Clinical importance of haptoglobin testing in diabetes

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Introduction

Diabetes affects nearly 400 million people worldwide and is expected to increase to nearly 600 million people by 2035. Patients with diabetes mellitus are often more susceptible to a host of multi-organ complications that arise as a result of microvascular and macrovascular dysfunction. These complications equate to the leading cause of morbidity and mortality in diabetic patients from accelerated atherosclerotic disease. Clearly, there are a variety of contributing factors leading to both diabetes and cardiovascular disease. Among these contributors, the most powerful are genetics, environment, and lifestyle. All three factors collide in the development of diabetes. At its heart, diabetes and all of its complications arise from biochemical mechanisms that are propagated by high glucose levels. For diabetes and subsequent complications to be averted, early glycemic control is paramount. This raises the concept of metabolic memory. Metabolic memory is a phenomena that suggests that microvascular damage that results from high glucose can be reversed if glucose levels can be controlled early after the initial hyperglycemic event [1,2]. The phenomena further shows that if glycemic control is not achieved for a period of time, vascular injury will still result even if glycemic control is eventually reached. One such example is the description of a study [3] where test subjects with diabetes were exposed to good glycemic control (5 years), poor glycemic control (5 years) and mixed (2.5 years poor control and 2.5 years good control) glycemic control immediately following a hyperglycemic event. What was found is that unless good glycemic control was achieved immediately after the hyperglycemic event retinopathy would develop within 5 years in both the poorly controlled and mixed. Early glycemic control can halt the pathological effects associated with reactive oxidative stress.

Those with diabetes are fully aware that complications such as heart attack and stroke are a significant concern. What may not be well known to those with the disease and those managing patients with diabetes is that an important inflammatory biomarker may foretell the likelihood of a heart attack. A report in the October 2015 issue of the Journal of American College of Cardiology reports that genetic testing for a specific biomarker may identify diabetic individuals who are at a greater risk for coronary heart disease [4].

Diabetes is accompanied by states of severe oxidative stress. This stress is characterized by lipid peroxidation arising from an excess of free reactive oxygen intermediates. The pathogenesis of diabetics is significantly impacted at the micro and macrovascular level by reactive species [5]. The counterpoint in the body to these reactive species is the haptoglobin (Hp) molecule. In nature, Hp binds to free Hb high affinity [6,7]. A variety of unfavorable consequences can arise when free iron is present in the circulation and in tissues. It is well known that iron overload can contribute to the development of diabetes and atherosclerosis [8,9]. Iron participates in the Haber-Weiss reaction to convert reactive oxygen species (ROS) such as superoxide and hydrogen peroxide to more powerful species such as hydroxyl radical [10]. Free iron can be a detrimental catalyst in lipid peroxidation because it can both initiate and amplify the process of lipid peroxidation.

Haptoglobin is an acute phase plasma protein with hemoglobin-binding properties that has the primary task of capturing and eliminating

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free heme circulating in the blood and tissues (reviewed in [11]). For this reason, Hp is considered a key antioxidant molecule in the prevention of hemoglobin-induced oxidative tissue damage. Recent evidence further suggests that acute phase proteins such as Hp have an association with the development of diabetes and the complications that arise from diabetes [12-15]. Each of us carries two copies of the Hp molecule expressed as oligomeric proteins that scavenge free hemoglobin following tissue injury or normal red blood cell recycling. Ample evidence suggests that Hp blood levels are increased in a variety of pathological and traumatic situations. In humans, Hp is expressed as three potential genotypes: Hp1-1, Hp1-2, and Hp2-2 representing 16, 37 and 47% of the U.S. population respectively. It is interesting to note that humans are the only organisms that express Hp2. Hp2, according to many reports was generated by a recombination event thousands of years ago that may have had a selective advantage to early hominids in the form of protection from certain infectious agents.

Clinical evidence suggests that 65% of diabetic patients will suffer a heart attack and a significant portion will perish from cardiovascular complications. These data are especially important to patients with uncontrolled diabetes. Several recent studies from groups around the world indicate that if a person carries the Hp1-2 or 2-2 genotype and has an A1C above 6.5, they have a significantly increased risk for a cardiovascular event [4,16]. Further, a recent study by Llaurado et al. suggests that Hp2-2 patients with type 1 diabetes suffer increased endothelial dysfunction independent of other classical cardiovascular risk factors [17]. Greater than 70% of the general Western population carries at least one copy of the Hp2 allele. Those with diabetes and the Hp1-2 genotype are THREE times more likely to have a cardiovascular event, while diabetics with the Hp2-2 genotype are FIVE times more likely to suffer a cardiovascular event. Although several studies have indicated that type 2 diabetic patients with Hp2-2 are at greater risk, a recently study suggests that associations exist but that Hp2-2 was still under investigation in type 1 diabetics with regard to cardiovascular heart risk [18]. Therefore, Hp1-2 and 2-2 directly predict heart disease in those with diabetes [15]. Clearly, the evidence supports the proactive testing of patients with diabetes for Hp genotype. Heart disease is the currently the number one and diabetes the number seven cause of death in the world. Advocacy for inclusion of the test into diabetic management programs and guidelines is imperative to the furtherance of better health and longevity of those with diabetes. In our current era of molecular medicine, a patient can be efficiently genotyped with a cost effective, simple, and non-invasive test that can empower patients with the knowledge of their risk of cardiovascular events. Although, heart disease is perhaps the most significant complication, diabetes is also a predisposing factor for soft tissue infections, nephropathies and neuralgias [5,19,20].

