



Journal of Biological Sciences

ISSN 1727-3048

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Assessment of Kidney Injuries and Other Diseases in Juvenile Diabetes

¹Hawazen Al-Amri, ²Sahira Lary and ²Taha Kumosani

¹King Fahad Medical Research Center, King Abdul Aziz University,
P.O. Box 80216, Jeddah 21589, Saudi Arabia

²Department of Biochemistry, Faculty of Science, King Abdul Aziz University,
P.O. Box 80203, Jeddah 21589, Saudi Arabia

Abstract: The increasing reports of Insulin Dependent Diabetes Mellitus (IDDM) among the children of Saudi Arabia are of due scientific concern. Random mid-day urine samples were obtained from 30 diabetic patients (15 males, 15 females) and 50 subjects (25 males, 25 females) not under medication and without clinical evidence of renal disease or diabetes mellitus were used as controls. Two urinary enzymes, N-acetyl-beta-D-glucosaminidase (NAG) and Alanine Aminopeptidase (AAP) were measured in the urine, together with microalbuminuria, total protein and Creatinine (Cr) concentrations. In diabetic patients, NAG, Microalbuminuria (MAU) and Total Protein (TP) were found to be elevated which indicated that these children were prone to kidney damage in the future. However, as these diabetic children had not yet developed any clinically measurable kidney damage, therefore other parameters such as, Alkaline Phosphatase (AP), AAP and urinary creatinine were not elevated. This elevation in the enzyme-levels was most probably due to dysfunction of their kidneys. Urinary NAG assay therefore could be used as an alternative measurement of renal dysfunction. The assay coupled to the logistic regression equation described herein, is expected to be a useful clinical tool for predicting incipient nephropathy in adults and children. A routine check-up focused on the markers mentioned in this study might help the patients in practicing caution to avoid any future health threat posed by diabetes and associated kidney damage.

Key words: Urinary N-acetyl- β -D-glucosaminidase, alanine amino peptidase, microalbuminuria, Insulin dependent diabetes mellitus, kidney injuries

INTRODUCTION

According to Crook (2006) Diabetes Mellitus (DM) is caused by an absolute or relative insulin deficiency. Type-1 diabetes is also called juvenile-onset diabetes or Insulin Dependent Diabetes Mellitus (IDDM) and accounts for only 5-10% of those with diabetes (American Diabetes Association, 2005). The prevalence of diabetes mellitus in the majority of developing countries is 1-2% (Bennet, 1983). In Saudi Arabia the prevalence of diabetes mellitus is very high and it is expected to become 40-50% by 2020 (El-Hazmi and Warsy, 1989). Especially, there is a high prevalence of obesity in school children (Al-Khader, 2001). Diabetic nephropathy occurs in approximately 1/3rd of individuals with insulin dependent diabetes mellitus and non insulin dependent diabetes mellitus (Rehman *et al.*, 2005). The prevalence of diabetic complication was low (66%) in diabetic patients with family history of diabetes as compared to diabetic patients without family history of diabetes (79%) (Hameed *et al.*, 2002). Renal complications have received little attention (El-Hazmi and Warsy, 1989) and detailed information on diabetes mellitus in Saudi Arabia is not yet available,

therefore, further studies are required (El-Hazmi, 1990). However, thrifty gene hypothesis, increased energy intake (kcal day^{-1}) and first cousin marriage could provide a reasonable explanation for this dramatic rise in DM in Saudi Arabia (Al-Khader, 2001). Evaluation of urinary enzyme is a variable tool in the diagnosis of impaired renal function (Dubach and Schmid, 1979; Jung *et al.*, 1992). Renal damage initially occurs in a specific region of the Nephron (Jung *et al.*, 1988) and in most instances the damage is irreversible (Price and Whiting, 1992). Most commonly used renal function tests are insensitive and fail to identify early lesion while biopsy sampling is not recommended in most of the cases except in the presence of a well-established renal disease. Therefore, a sensitive, non-invasive marker is required for early detection of renal injury (Price and Whiting, 1992).

Abnormality of the enzyme metabolism system leads to a number of metabolic diseases. It is shown that many diseases associate with many components of the enzyme metabolism systems are now widely applied in clinical examinations as special markers for diseases (Raja *et al.*, 2011). Urinary N-acetyl-beta-D-glucosaminidase (NAG) increases before the appearance of severe proteinuria in

