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Chitotriosidase Activity in Plasma and Mononuclear and Polymorphonuclear Leukocyte Populations

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In the general population, about 5% of individuals are homozygotic and 35% are heterozygotic carriers for chitotriosidase (ChT) deficiency. Activated macrophages are considered to be the main source of plasma ChT activity, which permits the biochemical characterization of homozygote deficients. However, in the case of detecting heterozygotic carriers, the results are often inconclusive. The activities of ChT in plasma and mononuclear (MN) and polymorphonuclear (PMN) leukocytes were determined in 169 control subjects (72 males and 97 females) with a mean age ($\pm$ SD) of 47.5$\pm$9.7 years (range 18–96 years). The specific enzyme activity was in PMN leukocytes > MN leukocytes > plasma, with a highly significant partial correlation being found between the activities of ChT in plasma and PMN leukocytes ($r = 0.578$, $P < 0.001$). A significant correlation was found between the age of the patients studied and plasma ChT activity ($r = 0.568$, $P < 0.001$). No significant correlation was found for enzyme activities in MN ($r = 0.105$) or in PMN leukocytes ($r = 0.043$). The results obtained suggest that, in normal physiological conditions, PMN leukocytes may secrete ChT to the plasma. Although the activities of ChT in MN and PMN leukocytes are not affected by demographic factors, it is not possible to use them for the biochemical detection of ChT-deficient heterozygotic carriers. J. Clin. Lab. Anal. 17:271–275, 2003.

Key words: chitotriosidase; mononuclear leukocytes; polymorphonuclear leukocytes; plasma; chitotriosidase deficiency

INTRODUCTION

Chitotriosidase (ChT), a member of the chitinase protein family, is mainly secreted by human macrophages to the extracellular medium as a 50 kDa active enzyme (1). Some of this enzyme, however, is proteolytically processed into a 39 kDa form that accumulates in the lysosomes (1). ChT was the first chitinase to be characterized in mammals, although a novel acidic mammalian chitinase has been recently identified (2). The macrophages are capable of producing large amounts of ChT under specific physiopathological conditions, such as Gaucher's disease (3–5), in which much higher increases of serum ChT are produced than for other macrophage activation markers (6). However, in other inherited lysosomal storage disorders, slight to moderate elevations of ChT activity were observed (4). Likewise, increases of plasma ChT have been described in patients affected by β-thalassemia (7,8), in children with acute plasmodium falciparum malaria (9), and in neonates with systemic candidiasis (10).

A recessive inherited deficiency for ChT activity, due to a 24-base pair duplication in the ChT gene (11), is found in about 5% of the population (3–6,12). This 24-bp duplication for ChT deficiency occurs panethnically and has a heterozygotic carrier frequency of about 35% (5,11,12). The deficient homozygotes may be easily identified by determining serum/plasma ChT activity (3–6,12). However, in the case of heterozygotic carriers for ChT deficiency, despite showing, on average, half of the serum enzyme activity for individuals with the wild-type gene (12), its biochemical identification through serum ChT activity is not possible in many individual cases.

Activated macrophages are considered to be the main or, indeed, the only source of serum/plasma ChT activity (1,3,13,14) that is widely influenced by the ChT genotype expression (12), although other factors such as age (4,5) and body mass (5) may introduce an additional source of interindividual variability.

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In human leukocyte lysates, chitinase activity is much greater than in serum (15), and Boussac and Garin (16) recently demonstrated that the neutrophils may express ChT. ChT in leukocytes may, perhaps, be less affected by demographic, anthropometric, and other factors than its enzymatic activity in serum.

This paper presents the results obtained for ChT activity in plasma and in populations of mononuclear (MN) and polymorphonuclear (PMN) leukocyte populations in healthy individuals.

MATERIALS AND METHODS

ChT activities were determined in plasma and MN and PMN leukocytes in 169 control subjects (72 male, 97 female), with a mean age ($\pm$SD) of 47.5$\pm$9.7 years (range 18–96 years). Cases with infections or systemic inflammatory diseases were excluded.

