Chitotriosidase: A New Inflammatory Marker in Diabetic Complications

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Introduction
Diabetes mellitus (DM) is a chronic metabolic disease, and its incidence is growing worldwide. Long-term hyperglycemia is the fundamental factor that promotes vascular lesions and dysfunction, leading to a variety of complications of DM [1]. DM evolves as a result of a combination of insulin resistance and pancreatic β-cell failure. Oxidative stress, ER stress, lipotoxicity, and glucotoxicity exacerbate insulin resistance and islet β-cell dysfunction. These cellular stresses induce an inflammatory response which is related with this pathophysiological state. In response to environmental stresses, macrophages, endothelial cells, and adipocytes release inflammatory cytokines including TNF-α, IL-1, IL-6, and chemokines (MCP-1, IL-8) that induce the production of acute-phase proteins, such as C-reactive protein and amyloid deposition in the pancreas [2]. Circulating levels of markers of inflammation generate a state of chronic low-grade inflammation, which confers an increased risk of macrovascular complications. In this context, chitotriosidase (CHIT1), the major member of the chitinase family and the best investigated human chitinase regarding its biological activity and association with various disorders, has recently been found to be involved also in the pathogen-
CHIT1 and DM

CHIT1 belongs primarily to the chitinase family [3], which represents a class of evolutionarily ancient enzymes that catalyze the hydrolysis of chitin to simple sugars. Chitin is a linear homopolymer of β-1,4-N-acetylglucosamine and is nearly the most abundant natural biopolymer known for being the structural component of protective structures in many eukaryotic organisms, but seems absent in vertebrates [4]. Although most mammals, including humans, do not synthesize their own chitin, their genomes encode several chitinase enzymes that can specifically degrade the chitin they encounter through ingestion or inhalation. CHIT1 has been widely implicated in a variety of diseases involving immune dysfunction [5]. Recently, CHIT1 has come under increasing scrutiny due to its excess secretion into the serum or overexpression in tissues which are chronically inflamed [6].

CHIT1 was the first mammalian chitinase to be discovered and characterized [6]. The CHIT1 gene is localized in chromosome 1q31-q32 and consists of 12 exons and spans approximately 20 kb of genomic DNA [6]. It is an enzymatically active chitinase that shows transglycosylation activity toward chitin [7] and is the major chitinase measured in disease states [8]. CHIT1 has been included as one of the secreted biomarkers for Gaucher’s disease [9]. The elevation of CHIT1 in these patients may reflect a particular state of activation of macrophages [10]. In a healthy population, CHIT1 activity is very low and originates in circulating polymorphonuclear cells [6]. Conversely, during the development of acute/chronic inflammatory disorders, the enzymatic activity of CHIT1 increases significantly [8]. During the effective maturation of monocytes into macrophages, CHIT1 levels increased significantly during the diverse stages of macrophage maturation and was modulated in polarized M1 and M2 macrophages, indicating a different role of this enzyme in the specialized macrophages [11] and underscoring that CHIT1 can be regarded not only as a marker of macrophage activation but also as an important regulator of inflammatory functions.

A conspicuous amount of evidence indicates that CHIT1 possesses an active role in disease states characterized by inflammatory response [12-14]. Nowadays, there is little evidence showing that serum CHIT1 activity is increased in patients with newly diagnosed, untreated, and uncomplicated type 2 DM (T2DM) [15, 16]. A recent study reported involvement of neutrophils in the pathogenesis of insulin resistance. Interestingly, it has reported a ‘chitinolytic’ profile of diabetic neutrophils indicating that neutrophil-derived chitinases represent a novel group of molecules, which may participate in metabolic disturbances and inflammatory pathways in the course of type 2 diabetes, especially connected with development of vascular complications. It has been suggested that levels of neutrophil-derived CHIT1 are independent of achievement of metabolic compensation of diabetes and its increase may be related to other biochemical pathways occurring in diabetes.

CHIT1 positively correlates with leukocyte elastase (LE) and increased progressively with increasing activity of LE, which is considered a marker of neutrophil activation. Increased activity of LE in diabetic neutrophils is associated with poor short-term glycemic control and development of diabetic angiopathies [17]. LE is able to degrade most components of the extracellular matrix, leading to destruction of the integrity of endothelial cells and damage of the vascular basement membrane. In this light, the relationship we observed of all examined GH18 proteins with LE may indicate their connection with progression of late vascular complications [16]. The results imply that increased CHIT1 activity may be a predictor of endothelial dysfunction [15].

