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A Review of Conventional and Emerging Biomarkers for Prognostication of Cardiovascular Disease.

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ABSTRACT

Cardiovascular disease (CVD) has remained a leading cause of death worldwide for the past several decades. It is a progressive pathological condition which gradually develops during the course of one's life at a rate which depends on the extent of interplay between its unmodifiable and modifiable risk factors. Its genesis lies in the initiation of atherosclerosis, which may remain asymptomatic in the subclinical state. Its gradual progression culminates in symptomatic CVD and its onset invariably predicts an adverse prognosis leading to multiple recurrent cardiac upheavals if uncontrolled. At this stage, clinicians largely rely on routine diagnostic procedures and physical examination for arriving at therapeutic decisions on patient management. Such evaluations are however bound by limitations, especially with irreversible changes having already set in at molecular and cellular levels, thus largely leading to ineffective treatment. Logically, disease management can be considered successful if undertaken before the onset of overt clinical manifestations which may conclusively require presymptomatic prognostication of the pathological condition. Biomarkers, in this regard, are gaining increasing interest, as they are, to some extent able to identify high risk subjects and predict the future development of CVD in them. Further, it is also interesting to note that ethnicity plays a very important role in determining the extent to which these markers can be implicated in cardiovascular risk prediction in specific populations. This is particularly true in case of Asian Indian populations wherein biomarkers, established for the Western population, are not so significant and certain other markers which are considered of low priority in Caucasians assume significance. This review focuses on different biochemical and immunological parameters, some of which have already been well established as biomarkers and routinely considered by clinicians for risk assessment. Certain additional novel parameters, which are slowly gaining importance as disease causal and consequential agents and throwing throw light on the progress of the disease are also highlighted.

Keywords: Cardiovascular disease, atherosclerosis, risk prediction, biochemical markers, Inflammatory markers

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INTRODUCTION

Cardiovascular Disease (CVD) is implicated worldwide as the most important cause of mortality[1]. Age, gender and genetic make up are its unmodifiable risk factors whereas its modifiable risk factors include lifestyle related parameters like tobacco abuse, low physical activity, a high fat and high salt diet, etc. Recently, some partially modifiable parameters like obesity, type A and D personalities and aggravated response to stress are also being recognized and granted due independent status. All the above parameters contribute to the development and progression of various forms of CVD [2-6]. Further, it is well established that the degree of adopting the lifestyle habits varies among ethnic groups and communities. Consequently, their common repercussions, like development of obesity, diabetes, hypertension and abnormal lipid profiles, are also highly variable among them[7,8]. The available epidemiological data suggests an interplay between age and gender, along with genetic, social and physiological factors, for evolving the final risk stratification in individuals, for development of CVD [9-11].

Currently, the treatment regimens for patients reporting with cardiac upheavals are based on their medical and family history, physical examination, ECG patterns and cardiac specific enzyme levels like CK-MB and LDH [12]. However, although the levels of such biomarkers provide first hand information about the degree of myocardial necrosis, they are indicative of acute coronary events which have already occurred. They are not precisely useful for prognosticating the occurrence of cardiovascular events in individuals. Hence it is imperative to identify biomarkers which serve as myocardial stress agents, so that these will not only help in early detection of CVD, but also be indicative of the probability of developing the disease decades later[13]. This will probably go a long way in designing the therapeutic and lifestyle advisory programs as part of personalized medical care. The clinical cardiac events can thus be avoided in individuals receiving such advice, which may be more beneficial than receiving therapeutic treatment after the actual onset of the disease eventually leading to myocardial ischemia.

