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Review Article

Serum Cystatin C: A Novel Marker of Diabetic Nephropathy and Other Diseases

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Abstract

Diabetes has become the most common single cause of end stage renal disease (ESRD) in most countries; this is due to the fact that diabetes, particularly type 2, is increasing in prevalence, diabetic patients now live longer and patients with diabetic ESRD are now accepted for treatment in ESRD programs where formerly they had been excluded. About 20 - 30% of patients with type 1 or type 2 Diabetes develop evidence of nephropathy. Recent studies have now demonstrated that the onset and course of DN can be ameliorated to a significant degree by several interventions but these interventions have their greatest impact if instituted at a point very early in the course of the development of this complication.

Keywords: *Serum Cystatin C; Diabetic Nephropathy; Glomerular Filtration Rate (GFR)*

Estimation of the glomerular filtration rate (GFR) is the most widely used test of renal function and reflects the kidney's ability to clear a particular substance from plasma. GFR is defined as the quantity of glomerular filtrate formed per unit time in all nephrons of both kidneys. The most precise and accurate methods for estimating GFR are based upon determinations of plasma clearance of substances like 51Cr-EDTA, iothalamate or iohexol. These so called "gold standard" methods require injection of an exogenous radioactive or contrast agent which are complex, laborious, expensive and impractical in a clinical setting as well as for larger research studies. Therefore, the measurement of endogenous blood substances to estimate GFR is common practice. For several decades clinicians have relied on measurements of serum creatinine as a rapid first-line test to determine GFR. This test is convenient and cheap, but results are affected by age, sex, muscle mass, diet, race and tubular creatinine secretion, particularly when GFR is reduced. Thus, there has been an ongoing search for suitable alternative endogenous markers of GFR.

Cystatin C is a relatively new marker for diabetic nephropathy and we intend to review the literature available about cystatin c in renal disease and other possible links with other diseases.

History and characteristics

Cystatin C is a non-glycosylated, basic, low molecular weight protein; it was initially described as a microprotein constituent of normal cerebrospinal fluid, and of urine from patients with renal failure [1]. The early literature referred to it by several different names such as γ-trace, post-γ-globulin, and gamma-CSF before it was sequenced. The amino acid sequence of cystatin C isolated from human urine was the first determined sequence of the cystatin superfamily [2]. The same protein was only two years later identified as an inhibitor of cysteine proteases. Cystatin C is the part of a superfamily of cysteine protease inhibitors. The cystatin superfamily is comprised of a large

