



CHANGES IN VARIOUS HEMATO-BIOCHEMICAL PARAMETERS AFTER PARTIAL AND COMPLETE NEPHRECTOMY IN RABBITS

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ABSTRACT

Some variations occur in hemato-biochemical parameters after surgical removal of a complete or a portion of kidney. There is very less data available on changes in hemato-biochemical profile in early post-operative days after partial or complete nephrectomy in rabbits. The current research study was designed to evaluate the effects of partial and complete nephrectomy on various hemato-biochemical parameters in rabbits in early post-operative days. We used twelve clinically healthy rabbits of either sex, age ranging between six months and one year, weight between 1.5 kg and 2 kg. Rabbits were divided into two groups named as group A and Group B. Group A underwent with partial nephrectomy and group B with complete nephrectomy. Blood samples were taken from jugular vein for hematology and biochemistry at day 0, 3rd and day 6th of surgery. General linear model with repeated measures design was applied to evaluate and compare the effects within groups as well as between groups. All mean values at day 3rd and 6th, were compared with baseline values which showed a significant difference of various hemato-biochemical parameters at day 3rd and 6th within groups. However, non-significant difference in various hemato-biochemical parameters was found between both partial and complete nephrectomised groups with some inconsiderable alterations. It is concluded that partial and complete nephrectomy causes mild changes on hemato-biochemical parameters in early post-operative days. Partial nephrectomy causes post-operative complications including kidney inflammation, congestion of abdominal cavity and death. Further study on larger sample size will be helpful for patients who require long term monitoring.

Keywords: electrolytes, hemato-biochemical, nephrectomy, rabbits

INTRODUCTION

Various bacterial, viral and parasitic renal ailments alter the renal function and affect kidney health. Some common diseased conditions include renal calculi, renal tumors, calcification, renal cyst, fatty infiltrations of kidneys and congenital defects like renal agenesis. These renal disorders eventually result in partial or complete loss of kidney. There are few uncommon diseased conditions like kidney abscesses, pyelonephritis, pyelitis and amyloidosis, also trigger loss of renal function (Margaret and Brown, 2013). If this loss is irreversible, there is need to remove kidney, partially or completely through surgical intervention.

From last two decades, there are two effective methods which are considered as prime choice to correct detrimental renal impairments like renal tumors, one is partial or non-sparing nephrectomy and other is complete or radical nephrectomy (Poon *et al.*, 2013). First partial nephrectomy was reported by Vincent Czerny in 1887 (Herr, 2005) and techniques of complete nephrectomy were systemically explained by Robson (Robson *et al.*, 1963).

Kidneys play a vital role in production of erythropoietin, a glycoprotein hormone in response to absence or low level of tissue oxygen. Main role of erythropoietin is production of red blood cells. Any renal disease condition can lead to decrease in the formation of erythropoietin which ultimately results in decrease in red blood cell production (Bieber,

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2001). Mostly when the kidney function is compromised, level of hemoglobin and packed cell volume fall down. This is because of reduction in erythropoietin (Khanam *et al.*, 2013). Withdrawal of waste products and toxins like blood urea nitrogen, serum creatinine and uric acid is also associated with kidney health. A damaged kidney is unable to excrete these waste products. Serum electrolytes such as sodium, potassium and chlorides provide better picture of patient suffering from kidney disorders. Bilirubin, a waste product is derived from breakdown of heme, excreted out from kidney in the form of urobilinogen (Stec *et al.*, 2012).

Some variations occur in hemato-biochemical parameters because of surgical removal of whole or partial kidney. This study was designed to evaluate those changes in various hemato-biochemical parameters before and after partial and complete nephrectomy in rabbits.

MATERIALS AND METHODS

This study was approved by Ethical Review Committee University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan on January 18th, 2019 vide letter number 220. Study was done in the Department of Clinical Medicine and Surgery in UVAS, Lahore, Pakistan. Twelve clinically healthy rabbits of either sex, age from six months to one year, weighing 1.5kg and 2kg were used in this study. Rabbits were divided into Group A and Group B comprising 6 rabbits in each group. In group A, partial nephrectomy was performed whereas in Group B, complete nephrectomy was performed. Statistical model for the experiment was as follows; $Y_{ij} = \mu + T_j + E_{ij}$ where Y_{ij} represents response of overall experiment effect, μ is overall mean, T_j is effect of nephrectomy procedure, and E_{ij} is the effect of error term.

