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The combinational effect of cardiac and biochemical markers in diabetic patients with cardiovascular disease

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ABSTRACT

Background: Clinicopathological correlations, as well as several angiographic studies, suggest that diabetic patients have more extensive atherosclerotic disease, affecting the coronary arteries in particular. We sought to examine the combinational effect of cardiac and biochemical markers in diabetic patients with cardiovascular disease. Method: The study population constituted 50 healthy subjects, 50 cardiovascular subjects with diabetes and 50 cardiovascular subjects without diabetes. The population was subjected to biochemical and cardiac marker analysis and the results were verified. Results and discussion: Studies suggest that glycated hemoglobin values in the abnormal range can identify persons at increased risk for coronary heart disease, stroke, and death before the diagnosis of diabetes, indicating that glycated hemoglobin is a useful marker of cardiovascular risk and death from any cause.

1. Introduction

Type 2 diabetes is associated with multiple abnormalities, all of which can contribute to vascular disease. The most notable of these abnormalities include obesity, insulin resistance, hyperglycemia, dyslipidemia, hypertension, and renal disease. Although a number of these disorders are often grouped together in an entity termed "metabolic syndrome," the increased risk for atherosclerotic disease in insulin-resistant patients correlates best with these abnormalities when each is considered individually. These abnormalities promote heart disease by inducing atherosclerosis, endothelial cell dysfunction, oxidative stress, inflammation, and vascular remodeling.

There could be several explanations for the different patterns of symptoms in patients with diabetes mellitus, including different thresholds of pain sensitivity, psychological denial, or the presence of autonomic neuropathy leading to sensory denervation. The latter seems to be more likely in diabetic patients, because autonomic neuropathy is a common feature of diabetes, and abnormalities of the autonomic nerve fibers were demonstrated histologically in diabetic patients who died after painless myocardial infarction. Furthermore, diabetic patients with silent myocardial ischemia show evidence of diffuse abnormalities in m-iodobenzylguanidine imaging, suggesting that abnormalities of pain perception may be linked to sympathetic denervation [1].
Cardiac markers are biomarkers measured to evaluate heart function. They are often discussed in the context of myocardial infarction, but other conditions can lead to an elevation in cardiac marker level. Cardiac marker tests identify blood chemicals associated with myocardial infarction (MI), commonly known as a heart attack. The myocardium is the middle layer of the heart wall composed of heart muscle. Infarction is tissue death caused by an interruption in the blood supply to an area.

Cardiac troponin T (cTnT) and troponin I (cTnI) are cardiac regulatory proteins that control the calcium mediatedinteraction between actin and myosin. The cardiac forms of these regulatory proteins are coded by specific genes and theoretically have the potential of being unique to the myocardium. Indeed, cTnI has not been identified outside the myocardium.Cardiac troponin T is expressed to a small extent in skeletal muscle; however, the current cTnT assay does not identify skeletal troponins [2].

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The measurement of serum cTnI and cTnT is superior in terms of sensitivity and specificity to cardiac muscle enzyme measurements in the identification of cardiac muscle damage.Raised cardiac troponin concentrations are now accepted as the standard biochemical marker for the diagnosis of myocardial infarction. Cardiac troponins are detected in the serum by the use of monoclonal antibodies to epitopes of cTnI and cTnT. These antibodies are highly specific for cardiac troponin and have negligible crossreactivity with skeletal muscle troponins. Cardiac troponins may not be detected in the serum for up to four hours after the onset of an acute coronary event and should be repeated after 12 hours if the troponin concentration on admission is not raised in an individual presenting with chest pain.

Creatine kinase is an enzyme responsible for transferring a phosphate group from ATP to creatine. It is composed of M and/or B subunits that form CK-MM, CKMB, and CK-BB isoenzymes. Total CK (the activity of the MM, MB, and BB isoenzymes) is not myocardial-specific. However, the MB isoenzyme (also called CK-2) comprises about 40% of the CK activity in cardiac muscle and 2% or less of the activity in most muscle groups and other tissues. In the proper clinical setting, MB is both a sensitive and specific marker for myocardial infarction. MB usually becomes abnormal three to four hours after an MI, peaks in 10–24 hours, and returns to normal within 72 hours. However, an elevated serum MB may occur in people with severe skeletal muscle damage (such as in muscular dystrophy or a crush injury) and renal failure. In such cases, the CK index (MB divided by total CK) is very helpful. If the index is under 4%, a nonmyocardial cause of a high MB should be suspected. CK-MB is considered the benchmark for cardiac markers of myocardial injury. Measurement of CK-MB may be performed via electrophoresis or immunoassays; the latter demonstrates better analytical sensitivity and better precision[3]

CK-MB forms can be used to determine whether thrombolytic therapy (such as treatment with tissue plasminogen activator to dissolve a blood clot in the coronary artery) has succeeded. MB forms are different molecular forms of MB found in the circulation. When MB is released into the blood, part of the M subunit is removed by an enzyme in the plasma. This results in a molecule called CK-2 1 . This is the prevalent form of MB in the blood. CK-2 2 is the unmodified cardiac form of MB. After successful thrombolytic therapy, the unmodified form of MB is rapidly flushed into the blood, causing it to become the dominant form .

