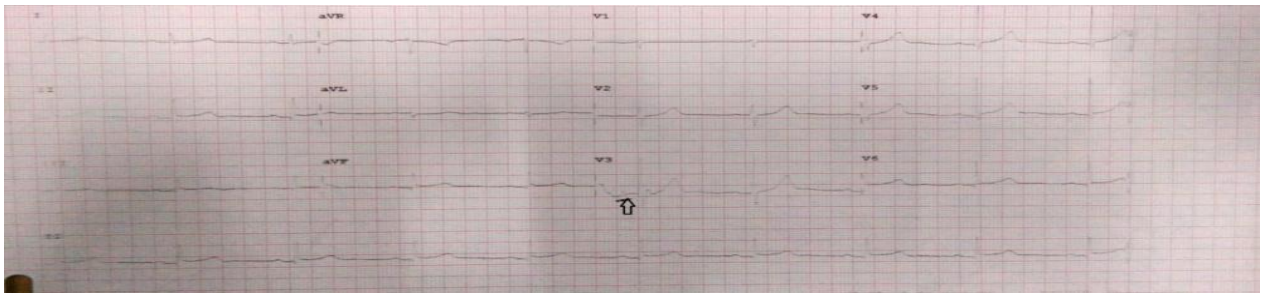


Fig: 2 Minimal ST elevations – heartattack

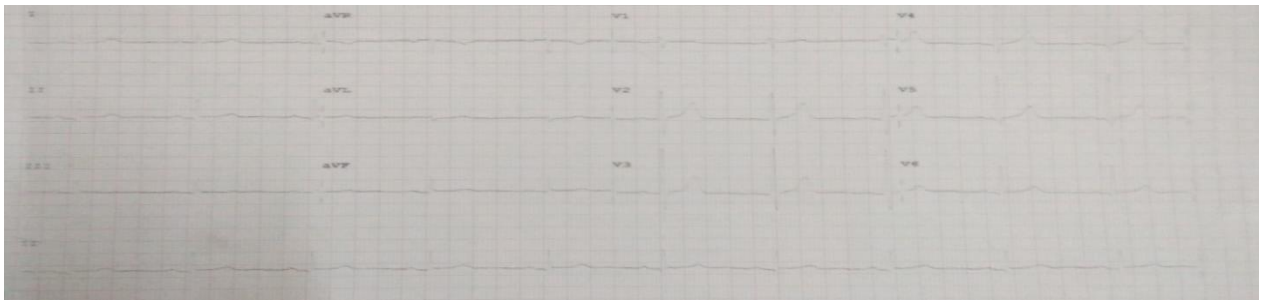


Fig: 3 Left atrial enlargement –Lead(s) II and V4 **(**biphasic p wave)

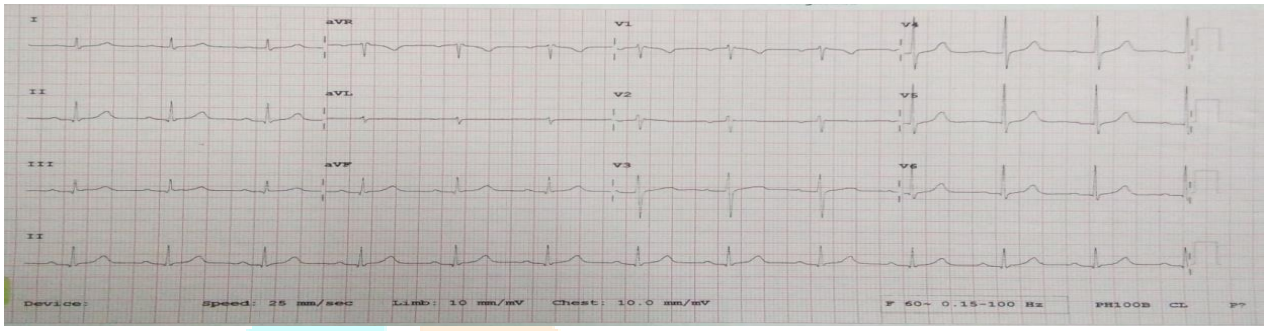
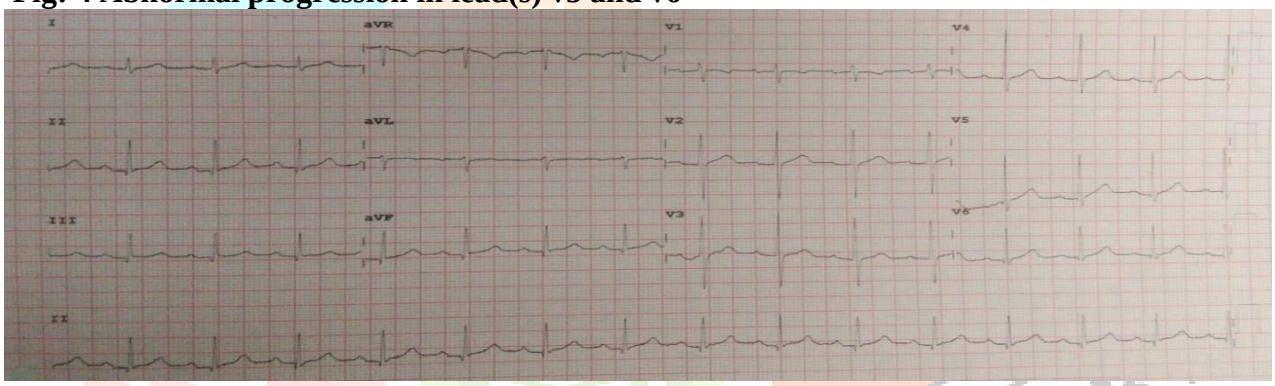


Fig: 4 Abnormal progression in lead(s) v5 and v6



Conclusion:

The biomarkers are released into the circulatory blood systems after myocardial infarction, cardiac necrosis and myocardial dysfunction. They play a significance role not only in the diagnosis of patients, risk stratification, selection of therapy, monitoring disease progression, and treatment efficacy. Troponin becomes a cardiac marker of choice for the patients with acute myocardial infarction. However; abnormal values should be explained properly.ECG (zero-crossing method or wavelet analysis) can be used to identify the high-risk group of patients.

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