The endothelium performs a crucial role in maintaining vascular integrity leading to whole organ metabolic homeostasis. Dysfunction of multiple aspects of the endothelium has been identified in DM. In human endothelial cells, modulation of plasminogen activator synthesis by insulin-like growth factor-1, epidermal growth factor, or acidic fibroblast growth factor is known to be influenced by diabetes [18]. Alterations in the synthesis and release of von Willebrand factor, an important factor for efficient platelet adhesion, are common in the diabetic endothelium [19]. Abnormal glycosylation of intracellular and plasma proteins occurs and affects endothelial function in diabetic patients [20].

CHIT1 and Atherosclerosis

Inflammation and endothelial dysfunction initiate the course of atherosclerosis, from the earliest steps of monocyte recruitment to the complicated phase of plaque rup-
Atherosclerotic lesion-related thrombosis is the major cause of myocardial infarction and stroke, which together constitute the principal cause of mortality worldwide. It is conceivable that the increased CHIT1 activity observed in T2DM patients may be another crucial mediator playing a detrimental role in mechanisms such as vascular inflammation, generation of ROS, and endothelial dysfunction, underlying the progression diabetes and its complications. The inflammatory response is considered as a major driving force in atherosclerotic plaque formation, growth, and progression towards instability and rupture. Macrophages are present in all phases of atherosclerosis, and are markers of atherosclerotic plaque formation (fig. 1) [22]. The consequent inflammation results in increased numbers of macrophages and lymphocytes, both of which are markers of the inflammatory response. A large body of evidence indicates that inflammation plays a central role at the beginning of the atherosclerosis process and in the mechanism underlying the development and progression of the atherosclerosis complications, plaque rupture, and subsequent thrombosis [23]. Being the most abundant cell type in atherosclerotic plaques, macrophages have a strong effect on plaque development and progression due to their overwhelming influence on intraplaque cholesterol homeostasis, inflammation, necrotic core initiation, and extracellular matrix degradation [24].

Macrophage accumulation localized in the supra-aortic and coronary vessels is associated with increased serum CHIT1 activity, which reflects the state of macrophage activation within atherosclerotic lesions, suggesting that CHIT1 augmentation could influence the synthesis of crucial components of the extracellular matrix in the vessel wall. CHIT1 produced by macrophages enhances atherosclerotic plaque formation and subsequent thrombosis [12].

Boot et al. [25] were the first to demonstrate a clear connection between CHIT1 expression and lipid-laden macrophages inside the human atherosclerotic vessel wall. Serum CHIT1 activity was shown to be related to the
severity of the atherosclerotic lesions, suggesting a possible role as a marker of atherosclerotic extension. High serum CHIT1 activity in patients with atherosclerosis revealed the presence of activated macrophages in these subjects. In other studies, patients with atherothrombotic stroke (ATS) and ischemic heart disease were reported to have significantly higher CHIT1 activity than controls [25]. However, ATS subjects had higher CHIT1 activity than ischemic heart disease subjects, suggesting that in the subjects with ATS the atherosclerosis process is wider than in the subjects with ischemic heart disease, whose atherosclerosis is localized more specifically in the coronary vessels. This was confirmed by the observation that, in the ATS group, CHIT1 activity was related to carotid stenosis, in accordance with a clinical picture featured by more widespread atherosclerosis [25]. It should be emphasized that the average CHIT1 activity in serum remained constant after 6 months of lipid-lowering treatment with either atorvastatin or bezafibrate, suggesting that LDL-cholesterol and triglyceride reduction obtained with both drugs did not modify the macrophage CHIT1 expression in these subjects [12]. Therefore, plasma lipid correction does not seem to interfere in the CHIT1 expression level in vivo, supporting the idea that CHIT1 activity cannot be used as a biological marker of atherosclerotic plaque modification related to hypolipidemic treatment [26].

In the CHIT1 gene, a 24-bp duplication in exon 10 activates a cryptic 39 splice site in the same exon, generating an abnormally spliced mRNA with an in-frame deletion of 87 nucleotides. The spliced mRNA encodes an enzymatically inactive protein that lacks an internal stretch of 29 amino acids; the resulting phenotype is an asymptomatic CHIT1 activity deficiency. However, mild enzymatic activity has been detected in heterozygous subjects [27].

This common natural genetic variation within the CHIT1 gene was strongly associated with human longevity and was also associated with several phenotypes of healthy aging [28]. This result was in agreement with the finding that augmentation of serum CHIT1 activity was age dependent. This phenomenon could be explained by the incessant accumulation of lipid-laden macrophages during the gradual progression of atherosclerosis in relation to age. Moreover, no detectable serum CHIT1 activity was found in the subjects homozygous for the defective allele. CHIT1 activity was significantly higher in homozgyous subjects for the major allele than in heterozygous subjects for the defective allele [29]. Furthermore, the CHIT1 produced by macrophages enhances atherosclerotic plaque formation and subsequent thrombosis [12].