Myoglobin may leak from muscle tissue into the blood circulation as a result of damage to skeletal or cardiac muscle and subsequently may appear in the urine because of its relatively low molecular weight.Myoglobinemia and myoglobinuria have been used in the diagnosis of myopathies and cardiopathies.The myocardium contains bundles of striated muscle fibers that are composed of cardiac-specific contractile proteins (actin and myosin), regulatory proteins (troponins and tropomyosin), and proteins that are required for the conversion of chemical energy into work (muscle contraction), i.e., myoglobin; and the enzymes creatine kinase and lactate dehydrogenase. Cardiac tissue injury may cause these proteins to be released into the circulation; they therefore serve as biochemical markers of cardiac injury.Biochemical markers provide clinicians with an important tool for the assessment of acute coronary syndromes. Biochemical surgeries or extensive medical treatments.

markers, including total creatine kinase (total CK), creatine kinase-MB (CK-MB), the MB isoforms, and myoglobin, as well as the troponins - cardiac troponin T (cTnT) and cardiac troponin I (cTnI) - are all used for assessment of the suspected acute myocardial infarction (AMI) patient. Myoglobin is released rapidly after tissue injury and may be elevated as early as 1 hour after myocardial injury. Myoglobin is also cleared rapidly by renal excretion, so abnormal levels may return to baseline values in six to twelve hours[4].

According to the American Heart Association, studies have shown that too much homocysteine in the blood is related to a higher risk of coronary heart disease, stroke, and peripheral vascular disease; and that it may also have an effect on atherosclerosis. High levels of homocysteine are the result of a lack of certain B vitamins, inheritance, or dietary excess and have been implicated in vascular-wall injury. Many risk factors, including family history of heart disease, smoking, obesity, lack of exercise, diabetes, high levels of low-density lipoprotein cholesterol (LDL or "bad" cholesterol), low levels of high-density lipoprotein cholesterol (HDL or "good" cholesterol), and high blood pressure have been documented to increase the risk of stroke and heart disease. With so many other risk factors, it has been difficult to determine whether high levels of homocysteine are an independent risk factor for the development these diseases. However, a substantial number of controlled, well-designed, and well-documented studies have shown that individuals who have high levels of homocysteine in the blood are at increased risk of developing blocked blood vessels, a condition known as occlusive arterial disease or at risk to worsen atherosclerosis ("hardening of the arteries").

Either BNP or NT-proBNP may be used to help diagnose heart failure and to grade the severity of that heart failure. Both BNP and NT-proBNP levels in the blood are used for screening, diagnosis of acute congestive heart failure (CHF) and may be useful to establish prognosis in heart failure, as both markers are typically higher in patients with worse outcome. The plasma concentrations of both BNP and NT-proBNP are also typically increased in patients with asymptomatic or symptomatic left ventricular dysfunction. There is no level of BNP that perfectly separates patients with and without heart failure. BNP accurately reflects current ventricular status The half-life of NT-ProBNP is 1 to 2 hours vs. 20 minutes for BNP [5].

D-dimer concentration may be determined by a blood test to help diagnose thrombosis. However, an elevated D-dimer does not always indicate the presence of a clot because a number of other factors can cause an increased level. Elevated levels may be seen in conditions in which fibrin is formed and then broken down, such as recent surgery, trauma, infection, heart disease, and some cancers or conditions in which fibrin is not cleared normally, such as liver disease Therefore, D-dimer is typically not used to rule out veneous thromboembolism (VTE) in hospitalized patients (inpatient setting).Pregnancyis also a condition in which fibrin is formed and broken down and may result in an elevated D-dimer level. However, if DIC is suspected in a woman who is pregnant or is in the immediate postpartum period, then the D-dimer test may be used, along with a PT, APTT, fibrinogen, and platelet countto help diagnose her condition. If the woman has DIC, her D-dimer level will be very elevated.D-dimer is recommended as an adjunct test [6].

Type 2 diabetes is present in 10-30% of patients presenting with MI and, given the expected doubling in the incidence of diabetes over the next 25 years, represents a major public health concern. In addition, MI may be associated with the first presentation of glucose intolerance or overt diabetes, early diagnosis providing an opportunity for appropriate intensive management and risk stratification.

