Is there an association between Diabetes Mellitus and Serum Creatinine Concentrations?

-Kassim Fawzi AbdulKareem,(MBCHB,M.Sc.) Pathology, FICMS Pathology, College /University of Basrah Iraq .
-Hassan Khalaf Ulaiwi,(BVMS/ DVM/ MSc.)Pathology, Veterinary Medicine College /University of Al-Qadissyia Iraq.
-Mohammed K. AbdulKareem, Medical Student 6th stage, Basrah University Iraq.

الخلاصة

الهدف من هذه الدراسةِ هو تقيّيمَ مستوى كرياتينين مصل الدم كإختبار تشخيصي لداء السكّري ولتَوضيح الإختلافِ في مستويات كرياتينين المصل بين الذكور والإناث المصابة بداء السكر.جلبت إثنان وثلاثون أرنبا مِنْ السوق المحليةِ، باوزان تتراوح من 1.2-2 كيلوغرام وإستعملت في التجربةِ. قُسّمتْ الحيوانات بالاعتمادً على الجنس إلى مجموعتين (ذكور واناث) وكُل مجموعة قُسّمتْ باعداد متساوية إلى مجموعة مصابة بداء السكر ومجموعة سيطرة.بينت النَتائِجُ إختلافَ هامً جداً في مستويات كرياتينين المصلَ بين المجاميع المصابة بداء السُكر ومجاميع السيطرةِ، وتبين ان مستويات كرياتينين المصلَ اخذة بالازدياد بشكل تدريجي بعد بدايةِ الاصابة بداء السكر، وكَشفَت النتائج أيضاً أنّ مستوى ارتفاع كرياتنن المصل في الذكور المصابة بداء السكر كَانَ اكثر اهمية احصائيا مِنْ المستوى في الإناث المصابة بداء السكر (تحت مستوى معنويةاقل من 0.05). ويُستَنتجُ من الدراسة بأنّ الزيادة غير الطبيعية لمستوى الكرياتنين تَحْدثُ في حالة اعتلال الكلى الناتج من الاصابة بداء السكركما ويرتفع مستوى كرياتنين المصل في الذكور إلى مستويات أعلى مِنْه في الاناث وذلك نتيجة كون كتلة العضلات في الذكور اكبر من الاناث.

Abstract

The aim of this study was to evaluate serum creatinine level as a diagnostic test in relation to diabetes mellitus and to clear the difference in serum creatinine concentrations between diabetic males and females. Thirty two rabbits purchased from the local market, weighing 1.2-2 kg were used in the experiment.

The animals were divided according to the gender into two groups and each was equally divided into diabetic and control group.The results revealed highly significant difference in the serum creatinine concentrations between diabetic groups and control groups, and the serum creatinine concentrations

gradually increased after onset of diabetes, also the result revealed that values in diabetic males was highly significant than diabetic females (P<0.05). It is concluded that abnormal results occur in diabetic nephropathy and males tend to have higher levels of creatinine because they have more muscle mass than females.

Introduction

Diabetes mellitus is a disease characterized by persistent hyperglycemia, resulting either from inadequate secretion of the hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors. Diabetes is a metabolic disease requiring medical diagnosis, treatment and lifestyle changes(Nathan *et al*., 2005).There are many causes and forms of diabetes known, the three most common patterns of diabetes have been recognized over the last thirty years as *type 1*, *type 2* and *gestational diabetes* (or *type 3*)(Sowers, 1995). Diabetes seems to be a group of different disorders but all diabetic patients seem to be subjected to the complications of diabetes including heart disease, blindness, cataracts, blood vessels damage , nerve disorders , and kidney damage (Kolat, 1979).Nephropathy is one of the most common microvascular complications of diabetes (Janssen, 2002). It is characterized by persistent proteinuria (preceded by microalbuminuria), declining renal function, an elevated arterial blood pressure, and a high risk of cardiovascular morbidity and mortality (Kelly *et al*., 2003). Diabetic nephropathy (DN) is a major complication of diabetes mellitus and is one of the main causes of death among humans, dogs and cats. Diabetic nephropathy can be diagnosed by several criteria like estimations of serum creatinine, urinary albumin (consider a microalbumin/creatinine level, which is more accurate) and Urine analysis (ADA, 1994 and 2004). Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass) (Gross *et al*., 2005).Creatinine is the end product of protein catabolism and its concentration in the blood and urine in the animals is not significantly influenced by diet (Coles, 1986).Creatinine is mainly filtered by the kidney, though a small

