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The Effect of Nephrectomy on Some Hormones in Adult White Rabbit's Male (New Zealand Strain)

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Abstract

THE of this research were to identify the most important hormonal changes resulting of left nephrectomy of New Zealand rabbits (*Oryctoagus cuniculus*), such as: angiotensin-II (Ang-II), Erythropoietin (EPO), and Vitamin D_3 (Vit.D_3), and to clarify the relationship between the left nephrectomy and the heart through the atrial natriuretic peptide (ANP) hormone. Therefore, this study was conducted at the University of Mosul /College of Science /Department of Biology, for the period from April (2023) to August (2023). For this study, 60 white New Zealand rabbits used, and divided into four groups, each group consisting of 15 rabbits. As a result, the study showed that the concentration of the AngII hormone was significantly increased in rabbit group one month after nephrectomy compared to the rest of the groups. Followed by a noticeable decrease in the concentration of this hormone in rabbit group tow months after nephrectomy, and increased decrease in the concentration of this hormone in rabbit group three months after nephrectomy, but it didnt reached to the concentration of control group, at the probability level ($P \leq 0.05$). On the other hand, there was a significant decrease in the concentration of the EPO, ANP hormones and Vit.D_3 in rabbit group one month after nephrectomy. Then the concentrations began to rise after two months and reached its peak in rabbit group three months after nephrectomy, but it did not reach the level of control group, at the probability level ($P \leq 0.05$). Conclusions: It has been concluded in this study that the renal hormones contribute extensively to the physiologic functions of the kidneys and in many cases, other organs. The removal of one of the kidneys caused some physiological changes in some hormones.

Keywords: Nephrectomy, Rabbits, Angiotensin-II, Erythropoietin, Vitamin D_3.

Introduction

The kidney is one of the most important organs in the urinary system [1] that helps to maintain homeostasis through a multi-step process that includes filtration, absorption, and secretion. In addition, the kidney controls the body's fluid and electrolyte balance, as well as the site of production of renin and erythropoietin, which regulate blood pressure and erythrocyte production, respectively [2]. Each kidney has millions of microscopic uriniferous tubules that consist of nephrons and collective ducts. The nephron is subdivided into renal corpuscle and renal tubules. Part of the renal corpuscle is the glomerulus, while, the higher magnification of the nephron showed afferent arteriole, glomerulus, Bowman's capsule, proximal tubules, and distal tubules [3].

The many physiological functions of the kidneys are largely regulated by the endocrine system. While the impact of extrarenal hormones on the urinary system is certain, recent research has identified a class of endogenous renal hormones that significantly affect renal hemodynamics, tubular electrolytes, glomerular filtration, and water processing [4]. While eliminating waste from the bloodstream is the kidney's principal function, it's vital to remember that the kidney is also a significant endocrine organ [5], that secretes several essential hormones, including prorenin/renin, erythropoietin (EPO), 1,25-dihydroxy vitamin D (1,25$(OH)_2$D), as well as urodilatin [6].

Juxtaglomerular cells in the kidney produce and secrete prorenin, an inactive precursor of renin. Renin is actively released from stored granules, whereas prorenin is released constitutively (6). Renin is a protease that initiates the rate-limiting step of the system known as renin-angiotensin (RAS), a system of hormones, enzymes, and active and inactive peptides that work in concert with the heart, blood vessels, and kidneys to regulate blood pressure and the balance of electrolytes and fluids [7]. Renin converts angiotensinogen produced by the liver into angiotensin-I, which activated the RAS [8]. The hormone angiotensin-converting enzyme (ACE), which is

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primarily located in the lungs, can convert the decapeptide angiotensin-I into the octapeptide angiotensin-II [7]. Angiotensin II also urges the adrenal cortex to secrete the hormone aldosterone Hence, it also called renin-angiotensin-aldosterone system (RAAS). Aldosterone triggers and causes an increase in the reabsorption of sodium and water through the renal tubules, whereas at the same time maintains electrolyte balance, leading to the secretion of potassium and then increasing the volume of extracellular fluid in the body, leading to high blood pressure [9].

Erythropoietin (EPO) is a hypoxia-induced erythropoietic hormone, produced primarily by renal EPO-producing cells in the adult kidney [6] that arise from fibroblasts in the renal cortex that regulates erythropoiesis, or red blood cells production [8] which in turn increases the bloods' ability to carry oxygen to be delivered to the exercised muscles, to produce energy that helps performance [10].

