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Review Article

Cystatin C: New Functional and Clinical Insights

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ABSTRACT
Cystatin C, previously named gamma-trace, is a low-molecular-weight protein that inhibits cysteine proteases and has been found in several human body fluids. It is encoded by the CST3 gene that is ubiquitously expressed at moderate levels. It is freely filtered at the glomerulus and almost completely reabsorbed and catabolized by tubular cells. Cystatins are involved in a number of normal and pathological conditions. They exert several immunomodulatory functions by controlling the activity of cysteine proteases. The serum levels of cystatin C have been reported to be unaffected by age, gender, or muscle mass. Cystatin C, a reliable marker of renal function, is not only associated with renal disease, but also linked to obesity, diabetes mellitus, metabolic syndrome, thyroid dysfunction, neurodegenerative diseases, ophthalmic diseases and tumors.

Keywords: Cystatin C, cysteine proteases, renal function, diseases, marker.

INTRODUCTION

Human cystatin C, previously named gamma-trace or post-gamma-globulin, is a low-molecular-mass protein that inhibits cysteine proteases and has been found in several human body fluids. The human cystatin family presently comprises of 11 identified proteins. Two of these, cystatin A and B, form the family 1 cystatin (stefins) and are mainly intracellular proteins, while the family 2 cystatins are comprised of cystatin C, D, E, F, S, SA and SN and are mainly extracellular and/or transcellular proteins. The family 3 cystatins, also known as high and low molecular weight kininogen, contain three cystatin domains and are primarily intravascular proteins, are also involved in the coagulation process and in the synthesis of vasoactive peptides, besides being inhibitors of cysteine proteases.[1]

Cystatin C is a cationic, low molecular weight (13 kDa) cysteine protease inhibitor, encoded by the CST3 gene that is ubiquitously expressed at moderate levels. It is freely filtered at the glomerulus and almost completely reabsorbed and catabolized by tubular cells. Cystatin C is an important extra and transcellular inhibitor and its monomeric form is present in all human body fluids, especially in cerebrospinal fluid, milk, saliva, tears, urine, seminal fluid, synovial fluid and blood plasma. Depending upon the analytical method used the concentration of

serum cystatin C in healthy adult individuals ranges between 0.8-1.2 mg/L.[2]

Discovery of Cystatin C
In 1961, Clausen reported occurrence of cystatin C in cerebrospinal fluid but he could not detect it in serum. In the same year, it was found in urine from patients with renal failure. The cystatin C concentration in human serum, urine, cerebrospinal fluid, saliva, semen, colostrum, ascitic fluid, and pleural fluid is very low and demands high analytical sensitivity and specificity of determination methods. Lofberg and Grubb developed the first enzyme immunoassay for quantifying cystatin C in human biological fluids in 1979 and later recommended this as a kidney function test. The assay was time consuming and had limited analytical sensitivity for detecting very low concentrations of cystatin C, as according to present standards. To improve the analytical reliability of the methods, simpler and more sensitive radio-, fluorescence-, and various enzyme immunoassays were developed. Perhaps, as a result of the general difficulties of standardizing immunological methods, the diagnostic value of cystatin C was not clearly confirmed by those investigators. The antigens and antibodies used in the previous tests were mostly prepared in-house, and the

difference in heterogeneity and purity attributable to the various isolation procedures used influenced the assay qualities.[3]

