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Clinical Research

EVALUATION OF SERUM CANAVANINE AS SUPPORT IN THE DIAGNOSIS OF CHRONIC KIDNEY DISEASE

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ABSTRACT

Objective. The purpose of the present study was to determine the relationship between the levels of serum canavanine, cystatin C and creatinine in patients with normal renal function and in patients with chronic kidney disease (CKD) and to observe if there is any difference in values obtained.

Material and methods. Were evaluated 100 blood samples from patients without renal disease and 100 samples from patients with CKD to determine the levels of L-canavanine with the Rosenthal technic, creatinine with an autoanalyzer Dimension RxL and cystatin C by a method of immunonephelometry in a BNProspec both of Siemens.

Results. The values found in patients without renal disease were, canavanine from 3 to 8.5 mg/dl, creatinine 0.2 to 1.2 mg/dl and cystatin C 0.6 to 0.95 mg/L and in patients with CKD the values were canavanine from 8.5 to 25 mg/dl. cystatin C 0.98 to 9.5 mg/L and creatinine 2.0 to 18.5 mg/dl. The reference values for canavanine were obtained with the values of cystatin C which is a standardized test and more specific than creatinine to evaluate renal function. Patients with CKD were considered to have serum creatinine higher than 2.0 mg/dl. The sensitivity obtained for canavanine is 95% with specificity of 93 %.

Conclusions. It is concluded that the evaluation of serum canavanine can be useful as a support to the diagnosis of CKD.

KEYWORDS : L-canavanine, Chronic Kidney Disease, creatinine, cystatin C, diagnosis

INTRODUCTION

Renal diseases have always been a problem because they are more frequent every day and their treatment is difficult until reaching the dialysis application, which implies a heavy financial cost for health institutions. Usually when the diagnosis is done, the disease is in advanced state because the symptoms are diverse and non specific; for this reason new laboratory methodologies have been sought to make a timely diagnosis of the disease and apply an adequate treatment to prevent the progression of the disease.

Leguminous plant contain several compounds that act as antinutritional factors, among these are L-canavanine which is an amino acid that is free in the seeds of some leguminous. L-canavanine is an analogous of arginine which exhibits antimetabolic properties in lowers animals up to higher animals; its toxic properties are due to its structure similar to arginine, interfering with its metabolism; inhibits the synthesis of proteins, interfering the synthesis of RNA and DNA and alters the synthesis of immunoglobulins. The association of disease with the ingesting alfalfa was observed for the first time in a volunteer who developed lupus erythematosus while ingesting alfalfa seeds for a hyperchole sterolemia study; this was later confirmed in monkeys fed alfalfa stems that developed lupus erythematosus and hemolytic anemia. By suppressing the intake of alfalfa the disease disappeared. The amino acid L- canavanine present in alfalfa was suspected to produce the disease (1). Some authors observed that approximately 60% of the known leguminous contain canavanine (2,3). In rats fed with L-canavanine they presented disruptions of the immune response, the total development was affected as well as the growth of some organs and in addition autoimmune complexes were deposited in the glomerular basement membrane (4). It has also been observed that the consumption of alfalfa in man can induce renal failure, so as a young athlete died of kidney failure after ingesting a bottle of alfalfa tablets for a week (5).

When administering canavanine in rats disturbances of the immune system take place; it has also been observed that immune complexes are deposited in the glomerular basement membrane. Canavanine is an analogous of arginine, therefore it is incorporated in the nascent polypeptide chain occupying the place of arginine. In leukocytes of patients with CKD, high levels of canavanine has been found in comparison with the levels of healthy subjects (6). Currently, to evaluate renal function it is done with the determination of creatinine in blood and urine. However when blood creatinine levels are increased, the kidney is already irreversibly damaged. The clearance of urea or creatinine may overestimate or underestimate the glomerular filtration rate. These methods require the collection of 24 hour urine which occasionally presents some drawbacks in its collection, especially in pediatric patients, for this reason, new laboratory methodologies have been sought.

A compound used in place of creatinine is Cystatin C which is a small protein that is produced consistently by most nucleated cells, it is eliminated only by glomerular filtration and is catabolized exclusively by the cells of the renal proximal tubule; therefore it is considered as a specific test and in addition, its levels are increased in advance to creatinine levels (7).