The blood samples were collected in Vacutainer tubes containing EDTA-K3 as anticoagulant. ChT activity in plasma and leukocyte lysates was determined in duplicate using the fluorogenic substrate 4-methyl-umbelliferyl-β-D-N,N′,N″-triacetyl chitotriose (Sigma-Aldrich Co., Madrid, Spain) as described by Hollack et al. (3). The separation of populations of MN and PMN leukocytes, as well as the preparation of cellular lysates, has been described in a previous publication (17).

Statistical analysis was carried out with the SPSS package for Windows (release 6.1). Skewness and kurtosis were used to check the distribution of the data. Parametric tests were used when the data had a Gaussian distribution (Student's *t*-test and Pearson's correlation coefficient); otherwise, nonparametric tests were used (Mann-Whitney U test and Spearman's correlation coefficient). Statistical significance was accepted at $P<0.05$.

RESULTS

Table 1 shows ChT activity in plasma and MN and PMN leukocytes in the group of individuals studied. The specific enzyme activity in PMN leukocytes is significantly higher than for MN leukocytes ($P<0.001$), with the specific activity in plasma being much lower (mean 0.69$\pm$0.43 nmol/h/mg protein, median 0.62 nmol/h/mg protein). No statistically significant differences were found with regard to sex. The interindividual variability for the ChT activity is slightly smaller in PMN leukocytes (CV = 59%) than in plasma (CV = 73%). This variability is greatest in the MN leukocytes (CV = 148%).

In examining plasma ChT activity, seven ChT deficient subjects were detected, representing 4.2% of the population. In the case of individuals less than 50 years of age (n = 93), five ChT deficient subjects were found (5.4%). In the case of individuals over the age of 50 years (n = 76), only two ChT deficient subjects were found (2.6%). However, no statistically significant difference was found between both frequencies (Fisher exact probability test). The results obtained in ChT deficient subjects for enzymatic activities in plasma and PMN leukocytes were highly concordant, although in MN leukocytes the enzymatic activity displayed less discriminatory power.

Figure 1 shows the relationship that exists between ChT activities in plasma and MN and PMN leukocytes, with statistically significant correlations being obtained in all cases. In the partial correlation of ChT activities in plasma and PMN leukocytes, keeping activity in MN leukocytes constant, statistical significance was maintained ($r = 0.578$, $P<0.001$). However, in the partial correlation between enzymatic activities in plasma and in MN leukocytes, keeping activity in PMN leukocytes constant, statistical significance was not attained ($r = 0.168$). No partial statistically significant correlation was obtained between ChT activities in MN and PMN leukocytes ($r = -0.044$). Similarly, the results shown in Fig. 1 reveal that ChT activities in MN and PMN leukocytes do not make it possible to discriminate heterozygotes for ChT deficiency among the general population.

Figure 2A indicates the correlation between plasma ChT activity and age, revealing a high level of statistical significance. By expressing plasma ChT activity in relation to the concentration of creatinine, statistical significance was maintained in its correlation with age (Fig. 2B). A poor correlation coefficient, although statistically significant ($r = 0.160$, $P<0.05$), was obtained between plasma ChT activity and the creatinine concentration. No statistical significance was obtained

TABLE 1. ChT activity in plasma and MN and PMN leukocyte lysates

	ChT wild-type			ChT deficiency		
	n	Mean$\pm$SD (median)	Range	n	Mean$\pm$SD (median)	Range
Plasma ChT (nmol/h/mL)	162	70.2$\pm$51.2 (56.6)	8.6–244.3	7	2.0$\pm$1.1 (1.7)	0.61–3.6
PMN ChT (nmol/h/mg protein)	95	495.2$\pm$293.2 (461.1)	31.2–1224.1	5	0.76$\pm$0.74 (0.33)	0.13–1.67
MN ChT (nmol/h/mg protein)	95	22.5$\pm$33.4 (9.2)	0.052–156.0	5	1.19$\pm$1.76 (0.038)	0.0–3.96

