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Significance of inflammatory bio-markers in detecting risk for CVD among type 2 diabetes mellitus

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Abstract:

CVD is a major complication of type 2diabetes and the leading cause of early death. About 65 percent of people with diabetes die from cardio vascular disease and stroke. People with diabetes are two to four times more likely to suffer from atherogenesis .From the pathological point of view, inflammation is the early stage for the initiation and growth of atherosclerotic plaque. Systemic inflammations can result in elevated levels of inflammatory markers that could be attributed to atherosclerotic cardiovascular disease. Increasing recognition of the inflammatory component of atherogenesis provides the biological plausibility for the *potential* use of inflammatory markers like : C-reactive protein, homocysteine, lipoprotein(a) and vitamin B_{12}as indicators of atherogenesis or as predictors of atherosclerotic complications among type 2 diabetes

Key words: Type 2 diabetes (T2DM), Cardiovascular disease (CVD), C-reactive protein (CRP), Homocysteine (Hcy), Lipoprotein (Lp(a)) , Vitamin B_{12} (Vit B_{12}).

Introduction

From a pathological point, all stages, that is initiation, growth, and complication of the atherosclerotic plaque is considered to be an inflammatory response [1]. The major factors that promote atherogenesis are cigarette smoking, hypertension, atherogenic lipoproteins, and hyperglycemia. These risk factors give rise to a variety of stimuli that promote the secretion of leukocyte soluble adhesion molecules, which facilitate the attachment of monocytes to endothelial cells, and chemotactic factors, which encourage the monocyte migration into the sub-intimal space. The transformation of monocytes into macrophages and the uptake of cholesterol lipoproteins initiate the fatty streak. Oxidized low-density lipoproteins may be one of several factors that contribute to loss of smooth muscle cells through apoptosis in the atherosclerotic plaque, and secretion of metalloproteinases and other connective tissue enzymes by activated macrophages may break

down collagen, weakening the muscle and making it liable to rupture. This disruption of the atherosclerotic plaque then exposes the atheronecrotic core to arterial blood, which induces thrombosis. Thus, every step in atherogenesis is believed to involve cytokines, other bioactive molecules, and cells that are characteristics of inflammation[2].

These pathophysiological events provide potential targets for the measurement of risk as a means to identify and monitor the ongoing inflammatory process. Potential targets for measurement include pro-inflammatory risk factors like oxidized low-density lipoproteins, proinflammatory cytokines (eg, interleukin-1, tumor necrosis factor-α), adhesion molecules (eg, intercellular adhesion molecule-1, selectins), inflammatory stimuli with hepatic effects (eg, interleukin-6) or the products of the hepatic stimulation, such as SAA, C-reactive protein (CRP), and a host of other acute-phase reactants[3]. It should be pointed out that this inflammatory cascade may have sources other than an atherosclerotic coronary artery, including atherosclerosis in other arteries, as well as systemic inflammation (eg, connective tissue diseases) and local infections (eg, gingivitis, prostatitis, bronchitis, urinary tract infections, and gastric inflammation). These systemic inflammations may result in elevated levels of inflammatory markers that may be incorrectly attributed to atherosclerotic CVD. Increasing recognition of the inflammatory component of atherogenesis provides the biological plausibility for the *potential* use of inflammatory markers as indicators of atherogenesis or as predictors of atherosclerotic complications[4].

Characteristics Desirable in a CVD Risk Predictor

The desirable characteristics of an inflammatory marker to be *potentially* used as a predictor of prevalent or incident CVD are .

- ★ The ability to standardize the assay and to control the variability of the measurement;
- ★ Independent from established risk factors;
- ★ Association with CVD clinical end points in observational studies and clinical trials;
- ★ The presence of population norms to guide interpretation of results;
- ★ Ability to improve the overall prediction beyond that of traditional risk factors;
- ★ Generalization of results to various population groups
- ★ Acceptable cost of the assays [5].

Prediction of Recurrent CVD Events and Death: Secondary Prevention

A growing number of studies have examined inflammatory markers as predictors of CVD risk, including short-term risk, long-term risk, and risk after revascularization procedures such as percutaneous coronary intervention (PCI)[5].The inflammatory biomarkers, identified are C-reactive protein, Homocysteine, Lipoprotein (a) and Vitamin B12.

