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Age and Gender Associated Changes in Cystatin C and β2-Microglobulin

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Abstract: Assessment of renal function in clinical medicine is of great importance. Various studies report that cystatin C (cysC) and β2-microglobulin are valuable markers of renal function. In this study, serum cysC and β2-microglobulin were measured in parallel with serum creatinine in a healthy population, and the characteristics of the relationship of cysC and β2-microglobulin to age and gender were compared.

Serum creatinine, cysC and β2-microglobulin were measured in 119 (86 female; 33 male, 6 to 69 years old) healthy subjects. They were divided into five different age groups: group 1 (6-15 years, n = 10), group 2 (16-30 years, n = 34), group 3 (31-45 years, n = 34), group 4 (46-60 years, n = 29) and group 5 (>61 years, n = 12).

Serum creatinine did not differ among groups and was not correlated with age. Creatinine values were significantly different (p = 0.004) between males and females. CysC values differed neither by gender nor by age in the groups. However, cysC exhibited a positive correlation with age (r = 0.212, p = 0.021). β2-microglobulin levels showed a significant difference between groups (p = 0.036). There was a positive correlation between serum β2-microglobulin and age (r = 0.188, p = 0.041). In conclusion, serum cysC and β2-microglobulin levels in healthy individuals increase with ageing, consistent with the decrease in GFR.

Key Words: Cystatin C, β2-microglobulin, glomerular filtration rate, ageing

Introduction

The most important clinical renal function to monitor with age is the glomerular filtration rate (GFR) (1). Its decline is the single most important physiological abnormality that accurately represents the function of the ageing kidney (2). Plasma creatinine concentration is the most commonly used test to evaluate an impairment in GFR. However, serum creatinine has significant disadvantages such as inability to measure renal function impairments of 50% or less (3). Moreover, because creatinine is not an inert substance and is secreted by the proximal tubules, elevating the true GFR by up to 30%, and because of inaccurately timed urine collections creatinine clearance measurements are of limited value (4). In addition, both creatinine clearance and serum creatinine values are affected by dietary protein and muscle mass, as well as the documented analytical interferences for creatinine with the Jaffé and less affected enzymatic methods (3,5). Thus, serum creatinine can only be used as a crude indicator of a significantly impaired renal function (3). Taken together, although creatinine is a cheap and simple test for the assessment of GFR, it is hampered by many biological and technical problems.

The measurement of plasma concentration of various low molecular weight (LMW) proteins has been proposed as a useful tool to evaluate the impairment of GFR (6-8). Among these, cystatin C (cysC) (MW 13,300) and β2-microglobulin (MW 11,800) have been suggested as better markers of GFR than creatinine (9-11).

CysC is a non-glycosylated, basic protein (pI = 9.2) that is a member of the cystatin superfamily of cysteine protease inhibitors (12). It is constitutively produced by all nucleated cells and therefore exhibits a stable production rate even in the presence of an acute inflammatory response (13). β2-microglobulin is associated with the histocompatibility antigen complex on the surface of nucleated cells and is shed from the cells during cellular turnover. It is filtered by the glomeruli and reabsorbed by the proximal tubular cells where it is metabolized. Its plasma concentration increases with decreasing renal function. Its production, however, is dramatically different in patients with lymphoproliferative syndromes, infections and autoimmune diseases (14).

Since there is a decrease in GFR with ageing, this study was performed to investigate serum cysC and β2-microglobulin levels in different age groups and to reveal the relationship of these LMW proteins with age and serum creatinine.

Materials and Methods

Subjects

One hundred and nineteen (86 female; 33 male) apparently healthy subjects referred to the outpatient clinical biochemistry laboratory participated in this study. The age of the subjects ranged from 6 to 69 years (mean female age, 39.07; mean male age, 34.24; mean overall age, 37.73). They were divided into five different age groups: group 1 (6-15 years, n = 10), group 2 (16-30 years, n = 34), group 3 (31-45 years, n = 34), group 4 (46-60 years, n = 29) and group 5 (>61 years, n = 12). Informed consent was obtained from the subjects (the university ethical committee gave its approval and the reported investigations were performed in accordance with the principles of the Declaration of Helsinki).