group of ancestrally related proteins active against papain-type proteases that are divided to three major families: the type 1 cystatins, which include stefins A and B, and are mainly intracellular; the type 2 cystatins, which include cystatins C, D, E/M, F, G, S, SN and SA, and are extracellular; and the type 3 cystatins, which include the kininogens, and are intravascular proteins. Cystatin C is the most potent inhibitor of cysteine proteases such as lysosomal cathepsins B, H, L and S, with apparent inhibition constants even below nanomolar range. Mature cystatin C is composed of 120 amino acid residues and it is synthesized as a preprotein with a 26-residue signal peptide [3]. The presence of a hydrophobic leader sequence in pre-cystatin C strongly indicates that the protein is normally secreted. Cystatin C gene is ubiquitously expressed in all tissues and cell types, and it is present in relatively high concentration in a number of body fluids, although mRNA levels vary several-fold between and among tissues. There are considerable differences in the extracellular levels of cystatin C; it is present at micromolar levels in cerebrospinal fluid and semen, while in serum, saliva, and tears its concentration is much lower. In some body compartments, as for instance in cerebrospinal fluid, cystatin C represents more than 90% of the total molar concentration of cysteine protease inhibitors, while in blood plasma, it only represents a few percent of the total cysteine protease inhibitory capacity. Cystatin C can inactivate their target enzymes either extracellularly or intracellularly, following reuptake. During intracellular trafficking cystatin C forms a dimmer. The dimerisation of cystatin C resulted in a complete loss of its biological activity. Although cystatin C gene has typical features of a constitutively expressed "housekeeping" gene, and is not an acute-phase protein, cystatin C levels are regulated in a manner that suggests a relationship to injury and immune responses. Additional function attributed to cystatin C is an efficient inhibition of legumain, also called asparaginyl endopeptidase (APE), which has, like cathepsin S, been proposed to be involved in the class II MHC presentation of antigens. A non-inhibitory mode of interaction between cysteine proteases and cystatin C, where cathepsin L cleaves cystatin C at Gly11-Gly12 bond upon complexation, was determined. Since both proteins are extracellular it was suggested that such cleavage could be of physiological importance by controlling the extent of inhibition of cysteine proteases, especially cathepsin B, at the site of inflammation. Independent of its effects on cysteine protease activity, cystatin C also regulates normal and transformed cell proliferation, inhibits melanoma cell motility, as well as antagonizes transforming growth factor ß signalling in normal and cancer cells. It was also suggested that cystatin C has a regulatory role in inflammatory processes by down-regulation of phagocytosis-associated respiratory burst reaction displayed by polymorphonuclear neutrophils and by down regulation of their chemotactic response. Another property unrelated to its potential of cysteine protease inhibitor is upregulation of nitric oxide release. These findings define novel cathepsin-independent functions for cystatin C. Another feature, which is common for type 2 cystatins, is N-terminal processing. A different N-terminal truncation of cystatin C was observed in urine from patients with nephrological disorders, purulent sputum, amyloid deposits in brain arteries, and in cerebrospinal fluid of patients with hereditary cystatin C amyloid angiopathy. The inhibitory effect of cystatin C on extracellular cysteine proteases may be reduced by the existence of truncated forms of the inhibitor as described by *in vitro* experiments with variety of protea. It is believed that N-terminal truncations are the result of proteolytic cleavage of the full-length cystatin C, since the N-terminal part is exposed in the outer side of the molecule and has no ordered structure in solution as revealed from X-ray crystallography and nuclear magnetic resonance spectroscopy. Indeed, such inactivation of cystatin C has already been observed *in vitro* following the action of papain, neutrophil elastase, cathepsin L, and gingipain. Leung-Tack., *et al.* [4] suggested the role of cystatin C, and N-terminal processing, in the modulation of neutrophil behavior. Cystatin C has been proposed to have a role as an intracellular modulator of MHC class-II-mediated antigen presentation in peripheral dendritic cells by controlling cathepsin-S-mediated degradation of the invariant chain. However, later results revealed that cystatin C expression can differ markedly between closely related cell types. This expression pattern is very striking, because cystatin C has generally been considered a ubiquitously expressed protein and its gene contains no obvious regulatory sequences that might explain this feature. Furthermore, it was also reported that cystatin C is neither necessary nor sufficient to control MHC II expression and antigen presentation in dendritic cells. Cystatin C is thought to play a role in periodontal disease, inflammation, cancer, multiple sclerosis, renal failure, asthma, HIV infection, bone remodeling, etc. Due to the widespread extracellular distribution of cystatin C and the antiviral function demonstrated for cystatins in experiments with polio, herpes simplex, and corona virus-infected cell cultures, one of suggested roles of cystatin C is a defence against microbial and viral infections. Despite the fact that the biological function of cystatin C is not yet fully determined, cystatin C concentrations in extracellular fluids have been shown to be of clinical importance.

