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THE DETECTIONOF CALCIFICATION IN KIDNEYS OF THE LAYING HENS*(GALLUS GALLUSDOMESTICUS)* BY USING THE HISTOLOGICAL AND IMMUNOHISTOCHEMICAL TECHNIQUES

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ABSTACT

The present research was planned to realize and determine the calcification in the kidneys of two ages (18 months and 24 months) of laying hens. There are no clear differences in histological appearance by using (H&E) stain between to ages, but there are higher significant differences in the height of epithelium cells and lower significant differences in the lumen of renal tubules in the kidneys of laying hens for (24 months) than in the (18 months). By using special dyes (Van Kossa) revelateddeposits of calcium salts and using immunohistochemistry method, the results showed presence of calcification in form of small foci in varying sizes bearing within them brown to dark color calcium deposits in the glomerulus, the epithelium layer and basement membrane of the renal tubules and between them. In general the calcified foci in different areas of kidneys in the 24 month age increase than in the 18 months age.

INTRODUCTION

In mammals and birds, the kidneys are main organ participate with keeping the stable nature of the internal environment by saving balancebetween glomerular filtration andvolume of renal tubular secretion in addition to the osmolality,ionic content and pH of the body fluids, there are high important role of the kidney in maintaining homeostasis [1].

In birds, the urinarysystem have large paired kidneys, located symmetrically at the sides of the vertebral column, the kidneys drained the secretion by ureters.The uerters open into the urodeum of the cloacae, urinary bladder is absent in birds [2].

There are tiny toughen structures with shoaly drooping between them covering the kidney. Each one of these structure is composed unite of cortical tissue of kidney [3]. About8 or 10 of the cortical unites consistence collection urine into one medullary

cone. These areas of kidney tissue are recognize as lobules composed from little elongated collectives of cortical tissue conjointly the one medullary cone to the urine stream. Medullary lobule is necessary etcher encompassed with connective tissue covering join to cortex at broad ending and related to the main urethral department at the other [4].

Avian kidney lobe is expressing cutting into lobules, the lobules had cortex and medulla [5,6,7]. The nephron is the active unite of the kidney and its structure has considerable varies amongst variant vertebrates; also its different between species. There are two types of nephron in birds (reptilian type and mammalian type). The reptilian type is smaller than the mammalian and have no loop of henle[8,2].

There are no differences in glomerulus between the avian and mammalian except in size, the mammalian glomerulus is larger and has complex system of capillaries loops. The proximal convoluted tubules(P.C.T) are variant from distal convoluted tubules(D.C.T) in height of epithelium cells which is approximately higher cuboidal than the P.C.T. and there are no brush border in DCT.[3,4].

Calcium is the metal which is consider highest concentration in laying hens, it's about 1.5% of the body weight. In the adult bird, the calcium is diversify higher than one third of all mineral [9]. The higher concentration of calcium ions is taking through the lifetime until reach to the egg production to begin decrease in concentration [10].The calcium salts are reabsorption through the proximal and distal tubules of nephrons in kidneys. There are participate in some parts of these structure as calcium phosphate in the epithelium and there are calcium carbonate in the renal interstitium[11].Egg laying and shell calcification need high quality ion calcium(metastasis). The calcium carbonate is compose 95% from the egg shell [12].

The incidence of calcification is occur by series of complex and regulation developments in parts of organs [13]. The principle of the causality of calcification include "age, hormone, nutrition calcium, vitamin D and the parathyroid hormone plays chief part in calcification [14].The broiler chicks which feeding 3.27% calcium in diet for 15 weeks starting with calcification in kidneys [15]

MATERIALS AND METHODS

Animals and tissue preparation:

Twelve birds of laying hens selected from poultry fields of the kut town. According to the age, the bird were classified into two groups (18 months and 24 months), six laying hens chicken in each class. These birds were well health and it's were not undergo from any disease. The birds were anesthized by using the chloroform, then the kidneys were rise and taken samples from different regions of it. The samples were fixated with 10% of neutral buffered formalin for (72 hours), after that the specimens washed by tap water 4-6 hours. The processes of the technical histology were utilized which include "dehydration, clearing, infiltration, embedding,

cutting, and staining". There were two dyes using hematoxylin and eosin (H&E) for showing the general structures, and Van Kossa stain torevelate the deposit of calcium salts in tissues of the kidney of laying hens[16].The morphometric measurements were done includes: diameters of PCT and DCT[17]."Statistical analysis was obtained as standard deviation and standard error for many parameters in kidneys. Analysis of T-test forstatistical differences of variables of two sets was done [18]".