Methods

CysC and β2-microglobulin were measured using latex particle-enhanced turbidimetry (PET) kits (Dako cystatin C PET kit code no:0071 and Dako β2-microglobulin PET kit code no:0052) on a Hitachi 704 automatic analyzer (Boehringer Mannheim GmbH, Mannheim, Germany). Creatinine was measured by the Jaffé method (15), performed on an automatic analyzer (Dax 48, Bayer Diagnostics, Toshiba, Japan).

Statistics

Statistical significance among groups was determined by the Kruskal-Wallis 1-Way ANOVA. Degrees of correlation were analyzed using Pearson correlation analysis. For the analysis of difference between two groups, the Mann-Whitney U non-parametric test was used and $p < 0.005$ was considered significant; $p < 0.05$ was considered significant for the other results.

Results

Serum triglyceride, urea, and transaminase levels were within the reference ranges in each group (data not shown).

Table shows the serum creatinine, β2-microglobulin and cysC levels of the five groups. While creatinine showed significant differences ($p = 0.004$) between male (1.22 ± 0.20) and female (1.09 ± 0.27) values, cysC (male: 1.21 ± 0.19, female: 1.17 ± 0.20) and β2-microglobulin (male: 1.93 ± 0.38, female: 2.10 ± 0.91) did not differ ($p = 0.374$ and $p = 0.178$, respectively).

Table. Serum creatinine, β2-microglobulin and cystatin C levels in different age groups (values are expressed as mean ± SD).

Age groups (years)	N	Creatinine (mg/dL)	β2-microglobulin (mg/L)	Cystatin C (mg/L)
1 (6-15)	Total: 10	1.20 ± 0.36	1.78 ± 0.33[1]	1.14 ± 0.08
	Female: 6	1.32 ± 0.43	1.90 ± 0.37	1.15 ± 0.10
	Male: 4	1.02 ± 0.07	1.62 ± 0.19	1.12 ± 0.06
2 (16-30)	Total: 34	1.13 ± 0.23	1.85 ± 0.40[2]	1.12 ± 0.17
	Female: 20	1.05 ± 0.18	1.78 ± 0.40	1.08 ± 0.19
	Male: 14	1.24 ± 0.26	1.94 ± 0.41	1.17 ± 0.14
3 (31-45)	Total: 34	1.10 ± 0.26	2.15 ± 1.20	1.20 ± 0.21
	Female: 28	1.05 ± 0.26	2.17 ± 1.32	1.18 ± 0.21
	Male: 6	1.32 ± 0.13	2.07 ± 0.29	1.29 ± 0.22
4 (46-60)	Total: 29	1.12 ± 0.31	2.12 ± 0.43[1,2]	1.25 ± 0.19
	Female: 23	1.09 ± 0.35	2.12 ± 0.44	1.23 ± 0.19
	Male: 6	1.24 ± 0.09	2.11 ± 0.42	1.31 ± 0.16
5 (>61)	Total: 12	1.13 ± 0.14	2.39 ± 1.07	1.20 ± 0.24
	Female: 9	1.11 ± 0.12	2.63 ± 1.15	1.22 ± 0.18
	Male: 3	1.20 ± 0.19	1.68 ± 0.21	1.11 ± 0.40

[1] Group 4 > Group 1 (p=0.024), [2] Group 4 > Group 1 (p=0.0037)

With creatinine, there were no significant differences between the groups. When split by gender, the female values were not significantly different across all the age groups, but the male values showed a small difference ($p = 0.0442$). However, the differences between groups 1 and 3, and groups 1 and 4 were not significant ($p = 0.0105$ and $p = 0.0103$, respectively). Serum creatinine levels were not correlated with age.

With cysC, there were no significant differences between the groups ($p = 0.0539$). However, group 4 represented significantly higher cysC concentrations compared with group 2 ($p = 0.0042$). Within each group there was no significant difference between the sexes. CysC exhibited a positive correlation with age in the whole group ($r = 0.212$, $p = 0.021$) and in females ($r = 0.258$, $p = 0.016$). There was no significant correlation between cysC and age in males.

With β2-microglobulin, the difference in levels between the age groups was significant ($p = 0.036$). Serum β2-microglobulin levels were higher in group 4 than in group 2 ($p = 0.0037$). Group 1 showed lower β2-microglobulin values than did group 4 ($p = 0.0243$). β2-microglobulin levels did not differ between males and females within groups. There was a weak positive correlation between serum β2-microglobulin and age in the whole group ($r = 0.188$, $p = 0.041$). When split by gender there were no correlations between age and β2-microglobulin levels in either sexs.

