Int J Pharm Bio Sci 2014 April ; 5 (2) : (B) 934 - 942

Research Article Biochemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

THE ROLE OF URIC ACID AND ALANINE AMINO TRANSFERASE IN TYPE 2 DIABETIC MELLITUS

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ABSTRACT

Type 2 diabetes mellitus is an independent risk factor for cardiovascular disease and the risk for cardiovascular disease is increased three to four fold in non insulin dependent diabetes mellitus as compared to non diabetic population. Lowering levels of uric acid may slow progression of renal disease, especially in patients with hyperuricemia. Alanine Amino Transferase to predict the development of type 2 diabetes independent of BMI and alcohol intake. numerical variable will be summarized as the mean, median and standard deviations for all subjects and student't' test significance of study parameters Altered uric acid levels observed in the type 2 diabetes subjects may be due to deterioration in glucose metabolism associated with hyper insulinemia, metabolic syndrome and its impact on the renal excretion of uric acid. Increasing evidence suggests that Levels of ALT and Uric acid were found to be altered in type 2 diabetic subjects.

KEYWORDS: **Diabetes mellitus, Alanine Amino Transferase, Uric Acid, Insulin, Hyperuricemia.**

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INTRODUCTION

Diabetes Mellitus (DM), a life-long progressive disease, is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of blood glucose, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Diabetes mellitus is an endocrine and complex multi factorial disorder involving both impaired insulin release leading to relative insulin deficiency and end organ insensitivity. Type 2 diabetes mellitus is an independent risk factor for cardiovascular disease and the risk for cardiovascular disease is increased three to four fold in non insulin dependent diabetes mellitus as compared to non diabetic population [2]. The prevalence of DM is increasing worldwide with an estimated projection of approximately 300 million patients worldwide by the year 2025[3]. Most type 2 diabetes patients are overweight, and most are diagnosed as adults. Although the genetic predisposition to type 2 diabetes is strong, no single genetic defect has been found. In addition to genetic influences, acquired risk factors for type 2 diabetes include obesity, advancing age, and an inactive lifestyle[4]. Uric acid is an organic compound, metabolic end product of purines and it is formed in liver. Uric acid is a non protein nitrogenous substance hence it should be eliminated in our body through the kidneys. An association of hyperuricemia with hypertension[5]. However, the putative association between serum uric acid levels and diabetes mellitus is not clear. Some studies reported that there is a positive association between high serum uric acid levels and diabetes[6]. Higher serum uric acid levels were inversely associated with diabetes mellitus after adjusting for age, sex, race/ethnicity, education, smoking, alcohol intake, body mass index, hypertension, and serum cholesterol[7]. Hyperuricemia is frequently encountered in hypertensive patients and may occur due to a defect in renal urate clearance[8]. Patients with hypertension and hyperuricemia have a 3- to 5-fold increased risk of experiencing coronary artery disease or cerebrovascular disease compared with patients with normal uric acid levels[9]. Uric acid has often been considered a part of the dysmetabolic syndrome or simply a marker of other coronary disease risk factors such as hypertension, dyslipidemia, obesity, glucose intolerance, and renal disease [10]. Increasing evidence suggests that uric acid may play a role in the metabolic syndrome. Historically, the elevated level of uric acid observed in the metabolic syndrome has been attributed to hyperinsulinemia, since insulin reduces renal excretion of uric acid[11]. Hyperuricemia, however, often precedes the development of hyperinsulinemia[12]obesity, and diabetes[13]. Hyperuricemia may also be present in the metabolic syndrome in people who are not overweight or obese[14]. The strongest evidence of a role for uric acid in the development of the metabolic syndrome has been from studies in animal models showing that decreasing uric acid levels can prevent or reverse features of the metabolic syndrome[15]. The mechanism has been suggested to explain how hyperuricemia might induce the metabolic syndrome that is related to the fact that glucose uptake in skeletal muscle depends in part on increases in blood flow mediated by the insulin-stimulated release of nitric oxide from endothelial cells. Features of the metabolic syndrome develop in mice lacking endothelial nitric oxide synthase. The observations that hyperuricemia can induce endothelial dysfunction in rats[16] and that treatment with allopurinol can improve endothelial function in patients with hyperuricemia[17] would support this mechanism. Both experimental and clinical studies suggest the possibility that an elevated level of uric acid itself can lead to kidney disease without the deposition of uric acid crystals. Experimental studies in rats have shown that raising uric acid levels can cause de novo kidney disease as well as accelerate existing kidney disease[18]. The principal lesions from increased uric acid in the rat are glomerulo sclerosis, interstitial fibrosis, and arteriolar disease, conditions similar to those observed in “gouty” nephropathy, except for the absence of intra renal urate crystals[19]. The mechanism of injury appears to be related to the