Atherosclerosis, the primary cause of CVD, sets in during childhood and clinically identified in adulthood[14]. Its insidious progression may be a consequence of genetic makeup of an individual or a response to lifestyle related and physiological factors. The course of its development is associated with many biochemical [15] and immunological [16] changes which may serve as valuable markers for predicting upcoming cardiac disease. The genetic basis of developing this disease is also being identified through the still nascent area of genomics [17-19]. Research is underway to study the single nucleotide polymorphisms (SNP's) which can make the prognosis of CVD in individuals [20]. Apart from this, the developing field of metabolomics [21], which involves identification and analysis of metabolites arising as a consequence of these SNP's may also prove to be invaluable marker to predict the occurrence of CVD in supposedly normal individuals. The integration of all these markers onto a common scale may be extremely useful to study the relationships and interactions between them [9]. This may subsequently form the basis for arriving at a final risk score for individuals for personalized treatment and therapy.

In this review, the biochemical and inflammatory markers which are being used to predict the development of CVD in individuals is discussed. The risk prediction with respect to established markers, and the ones which are still under trials are elaborated for final risk stratification. The extent to which these markers are influenced by the ethnicity of the population and individuals is also explored.

Biochemical Markers for cardiac risk prediction

Lipids, lipoproteins and related parameters

Many biochemical markers are routinely used by clinicians to evaluate risk factors which are traditionally associated with CVD and the prevalence of such biochemical and clinical markers has been discussed [22]. Among them, the most common ones include serum cholesterol and triglycerides which exhibit a very strong positive correlation with CVD [3, 23].The perspectives of dyslipidemia, along with the impact of lipid lowering therapy, specifically in women have been reviewed [24]. Increases in low density and very low density lipoprotein cholesterol (LDL-C and VLDL-C) also predict the possibility of onset of myocardial events [25]. However, oxidized LDL[26] and lipoprotein associated phospholipase A2 (Lp-PLA2)[27] levels do not qualify as potential independent biomarkers for development of CVD in a general population at this stage. Further research may be required to evaluate their roles in sub populations selected for more traditional risk

factors. High Density Lipoprotein (HDL-C) is reported to negatively correlate with CVD and its anti atherogenic properties are so well documented that HDL therapeutic methodologies are currently being adopted for the treatment of atherosclerosis [28, 29]. The multi facets of HDL particle biology have also been reviewed [30]. Further, an increased LDL-C / HDL-C ratio may be associated with the initiation of atherosclerosis and may be more useful for risk assessment than LDL-C alone [31].

Paraoxonase (PON), an enzyme initially of interest in toxicology, is found to be associated with HDL and part of HDL's protective action has been attributed to its linkage with this enzyme. PON has thus been suggested to be protective against atherosclerosis, possibly by preventing the oxidative modifications of lipoproteins, and by causing hydrolyses of phospholipids and cholesteryl ester hydroperoxides [32]. Lipoprotein trends are reflected in apolipoprotein levels which show similar risk profiles [33, 34]. Another interesting biochemical parameter which is predicted as a potential marker for underlying atherosclerotic plaque instability is the erythrocyte membrane cholesterol. Patients with primary hypercholesterolemia, which is a strong positive risk factor for the development of CVD, have been reported to have increased levels of red cell membrane cholesterol compared to normo cholesterolemic subjects [35]. Hence the possible contributions of this parameter to the development of clinical symptoms of CVD have been stressed.

Lipoprotein (a) is known to be an independent risk factor whose synthesis is under genetic control [36]. Its levels remain unaltered through diet, exercise and lipid lowering strategies, however, it is reported that sex hormones may serve to protect females against the deleterious effects of Lp(a)[37]. The plasma concentration of Lipoprotein lipase and Lecithin Cholesterol Acyl Transferase (LCAT) has been studied and these were found to be in putative association with female gender and alcohol use [38]. However, both these enzymes do not appear as direct significant risk parameters for the atherosclerotic score and their statistical contributions remain indirect and diluted.

