

nephropathy), and control participants who appeared to be in good health. A total of 150 participants were involved in this study. Three groups of 50 patients each who had diabetes mellitus, diabetic nephropathy, and control subjects who appeared to be in good health. The Reitman and Frankel technique of analysis was used to measure ALT and AST activity.

Results: Diabetic nephropathy was associated with a substantial increase in enzyme activity above the control value ($P < 0.05$). While ALT activity was not significant [ALT ($P > 0.05$)], diabetes mellitus was also associated with a little increase in AST activity above the control value. Patients with diabetic nephropathy had mean ALT and AST activity that was statistically significant ($P < 0.05$) when compared to patients with diabetes mellitus who did not have nephropathy.

Conclusion: The study demonstrated that both ALT and AST levels were significantly elevated in patients with diabetic nephropathy and diabetes mellitus compared with control subjects

Key words: diabetic nephropathy, Alanine aminotransferase, diabetes mellitus

INTRODUCTION

Diabetic nephropathy is one of the most serious microvascular complications of diabetes mellitus and remains a leading cause of chronic kidney disease and end-stage renal failure worldwide. It is characterized by progressive deterioration of renal function due to persistent hyperglycaemia, leading to structural and functional changes in the kidneys. In addition to renal impairment, diabetic nephropathy has also been linked to alterations in liver function, suggesting a broader metabolic disturbance affecting multiple organ systems [1,2]. Published clinical evidence has reported that biochemical markers of liver integrity, particularly the transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST), may be affected in diabetic conditions. However, there is limited or no documented study specifically

investigating these biochemical alterations among patients with diabetic nephropathy and diabetes mellitus attending Iyi-Enu Mission Hospital, Anambra State, Nigeria.

ALT and AST are intracellular enzymes that play essential roles in amino acid metabolism and are widely distributed in the liver, heart, kidneys, skeletal muscles, and other tissues. ALT is primarily localized in the liver and is considered a more specific indicator of hepatocellular injury, while AST is found in both hepatic and extrahepatic tissues, making it a less specific but still valuable marker of tissue damage [3,4]. Under normal physiological conditions, these enzymes are present in low concentrations in the blood. However, when there is cellular injury or increased membrane permeability, such as in diabetes mellitus and its complications, elevated levels may be detected in the serum. This makes ALT and AST useful biochemical

markers in assessing metabolic and organ dysfunction.

The relationship between diabetes mellitus, diabetic nephropathy, and altered transaminase activity is an important area of clinical investigation^[5,6]. Chronic hyperglycaemia can induce oxidative stress, inflammation, and metabolic derangements that may affect both hepatic and renal tissues. These pathological processes can lead to leakage of intracellular enzymes into the bloodstream, resulting in abnormal serum levels of ALT and AST^[7]. Understanding these alterations is important for early detection, monitoring, and management of diabetic complications.

Given the increasing burden of diabetes mellitus and its complications in developing countries such as Nigeria, there is a need for localized studies that provide region-specific data. Investigating the activity of transaminases among patients with diabetic nephropathy and diabetes mellitus attending Iyi-Enu Mission Hospital will help bridge this knowledge gap. Such data will not only contribute to existing scientific literature but will also provide valuable insight for clinicians managing diabetic patients in the region.

The aim of this study is to assess the activity of transaminases (ALT and AST) in patients with diabetic nephropathy. The specific objectives are to investigate the levels of ALT and AST in diabetic nephropathy, determine transaminase activity in diabetes mellitus, and compare the differences in transaminase activities between diabetic nephropathy and diabetes mellitus. The

findings from this study are expected to demonstrate the prevalence of abnormal enzyme levels and provide empirical evidence that may assist in improving the clinical management and monitoring of diabetes mellitus and diabetic nephropathy.

The study was designed to investigate the levels of these transaminases in individuals diagnosed with diabetic nephropathy in order to understand possible biochemical alterations associated with the condition. In addition, it seeks to determine the activity of ALT and AST in patients with diabetes mellitus, thereby providing a comparative baseline for enzyme levels in uncomplicated diabetic cases.

Furthermore, the study compared the differences in transaminase activities between patients with diabetic nephropathy and those with diabetes mellitus. This comparison is intended to highlight any significant variations that may be associated with the progression of diabetic complications, particularly renal involvement. By achieving these objectives, the study will contribute to a better understanding of the relationship between liver enzyme activity and diabetic disease states, which may be useful in clinical monitoring and management.