After surgical site preparation and positioning of rabbits at dorsal recumbency, anesthesia was administered by using ketamine (30 mg/kg) intramuscularly and xylazine (5 mg/kg) intramuscularly (Quesenberry and Carpenter, 2011).

Group A underwent with surgical procedure, partial nephrectomy. A cranial ventral midline approach was followed for partial nephrectomy. After standard laparotomy procedure, kidney was exposed and two straight needle technique threaded with chromic gut #2 was applied. After performing this standard technique, kidney was placed back to its original

anatomical position and laparotomy incision was closed with standard technique. In group B, after exposing kidney, ligation of blood vessels and ureter done and complete kidney was removed surgically (Slatter, 2003). Flunixin meglumine with dose rate of 2 mg/kg intramuscularly was used as an analgesic agent post-operatively (Quesenberry and Carpenter, 2011) and enrofloxacin with dose rate of 5 mg/kg intramuscularly was used as an antibiotic post-operatively (Varga, 2013).

Blood sampling was carried out at day 0, 3rd and day 6th of surgeries to evaluate hemato-biochemical parameters performing various tests such as hematological studies (red blood cells, white blood cells, granulocytes, lymphocytes, monocytes and packed cell volume), renal function tests (blood urea nitrogen and serum creatinine), liver function tests (total bilirubin and total proteins), and serum electrolytes (Na, K, Cl). A total 2ml blood sample was withdrawal from jugular vein and 1ml transferred to an EDTA vacutainer for complete blood count (CBC) and liver function tests (LFTs) and 1ml was transferred to gel vacutainer to get serum for renal function tests (RFTs) and serum electrolytes before any anesthetic administration. This sample was collected to establish baseline for CBC, RFTs LFTs, and serum electrolytes. Similarly, blood tests were performed on day 3rd and day 6th postoperatively. All blood samples were processed as mentioned by Benjamin (1983).

Data was analyzed by statistical analysis tool, general linear model repeated measures design by using statistical analysis software SPSS 20.0 version (Daniel, 2010). The data are described as mean $\pm$ standard deviation with $p < 0.05$ which was regarded as significant.

RESULTS

Blood samples with baseline laboratory values which were taken at day 0 pre-operatively, were compared with the values taken by post-operatively. After partial and complete removal of kidney, reduced or enhanced concentration of various endogenous substances happened in blood. Hematological picture showed decline of red blood cells at day 3rd and day 6th in both groups. Using general linear model repeated measures design, mean $\pm$ S.D pre-operative RBCs count significantly decreased from 6.14 ± 0.7 to 5.55 ± 0.8 in group A and 6.14 ± 0.6 to 5.09 ± 0.2 in group B ($P < 0.05$). Pre-operative white blood cells values were with a mean $\pm$ S.D of 7.99 ± 1.2 and 7.24 ± 0.8 in group A and B

respectively. Increase in WBCs values up to 8.48 ± 1.2 and 7.64 ± 0.7 has been recorded at day 6th post-operatively but this increase was non-significant ($P > 0.05$).

In group A, mean $\pm$ S.D baseline values of granulocytes and lymphocytes were 0.62 ± 0.2 and 3.30 ± 1.1 respectively. In group B, these values were 0.93 ± 0.3 for granulocytes and 3.64 ± 1.1 for lymphocytes. A significant increase of granulocytes with mean $\pm$ S.D of 1.17 ± 0.4 in group A and 1.60 ± 0.6 in group B was recorded. Decrease in lymphocytes with mean $\pm$ S.D of 2.92 ± 1.1 in group A and 3.32 ± 1.1 in group B was observed.

Hemoglobin showed significant decrease within as well as between groups ($p < 0.05$) with mean $\pm$ S.D of 13.16 ± 0.8 and 13.28 ± 0.9 in group A and B at day 0 respectively. Decrease in hemoglobin with mean $\pm$ S.D of 12.15 ± 0.4 in group A and 12.33 ± 0.4 in group B was measured. In group A, pre-operative mean $\pm$ S.D packed cell volume was 38 ± 4.8 and in group B, mean recorded was 37.6 ± 2.1 . It started decreasing up to 32 ± 2.5 in group A and 34.6 ± 2.1 in group B ($P < 0.05$).

Total bilirubin level at day 6 was found greater with mean $\pm$ S.D of 1.03 ± 0.3 in group A and 0.96 ± 0.2 in group B than that of baseline value at day 0 with mean $\pm$ S.D of 0.43 ± 0.1 and 0.30 ± 0.1 in group A and B respectively ($P < 0.05$). Similarly, increase in total protein from 5.91 ± 0.6 up to 7.20 ± 0.8 in group A and from 6.18 ± 0.5 up to 7.56 ± 0.4 in group B.