Hypertension and diabetes together result in more cardiac fibrosis than when either occurs alone. Endothelial dysfunction may impair coronary perfusion at the microvascular level, resulting in ischemia. Although the heart utilizes free fatty acids as its major source of energy, ischemia results in greater expression of GLUT4 transporter proteins, facilitating glucose entry and glycolysis, a major source of myocardial ATP in anaerobic conditions.In diabetes, however, ATP generation is less efficient, because relative insulinopenia results in increased lipolysis, elevated plasma levels of free fatty acids, and increased fatty acid oxidation as glycolysis and glucose oxidation are suppressed.In addition, despite the hyperglycemia most diabetics experience in acute MI, glucose is unavailable as an energy source, because myocardial GLUT4 transporter protein levels may be depressed. These metabolic perturbations result in depressed ATP production, generation of oxygen free radicals, increased myocardial oxygen consumption, and myocardial contractile dysfunction. It is not surprising that additional myocardial damage results in heart failure out of proportion to infarct size in patients with diabetes [7].

Hemoglobin A1c (HbA1c), also known as glycosylated hemoglobin, is a measure of the average sugar level in the blood over an extended period of time. It is considered to be a better measure of general blood glucose levels than self-reported blood sugar readings. Diabetics with HbA1c numbers that are elevated are at increased risk of numerous vascular complications, which may effect the heart, kidneys, eyes and feet, among other organs. Those with raised HbA1c values are at increased risk of requiring

2.Materials And Methods

2.1.Study population

The population consisted of 150 male subjects divided into two groups, 50 subjects (mean age 45 – 60 years) had evidence of AMI with diabetes and 50 AMI patients without diabetes. The other 50 subjects age and sex matched healthy subjects were studied as controls. All patients had been admitted to the Coronary Care Units (CCU) of K.G. Hospital and Postgraduate Medical Institute, Coimbatore, Tamil Nadu, India, between July 2009 and March 2010. The diagnosis of AMI was based on a history of prolonged ischemic chest pain, characteristic electrocardiogram (ECG) changes and elevated creatine kinase isoenzyme MB (CK-MB) and troponin T within 12 h after the onset of pain.

Cardiovascular disease was diagnosed by angiography, ECG, sintigraphy and effort test, or self-reported use of a β-blocker, angiotensin I converting enzyme (ACE) inhibitor and/or diuretic drug. The patients who had total cholesterol level of >220 mg/dL or triglycerides concentration >200 mg/ dL, or receiving lipid lowering drugs were defined as having hyperlipidemia. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was≥126 mg /dL or if the patient was treated with insulin or oral hypoglycemic agents. Oral consent was obtained from the patients' relatives and normal subjects, prior to study.

2.2. Biochemicalanalysis

Biochemical investigation including Fasting Plasma glucose,total cholesterol, triglycerides, HDL-C, LDL-C ,Glycosylated Haemoglobin,were determined using fully automated clinical chemistry analyzer (HIT-912, Boehringer, Mannheim, Germany).Serum VLDL-C was calculated according to Friedewald et al.Estimation of Troponin-I level measured by automated Immunoassay analyzer (AXSYM System-Abbott Laboratories,Abbott Park,USA).Troponin T ,Pro BNP,D-dimer was measured by Roche Elecsys 2010 Immuno assay analyzer,USA.

3. Results And Discussion

Diabetes mellitus and heart failure (HF) are major health problems. . In individuals free of HF, elevated HbA1C has been associated with an increased risk of adverse cardiovascular outcomes, including increased risk of incident HF. Despite these data, studies examining the association between HbA1C and outcomes in diabetic patients with established HF have been limited and have reported discrepant results.

Table 1.Shows the demographic characteristics of study population in healthy, cardiovascular patients with and without diabetes. The entire studied group had similar mean age and mean body weight. The fasting plasma glucose level and Glycosylated Haemoglobin (HbA1c) levels were elevated in cardiac patients with and without diabetes as compared to healthy subjects. From the lipid profile,total cholesterol,triglycerides,VLDL,LDL-C ,Cardiac troponins (cTn I and cTnT)levels were significantly higher in cardiac patients with and without diabetes as compared to control subjects. The Pro BNP,D-dimer level was also significantly raised in cardiac patients with diabetes as compared to the control subjects.HDL level was lower in cardiac patients with diabetes as compared to the control subjects.