The physiologically active form of vitamin D, $1,25(OH)_2D$, is essential for preserving calcium homeostasis and controlling the growth of new bone. It is derived from 25(OH)D, an inactive form of vitamin D that is synthesized in proximal tubular cells and produced in the liver [11]. The human skin is primarily responsible for producing it following extended exposure to Ultraviolet B (UVB) light. The skin's 7-dehydrocholesterol is transformed into cholecalciferol, or Vit.D_3, an inactive precursor. Skin exposure to sunlight generates 90% of the total amount of circulating vitamin D, with the remaining 10% coming from diet (fatty fish, cod liver oil, egg yolk, and fortified dairy products [12].

Urodilatin is produced by distal tubular cells and secreted in urine, unlike the other previously mentioned hormones, which are released into the circulation [6].

By various kinds of physiological crosstalk among these organs, the relationship between heart and kidney function is crucial to preserving homeostasis. It has been proposed that, as in the case of patients with cardiorenal syndrome, either acute or ongoing dysfunction in one organ causes dysregulation in another [13]. An essential hormone called an atrial natriuretic peptide (ANP) controls a wide range of physiological and pathological processes, such as blood volume and pressure, electrolyte and body fluid balance, cardiac conduit activity and function, inflammatory reaction, lipid metabolism, vascular remodeling [14]. ANP also inhibits the reabsorption of sodium and water at different levels of the nephron and raises the glomerular filtration rate in the kidney by increasing both efferent arteriolar constriction and afferent arteriolar dilation. Atrial natriuretic peptide then inhibits renin secretion and aldosterone production, which is how it antagonizes the renin-angiotensin-aldosterone system (RAAS) [15].

This study aims to assess the efficacy of the right kidney safe life of rabbits and to determine the most important physiological effects of removing the left kidney on 60 male New Zealand rabbits. The study confirmed a significant decrease in the concentration of the hormones Ang-II, EPO, ANP, and Vit.D_3 during the eradication period (one, two, and three months) until it reached its peak. Also, identifying the reasons for the increase and decrease in hormones levels compared to the level of the control group.

Material and Methods

Study Area

The study was conducted in the Biology Department / College of Science/ Mosul University, Iraq.

Animals

The study was performed on 60 healthy adults, New Zealand white Rabbits male (*Oryctoagus cuniculus*), Obtained from local markets, aged (9-12) months, weighting (1250-1500) g. The rabbits were housed in wooden crates with bedding that complied with present regulations. The cages were located in an enclosed room with forced air, a water supply, and nibble drinkers. Ten hours of darkness and fourteen hours of light were imposed upon the animals.

The usual temperature was between (25-28)°C, and the humidity was controlled between (60-70)%. The animals were constantly receiving veterinary care and had access to water, and granulated green fodder. Based on the requirements of the animals, the cages and feeders underwent a methodical cleaning and disinfection process. The rabbits received a scabies vaccination.

For the entire month, animals were kept under close observation to prevent illness and were treated with anthelminthic agents to prevent the infestation of both internal and external parasites. For this, ivermectin 1% was administered subcutaneously at a dose of 0.2 mg/kg body weight [16]. Moreover, Hay and grain were withheld for 12 hours before surgery, but free access to water was allowed. The treated animals were put under observation postoperatively.

Experimental design

Experimental animals were divided randomly into four groups: Control Group without nephrectomy (time zero) and three groups of equal treatment, all of them were removed from the left kidney at the same time. Then euthanasia of these animals was performed by decapitation successively, after 1, 2 and 3 months. Blood samples were collected from all groups before euthanasia to assess each of the Angiotensin-II, Erythropoietin, ANP hormones and Vit.D_3.

Surgical techniques

Rabbit undergoing protocol of general anesthesia including a mixture of Ketamine hydrochloride 10%

(Rotexmedica, Germany) 35 mg/kg body weight and Xylazine hydrochloride 2% (Interchemie, Holland) 5 mg/kg body weight. Intramuscularly, the dose is repeated if necessary [17]. The operation site was shaved, prepared aseptically with iodine tincture, and cut. A five cm full-thickness ventral midline laparotomy incision was made. The left kidney was carefully identified through elevation mesocolon from the abdominal cavity, the left renal artery and vein were identified and ligated by double ligature using absorbable suture material, then the ureter was also ligated separately, cutting done between two ligatures, the kidney exteriorized out of abdominal cavity then the muscle, subcutaneous and skin closed routinely [18, 19].

