Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (O)**
Sch. J. App. Med. Sci., 2013; 1(3):205-208 **ISSN 2347-954X (P)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2013.v01i03.011

Research Article

Study on Acetylhydrolase Enzyme Activity in Urine of Diabetics

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Abstract: The activities of the enzyme acetylhydrolase was studied in urine samples of diabetics and observed to exacerbate diabetic conditions. Acetylhydrolase catabolizes Platelet Activating Factor (PAF) produced by the kidneys by deactivation. Making use of radioimmunoassay technique to evaluate activities in urine samples from non diabetics and diabetics, activity of the enzyme was detected in normal urine with a mean of ±2D = 0.66 nmol/30min/ml. Higher values were however observed among diabetics, specifically relating raised hydrolase activity to this dysfunctional metabolic state in diabetes mellitus.
Keywords: Acetylhydrolase, Urine, Diabetics

INTRODUCTION

Acetylhydrolase is a platelet activating factor exhibiting functional similarity with lipoprotein – associated phospholipase A_2. It carries out its action by hydrolyzing the SN – Zester bond yielding biologically inactive lyso –platelet – activating factor. It has specificity for phospholipid substrates with short chain residues at the Sn–2 position. In several conditions especially inflammatory disorders and allergic conditions wether systemic or localized, Acetylhdrolase has been shown to mediate increased vascular permeability associated with hypertension.[1] The process of production of Acetylhydrolase in the kidney takes place in the glomerular messangial and medullary interstitial cells which provides the origin of the circulating enzyme.[2]

The roles to be played by Acetylhydrolase in diabetic conditions need to be further clearly defined and buttressed. As shown by Lee et al the presence of specific acetylhydrolase (AH) which inactivates to its Lyso form is known to play a significant role regulating the activity of the enzyme. To clarify doubts as to wether changes in the activity of the enzyme is reflective of diabetic condition, urine samples were obtained from patients and studied for its activity.[3]

MATERIALS AND METHODS

Glucose Reagent was purchased from Randox Laboratories Limited (UK) and Meditest (Germany). A product of Calbiochem (San Diego) an unlabelled 1 – 0 – hexadecyl – 20 acetyl – glycerol – 3 – phosphocholine was used for radio – immunoassay. Sephadex – G – 25 Columns used for chromatography was a product of Isolab (Akron, Ohio). Other reagents were sourced and obtained from Sigma Chemical Company Ltd. (St. Louis, M O).

Treatment of Samples:

The urine samples were collected from Fifty patients attending the adult diabetic clinic in the Federal Medical Center, Yenagoa, Bayelsa State, Nigeria. They all had serum fasting glucose values greater than 10.0mmol/L (i.e above threshold). Control samples were obtained from Fifty individuals aged between 30–60 years with no known diabetic disease and no glucose in their urine as shown by the reagent strip assay (Combur 9, Meditest), and fasting serum glucose within reference range (3.5 – 7.0mmol/L) by the glucose oxidase method produced by Randox. Chromatography of the Urine was done after 2 hours collection on sephadex G – 25 mini – columns and 0.7mmol/L HEPES buffer 7.2 utilizing the method of Horak et al. [4]

Enzyme Activity Determination

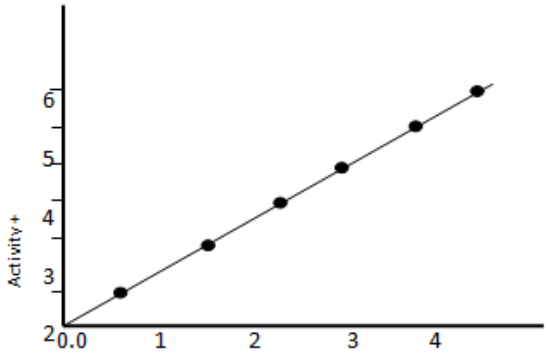
Enzymatic activities were determined with the chromatographed urine for the Acetylhydrolase adopting the method of Staforini et al with slight modification where in 5mmol/EDTA was added.[5] Comparison of an eluted radioactivity of 3H – acetyl Platelet Activating Factor that has been completely hydrolysed by incubation in 0.33mmol/L KOH was made with 3H – acetyl PAF substrate dissolved in the HEPES buffer which contained 1.0mg/ml serum albumin present at a concentration of 4nmol per assay in a total volume of 50µl. Measurement was actualized from c–18 phased catridge.