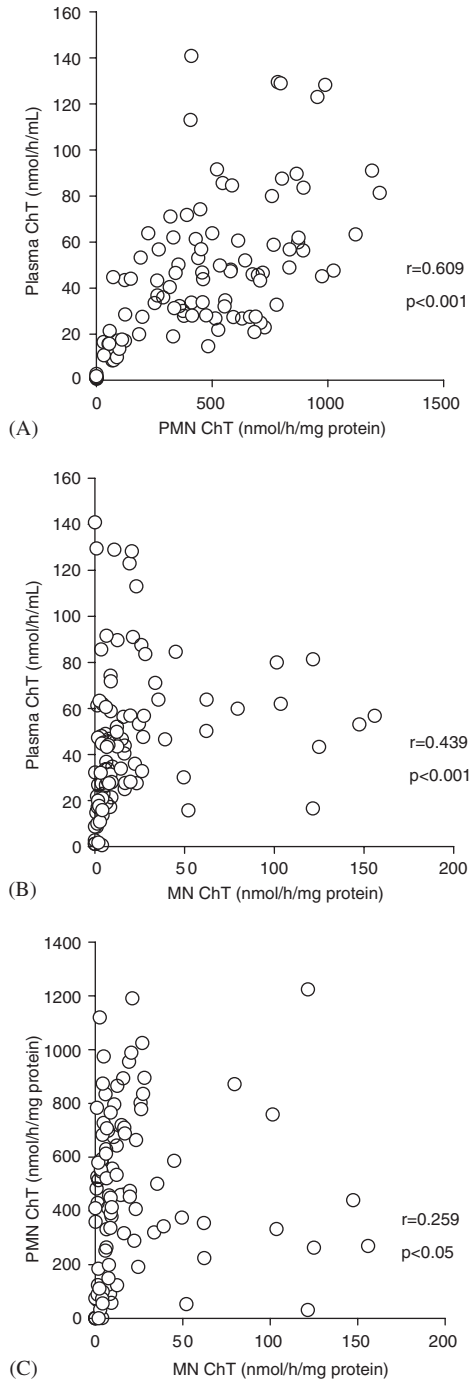

Fig. 1. Relationship between ChT in plasma and MN and PMN leukocytes.

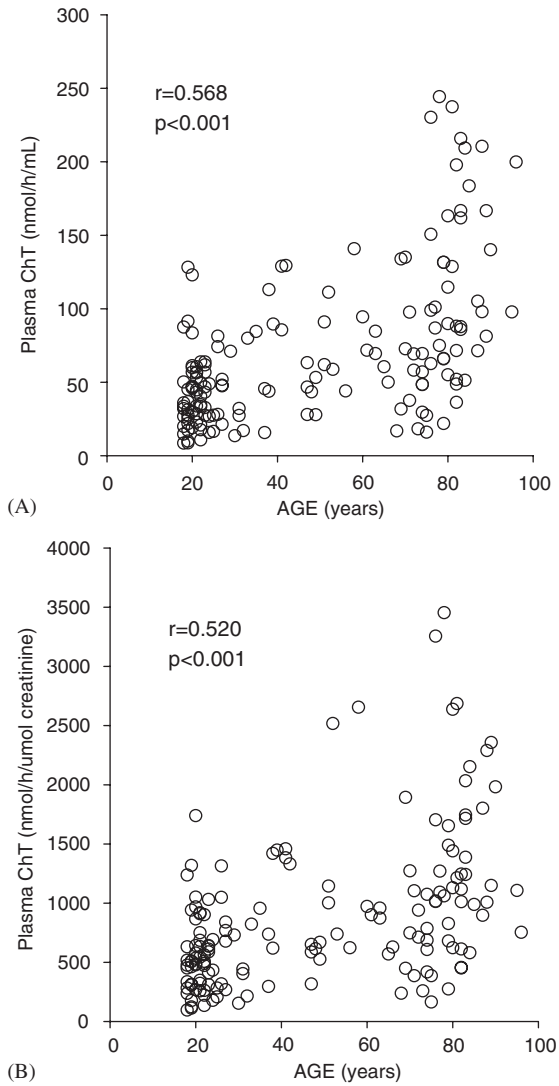

Fig. 2. Relationship between plasma ChT activity and age (ChT deficient subjects were excluded).

in the correlation between the activity of ChT in MN ($r = 0.105$) or PMN leukocytes ($r = 0.043$) with age.