Discussion

There is a gradual decrease in kidney functions with advancing age. The number of glomeruli decrease, together with a fall in the mass of juxtamedullary nephrons. The result is a decrease in the filtration area of the glomerular basement membrane, decreased permeability and therefore reduced GFR. Early studies showed that kidney size decreases 20-30% after the fourth decade and kidney volume by 40% by the eighth decade (16). Furthermore, renally excreted medications need dose adjustment as GFR changes. The clinical methods utilized to measure renal function ideally should be accurate, reproducible, simple, safe, inexpensive and free of cumbersome features. Standard clearance techniques require timed urine collections, which are not only time consuming but subject to erroneous and inaccurate collection. While single-shot clearance techniques may be more accurate and less cumbersome, the injection of agents is not practical if repeated measurements are required and involve radiation exposure (17). In addition, predicting GFR from serum creatinine by the Cockcroft-Gault equation (18) was found to be inaccurate (19). For these reasons, many attempts to find better markers have been undertaken.

The use of endogenous markers is thus of great advantage due to their speed and simplicity. In clinical practice, serum creatinine and creatinine clearance are widely used as indirect markers of GFR. It was demonstrated that cysC and β2-microglobulin had diagnostic accuracies very similar to that of creatinine as markers of GFR (6,20).

In this study, serum cysC and β2-microglobulin were measured in parallel with serum creatinine, as the most commonly used test to evaluate an impairment of GFR, in a clinically healthy population, and the characteristics of the relationship of cysC and β2-microglobulin to age and gender were compared.

We have shown that there is no correlation between creatinine and age. The decreasing GFR with ageing is not apparently mirrored by increasing creatinine. While serum creatinine would increase with decreasing GFR, it remains stable due to the concomitant decrease in muscle mass that also occurs with age. Thus, with serum creatinine remaining in the middle age reference range, any decline in renal function may not be recognized until it is well established. As expected, serum concentrations of creatinine differed between sexes due to muscle mass. Cameron and Greger believe that for ordinary clinical practice, the plasma creatinine concentration measured by a rate-dependent method is adequate for assessing glomerular function when it is over 200 mmol/L; below that level, an isotopic method should be used (21). Furthermore, there is evidence to suggest that a small loss in glomeruli with age can be compensated for by physiological changes in the remaining healthy nephrons. Thus, since serum creatinine allows only crude estimation of renal function, as it is hampered by muscle mass and nutrition; it may not show slight differences in GFR that may occur with ageing.

Our data support other investigators who observed a slight increase in serum cysC values with increasing age (22-24). This reflects what is known about decreasing GFR and function of the kidneys with advancing age. In this study no sex difference between cysC levels could be

found, in agreement with previous studies (23,24). We have shown that there are no sex differences between different age groups, but values do significantly differ with age, especially between groups 2 and 4.

To be of use for monitoring renal function, any parameter is expected to have a low intraindividual variation. It has been reported that cys-C possesses a higher intraindividual variance but a lower interindividual variance than serum creatinine, allowing earlier detection of impaired renal function (25). Although cysC may be potentially better for detecting the onset of an abnormal GFR, it is said to be not as sensitive as serum creatinine for detecting changes within the same individual (25). However, creatinine levels do not change until significant impairment of the renal function occurs.

Serum β2-microglobulin is also said to be a good endogenous marker of GFR, better than serum creatinine (11). With declining renal function, a larger and earlier increase in serum β2-microglobulin than serum creatinine has been demonstrated (11,26). Serum β2-microglobulin concentrations were significantly different between the age groups. Values were significantly high in the 46-60 age group than in the 16-30 year old individuals. There was a positive correlation with age. These findings again reflect decreasing GFR and function of the kidneys with advancing age.

In conclusion, serum cysC and β2-microglobulin levels in healthy individuals increase with ageing, consistent with the decrease in GFR. However, these findings have to be reaffirmed further by studies comparing a gold standard method with the serum measurements of these parameters.