Antioxidant enzymes and scavengers

Oxidative stress, leading to the formation of reactive oxygen species plays an important role in the development and progression of atherosclerosis [39,40] and hence antioxidant enzymes and scavengers, which serve to retard the process, have been extensively studied. Antioxidants are known to inhibit oxidant formation, intercept formed oxidants and also repair oxidant induced injury. These points of oxidant intervention are especially important to regress atherosclerosis. The role of antioxidants in cardiovascular health has been reviewed [41]. Meta analyses of observational studies focusing on antioxidant enzyme activity and coronary heart disease show an inverse relationship between levels of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) with development of CVD. This analyses is based on computer aided and manual searches of literature between January 1966 to January 2008 [42]. Epidemiological studies carried out in different countries reflect a similar trend [43-46].

GPx is the key antioxidant enzyme in most cells and its low erythrocyte concentration identifies those at risk for cardiovascular events. These subjects may benefit from antioxidative treatments [47]. Its levels are also reduced in obese individuals indicating defective protective mechanisms against atherosclerosis and oxidative stress [48]. SOD is the first line of enzymatic defense in cells and regulates the vascular levels of superoxide anion which has been implicated in the pathogenesis of CVD, and hence there has been substantial interest in this enzyme [49]. Its activity is found to decrease in clinical conditions like diabetes mellitus and hypercholesterolemia, which are strong risk factors for CVD [50, 51].

The mechanistic data regarding the biochemical effects of Vitamin E along with Vitamin C, and their relevance to atherosclerosis has been discussed [52]. The levels of these vitamins are shown to be lowered in CVD patients compared to controls in several studies [53,54]. The role of Vitamin E, the main lipid soluble antioxidant scavenger, in the prevention of atherosclerosis has also been reviewed[55] and it has been reported that in Tunisian CHD patients, lower lipid adjusted vitamin E values were associated with enhanced LDL susceptibility to oxidation but without alteration of serum fatty acid profile [56]. However, evidences also indicate that in addition to inhibition of oxidative modification of LDL, Vitamin E may inhibit atherogenesis through several other mechanisms at the molecular and cellular levels, which also include its non-antioxidant functions [57]. The relationship between Vitamin C and cardiovascular disease has been discussed and evidence, indicative of a protective effect of Vitamin C on lipid peroxidation has been examined [58]. However, evidence linking vitamin C to human cardiovascular disease is largely circumstantial, but taken in total, is

suggestive of an association. Conclusively, further research is needed to substantiate these claims, which presently appear inconsistent.

Other novel biochemical markers

A positive correlation has been found between plasma plasminogen levels in women diagnosed with Ischaemic Heart Disease, which increases with triglycerides and glucose levels [59]. Its levels however decreases with advancing age in males, is higher in current smokers and positively correlates with Total and LDL Cholesterol. As dietary glycemic load is a determinant of hyperlipidemia, it may be a risk factor for CVD too. A population based follow up study indicated that the risk increases among middle aged women consuming a high glycemic diet, particularly in overweight ones [60]. An analysis among cohorts of Asia Pacific region shows a positive continuous association between blood glucose and CVD risk in males and females, across all age subgroups [61].The overall associations for non-fasting glucose are weaker than those for fasting glucose. The importance of identification and quantification of plaque burden, in terms of coronary calcium scores, measured by electron beam tomography, has been stressed [62]. Coronary calcium may also be a predictor of coronary events across racial and ethnic groups[63] which can be useful to treat patients at greater risk for developing cardiac complications. Further, women seem to benefit more from the prognostic value of predicting coronary calcification than men [64, 65]. A prognostic value has also been attributed to myeloperoxidase, a haemprotein, in patients with chest pain [66]. Its plasma concentrations may predict the mortality, probability after the occurrence of acute coronary events [67, 68].

Elevated levels of homocysteine have been linked to both atherothrombosis and thrombogenesis [69, 70]. Further, endothelial function seems to be hampered in older hyper homocysteinemic subjects compared to younger ones [71]. Higher levels of homocysteine, seen in their ATTICA epidemiological study, has been attributed to the lack of Vitamin B12 and Folic acid [22]. It may probably be essential to consider homocysteine as a biomarker for the final risk stratification in general and specifically for Indian patients with a strong genetic predisposition to the disease as inferred from a study conducted among the rural population in Maharashtra, India[72]. Metalloproteinase (MMP-9) enzymes, which are involved in the degradation and reorganization of extracellular matrix, have been implicated in the development of CVD [73, 74]. The biochemical and molecular genetic studies on MMP-9, performed in this pathological condition, is summarized[75]. Plasma von Willebrand factor, which plays a major role in platelet adhesion and aggregation, is also shown to influence thromboembolic cardiovascular events [76].