Mechanism of action

In the 20th century, lysosomal cysteine proteases, generally known as cathepsins (Cats), were discovered. Most endosomal proteases belong to 3 distinct families. There are 11 human Cats (Cats B, C, F, H, K, L, O, S, V, W, and X) that belong to the papain subfamily of cysteine proteases (PLCPs). Alongside these there are the aspartyl proteases related to pepsin: cathepsins D and E. Another cysteine protease known as asparaginyl endopeptidase (AEP) or legumain is more closely related to the caspases. Each of these 3 classes of cysteine proteases can be inhibited by distinct and non-overlapping small molecule inhibitors. But *in vivo* inhibition, or knockout, of these proteases often shows limited or no phenotype, mainly due to functional redundancy. Cats have been known to synthesize proenzymes with an N-terminal signaling peptide that targets the protein to the lumen of the endoplasmic reticulum. Cat activity is regulated intracellularly by stefins (stefin A and B) and extracellularly by cystatins (cystatin C, or CystC) and kininogens. Through their combined activities, Cats degrade almost all intracellular and extracellular proteins. The expression patterns of cathepsin are different in cardiovascular and CVD-related. The cysteinyl Cats are predominantly endopeptidases located intracellularly in endolysosomal vesicles. However, Cats act in the extracellular space as well as in the cytosol and nucleus. The overexpression of elastolytic and collagenolytic Cats S, K, B, H, and L are seen in failing cardiac and atherosclerotic vessel tissues from humans and animals but shown no changes in levels of their endogenous inhibitor, cystatin C.[4] It was postulated that inhibiting all three families of endosomal proteases would provide a powerful tool for modulating endosomal/lysosomal function. Cystatin C potently inhibits PLCPs and AEP. The cystatins inhibit PLCPs with sub-nanomolar affinity. They are present in the bloodstream and are supposed to play a role in the mopping up of proteases released during physiological and pathological responses. Thus, for the synthesis of a pan-endosomal protease inhibitor, Cystatin C represents an excellent scaffold.[5]

Functions of Cystatins

Cystatins are involved in a number of normal and pathological conditions. They exert several immunomodulatory functions by controlling the activity of cysteine proteases or by other mechanisms not related to their inhibitory function[6] (Table-1).

Reference range of Cystatin C

The cystatin C range differs according to the analytical method used (Table 2). The different cystatin C ranges found by Kyhse-Anderson (0.61-1.21 mg/L), Norlund (0.70-1.21 mg/L) and Erlandsen (0.54-1.21 mg/L); these three groups used the Dakopatts method. The ELISA, Dakopatts and Dade Behring assays use different antibodies, calibrants and technologies and therefore give different reference ranges. This new marker requires an internationally agreed reference preparation in order to allow direct comparison of methods and reference ranges.

Cystatin C and disease states: General considerations

Serum cystatin C, marker of renal function, has been proposed as potentially superior to serum creatinine level for estimating renal function, because it is produced at a constant rate by most nucleated cells. Moreover, cystatin C production has been reported to be unaffected by age, gender, or muscle mass. Cystatin C is freely filtered at the level of the glomerulus and virtually all is reabsorbed and metabolized by the proximal tubular cells, with a half life of 1.5 hour.[16] Cystatin C has been proposed as a more reliable marker of renal function than serum creatinine, as it detects small reductions in GFR. Cystatin C is considered to reveal microvascular renal dysfunction. It has been associated with increased levels of coagulation markers, increased levels of inflammatory markers, and severity of coronary artery disease. In addition, when compared with creatinine-based estimation of GFR in both elderly and in coronary artery disease populations, cystatin C significantly improves risk stratification. It has become evident that disturbances in expression and localization of cystatins may be implicated in several pathological processes, such as inflammatory skin diseases, age-related macular degeneration and neurodegenerative disorders.[6] Serum cystatin C has also shown to be associated with corticosteroid administration and cigarette smoking.[16,17] Higher cystatin C levels were significantly associated with hip fracture in women but not in men.[18] Cystatin C was also associated with more rapid loss of bone mineral density at the hip using DEXA, especially in men.[19] After adjusting for creatinine clearance, higher serum cystatin C levels were independently associated with older age, greater weight, greater height, male gender, higher serum C-reactive protein levels and current cigarette smoking.[16] Donahue et al suggested that elevated levels of serum cystatin C are associated with prediabetes.[20] Magnusson et al found that increased baseline serum levels of circulating

cystatin C predict new-onset metabolic syndrome (MetS) (according to the NCEP-ATP III guidelines), independently of MetS risk factors during 16 years of follow-up, suggesting that high cystatin C is related to MetS development. They further concluded that high levels of cystatin C independently correlate with long-term waist progression, as well as incident abdominal obesity. These findings highlight the influence of cystatin C on abdominal obesity, thereby signifying a potential mechanism for the cystatin C-derived risk of future MetS and CVD development.[21]

