A study of urinary amino acid patterns in type 2 diabetes

R.Krishnaprasad, Former Assistant Professor, Dept of Biochemistry, AG Unnikrishnan, Harish Kumar, Professors, Dept of Endocrinology Amrita Institute of Medical Sciences, Cochin, Kerala, India


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ABSTRACT

In the blood, amino acid levels are maintained at fairly constant levels. Urinary amino acid patterns are well known to reflect changes in the blood. This is also true in diabetes mellitus, where it has been proposed that hyperglycemia leads to alterations in the urinary amino acid patterns. Hence, this study was conducted to assess urinary amino acids in subjects with type 2 diabetes. Our results show that the average amount of amino acids lost in the urine was 82.4±25 mg/dl. There was poor correlation between the amount of amino acid lost in the urine and the blood glucose levels. Aminoaciduria showed an increasing trend with glucosuria. To conclude, our study on type 2 diabetic subjects with well-controlled glucose levels and no metabolic complications shows subtle abnormalities in urinary amino acid patterns. Further studies are required before any significance can be attributed to these changes.

INTRODUCTION

Amino acid levels in blood are maintained at fairly constant levels, and urinary amino acid patterns are well known to reflect changes in the blood. This is also true in diabetic subjects, in whom it has been proposed that hyperglycemia leads to alterations in the urinary amino acid patterns. Hence, this study was conducted to assess urinary amino acids in subjects with type 2 diabetes. We chose subjects who had not yet developed microalbuminuria, in order to find out if there are subtle urinary amino acid pattern alterations that antedate the onset of microalbuminuria. We chose only subjects taking insulin and oral drugs as there is evidence that untreated hyperglycemia per se leads to urinary amino acid pattern alterations.

Methods

We studied urinary amino acid patterns in 45 type 2 diabetic subjects. Only patients whose diabetic status was under control, as defined by a fasting plasma glucose of <110 mg/dl were selected and asked to collect a single fasting urine sample.
Subjects with overt nephropathy and renal failure were excluded. All diabetics were on treatment with insulin or oral hypoglycemic agents.

Urine amino acid levels were studied by chromatography as follows: The urine was deproteinised by the addition of ethanol. Deproteinisation is needed as only free amino acids were being evaluated. Total amino acid level in the urine was estimated in the deproteinised sample by the ninhydrin method. Amino acid levels were expressed as mg/dl.

The excretory pattern of amino acids in diabetics showed that the amino acid most commonly encountered were Cysteine, followed by Arginine, Serine, Threonine, Isoleucine, Proline, Leucine, Alanine, Glycine, Glutamic acid, Methionine, Tyrosine and Lysine in that order of frequency (see figure 1).

Figure 1. Shows the percentage of type 2 subjects excreting the individual amino acids in the urine

The average amount of amino acids lost in the urine was 82.4± 25 mg/dl. While a trend of increasing amino acid loss with high blood glucose was noted, there was poor correlation between the amount of amino acid lost in the urine and the blood glucose levels (R= 0.245). The aminoaciduria showed an increasing trend with glucosuria (R=0.568). No correlation between urine ketones or urine protein with the amino acid excretion was noted.

Thus, in this group of patients with type 2 diabetes the amino acid excretion pattern showed that a greater proportion of the diabetic subjects excreted Cysteine, Arginine and Isoleucine. This contrasts sharply with the pattern in normal people where Alanine and Glycine are the main constituents.
The aminoaciduria did not correlate with the urine protein in the patients who were positive for either. This is because amino acids in the urine are from the blood. Following protein catabolism they are filtered at the glomerulus and reabsorbed in the PCT.

The mechanism of aminoaciduria correlating with glucosuria in our subjects is not clearly known. One explanation is that glucose in the renal tubules is believed to depolarize and dissipate the electrical gradient of the sodium dependent glucose transporters. This would result in generalized aminoaciduria, according to a recent report, where 25 type 2 diabetic subjects were studied and compared with HNF-1alfa mutation carriers. The study also showed that HNF-1alfa mutation carriers have a specific defect in renal glucose transport. The type 2 diabetic subjects that we studied (n=45) did not have a generalized aminoaciduria. However, in contrast to the type 2 diabetics in that study, where the HbA1c was 7.6%, none of our subjects had current hyperglycemia, as we chose only well controlled type 2 diabetics. Is it possible that the differences in amino acid excretion patterns could be related to differences in blood glucose levels? This would mean that a high degree of glucosuria (as in the setting of hyperglycemia) would result in a generalized aminoaciduria, while a specific aminoaciduria might be unmasked when glucose control is achieved. Alternatively, it is also possible that the amino acid excretion patterns in the two studies are due to small sample sizes in both of them, and our report justifies a need for a larger study. Another limitation of our study was that we did not study controls.

We propose two alternative explanations for the altered amino acid pattern in these subjects: firstly, these alterations could represent subtle evidence of early renal tubular or glomerular damage in the hyperfiltration stage itself, and might predate the onset of future renal insufficiency in these subjects. This can be confirmed only by identifying and following up newly diagnosed subjects. Indirect evidence for this is the association between glucosuria and aminoaciduria (despite normoglycemia) in our subjects. The association between glucosuria and aminoaciduria in non-diabetic subjects indicates renal tubular dysfunction and has been well described earlier in diverse settings, including lead poisoning, myelomatosis and Fanconi’s syndrome. Thus it is possible that urinary amino acid pattern in our subjects is due to renal tubular dysfunction in diabetic subjects and that urine amino acid patterns could serve as an inexpensive marker for the development of nephropathy. To our knowledge the use of amino acid patterns as a marker of renal tubular damage in diabetics (who have predominantly glomerular disease) has not been reported earlier.

Another explanation for the abnormal pattern could be the metabolic milieu that is characteristic of the diabetic state itself. Indeed sugar infusions per se can cause aminoaciduria. Gluconeogenesis occurs continuously in diabetes, contributing to the hyperglycemia and constant breakdown of proteins and fat. The main gluconeogenic amino acids are alanine and glycine. Diversion of these towards glucose production would lead to a reduced excretion into urine. This would explain the relative absence of alanine and glycine. Interestingly, the perfusion of alanine and glycine has been shown to be nephroprotective. A unifying hypothesis, highly speculative at present, would be that the altered amino acid pattern, which is associated with deficiency of alanine and glycine, would increase vulnerability to kidney damage, and that therefore, this pattern could be causally linked with renal damage.
CONCLUSION

To conclude, our study on type 2 diabetic subjects with well-controlled glucose levels with no metabolic complications shows subtle abnormalities in urinary amino acid patterns. We further need to determine the significance of these changes.

CONSENT

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

COMPETING INTERESTS

The author(s) declare that they have no competing interests'.

REFERENCES