Immunohistochemistry technique:

The standard biotin free one- step HRP polymer anti-mouse, rat and rabbitIgG (H+L) with DAB immunostaining procedure were used to detect calcium deposits inthe kidney. The dewaxed, hydration of the series of alcohol solution and washed up time of distilled waterwere evented for sections of embedded in laying hens. Antigen retrieval by submerge slides in jar having "citrate buffer solution". Slides were washed with "phosphate buffer saline with pH 7.2 for 5 minute". "The sections were incubated with peroxidase block for 5-10 minutes at room temperature RT, and then were washed with distilled water 3times, also slides were washed with PBS. Subsequently, sections were incubated with protein blocking solution 5-10 minutes at RT, and then incubated with primary antibody (diluted of 1:500) for 30 minutes at RT. Slides were washed with PBS 5-7 times, and then incubated with one -step HRP polymer for 30 minutes at RT, then slides were washed with PBS, also slides were washed with distilled water 2-3 times afterward, add few drops of ready to use DAB reagent on tissue slides (was used by mixed well 1 ml of reagent buffer & substrate (BS) and 50 microliter of reagent C chromogen) for 6-10 minutes at RT, then washed with PBS and were washed in distilled water. Later, section was incubated with hematoxylin stain 30- 60 seconds subsequently; slides were washed with distilled water and mounted with D. P. X. mounting medium"[19]and the photographs were takenbya canon digital camera with 18.0 mega pixel

RESULTS AND DISCUSSION

Histological study:

In the cross section of kidneys in the two groupsof the laying hens, the microscopic examination were showed that the kidneys covered by capsule composed of smooth muscle with some of collagen fibers. The kidneys had many lobules, these lobules had two layers (medullary tissue and cortical tissue) the delineation between cortical and medullary tissue are no cleared as in mammalian. There are found the cortical type and medullary type of nephrons (Fig.1, 2).The same finding was showed by [20].

The current result revealed that the nephrons had renal corpuscles composed of the glomerulus surrounding with Bowmans capsule which is separated from it by Bowman space. The distal convoluted tubules(DCT) were showed different from proximal convoluted tubules(PCT) which the epithelium lining without brush border and the cells was lighter staining, the shape of the DCT was approximately cuboidal. The limbs of henle lining with simple cuboidal epithelium cells, the thick and thin limbs of henle passed between the collecting ducts(Fig.1, 2).This finding was in agreement with that of [21].

In both ages, our results showed with Van kossa stain, there were presence of calcification as few small blot deposited in epithelium, basement membranes of renal tubules and between them, also can be observed in the glomerulus. This calcification represented the calcium salts which has brown to dark in color. The calcification in the 18 age relatively decrease than in the 24 age(Fig.3, 4, 5, 6).This is may be because increase intake of calcium in fed of laying hens for long period lead to calcification in kidney, this opinion was concordant to what has been mentioned by many authors as [22] that the broiler chicks which feeding 3.27% calcium in diet for 15 weeks starting with calcification in kidneys,[23] remained that the nephrocalcinosis in young ostriches occur when intake (13.48% calcium & 0.82% phosphorous) this is diagnosis through radio graphically and appeared as multiple radio opacities throughout the renal parenchyma. For verification, [24] observed that the high dietary calcium and low dietary phosphorous diet of pullet and laying hens cause calcification &urolithiasis.

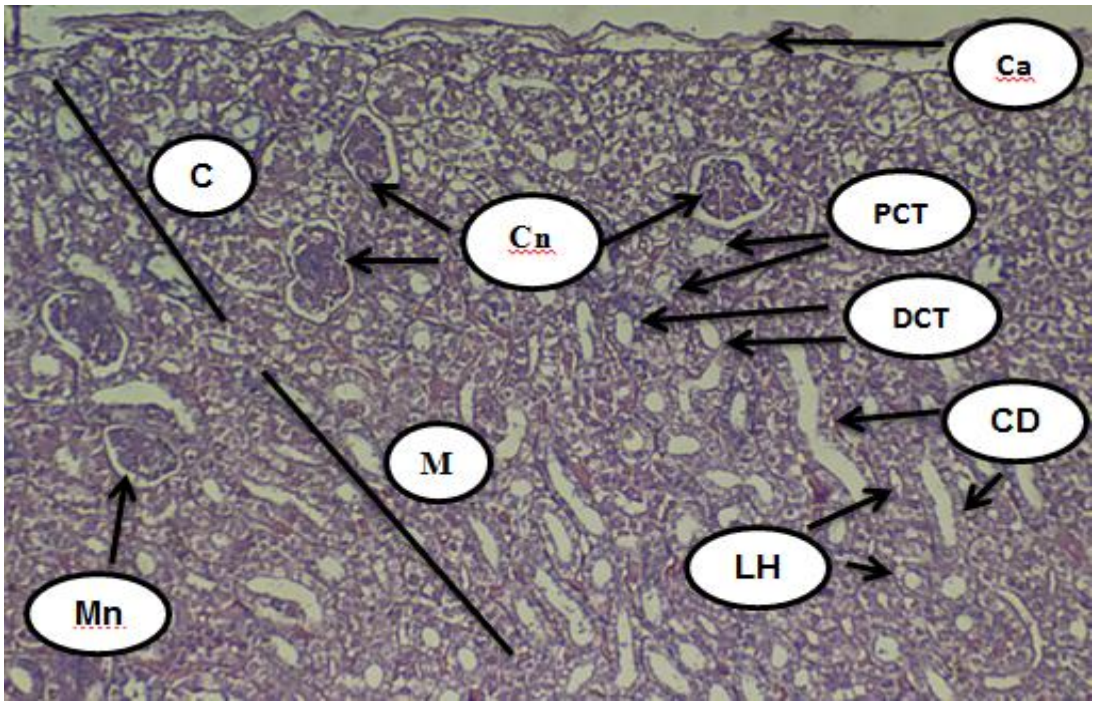
The diameters of tubules and height of epithelial cells of proximal convoluted tubules and distal convoluted tubules in 18 months age of laying hens were higher significant differences than in 24 months age(Table 1). We suggest that the presence of calcification in the epithelium and basement membrane of the tubules which more abundant in the 24 month age than the 18 month age lead to thickening in these parts, this cause decrease of the lumens of tubules. This suggestion agree with [25] who mention that the occurrence of calcification in kidney of mice after giving the calcium gluconate caused thickening of epithelium and basement membrane of renal tubules and there are no presence of calcification in the nucleus of cells.