Total blood urea nitrogen in partial nephrectomised group showed difference between day 0 with mean $\pm$ S.D of 45 ± 9.8 and day 6th with mean $\pm$ S.D of 67.6 ± 8.4 . Likewise, complete nephrectomised group showed increase in blood urea nitrogen from baseline value with mean $\pm$ S.D of 44 ± 11.6 to 69.6 ± 6.7 post-operatively.

Serum creatinine values in group A were comparable to those in group B from day 0 to 6th day (mean $\pm$ S.D 0.85 ± 0.2 and 1.20 ± 0.5 in group A at day 0; 2.49 ± 0.6 and 2.36 ± 0.3 in group B at day 6th). These values were found significant within groups ($P < 0.05$) and non-significant between partial and complete nephrectomised groups ($P > 0.05$) as similar result values were recorded in both groups.

In group A, mean $\pm$ S.D of serum electrolytes at 0 day were 143 ± 4.9 (sodium), 5.20 ± 1.0 (potassium) and 107.3 ± 2.4 (Chloride). In group B, mean $\pm$ S.D of these values were 143 ± 3.3 (sodium), 2.86 ± 0.7 (potassium) and 104.3 ± 2.5 (chloride). At day 6th, they significantly

decreased up to 138 ± 2.5 , 5.20 ± 1.0 and 107.3 ± 2.4 in sodium, potassium and chloride respectively within in group A and 134 ± 2.1 , 2.86 ± 0.7 and 98.1 ± 2.6 in sodium, potassium and chloride respectively within group B ($P < 0.05$). Between groups, they were found non-significant ($P > 0.05$). However, serum calcium showed a significant difference within groups as well as between groups ($p < 0.05$). In group A, mean value $\pm$ S.D of serum calcium at day 0 was 12.01 ± 0.5 which decreased up to 11.1 ± 0.2 at day 6 while in group B, mean $\pm$ S.D values of serum calcium were 12.2 ± 0.8 and 7.7 ± 0.6 at day 0 and 6, respectively. It shows that in Group B, serum calcium has decreased more than that of Group A. Two rabbits of partial nephrectomy group died after 6th day of follow up period. All results are shown in (Tables 1 and 2).

Table 1. Various hemato-biochemical parameters (Mean $\pm$ S.D) at day 0 (Pre-operative), their comparisons with day 3rd (Post-operative), day 6th (Post-operative) and normal clinical values (Fielder, 2010) are illustrated in (Tables 1 and 2)

Group A (Partial nephrectomy)				
Parameters (Units)	Normal values	Day 0 (Mean $\pm$ S.D) (Pre-operative)	Day 3 (Mean $\pm$ S.D) (Post-operative)	Day 6 (Mean $\pm$ S.D) (Post-operative)
Red blood cells ($10^{12}/L$)	5-8	6.14 ± 0.7	5.48 ± 0.8	5.55 ± 0.8
White blood cells ($10^9/L$)	5.2-12.8	7.99 ± 1.2	8.26 ± 1.1	8.48 ± 1.2
Granulocytes ($10^9/L$)	0-2.8	0.62 ± 0.2	1.12 ± 0.3	1.17 ± 0.4
Lymphocytes ($10^9/L$)	2-9.1	3.30 ± 1.1	3.07 ± 1.1	2.92 ± 1.1
Hemoglobin (g/L)	10-17	13.16 ± 0.8	11.56 ± 0.5	12.15 ± 0.4
Packed cell volume (%)	33-50	38 ± 4.8	31.6 ± 3.6	32 ± 2.5
Total bilirubin (mg/L)	0-0.7	0.43 ± 0.1	1.08 ± 0.4	1.03 ± 0.3
Total protein (g/dL)	5.4-7	5.91 ± 0.6	7.05 ± 0.5	7.20 ± 0.8
Total blood urea nitrogen (BUN) (mg/dL)	20-65	45 ± 9.8	68.1 ± 11.6	67.6 ± 8.4
Serum creatinine (mg/dL)	0.5-2.5	0.85 ± 0.2	1.97 ± 0.4	2.49 ± 0.6
Serum sodium (mEq/L)	138-150	143 ± 4.9	134 ± 2.7	138 ± 2.5
Serum potassium (mEq/L)	3.5-6.9	5.20 ± 1.0	3.43 ± 0.6	3.31 ± 0.4
Serum chloride (mEq/L)	100-115	107.3 ± 2.4	98.5 ± 2.4	101.6 ± 1.7
Serum Calcium (mEq/L)	11-14	12.01 ± 0.5	11.3 ± 0.3	11.1 ± 0.2