C-REACTIVE PROTEIN

C-reactive protein (CRP) is a, pentameric protein found in blood plasma, levels of which would rise in response to inflammation[6]. It is synthesized by the liver in response to inflammatory factors released by macrophages [7]. It belongs to the family of proteins known as pentraxin. The first pattern recognition receptor (PRR) to be identified was C-reactive protein. Levels of CRP increase very rapidly in response to trauma, inflammation, and infection and decrease just as rapidly with the resolution of the condition. CRP has evolved as an ideal marker of inflammation due to its precise response[8].

Inflammation, Endothelial Dysfunction, and Insulin Resistance

In response to the development of the euglycemic - hyperinsulinemia the major site of insulin resistance in patients with type 2

diabetes is skeletal muscles . A study by Randle et al. showed that there is an inhibitory effect of lipid accumulation in skeletal muscle on glucose metabolism which ultimately affects the insulin-sensitive glucose uptake. Adipose tissues were involved in modulating muscle insulin sensitivity by releasing adipocytokines that promote insulin resistance. Endothelial nitric oxide synthase (eNOS) produced by endothelial muscles normally regulates blood pressure and increases muscle blood flow. This was the first evidence on the importance of skeletal muscle blood flow in insulin sensitivity[9].

★ CRP is synthesized in liver in response to acute inflammation. Elevated CRP levels impair both insulin-induced skeletal muscle blood flow and muscle glucose delivery, ultimately resulting in insulin resistance was shown by a study done by Tanigaki and also showed that CRP loses the power to induce muscle insulin resistance .Inflammatory status activates the CRP and causes impaired insulin endothelial action in skeletal muscle and thus impairs muscle glucose delivery, and insulin resistance. Muscle glucose uptake is important for the whole-body glucose disposal. It is the combination of muscle insulin action, muscle contraction, and vascular endothelium-induced muscle blood flow that together promotes muscle glucose uptake. This study demonstrates that muscle blood flow and insulin sensitivity can be impaired by the production of CRP[10].

Role of CRP as an inflammatory bio-marker

CRP, has been recognized as a golden biomarker of inflammation by the invention of high-sensitivity assays for determining CRP, it has emerged as one of the most powerful independent predictors of cardiovascular disease. CRP levels increases in acute coronary syndromes, has proven to be of value in predicting the complications related to CVD [11].

There is a major role of CRP in the pathophysiology of atherosclerosis, including the potential mechanisms of action in circulation. The role of CRP to activate and complement the inflammatory process is an important characteristic that links CRP and inflammation with atherosclerosis. Recent research has suggested that, in addition to pentameric CRP, monomeric CRP has an active role to play in atherosclerosis[12] . Loss of pentameric CRP gets associated with bioactive material in monomeric CRP that enhances neutrophil localization and activation at the inflamed vascular sites. The physiological effect of CRP on the development of atherosclerosis is a complex network of interactions which acts on immunity and speeds up the inflammatory process. CRP is also directly involved with the cells involved in lesion growth and development[13]. There are evidences that suggest CRP functions as a pro-atherogenic factor and also an indicator for atherosclerotic and metabolic events. There are various studies that implicate CRP as a novel risk marker for cardiovascular and other metabolic diseases. It also acts as a mediator of these events as it contributes to the underlying lesion in the formation of plaque, plaque rupture, and coronary thrombosis [14].