insulin-dependent diabetes mellitus. Urinary NAG is related to blood glucose levels and it decreases to normal levels when the blood glucose level is well controlled (Hsiao *et al.*, 1996). Some authors have observed that urinary NAG did not change with recombinant human growth hormone treatment in short children who suffered from growth hormone deficiency. On the other hand, some studies have reported increased levels of urinary NAG when proximal tubular cells are injured due to any disease process including glomerular proteinuria, hyperglycemia and transplant rejection (Sasaki *et al.*, 1996; Kavukcu *et al.*, 2002). Increased NAG enzymatic activity in urine has been reported to be associated with various kidney injuries (Price, 1992a). Furthermore, it is considered to be a sensitive marker of renal diseases, an early warning of rejection after renal transplantation (Simeoni *et al.*, 1994) and a sign of drug nephrotoxicity (Wellwood *et al.*, 1975). Blood glucose and urinary NAG excretion have also been reported to be increased in patients associated with poor glycemic control (Stefanovic *et al.*, 2003). In previous studies, urinary NAG was found to be responsive to acute changes in blood glucose levels over 7 days before the collection of urine (Stefanovic *et al.*, 2003). Alanine aminopeptidase (AAP) is a superficial component of the luminal plasma membrane and it is located in the brush border membrane along the nephron (Price and Whiting, 1992). AAP is used as a specific biomarker for kidney damage and may be as a tool to diagnose kidney disorders. This is because it is found in high levels in the urine when the tubular cells are damaged (Jung *et al.*, 1988). Urinary NAG was found to be highly elevated in normotensive as well as hypertensive patients, while urinary AAP was found to be more elevated in hypertensive patients compared to normotensive patients (Lary, 2004). Alkaline phosphatase are a group of isoforms which hydrolyse many types of phosphate esters. In both humans and animals, the major sources of ALP are the liver, bone, kidney and placenta (Raja *et al.*, 2011). Two distinct isoenzymes of Alkaline Phosphatase (AP) are found in the kidney cortex but not in medulla (Burdmann *et al.*, 1994). Alkaline phosphatase AP is present in the luminal plasma membrane in the proximal tubule (Cohn and Roth, 1996).

In the current study, Urinary N-acetyl-beta-D-glucosaminidase with other urinary enzymes that provide an early indication of renal damage was assayed. The commonly used parameters indicative of the presence of diabetes were measured as well.

MATERIALS AND METHODS

The present study was conducted on a total of 80 subjects. Ethical approval was granted to this work by

Directorate of Health Affairs Jeddah, Ministry of Health, Kingdom of Saudi Arabia, (Dated: 8 April 2008). The protocol followed for the study was in conformity with the latest revised draft (2008) of the 'Declaration of Helsinki'. The study subjects were divided into 2 groups, as follows:

- **Group I: Non-diabetic subjects:** The control group consisted of fifty normal subjects (25 males and 25 females), aged 8.34 ± 2.85 years, who were school students without any clinical evidence of renal disease or diabetes mellitus. None of these controls were taking drug medication. The body mass index was 16.50 ± 6.53 kg m⁻² among males and females
- **Group II: Diabetic patients:** Thirty IDDM patients were studied including 15 males and 15 females. The age of the diabetic subjects was 10.10 ± 2.37 years. The patients were attending the Al-Aziziah Maternity and Children Hospital. The body mass index was 17.66 ± 5.98 kg m⁻². The HbA1c values were 10.88 ± 3.19 mg dL⁻¹ among males and females

Methods: The urine samples were divided into three aliquots; one (5 mL) was stored at -20°C until required, while the second (3 mL) was stored in 30% glycerol for the assay of alanine aminopeptidase (AAP) and the third sample was used immediately for urinary NAG activity ($\mu\text{mol/MNP released/h/L}$) assay. The third sample was assayed for NAG activity without freezing (Yuen *et al.*, 1984), using a commercially available colorimetric kit based on MNP-GlcNAc substrate (PPR Diagnostics Ltd., London E1 9AT, UK). Urinary creatinine concentration (mmol L^{-1}) was determined using the Bonsens and Taussky method (Bonsnes and Taussky, 1945) based on the Jaffe' reaction (Bradford, 1976). The determination of creatinine was carried out in order to correct for variations caused by changes in urine flow (Wellwood *et al.*, 1975). The AAP activity (μmol^{-1} Cr) was determined according to the method of Mattenheimer *et al.* (1992). Alkaline phosphatase was measured using the modified method described by Pollard *et al.* (1990). Urinary total protein (mg mmol^{-1} Cr) was assayed according to the method of Bradford (1976) using Coomassie Brilliant Blue G-250 (Merck Chemicals Ltd., Poole, England). The microL test strips (Boehringer Ltd.) were used to detect microalbuminuria (mg L^{-1}). This test allows specific detection of human albumin (<200 mg L⁻¹). The intensity of the dye is checked exactly after five minutes, the intensity being directly proportional to the albumin (mg L^{-1}) content of the urine. Urine pH and glucose (mg L^{-1}) were detected using Combur-Test strips (Boehringer Ltd.). Glycosylated hemoglobin (HbA1c) was determined using an Abbott IMX glycated hemoglobin test (Little *et al.*, 1986; Middle *et al.*, 1983).

Statistical analysis: Significance of the association of study variables with status of the subjects (normal subjects versus diabetic subjects) was tested by Student's t-test or Fisher's exact test as appropriate. A two tailed p-value of <0.05 was taken as significant. Statistical analysis was performed by SPSS version 14.

RESULTS

The clinical and biochemical features of the studied groups are shown in Table 1. Urine samples from healthy Saudi subjects without any history of renal disease, hypertension or diabetes mellitus were found to be free of glucose, had normal PH, as well as NAG, AAP and AP activities. There was no microalbuminuria or overt proteinuria (Table 1) present. No significant differences were found between males and females and therefore the data was pooled for the whole group.