development of preglomerular arteriolar disease that impairs the renal auto regulatory response and thereby causes glomerular hypertension[20]. Similar histologic findings are also present in the hereditary human disease familial juvenile hyperuricemic nephropathy.

Recent studies suggest that lowering levels of uric acid may slow progression of renal disease, especially in patients with hyperuricemia. The treatment of asymptomatic hyperuricemia in patients with mild renal disease (chronic kidney disease at stage resulted in delayed disease progression[21]. The treatment of asymptomatic hyperuricemia improved renal function[22]. An used a different approach in which they withdrew allopurinol from a group of patients with chronic kidney disease who were in stable condition. This withdrawal resulted in worsening of hypertension and acceleration of kidney dysfunction in the patients who were not taking angiotensin-converting–enzyme inhibitors[23]. Alanine amino transferase is a liver enzyme that catalyzes the transfer of an amino group from alanine to alpha-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. Elevation of transferases within three times the upper limits of normal is not a contraindication for starting oral antidiabetic or lipid-modifying therapy. In contrast, anti diabetic agents have generally been shown to decrease alanine amino transferase levels as tighter blood glucose levels are achieved. Alanine amino transferase is present in higher concentration in liver than in muscle. Consequently an elevation is more specific for liver disease. Both SGOT and SGPT become elevated whenever liver cells are damaged as, for instance, in viral hepatitis. Individuals with type 2 diabetes have a higher incidence of LFT abnormalities than individuals who do not have diabetes. The most common abnormality is elevated ALT[24]. The precise genetic, environmental, and metabolic factors and sequence of events that lead to the underlying insulin resistance, however, is not fully understood[25]. The excess free fatty acids found in the insulin-resistant state are known to be directly toxic to hepatocytes. Putative mechanisms include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism[26]. The above theories all attribute elevated transaminitis to direct hepatocyte injury. It is also hypothesized that elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate an impairment in insulin signaling rather than purely hepatocyte injury [27]. The liver has a major role in glucose homeostasis and, in liver diseases, hepatic carbohydrate metabolism is commonly disturbed[28]. Altered portal insulin levels and the insulin/glucagon ratio may influence hepatocyte function and integrity in diabetic patients[29] and predispose them to various hepatic disorders . Although no specific liver disease is known to be associated with diabetes mellitus[30], altered hepatic glucose metabolism may be involved in the pathogenesis of non-insulin-dependent diabetes[31]. Disturbances in liver function tests (LFT) are well recognized in some diabetic patients[32] especially in acute metabolic decompensation [33,34]. However, in diabetic patients, the prevalence of abnormal LFT results and their relationships to clinical findings and diabetes, as well as to pathologic changes in liver structure, are controvers [35]. Hepatic dysfunction resulting from the insulin resistance syndrome may contribute to the development of type 2 diabetes [36]. Alanine aminotransferase (ALT) is the most specific marker of this hepatic pathology. Glutamyl transferase (GGT) is considered to be a sensitive indicator of liver damage but is not specific. Obesity also has major effects on GGT [37]. A number of prospective studies[38] have shown raised GGT or ALT to predict the development of type 2 diabetes independent of BMI and alcohol intake. In our earlier study, raised GGT level was shown to be an independent risk factor for type 2 diabetes, and we hypothesized that GGT might be a marker for visceral and hepatic fat deposition (steatosis) and, by inference, a marker of hepatic insulin resistance.