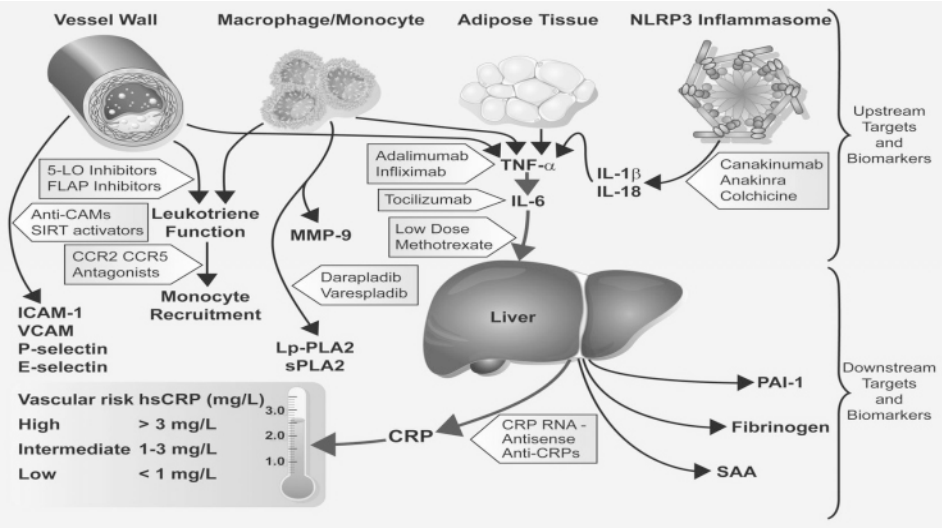
A CRP that is incapable of binding to complement proteins provides a tool to assess interactions of CRP with the other biologically significant ligands, to further review the functions of CRP with inflammation. Few studies indicate that CRP can modify the autoimmune disease, by preventing the exposure of nuclear antigens to the immune system[15]. A relationship between leptin and CRP also supports that the adipokine (leptin) also has a role in inflammation and atherothrombosis. Several evidences has proved CRP as a direct mediator of endothelial dysfunction [16].

Impaired endothelium-dependent vasodilatation, suggests that elevated levels of CRP is found in the blood and acts as a useful clinical tool for detection of CVD risks . CRP directly promotes activation of monocytes activation stimulating the release of cytokines, such as IL-1b, IL-6, and TNF-α . There are evidences that shows that CRP is deposited in the arterial intima, atherogenesis[17]. CRP pro-atherogenic in nature and is present as macrophages, because it increases tissue factor expression, promotes adhesion to endothelial cells, releases reactive oxygen species and matrix metalloproteinase-1, and increases the uptake of oxidized low-density lipoprotein, leading to increased foam cell formation[18].

CRP and cardiovascular risk

C-reactive protein was linked to future cardiovascular events over the past few years . Alternative biomarker has not been proved to be a superior for vascular inflammation in the prediction of global cardiovascular risk. This observation is re-affirmed in population-based prospective cohort data from the Danish Research Centre of Prevention and Health has reassured the same Among the two biomarkers studied, upstream C-reactive protein and downstream TNF-α and proved to be significant predictors for future vascular risk A study done by Emerging Risk Factors Collaboration on C-reactive protein for predicting CVD risk was found to be comparable in magnitude to that of total and high-density lipoprotein cholesterol[19].

The research on both 'upstream' and 'downstream' biomarkers of inflammation well associate with vascular risk and supports the targeting causal pathways of inflammation more than any other biomarker. The prominent pathways like interleukin-1 (IL-1), TNF-α, IL-6 pathway, results in elevated levels of elevated levels of C-reactive protein, fibrinogen, and plasminogen. Further several data supports a relationship between classical inflammatory activation and future plaque rupture. The association between CRP and cardiovascular risk is principally identified by the systemic inflammation. Mendelian randomization studies have revealed same results. CRP levels of 1, 1 to 3, and 3 mg/L have been classified as low, moderate, and high-risk groups using available high-sensitivity assays to avoid future cardiovascular events[20].



Diet and CRP levels

C-reactive protein (CRP) has emerged as one of the most powerful predictors of cardiovascular disease, based on the results of several prospective epidemiological studies. This marker provides valuable information on cardiovascular disease, stable angina, acute coronary syndromes and peripheral vascular disease. Elevated C-reactive protein levels acts as a marker to atherogenesis / hardening of the arteries, endothelial dysfunction, atherosclerotic-plaque formation, plaque maturation, plaque destabilization and eventual rupture. Levels of C-reactive protein can be decreased by increasing consumption of fruits and vegetables containing lycopene and vitamin C [21].

