ASSESSMENT OF SEX DIFFERENCES IN SERUM UREA AND CREATININE LEVELS FOLLOWING AN ACUTE SPINAL CORD INJURY IN ALBINO RAT MODELS

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ABSTRACT
Background: One of the most serious consequences of spinal cord injury (SCI) is progressive deterioration of renal function mostly as a result of urine stasis and ascending infection of the paralyzed bladder. Several studies have reported variable changes in serum urea and creatinine especially in people with chronic SCI. However, there is paucity of information on sex related differences in these parameters in response to SCI.
Methods: A total of 24 adult albino rats weighing above 150g were divided equally into two groups, a control and experimental group (n = 12) each containing equal number of male and female rats. The experimental group animals were paralyzed by complete transection of spinal cord below T4 level after deep anesthesia with ketamine 75mg/kg. Blood samples were collected from both groups 5 days post SCI for analysis. Mean values of serum urea (mmol/L) and creatinine (µmol/L) for both groups were compared. $P < 0.05$ was considered as significant.
Results: The results showed significantly higher levels ($P < 0.05$) of serum urea and creatinine in the male SCI models with mean values of 92.12 ± 0.98 and 2573 ± 70.97 respectively compared with their controls where the mean values for serum urea and creatinine were 6.31 ± 1.48 and 476. 95 ± 4.67 respectively. In the female SCI models, serum urea 13.11 ± 0.81 and creatinine 519.88 ± 31.13 were not significantly different from that of female controls with serum urea and creatinine levels of 11.71 ± 1.43 and 493.69 ± 17.10 respectively ($P > 0.05$).
Conclusion: Spinal cord injury caused a significant increase in serum Urea and Creatinine levels in the male models compared to the females. This indicated that males might have higher risk of renal dysfunction following SCI.

INTRODUCTION
Spinal cord injury (SCI), a medically complex and life disrupting ailment, has remained a terminal condition especially in developing countries where people with SCI die within a few years of injury (Nwadinigwe *et al.*, 2013). Even in developed countries SCI and paralysis remain a significant cause of disability (Malhotra *et al.*, 2010). It is an important public health problem requiring lifelong treatment and high cost care which negatively affect the patient, his/her family and their community at large. Unfortunately, there is still no single accepted universal treatment for SCI to date. This is why many studies are still on going on the pathophysiological changes taking place in different tissues of the body post SCI (Yılmaz *et al.*, 2014). One of the most serious consequences of spinal cord injury is progressive deterioration of renal function as a result of the ascending infection of the paralyzed bladder. Although the advances made in the management of paraplegics since World War II have dramatically reduced the early high mortality from renal infection experienced earlier, there is still high mobidity and mortality in the late

stages related to impaired renal function and failure (Doggart *et al.* 1964).

Renal function is a predictor of survival in the general population and in patients suffering from cardiovascular diseases such as heart failure, myocardial infarction, and those undergoing surgical procedures (Mostofsky *et al.*, 2009). Individuals who have spinal cord injury (SCI) are at increased risk of developing renal insufficiency, because of neurogenic bladder dysfunction (Elmelund *et al*., 2014).

There are many studies that reported decrease in Glomerular filtration rate (GFR), in patients with chronic spinal cord injury (Rodríguez-Romero *et al*., 2013). Some studies conducted on plasma creatinine and urinary excretion of creatinine in patients with chronic spinal cord injury reported a decrease in plasma creatinine when renal function is normal. This is attributed to the decrease in muscle mass due to prolonged disuse atrophy. Urinary creatinine was also said to be reduced in chronic spinal cord injury due to decrease production, provided the renal function is normal or less severely impaired (Burr *et al.* 1993). Despite the fact that serum creatinine level is affected by muscle mass and metabolism, its measurement has still remained a commonly accepted method in assessing renal function (Elmelund *et al*., 2014).

Despite the devastating effects of SCI, many researchers focus more on changes in serum urea and creatinine months and years after the injury. There is also paucity of information on sex related differences associated with such changes following SCI.