Cystatin C and renal disease
Serum creatinine level is commonly used for the estimation of renal function. Creatinine level in serum is determined by its renal excretion, its production in muscular tissue, which further depends on age, weight, and gender. So, while using serum creatinine level to estimate renal function one needs to adjust these factors. Hence, for estimating renal function, these parameters are integrated into the Cockcroft-Gaultformula[16].

Although micro albuminuria is the first detectable functional abnormality, glomerular filtration rate (GFR) is the critical index of renal function.[22] The "gold standard" for determining GFR is to measure clearance of exogenous substances such as inulin, ^{51}Cr EDTA, iohexol, or ^{125}I-labeled iothalamate. These techniques, however, are time-consuming, labor-intensive, expensive, and require administration of substances that make them incompatible with routine monitoring. So, assuming constant cellular production, serum cystatin C level has the potential to be an exceptional substitute marker of GFR. Recently, urinary biomarkers, like kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and neutrophil gelatinase-associated lipocalin (NGAL), have been used for the early detection of acute kidney injury (AKI). However, these markers have disadvantage of collection of urine, that is costly and may delay the initiation of the treatment in critically ill patients. Some authors suggested that plasma cystatin C was superior to plasma creatinine as an early predictor of AKI in the intensive care unit (ICU) population.[23] Hence serum cystatin C, is a stronger predictor of the risk of cardiovascular events and death in elderly persons than is creatinine. Its serum concentrations are mainly determined by GFR. The evidence accumulates for the value to estimate GFR by means of cystatin C in both patients with and without diabetes. The different cystatin C–based formulae have been developed. Irrespective of the formula used, when compared with creatinine-based formulae, the accuracy and precision by cystatin C methods, seems to be better.[24]

For diabetes patients, Pucci et al observed a higher predictive value in the early detection of reduced renal function (determined by the iohexol plasma clearance method) when the GFR was estimated by cystatin C compared with GFR estimation by the creatinine-based Modification of diet in renal disease (MDRD) and Cockroft-Gault formulae.[25] Particularly poor precision and accuracy of creatinine-based eGFR equations with respect to the range of 60–89 mL/min/1.73 m^2 (determined by the isotopic 51-creatinine EDTA method) have also been described by others. Nevertheless, the evidence accumulates for better accuracy and precision of eGFR-Cystatin C compared with eGFR based on creatinine levels in subjects with diabetes, especially in early changes of kidney function.[26] Orlando et al found that plasma cystatin C concentration is an accurate GFR marker in cirrhotic patients. As their reference values vary with the severity of the liver disease, plasma creatinine concentration and calculated creatinine clearance are of no practical value.[27] Zhu et al and other authors concluded that the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2012) serum creatinine-cystatin C based equation appeared more accurate and less biased in overall participants. In senior participants with moderately to severely injured GFR neither of the new CKD-EPI equations achieved ideal accuracy.[28]

CKD-EPI Equations that Incorporate Creatinine, Cystatin C, or Both
2009 CKD-EPI creatinine[29]

eGFR = 141 × min(SCr/K,1) × max(SCr/K,1)$^{-1209}$ × 0.993^{age} [× 1.018 if female] [x1.159 if black]
If female: K = 0.7,   =−0.329
If male: K = 0.9,   =−0.411

2012 CKD-EPI cystatin[30]

eGFR = 133 × min(SCysC/0.8, 1)$^{-0.499}$ × max(SCysC/0.8, 1)$^{-1328}$ × 0.996^{age} [× 0.932 if female]

