Newly developed method for quantification of tryptophan and its metabolites: Clinical application

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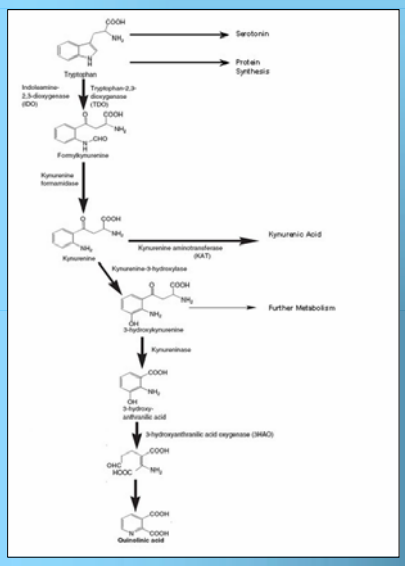

Figure 1 Metabolic pathway illustrating tryptophan metabolism.

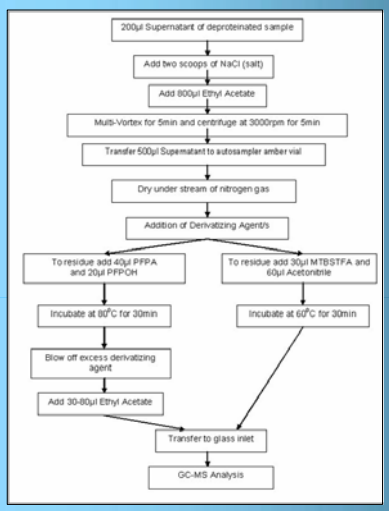

Figure 2 Flow chart illustrating preparation of samples

Table 1 Precision data (CV = coefficient of variance)

Between day Precision (CV in %)	High (4.44mg/L)	Low (0.56mg/L)
Tryptophan	4.8	5.5
Kynurenine	9.9	13.4
Quinolinic acid	8.2	10.2

Table 2 Recovery data for quality controls

Average Recovery	High Control	Low Control
Tryptophan	98.94 %	88.16 %
Kynurenine	87.12 %	72.63 %
Quinolinic Acid	98.04 %	75.80 %

Introduction:

Tryptophan, an essential amino acid, follows important metabolic pathways for protein, niacin and serotonin synthesis (Fig 1). Tryptophan depletion and accumulation of some of its neurotoxic metabolites have both physical and psychological implications. The kynurenine pathway is a major pathway for tryptophan degradation and for the synthesis of the metabolites kynurenine and quinolinic acid. Assessment of tryptophan metabolism is generally hampered by the non availability of analytical techniques for these substances.

Aim:

- To develop and validate a suitable method for the simultaneous quantification of tryptophan, kynurenine and quinolinic acid in blood.
- The second aim was to demonstrate the clinical application of this method by quantification of the said metabolites in the blood of chronic renal failure (CRF) patients and to compare the levels in patients on different renal replacement modalities and to controls.

Methods:

Various chromatographic methods employing different derivatization agents were assessed (Fig 2). Blood was collected from 15 haemodialysis (HD) and 15 peritoneal dialysis (PD) patients at initiation of treatment. The ethical clearance number was S168/2006.

Results:

A sensitive, selective and repeatable method was developed that employed gas chromatography coupled to mass spectrometry (GC-MS). The gas chromatographer was a Hewlett Packard HP GC 6890 series instrument coupled to a MS 5973 series mass spectrometer. Separation of the analytes was achieved on a DB-5MS GC column with a nominal length of 30 metres, a diameter of 250.0 µm and film thickness of 0.10 µm. Analytes were derivatized with pentafluro propionic anhydride (PFPA) and pentafluro propanol (PFP). Method validation results (Tables 1,2,3) in terms of accuracy; precision; sensitivity; stability and recovery fell within the international acceptance criteria for newly developed methods. Table 4 shows the results of the patient and control groups. Control values were in agreement with international published values by other techniques. Tryptophan levels were significantly lower ($p<0.05$) and kynurenine and quinolinic acid higher in the patient groups than in controls ($p<0.05$). Kynurenine levels differed significantly ($p<0.05$) between HD and PD patients.

Conclusions:

GC-MS is suitable for simultaneous assessment of tryptophan, kynurenine and quinolinic acid. Significant tryptophan depletion and accumulation of the metabolites kynurenine and quinolinic acid occurs in CRF patients on both haemodialysis and peritoneal dialysis treatment.

Acknowledgements:

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Table 3 Sensitivity data (LOQ – limit of quantification; LOD – limit of detection)

Analyte	LOQ	LOD
Tryptophan	0.343 µM	0.0428 µM
Kynurenine	0.336 µM	0.084 µM
Quinolinic Acid	0.105 µM	0.0262 µM

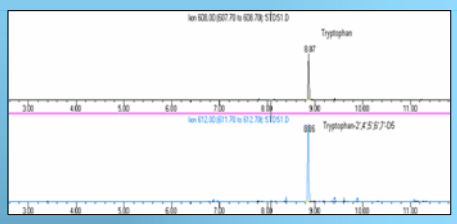

Figure 3 Typical chromatogram obtained for tryptophan

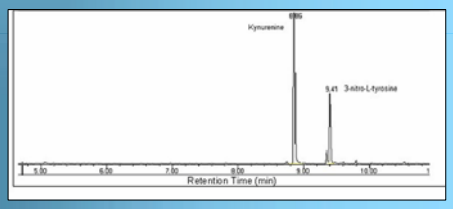

Figure 4 Typical chromatogram obtained for kynurenine

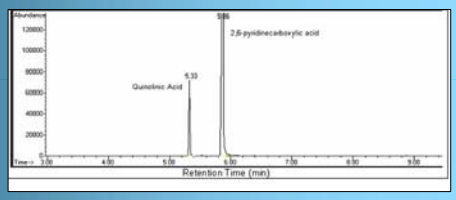

Figure 5 Typical chromatogram obtained for quinolinic acid

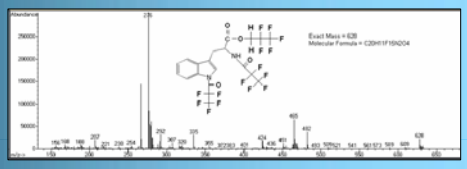

Figure 6 Electron impact mass spectrum obtained for tryptophan

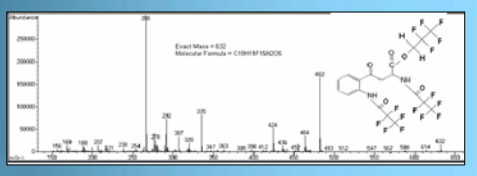

Figure 7 Electron impact mass spectrum obtained for kynurenine

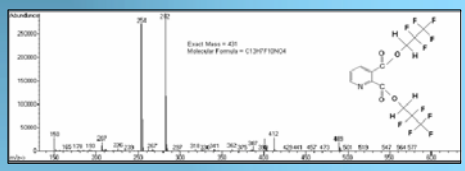

Figure 8 Electron impact mass spectrum obtained for quinolinic acid

Table 4 Results for patient and control groups

Group	Result	Tryptophan (µM)	Kynurenine (µM)	Quinolinic Acid (µM)
HD Patients (n=15)	Mean	5.3	4.7	4.9
	SD	5.04	1.9	2.03
PD Patients (n=15)	Mean	6.7	2.9	2.8
	SD	3.2	0.8	2.03
Control group (n=12)	Mean	28.4	2.1	0.3
	SD	4.3	0.6	0.15