This occurrence of increasing of calcification in the kidneys of laying hens through forward of aging is agree with [15]who show the calcification is increase with the progression of aging in many organs(heart, trachea and kidneys) of chicken and ostrich, and there are many authors refers for that as [26] whom appeared that the calcification in the trachea increasing with the progression of aging in laying hens by using the immunohistochemical methods.

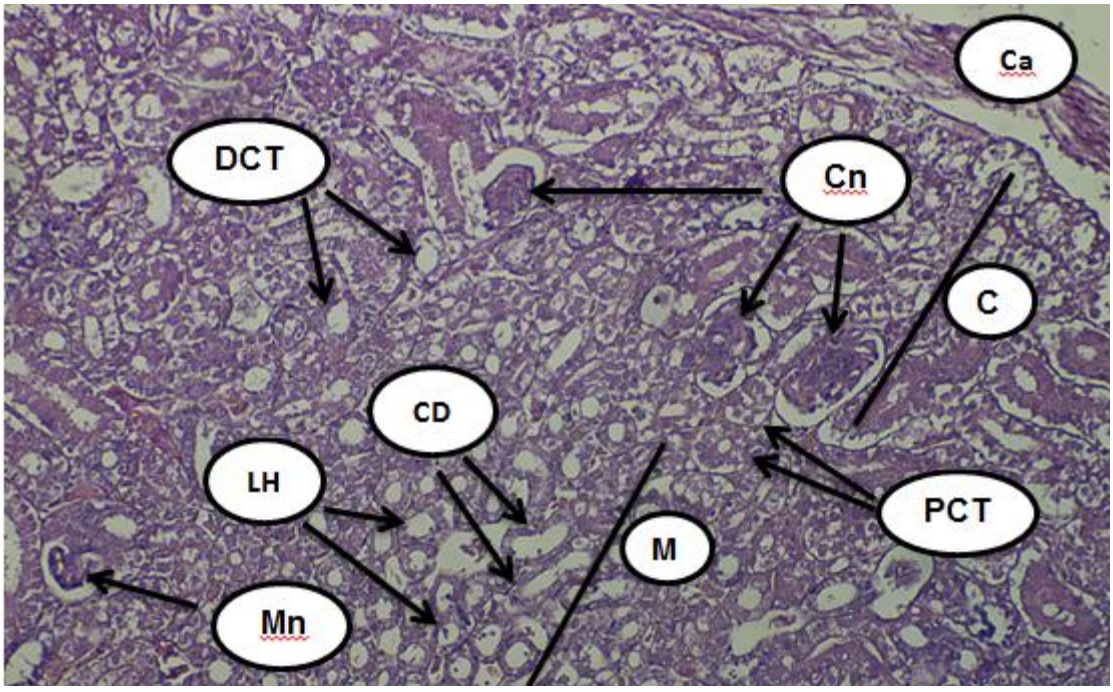
Table(1): Show diameter of PCT and DCT with the height of epithelial lining inthe two ages of laying hens:

Aging		18 month	24 month
Diameter of tubule (μm)	PCT	45.15 ± 1.132 A	42.12 ± 1.211 B
	DCT	29.23 ± 1.211 A	25.34 ± 1.113 B
Height of epithelial (μm)	PCT	11.32 ± 0.145 A	14.16 ± 0.121 B
	DCT	7.422 ± 0.312 A	9.532 ± 0.226 B