DISCUSSION

The results obtained for ChT in plasma and in MN and PMN leukocyte lysates (Table 1) are in agreement with those obtained by Escott and Adams (15) for chitinase activity, with specific activities in PMN leukocytes > MN leukocytes > plasma. The values obtained for ChT deficients for the activity of ChT in plasma and in PMN leukocytes were highly concordant in all cases. In MN leukocytes, however, the enzymatic activity in two deficients was similar to the low activity levels found in non-ChT deficients. Guo et al. (4) have also shown that fibroblasts of ChT deficients had normal ChT activity. The possible effect of the lysozyme, which has some catalytic activity on the substrate 4-methylumbelliferyl-β-D-N, N′, N″-triacetyl chitotriose (3) or another chitinase, may be considered.

At present, the exact physiological function of ChT is not fully understood, although a role in host defense against chitin-containing cell wall pathogens has been

suggested (2,11,15,16). The high levels of ChT in PMN leukocytes may reflect the fact that the enzyme makes a contribution to the destruction of pathogens. However, the high prevalence of enzyme deficiency suggests that ChT is not an important defense function, or that other mechanisms may compensate for the lack of ChT (2). Additional prospective studies are needed to verify the clinical importance of ChT deficiency in humans. The frequency of ChT deficiency in the whole group of individuals studied (4.2%) is similar to that reported by other authors (3–6,12). Due to the size of the population sample, no statistically significant difference was found between the frequency of ChT deficients in the 93 individuals under 50 years of age (5.4%) and in the 76 individuals over 50 years of age (2.6%). Further studies into this issue (previously observed by Guo et al. [4]), would be of interest. The nature of the ChT deficiency is a 24-bp duplication that activates a cryptic 3′ splice site in the same exon (11), and a similar mutation affecting splice site selection has been reported for the for the β subunit of β-hexosaminidase (18). In the ChT deficient individuals studied, the activities of plasma and leukocyte β-hexosaminidase and its isoenzyme profile were comprised in the reference ranges (data not shown).

The ChT may be synthesized by the neutrophils and stored in granules until degranulation. Boussac and Garin recently revealed that the activated neutrophils may secrete ChT with a high molecular mass heterogeneity to the extracellular medium (16). In our study, we found a highly significant correlation between the activity of ChT in plasma and in PMN leukocytes, with statistical significance being maintained in the partial correlation between these two biochemical variables. These results suggest that plasma ChT in healthy individuals may be, at least partially, the result of the enzyme being secreted by circulating PMN leukocytes.

The isoform of the ChT present in plasma has a molecular mass of 50kDa (1) or even less (16), and, consequently, would be partially filtered at the glomerular level (19). The significant correlation between the plasma activity of ChT and age (Fig. 2A) has already been indicated by other authors (4,5), with statistical significance being maintained on expressing enzyme activity in relation to the concentration of serum creatinine (Fig. 2B). The level of creatinine in serum increases with age, muscular mass, size, and body mass (20). As the plasma ChT/creatinine relationship presented a similar correlation with age as ChT activity expressed by volume, this may suggest that the previously described significant correlation between plasma ChT and body mass (5) could, in fact, be due to a misleading effect of age. It is worth indicating that, in line with previous results (4), the plasma activity of ChT did not show any significant differences according to sex.

ChT activity in MN and PMN leukocytes did not present any significant correlation with age in the patients studied, confirming the proposed hypothesis regarding its possible independence from demographic factors. However, the determination of ChT activity in leukocyte lysates does not permit the biochemical identification of heterozygotic carriers for the ChT deficiency, contrary to the situation described for other lysosomal enzymes.

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