MATERIALS AND METHODS

Subjects

A total number of one hundred and fifty (150) adult subjects aged 18-70 years, were randomly selected. fifty (50) were already diagnosed diabetes mellitus patients and the rest fifty (50) were diabetic kidney disease patients attending Iyi-Enu Mission Hospital

(IEH), Anambra State with their 50 control individuals.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of Iyi-Enu Mission Hospital Ethics Committee before the commencement of the research. The study was conducted in accordance with established ethical principles guiding research involving human participants. Informed consent was obtained from all participants prior to sample collection and data acquisition. Participants were adequately informed about the purpose of the study, the procedures involved, and their right to withdraw from the study at any stage without any consequences. Confidentiality and privacy of participants' information were strictly maintained throughout the study, and all data obtained were used solely for research purposes.

Inclusion Criteria

Already diagnosed diabetes mellitus patients, diabetic nephropathy patients and apparently healthy volunteered male and female adults who are not diabetic were recruited for the study.

Exclusion Criteria

Non-adult diabetes mellitus patients and non-adult control subjects were excluded from the study.

Sample Collection

venous blood sample (4mls) was collected from each subject by vein puncture from antecubital vein using a 5mls sterile

disposable syringe. The area was first swabbed with cotton wool dipped in 70% alcohol. The blood was delivered into properly labelled lithium heparin bottle with the subject's name, age and sex. It was capped and mixed well with the anticoagulant by gentle inversion. The blood was spun for 5 minutes at 3000 rpm. The serum was separated from the red cells using a dry clean pipette into a dry clean plain specimen container. The serum was then stored at -70°C . Enzyme activity for AST and ALT were estimated.

Analytical Methods

The tests were performed in batches of samples daily alongside standards. Each test sample, the standard, and the blank had their respective tubes.

Estimation of Plasma ALT AND AST (Reitman and Frankel, 1957)

Analytical Procedure

For the determination of alanine aminotransferase (ALT) activity, two test tubes were prepared for each specimen and appropriately labelled as T (test) and B (blank). Into each tube, 0.5 ml of buffer solution containing the substrate mixture composed of L-alanine and α -oxoglutarate was carefully pipetted. Subsequently, 0.1 ml of the sample was added only into tube T.

The contents of both tubes were mixed thoroughly and incubated at 37°C for exactly 30 minutes to allow the enzymatic reaction to proceed. At the end of the incubation period, 0.5 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent was

added into both tubes to terminate the reaction and facilitate colour development. Thereafter, 0.1 ml of the sample was also introduced into tube B, after which both tubes were mixed again and allowed to stand for exactly 20 minutes at room temperature (20–25°C).

Following incubation, 5 ml of 0.4N sodium hydroxide (NaOH) solution was added to each tube and mixed thoroughly. The absorbance of the resulting solution was then measured spectrophotometrically after 5 minutes at a wavelength of 546 nm, using the blank as reference. The activity of ALT in the sample was subsequently determined by referring to the standard table provided in the manufacturer's manual.

Analytical Procedure

The determination of aspartate aminotransferase (AST) activity was carried out using a colorimetric method under carefully controlled laboratory conditions. For each specimen analysed, two clean test tubes were prepared and appropriately labelled as T (test) and B (blank). Into each tube, 0.5 ml of buffer solution containing the substrate mixture composed of L-aspartate and α -oxoglutarate was carefully pipetted. Thereafter, 0.1 ml of the patient's sample was added only into tube T, while tube B was initially left without the sample to serve as a control for background correction.

Both tubes were thoroughly mixed to ensure proper interaction of the reagents and then incubated at 37°C for exactly 30 minutes to allow the enzymatic reaction to occur. At the end of the incubation period, 0.5 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent was

added to each tube to terminate the reaction and facilitate the formation of a coloured complex. Subsequently, 0.1 ml of the sample was also added into tube B, and both tubes were mixed again and allowed to stand at room temperature (20–25°C) for exactly 20 minutes.

After the standing period, 5 ml of 0.4N sodium hydroxide (NaOH) solution was added to each tube to develop the final colour. The contents were mixed thoroughly, and the absorbance of the test solution was measured spectrophotometrically after 5 minutes at a wavelength of 546 nm, using the blank as reference. The AST activity in each sample was finally determined by comparing the absorbance values with standard reference tables provided in the manufacturer's manual.

Data Analysis

The results obtained in this study were analyzed statistically. The mean and standard errors of mean values were calculated in each case. Students' t-test statistical method was employed for comparisons. A p-value equal less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Table 1: show the mean ALT activity of patients with diabetes Nephropathy was found to be 11.80 ± 1.29 U/L and AST activity was 20.40 ± 3.90 U/L. That of the control subjects was found to be 9.80 ± 0.80 for ALT and 7.60 ± 0.46 L for AST. This table also reveals that a comparison of the

mean transaminase activities of patients with diabetic nephropathy and the control subjects was statistically significant ($P < 0.05$).