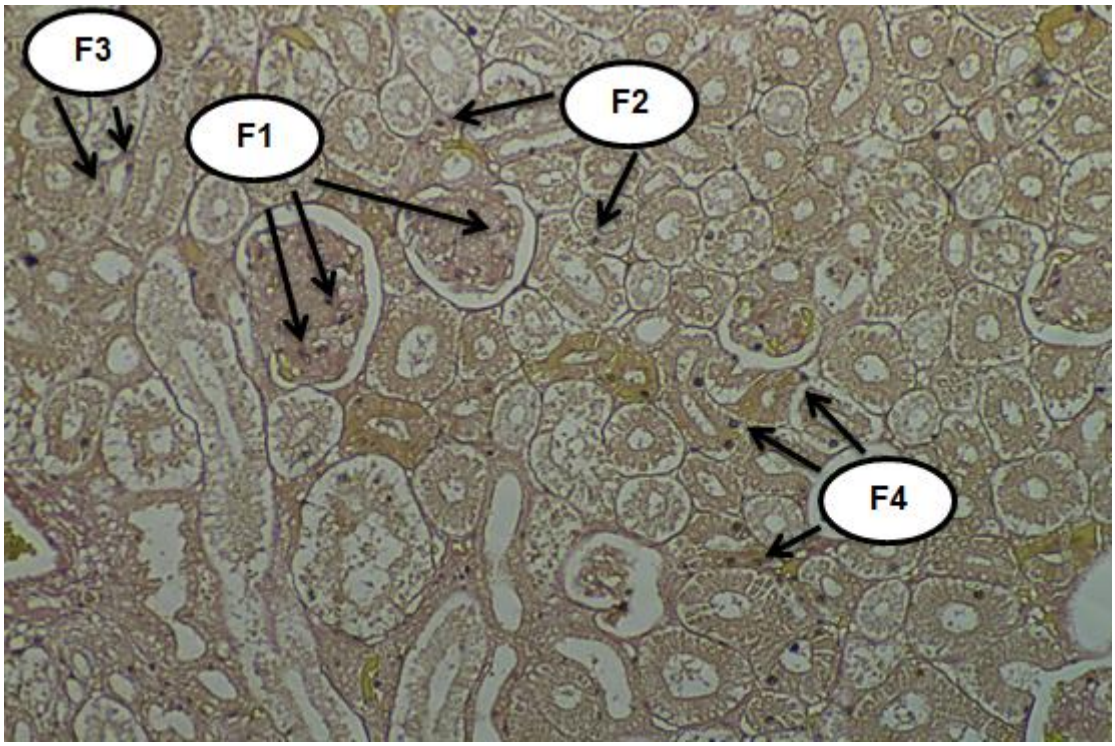
"The values represent the Mean ± Standard Error S.E.) The different capital letters mean significant differences at level of p<0.05"



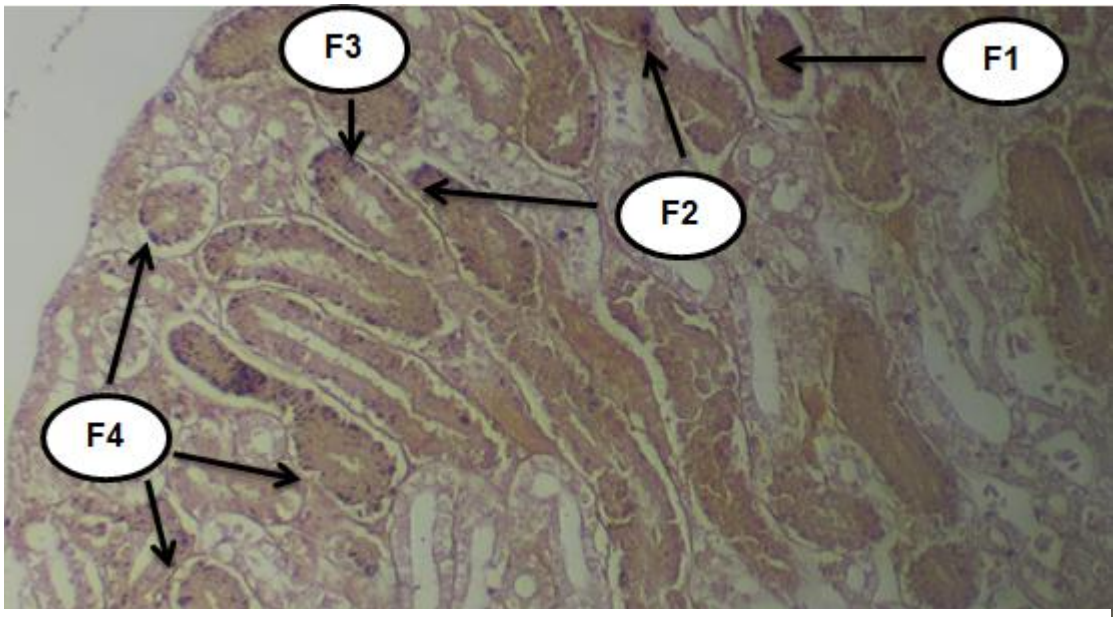
Fig(1): Histological section of kidney in laying hens (at 18 months age) showno cleared delineation of cortical and medullary layers. capsule (Ca) Cortex (C), medulla (M) cortical nephrons (Cn) medullary nephrons (Mn), proximal convoluted tubules (PCT),distal convoluted tubules (DCT), collecting duct(CD) and loop of henle (LH). (H & E stain 20 X).