When Urine samples from 30 diabetic subjects were analyzed and compared with the control group (Table 2). NAG activities were found to be increased four-fold. AAP and AP activities displayed a three-fold increase each while microalbuminuria displayed a five-fold increase. Significant differences in levels of urinary NAG, TP, urinary glucose and HbA1c ($p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) were observed when compared diabetic group with non-diabetic control subjects.

Regression

Linear regression: Analysis for each group was carried out between NAG/Cr (as an independent variable) and AAP/Cr, AP/Cr, MAU/Cr and TP/Cr (as a dependent variable).

NAG/Cr and AAP/Cr: In normal subject the relationship is not significant ($p > 0.05$) and coefficient of determination is very low ($R^2 = 0.071$) that is to say that any increase in NAG/Cr value is not associated with an increase in AAP/Cr value. The data shows that the relationship of NAG/Cr and AAP/Cr in IDDM group is stronger than in increase levels of AAP/Cr in diabetic group whereas, $p < 0.05$ and coefficient of determination is high ($R^2 = 0.68$) (Fig. 1).

Table 1: Clinical and biochemical features of the control group (n=50) and diabetic patients (n=30), which is collected from the hospital files

Clinical and biochemical features	Control subjects	Diabetic patients
Body mass index (kg m ⁻²)	16.50±6.35	17.66±5.98
HbA1c (mg dL ⁻¹)	4.2-6.2	10.88±3.19
Retinopathy (%)	-ve	1
Neurological disease (%)	-ve	-ve
IDDM (%)	-ve	30
Glucose (mg dL ⁻¹)	70-180	356.57±173.24
Ca ⁺⁺ (mg dL ⁻¹)	7.6-11	8.79±0.057

NAG/Cr and MAU/Cr: In control subjects there is no significant difference between the two parameter ($p > 0.05$)

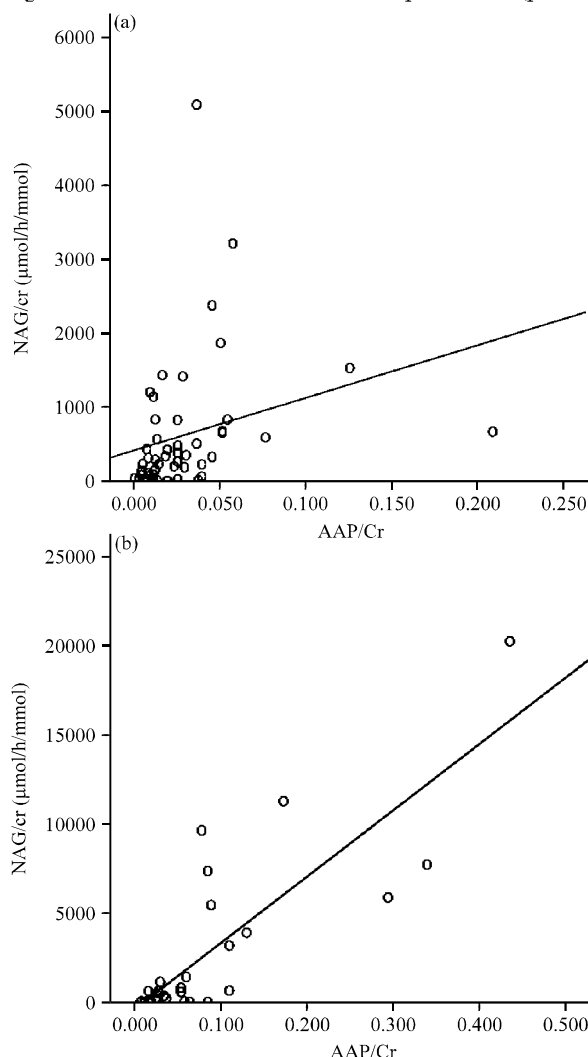


Fig. 1(a-b): Scattered plots of NAG/Cr vs. AAP/Cr in (a) Control subjects (p -value = 0.06, $R^2 = 0.07$) and (b) IDDM group (p -value = 0.001, $R^2 = 0.68$)

Table 2: Comparison between group I: (normal population) versus group II: (diabetic population)

Parameters	Group I	Group II	p-value
	n = 50	n = 30	
NAG (μmol/h/mmol creatinine)	628.03±911.78	2762.45±4581.74	0.001
AAP (U mmol ⁻¹ creatinine)	0.03±0.03	0.08±0.10	0.254
AP (U mmol ⁻¹ creatinine)	0.04±0.04	0.10±0.09	0.222
Total protein (mg mmol ⁻¹ creatinine)	0.00±0.00	0.10±0.01	0.001
Microalbuminuria (mg mmol ⁻¹ creatinine)	3.87±6.75	18.77±46.61	0.461
Glucose (mg dL ⁻¹)	All < 50	1998.20±4934.73	0.001
Urinary creatinine (mmol)	3.35±3.19	2.35±3.16	0.156
pH	6.04±1.12	5.53±0.73	0.022

and coefficient of determination is very low ($R^2 = 0$) so, the relationship is not linear at all, this means that there is no significant relationship between the two parameters. As for diabetic patients the linear regression may be considered fairly linear i.e., the relationship is not significant and coefficient of determination is low so, the relationship is not strong enough and increase in NAG/Cr was not associated with an apparent increase in MAU/Cr whereas, ($p > 0.05$ and $R^2 = 0.02$) (Fig. 2).