Diabetic patients have a greater risk of systemic infection when compared to non-diabetics [21]. Variation in Hp genotype is also implicated as a contribution to mortality from both extracellular and intracellular pathogens [22]. For example, several studies have demonstrated that patients can have a greater susceptibility to soft tissue infections and the development of severe complications depending on their Hp genotype. Diabetic patients are especially susceptible to bacterial infections, especially, urinary tract, respiratory and soft tissue infections. *Staphylococcus aureus* is known to a leading cause of bacterial nephritis and represents the most common soft tissue infection of diabetics. *S. aureus* requires iron for growth, are known have transporter mechanisms to move iron into the organisms and have been found to take advantage of the Hp-Hb complex for their metabolic activity [23]. The reduced activity of Hp2 to clear free Hb may allow for these bacteria to utilize this circulating iron source enhancing colonization and growth.

Diabetic nephropathy is the leading cause of end stage renal disease and dialysis worldwide. Despite a focus on this subject, 40% of all patients will still require replacement therapy. Several studies have showed a graded risk relationship to the number of haptoglobin 2 alleles in both type I and type II diabetic subjects [24,25]. The data presented from these studies supports the hypothesis that the haptoglobin phenotype of a patient has an influence on and is a major susceptibility gene for the development of diabetic nephropathy.

In addition to aforementioned risks and complications, Hp genotype also correlates to increased risk for diabetic retinopathy in people with type 2 diabetes. In patients with normal blood pressure, Mogarekar and Hampe

showed an elevated risk of severe retinopathy in those with the Hp2-2 genotype [26]. When compared to other genotypes, they showed a graded risk relationship with the number of Hp2 alleles. Moreover, Hp2 carriers face risk of developing increased cholesterol levels, increased risk for aneurysms, as well as elevated arterial inflammation—a known contributing factor to having a stroke. Patients who carry the Hp2 gene will require more treatment support and follow-up than normal patients.

According to the American Diabetes Association, International Diabetes Federation, and the CDC there are an estimated 30 million Americans suffering from diabetes and 80 million with prediabetes. In Europe there are an estimates 55 million with diabetes. It is clear that the growing crisis with diabetes will result in significant morbidity and mortality. Given the involvement of Hp in the pathogenesis of cardiovascular, neurological, infectious, and inflammatory conditions, it would be beneficial to patients to have a diagnostic tool that can quickly identify Hp genotype so proper therapy can be implemented.

A highly reliable and rapid test is available to identify which patients carry the Hp2-2 genotype. With accurate testing information, a physician can initiate a course of therapy such as vitamin E to minimize the risk of a cardiovascular event by as much as 30-40% [27]. The implementation of 400 IU of vitamin E has been shown to be effective in reducing cardiovascular events [27,28]. Vitamin E neutralizes the oxidative capacity of free heme and serves as a surrogate for Hp activity [5]. Free heme can result in the oxidation of LDL particles and lead to the growth and instability of the atherosclerotic plaque. Further, moderate doses of vitamin E can be effective in stabilizing oxidative stress and reducing the levels of circulating free radicals. Novel molecular methods for high-volume patient genotyping are available to allow for rapid screening and early implementation of antioxidant therapy and provide a tremendous benefit people with diabetes.

In conclusion, although the risk is higher for those with Hp2-2, there are several ways to reduce this risk back to baseline cardiovascular risk for patients with diabetes. The administration of exogenous antioxidants such as vitamin E can mitigate the deleterious effects of an Hp2-2 genotype. Thus, it is crucial for the providers to order a genotype test for each and every uncontrolled diabetic that they manage to ensure that all steps to mitigate and reverse potential oxidative injury are taken. Compared to a heart attack or stroke the cost of testing is minimal and the benefits are tremendous not only for the quality of life of the patient but also for the fiscal wellbeing of the healthcare system.

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