Cystatin C and renal function

Assessment and follow-up of renal dysfunction is important in the early detection and management of chronic kidney disease. The glomerular filtration rate (GFR) is considered the most accurate measurement of kidney disease and is reduced before the onset of clinical symptoms. GFR is measured or predicted according to different methods. The most precise and accurate method for evaluation of kidney function is the measurement of GFR using particular exogenous substance. However, the use of these filtration markers makes measurement of GFR costly and incompatible with routine monitoring [5]. Use of endogenous substance creatinine as markers of GFR is the most commonly used biochemical parameter, which provides an approximate index of the level of GFR. Intra-individual variation of creatinine is low, but its concentrations differ considerably between people because factors such as muscle mass, protein intake, age, sex, race and extrarenal metabolism can influence creatinine production, and lead to an inaccurate estimation of GFR. Creatinine clearance gives a better estimate of GFR than does serum creatinine but requires the inconvenience of urine collection. Serum creatinine is of limited value in early detection of renal impairment; while creatinine clearance overestimates true GFR because creatinine is not only filtered by the renal glomeruli but is also secreted by the tubules. According to National Kidney Foundation-K/DOQ1 clinical guidelines for chronic kidney disease, serum markers should not be used alone to assess GFR. Clinical laboratory should report estimate GFR using prediction equations. Knowledge of GFR is of crucial importance in the management of patients to allow correct dosage of drugs cleared by the kidneys, to detect early impairment of renal function, to prevent further deterioration, to manage renal transplant patients and for use of potentially nephrotoxic radiographic contrast media. In specific patient populations where creatinine measurements are crude and can often be misleading, there is a need for more reliable measurement of GFR [6]. The fact that cystatin C exhibits a stable production rate in all nucleated cells, that it is freely filtered in renal glomeruli and almost completely reabsorbed and catabolised in proximal tubules, suggested that its plasma or serum concentrations might be a potentially good marker for GFR. Indeed, many studies during the last few years observed a correlation between serum cystatin C and GFR [7-10]. Serum cystatin C has been shown to be at least as good a measure of GFR as serum creatinine and several advantages to the use of cystatin C were cited. According to the temporal analysis the time of serum sampling in healthy subjects did not compromise the results of quantitative measurements of cystatin C over the course of a day [11], supporting its usefulness for clinical evaluation as diagnostic tool. It also appears that the disease and the type of therapy did not influence the circadian characteristics of serum cystatin C, which was demonstrated in a clinical study that included asthma patients receiving methylprednisolone therapy. Several studies have revealed the normal population range of cystatin C, which after the age of one year is remarkably constant. Cystatin C is higher before age of three months and after the age of seventy. Multiple studies have validated the use of cystatin C as a renal marker to perform at least as well as serum creatinine in the population at large and that it is likely to be superior in specific patient populations [12]. Cystatin C was found to be a more sensitive marker than serum creatinine for small changes in GFR, and for earlier indication of mild renal failure. Several cystatin C-based prediction equations for calculation of GFR have been published in the last years for adults and children. These formulas were evaluated with different cystatin C assays and may reveal inaccurate GFR estimates if inappropriate formula is used for a cystatin C result, therefore, the availability of the internationally standardized methods for cystatin C is important [12].

Cystatin C in pediatric population

In pediatric populations cystatin C may have advantage over routinely used endogenous markers of GFR because of the low muscle mass in children, which leads to very low serum creatinine values and thus to increased assay imprecision. In pediatric patients the urine collection is impossible, as well as in neonates where cystatin C, in contrast to creatinine, does not cross the feto placental barrier. Another advantage is that cystatin C concentration appears to be rather constant in children after the age of one and similar to that of adults. In addition, it has been shown that cystatin C is the only marker of GFR that is reliable in patients with spina bifida or spinal cord injury [13]. Serum cystatin C was confirmed as easy and useful marker, better than creatinine, to detect acute kidney injury in critically ill children [14].

Cystatin C in elderly population

The limitations of serum creatinine, namely low muscle mass and age, for this population are similar to pediatric population. Serum cystatin C was shown to be a superior marker for the early detection of renal impairment in the elderly compared to creatinine.

Cystatin C in pregnancy

Increased cystatin C concentrations were found in healthy women at term pregnancy when compared to healthy controls [15] and significantly lower cystatin C concentrations were detected in serum of patients with recurrent miscarriage. Cystatin C was shown to be a more sensitive indicator of early renal function decline in women with pre-eclampsia. At the same time serum cystatin C was suggested as a marker of endotheliosis, where higher concentrations have been associated with the degree of endotheliosis in women with pre-eclampsia [16]. It therefore appears that monitoring of the degree of endothelioses by determination of serum cystatin C might considerably reduce the need for renal biopsy in pre-eclampsia. More results are needed to establish cystatin C as a clinical marker of renal disease in pregnancy.

Cardiovascular diseases

Renal impairment is associated with bad prognosis. Even moderately reduced renal function represents increased risk of cardiovascular morbidity and mortality.