HOMOCYSTEINE

Homocysteine is a non-protein □-amino acid. This amino acid occurs in the body as an intermediate product in the metabolism of methionine and cysteine with the help of certain B-vitamins . high level of homocysteine is a risk factor for heart disease. It's associated with low levels of vitamins B6, B12, and folate, as well as renal disease. Research has shown, however, that getting your homocysteine levels down with vitamins doesn't reduce your chance of having heart disease [22].

Homocysteine and Diabetes

There is a strong relationship between T2DM and homocysteine levels. Diabetic patients with hyperhomocysteinemia (Hcy levels above 14 µmol/L) have higher risk of mortality by CVD than other diabetic patients (Hcy below 14 µmol/L). It is established that each 5 µmol/L increase in serum tHcy, increases mortality by 60% among the diabetics . A relationship between gestational diabetes and homocysteine levels has also shown several types of birth defects[23].

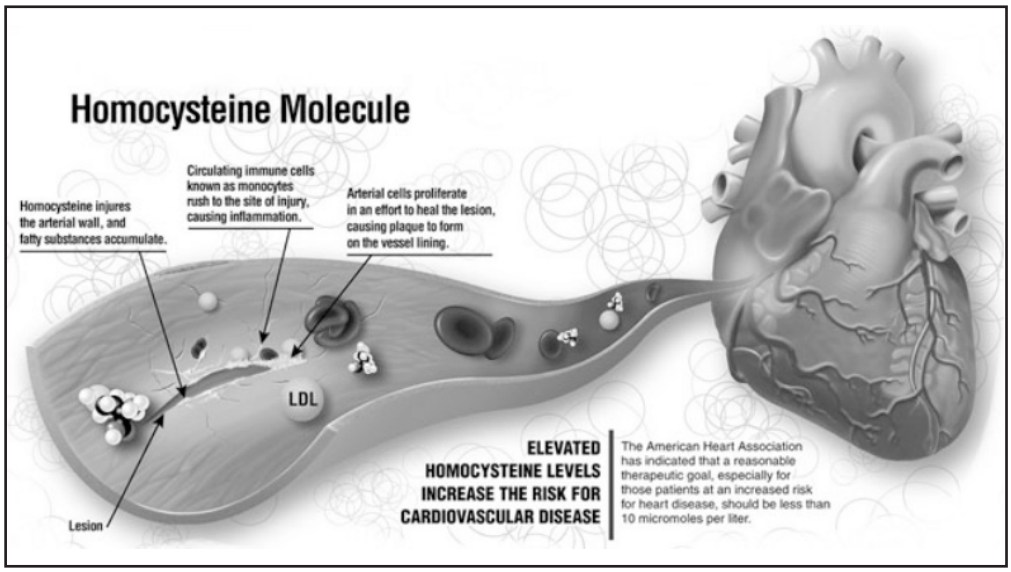
Improved glycemic control has shown to lower homocysteine levels in diabetic patients. In a study which involved 95 type II diabetics followed for 3 years, with improving glycemic control measured by glycosylated hemoglobin (%HbA1c) had lower homocysteine levels than those with increased HbA1c levels after 3 years. Metformin, an oral hypoglycemic drug used to treat type II diabetics, decreases plasma folate and vitamin B12 levels and increases homocysteine levels [24].

Homocysteine levels >100 µmol/l is known as homocystinuria or severe hyper-homocystenemia a condition caused by rare genetic disorder. Normal levels of homocysteine are <11.4 µmol/l for men and <10.4 µmol/l for women [25].

The following mechanisms result due to higher levels of homocysteine .

- ★ Reaction of proteins with LDL cholesterol, leads to formation of foam cells resulting in plaque build-up.
- ★ Increases the thickness of platelets, which leads to clotting.
- ★ Promotes collagen deposition in the arteries, leading to fibrosis in the vascular.
- ★ Nitric oxide production is decreased due to excess oxidative stress.
- ★ Glutathione levels are suppressed by inhibiting the peroxidase enzymes.
- ★ It causes damage to the mitochondria inside endothelial cells
- ★ The binding of lipoprotein (a) are increased to fibrin, causing increased clotting and development of atherosclerosis.
- ★ Cause decreases in hydrogen disulfide production
- ★ Free radical damage to blood vessels increases and causes hypertension [26].