amount is actively secreted. There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, blood levels rise. As a result, creatinine blood levels may be used to calculate creatinine clearance (ClCr), which reflects the glomerular filtration rate (GFR). The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be *overestimated* because the active secretion of creatinine will account for a larger fraction of the total creatinine cleared (ADA, 2oo7). Because it is a non threshold substance as far as the kidney is concerned, i.e. it is infiltered by the glomerulus and not reabsorbed by the tubules, when there is severe renal damage a rise in blood creatinine occurs. The degree of this rise can be more accurately correlated to the extent of the kidney damage in chronic nephritis than in acute renal impairment ,in which excessive protein catabolism will artificially elevate the blood creatine value (Coles, 1986). Because Creatinine is a byproduct of muscle use, its production is expected to vary with body composition and activity. Edwards and Whyte (1959) reported a correlation between lean body mass and urinary CR of 0.65 from a group of 31 men and women .Belier and Schedl (1962) found the same measure to be 0.47–0.48 in 11 women and 0.53–0.55 in 51 men and reported correlations between urinary CR and weight and body surface area in24-h specimens. Muscularity also contributes to observed differences in CR concentrations. Men produce more CR than women and have a higher clearance rate *(*James *et al.,* 1988). Diets with substantial amounts of particular kinds of meat, such as beef, can also affect urinary CR concentrations. (Miller *et al.,* 2004). Meat contains creatine, the precursor of CR, which is quickly excreted and can cause considerable short-term CR increases in the hours after ingestion (Miller *et al.,* 2004). AL-Khafajy showed in his study blood urea and Creatinine levels increased significantly in the diabetic rabbit compared with he non-diabetic animals(AL-Kafajy, 1996).Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function (Gross *et al*., 2005). A rise in blood creatinine levels is observed only with

marked damage to functioning nephrons. A better estimation of kidney function is given by the creatinine clearance test(Vinik *et al*., 2004).Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race as suggested by the American Diabetes Association without a 24 hour urine collection(ADA, 2000).

Animals and Experimental Design

Thirty two rabbits (White Newzealand Rabbits) purchased from the local market, weighing 1.2-2 kg were used in the experiment. Animals were housed in cages with dimension (130×100×70) under 12/12 h light/dark cycle at 25±2 c & 60% relation humidity with standard granulated food, & water available *ad libitium.* The animals were divided according to the gender into two equal groups:

1. Male group: includes (16) rabbits , which are subdivided into two subgroups:

A. Diabetic group denoted by (Dm) includes (8) rabbits.
B. Control groups denoted by (Cm) includes (8) rabbits.

2. Female group: includes (16) rabbits , which are subdivided into two groups:

A. Diabetic group denoted by (Df) includes (8) rabbits.
B. Control group denoted by (Cf) includes (8) rabbits.

Animals were left (1) month for adaptation. The animals were given Clopidol 0.06 mg/kg with feed as a prophylactic drug against coccidiosis during adaptation period.

Induction of Diabetes Mellitus

Diabetes mellitus was induced in over night fasting rabbits by a single injection of alloxan (alloxan monohydrate) at dose 100 mg /kg via marginal ear vein .Each 100 mg of alloxan was diluted in 1 ml of 0.9% normal slain (Lukens , 1948).Immediately, after

alloxan injection, 10 ml of 20% glucose i.v & 5 ml of 20% glucose i.p was given to the rabbits in order to overcome sudden decrease in blood glucose level (hypoglycemia).The rabbits were prevented from feeding for 12 h and the drenching water replaced by 5% glucose for 24h.The procedures of administrations and blood collection made under sedation of animals by using kitamin 44 mg /kg and xylazine 5 mg/kg. The control groups were I.V injected with 1 ml of 0.9 % of normal slain (EFPIA & ECVAM, 2000).

Blood Collection

The blood was collected according to the following equation: Total blood volume (TBV) =6% of lean body weight, maximum blood collection =20%of total blood volume every two weeks (McGill and Roman, 1989). {Animal weight in kg×o.o6×o.o2×1000= () ml.}

The blood collected at the following periods: Zero day (before the injection of alloxan), three days, ten days, twenty days, thirty days and forty days after injection of alloxan.

The blood was collected in a sodium fluoride containing test tubes from marginal ear vein with empty stomach. The collected blood was centrifuged to obtain serum. The serum used immediately for checking Fasting serum glucose (FSG); the remaining serum was kept in deep freezing (20- c) for checking serum Creatinine.