In the urine of uremic patients a low molecular weight substance was isolated, which is toxic to cells grown in culture. This toxic substance appears to be the guanidinosuccinic acid which when injected into laboratory animals, it causes twitching, hemolysis of red blood cells and gastroenteritis. It is clear that this compound it accumulates due at block the functioning of the urea cycle .This acid comes from canavani nosuccinic acid to be metabolized produces canaline and guanidine-acetic acid which is elevated up to 4 times more than its normal value; it is toxic in cell cultures and also interferes with the absorption of potassium in erythrocytes (8). Canavanine is a substrate for the arginase enzyme and when metabolized produces urea, guanidine-acetic acid and canaline. Canaline when injected into rats results in renal failure (9). In a previous research were found increased values of canavanine in leukocytes of patients with CKD (6). With these data we were interested in research the levels of canavanine in blood of patients with CKD and also without renal disease to know their levels and observe if they may have some utility in support of the diagnosis of the disease.

MATERIAL AND METHODS

The levels of creatinine, cystatin C and canavanine were evaluated in 100 blood samples of patients with CKD and 100 samples of patients without renal damage, both sexes and age 5 to 17 years. The creatinine levels were evaluated in a autoanalyzer "Dimension RxL" from Siemens (10), cystatin C by a method of immunonephelometry in a BNProspec equipment from Siemens (11) and the canavanine was quantified with the Rosenthal technic (12). To perform this study blood samples with creatinine values higher than 2.0 mg/dl were used as samples from patients with CKD. The results are expressed as mean, standard deviation, p value of <0.05 was considered statistically significant . The

diagnostic sensitivity and specificity were analyzed by the graph characteristics. The cystatin C levels will be used as a reference method to obtain the reference values of serum canavanine.

RESULTS

The values of canavanine obtained in patients without renal disease were of 3 to 8.5 mg/dl mean of 5.8 and standard deviation of ±1.5, creatinine of 0.2 to 1.2 mg/dl mean of 0.5 and standard deviation of ± 0.19 and cystatin C 0.6 to 0.95 mg/L, mean 0.77 and standard deviation ± 0.11; the values for patients with CKD were canavanine of 8.5 to 25 mg/dl, mean of 13.2 and standard deviation ±2.4 presenting a difference statistical significant of $p<0.01$, creatinine of 2.0 to 18.5 mg/dl mean of 5.4 and standard deviation ±5.2 and cystatin C 0.98 to 9.5 mg/L, mean 3.7 and standard deviation ± 2.6 For cystatin C a sensitivity of 90% was obtained with specificity of 89%; the sensibility of the test for canavanine determination is 95% and specificity is 93%. It was observed that the values of canavanine are increased in advance to the values of creatinine and cystatin C (see graph 1). The reference values obtained for cystatin C were the same reported in the medical literature.

DISCUSSION

The toxicity of the non-protein amino acids is due to the fact that they are incorporated in the proteins instead of the normal homologous amino acids. Approximately 500 varieties of leguminous plants contain the amino acid L-canavanine which, when ingested by animals, is metabolized by the enzyme arginase producing canaline and urea. Among these leguminous plants is the common bean, soy beans, lentils, chick pea, broad beans which are frequently consumed as food by the human population.

Canavanine is an amino acid that when ingested by an animal or a person due to its structural similarity with arginine is incorporated in the nucleus of the cell, in cytoplasm and in some proteins it also interferes with the helix of DNA and RNA in formation and during the synthesis of proteins (13). When alfalfa seeds were administered to monkeys, they developed hemolytic anemia. In rats they were also given a diet based on canavanine,, observing that the immune system was altered and some organs were affected. In the blood serum of rats and monkeys, canavanine was found, as well as anti-DNA antibodies and it was observed that autoimmune complexes were deposited in the glomerular basement membrane and decrease the number of leukocytes and erythrocytes. In normal cellular metabolism arginine is catalyzed by means of the enzyme arginase and converted into ornithine which by means of the enzyme ornithine-decarboxylase is transformed into polyamines; these compounds play a very important role in the processes of growth, multiplication and cell differentiation (14). Canavanine inhibits the functions and effects of arginine through its potent metabolite L-canaline, which acts as an antagonist of ornithine. On the other hand, it has been observed that the arginyl-RNAsynthetase enzyme easily incorporates canavanine into the nascent peptide chains, resulting in structurally and functionally altered proteins; these alterations are due to the fact that the positive charges of canavanine are different of these of arginine . On the other hand it decreases the synthesis speed of some enzymes, mRNA levels and also inhibits histone synthesis. The canaline functions as a lysine antagonist, in addition it decreases the blood levels of aspartic acid and glutamic acid. In pig kidney the concentration of glutamic acid decreases 27% and the activity of ornithine-aminotransferase decrease 90% (15). The toxic effect of canavanine occurs when the ingestion is for a long time, with a high content of the amino acid in the blood and in the tissues. The pharmacological and physiological properties of canavanine are of great importance because it presents a range of involvement at different tissue levels (16).