A meta-analysis done in the year 2015 has shown that elevated levels of Hcy are linked to increased risk of developing CVD. Ensuring low homocysteine levels with adequate B-vitamin supplementation along with healthy lifestyle would prevent levels from rising and lower the risk of CVD [27].



Homocysteine levels an inflammatory biomarker for CVD risk

Kilmer McCully and his colleagues reported , that elevated levels of homocysteine were directly responsible for the various vascular lesions and he further postulated that moderately elevated levels are due to heterozygous mutations in homocysteine related genes and poor vitamin status , all of which leads to increased risk of CVD. By the year 1990, elevated homocysteine was considered as an independent risk factor for CVD risk along with cholesterol and other lipid markers, age, gender, smoking status, obesity, hypertension and diabetes. A prospective study in 1992 found that acute myocardial infarction (MI) or death due to coronary disease was statistically related to increased homocysteine levels, after adjusting for other risk factors [28].

LIPOPROTEIN (a)

Lipoproteins are complexes that consists a lipid core of triglycerides and cholesterol esters surrounded by a surface monolayer of phospholipids, free cholesterol and specific protein components named apolipoproteins. Most of the apolipoproteins undergo complex exchange reactions which serve many metabolic functions including transport, enzyme cofactors and receptor ligands. Lipoprotein disorders are often associated with cardiovascular disease (CVD) like atherosclerosis and other organ dysfunctions. Abnormal concentrations of lipoproteins (a) can result from changes in production, conversion or catabolism of lipid particles [29].

Lp(a) exists mainly in plasma. It contributes to the process of atherogenesis. It enhances the coagulation by inhibiting the function of tissue factor pathway inhibitor . Lp(a) carries cholesterol which binds with atherogenic proinflammatory oxidized phospholipids, that acts as a preferential carrier to oxidized phospholipids in human plasma which attracts the inflammatory cells to the walls of the smooth muscle that leads to cell proliferation[30]. Lp(a) is also said to be involved in wound healing and tissue repair [31].

Lipoprotein levels and cardio vascular risk

There are various studies which have suggested role of cholesterol in the

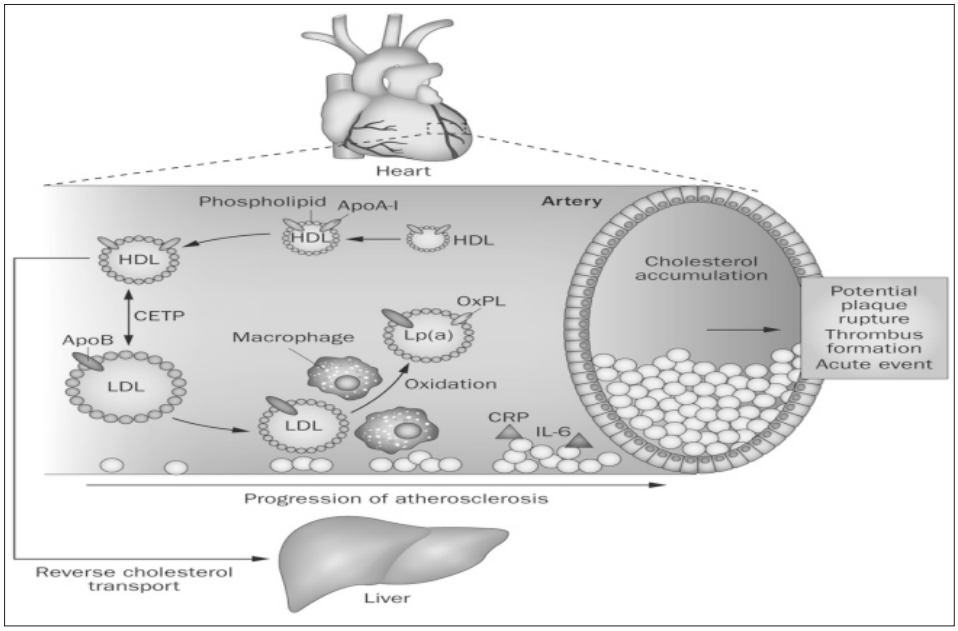
pathogenesis of atherosclerotic cardiovascular disease (CVD). There are findings which show of a curvilinear relationship between systemic levels of LDL cholesterol (LDL-C) and prospective cardiovascular risk in various population studies [32].

