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Cystatin-C levels in healthy children and adolescents: Influence of age, gender, body mass index and blood pressure

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ABSTRACT

Objectives: Cystatin-C is considered a more sensitive and specific marker of kidney function than creatinine since it can diagnose patients with earlier-stage of renal dysfunction. The aim of this study is to determine the levels of Cystatin-C in healthy children and adolescents as well as any correlations to age, gender, body-mass index (BMI) and blood pressure (BP).

Design and methods: Cystatin-C was measured in 536 healthy Greek children and adolescents (295 males and 241 females) using a nephelometric immunoassay. Additionally, the age, body mass index and blood pressure was recorded for each subject.

Results: Overall, the mean serum Cystatin-C level was 0.79 ± 0.10 mg/L. Cystatin-C was found to be statistically significantly lower in females than in males ($p < 0.001$) as well as in prepubertal children compared to adolescents ($p < 0.001$). Higher values of Cystatin-C were observed in subjects with increased BMI ($p < 0.001$). Neither systolic nor diastolic blood pressure was found to significantly affect Cystatin-C levels.

Conclusions: The levels of Cystatin-C were statistically significantly higher in males, compared to age-matched females and also positively correlated with age and BMI.

1. Introduction

Cystatin-C is a non-glycosylated, low molecular weight (13.250 Da) basic protein and a member of the super family of cysteine protease inhibitors [1–3]. It is made up of 120 amino acids and is produced at a constant rate by all cells that have a nucleus. It is excreted by the kidneys using two distinct processes, the "free glomerular filtration" and the "complete tubular reabsorption and degradation" [4–6]. In healthy individuals Cystatin C is completely reabsorbed and degraded in the tubules.

It has become apparent in recent years that its usefulness as a marker in certain biological functions such as in glomerular filtration rate and in renal function, is very important. Cystatin-C proves to be a very useful marker, helping in the detection of early kidney damage, overcoming some of Creatinine's diagnostic limitations, especially when age and body size are considered [7,8]. It has also been found to be a lot more sensitive and specific as a marker of renal function in both children and adults, when compared to Creatinine [9–12]. For the above reasons it is very important to further study the physiology of Cystatin-C, especially in relation to demographic and anthropometric characteristics in young populations, in order to establish a useful baseline.

The objective of this study was to determine the levels of Cystatin-C, in healthy children and adolescents (4–17 years old) and to evaluate any correlations that may exist with age, gender, body-mass index (BMI) and blood pressure.

2. Materials and methods

2.1. Subjects

536 healthy individuals (311 prepubertals and 225 adolescents) participated, 295 males and 241 females with an age range of 4–17 years and a mean age (MA) of 10.8 ± 3.4 years (Table 1). All participants were recruited from the 2nd Department of Pediatrics, Medical School, University of Athens, Children's Hospital "P & A Kyriakou', Athens, Greece. Consent forms, approved by the Ethics Committee of the Children's Hospital "P & A Kyriakou", were signed by all parents whose children participated in the study.

Exclusion criteria taken under consideration in the recruitment of the study group consisted of chronic diseases, especially diabetes,

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Table 1
Anthropometric characteristics of study population.

Variable	Category	Descriptive
Gender	Girls	55.0%
	Boys	45.0%
Age (years)		10.8 (SD 3.4)
Puberty	Yes	57.8%
	No	42.2%
BMI (kg/m^2)	Normal	68.2%
	Increased	31.8%
SBP (mm Hg)	Normal	79.6%
	Increased	20.4
DBP (mm Hg)	Normal	94.1%
	Increased	5.9

*BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure.

cancer, thyroid dysfunctions and factors that might influence the levels of Cystatin-C such as the use of anti-inflammatory agents, diuretics, anticonvulsants and antibiotics.

2.2. Cystatin-C

Levels of Cystatin-C were measured in fasting serum, using an automated particle-enhanced nephelometric immunoassay (PENIA) on a Siemens Behring Nephelometer BN II system. The system was programmed and the measurements were carried out according to the manufacturer's instructions. All samples were measured in duplicates and by the same batch of reagents. The detection limit of the assay was 0.05 mg/L, with 2.6% CV (within assay precision) and 2.9% CV (between assay precision), according to the operations manual (N Latex Cystatin C-Siemens). The reference interval was determined to be 0.006 mg/L. The specificity and the sensitivity of N Latex Cystatin-C were 82% and 94% respectively, compared to 88% and 81% for Creatinine.