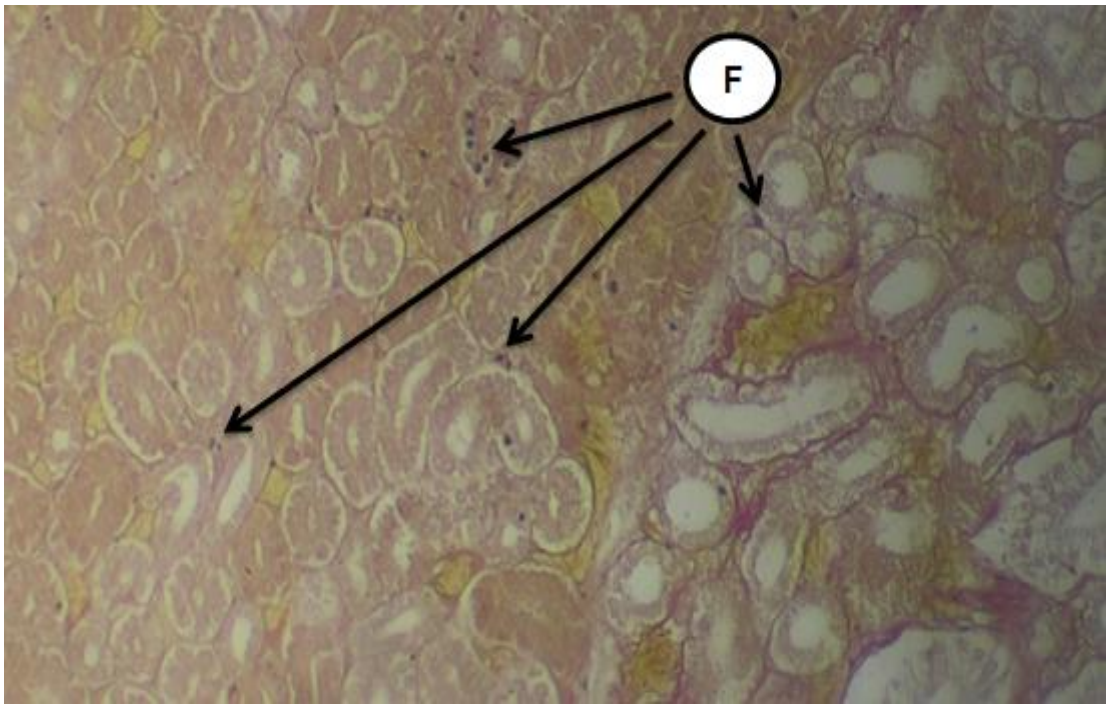
Fig(2): Histological section of kidney in laying hens (at 18 months age) showno cleared delineation of cortical and medullary layers. capsule (Ca), Cortex (C), medulla (M) cortical nephrons (Cn), medullary nephrons (Mn) proximal convoluted tubules (PCT) ,distal convoluted tubules (DCT), collecting duct(CD) and loop of henle (LH). (H & E stain 20 X).



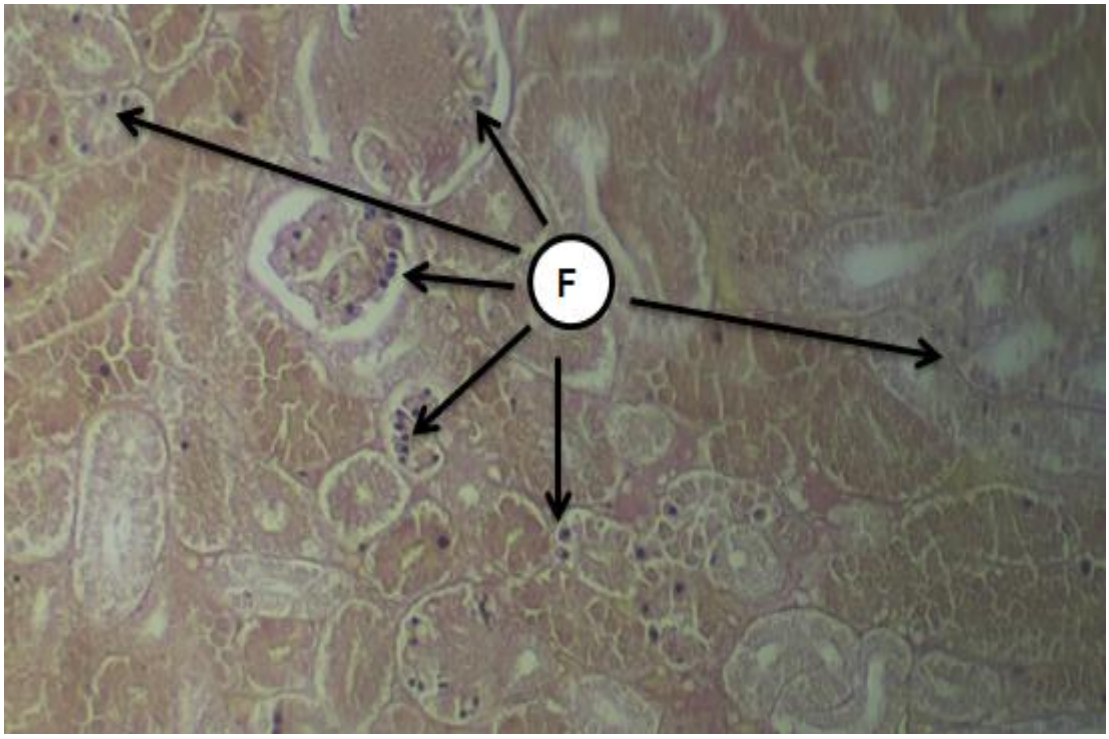
Histological section of kidney in laying hens (at 18 months age) showcalcification in form of group's of small foci (F): in glomerulus (1),epithelium and basement membrane of proximal convoluted tubules (2), epithelium and basement membrane of distal convoluted tubules (3) and between renal tubules (4) (Von Kossa 20 X).