NAG/Cr and AP/Cr: In control subjects the relationship is not significant ($p > 0.05$) and coefficient of determination ($R^2 = 0.01$) is very low that is to say that any increase in NAG/Cr value is not associated with an increase in AP/Cr. The data shows for IDDM group that the relationship is linear and any increase in NAG/Cr is associated with an increase of AP/Cr whereas, $p < 0.05$ and $R^2 = 0.76$ (Fig. 3).

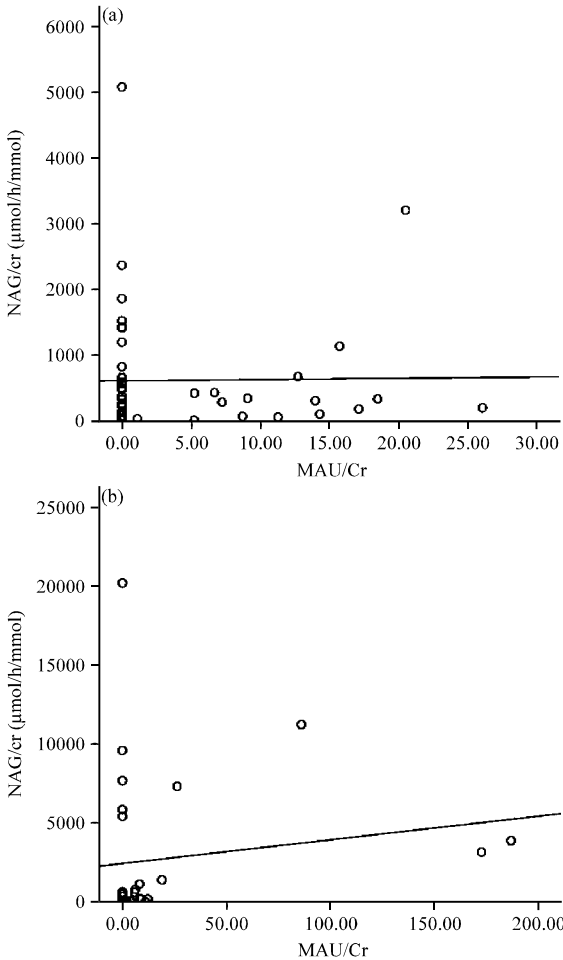


Fig. 2(a-b): Scattered plots of NAG/Cr vs. MAU/Cr in (a) Control subjects (p -value = 0.92, $R^2 = 0$) and (b) IDDM group (p -value = 0.42, $R^2 = 0.02$)

NAG/Cr and TP/Cr: In control group there is no significant association between the two parameters ($p > 0.05$) and coefficient of determination is very low ($R^2 = 0.05$) so, the relationship is not linear at all. This means that there is no strong relationship between the two parameters. As for diabetic patients the relationship is significant ($p > 0.05$) and coefficient of determination was very low ($R^2 = 0.05$) i.e., the relationship between the NAG/Cr and TP/Cr was not strong relationship and any increase in NAG is not associated with an apparent increase in TP/Cr (Fig. 4).

Logistic regression: This analysis can be adapted to include qualitative dependent variables (NAG) to