Table 2. Group B (Complete nephrectomy)

Parameters (Units)	Normal values	Day 0 (Mean $\pm$ S.D) (Pre-operative)	Day 3 (Mean $\pm$ S.D) Post-operative)	Day 6 (Mean $\pm$ S.D) (Post-operative)
Red blood cells ($10^{12}/L$)	5-8	6.14 $\pm$ 0.6	5.13 $\pm$ 0.3	5.09 $\pm$ 0.2
White blood cells ($10^9/L$)	5.2-12.8	7.24 $\pm$ 0.8	7.58 $\pm$ 0.4	7.64 $\pm$ 0.7
Granulocytes ($10^9/L$)	0-2.8	0.93 $\pm$ 0.3	1.88 $\pm$ 0.5	1.60 $\pm$ 0.6
Lymphocytes ($10^9/L$)	2-9.1	3.64 $\pm$ 1.1	3.33 $\pm$ 1.1	3.32 $\pm$ 1.1
Hemoglobin (g/L)	10-17	13.28 $\pm$ 0.9	11.10 $\pm$ 0.6	12.33 $\pm$ 0.4
Packed cell volume (%)	33-50	37.6 $\pm$ 2.1	31.6 $\pm$ 3.3	34.6 $\pm$ 2.1
Total bilirubin (mg/L)	0-0.7	0.30 $\pm$ 0.1	1.15 $\pm$ 0.3	0.96 $\pm$ 0.2
Total protein(g/dL)	5.4-7	6.18 $\pm$ 0.5	7.35 $\pm$ 0.4	7.56 $\pm$ 0.4
Total blood urea nitrogen (BUN) (mg/dL)	20-65	44 $\pm$ 11.6	76 $\pm$ 9.2	69.6 $\pm$ 6.7
Serum creatinine (mg/dL)	0.5-2.5	1.20 $\pm$ 0.5	2.65 $\pm$ 0.7	2.36 $\pm$ 0.3
Serum sodium(mEq/L)	138-150	143 $\pm$ 3.3	132 $\pm$ 2.8	134 $\pm$ 2.1
Serum potassium (mEq/L)	3.5-6.9	4.78 $\pm$ 0.9	2.93 $\pm$ 0.7	2.86 $\pm$ 0.7
Serum chloride (mEq/L)	100-115	104.3 $\pm$ 2.5	95.6 $\pm$ 2.1	98.1 $\pm$ 2.6
Serum Calcium (mEq/L)	11-14	12.2 $\pm$ 0.8	10.1 $\pm$ 0.9	7.7 $\pm$ 0.6

DISCUSSION

Various hemato-biochemical parameters play a vital role to evaluate total renal function, blood cells, haemoglobin, serum creatinine, blood urea nitrogen and serum electrolytes (Na, K and Cl) are the worthwhile clinical findings to assess renal function. Subsequent to nephrectomy, the remaining renal function depends on loss of renal parenchyma and post-operative time span. However, maintenance of renal function in contra lateral kidney is yet under active area of study (Antoniewicz *et al.*, 2012). After nephrectomy, the remnant kidney sustains a steady glomerular filtration rate from 70% to 75% (Buccianti *et al.*, 1993). Furthermore, microalbuminuria and hypertension has been reported in spite of fact that kidney function was well maintained up to 18 years post nephrectomy (Novick *et al.*, 1992). It has been recently documented that renal function persists stable after removal of a portion of renal mass even though these patients suffer hypertension, protein urea, alterations in blood profile and ultimately renal failure (Rutsky *et al.*, 1991).

In this research study, we eventually observed significant changes in hemato-biochemical parameters within groups and insignificant alterations were recorded between

groups regardless of some inconsiderable variations. Within group A and group B, there was significant decrease in RBCs count at day 3rd and gradual increase in RBCs count at day 6th after surgery. Decreases in erythropoietic stimulating factor (ESF) occur mostly after loss of renal tissues during kidney surgeries such as nephrectomy. Decrease in erythropoietic stimulating factor ultimately results in decrease in RBCs production. Kidney produces erythropoietin, a hormone which is responsible of producing RBCs (Jelkmann, 2011).