Post-operative Care

After the nephrectomy, animals were housed in a clean and excellent environment for recovery. On the first post-operation day, the animals will be kept only on fluid therapy, on the second and third days; they fed on green grass, and return to their normal fodder. Every group was closely monitored to ensure that there were no issues or health concerns. Animals received intramuscular antibiotic injections as a preventative measure, administered for four days following surgery, at doses of 20000 I.U. and 20 mg/kg B.W., respectively, of penicillin and streptomycin. Furthermore, the heart rate, respiration, and rectal temperature were recorded every day for five days following surgery.

Samples collection and preparation

5 milliliter rabbit blood sample was taken directly from the heart and placed in a sterile tube. Each animal's blood sample, was allowed to coagulate and serum was collected by leaving it for (10-30) minute at room temperature. The samples were then centrifuged for 15 minutes at 3000 revolutions per minute (rpm), using an Indian-made Remi Motor benchtop centrifuge. Serum was prepared in plain tubes for later hormonal testing and every sample was labeled with the name of the group and assigned a serial number.

Determination of serum hormonal concentrations and Vit. D_3

To remove sediment, tests were defrosted at 37 °C and centrifuged at 4000×g for 5 minutes. The hormone's serum concentration levels of Ang-II, EPO, and ANP were determined using enzyme-linked immune sorbent assay (ELISA) kits (Biotech, Shanghai, China). This kit is based on the double antibody sandwich technology to assay the hormone. Procedures were based on the manufacturer's instructions. Reading is performed at (450 ± 10) nm. The concentration of each hormone was calculated from the standard curve. While total serum Vit.D_3 concentration can be measured by competitive binding assay (Roche, Mannheim, Germany). This assay is a competitive electrochemiluminescence protein binding assay intended for the quantitative determination of total Vit.D_3 in serum.

Statistical analysis

Following a completely randomized design and statistical analysis by the simple experimental system, at the probability level ($P \leq 0.05$) when the variances between the groups were tested using Duncan's multiple range test [20], using the SAS statistical program [21].

Results

Fig. 1 shows a significant decrease in the concentration of Angiotensin-II (Ang-II) hormone in a group of rabbits three months after kidney removal compared with the rest of the groups at a probability level ($P \leq 0.05$) and an arithmetic mean of (75.26 ± 0.10) IU/L, but it did not reach the level of the control group, which reached the arithmetic mean of it (70.36 ± 0.11) IU/L for a group of rabbits whose kidneys were removed after two months is (76.39 ± 0.23) IU/L. The arithmetic means of a group of rabbits one month after removal of the kidney was (92.43 ± 0.30) IU/L

Fig. 2 shows a significant increase in the concentration of erythropoietin hormone in a group of rabbits three months after the removal of the kidney compared to the rest of the groups, but it did not reach the level of the control group and at the level of possibility ($P \leq 0.05$) with an arithmetic mean of (55.36 ± 0.09) mIU/mL, while the arithmetic mean of the control group was (55.87 ± 0.07) mIU/mL, while the arithmetic means of the group of rabbits that had the kidney removed after two months was (50.44 ± 0.22) mIU/mL, and the arithmetic mean of the group of rabbits one month after kidney removal was (43.58± 0.20) mIU/mL.

There are 60 rabbits, and values are presented as mean standard deviation. At the probability level ($P \leq 0.05$), shapes and various letters indicate a significant difference.

Fig. 3 shows the occurrence of a significant increase in the concentration of Atrial Natriuretic Peptide hormone at a probability level ($P \leq 0.05$) in the rabbit group three months after the removal of the kidney compared with the rest of the groups, but it did not reach the level of the concentration of the control group with an arithmetic mean of (101.18 ± 0.15) Pg /ml. The arithmetic mean for the control group was (126.24 ± 0.19) Pg /ml, while the mean for the rabbit group two months after kidney removal for this hormone was (94.62 ± 0.25) Pg/ml, while the mean for the rabbit group was one month after kidney removal for this hormone (36.38 ± 0.21) Pg/ ml.