Estimation of Fasting Serum Glucose

After 3 days of alloxan injection the animals were fasting overnight and bled for checking the hyperglycemia .Fasting serum blood glucose (FSG) was measured by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), then the (FSG) concentrations were checked every 10 days .

Estimation of Serum Creatinine (by Jaffes method)

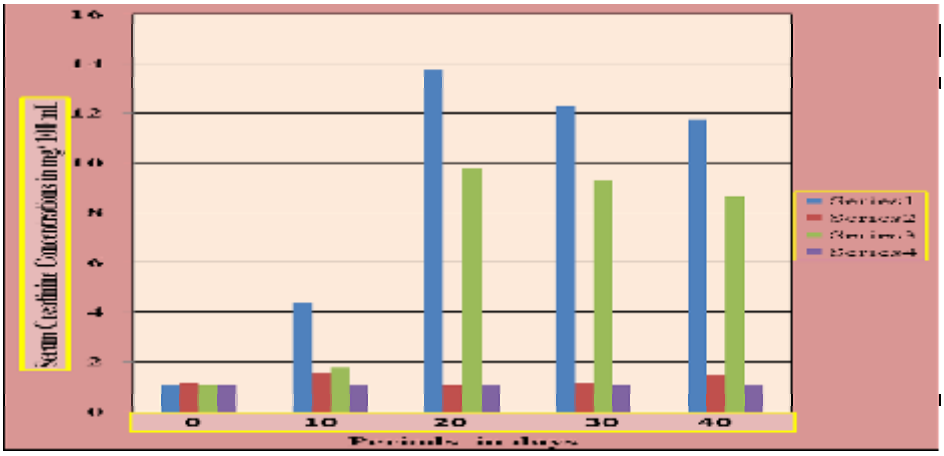
Serum Creatinine was estimated every 10 days intervals from the beginning of the experiment by using Jeffers methods. (Dandekar & Rane, 2004).

Results

The initial normal fasting serum glucose concentration had an average value of 91.37±8.01mg/dl in males and 93.56±8.20 mg/dl in females. On day ten after alloxan injection the concentration had increased to 297.25±8.13 mg/dl in males and 164.58±90.09 mg/dl in females. It reached its peak level of 443.04±37.32 mg/dl in males and 327.09±255.35mg/dl in females, on the thirty day. The level decreased to 335.19±80.41in males and 186.86±111.92 in females, on day forty.

The serum Creatinine concentrations in control groups had the total average value of 1.13±0.31 mg/100 ml in males and 1.06±0.33 mg/100 ml in females. The initial concentrations of diabetic groups in zero days were 1.13±0.33 mg/100 ml in males and 1.09±0.30 mg/100 ml in females. Ten days later the concentrations were 4.37±1.90 mg/100 ml in males while remained in the normal values 1.76±0.99 mg/100 ml in females. Twenty day the values reached 13.75±3.4 mg/100 ml in males and 9.80±9.16 mg/100 ml in females. Thirty day later the values were 12.25±0.50 mg/100 ml in males and 9.32±8.90 mg/100 ml in females. Forty day later the values were 11.75±1.76 mg/100 ml in males and 8.62±8.51 mg/100 ml in females.

The result revealed that there were highly significant difference in serum Creatinine concentrations between males and females at ($P<0.000$), the total average values in males was 7.09±5.61 mg/100 ml and 4.85±6.88 mg/100 ml in females. Also the result revealed significant difference between the groups and days at ($P<0.000$). The result revealed that the average values reached its peak in the twenty day intervals.



Serum Creatinine Concentrations in (Dm,Cm,Df and Cf) in mg/100 mL .

Series 1: Represente diabetic males (Dm).
Series 2: Represente control males (Cm).
Series 3: Represente diabetic females (Df).
Series 4: Represente control females (Cf).