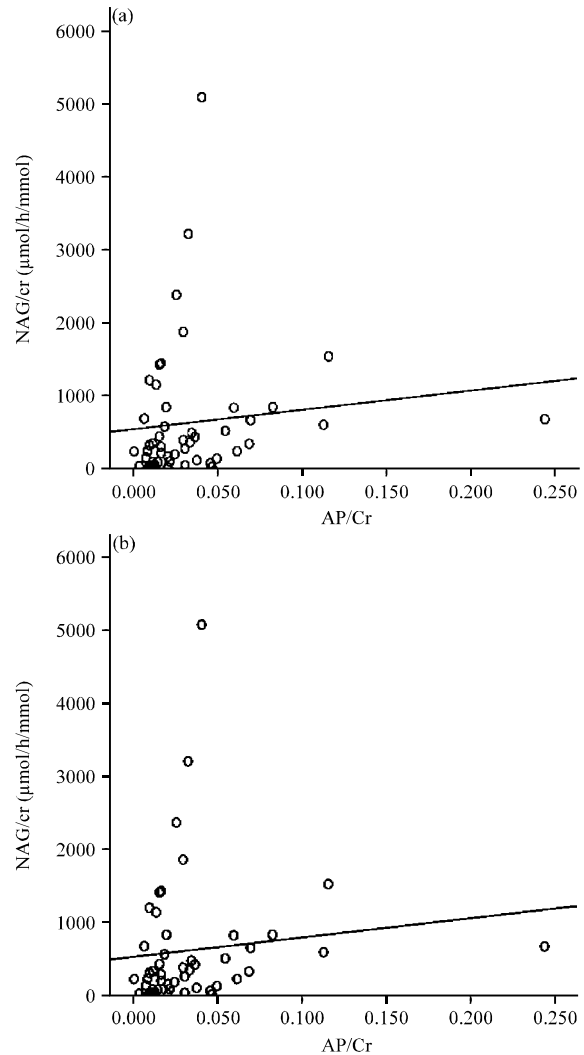


Fig. 3(a-b): Scattered plots of NAG/Cr vs. AP/Cr in (a) Control subjects (p -value = 0.42, $R^2 = 0.03$) and (b) IDDM group (p -value = 0.001, $R^2 = 0.76$)

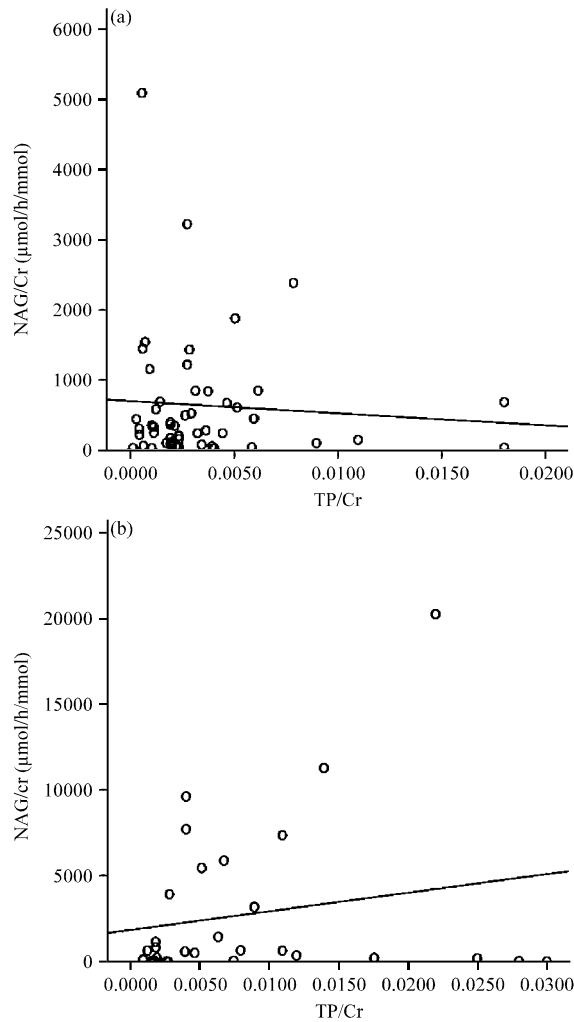


Fig. 4(a-b): Scattered plots of NAG/Cr vs. TP/Cr in (a) Control subjects (p-value = 0.62, R² = 0.05) and (b) IDDM group (p-value = 0.29, R² = 0.03)

Table 3: The values of coefficients (b-constant) for all variables, p<0.05

Variables	Logistic regression coefficients (b)
Age	-0.45
Urinary creatinine	-0.29
NAG	-0.00
AP	24.33
AAP	31.01
MAU	0.06
TP	-734.86
pH	-0.33
UGLu	0.00
Albumin	0.57
BMI	0.23
HbA1c	-0.01
Constant (a)	-54.84

dependent variables as seen below in order to predict the presence or absence of a characteristic or outcome based on the values of these dependent variables on

the other hand, it is also to see the probability of how much the individuals will be affected by a particular diseases.

$$\rho = e^{\hat{Y}} / (1 + e^{\hat{Y}})$$

So, The predicted \hat{Y} value for this individual using the values of the partial regression coefficients (b) for the corresponding variables and (a) is a constant which is equal -54.84 (Table 3) whereas, ρ has a value of 1 for the presence the disease but if ρ is equal zero the individual is normal.

DISCUSSION

Renal damage is an important and serious diabetic microvascular complication and is the leading cause of end stage renal disease (Ibrahim and Vora, 1999). In the present study, numbers of markers for diabetic nephropathy have been assayed in the urine of Saudi control group and Saudi patients suffering from insulin dependent diabetes mellitus. The parameters used for investigation are considered to be useful markers for diseases and are used for the first time among the Saudi children population. To our knowledge, this is the first report which describes possible early markers for assessment of diabetic nephropathy in Saudi children.