2.3. Anthropometric and blood pressure measurements

Anthropometric measurements were recorded using a standard portable scale. Body weight (Kg) and height (cm) were recorded with subjects barefoot and wearing light clothing in the morning. The Body mass index (BMI) was calculated as weight in kilograms/height square meters (kg/m^2). Participants were defined as being overweight or obese based on age- and sex-specific cutoff points of BMI on the percentile curves of an international reference population [13]. In addition, three blood pressure (BP) measurements were taken from each subject after a 5 min rest interval while seated and with a one min interval between measurements using a standard sphygmomanometer. Appropriate cuff size was implemented according to the individual's arm circumference. The average of all three BP measurements of each individual was used in the analysis (Table 1).

2.4. Serum blood samples

Three ml of blood was drawn from each subject and left to clot for 30 min. It was then centrifuged at 1600 g for 10 min. The supernatant serum was collected in aliquots of two in 1.5 ml polypropylene eppendorf tubes and was frozen immediately in a −75 °C deep freezer, until the day of the analysis.

2.5. Statistical analyses

Mean values and standard deviations were used to describe continuous variables while relative and absolute frequencies were used for categorical ones.

In order to investigate the effect of several risk factors on the levels of Cystatin-C, a linear regression model was fitted. Assumptions of best fit were first checked. Potential risk factors that were introduced into the model were sex, age (in a variable category that indicates the existence of puberty), body mass index (in normal and overweight/obese categories) as well as the presence of increased systolic and diastolic pressure.

Data were analyzed with the statistical program SPSS 20 for Windows OS and a value of $p < 0.05$ was considered as statistically significant.

3. Results

Distribution of Cystatin-C levels was normal (Fig. 1). Cystatin-C mean serum levels in all children were 0.79 ± 0.10 mg/L (Median: 0.78 mg/L, 25th percentile: 0.72 mg/L and 75th percentile: 0.85 mg/L, min. 0.55 mg/L, max. 1.37 mg/L) (Table 2).

Gender and puberty seem to affect significantly the levels of Cystatin-C when these are adjusted by other potential confounders. Cystatin-C was found to be statistically significantly lower in females than in males (0.76 ± 0.09 vs 0.80 ± 0.11, $p < 0.001$) and also in prepubertal children(4–12 years old) compared to adolescents (12–17 years old) (0.76 ± 0.09 vs 0.82 ± 0.11, $p < 0.001$) (Table 2).

Higher values of Cystatin-C levels were observed in children with increased BMI (0.82 ± 0.08 vs 0.78 ± 0.10, $p < 0.001$). As shown in the results, an overweight/obese child has an average of 0.03 more Cystatin-C. However, there were no significant differences in the levels of Cystatin-C when the diastolic (DBP) and the systolic blood pressure (SBP) were taken into consideration ($p = 0.171$ and $p = 0.385$ respectively) (Table 3).

4. Discussion

In the current study, the mean and median Cystatin-C levels in healthy Greek children and adolescents are 0.79 mg/L and 0.78 mg/L respectively. Moreover, Cystatin-C level is associated with gender, puberty status and excess weight.

Linear regression analysis shows that Cystatin-C level is statistically significantly lower in females than in males. This result agrees with several studies in adults, where gender levels are compared and nephelometric assays are used, showing that males can have up to 8.1% higher Cystatin-C levels than females [14]. Pergande et al., also showed that males can have up to 17% higher Cystatin-C levels than age-matched females [15]. Similarly, in the study by Galteu et al., males had up to 12% higher Cystatin-C levels than females [16]. A sex difference has also been reported in adolescents with type 1 diabetes, but not in controls

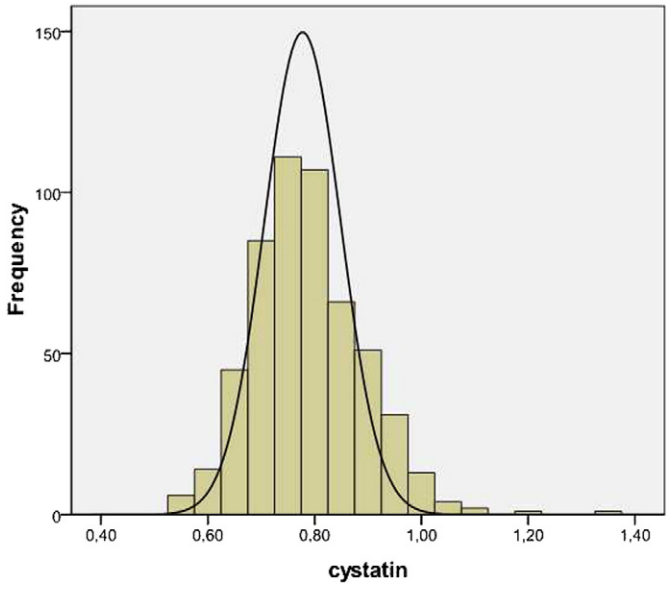

Fig. 1. Distribution of Cystatin-C levels in Children (4–17 yrs) in mg/L.