The polarization of macrophages towards a specific phenotype has been reported to be positively affected by lipids, growth factors, and cytokines; the M1 macrophages that are classified by means of classical methods may result in plaque vulnerability, whereas the M2 macrophages which are activated by alternative methods may increase plaque stability [30]. The phenotypes of M1/M2 macrophages can be exchanged depending on the conditions of their microenvironment [31]. Noteworthy, CHIT1 levels increased significantly during the diverse stages of macrophage maturation, and were modulated in polarized M1 and M2 macrophages, indicating a different role of this enzyme in the specialized macrophages [11].

In response to various signals, macrophages may undergo classical M1 activation (stimulated by TLR ligands and IFN-γ) or alternative M2 activation (stimulated by IL-4/IL-13); these states mirror the Th1-Th2 polarization of T cells [32, 33]. It has been reported that IL-4 promotes a M2 phenotype in regressive atherosclerotic lesions [34], providing in such a way a potential explanation of CHIT1 activity associated with the presence of atherosclerosis [12]. Paradoxically, it was found that in monocytes IL-4 treatment induced a significant increase on mRNA CHIT1 expression [11]. IL-4 is a well-appreciated antagonist of the M1 response and macrophage proinflammatory properties [35]. IL-4 stimulating monocyte/macrophage alternative activation is able to activate peroxisome proliferator-activated receptor-γ [36] and -δ [37], which are ligand-activated transcription factors that regulate genes important in cell differentiation and various metabolic processes, especially lipid and glucose homeostasis. Experimental studies in animal models of metabolic disease have demonstrated their effects on improving lipid profile, insulin sensitivity, and reducing inflammatory responses. Peroxisome proliferator-activated receptor-γ and -δ are also expressed in the vasculature and their beneficial effects have been examined in various cardiovascular disease models such as atherosclerosis, hypertension, diabetic vascular complications, etc., using pharmacological ligands or genetic tools including viral vectors and transgenic mice. Therefore, it would be interesting to deeply investigate the interaction between peroxisome proliferator-activated receptor and CHIT1, and their role in DM and endothelial dysfunction.

New findings have reported that CHIT1 is expressed differently during monocyte-derived dendritic cell (DC) differentiation and maturation [38]. DCs, which are antigen-presenting cells, display a variety of antigens to T cells in addition to initiating and supporting immune responses as well as inhibiting the activation of T cells. The
capacity of DCs in the activation or inhibition of T cells depends on their cytokine production profile and expression of cell surface costimulatory molecules. DCs are transformed by activated innate immune receptors, such as the TLR, into antigen-presenting cells that activate T effector cells, whereas immunological tolerance is produced by antigen presentation which develops when TLR activation is lacking. Therefore, DCs play a critical role as a connector between innate and adaptive immune responses [39].

Unlike with healthy vessels, most of the DCs in atherosclerotic aortas are localized in the intima [40]. Mature DCs are more abundant in advanced lesions. A high level of expression of CHIT1 was detected in mature DCs [38], indicating that CHIT1 could have a crucial role as one of the local mediators, which together with cytokines contribute essentially to the acquisition of the functional specialization of monocyte-derived DCs formed at inflammatory foci induced by immune responses. It has been reported that once DCs are activated by oxLDL in the plaque, they move to secondary lymphoid organs and initiate the clonal proliferation of the T cells that are specific to oxLDL [30]. Therefore DCs, as important mediators of immune responses, may also act as the regulators of innate or adaptive immunity against the potential antigens that are engaged in atherosclerosis. The effect of CHIT1 expression in DCs in atherosclerosis can influence the induction of chemokines and cytokines, presentation of antigens, and lipid absorption that might trigger inflammation or promote tolerance.