MATERIALS AND METHODS

Subjects were chosen from type 2 diabetes and apparently healthy population who

visited Sri Ramachandra Medical centre, Chennai for master health check up. With the consent of the subjects and the approval of medical officers and ethical committee, access to values on biochemical tests, BMI and Duration of diabetes and an aliquot of blood sample of the subjects were obtained for the study. All subjects were recruited from the outpatient division of Sri Ramachandra Medical centre, Chennai. People with carcinomas and patients who have undergone treatment for cancer or any other surgical procedures are excluded. Subjects without any known chronic or acute diseases or illness were considered for the study. 90 subjects (60 type 2 diabetic subjects, 30 healthy subjects) in an age group of 30 – 75 years satisfying the above criteria were selected for the study. Fasting and post prandial blood was collected between 8 am and 12 pm from all recruited patients. About 5 ml peripheral blood sample (by a standard venous puncture) was collected by the technical staff of Sri Ramachandra Medical Centre, Chennai in a clean and sterilized plain vacutainer. The serum was separated by centrifuging the samples at 2500 rpm for 10 minutes and collected in microfuge tubes. FBS, Uric acid, ALT and AST, GGT were measured using a quantitative enzymatic method in an automatic analyzer Dimension – R in the Hospital Central Laboratory, Sri Ramachandra Hospital, Chennai.

Uric acid

The URCA method for the Dimension-R clinical chemistry system is an in vitro diagnostic test intended for the quantitative determination of uric acid in human serum, plasma and urine. The uric acid method is a modification of the uricase mthod first reported by Bulger and Johns, later modified by Kalckar. Measurement of uric acid by monitoring the loss of adsorbance at 293nm following uricase treatment is generally recognized as being more specific and less subject to interference than other, indirect method. Uric acid, which absorbs light at 293, is converted by uricase to allantionin, which is non absorbing at 293nm. The change in absorbance at 293nm due to the disappearance or uric acid is directly proportional to the concentration of uric acid in the sample and is measure using a bichromatic (293,700nm) endpoint technique.

Alanine amino transferase

The ALT method for the Dimension-R clinical chemistry system is an in vitro diagnostic test intended for the quantitative determination of alanine amino transferase in human serum or plasma. ALT catalyses the transamination from L-alanine to α-ketoglutarate, forming L-glutamate and pyruvate..

Aspartate amino transferase

The AST method for the Dimension-R clinical chemistry system is an in vitro diagnostic test intended for the quantitative determination of aspartate aminotransferase in human serum or plasma. AST catalyses the transamination from L-aspartat to α-ketoglutarate, forming L-glutamate and oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase with simultaneous oxidation of reduced NADH. The change is absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity is measured using a bichromatic (340-700nm) rate technique.

Gamma glutamyl transferase

The GGT method for the Dimension-R clinical chemistry system is an in vitro diagnostic test intended for the quantitative determination of gamma glutamyl transferase in human serum or plasma. GGT catalyse the transferase of the glutamyl moity from gamma glutamyl 3-carboxyl-4-nitranilide (GCNA) to Glycyl-Glycin there by releasing 5-amino-2-nitrobenzoite which absorbs at 405nm. This change is proportional to the GGT activity and is measured by using a bi chromatic (405, 600nm) rate technique. Statistics for each numerical variable will be summarized as the mean, median and standard deviations for all subjects and student ‘t’ test (two tailed) are to be used to test the significance of study parameters using Micro soft Excel software (version). Correlation studies were performed using Pearson correlation.

RESULTS

Table 1

Biochemical parameters in Type-2 diabetes and Healthy controls.

Biochemical parameters	Type-2 diabetes NO=60 (MEAN ± STD)	Controls NO=30 (MEAN ± STD)	P.value
Fasting Glucose (mg/dl)	146.53±49.72	95.6±9.05***	< 0.001
Postprandial Glucose (mg/dl)	216.73±71.90	120.23±18.60***	< 0.001
Uric acid (mg/dl)	4.94±2.51	5.01±1.23	NS
Alanineamino transferase (IU/L)	45.23±15.22	44.9±13.73	NS
Aspartateamino transferase (IU/L)	26.16±15.18	25.4±7.19	NS
Gamma glutamyl transferase (IU/L)	48.56±47.77	36.04±14.93	NS

Table 2

Correlation co- efficient [r] of Uric acid with other parameters in patient population based on BMI

Parameters		Group-I	Group-II	Group-III
Age		-0.015	-0.023	0.50
Blood pressure (mm/hg)	Systolic	0.08	0.172	0.20
	Diastolic	-0.01	0.173	0.21
Duration		-0.07	-0.119	0.42
Risk ratio		-0.25	0.107	-0.43
HbA_1C		-0.20	0.018	-0.65