Fig. 4 shows a significant decrease in the Vit.D3 level in the group of rabbits one month after nephrectomy compared to the rest of the groups, with an arithmetic average of (12.32 ± 0.08) nmol/L and at the probability level ($P \leq 0.05$). The arithmetic mean of the control group was (22.35 ± 0.11) nmol/L in the group of rabbits that had the nephrectomy after two months with an arithmetic mean of (13.15 ± 0.08) nmol/L while the arithmetic mean of the group of rabbits three months after nephrectomy was (21.20 ± 0.15) nmol/L but it did not reach the control group.

Discussion

The Angiotensin-II (Ang-II) hormone, is a peptide hormone of great physiological importance that contributes to the regulation of blood pressure, electrolyte homogenization, and aldosterone release, as overactivity of the RAS may lead to high blood pressure [22]. One month after nephrectomy, there was an increase in this hormone as a result of a decrease in systolic blood pressure, so the cells near the glomeruli in the remaining kidney began to release the renin enzyme into the bloodstream. Renin which breaks down angiotensinogen produced by the liver to give one of the derivatives of angiotensin, which is Ang-I that considered inactive large protein, which in turn is divided into parts by Angiotensin Converting Enzyme (ACE) [23]. One of these parts is Ang-II, which works to contract the muscles of the walls of small arteries and raise blood pressure, also stimulates the adrenal cortex to release aldosterone and the anti-adhesive hormone ADH, which is secreted from the posterior lobe of the pituitary gland. However, after three months of kidney removal, blood pressure began to gradually rise and return to normal, which led to a decrease in the secretion of this hormone and a decrease in its concentration in the blood serum [24].

This study indicates the occurrence of a significant decrease in the concentration of the EPO hormone one month after the removal of the left kidney in male rabbits. Compared with other groups. While the increase in this hormone appeared after the second month and reached its peak in the third month after eradication, it did not reach the level of the control group. On the other hand, no studies were found to support the results reached in this research, so the results will be deliberately interpreted according to what was mentioned in approved and published sources, which requires a great effort in research and investigation to prove or deny their validity and evaluate them. Erythropoietin is one of the glycoprotein hormones secreted by the kidneys and works to stimulate the bone marrow to produce more red blood cells [25], and this would have an effect by increasing the capacity to carry and transport oxygen. In addition, the kidney cells responsible for manufacturing this hormone are characterized by their sensitivity to a lack of oxygen in the bloodstream, which travels through the kidney and nourishes it.

These cells produce the erythropoietin hormone and secrete it in response to a lack of oxygen from its normal levels in the blood, this decrease in the level of oxygen may be an indicator of a deficiency of red blood cell numbers and the lack of hemoglobin molecules are responsible for carrying oxygen and transporting it through the blood, as this hormone is responsible for the formation of RBCs. It can also lead to a decrease in this hormone after the removal of the left kidney due to the loss of cells in the removed kidney, which are responsible for detecting oxygen. However, three months after the removal, there was a doubling in the number of cells that sense the level of oxygen in the remaining kidney to compensate for the deficiency in this hormone, and this is what was indicated by [26].

In addition, the results of this study also showed a significant decrease in the concentration of Vit.D_3 after the resection of the left kidney, one month after the resection procedure, while the concentration of Vit.D_3 began to rise two months after the resection procedure, and after the third month its concentration reached higher than the previous groups, but it did not rise to the level of the control group. Vit.D_3 is very necessary for humans and animals and is produced internally through a photochemical reaction. The final step to activate Vit.D_3 occurs in the kidneys, which work to convert it from the inactive form to the biologically active form of cholecalciferol. Vit.D_3 is mostly produced through the skin after exposure prolonged exposure to sunlight [27]. As a result, 90% of the total amount of Vit.D_3 is in the blood through exposure of the skin to sunlight while the remaining 10% is obtained through the diet [28]. Also, when the left kidney is removed, there is a decrease in the concentration of Vit.D_3 a month after the removal as a result of the loss of kidney cells responsible for activating Vit.D_3. However, an increase occurs after two months and peaks three months after the removal, which may be due to an expansion and an adaptation of the remaining kidney cells, since the expanding kidney cells grow rapidly and divide to perform double functions to compensate. The loss of the resected kidney, which converts inactive Vit.D_3 to the active form, plays a major role in gene expression and enhancing immune procedures [29]. This is the reason for the significant decrease in the concentration of the ANP hormone in the group of rabbits one month after the resection of the left kidney, and then an increase in the concentration of the ANP hormone two months and three months after the nephrectomy but it did not reach the level of the control group maybe it will come back to adaptation in the remaining kidney tissue after nephrectomy, and we noticeable increase in the concentration of the ANP hormone was observed after three months. The researchers explained that natriuretic peptides contribute to functional adaptation and protection of the barrier in the kidneys [30].