In clinical trials, decrease in creatinine clearance, increase in creatinine clearance and especially appearance of microalbuminuria are keys for diagnosis and treatment of diabetic nephropathy (Hong and Chia, 1998; Ruggenti *et al.*, 2004). But these markers are insensitive, unreliable, non specific and there is a time delay between renal injury and detection. Thus, if other biomarkers are found with improved specificity and sensitivity, this could reverse or prevent the onset of renal damage. For several years, many studies including *in vitro* and *in vivo* have demonstrated that excreted urinary enzymes are useful biomarkers for evaluation and diagnosis of tubular dysfunction or injury, especially NAG (Uslu *et al.*, 2005; Price, 1992b; D'Amico and Bazzi, 2003). Hence, tubular damage most likely precedes glomerular damage so, urinary enzyme excretion can be used (Uslu *et al.*, 2005). Our study showed that urinary NAG and TP were excreted higher in diabetic patients compared with control group (p<0.001 and p<0.01, respectively). This data agrees with (Fathy *et al.*, 2009; Basturk *et al.*, 2006; Calatlano *et al.*, 1996; Uslu *et al.*, 2005; Karakani *et al.*, 2007) which demonstrated an increase in urinary NAG in diabetic patients that indicate these diabetic patients had tubular

damage and may identify early diabetic nephropathy. Urinary NAG should be as useful in children as it is in adults for the detection of renal tubular disease, provided an accurate normal range is used. Increase in NAG excretion has been reported by several authors in diabetic patients (Jung *et al.*, 1988; Salem *et al.*, 2002; Piwowar *et al.*, 2006).

Microalbuminuria refers to the appearance of low but abnormal levels = 30 mg day⁻¹ or 20 µg min⁻¹ of albumin in the urine or an Albumin Creatinine Ratio (ACR) 30 = mg g⁻¹ in spot urine. Patients with microalbuminuria are referred to as having incipient nephropathy (American diabetes association, Inc., 2004). Several studies has been identified a number of children with diabetes developing MAU before the onset of puberty (Moore *et al.*, 2000; Schultz *et al.*, 1999). In this study we found no significant difference in MAU between control group and diabetic patients (p>0.05). The presence of elevated NAG and microalbuminuria in all diabetic patients investigated, suggests that they have a high risk of developing diabetic nephropathy with the possibility of progressing to ESRD (UK perspective diabetes study IX, 1993). Microalbuminuria is also considered as an important marker of greatly increasing cardiovascular morbidity and mortality among the patients with type 1 diabetes and type 2 diabetes.

One finding in the current study was different remarkably from other reports. Marked decrease was encountered in AAP and AP due to the storage condition as these markers are very sensitive. The present study showed that no significant difference in AAP and AP (p>0.05, p>0.05, respectively) in diabetic patients compared with control group. Alanine aminopeptidase and alkaline phosphatase were not elevated because the kidney damage had not progressed yet.

The results obtained in the study for pH, urinary creatinine and the activity of N-acetyl-beta-D-glucosaminidase enzyme were in close agreement with the international reference ranges. Also no sex difference was recorded between males and females because the sex hormones are not active yet. It is a genuine observation that urinary glucose, HbA1c and plasma glucose increased in diabetic children because the pancreas fails to form and excrete insulin and this leads to accumulation of glucose in the body.

The parameters investigated may not be useful in diagnosing late stage of kidney failure but the parameters might be useful for monitoring the progress of the disease and the effect of the treatment of kidney failure in normal children and diabetic children. By using the logistic regression equation we can predict the percentage of population that might be expected to suffer from the disease in the future. In the logistic regression \hat{y} is calculated by adding all parameters and each multiplied by its own constant. In

order to calculate the probability of effect, the following equation is plotted:

$$\rho = e^{\hat{y}} / (1 + e^{\hat{y}})$$

were as ρ is a probability of effect. This equation can predict the percentage for occurrence of the disease in 20, 30 or 100%. The use of linear regression and according to coefficient of determination and p-value, there was a strong relationship between NAG and some other parameters, such as: AAP and AP in diabetic subject i.e., the increased level of NAG was associated with an increased level of AAP and also, increased the level of activity of AP in the body. In control subjects, no strong relationship was found between NAG and other parameters such as AP, TP and MAU.

CONCLUSION

Urinary NAG assay therefore could be used as an alternative measurement of renal dysfunction. The assay coupled to the logistic regression equation described herein, is expected to be a useful clinical tool for predicting incipient nephropathy in adults and children. A routine check-up focused on the markers mentioned in this study might help the patients in practicing caution to avoid any future health threat posed by diabetes and associated kidney damage.

ACKNOWLEDGMENTS

We acknowledge the support of Dr. Khairya Mohammed Moussa, (internal consultant at the Al-Aziziah Maternity and Children Hospital) for her continuous help and support and Dr. Rajaa Mohammad Al-Raddadi (Consultant Community Medicine) for his help. We, also acknowledge Professor R. Price, King's College London for his support.

REFERENCES

- Al-Khader, A.A., 2001. Impact of diabetes in renal diseases in Saudi Arabia. *Nephrol. Dialysis Transplantation*, 16: 2132-2135.
- American Diabetes Association Inc., 2004. Nephropathy in diabetes. *Diabetes Care*, 27: S79-S83.
- American Diabetes Association, 2005. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 28: S37-S42.
- Basturk, T., Y. Ituntas, A. Kurklu, L. Aydin, N. Eren and A. Unsal, 2006. Urinary N-acetyl-beta-D-glucosaminidase as an earlier marker of diabetic nephropathy and influence of low-dose perindopril/indapamide combination. *Ren. Fail*, 28: 126-128.