MATERIALS AND METHODS

Animals and Conditions

A total of 24, comprising of 12 males and 12 females adult albino rats, weighing 150 to 200 grams, ages ≥ 120 days, were obtained from the animal house, Department of Human Physiology, Bayero University Kano. All the rats were housed in plastic cages with length, width and height of 41.5cm, 33.3 cm and 13 cm respectively, four rats per cage, with adequate ventilation and maintained at temperature of between 27^0C to 32^0C inside the animal house. They were allowed free access to standard laboratory rat chaw and tap water ad libitum throughout the length of the study. All the procedures involving the handling of the animals were performed according to the ethical guidelines for animal care and handling (IACUC, 2011).

The rats were randomly divided into two groups containing equal number of males and females.

Group 1: (Control) comprising of 6 males and 6 females albino rats weighing 150 to 200 grams aged ≥ 120 days and Group 2: (SCI models) also comprising of equal number (n = 6) of males and females albino rats with the same weights and ages as in group one. Each group is further subdivided into two subgroups (a) and (b) based on their sex as females and males respectively. The animals in group (2) were subjected to complete transection of spinal cord below T4 level.

Spinal Cord Injury Model

The rats were allowed to acclimatize to the environmental condition for one week before the induction of SCI. They were allowed access to food and water until one hour prior to the anaesthesia (IACUC, 2011). At the time of the procedure, the rats in group 2 were anaesthetized with Ketamine 75mg/kg body weight via intra-peritoneal injection. The dorsum of each animal was then shaved and a longitudinal incision was ensured through the skin. The dorsal surface of the spinal cord was then exposed and the spinal cord was transected completely below T4 with a sharp blade using a magnifying lens (Yang *et al*., 2015). After the procedure, the overlying muscles were sutured with 4 – 0 silk.

Post-Operative Care

The rats were placed in warmed and clean cages for recovery 1 rat/cage. They were given Carprofen 5mg/kg S.C 12 hourly for 48 hours (IACUC, 2011). They were then monitored 2 – 3 times daily for general health, coat quality, mobility within the cage and signs of paralysis such as; hind limb paralysis, tail flaccidity and uncoordinated movements (Sharp *et al*., 2014).

Urinary bladder manually voided 2 - 3 times daily until recovery of function as needed (Yang *et al*., 2015). After recovery from anesthesia, the rats were allowed free access to water and food as before the procedure.

Five days following SCI induction; the rats were anaesthetized using Ketamine as described earlier. Blood samples were collected in plane bottles for serum urea and creatinine analysis.

Urea was measured using the Randox assay kits. The assay was based on Berthelot's reaction, in which ammonia liberated or released from urea by Urease react with phenol and hypochloride to produce a blue colored solution (Indophenol). First, urea is hydrolyzed in the presence of urease to form ammonia and carbon dioxide. The liberated ammonia then reacts with hypochlorite and phenol to form indophenol (blue colored solution). The absorbance of the solution was then read at 630nm (Fawcett and Scott, 1960).

Urea + H_2O = $2NH_3$ + CO_2.

NH_3 + hypochlorite + phenol = indophenol (blue compound).

Creatinine was also estimated using Randox kits based on Jaffe's reaction. The principle was based on the reaction of Creatinine with alkaline picric acid to form a coloured complex, which is directly proportional to the concentration of Creatinine (Jaffe, 1886)

Statistical Analysis

Data were presented as mean ± SEM and analyzed using SPSS version 20.0 (SPSS Inc, Chicago, U.S.A). Independent sample t test was used to compare the mean serum urea and creatinine levels between group 2(a) (female SCI models) and 1(a) (female controls) as well as between group 2(b) (male SCI models) and 1(b) (male controls). Values $P < 0.05$ were considered as significant.

Table 1: Serum Urea and Creatinine levels 5 days after Spinal Cord Injury (SCI) in Albino Rats (Mean ± S.E.M)

Parameters	Group 1 (Controls)	Group 2 (SCI models)	P value
Serum urea (mmol/L)			
(a) Females	11.71 ± 1.43	13.11 ± 0.81	0.43
(b) Males	6.31 ± 1.48	92.12 ± 0.98[s]	0.000
Serum creatinine (µmol/L)			
(a) Females	493.69 ± 17.10	519.88 ± 31.13	0.48
(b) Males	476.95 ± 4.67	2573 ± 70.97[s]	0.000

[s] = significant compared to the control ($p < 0.05$)

The results of serum urea (mmol/L) and creatinine (µmol/L) assessment obtained from the albino rats five days post SCI, were displayed in table above. It shows that, the mean serum urea and creatinine level (mmol/L) were significantly higher ($P < 0.05$) in group 2(b) (male SCI models) compared to group 1(b) (male controls) In the female rats, the serum urea and creatinine obtained from 2(a) (female SCI models) were not significantly different from that of 1(a) female controls.