Circulating inflammatory biomarkers for predicting upcoming cardiac events

Inflammatory stress has been identified to play a central role in the pathobiology of atherosclerosis. The lesions developed here may possibly be a consequence of specific cellular and molecular responses to low level inflammatory activity. Hence circulating factors which are released into the blood stream as a consequence of inflammation may predict the progression of this disease along with prognosticating its extreme events like myocardial ischaemia. It is well established that lipid peroxidation accelerates atherosclerosis. The activated monocytes may cause the oxidation of LDL, and consequently, the oxidised LDL stimulates the efflux of monocyes and also induce their adhesion. The release of cytokines may be a companying phenomenon [77].

The American Heart Association and U.S Centre for Disease, Control and Prevention has recommended the request for inflammatory markers. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines has suggested some of the emerging risk factors for cardiovascular diseases which include C-reactive protein, Interleukins, Serum amyloid A, Fibrinogen, D-Dimer, Vascular and cellular adhesion molecules, soluble CD40 ligand, Leukocyte count, Plasminogen activator inhibitor 1, Tissue plasminogen activator, Factor VIII, microalbuminuria, creatinine (glomerular filtration rate), cystatin C, infectious agents, and von willebrand factor antigen [78].

C-reactive protein (CRP) is a systemic marker, which is elevated due to accumulation of macrophages and T lymphocytes, in atherosclerosis lesions [79]. It is a pentraxin which is synthesized in liver after a stimulus like tissue injury, inflammation and/or infection, and plays a pivotal role in the innate immune response. It is been identified among both men and women with no previous history of cardiovascular disease. High plasma levels of CRP are found to be positively associated with the risk of CVD [80]. It can maybe predict the

development of vascular events and prognosticate these upheavals beyond LDL levels [81]. Ultra-sensitive CRP tests detect an increase in its levels at low concentrations in healthy persons who are metabolically stable and free of infection or acute illness. A value of less than 1 mg/L puts the patient at low risk, between 1 to 3 mg/L, at moderate risk and at greater than 3 mg/L, at high risk [82]**.** The highest risk is seen at levels > 5 mg/L. However, in patients with stable CVD, a concentration of CRP greater than 1mg/L is a significant predictor of adverse cardio vascular events, independently of baseline characteristics [83]. With more data coming in, confirming the diagnostic role of CRP in predicting cardiovascular events, it may soon join the group of classical risk factors which are routinely used to treat and manage this disease.

Erythrocyte sedimentation rate (ESR), also known sed rate or sedimentation rate, is a widely used laboratory test to determine infections, tumors and inflammation as it is found to be elevated in many acute and chronic disease states characterized by tissue necrosis and inflammation. Along with CRP, ESR was found to be a weak predictor for the development of CVD[84]. The Reykjavik Study has explained the association of ESR with CVD risk. In both men and women ESR came across as a long-term independent predictor of CHD, which supports the role of an inflammatory process in CVD [85].

In elderly subjects above 65 years, Interleukin 6 (IL 6), TNF α, and Interleukin 10, also proved to be more appropriate predictors of CVD than CRP. The latter may hence not be a strong marker in case of elderly subjects [86]. IL- 6 can participate in the destabilization of atherosclerotic plaque and thus act as an important immune cell activator. A strong association of TNF α with CVD has also been reported, with several studies reporting a better correlation than CRP [87,88]. Similarly, few anti-inflammatory cytokines show the ability to inhibit the production of several inflammatory cytokines such as IL2, and IFN γ, which are associated with acute ischemic syndrome [89, 90]. The role of inflammatory markers on the cardiovascular health of elderly subjects has been reviewed [91].