- Bennet, P.H., 1983. Diabetes in Developing Countries and Unusual Population. In: Diabetes in Epidemiological Perspective, Mann, J.I., K. Pyorala and A. Teuscher (Eds.). Churchill Livingstone, Edinburgh, UK., pp: 43-57.
- Bonsnes, R.W. and H.H. Taussky, 1945. On the colorimetric determination of creatinine by the *Jaffe* reaction. *J. Biol. Chem.*, 158: 581-591.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- Burdmann, E.A., T.F. Andoh, J. Lindsley, J. Russel, W.M. Bennett and G. Porter, 1994. Urinary enzymes as biomarkers of renal injury in experimental nephrotoxicity of immunosuppressive drugs. *Renal Failure*, 16: 161-168.
- Calatlano, C., P.H. Winocour, S. Parlongo, S. Gillespie and K.S. Alberti, 1996. Treasures of tubular function in normal albuminuria in insulin dependent diabetic patients and their relationship with sodium lithium co-center transport activity. *Nephron*, 73: 613-618.
- Cohn, R.M. and K.S. Roth, 1996. *Biochemistry and Disease: Bridging Basic Science and Clinical Practice*. Williams and Wilkins Inc., New York, USA., ISBN-13: 9780683020496, pp: 202-206.
- Crook, A.M., 2006. *Carbohydrate Metabolism in Clinical Chemistry and Metabolic Medicine*. 7th Edn., Hodder Arnold, London, pp: 174-197.
- D'Amico, G. and C. Bazzi, 2003. Urinary protein and enzyme excretion as markers of tubular damage. *Curr. Opin. Nephrol. Hypertens*, 12: 639-643.
- Dubach, U.C. and U. Schmid, 1979. *Diagnostic Significance of Enzymes and Proteins in Urine*. Huber, Bern, Switzerland.
- El-Hazmi, M.A.F. and A.S. Warsy, 1989. Hyperglycaemia in Saudi population: A comparative study in different regions. *Ann. Saudi Med.*, 9: 435-438.
- El-Hazmi, M.A.F., 1990. Diabetes mellitus present state of the Arab Saudi. *Med. J.*, 11: 10-17.
- Fathy, M.A., M.M. Elkady, H.A. Fathy, S.A. Awad and A.A. Elmenshawy, 2009. Estimation of renal tubular markers for predicting early stage diabetic nephropathy in Egyptian children with Type I diabetes mellitus. *Res. J. Med. Med. Sci.*, 4: 207-211.
- Hameed, A., S.A. Malik, F. Rabbi, A. Sharif and N. Ahmad *et al.*, 2002. Diabetic complications influence of age, sex, family history, glycemic control and obesity. *J. Biol. Sci.*, 2: 710-714.
- Hong, C.Y. and K.S. Chia, 1998. Markers of diabetic nephropathy. *J. Diabetes Complications*, 12: 43-60.
- Hsiao, P.H., W.S. Tsai, W.Y. Tsai, J.S. Lee, Y.K. Tsau and C.H. Chen, 1996. Urinary N-acetyl- β -D-glucosaminidase activity in children with insulin-dependent diabetes mellitus. *Am. J. Nephrol.*, 16: 300-303.
- Ibrahim, H.A. and J.P. Vora, 1999. Diabetic nephropathy. *Baillieres Best Pract. Res. Clin. Endocrinol. Metab.*, 13: 239-264.
- Jung, K., H. Mattenheimer and U. Burchardt, 1992. *Urinary Enzymes in Clinical and Experimental Medicine*. Springer-Verlag, London, UK., ISBN-13: 9780387531885, pp: 10-16.
- Jung, K., M. Pergande, E. Schinrte, K.P. Ratzmann and A. Lius, 1988. Urinary enzymes and low molecular mass proteins as indicators of diabetic nephropathy. *Clin. Chem.*, 34: 544-547.
- Karakani, A.M., S.A. Haghighi, M.G. Khansari and R. Hosseini, 2007. Determination of urinary enzymes as a marker of early renal damage in diabetic patients. *J. Clin. Lab. Anal.*, 21: 413-417.
- Kavukcu, S., A. Soylu and M. Turkmen, 2002. The clinical value of urinary N-acetyl- β -D-glucosaminidase levels in childhood age group. *Acta Med. Okayama*, 56: 7-11.
- Lary, S.A., 2004. Urinary enzymes and microalbuminuria as indicators of renal involvement in patients with diabetes mellitus in Saudi Arabia. *Saudi J. Kidney Dis. Transplantation*, 15: 18-26.
- Little, R.R., J.D. England, H.M. Wiedmeyer, E.M. McKenzie and R. Mitra *et al.*, 1986. Interlaboratory standardization of glycated hemoglobin determinations. *Clin. Chem.*, 32: 358-360.
- Mattenheimer, H., K. Jung and Grlsch, 1992. *Methods for Measuring Urinary Enzyme Activities*. In: *Urinary Enzymes in Clinical and Experimental Medicine*, Jung, K., H. Mattenheimer and Burchardt (Eds.). Springer-Verlag, Berlin, Germany, pp: 99-109.
- Middle, F.A., A. Bamister, A.J. Bellingham and P.E. Dean, 1983. Separation of glycosylated haemoglobins using immobilized phenylboronic acid. Effect of ligand concentration, column operating conditions and comparison with ion-exchange and isoelectric-focusing. *Biochem. J.*, 209: 771-779.
- Moore, T.H. and J.P. Shield, 2000. Prevalence of abnormal urinary albumin excretion in adolescents and children with insulin dependent diabetes: The MIDAC study. Microalbuminuria in Diabetic Adolescents and Children (MIDAC) research group. *Arch. Dis. Child.*, 83: 239-243.