Statistical Analysis

The Data obtained from the study were expressed as nmol/PAF hydrolysed per minute per ml of urine. One way and Two way analysis of variance (ANOVA) was the statistical test used. Significance level was maintained at $P>0.05$.

RESULT

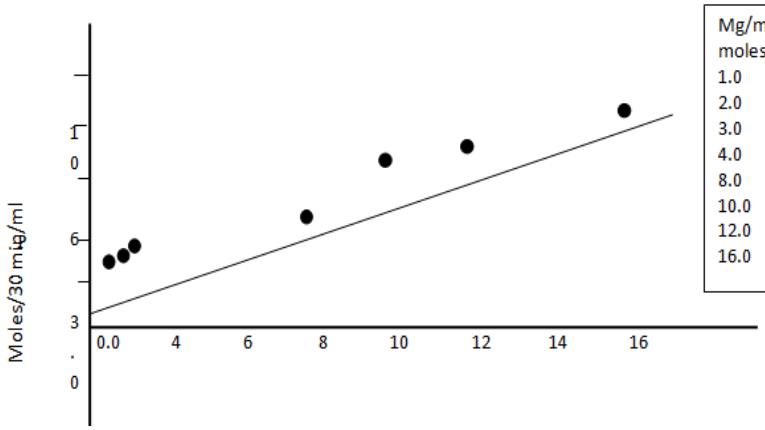
We have studied the effect of EDTA on Acetylhydrolase and determined the relationship between protein and Acetylhydrolase alongside the affinity of acetylhydrolase with glucose. To elucidate the degree of acetylhydrolase activity in urine the assay method.



+EDTA	-EDTA
0.0	0.00
1.0	1.0
2.0	1.97
3.0	2.96
4.0	4.0
5.0	5.0
6.0	6.0

Fig 1: Effect of EDTA On Acetasyhyprolase

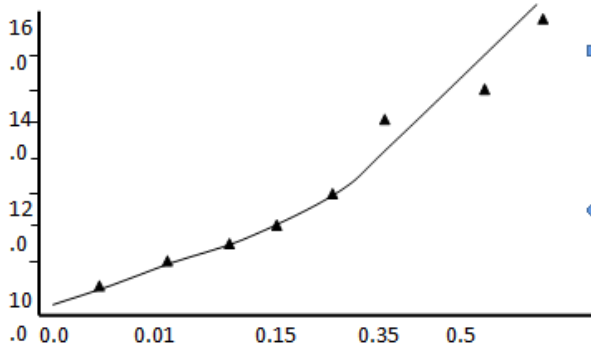
Activity was determined in the presence and absence of EDTA. There is no significant difference in the mean values. The best fit correlation equation is. Y=0.93x+0.148 (r^2=0.97)



Mg/ml	moles/30min/ml
1.0	0.7
2.0	0.8
3.0	0.9
4.0	0.99
8.0	1.3
10.0	3.3
12.0	4.6
16.0	8.3

Fig. 2: Correlation between proteinuria and Acetylhydrolase .

Best fit equation is log (Acetylhydrolase) = 0.094 (Protein) – 0.554 (r^2=0.48, P<0.001)



AH nmol/30min/ml glu.mmol/l	
1.0	0.01
2.0	015
3.0	0.35
4.0	0.5
6.0	0.75
10.0	6.3
12.0	9.4
16.0	10.3

Fig. 3: Relationship of Acetylhydrolase with glucose in IDDM

It is shown in fig. 1 that acetylhydrolase activity was not affected in the presence of the EDTA, a calcium chelator. This is in tandem with the fact that there no dependence of specific acetylhydrolase on Ca^{2+} and the absence of Ca^{2+} dependent enzyme in the urine sample which is capable of hydrolyzing the c–2 acetyl group of acetylhydrolases.