A number of lipid risk factors have been proposed to be independently associated with CVD. Lipoprotein contains apolipoprotein (apo)B-100 which is linked by a disulfide bond to apo(a), which foms lipoprotein (a), that possesses potent atherogenic and thrombogenic properties. The presence of a kringle structure with the presence of apo B-100 alters the hemostatic activity [33].

Epidemiological studies have reported relationship between Lp(a) levels and cardiovascular risk (Bennet.etal,2008). It was reported that genetic polymorphism is associated with elevated levels of Lp(a) and is associated with CVD risk . In addition, Erqou suggests a relationship between apo(a) isoform size and both Lp(a) levels and cardiovascular risk [34].

Many lipidologists consider a Lp(a) level < 10.0 mg/dL to be indicative of lower cardiovascular risk, with higher levels associated with an increase in cardiovascular risk [35].A study by Davidson, states that the odds ratio for significant coronary heart disease on angiography in patients with a Lp(a) level greater than 30 mg/dL versus a level less than or equal to 5 mg/dL increased from 1.67 to 6.0, as LDL concentrations increased as well . A number of prospective trials have also demonstrated that Lp(a) predicts risk and also increases in cardiovascular risk [36].

Lp(a) has shown a strong association with angiographically documented coronary artery disease .During atherogenesis and inflammation oxidized phospholipids have a role in maintaining general health as well as vascular health. In this regard, Lp(a) lipoprotein acts in a similar way to CRP [37]. When low levels



Lipoprotein(a) as a inflammatory biomarker

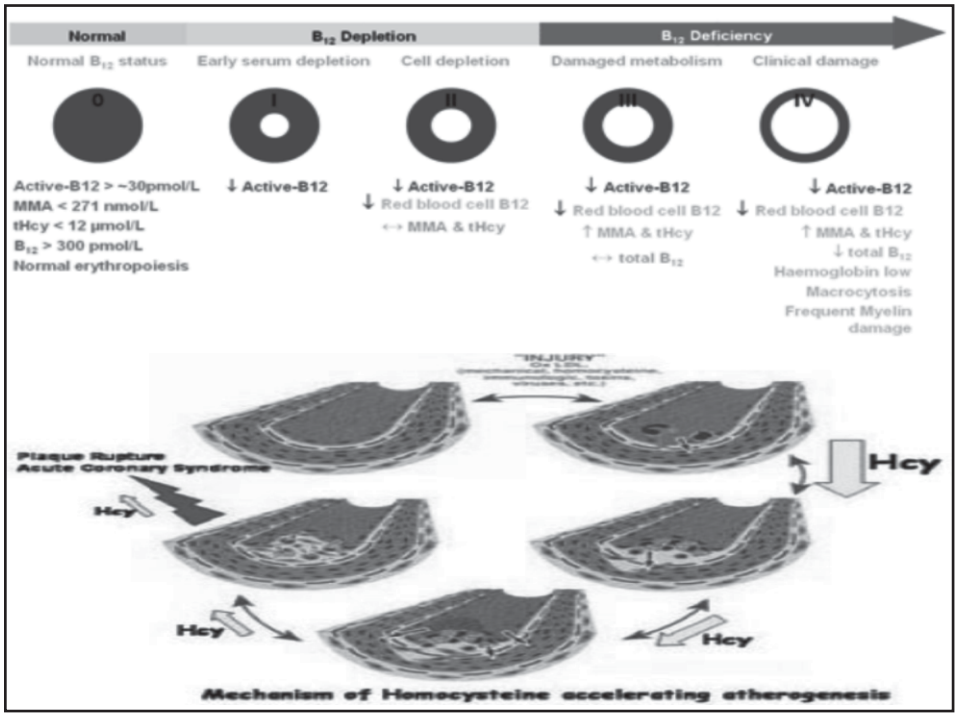
of Lp(a) are present it serves as protective function by binding and participating in the transfer and degradation of oxidized phospholipids that are formed during homeostasis or during any stress. But when Lp(a) levels are elevated it has enhanced binding ability to the extracellular matrix of the artery wall and results in CVD risks. These provide essential details that, elevated Lp(a) levels, a proinflammatory marker may predominate and contributes to the clinical expression of CVD[38].