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References

1. Lindeman RD. Assessment of renal function in the old. Clin Lab Med 13:269-277, 1993.
2. Meyer BR, Bellucci A. Renal function in the elderly. Cardiol Clin 4:227-234, 1986.
3. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 38:1933-1953, 1992.
4. Payne RB. Creatinine clearance: a redundant clinical investigation. Ann Clin Biochem 23:243-250, 1986.
5. Spencer K. Analytical reviews of clinical biochemistry: the estimation of creatinine. Ann Clin Biochem 23:1-25, 1986.
6. Grubb A, Simonsen O, Storfelt G, Truedsson L, Thysell H. Serum concentration of cystatin C, factor D and beta 2-microglobulin as a measure of glomerular filtration rate. Acta Med Scand 218:499-503, 1985.
7. Schardijn GHC, Statius van Eps WE. Beta 2-microglobulin: its significance in the evaluation of renal function. Kidney Int 32:635-641, 1987.
8. Tramonti G, Donadio C, Ferdeghini M, Annichiarico C, Norpoth M, Bianchi R, Bianchi C. Serum tumour-associated trypsin inhibitor (TATI) and renal function. Scand J Clin Lab Invest 56:653-656, 1996.
9. Kyhse-Andersen J, Schmidt C, Nordin C, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 40:1921-1926, 1994.
10. Le Bricon T, Thervet E, Benlakehal M, Bousquet B, Legendre C, Erlich D.Le Bricon T, Thervet E. Changes in plasma cystatin C after renal transplantation and acute rejection in adults. Clin Chem 45:2243-2249, 1999.
11. Bianchi C, Donadio C, Tramonti G, Consani C, Lorusso P, Rossi G. Reappraisal of serum beta2-microglobulin as marker of GFR. Ren Fail 23:419-29, 2001.
12. Henskens YMC, Veerman ECI, Nieuw Amerongen AV. Cystatins in health and disease. Biol Chem Hoppe-Seyler 377:71-86, 1996.
13. Abrahamson K, Olafsson I, Palsdottir A, Ulvsback M, Lundwall A, Hensson O, Grubb A. Structure and expression of the human cystatin C gene. Biochem J 268:287-94, 1990.
14. Henne V, Frei P, Bürgisser P. Beta2-microglobulin– a rapid and automated determination for a broad range of clinical applications. Anticancer Res 17:2915-2918, 1997.
15. Larsen K. Creatinine assay by a reaction-kinetic approach. Clin Chim Acta 41:209-217, 1972.
16. Dunnill MS, Halley W. Some observations on the quantitative anatomy of the kidney. J Pathol 110:113-121, 1973.
17. Swan SK. The search continues– an ideal marker of GFR. Clin Chem 1997; 43:913-4.
18. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 16:31-41, 1976.

19. Baracskay D, Jarjoura D, Cugino A, Blend D, Rutecki GW, Whittier FC. Geriatric renal function: estimating glomerular filtration in an ambulatory elderly population. Clin Nephrol 47:222-228, 1997.

20. Donadio C, Lucchesi A, Ardini L, Ardini M, Giordani R. Cystatin C, β2-microglobulin, and retinol-binding protein as indicators of glomerular filtration rate: comparison with plasma creatinine. J Pharm Biomed Anal 24:835-842, 2001.

21. Cameron CS, Greger R. Renal function and testing of function. In: Davidson AM, Cameron JS, Grünfeld J-P, et al. Oxford Textbook of Clinical Nephrology. Oxford: Oxford University Press, 1998; 1.3:39-69.

22. Finney H, Bates CJ, Price CP. Plasma cystatin C determinations in a healthy elderly population. Arch Gerontol Geriatr 29:75-94, 1999.

23. Norlund L, Fex G, Lanke J, von Schenke H, Nilsson JE, Leksell H, Grubb A. Reference intervals for the glomerular filtration rate and cell-proliferation markers: serum cystatin C and serum β2-microglobulin/cystatin C ratio. Scand J Clin Lab Invest 57:463-470, 1997.

24. Erlandsehn EJ, Randers E, Kristensen JH. Reference intervals for serum cystatin C and serum creatinine in adults. Clin Chem Lab Med 36:393-397, 1998.

25. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. Clin Chem 44:1535-1539, 1998.

26. Nolte S, Mueller B, Pringsheim W. Serum a1-microglobulin and beta2-microglobulin for the estimation of fetal glomerular renal function. Pediatr Nephrol 5:573-7, 1991.