2012 CKD-EPI creatinine-cystatin C[30]

eGFR = 135 × min(SCr/K, 1)$^-$ × max(SCr/K, 1)$^{-0601}$ × min(SCysC/0.8, 1)$^{-0375}$ × max(SCysC/0.8, 1)$^{-0.711}$ × 0.995^{age} [× 0.969 if female] [× 1.08 if black]
If female: K = 0.7,   =−0.248
If male: K = 0.9,   =−0.207

SCysC, serum cystatin C; SCr, serum creatinine; min, minimum; max; maximum; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Cystatin C and obesity

The mRNA of cystatin C is expressed in subcutaneous and omental adipose tissue, at twice higher levels in non adipose cells as compared to adipose cells. There is two-to-threefold increase gene expression and cystatin C release by adipose tissue explants in obesity.[31] Cathepsins play important roles in human pathobiology. Some members, including cathepsins S, L, and K, have been implicated in atherogenesis. These three proteases were initially found to be abundantly expressed in atherosclerotic lesions in humans and were thought to contribute to plaque progression through proteolysis of extracellular elastins and collagens. Their proatherogenic role has been confirmed in genetically modified mice with targeted deletion of cathepsins S, L, or K. Previously, cathepsin S was identified as an adipose gene whose expression increases with obesity.[32] Researchers found that cathepsin S mRNA and serum levels were positively correlated with BMI and decreased after gastric surgery- induced weight loss. Other groups have reported that cathepsin K is expressed in adipose tissue and increased in human and mice models of obesity. More recently, cathepsin L was detected in the adipose tissue of obese mice and in human adipocytes.[33] The pro-atherogenic cysteine protease Cathepsin S is increased in obese subjects. Cathepsins' enzymatic activity is regulated by endogenous inhibitors, the most abundant being cystatin C.[31]

Cystatin C and cardiovascular disease

Cystatin C is strongly associated with cardiovascular mortality as well as with myocardial infarction, stroke, peripheral arterial disease and incident hypertension. Michael G and other authors had evaluated the levels of cystatin C with cardiovascular risk as low risk, intermediate risk, and high risk corresponding to cystatin C levels of less than 1.00 mg/L, 1.00 to 1.28 mg/L, and more than 1.29 mg/L.[34,35] Many studies showed that cystatin C is strongly and independently associated with subsequent CVD risk [35,36] and has predictive and prognostic value in CVD, including chronic heart failure. Lassus et al in their study found that cystatin C is a strong predictor of mortality in patients hospitalized for acute heart failure.[37] Bjurman et al found correlation between cystatin C and infective endocarditis prognosis.[38] Cysteinyl Cats K, L, and S are among the most potent mammalian elastases, and high levels of these proteases are seen in human atherosclerosis and AAA lesions. The growth and rupture of abdominal aortic aneurysms (AAAs) result from increased elastin turnover, which critically depends upon specific elastases that cleave multilayer elastic laminas. In contrast, their endogenous inhibitor cystatin C is deficient in these lesions. Aortic tissue extracts of AAA patients had higher levels of Cat-dependent elastolytic and collagenolytic activities than did those of patients with aortic occlusion diseases, but cystatin C levels were regulated inversely. Recently, using gene deletions of Cat K or Cat L, Sun and colleagues demonstrated clearly that both Cats contribute to AAA formation by several mechanisms[5] (Figure-1).

Recently, the significance of cystatin C in an atrial fibrillation (AF) population was reported in the ARISTOTLE and RE-LY biomarker substudies. Increased rates of stroke or systemic embolism, mortality, and major bleedings, were independently associated with rising cystatin C levels and yielded an better risk stratification and risk prediction. The renal impairment constitutes a major risk factor for thrombo-embolic and cardiovascular events in AF, though it is not represented in current risk stratification models. The major problem as with renal dysfunction patients is that they tend to be undertreated with oral anticoagulation therapy due to the increased risk of bleeding when treated with vitamin-K antagonists.[39]