Serum Amyloid A (SAA) is an acute phase inflammatory marker which exists in human body as a complex with HDL_3. SAA, which is synthesized in liver, is regulated by the synergistic action of cytokines, especially Tumor Necrosis Factor (TNF) α, Interleukin (IL) 1α, and IL 6 which are released by activated macrophages [92]. It is reported that like CRP, elevations in the SAA baseline identifies a population of patients at risk for developing acute coronary syndrome [93]. This was the conclusion in case of women based study also [94].

Plasma Fibrinogen is a coagulating protein that is present in blood, and helps in determining the blood viscosity and platelet aggregation. Studies showing its strong association with CVD risk by increasing platelet activity have been reported [95]. However, it is not still very clear whether the elevation in the levels of fibrinogen is due to cardiovascular disease or an effect of it. A case study showed that γ fibrinogen level is elevated in the acute phase of Ischemic stroke[96]. In the same context, another advanced recent report showed that γ fibrinogen chain has 2 isoforms, the γ A (simply γ) isoform and the γ' (γ B) isoform, among which γ' showed perfect utility in determining CVD risk [97]. Apart from this, other homeostatic factors found to be linked with CVD risk are Fibrin D-Dimer, factor VIIIc, and antiplasmin (PAP) complex. These are found to be associated independently with increased risk of coronary vascular deaths [98, 99].

Among the soluble adhesion molecules, vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1) and endothelial-leukocyte adhesion molecule-1 (sE-selectin), play a significant role in characterizing the risk for CVD. The sICAM-1, an immunoglobulin-like molecule, is characterized by a low level of expression in in endothelial cells, which markedly increases upon exposure to inflammatory cytokines. sVCAM-1 has been shown to be involved in accumulation and adhesion of leukocytes. Increase in concentrations of soluble adhesion molecules sVCAM-1, sICAM-1, and sE-selectin were significantly related to future death from cardiovascular causes among patients with documented CVD [100]. A number of inflammatory markers as a criteria for analysing the risk factors in the elderly has been studied and IL-6, CRP, D-dimer, homocysteine, WBC, FVIIIc and Lp(a) been identified as the major parameters. Fibrinogen is implicated as a risk factor in males and sICAM -1 in females [101].

The factors which influence the levels of some inflammatory markers acting as risk factors for the development of CVD are tabulated in Table 1.

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Table 1: Factors influencing the levels of inflammatory markers

S. No	Markers	Risk Factors and Effects [Elevated levels ↑, Decrease levels ↓]	References
1	Fibrinogen	[S, E, BP, PF] ↑, [A] ↓	102
2	CRP/hs-CRP/ us-CRP	[S, E, BP, PF] ↑, [A] ↓	84
3	WBC Count	[S, E] ↑, [A] ↓	102
4	Plasma Viscosity	[S, E] ↑, [A] ↓	103
5	ESR	NOT AVAILABLE	103
6	Albumin	[S, E] ↓	102
7	VWF	[E] ↑, [A] ↓	84
8	t-PA	[S, E] ↑,	104
9	D - Dimer	[S, E] ↑, [A] ↓	105
10	SAA	[PF] ↑,	103, 106
11	Factor VIII	[E] ↑, [A] ↓	104
12	V CAM I	NOT AVAILABLE	107
13	I CAM I		107
14	E – Selectin		107
15	P – Selectin		107
16	IL 6, IL 8, IL 18, CD 40, CD 48		108, 109,

[Smoking-S, Alcohol-A, Exercise-E, Blood Pressure-BP, Psychosocial Factors-PF]

CONCLUSION

The use of biomarkers for predicting the development, progression and outcomes of CVD is slowly becoming a norm in personalized patient management. Although some of them have been established well as risk markers and others are still undergoing the evaluation process for positive risk stratification. A systematic approval is expected to evolve soon integrating all of these markers to arrive at a new strategic approach for prognostication of CVD in individuals.

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