When canavanine is administered in rats, high concentrations of guanidinoacetic acid are eliminated in the urine. On the other hand, the arginase enzyme hydrolyses canavanine producing canaline and urea (17), In rats the toxic effect of canavanine is severe, this amino acid is incorporated into the tissues of the organ such as the spleen, heart, lungs, salivary glands, and brain. In histological sections of the pancreas, damage is observed in the acinar cells, a site where digestive enzymes are produced (18). It has been observed that canavanine replaces arginine in the collagen protein causing an incomplete synthesis of this protein and also converting it in a non-functional protein. The glomerular basement membrane is constituted by collagen and it has been observed that autoimmune complexes are deposited in this membrane, therefore it is possible that canavanine is included in the peptides chain of the collagen. Therefore the glomerular basement membrane loses its functionality because the polarity of canavanine is very different to the polarity of arginine and possibly this is the reason why patients with CKD pass through the basement membrane the albumin towards the urine. This does not happen at normal conditions. On the other hand it has been observed that with highs doses of canavanine the ammonium excretion is disturbed because its metabolic derivatives are competitive inhibitors of the metabolic compounds of the urea cycle causing accumulation of ammonium in the blood. The degradation products of canavanine are: O-ureido-homoserine which is an analogous to citrulline and canavanine-succinic acid which is an analogue of arginine-succinic acid (15) (figure 1) . This causes the urea cycle to be altered and furthermore the creatinine production to be greater due to the fact that creatinine is synthetized from the transamidination reaction of glycine with canavanine (fig. 2). For this reason, blood levels of creatinine are increased in patients with CKD. On the other hand, due to the deposition of autoimmune in the glomerular basement membrane a thickening of the basement membrane is observed and it is probable that this thickening contributes to the decrease in the filtration rate of renal glomerulus. Therefore it is very probable that the consumption of leguminous plants containing canavanine is the cause of exacerbation of the disease.

The composition of collagen by amino acids is characteristic being approximately 30% glycine, 8% arginine and 4% lysine; also contains 14% proline and 12% hydroxyproline. The amino acids proline and hydroxyproline are bulky and relatively inflexible; these amino acids give the collagen its structural and stiff properties to the whole protein (19) .In patients with CKD, the urinary excretion of proline, hydroxiproline and glycine peptides is more than 3 times of the normal values therefore it is very probable that the accumulation of canavanine in organs and tissues of patients with CKD is the cause of the progression of kidney failure until its terminal state; therefore it is thought that would be convenient to restrict or permanently prohibit patients with CKD from consuming any food that containing canavanine.

The glomerular filtration in the kidney is regulated mainly by two factors

1.- The negative and positive charges of the basement membrane.
2.- The size of the pores of the basement membrane

In this way the passage of albumin through the glomerular basement membrane to the urine is explained; however hemoglobin does not cross the basement membrane despite being a smaller protein. The positive charges of canavanine are different from the positive charges of arginine. The canavanine has a pK value of 7.04 and an isoelectric point close to neutrality while the pK of arginine is 10.48 being a more basic amino acid with isoelectric point of 12.48, therefore at physiological conditions arginine is fully protonated whereas canavanine is not. For this reason, when canavanine is included in the collagen protein it undergoes alterations, ceasing to be functional and the excretion of tripeptides consisting of proline, hydroxyproline and glycine is observed in the urine. These peptides with hydroxyproline are exclusive of the collagen protein and their concentration in the urine of patients with CKD is increased more than 3 times of their normal values, which makes us think that the collagen of the renal glomerulus is damaged (20). In studies done in rats to which a dose of canavanine of 2g/kg of weight was administered; 2% was eliminated by feces, in the tissues examined at 24 hours 0.3% was found, 1% was incorporated into proteins and 5% was excreted in 5 days in the urine. In the urine were found the metabolites of canavanine which are: urea, guanidinoacetic acid and methylguanidine. The canaline was not found in the urine but 0.2% was found in the kidney (17).

Cystatin C is a small protein of low molecular weight reabsorbed and catabolized exclusively in the cells of the renal proximal tubule . Due to its constant production and elimination through the kidney it is considered a good marker of glomerular filtration, its concentration increases when there is kidney damage in advance to creatinine levels and is independent of height, age, sex, diet, muscle mass and nutritional status, therefore it is considered as a specific test. By this reason it was used as reference method to evaluate the levels of serum canavanine and also the creatinine values are reported based on values of cystatin C. In the values obtained it was observed that canavanine levels increase simultaneously with creatinine values; in addition it can be observed in figure number1 that the values of canavanine are increased in advance to the values of creatinine and