Table 3

Correlation co- efficient [r] of Alanine amino transferase with other parameters in patient population based on BMI

Parameters		Group-I	Group-II	Group-III
Age		-0.46	-0.08	0.414
Blood pressure (mm/hg)	Systolic	-0.39	o.38	0.104
	Diastolic	-0.288	-0.110	0.04
Duration		-0.21	-0.17	0.522
Risk ratio		-0.35	-0.07	-0.21
HbA_1C		-0.31	-0.041	-0.45

Figure 1
Correlation graph between Uric acid and age in GroupIII

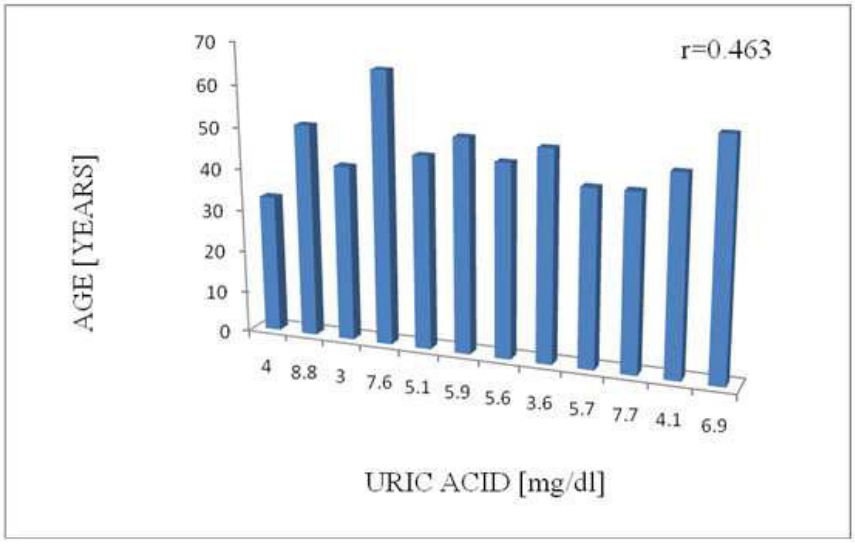


Figure 2
Correlation graph between Uric acid and duration in Group III

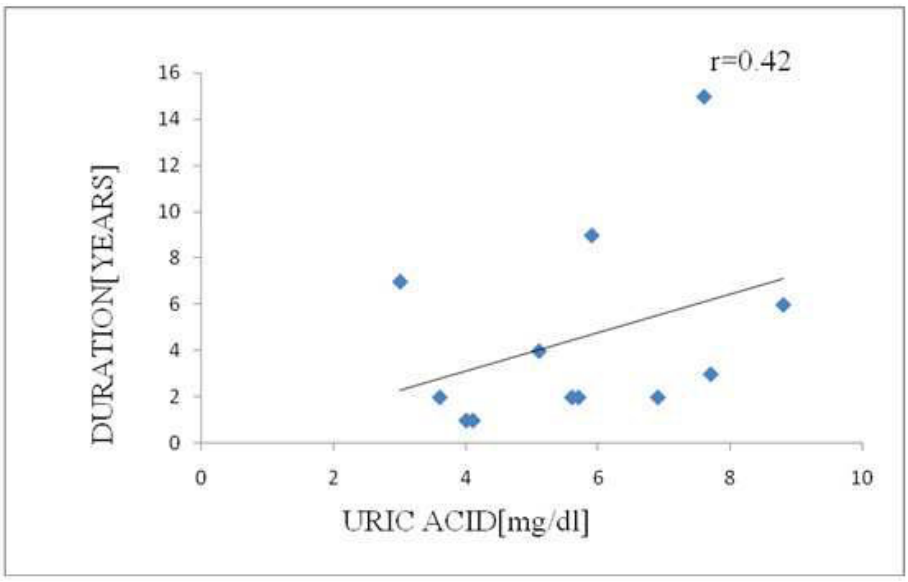


Figure 3
Correlation graph between Alanine amino transferase and age in GroupIII

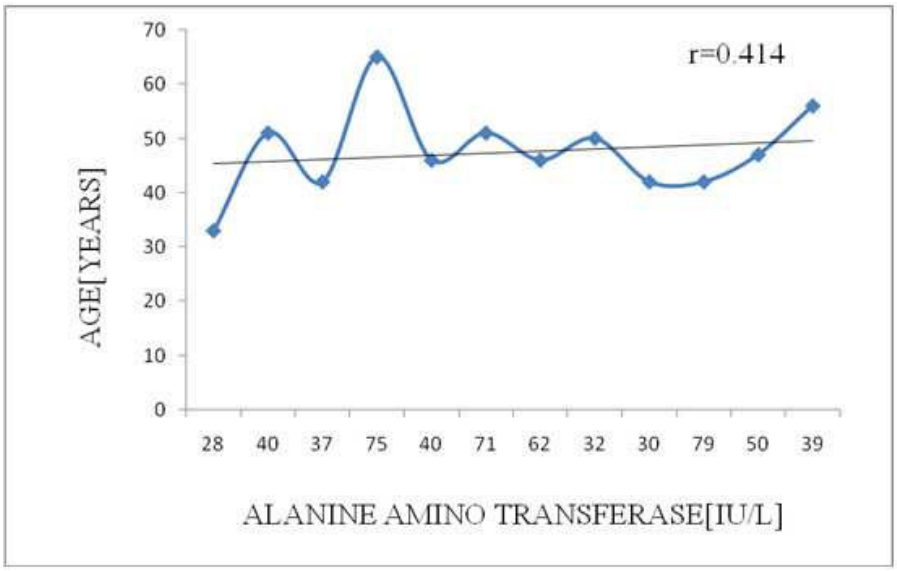
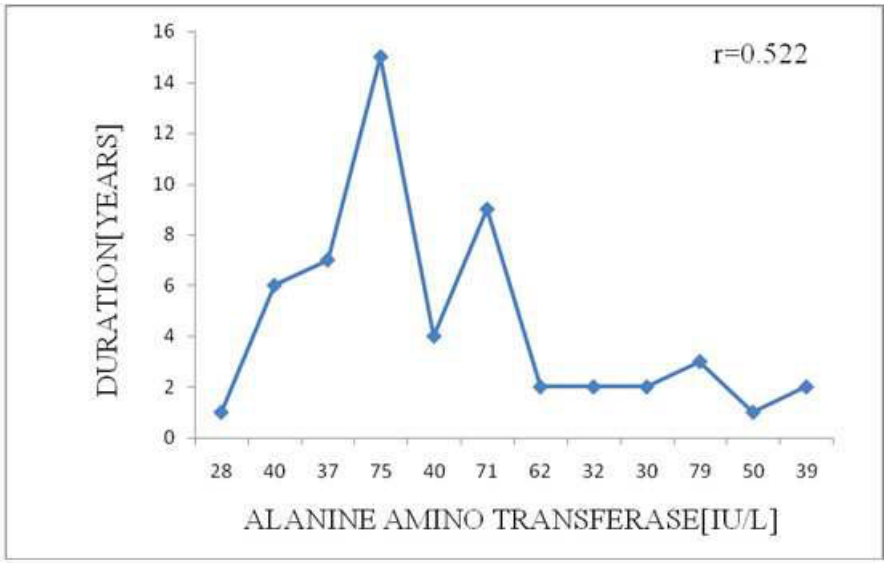


Figure 4
Correlation graph between Alanine amino transferase and duration in GroupIII



CONCLUSION

In the present study, the uric acid levels were found to be decreased although not statistically significant compared to controls. The markers of liver injury, including elevated concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), suggest a risk of type 2 diabetes. Levels of AST and ALT were positively associated with type 2 diabetes and are known to independently predict type 2 diabetes. Altered portal insulin levels and the insulin/glucogon ratio may influence hepatocyte function and integrity in diabetic patients and predispose them to various hepatic disorders. Although no specific liver disease is known to be associated with diabetes mellitus, altered hepatic glucose metabolism may be involved in the pathogenesis of non insulin dependent diabetes. Disturbances in liver function test (LFT) are well recognized in some diabetic patients. Altered uric acid levels observed in the type 2 diabetes subjects may be due to deterioration in glucose metabolism

associated with hyper insulinemia, metabolic syndrome and its impact on the renal excretion of uric acid. Increasing evidence suggests that uric acid may play a role in the metabolic syndrome. Levels of ALT and GGT, and URIC acid found were found to be altered in type 2 diabetic subjects.

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