Due to the close connection between the heart and the kidneys from a functional standpoint and to the ANP hormone secreted from the heart cells, when the left kidney is removed, there is a decrease in the concentration of this hormone after a month of removal, but the remaining kidney cells begin to adapt after two months and the peak of adaptation reaches three months after the nephrectomy. The cells and tissue of the remaining kidney adapt to lead it to perform a double function to compensate for the deficiency resulting from the loss of the removed kidney, which is reflected in the increased secretion of this hormone from the heart three months after removal [31].

Based on what was mentioned above, this study is the first in this field. For this reason, the discussion of our results will depend mainly on the results that have been adopted in published research as important sources from the reality of their application in that research and its relationship to other research that has a relationship in this field. For this reason, this study is considered to have a modern nature that was the result of conducting live operations, especially after it was discovered that the results of some related work are hypotheses without application, that is, without scientific evidence. For this reason, the work of this research and its results are considered distinctive, especially since it started from scratch and the suffering was great due to the lack of sources that can help us learn or obtain results that serve this work.

Conclusion

It has been concluded in this study that the renal hormones contribute extensively to the physiologic functions of the kidneys and in many cases, other organs. The removal of one of the kidneys caused some physiological changes in some hormones. Where the Ang-II hormone concentration increased a month after eradication, and its concentration began to gradually decrease after two months and reached its peak three months after nephrectomy, but it did not reach the control group. While the hormones concentration EPO, ANP, and Vit.D_3 decreased a month after the removal, then they began to rise after two months and reached their peak three months after the removal of the kidney.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical considerations

The study had been approved by the Institutional Animal Care and Use Committee of the University of Mosul. College of Veterinary Medicine (Ref: UM. VET. 2023.037 / Date: 15-3-2023).

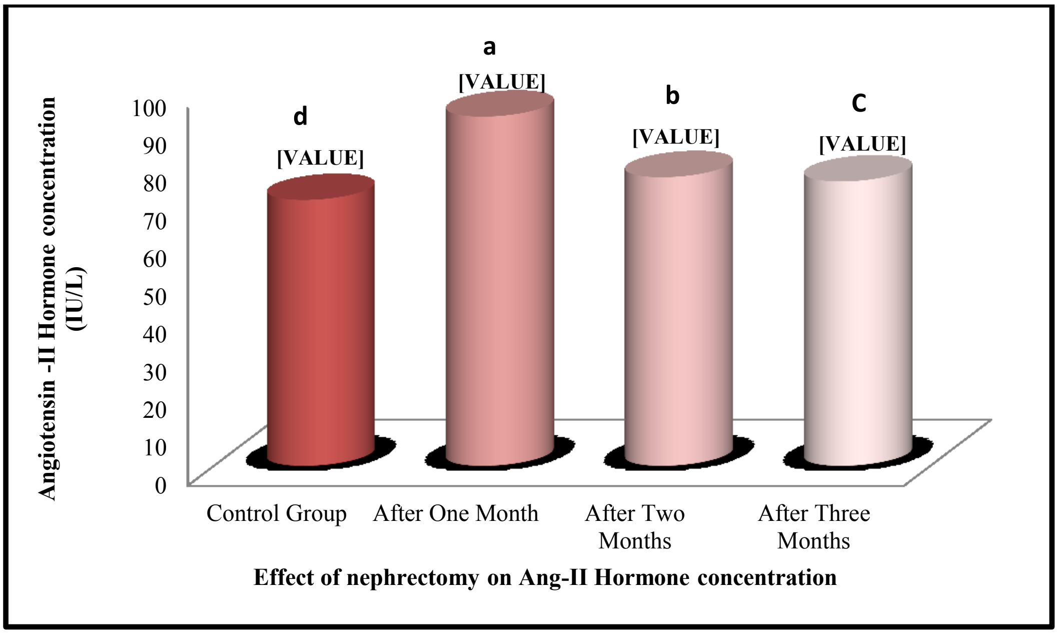

Fig. 1. The Effect of nephrectomy on Angiotensin-II (Ang-II) Hormone concentration in multiple periods