**CHIT1 and Cerebrovascular Dementias**

A recent study showed that over a maximum of 11 years of follow-up, diabetic patients experienced a higher incidence of Alzheimer’s disease (AD) than nondiabetic subjects [41]. Thus, AD could represent a neuroendocrine disorder that resembles a unique form of T2DM accompanied by neurodegeneration, which is sometimes considered type 3 diabetes [42]. Previous investigations suggested that CHIT is also involved in cerebrovascular dementias in which the inflammatory process is activated [43]. A feature in the brain of AD patients is the presence of activated microglia and astroglia, which release proinflammatory cytokines and chemokines [44]. Endothelial dysfunction also represents a key etiological factor leading to moderate-to-severe vasculopathies that are also observed in AD patients. Type 2 diabetes provokes an immunologically mediated chronic proinflammatory state involving deleterious effects of leukocyte-derived cytokines and endothelial-derived chemotactic agents leading to vascular and whole organ dysfunction. The long-term negative consequences of vascular proinflammatory processes on the integrity of CNS basal forebrain neuronal populations mediating complex cognitive functions establish a striking temporal comorbidity of AD with type 2 diabetes [45]. Extensive biomedical evidence supports the pivotal multifunctional role of constitutive nitric oxide production and release as a critical vasodilatory, anti-inflammatory, and antioxidant mechanism within the vascular endothelium. Therefore, the presence of inflammatory mediators highly expressed in the vicinity of Aβ deposits in the brain of AD patients strongly indicates the contribution of inflammation in the pathogenesis of AD [46]. As well as AD, the pathophysiological events involved in ischemic cerebrovascular dementia in the development of brain ischemia and multi-infarct cognitive impairment appear due to secondary mechanisms substantially mediated by inflammation [47]. The evidence that cytokines such as IL-16 and IL-18 are markedly elevated in cerebrovascular dementia and AD patients are significantly and positively correlated with CHIT1 suggests that this enzyme is also involved in the pathogenesis of these diseases in which the inflammatory process is activated. Moreover, the evidence that IL-16 has a chemotactant and immunomodulatory role in chronic inflammatory disorders in the brain [48] could explain why chemotacttracted macrophages are activated to produce CHIT1 (fig. 1). Further evidence that CHIT1 reflects the severity of inflammation in AD [48] arises from the finding that the expression of TGF-β in AD patients was inversely correlated with CHIT1 expression. Interestingly, increased activity of CHIT1 was also shown in patients with ischemic stroke, which directly correlates with stroke severity [49].

**CHIT1 and Nonalcoholic Steatohepatitis**

Nonalcoholic steatohepatitis (NASH) is a clinicopathological condition characterized by a necroinflammatory disorder with fatty infiltration of hepatocytes. This disorder can occur in association with inherited metabolic disorders including obesity, dyslipidemias, type 2 diabetes, and other forms of the insulin-resistance syndrome [50]. A crucial event in the initiation of NASH involves lipid accumulation and lipid peroxidation in the hepatocytes, followed by Kupffer cell and hepatic stellate cell (HSC) activation. Since Kupffer cells possess scavenger receptors, they can be activated by exposure to products of lipid peroxidation [51]. Interestingly, it was reported that CHIT1 increases significantly in NASH [52]. Kupffer cells were found to be the only hepatic source responsible for CHIT1 production and secretion in vivo.
for CHIT1 mRNA expression (fig. 1) [52], confirming that CHIT1 activation could be associated with fatty acid accumulation [8].

In response to liver cell injury/inflammation, activated Kupffer cells are a source of proinflammatory cytokines [53], ROS production, and lipid peroxides levels which could induce CHIT1 production. Cytokines released by hepatocytes and Kupffer cells are an additional factor for the progression of NASH. Since the biological effects of CHIT1 are regulated by the release of cytokines [14], it could contribute to the activation of nonparenchymal cells. CHIT1 appears to have organ- as well as cell-specific effects in the context of inflammatory disorders.

CHIT1 levels are increased in polarized M2 macrophages [32]. Macrophages are involved in both generation of fibrosis and its resolution. Since M2 polarization generates a positive feedback loop during resolution of inflammation, it is unclear what events influence tissue repair/remodeling as well as fibrotic outcomes. It is possible that CHIT1 found in M2 macrophages could be involved in the modulation of the extracellular matrix, affecting cell adhesion and migration during the tissue remodeling processes that take place in fibrogenesis [54].

Additionally, the correlation of CHIT1 levels with α-smooth muscle actin expression strongly confirms that CHIT1 could be involved in the modulation of the extracellular matrix [55] in hepatic tissue during fibrogenesis [56]. These data were consistent with in vitro studies showing that Kupffer cell medium stimulates collagen production and proliferation by isolated HSCs and elicits an activated morphology [57]. The parallel increases of CHIT1 levels in Kupffer cells and of α-smooth muscle actin in HCSs, respectively, indicate that this enzyme can be regarded as a mediator of HSC activation in the liver of NASH patients. CHIT1 released by activated Kupffer cells could activate HSCs to synthesize collagen whose overproduction leads to hepatic fibrosis and cirrhosis [54].