We observed a significant correlation between the degree of proteinuria and the log of acetylhydrolase ($r^2 = 0.4$ $P<0.001$). See fig 2.

DISCUSSION

The samples collected from the subjects were used to demonstrate the presence of acetylhydrolase activities in both non-diabetic and diabetic patients. Results elucidated the fact that Acetyhydrolase activity is not dependent on calcium evidenced by the non release of 3H from substrate following inclusion of EDTA. This observation tallies with the work of Kujiroaka et al. [6] It is understood that diabetic nephropathy ultimately progress to glomerular sclerosis resulting in renal dysfunction. It would appear that factors such as metabolic and haemodynamic response may modulate the process leading to stimulation of cytokines and growth factor in the glomeruli and tubules from the diabetic kidney. Moreover, the renal cortex is known to contain a hydrolase functioning uniquely for the deacetylation of PAF at the C–2 location, Serban et al. [7] Diabetes is connected with abnormalities of platelets functions. As shown by this study the elevation of Acetylhydrolase was concomitant with that of glucose.

Platelet Activating factor (PAF) Acetylhdyrolase is known to be a strong inducer of platelets aggregation and is elevated in insulin dependent diabetes as shown by measurement of the plasma PAF acetylhydrolase and lipoprotein level in both diabetic and non- diabetic patients. Data show Acetylhydrolase activity was markedly decreased in IDDM. This has been supported by the work of de Castro et al. [8] Similar to other renal enzymes present in urine, urinary acetylyhydrolase are believed to emanate from renal cells which is a reflection of the degree of physiological stimulation of tissue PAF degradation activity or other underlying pathophysiological state resulting in inflammation with a resultant decrease in PAF.

It is now known that even in the absence of aggressive glomerulonephritis or any systemic illness there is a consistent high excretion as confirmed by its activity thus, buttressing the fact that there is an intra-renal source of Acetyhydrolase production and inactivation. Previous studies have suggested that oxygen radicals may act synergistically with platelet activating factor to potentiate tissue injury. Since concomitant production of PAF and oxygen radicals can occur in various forms of tissue injury, inactivation of acetylhydrolase might represent one mechanism by which oxygen radicals may potentiate and prolong the inflammatory effect of PAF.

We observed significant proteinuria among the diabetics which correlated positively with the log Acetylhdrolase among diabetics with a significant correlation between proteinuria and log Acetylhydrolase activity (r^2=0.75 and 0.48) respectively. Heightened acetylhydrolase activity may be accountable due to response to the PAF – independent mechanism which promotes hypertension or may be a result of hypertensive related damage in the cells. We estimated the creatinine levels and correlated with log acetyl hydrolase ($r^2 = 0.30$). the mean value of creatinine for the diabetic subject was however 344 umol/L (not shown) which was far higher than the reference range (60-120µmol/L). Recent discovery of platelets playing significant roles in acid base balance and thrombosis as well as key regulators in inflammation tends to lend credence to the fact that platelets stored for transfusion has the ability to produce pro-thrombic and proinflamtory mediators implicated in adverse transfusion reaction, a fact supported by previous workers such as Mclntyre et al , Mello et al and Wen et al . These mediators are key players in the pathological condition including vascular attack which is a major cause of death in diabetes. [9-11]

We may deduce here that the study of acetylyhydrolase activity may elicit the prognosis of an uncommon group of diabetic condition in whom specific pathological and functional role for acetyhydrolase can be highlighted. As earlier suggested by Kasarawa et al it may be possible to identify different pathological condition from PAF–Acetyledrolase. [12]

CONCLUSION

In conclusion, our studies have demonstrated increased acetylhyrolase activity in diabetics and suggests that urinary Acetylhydrolase activity may be a driving force that may exercise a role in the response by the Kidney to PAF stimulation and provide a measure of acetylhydrolase involvement in diabetes mealitus complications.

Acknowledgement:
The authors wishes to thank the Research Ethics committee of the Federal Medical Centre, Yenagoa, Bayelsa State Nigeria for granting our proposal for this study. We also recognize Mr. Fiezibe Ogboto and staff of Geotech Medical Laboratory, Yenagoa for their technical assistance.

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