- Piwowar, A., M. Knapik-Kordecka, I. Fus and M. Warwas, 2006. Urinary activities of cathepsin B, N-acetyl-beta-D-glucosaminidase, and albuminuria in patients with type 2 diabetes mellitus. *Med. Sci. Monit.*, 12: CR210-CR214.
- Pollard, J.W., M. Jahan and P.J. Butterworth, 1990. Characterization and expression of uterine and placental alkaline phosphatase in the mouse. *Reprod. Fer.*, 89: 735-742.
- Price, R.G. and P.H. Whiting, 1992. Urinary Enzymes and Nephrotoxicity in Humans. In: *Urinary Enzymes in Clinical and Experimental Medicine*, Jung, K., H. Mattenheimer and Burchardt (Eds.). Springer-Verlag, Berlin, Germany, pp: 203-216.
- Price, R.G., 1992a. Measurement of N-acetyl-D-glucosaminidase and its isoenzymes in urine: Methods and clinical applications. *Eur. J. Clin. Chem. Biochem.*, 30: 693-705.
- Price, R.G., 1992b. The role of NAG (N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. *Clin. Nephrol.*, 38: S14-S19.
- Raja, M.M.M., A. Raja, M.M. Imran, A.M.I. Santha and K. Devasena, 2011. Enzymes application in diagnostic prospects. *Biotechnology*, 10: 51-59.
- Rehman, G., S.A. Khan and M. Hamayun, 2005. Studies on diabetic nephropathy and secondary diseases in type 2 diabetes. *Int. J. Diab. Dev. Ctries.*, 25: 25-29.
- Ruggenenti, P., A. Fassi and A.P. Ilieva, S. Bruno and I.P. Iliev *et al.*, 2004. Preventing microalbuminuria in type 2 diabetes. *N. Engl. J. Med.*, 351: 1941-1951.
- Salem, M.A., S.A. El-Habashy, O.M. Saeid, M.M. El-Tawil, and P.H. Tawfik, 2002. Urinary excretion of n-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. *Pediatr. Diabetes*, 3: 37-41.
- Salem, M.A., S.A. El-Habashy, O.M. Saeid, M.M. El-Tawil, and P.H. Tawfik, 2002. Urinary excretion of n-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. *Pediatr. Diabetes*, 3: 37-41.
- Sasaki, H., N. Takeda, I. Kawamura, Y. Nishida, K. Kikuchi and M. Tanaka, 1996. Urinary albumin excretion in short children treated with recombinant human growth hormone. *Clin. Nephrol.*, 46: 6-9.
- Schultz, C.J., T. Konopelska-Bahu, R.N. Dalton, T.A. Carroll and I. Stratton *et al.*, 1999. Microalbuminuria prevalence varies with age, sex and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. Oxford regional prospective study group. *Diabetes Care*, 22: 495-502.
- Simeoni, U., B. Schnitzler, T. Massfelder, C. Hirt and C. Koehl *et al.*, 1994. Specific developmental profiles of lysosomal and brush border enzymuria in the human. *Biol. Neonate.*, 65: 1-6.
- Stefanovic, V., M. Rajic, S. Antic, M. Mitic-Zlatkovic, S. Stojiljkovic, M.A. Ivic and P. Vlahovic, 2003. Urinary PC-1 activity in patients with type 1 diabetes mellitus. *Ann. Clin. Biochem.*, 40: 235-238.
- Uslu, S., B. Efe and O. Alatas, N. Kebapci, O. Colak, C. Demirustu and A. Yoruk, 2005. Serum cystatin C and urinary enzymes as screening markers of renal dysfunction in diabetic patients. *J. Nephrol.*, 18: 559-567.
- Wellwood, J.M., B.G. Ellis, R.G. Price, K. Hammond, A.E. Thompson and N.F. Jones, 1975. Urinary N-acetyl-beta-D-glucosaminidase activities in patients with renal disease. *Br. Med. J.*, 3: 408-411.
- Yuen, C.T., P.R. Kind, R.G. Price, P.F.G. Prall and A.C. Richardson, 1984. Colorimetric assay for N-acetyl-beta-D-glucosaminidase (NAG) in pathological urine using the omega-nitrostyryl substrate: The development of a kit and the comparison of manual procedure with the automated fluorimetric method. *Ann. Clin. Biochem.*, 21: 295-300.