Diabetes mellitus is commonly associated with both microvascular and macrovascular complications. Increasing evidence supports that atherosclerosis is a co-morbid condition in the diabetic patients. Impairment of vascular endothelial function is an initial step in the development of cardiovascular problems. Recently the important contribution of inflammation and oxidative stress to the pathogenesis of accelerated atherosclerosis in diabetic patients has been emphasized. Hypercholesterolemia causes focal activation of endothelium by infiltration and retention of LDL -cholesterol in arteries causing inflammatory response and activation of reactive oxygen species (ROS). Modification of LDL, through oxidation and enzymatic activity causes LDL oxidation. OxLDL when recognised by macrophages is converted into foam cells, which is a key event in atherogenesis. The central role of dyslipidemia in causing progression of atherosclerosis in adults with diabetes has been elucidated. There are a few researcher who have reported higher levels of total cholesterol, LDL-cholesterol and triglyceride with higher HbA1c concentrations in diabetic patients [8].

Recommendations for the diagnosis of diabetes are based on the relations of fasting glucose and glycated hemoglobin with micro vascular disease, typically retinopathy. Nonetheless, cardiovascular disease is the leading cause of illness, death, and hospitalization in persons with diabetes. Studies suggest that glycated hemoglobin values in the normal range can identify persons at increased risk for coronary heart disease, stroke, and death before the diagnosis of diabetes, indicating that glycated hemoglobin is a useful marker of cardiovascular risk and death from any cause. The J-shaped relation between the glycated hemoglobin value and the risk of death from any cause suggests that further exploration of the health risks associated with the low-normal glycemic state and possible nonglycemic determinants of glycated hemoglobin is warranted.

Table1: Demographic characteristics and biochemical and cardiac marker in healthy controls and cardiovascular patients with and without diabetes.

Parameter	Control Subjects	CV patients without diabetes	CV patients with diabetes
No.of subjects	50	50	50
Mean Age (Years)	42±8.7	41±7.4	43±5.6
Weight (mean±SD;Years)	55±9	57±9	60±5
Hypertension	-	70.5%	82.2%
Cholesterol (mg/dl)	154±2.7	221±18.5	119±12.2
Triglycerides (mg/dl)	92±10.5	234±18	226±22
HDL Cholesterol (mg/dl)	48±3	32±6	31±7
LDL Cholesterol (mg/dl)	77±7	142±13	138±13
VLDL Cholesterol (mg/dl)	24±4	50±5	48±7
Fasting plasma Glucose (mg/dl)	91±8	102±6	160±6
(HbA1c) (%)	5.0±0.6	5.3±0.6	6.9±0.4
Mean Plasma Glucose (MPG)(%)	106±5	130± 5	180±15
Troponin T(ng/ml)	0.000±0.000	0.85±0.03	0.92±0.06
Troponin I (ng/ml)	0.000±0.000	1.02±0.093	1.06±0.086
D-dimer (µg/ml)	0.0±0.0	1.0±0.2	0.9±0.3
ProBNP (pg/ml)	88±10	2325±86	2400±86

Values are given as mean ±S.D from 50 subjects in each group.

Therefore, we sought to determine the association between HbA1C and total mortality or HF hospitalization in a large, national cohort of ambulatory diabetic patients with established HF. Glycated hemoglobin (HbA1c), even at levels considered in the "normal" range, emerged as an independently significant predictor of heart-disease events, stroke, and death over more than a decade in an analysis from the Atherosclerosis Risk .The risks of incident diabetes, coronary heart disease events, and death were significantly increased for all HbA1c values higher than the reference range of 5.0% to <5.5%; the ischemic stroke risk went up significantly at levels >6.0%.

While the risk of diabetes fell and the CV and stroke risks were unchanged at HbA1c levels <5.0%, compared with the reference range, all-cause mortality went up significantly at the lower levels.

Studies have demonstrated that there is an increased inflammatory and oxidative damage of coronary vessels in type II diabetic patients. HbA1c is a marker of long-term glycaemic control and for every one-percent increase in HbA1c, increase the relative risk for cardiovascular events increase.20 Thus the measurement of HbA1c levels is important not only for monitoring of diabetes but also for assessment of the risk of CHD in diabetics [9].

4.Conclusion

A large body of epidemiological and pathological data documents that diabetes is an independent risk factor for CVD in both men and women. The future glycometabolic profile of patients suffering AMI without diabetes can be predicted in the hospital phase. There is also a correlation between blood glucose

on hospital admission for AMI and long-term mortality in patients with or without known diabetes. Moreover, hyperglycemia in patients with ST elevation MI was found to be an important predictor of impaired epicardial flow. In acute coronary syndromes, glucose metabolism is modified, and stress hyperglycemia commonly occurs secondary to increased catecholamine levels. In short, Hyperglycemia during acute myocardial infarction (AMI) is associated with a poor prognosis, and blood glucose level is an independent predictor of mortality in patients with or without known diabetes.

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