Studies with genetically modified mice demonstrated that CHIT1 enhances TGF-β1 receptor expression and signaling, suggesting a role in initiating or amplifying the response to organ injury [58]. In addition, it was demonstrated that heterozygosis for a 24-bp duplication in the CHIT1 gene protects from nonalcoholic fatty liver disease progression [59]. Therefore, it is conceivable that CHIT1 inhibition might have beneficial effects on the expression of genes associated with tissue remodeling processes in fibroblastic hepatic tissue [54].

**CHIT1 and Skeletal Fragility**

Increased fracture risk, habitually related with type 1 diabetes, has currently been of great distress in patients with type 2 diabetes [60]. The presence of diabetic complications, longer disease duration, insufficient glycemic control, insulin use, and increased risk for falls are all reported to increase fracture risk. Recently, alterations in bone material properties seem to be the predominant defect leading to increased bone fragility. Accumulation of advanced glycation end products and changes in collagen cross-linking along with suppression of bone turnover seem to be significant factors impairing bone strength. Additionally, the clinical literature reporting the influence of DM on osteoarthritis (OA) and its therapeutic consequences suggests that DM may increase the risks of development and severity of OA.

OA represents a major clinical and scientific challenge for clinicians and biologists due to the limited repair capacity of articular cartilage, which is rich in matrix proteins but also avascular [61]. Articular cartilage defects cause pain, reduced joint function, and significant disability [62]. OA and DM frequently coexist simply by chance due to their high prevalence and shared risk factors. Nearly half of the patients with DM have some form of arthritis [63]. The presence of comorbid conditions usually increases the care needs of individual patients, decreases the effectiveness of care, and escalates health-related costs. In addition, therapeutic strategies that emphasize personalized medicine and take into account comorbid conditions may result in improved outcomes for patients with OA [64]. The development of OA may also complicate DM. Moreover, there is increasing evidence that OA adds to the burden of cardiovascular disease, which is higher than average in DM patients [65].

The latest finding demonstrated that CHIT1 production is not a macrophage peculiarity and that CHIT1 may play an important role in the process of bone remodeling (fig. 1). This evidence strongly supported an evolving concept regarding the roles of CHIT1 in osteolysis [66]. The high expression of CHIT1 observed in osteoclasts could have a detrimental role in the osteolytic processes occurring in DM. Further study demonstrated that CHIT1 was expressed in an osteoarthritic rat cartilage model. These findings suggest that patients with elevated serum levels of CHIT1 may have increased osteolytic activity and a faster progression of the disease [63]. Indeed, silencing CHIT1 with siRNA resulted in a significant decrease in bone resorption activity and transfection with CHIT1 siRNA, and cotransfection with both decreased the levels of the prodifferentiative marker MMP9 [66].
Therefore, patients with elevated serum levels of CHIT1 may have increased osteolytic activity and a faster progression of degenerative skeletal diseases in DM.

Conclusion

The study of CHIT1 functions in metabolic and degenerative diseases is a fascinating area of research due to its roles in the fine-tuning of many pathophysiologic processes and its immune-modulation in human diseases. CHIT1 has emerged as a new molecule that may contribute to numerous aspects and phases of diabetes and its complications, including the impaired endothelial dysfunction and wound healing observed in DM. CHIT1 can play diverse roles such as activating specific signaling pathways and upregulating or downregulating the expression of certain genes, depending on the stimuli. It has become clear that this enzyme may provide valuable information within a clinical setting, potentially acting as a screening tool for high-risk patients, becoming an early predictive diagnostic tool, and helping in the treatment decision-making process. Many of the results obtained so far on the involvement of CHIT1 in degenerative diseases have been the result of large screening studies or have been performed in ‘in vitro’ cell systems. Therefore, many of the CHIT1 functions need to be validated in ‘in vivo’ settings, where the promiscuity and diversity of interac-
tions may pose some serious problems and invalidate clinical applications. CHIT1 accumulating in diabetic tissues could affect biomechanics. Most importantly, we still lack critical knowledge of the pathways involved in CHIT1 activation. We have only seen a small fraction of the ‘big picture’ since CHIT1 exerts different functions according to the tissues and conditions where activated macrophages are resident. Thus, maintaining CHIT1 equilibrium, both in activity and in expression, is mandatory to prevent or delay DM complications. Gaining more insight into the mechanism(s) by which CHIT1 is regulated will offer new tools for harnessing risk factors underlying complex (multifactorial) diseases such as type 2 diabetes and its complications. Altogether, there is a rationale to use CHIT1 evaluation as an emerging integrative biomarker with improved predictive value for vascular complications.

Disclosure Statement

We declare that we do not have any conflict of interest.
41 Bilzer M, Roffel G, Gerbes AL: Role of Kupffer cells in host defense and liver disease. Liver Int 2006;26:1175–1186.