VITAMIN B_{12}

Vitamin B12 or cobalamine is a water soluble vitamin that plays an important role in DNA synthesis, optimal haemopoesis and neurological function. Vitamin B_{12} deficiency acts as a possible comorbidity many diabetic subjects are at risk. The main cause for this may be due to metformin, that lowers serum vitamin B_{12} levels [39]. In addition, most of the diabetics are in an age group (>50yrs), in which the prevalence of B_{12} deficiency ranges from 12% to 23%. Because of these risk factors, screening the prevalence of this vitamin deficiency in the diabetic population could help determine the risk factors associated with this vitamin deficiency. According to the World Health Organization's screening for this deficiency could help in detecting conditions at an early, treatable stage and reduce the morbidity status among diabetics [40].

Physiological roles of vitamin B12 leading to CVD risk

Vitamin B12 exerts physiological effects by mediating two important enzymatic pathways ; the methylation process of



Vitamin B12 deficiency : a comparison between with type 2 diabetes mellitus and healthy population

homocysteine to methionine and the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. Vitamin B_{12} as a co-factor promotes the methylation of homocysteine to methionine which is activated to S-adenosyl-methionine that donates its methyl group to methyl acceptors such as myelin, neurotransmitters and membrane phospholipids. Vitamin B_{12} deficiency , therefore will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine and results in hyperhomocysteinemia. This condition is found to have adverse effects on neurones and the vascular endothelium[41]. There are several experiments that demonstrate that hyperhomocysteinemia is associated with an increased risk of cardiovascular events due to its cellular and vasculo-toxic effects[42].

Several cross sectional studies and case reports have reported an increased prevalence of vitamin B_{12} deficiency among type 2 DM (T2DM) subjects. Metformin use has been demonstrated as the prime factor associated with vitamin B12 deficiency among diabetics[43]. In a cross sectional study by Pflipsen et al. on 203 outpatient with type 2 diabetic patients at a large military primary care clinic in USA, vitamin B12 deficiency was defined as serum vitamin B12 concentrations of <100 pg/ml or elevated serum methylmalonic acid of >243 nmol/L or homocysteine concentrations of >11.9 nmol/L if serum vitamin B12 concentrations were between 100 to 350 pg/mL[44]. Reinstatler et al. in the National Health and Nutrition Examination Survey of 19992006 in the USA defined biochemical vitamin B12 deficiency as serum vitamin B12 concentrations of ≤148 pmol/l and >148-221 pmol/l respectively[45]. In one cross sectional study that reported a high prevalence of vitamin B12 deficiency of 33% among adult patients with T2DM by Qureshi et al., vitamin B12 deficiency was defined as serum vitamin B12 concentrations <150 pg/ml[46] .Subjects recruited for this study were those who were on high dose (>2 g/day) and long-term (4 years) metformin treatment, both factors known to be associated with vitamin B12 deficiency[47].

Various studies have demonstrated inverse association between plasma homocysteine levels with blood levels of vitamins B6, B12, and folate. Human experimental studies have shown supplementation of these vitamins (especially folate) reduces elevated homocysteine levels and epidemiologic studies have found inverse associations of the vitamins with vascular disease[48].

Conclusion

Strategies to prevent morbidity and mortality of diabetics due to CVD should be a multifactorial approach. This includes, improved glycemic control, assessing inflammatory bio-markers in the blood to identify CVD risk and early treatment for CVD. Nutrition modulation through effective counselling, importance in life style changes and importance of micronutrients, help control elevated levels of inflammatory bio-markers and glucose levels.

Further work in this area with nutrition intervention would provide data that could be useful in reducing the CVD morbidity and mortality rates among diabetics.

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