Cystatin C and neurodegenerative diseases

Cystatin C is also involved in the process of neuronal degeneration and repair of the nervous system. Any imbalance between active proteases and their endogenous inhibitors may lead to uncontrolled proteolysis, which has been related with different neurological diseases. The greater part of cystatin C in the CSF is produced by the choroid plexus. Cystatin C levels in CSF were found to be five times greater than plasma levels, which is suggestive of a potential physiological role of cystatin C in the brain. In neurodegenerative diseases, alterations in Cystatin C levels in the CSF have been observed. A great diagnostic potential of Cystatin C is seen, as a biomarker for Amyotropic Lateral Sclerosis (ALS), a fatal neuromuscular disease characterized by progressive motor neuron degeneration. In ALS patients, significantly reduced cystatin C levels in CSF were observed as compared to healthy controls. In addition, the rate of ALS disease progression was correlated with the direction of the longitudinal change in CSF cystatin C levels, and initial CSF cystatin C levels were predictive of patient survival, signifying that cystatin C may act as a surrogate marker of progression of disease and survival. Cystatin C is also linked to ALS histopathologically. It is one of two known proteins that localize to Bunina bodies (small intraneuronal inclusions present in degenerating motor neurons), which are a specific

neuropathologic feature of ALS.[6] It was found that lower cystatin C levels in the CSF of Alzheimer's disease (AD) patients were seen than non-demented individuals. An enhanced cystatin C expression was showed by specific neuronal cell populations in the brains of AD patients. The secreted protein, cystatin C, plays an important role in various neurodegenerative diseases. An enhanced cystatin C expression in the neurodegenerative states puts forward two conflicting theories whether augmented expression of cystatin C is causing or promoting already initiated neurodegenerative changes or, instead, an endogenous neuroprotective response to the disease process.[40]

Cystatin C co-localizes with amyloid-beta (A) in amyloid-laden vascular walls and in senile plaque cores of amyloid as shown in immunohistochemical studies. A immunoreactivity and cystatin C co-localizes in a specific group of pyramidal neurons that is susceptible to neurodegeneration in AD. The binding of cystatin C to A and prevents A oligomerization, fibril formation, and amyloid deposition as shown in biochemical studies. The linkage of a cystatin C gene polymorphism with AD is associated with reduced secretion of cystatin C, found in genetic study. Alteration in cystatin C trafficking by two FAD-linked presenilin2 gene mutations causes reduced cystatin C secretion. Low concentrations of cystatin C were found in plasma and CSF of AD patients. Thus, cystatin C could protect the brain from amyloid-induced toxicity and may have therapeutic implications for AD. The slight increase in the expression of cystatin C, activates various mechanisms of protection like inhibition of cysteine proteases, prevention of amyloidogenesis, induction of cell division and autophagy.[40]

The changes in cystatin C expression and localization have been related with various other neurodegenerative diseases. A point mutation in the cystatin C gene, leading to substitution of Leu to Gln, is responsible for the hereditary cystatin C amyloid angiopathy (HCCAA), a dominantly inherited icelandic type of amyloidosis. Highly reduced levels of cystatin C in cerebrospinal fluid were seen in patients of multiple sclerosis (MS) when compared with healthy individuals. The impairment in the inhibition of cysteine proteases occurs in MS, as suggested by the low concentration of cystatin C in these patients. Hence, higher activity of cysteine proteases could initiate or increase of myelin breakdown. Enhanced expression of cystatin C has been observed in response to different types of insults to the brain, such as ischemia and epilepsy, which shows that it has an important role in brain injuries.[6]

Cystatin C and endocrine function
The impact of thyroid dysfunction on serum cystatin C was also investigated. In patients with hyperthyroidism, GFR is underestimated, and, in patients with hypothyroidism, GFR is overestimated, when considering serum cystatin C concentrations. Because of the influence of the thyroid state on general metabolism, there is increased or decreased production of cystatin C in hyper- and hypothyroidism as stated by Den Hollander. Higher serum concentrations of cystatin C and transforming growth factor 1 (TGF- 1) were found in patients with hyperthyroidism, and a positive correlation between serum cystatin C, thyroid hormones, and TGF- 1 was observed. There is decrease in serum cystatin C and TGF- 1 after treatment decreased. An increase in TGF- 1 concentrations in hyperthyroidism and a stimulatory effect of thyroid hormones and TGF- 1 on cystatin C production was suggested by *in vitro* findings.[41]