also before the cystatin C values (it is marked with arrows in the graph), which makes the canavanine more advisable to evaluate the renal glomerular function. The values obtained for cystatin C in patients without renal damage are the same as those reported in the medical literature (0.6 to 0.95 mg/L) and the values of canavanine were 3 to 8.5 mg/dl and the levels in patients with CKD were cystatin C 0.96 to 9 mg/L creatinine 1.0 to 18.5 mg/dl and canavanine 8.5 to 25 mg/dl with a statistically significant difference of $p<0.01$. On the other hand in this study for cystatin C was found a diagnostic sensitivity of 90% and specificity of 89% and for canavanine the sensitivity is 95% with specificity of 93%; therefore it is considered that the determination of serum canavanine is a specific test to evaluate renal function. Furthermore, the range of values in healthy people is quite wide from 3 to 8.5 mg/dl and for patients with CKD is 8.5 to 25 mg/dl which allows without any difficulty to differentiate between a healthy people and another with CKD. Apparently the values of serum canavanine do not depend on height , sex or muscle mass. On the other hand it has been observed that the levels of cystatin C can be increased in some liver diseases, in thyroid dysfunction and when glucocorticoids are administered. This information makes the evaluation of serum canavanine more advisable as a laboratory test very useful to evaluate glomerular renal function. To evaluate the renal function, the determination of creatinine in blood and urine is currently used, however when the creatinine levels are increased, the kidney is already irreversible damaged.

On the other hand, it has been observed that the highest incidence of patients with CKD worldwide occurs in some countries of Central America which is possibly due to the sword beans a tropical leguminous consumed as a substitute for mashed potatoes, seeds also are used as a coffee substitute. Sword beans have high protein content, but also containing hemaggluitinins, protease inhibitors, hydrocyanic acid, tannins and canavanine 4.1%. (21). Canavanine is an amino acid of vegetable origin that has not been explored in humans; this is the third study carried out (22). According to the results obtained it was observed that levels canavanine in blood increased before creatinine; therefore it can be very useful to make an early diagnosis of the disease but is necessary to do more studies in blood and tissue samples of 'patients with CKD in a larger population to confirm the usefulness and importance of this finding.

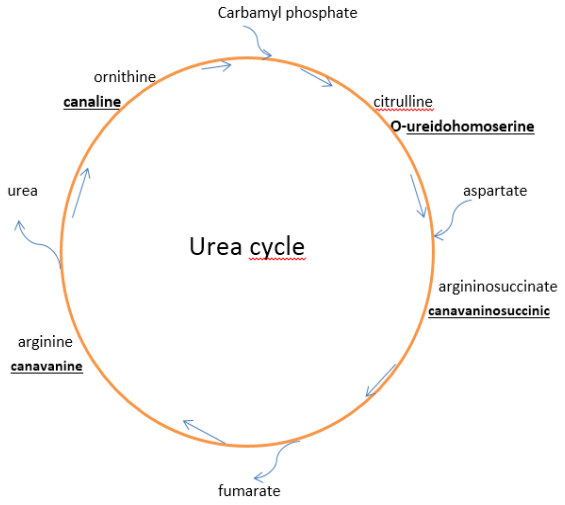

Figure 1. Urea cycle with L-arginine and with its **L-canavanine** analogue

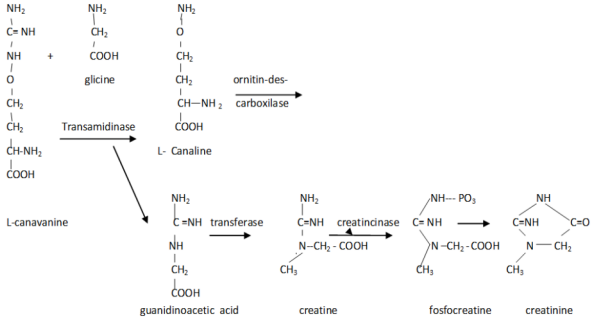

Figure 2. Creatinine synthesis from L-canavanine

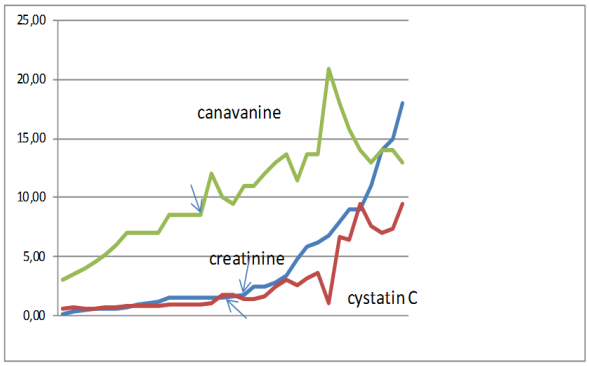

FIGURE 3. Relationship between levels of serum cystatin C creatinine and canavanine of blood samples analyzed

The arrows indicate the point where the analytes values begin to increase. It is observed that the first one to increase is canavanine

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