Fig(4): Histological section of kidney in laying hens (at 24 months age) showcalcification in form of group's of small foci (F): in glomerulus (1),epithelium and basement membrane of proximal convoluted tubules (2), epithelium and basement membrane of distal convoluted tubules (3) and between renal tubules (4) (Von Kossa 20 X).



Fig(5): Histological section of kidney in laying hens (at 18 months age) showcalcification in form of group's of small foci (F) in different areas of kidney (Von Kossa 20 X).



Fig(6): Histological section of kidney in laying hens (at 24 months age) show increase of calcification than in (18 months age). The calcification was in form of group's of small foci (F) in different areas of kidney. (Von Kossa 20 X).

Immunohistochemistry study:-

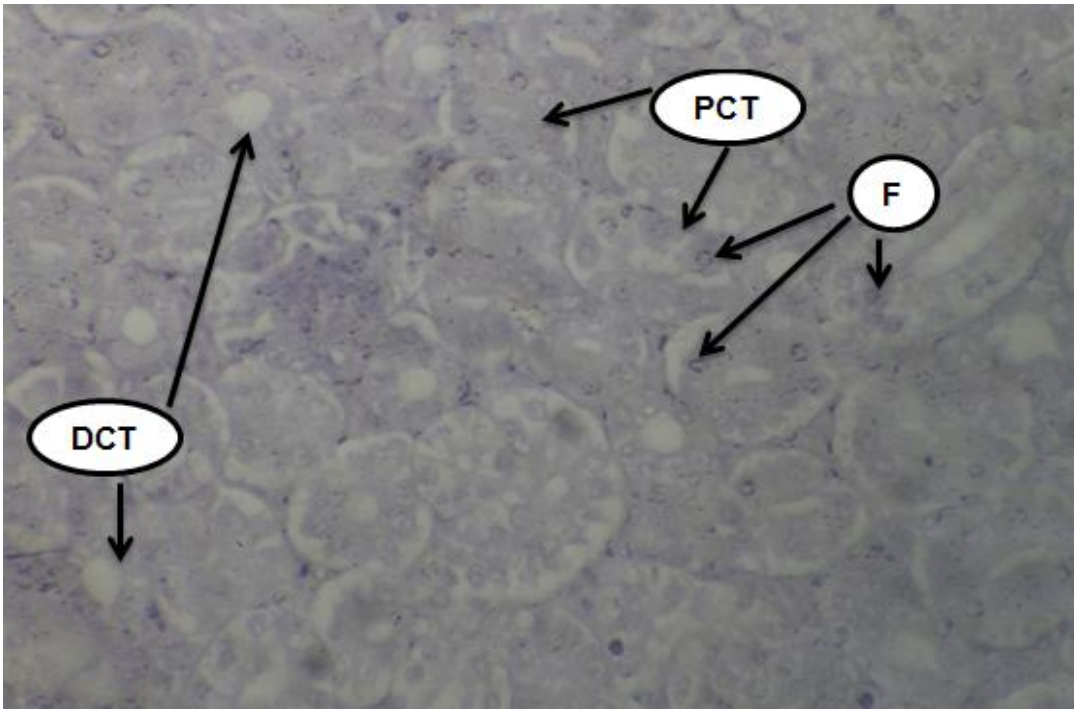
A- At 18 months age:

At this stage, the current study revealed that the calcification was detected in the glomerulus of Bowmans corpuscles in form of foci has brown to dark deposits calcium salts inside it(Fig.7, 8). These resultant of calcification in the glomerulus of Bowman capsules relatively was appreciated [22]. On the other hand, these foci of calcium salts was scattered in the epithelium, basement membrane of proximal convoluted tubules and distal convoluted tubules and between these tubules. The amount of these areas relatively valued (++)