Increase in WBCs, granulocytes and decrease in lymphocytes were found within groups (Latif *et al.*, 2007). Increase in WBCs is associated with decrease in post-operative infectious complications (Garbens *et al.*, 2017). Some major surgeries of kidney such as nephrectomy induce stress on endocrine tissues as a result plasma adrenaline, serum cortisol and noradrenaline increases. Subsequently, this surgical stress involves in granulocytosis and lymphopenia (Toft *et al.*, 1993). Packed Cell Volume values were found to having significant difference within group A as well as in group B. A slight decrease in PCV at day 3rd and at day 6th was observed but this change was not significant with respect to reference value. It was according to findings of Gibb and Hamilton, (1985) in which decrease in PCV was observed. Similarly, in case of hemoglobin estimation, there was significant difference between groups as well as within groups. Values of hemoglobin decreased up to day 3rd then gradual increased up to day 6th in partial as well as complete nephrectomised groups. All fluctuations in value of hemoglobin were in reference range. Such findings have also been recorded by Darby *et al.* (1993). These results are also accredited with the results of Latif, *et al.* (2007). Mostly when the kidney function is compromised, level of hemoglobin and packed cell volume become lower than that of control groups. This is because of reduction in erythropoietin (Khanam *et al.*, 2013). Reduction in packed cell volume is also attributed to blood loss during surgical intervention (Robertson and Gourlay, 2017). Biochemical tests of total protein and bilirubin showed increase in values up to day 3rd and day 6th. There was a significant difference within groups. Such results have also been reported by Fortyn *et al.* (1985). Renal total proteins (albumin and globulin) are good markers of kidney health. Increase in total proteins often occurs when kidney is not functioning properly (Wu *et al.*, 2012).

Blood chemistry of serum creatinine and blood urea nitrogen were found significant within groups ($P < 0.05$). Increase in serum creatinine and BUN was recorded up to day 3rd and day 6th. Serum creatinine and blood urea nitrogen are a mostly used parameter to judge renal function. These are cleared from the body only from kidneys. Decreased clearance by kidneys results in increased serum creatinine and blood urea nitrogen level (Clark *et al.*, 2001). Such findings also have been reported by Fleck *et al.* (1992). After nephrectomy, blood urea nitrogen (BUN) level has been recorded. In previous studies, Park *et al.* (2017) reported that incidence of onset of hyponatremia after urologic surgery was 15.4%. Post-operative hyponatremia is associated with improper prognosis. Kardalas *et al.* (2018) found that excessive excretion of potassium occurs due to use diuretic drugs, renal disorders, endocrine ailments such as hyperaldosteronism and genetic syndromes which ultimately results in reduction in serum potassium. Reasons behind hypochloric conditions have not been researched sufficiently. However, Tani *et al.* (2012) reported that abnormal chloride level in critically ill patients was 25.4%. In present study, decrease in serum sodium level (hyponatremia), serum potassium level (hypokalemia) and serum chloride level (hypochloremia), were found in both groups and results were significant within groups. However, serum calcium showed decrease in readings at day 3 and at day 6. Results were significant within groups as well as between groups. Decrease in serum calcium is associated with improper tubular re-absorption of calcium from kidney because balance of calcium, magnesium and phosphate is well maintained by tubular re-absorption and urinary excretion (Blaine *et al.*, 2015). Two rabbits of partial nephrectomy died at the end of follow up period. Post-mortem showed severe inflammation of kidney and congestion in abdominal cavity.

There are some limitations of this study including small number of total animals in both groups and short follow up length. Considering these factors, further investigations are required to obtain comprehensive results.

Our research study has various distinguishing features. So far, detailed hemato-biochemical parameters just after nephrectomy has not been studied in any animal. The very few studies have analysed these parameters individually. Substantial number of repeated measurements enables us to evaluate

thoroughly various parameters at different time intervals.

CONCLUSION

In conclusion, we have observed that early post-operative days after partial or complete nephrectomy are very critical to judge kidney health. Partial and complete nephrectomy does not cause detrimental variations in hemato-biochemical profile after first week of surgery. However, partial nephrectomy has some complications such as fever, congestion and inflammation of kidney and death. Study with long term follow up is required for further evaluation of alterations in various haematological and biochemical parameters.

CONFLICT OF INTEREST

We certify that there is no conflict of interest regarding anything associated with this manuscript.

AUTHOR'S CONTRIBUTION

F. Rehman: Conducted Research.

H. B. Rashid: Supervised Research.

M. Ali: Assisted in write up.

W. Hasan: Assisted in formatting.

M. Asif: Assisted in Surgeries and sample collection.

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