Growth hormone (GH) also appears to affect cystatin C levels independent of renal function. Increase in GH leads to increase in cystatin C which is in accordance to the physiological condition of normal puberty, where GH activity is enhanced. Cystatin C levels were found to peak at the age of 12 in females, and at the age of 14 in males in the NHANES study, apparently in the absence of changes in the GFR. Some authors stated that cystatin C levels could be affected by growth in adolescents, as its levels rise in parallel with peak height velocity, and the age-related variation in cystatin C levels was likely related to variation in cystatin C production.[42]

Cystatin C and ophthalmic diseases
Cystatin C is amongst the most abundantly expressed proteins of the retinal pigment epithelium (RPE). Cystatin C is involved in proteolysis events that regulate the maintenance of the bruch's membrane and cell-signaling processes on the basolateral side of RPE. The age-related macular degeneration (AMD) associated changes, such as disruption in the stability and function of the bruch's membrane, and increased permeability of RPE can be due to reduced protein level in this region as a result of its decreased secretion. Cystatin C can interact with complement component and may inhibit the complement cascade. So, there is a possibility that reduced levels of cystatin C secretion can contributed to the increase in complement activity often associated with AMD pathogenesis.[43]

Table 1
Functions of Cystatins

• **Proteolytic processing of progranzymes and other substrates**
• **Major histocompatibility complex class II antigen presentation**
• **Maturation of dendritic cells**
• **Modulation of integrin function**
• **Formation of skin barrier**

Table 2
Published reference ranges for cystatin C

Author(s)	Method	Calibrant (cystatin C protein)	Reference intervals (mg/L), mean±SD (or range)	Number of subjects
Kyhse-Anderson et al[7]	PETIA	Recombinant human protein in cystatin C-free normal human serum	(0.61-1.21)	27
Lofberg and Grubb[8]	RID	Purified human urinary protein in PBS (1º), purified human CSF protein (2º)	1.3 ± 0.26(0.72-1.7)	46
Lofberg and Grubb[8]	EIA	Purified human urinary protein in PBS (1º), purified human CSF protein (2º)	1.1 ± 0.42(0.63-2.5)	30
Poulik et al[9]	RIA	Purified human urinary protein in PBS	0.96 ± 0.20(0.6-1.7)	100
Cattaneo et al[10]	EIA	Not stated	1.10 ± 0.15	20
Ishiguro et al[11]	EIA	Purified protein from human hepatoma cell line	0.75 ± 0.65, 20 years 1.34 ± 0.95, > 20 years	85 189
Colle et al[12]	EIA	Purified human urinary protein in PBS (+BSA)	1.25 ± 0.22 (0.86-1.7)	50
Pergande and Jung[3]	EIA	Purified human urinary protein in PBS	1.78±0.26, women 2.14 ± 0.31, men	33 33
Norlund et al[13]	PETIA	Recombinant human protein in cystatin C-free normal human serum	(0.70-1.21), 20-50 years (0.84-1.55), > 50 years	242
Erlandsen et al[14]	PETIA	Recombinant human protein in cystatin C-free normal human serum	(0.54-1.21),20-65 years (0.56-1.29), women (0.42-1.39), men	270 135 135
Finney et al[15]	PENIA	Purified human urinary protein	(0.53-0.92), 19-49 years (0.58-1.02), >50 years (0.49-0.94), women aged 19-67 years (0.56-0.98), men aged 19-67 years	258 51 155 154