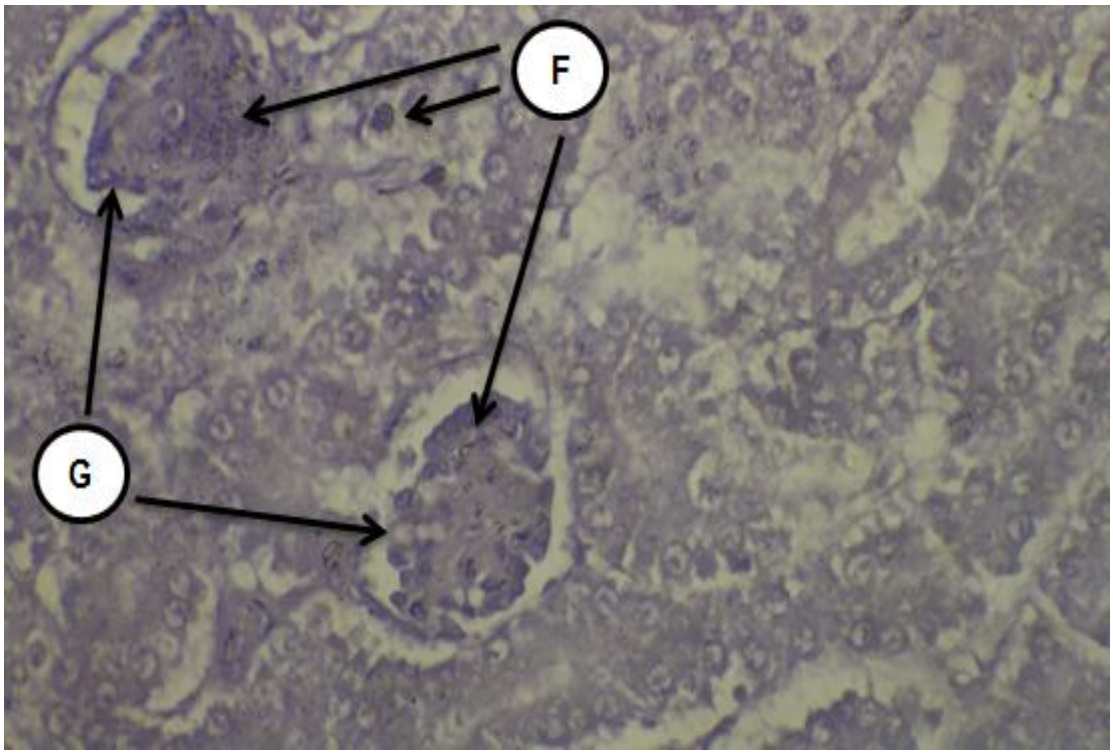
B- At 24 months age:-

In this precede age, the present study found that the calcification was demonsted more proportion from previous age in all regions of kidneys which occurrence the calcification(Fig.9, 10)..Comparatively with the previous age can appreciated the calcification in this age at (+++)in glomerulus, (+++)in proximal convoluted tubules and distalconvoluted tubules

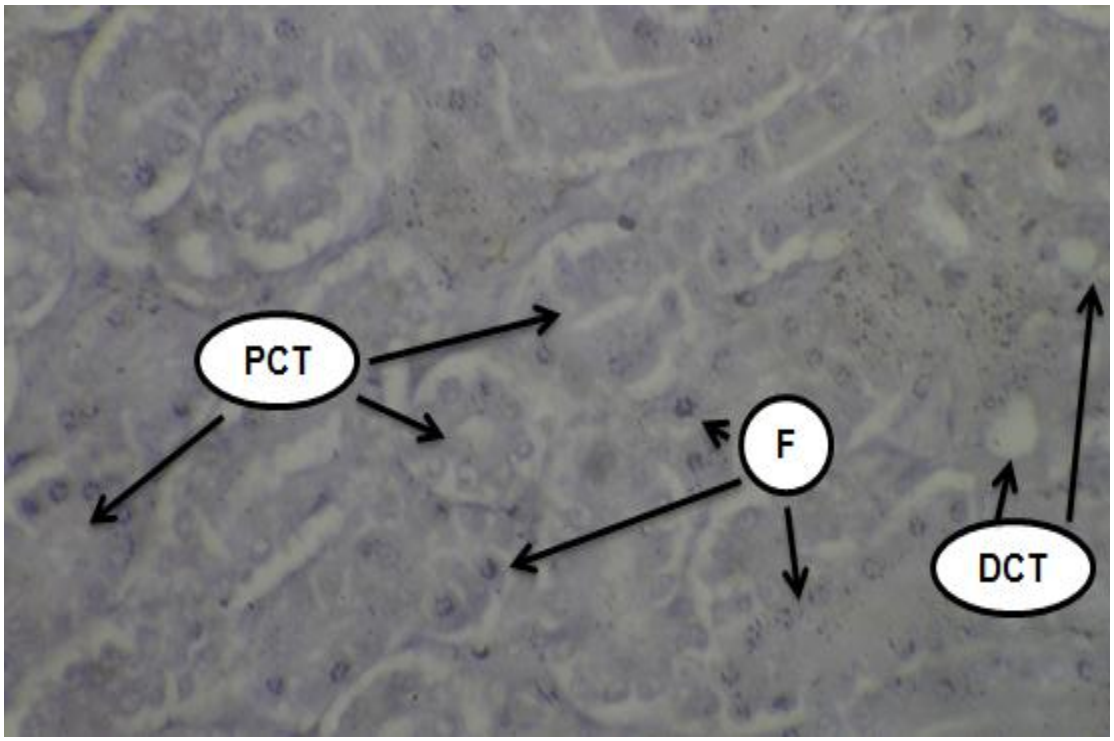
This occurrence of increasing of calcification in the kidneys of laying hens through forward of aging is agree with [15]who show the calcification is increase with the progression of aging in many organs(heart, trachea and kidneys) of chicken and ostrich, and there are many authors refers for that as [24] whom appeared that the calcification in the trachea increasing with the progression of aging in laying hens by using the immunohistochemical methods