DISCUSSION

The results obtained in this study indicated slightly higher levels of urea and creatinine (not significant) in the female SCI models. This might be due to catabolism of the paralyzed muscles causing increase in creatinine production which is a by-product of muscle catabolism (Scelsi, 2001; Gorgey *et al*., 2014). Slightly higher levels of urea might be as a result of increased turnover of amino acids resulting from muscle tissue catabolism. This might cause increase in ammonia (NH_3) production via deamination of amino acids and finally increased urea production in the liver through the entry of ammonia into urea cycle. The mean serum urea and creatinine in the female SCI models were not significantly different from that of the control. This has indicated that, within the first week following complete transection of spinal cord, the female rats had no or less impairment in renal function.

Contrary to the findings in the female SCI models, the male SCI models had significantly higher levels of serum urea and creatinine than their controls. It is expected that the males might have higher levels of creatinine and urea because of more muscle bulk and therefore more creatinine production through catabolism and more urea production through amino acid turn over as explained above. However, it is not expected that these levels will be significantly different up to 5 and 15 times higher than that of the controls (normal male rats) in case of serum creatinine and urea respectively. Many studies have reported that, despite increase in muscle breakdown in individuals with SCI the serum creatinine remains normal or even decreased in chronic SCI provided there is no or less renal impairment (Burr *et al.* 1993; Elmelund *et al*., 2014). Therefore, our results indicated that the male SCI models might have compounding impairment in renal function which contributed to these higher levels of serum urea and creatinine. It has been found that Patients with acute traumatic spinal cord injuries (SCIs) are at risk of developing acute kidney injury (AKI) secondary to rhabdomyolysis, (Galeiras *et al*., 2016) due to high-impact injuries, immobilization, thermal dysregulation, and hemodynamic instability. The kidney injury associated with rhabdomyolysis results from vasoconstriction with decreased renal perfusion and intraluminal deposits of myoglobin, its breakdown products and of uric acid, which causes obstruction and tubular damage (Ward, 1988). Therefore, the males having higher muscle mass and higher rate of muscle catabolism are likely to be more predisposed to AKI resulting from deposition of muscle breakdown products in the glomeruli and renal tubules.

In addition, several studies have reported that sex hormones testosterone and estrogen affect renal injury. It has been shown that estrogen or administration of estradiol play a protective role against renal injury. Estrogen is said to improve renal blood flow and GFR through activation of nitric oxide synthase and therefore increasing nitric oxide production. Estrogen is also reported to play a protective role against renal vascular damage and renal ischemia via suppression of Endothelin 1 over production (Nematbakhsh, *et al*., 2015). Therefore, the males lacking such protection might be more predisposed to have elevated levels of serum urea and creatinine following SCI due to increase production and possible impairment in renal function. This findings agreed with the findings of Doggart *et al.* (1964), Malhotra, *et al.* (2010), and Rodreguez, *et al.* (2013). Both reported an impaired and decreased in renal function in individual suffering from SCI resulting mostly from ascending urinary tract infection due to stasis of urine in the paralyzed bladder. On the other hand, these findings contradicted that of Burr, *et al.* (1993) and that of Elmelund*et al.* (2014). The later, agreed to the fact that individuals suffering from SCI are at more risk of renal insufficiency due to neurogenic bladder dysfunction.

However, in their fifty year follow up of patients with SCI, they reported that serum creatinine level remained steady in the first 30 years following SCI and significantly reduced thereafter. The possible reason for this finding may be attributed to the fact that, despite the increase in creatinine production in the early phase of SCI resulting from increased muscle catabolism (Scelsi, 2001; Gorgey *et al*., 2014), the serum creatinine is likely to remain normal provided that the renal function is normal or less severely impaired. In such case, urinary excretion of creatinine will be increased by the kidney thus preventing the body from elevated creatinine levels.

CONCLUSION

Based on our findings we concluded that, SCI caused a significant increase in serum urea and creatinine levels in the male albino rat models compared to the females. This indicated that males might have higher risk of renal dysfunction following SCI.

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