Discussion

A significant difference was found in the serum Creatinine concentrations between diabetic groups and control groups. Our results are in accordance with the results of Al-Khafajy, (1996) and Al-Mashhaddany, (1999) that showed significant difference in serum Creatinine levels between diabetic groups and control groups. The results also revealed significant difference between males and females. Creatinine is water-soluble and distributes throughout the body water, equilibrating between various fluid regions in approximately 4 hours with the help of Na/Cl transporters on cell membranes. This equilibration time is longer than that for urea, which equilibrates within 1½ hours (Latimer *et al*., 2003).Creatinine is freely filtered by the renal glomerulus (Stockham and Scott, 2002). The serum creatinine concentration can vary based on a number of factors including an animal's diet, muscle mass, and gender (Watson and Lefebvre, 2003). Diets that contain high concentrations of muscle offer a large pool of creatine and creatinine that are absorbed in the small intestine and contribute to the serum concentration of creatinine (Thoresen

et al., 1992). Muscle mass harbors the precursor of creatinine, phosphocreatine as an energy source. This compound is often mistakenly referred to as "phosphocreatinine." A constant amount of phosphocreatine is spontaneously, irreversibly and nonenzymatically converted to creatinine daily and utilized by the body. This amount is directly proportional to the individual's muscle mass. Therefore, a stable amount of creatinine is presented to the kidneys daily for excretion (Kaneko *et al.*, 1997). An increasing muscle mass from conditioning or exercise will result in an increase of phosphocreatine and serum creatinine. Males often have higher creatinine values than females, as well (Latimer *et al*., 2003). This finding is most likely due to their typically increased muscle mass as compared to females. Serum creatinine values also depend on the kidney's ability to excrete creatinine. An elevation in creatinine is called azotemia and usually occurs simultaneously with an increase in blood urea nitrogen, a compound that is also freely filtered by the glomerulus. This can be due to prerenal, renal or postrenal processes causing a decrease in glomerular filtration rate (GFR). Disease processes like pyometra, gastric dilatation / torsion, diabetes mellitus and hypocalcaemia of malignancy also can create secondary renal injury and elevate serum creatinine levels (Braun *et al.*, 2003). Stack, (2004) was indicated that the most common cause of end-stage renal disease (ESRD) is diabetes mellitus. Hyperglycemia is an independent risk factor for diabetic nephropathy. The pathophysiology of diabetic nephropathy is a process that involves both hemodynamic and glucose-dependent factors, including the accumulation of glycated products, endothelial dysfunction, and loss of intraglomerular BP regulation (Snively, 2004). In the early stages of diabetic nephropathy, the kidney adapts by increasing the filtration rate in the noninjured nephrons. This adaptive hyperfiltration allows for normal or near-normal serum creatinine (sCr) levels and is asymptomatic. The American Diabetes Association revealed that Serum creatinine is very important test for the estimation of GFR and was defined cutoff values for the spot urine albumin-to-creatinine ratio for microalbuminuria as 30 mg of albumin to 1 g of creatinine and for

the spot urine albumin-to-creatinine ratio for albuminuria as 300 mg of albumin to 1 g of creatinine (ADA, 2003). A declining glomerular filtration rate (GFR) correlates with decline in renal function. The GFR is the measurement of the total filtration rate of all the renal nephrons (Manjunath *et al.*, 2001). Chronic Kidney Disease is defined as a GFR less than 60 mL/min/1.73 m^2 for 3 months or more (Remuzzi and Bertani, 1998). This GFR corresponds to a sCr concentration higher than 1.5 mg/dL in men and higher than 1.3 mg/dL in women (Snively and Gutierrez, 2004). Progressive decline in renal function occurs when renal injury raises the sCr level to 1.5 to 2 mg/dL (Neal and Greene, 2002).Creatinine production is expected to vary with body composition and activity. Muscularity also contributes to observed sex differences in Creatinine concentrations. Men produce more Creatinine than women and have a higher clearance rate (Miller *et al.,* 2004). Maeda, (2003) revealed that plasma Creatinine and blood urea nitrogen concentrations gradually increased after onset of diabetes and this accordance with our result. Creatinine can give men abnormal amounts of bloating and gas. Men tend to have higher levels of creatinine because they have more skeletal muscle than women (ADA, 2007). Gross *et al*.(2005) indicated that the typical reference ranges are 0.5 to 1.0 mg/dL (about 45-90 µmol/l) for women and 0.7 to 1.2 mg/dL (60-110 µmol/l) for men, while a baseline serum creatinine of 2.0 mg/dL (150 µmol/l) may indicate normal kidney function in a male body builder, a serum creatinine of 0.7 mg/dL (60 µmol/l) can indicate significant renal disease in a frail old woman. Danderkar and Rane (2004) revealed that a creatinine clearance test measures how well creatinine is removed from your blood by your kidneys. A creatinine clearance test gives better information than a blood creatinine test on how well your kidneys are working. A creatinine clearance test is done on both a blood sample and on a sample of urine collected over 24 hours (24-hour urine sample). (Maschio *et al*., 1996).

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