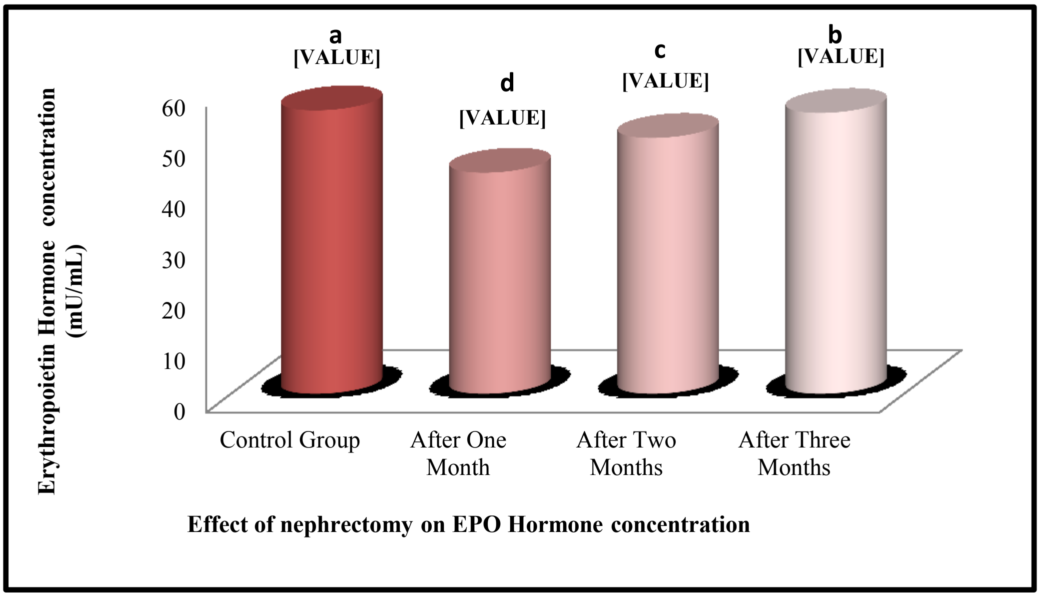

Fig. 2. The effect of nephrectomy on Erythropoietin (EPO) Hormone concentration in multiple periods

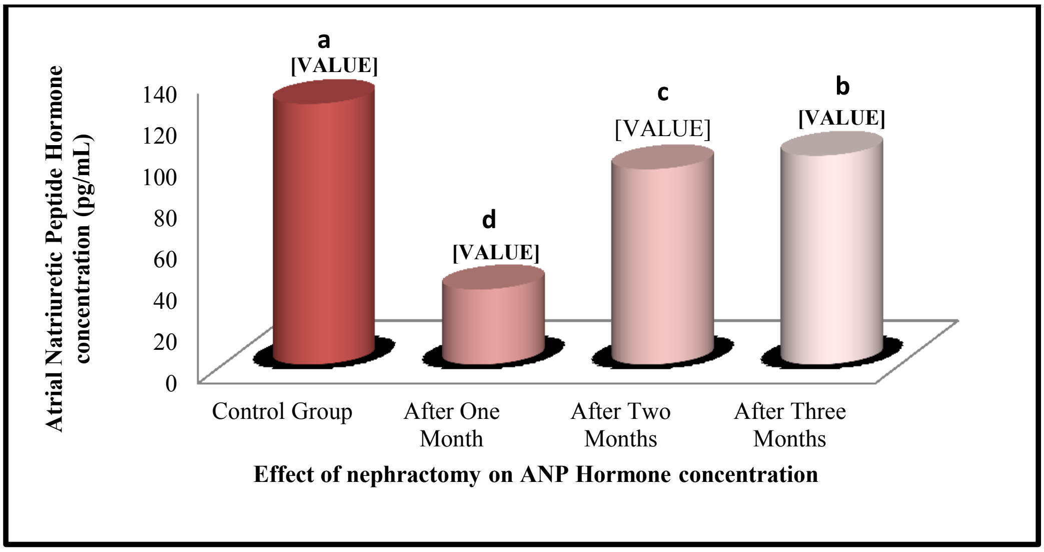

Fig. 3. The effect of nephrectomy on Atrial Natriuretic Peptide (ANP) Hormone concentration in multiple periods