BSA = bovine serum albumin; CSF = cerebrospinal fluid; EIA = enzyme immunoassay;   PETIA = particle-enhanced turbidimetric immunoassay; PENIA = particle-enhanced nephelometric immunoassay; PBS = phosphate-buffered saline; RIA = radioimmunoassay; RID = radial immuno-diffusion; SD = standard deviation; 1º = primary; 2º = secondary.
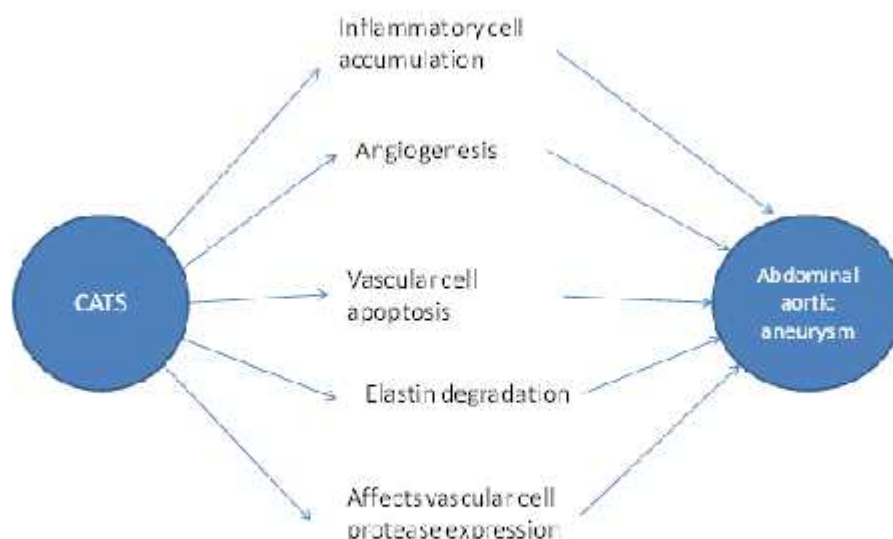
Figure-1
Role of CATs in AAA

Cystatin C and tumors
Type II cystatins, cystatins C and E/M are usually down-regulated in tumors. Although they have a protective role, but their lower levels might allow a surplus of harmful tumor associated proteolytic activity. Higher levels of type II cystatins outside the cells may impair extracellular activity of cysteine proteases, connected directly or indirectly with the degradation of extracellular matrix and thus, resulting in tumor cell invasion and metastasis. Still, higher levels of cystatins in body fluids have been associated with poor prognosis in cancer patients, hence supporting their role in regulation of proteases involved in the tumor regression. Increased extracellular levels of cystatin C as well as stefins significantly correlated with high risk of adverse outcome in patients of melanoma and colorectal cancer. An imbalance between the enzyme and its inhibitor in cancer patients is depicted by less abundant cathepsin B/cystatin C complex in sera of tumor patients.[6] Cystatin C is also a good marker of renal function to predict dose of renally excreted drugs in cancer.[44]

Cystatin C in pediatrics
The cystatin C concentrations are higher in the first year of life. Bökenkamp et al in their study conducted in 258 children, aged 1 day to 18 years, without kidney disease, found that cystatin C concentration was highest on the first days of life, with a rapid decrease during the first 4 months. The cystatin C concentration was constant after first year of life. Serum cystatin C was found to be a superior biomarker to serum creatinine in the assessment of GFR in premature infants in a recent study. The higher levels of cystatin C in the first year of life possibly reflect the low GFR of neonates and infants.[45] Interestingly, in a study conducted by Kolialexi et al, cystatin-C was associated with subsequent pre-eclampsia development.[46]

CONCLUSION
Cystatin C has a role in various normal and pathological processes. Its reference interval in various biological fluids, and the various derived formulas need to be further streamlined. The evidence suggests the relationship of cystatin C with various disease states like renal disease, obesity, diabetes mellitus, metabolic syndrome, thyroid dysfunction, neurodegenerative diseases, ophthalmic diseases and tumors. In the future years, there is possibility that certain new diagnostic, prognostic and therapeutic aspects about this cysteine protease inhibitor can come into light.

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