Cystatin C was determined as a stronger predictor of mortality than creatinine in elderly persons with heart failure. Recently, it was concluded on the bases of eight years long study including 4384 persons, 65 years of age or older, and without previous heart failure that serum cystatin C concentration is an independent risk factor of heart failure in older adults [17]. In addition, serum cystatin C concentration appeared to be a better predictor of the risk of death and cardiovascular disease than serum creatinine concentration. Thus, cystatin C was shown to be not just a useful alternative measure of renal function, but also an effective prognostic tool. The mechanism by which cystatin C concentration predicts risk of heart failure remains unclear. Koenig., *et al.* [18] demonstrated that increased cystatin C concentrations are strongly associated with future secondary cardiovascular events, indicating cystatin C as a promising and clinically useful marker for risk determination in patients with coronary heart disease. It was also published that cystatin C detects reduce GFR more reliably and at earlier stage in patients undergoing cardiac catherization allowing a better identification of patients with renal dysfunction and those at risk of contrast damage.

Renal transplantation

Several studies gave varying results of the use of cystatin C as a marker for GFR in renal transplant patients. For some authors, serum cystatin C was a better GFR marker than creatinine in renal transplant patients [19] for others, serum cystatin C was not a reliable marker because it was observed to be falsely increased in transplant patients compared with nontransplant patients with a similar GFR. On the other hand, the largest study with 110 kidney recipients demonstrated that serum cystatin C accurately reflected creatinine clearance over the entire range of transplant function and was as efficacious as serum creatinine.

Complicating the value of cystatin C in renal transplant patients is our study in asthmatic patients demonstrating that corticosteroids can increase cystatin C whereas cyclosporine can decrease cystatin C values, both of which most transplant patients will receive [20].

Liver and other diseases

Cystatin C was proposed as superior marker from creatinine in patients with liver cirrhosis or after liver transplantation where determination of GFR by creatinine clearance fails because of their decreased muscle mass and increased tubular secretion of creatinine. Cystatin C was demonstrated as better marker of GFR prediction than creatinine in patients with diabetes, and rheumatoid arthritis patients. Cystatin C was reported to be an accurate marker of subtle changes in GFR of critically ill patients.

Non-renal factors altering cystatin C production

So far in most reports serum cystatin C concentrations were uninfluenced by age, gender or muscle mass. However, just recently Knight., *et al.* [7] concluded from a cross-sectional study of 8058 inhabitants in Netherlands that serum cystatin C appeared to be influenced by nonrenal factors such as age, gender, weight, height, current cigarette smoking, and C-reactive protein (CRP), which is in line

with results obtained in another large population of 1033 patients with coronary heart disease. The observed association between levels of cystatin C and that of CRP was first reported in an apparently healthy elderly population which could relate cystatin C concentrations to the inflammatory condition.

Another non-renal factor which was found to alter serum cystatin C concentrations in different diseases is corticosteroid therapy. Corticosteroid increase of cystatin C concentrations was probably due to a promoter-mediated increase in transcription of the cystatin C gene which was observed in glucocorticoid induced HeLa cells [21].

Thyroid function is another condition which has to be considered when cystatin C is used as a marker of kidney function [22]. Even mild thyroid dysfunction is associated with significant changes in cystatin C where hypothyroidism leads to lower cystatin C concentrations and hyperthyroidism leads to higher cystatin C concentrations. Several investigators have demonstrated increased cystatin C concentrations in sera, pleural effusions, and ascetic fluids collected from cancer patients. However, there are also contradictory reports claiming that malignancy had no effect on serum cystatin C concentrations. Significant correlation between increased serum cystatin C and malignant progression in melanoma and colorectal cancer in the absence of any changed creatinine values have been revealed [23]. Additional prospective studies providing actual determination of GFR by clearance of creatinine or exogenous substances such as inulin will be necessary to assess the utility of cystatin C for early detection of reduced renal function in cancer and metastatic disease patients.

Conclusion

Serum Cystatin C can be a reliable marker for detection of early nephropathy along with albumin-creatinine ratio. Currently many researchers are evaluating the role of cystatin c in other diseases like pre-eclampsia and cancer detection. We can expect this biochemical parameter to play an important role in detection of various diseases in future.

Bibliography

1. Clausen J. "Proteins in normal cerebrospinal fluid not found in serum". *Proceedings of the Society for Experimental Biology and Medicine* 107 (1961): 170-172.