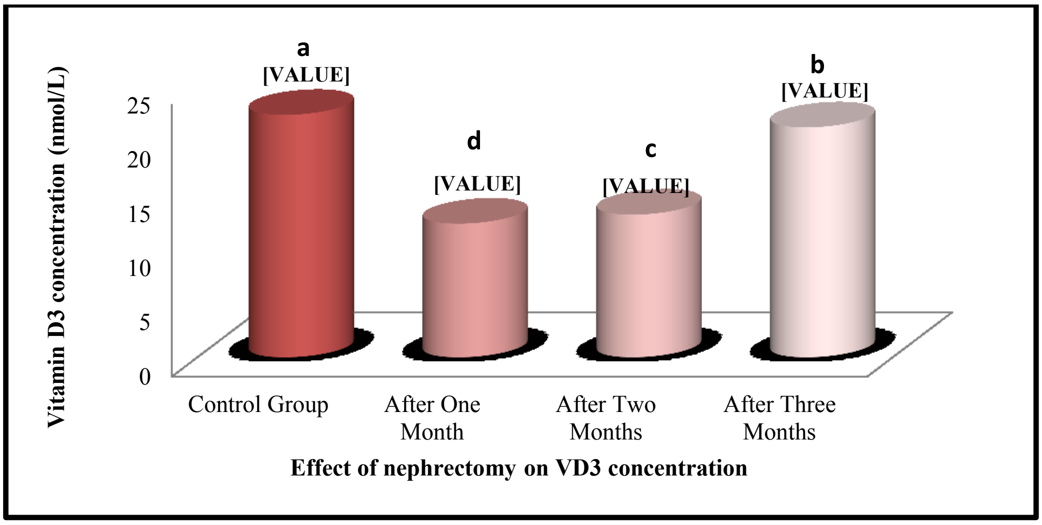

Fig. 4. The Effect of nephrectomy on Vit.D_3 concentration in multiple periods

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تأثير استئصال الكلية على بعض الهرمونات في ذكور الأرانب البيض البالغة

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الملخص

يهدف هذا البحث إلى التعرف على أهم التغيرات الهرمونية الناتجة عن استئصال الكلية اليسرى للأرانب النيوزلندية (*Oryctoagus cuniculus*)، مثل: الأنجيوتنسين-II (Ang-II) ،الإريثروبويتين (EPO)، وفيتامين D_3 (Vit. D_3)، وتوضيح العلاقة بين استئصال الكلية اليسرى والقلب من خلال هرمون البتيد الأذيني المدر للصوديوم (ANP). لذا أجريت هذه الدراسة في جامعة الموصل / كلية العلوم / قسم علوم الحياة، للمدة من نيسان (2023) الى آب (2023). تم في هذه الدراسة استخدام 60 أرنب نيوزيلندي أبيض. وتم تقسيمهم إلى أربع مجموعات، كل مجموعة مكونة من 15 أرنباً. ونتيجة لذلك أظهرت الدراسة أن تركيز هرمون Ang-II ارتفع بشكل ملحوظ في مجموعة الأرانب بعد شهر واحد من استئصال الكلية اليسرى مقارنة ببقية المجاميع، تلا ذلك انخفاض ملحوظ في تركيز هذا الهرمون في مجموعة الأرانب بعد شهرين من استئصال الكلية، وازداد انخفاض تركيز هذا الهرمون في مجموعة الأرانب بعد ثلاثة أشهر من استئصال الكلية، إلا أنه لم يصل إلى تركيز مجموعة السيطرة عند مستوى احتمال ($P \leq 0.05$). ومن ناحية أخرى، حدث انخفاض معنوي في تراكيز كل من هرمونات EPO، Vit. D_3 وANP في مجموعة الأرانب بعد شهر واحد من استئصال الكلية، ثم بدأت التراكيز في الارتفاع بعد شهرين ووصلت إلى ذروتها بعد ثلاثة أشهر من استئصال الكلية، إلا أنها لم تصل إلى مستوى مجموعة السيطرةعند مستوى احتمال ($P \leq 0.05$). الاستنتاج: وقد خلصت هذه الدراسة إلى أن الهرمونات الكلوية تساهم بشكل كبير في الوظائف الفسيولوجية للكلى وفي كثير من الحالات للأعضاء الأخرى. وتسببت عملية استئصال إحدى الكليتين في حدوث بعض التغيرات الفسيولوجية في بعض الهرمونات.

الكلمات المفتاحية: استئصال الكلية، الارانب، ألانجيوتنسين-II، الإريثروبويتين، البتيد الأذيني المدر للصوديوم، فيتامين D_3.