Table 2: reveals the mean Transaminase activities of patients with diabetes mellitus and the control subjects. From this table, the mean ALT activity of patients with diabetes mellitus was found to be 11.80 ± 1.40 U/L and the mean AST activity was $20.40 \pm$

3.10 U/L . While that of the control subjects was found to be 9.20 ± 0.92 U/L for ALT and 7.60 ± 0.46 U/L for AST.

Table 3: indicates the mean Transaminase activities of patients with diabetes mellitus and diabetic nephropathy. This table reveals that a comparison of the mean Transaminase activities of patients with diabetes mellitus and diabetic nephropathy was statistically significant ($P < 0.05$).

Table 1: Shows the mean Transaminase Activities in Patients with Diabetic Nephropathy and Control Subjects

	Diabetic (n=50)	Nephropathy	Control (n=50)	t-Value	P-Value
ALT(U/L)	32.90 ± 7.10		9.80 ± 0.84	4.0	$P < 0.00$
AST(U/L)	45.0 ± 6.20		7.60 ± 0.46	7.0	$P < 0.00$

Table 2: Mean ALT and AST Activities in Diabetes Mellitus patients and the Control Subject

Parameter	Diabetes Mellitus	Control Subjects	t-value	P-value
	n=50	n=50		
ALT (U/L)	11.80 ± 1.40 U/L	9.20 ± 0.92 U/L	1.32	$P > 0.00$
AST (U/L)	20.40 ± 3.10 U/L	7.60 ± 0.46 U/L	4.4	$P < 0.01$

Table 3: Mean ALT and AST Activities of Diabetes Mellitus patients and Diabetic nephropathy patients

Parameter	Diabètes Mellites patients (n=50)	Diabetic nephropathy patients (n= 50)	t-value	P-value
ALT (U/L)	11.80 ± 1.40 U/L	32.00 ± 7.20 U/L	3.34	P < 0.01
AST (U/L)	20.40 ± 3.10 U/L	45.04 ± 6.11 U/L	3.65	P < 0.00

DISCUSSION

Persistent albuminuria, a steadily declining glomerular filtration rate (GFR), and high arterial blood pressure are the hallmarks of diabetic nephropathy, a clinical illness. This study's main goal was to evaluate transaminase activity in diabetic nephropathy. Among the transaminases are aspartate amino transferase (AST) and alanine aminotransferase (ALT)^[6,15].

The test group (diabetic nephropathy) showed elevated ALT and AST activity. This indicates that diabetic nephropathy has an excitatory influence on the transaminases' plasma level by raising their activities over the reference range's upper limit and control values^[11,14,16]. It can be the result of kidney injury. Damage to cells increases permeability, which allows cytosolic and mitochondrial enzymes to leak into the interstitium and then into the peripheral

circulation. When a kidney with high amounts of ALT and AST is injured, these enzymes can seep into the bloodstream^[6].

In diabetic mellitus (without nephropathy), there was a little increase in transaminases above the control value. While AST was substantial (P<0.05), the increase in ALT was not significant (P>0.05)^[9,10,11,12]. Increased transaminase activity in diabetes mellitus is thought to be an indication of impaired insulin signalling rather than only hepatocyte dysfunction. or possibly as a result of increasing hepatocyte injury. Hepatocytes are known to be directly harmed by the excess free fatty acids present in the insulin-resistant state, and proinflammatory cytokines such tumour necrosis factor α (TNF ALPH) may also be involved in hepatocellular damage^[1,14,15,17,18,19].

Because ALT and AST activities were higher in diabetic nephropathy than in diabetes mellitus, there was a substantial difference in transaminase activities between the two conditions. One explanation could be that the enzymes leak from both renal damage and hepatocellular injury, leading to a marked rise in the enzymes' plasma activity.

The results suggest progressive hepatic and metabolic involvement in diabetic conditions. These findings align with reports that diabetes mellitus is associated with altered transaminase activity due to metabolic stress and tissue damage. Monitoring these enzymes may therefore be useful in assessing disease progression and complications in diabetic patients.

CONCLUSION

In conclusion, the study demonstrated that both ALT and AST levels were significantly elevated in patients with diabetic nephropathy and diabetes mellitus compared with control subjects, with AST showing a more pronounced increase.

RECOMMENDATIONS

Transaminase testing is crucial for patients with diabetic nephropathy in addition to urine albumin testing. This is advised for the early detection of nephropathy in patients. Additionally, to evaluate the disease's course and response to treatment.

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