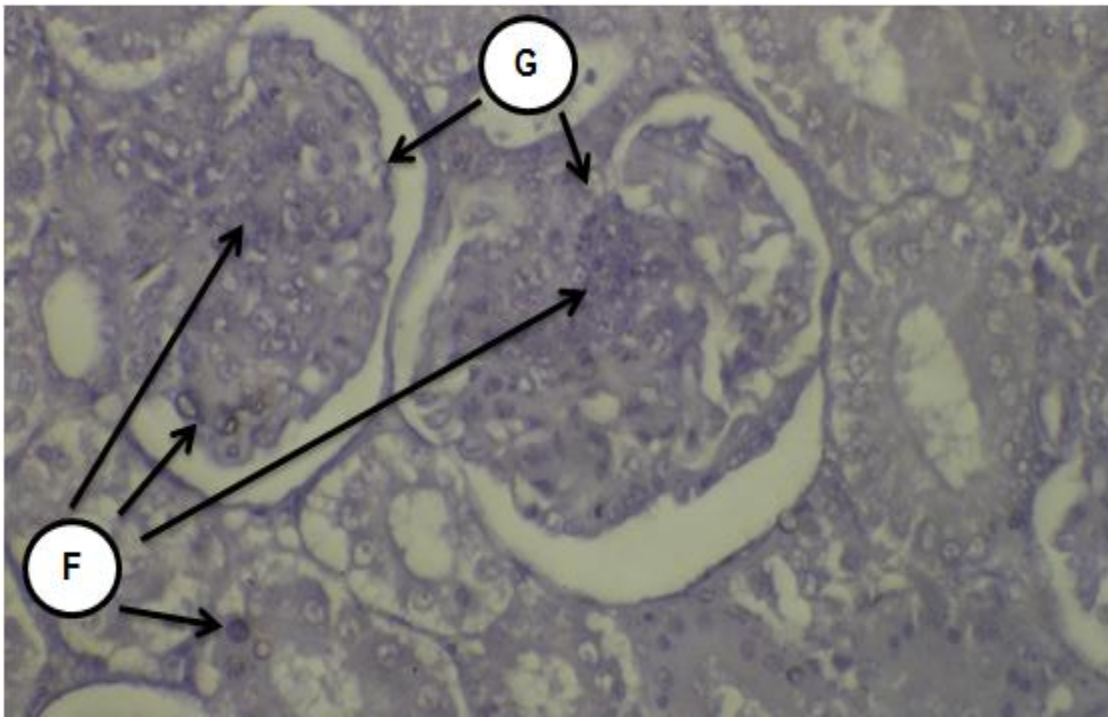
(7):immunohistochemicalsection of kidney in laying hens (at 18 months age) appeared calcification in form of group's of small foci (F) in different areas of kidney.Proximal convoluted tubules(PCT) and dital convoluted tubules(DCT).(20 X).



Fig(8): Immunohistochemicalsection of kidney in laying hens (at 18 months age) appeared calcification in form of group's of small foci (F)in different areas of kidney.Glomerulus (G).40



Fig(9): Immunohistochemicalsection of kidney in laying hens (at 24months age) appeared relatively valued calcification more than in the previous age. The calcification in form of group's of small foci (F) in different areas of kidney.Proximal convoluted tubules(PCT) and dital convoluted tubules(DCT). 20 X



Fig(10): Immunohistochemicalsection of kidney in laying hens (at 18 months age) appeared calcification in form of group's of small foci (F) in different areas of kidney.Glomerulus (G)40X

الكشف عن التكلس في كلية الدجاج البياض*(Gallus gallusdomesticus)* بواسطة استخدام التقنيات النسيجية والكيمياء النسيجية المناعية

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الخلاصة

صمم هذا البحث للتحقق من التكلس في كلية الدجاج البياض وتحديده للعمرين(18 شهر و 24 شهر). أظهرت النتائج عدم وجود اختلافات بالمظهر النسيجي في كلا العمرين عند استخدام صبغة الهيموتوكسلينوالايوسينفي حين وجد فرق معنوي في ارتفاع الخلايا الظهارية للنبيبات البولية في كلية الدجاج البياض عند عمر 24 شهر اعلى مما هي عليه في عمر 18 شهر بينما سجلت النتائج فرق معنوي في اقطار النبيبات البولية في عمر 18 شهر اعلى مما هي عليه في عمر 24 شهر. تم استخدام صبغات خاصة(صبغة الفان كوسا) وطريقة الفحص بالكيمياء النسجية المناعية للكشف عن ترسبات املاح الكالسيوم، اظهرت النتائج وجود تكلسات على شكل بؤر صغيرة مختلفة الاحجام وذات لون بني الى اسود مترسبة في الكبيبة وفي الطبقة الطلائية والغشاء القاعدي للنبيبات الكلوية وبين تلك النبيبات. بصورة عامة كانتالبؤر المتكلسة في المناطق المختلفة من الكلية في عمر 24 شهر اكثر مما هي عليه بعمر 18 شهر.

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