2. Grubb A and Lfberg H. "Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis". *Proceedings of the National Academy of Sciences of the United States of America* 79.9 (1982): 3024-3027.

3. Abrahamson M., *et al*. "Molecular cloning and sequence analysis of cDNA coding for the precursor of the human cysteine protease inhibitor cystatin C". *FEBS Letters* 216.2 (1987): 229-233.

4. Leung-Tack J., *et al*. "Neutrophil chemotactic activity is modulated by human cystatin C, an inhibitor of cysteine proteases". *Experimental Cell Research* 188 (1990): 16.

5. Thomas L and Huber AR. "Renal function - estimation of glomerular filtration rate". *Clinical Chemistry and Laboratory Medicine* 44.11 (2006): 1295-1302.

6. Grubb A., *et al*. "Simple cystatin C-based prediction equations for glomerular filtration rate compared with Modification of Diet in Renal Disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children". *Clinical Chemistry* 51.8 (2005): 1420-1431.

7. Knight EL., *et al*. "Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement". *Kidney International* 65.4 (2004): 1416-1421.

8. Cimerman N., *et al*. "Twenty-four hour variations of cystatin C and total cysteine protease inhibitory activity in sera from healthy subjects". *Clinica Chimica Acta* 291.1 (2000): 89-95.

9. Randers E., *et al*. "Reference interval for serum cystatin C in children". *Clinical Chemistry* 45.10 (1999): 1856-1858.

10. Laterza OF., *et al*. "Cystatin C: an improved estimator of glomerular filtration rate?" *Clinical Chemistry* 48.5 (2002): 699-707.

11. Roos JF., *et al*. "Diagnostic accuracy of cystatin C compared to serum creatinine for estimation of renal dysfunction in adults and children – a meta-analysis". *Clinical Biochemistry* 40.5-6 (2007): 383-391.

12. Grubb A. "Cystatin C as estimator of glomerular filtration rate: needs for its definitive implementation". *Clinical Chemistry and Laboratory Medicine* 45 (2007): S57.

13. Pham-Huy A., *et al*. "Measuring glomerular filtration rate with cystatin C and beta-trace protein in children with spina bifida". *Journal of Urology* 169.6 (2003): 2312-2315.

14. Herrero-Morin JD., *et al*. "Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children". *Critical Care* 11.3 (2007): R59.

15. Cataldi L., *et al*. "Cystatin C in healthy women at term pregnancy and in their infant newborns: relationship between maternal and neonatal serum levels and reference values". *American Journal of Perinatology* 16.6 (1999): 287-295.

16. Strevens H., *et al*. "Serum cystatin C reflects glomerular endotheliosis in normal, hypertensive and pre-eclamptic pregnancies". *BJOG: An International Journal of Obstetrics and Gynaecology* 110.9 (2003): 825-830.

17. Sarnak MJ., *et al*. "Cystatin C concentration as a risk factor for heart failure in older adults". *Annals of Internal Medicine* 142.7 (2005): 497-505.

18. Koenig W., *et al*. "Plasma concentrations of cystatin C in patients with coronary heart disease and risk for secondary cardiovascular events: more than simply a marker of glomerular filtration rate". *Clinical Chemistry* 51.2 (2005): 321-327.

19. Risch L., *et al*. "Assessment of renal function in renal transplant patients using cystatin C. A comparison to other renal function markers and estimates". *Renal Failure* 23.3-4 (2001): 439-448.

20. Macisaac RJ., *et al*. "The accuracy of cystatin C and commonly used creatinine-based methods for detecting moderate and mild chronic kidney disease in diabetes". *Diabetic Medicine* 24.4 (2007): 443-448.

21. Bjarnadottir M., *et al*. "Promoter mediated, dexamethasone induced increase in cystatin C production by HeLa cells". *Scandinavian Journal of Clinical and Laboratory Investigation* 55.7 (1995): 617-623.

22. Jayagopal V., *et al*. "Paradoxical changes in cystatin C and serum creatinine in patients with hypo- and hyperthyroidism". *Clinical Chemistry* 49.4 (2003): 680-681.

23. Kos J., *et al*. "Cathepsins B, H, and L and their inhibitors stefin A and cystatin C in sera of melanoma patients". *Clinical Cancer Research* 3.10 (1997): 1815-1822.

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