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Clinical and Histopathological Study on Dog's Tumors in Iraq

Huda. Hameed Alabbody[*1], Mohammed Jwaid Alwan[2] , Mohummed Mushgil Zenad [3], Abdulraheem Abduljaleel Wali[4]

[1] Iraqi National Cancer Research Center, University of Baghdad, Baghdad, Iraq.

[2] Department of Pathology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

[3] Department of Intarnal and Prevantive Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

[4] Veterinary Hospital in Aden Square ,Public Company of Veternary, the Ministry of Agriculture, Baghdad ,Iraq.

Abstract

The study was conducted on twenty dogs from variety breeds to estimate the incidence of tumor mass and determine the risk factors of survey to cases of a year in veterinary hospital in Baghdad. The most common clinical signs were, ulceration, bleeding into lesions in addition to drowsiness, anorexia, fever and the others were depended tumor's location in dog's body like lameness, lacrimation and bloody constipation etc.

The results showed 70% of the infected dogs were working with military forces and 30% of them were pet dogs and we found that the highest percentage of tumor accrued in dogs aged more than 10 years and the females recorded 60% of infection. Terrier breed had the highest percentage of infection (30%) followed by German shepherd (25%). the most tumor affected part of the body were mammary glands in females and the limbs in both gender (25% each one) and followed by the other sites, the histopathology picture had recorded seven types of malignant tumors in (skin, intestine and mammary glands) more frequent was Sequmas cell carcinoma 35% Adenocarcinoma 20%. Some dogs had more than one type of cancer, and some cases had recorded of benign tumors other cases had only transformed tissue but not cancer.

Keywords: dogs, tumors, risk factors, clinical findings, histopathology

دراسة سريرية و نسيجية لاورام الكلاب في العراق

هدى حميد العبودي[1]*، محمد جويد علوان[2]، محمد مشجل زناد[3]، عبد الرحيم عبد الجليل والي[4]

[1]المركزالوطني الريادي لبحوث السرطان، جامعة بغداد، بغداد، العراق.

[2]قسم علم الامراض، كلية الطب البيطري، جامعة بغداد، بغداد، العراق.

[3]فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة بغداد، بغداد، العراق.

[4]المستشفى البيطري في بغداد، هيئةالبيطرة ، وزارةالزراعة، بغداد، العراق.

* Email: hudaalabbody@gmail.com

الخلاصة

اجريت الدراسة على عشرين كلب من سلالات مختلفة مصابة بكتل ورمية في مناطق عدة من الجسم لغرض تقييم حدوث الاورام وتحديد عوامل الخطورة خلال عام في المستشفى البيطري في بغداد . كانت اكثر العلامات الظاهرةعلى الحيوان تقرح ونزف الافة الورمية فضلا عن الخمول وفقدان الشهية و الحمى ، وقد تباينت العلامات توافقا مع قربها من موضع الورم مثل عرج الطرف وتدمع العين و الامساك المدمى في ورم الامعاء .. الخ. اظهرت النتائج ان ٧٠% من الكلاب المصابة كانت كلاب عاملة مع القوات العسكرية و30% منها كانت كلاب منزلية كما واظهرت النتائج بان اعلى نسبة للاورام كانت تحدث بالكلاب المسنة والتي تزيد عن ١٠ سنوات والنسبة الاقل بعمر دون السنة وقد سجلت الاناث نسبة 60% من الاصابة ، كما و سجلت كلاب سلالة التيرر اعلى نسبة اصابة (30%) ثم تلتها سلالة الجيرمن شفيرد (25%)، اما اكثر المناطق عرضة للاصابة فكانت الغدة الثدية في الاناث والاطراف في كلا الجنسين بنسبة (25%) لكل منهما، ثم تلتها بقية المناطق اما الصورة النسيجية فقد سجلت سبعة انواع من الاورام السرطانية الخبيثة في كل من الاثداء والجلد والامعاء ابرزهم سرطان الخلية الحرشفية (35%) وسرطان الادينوكارسينوما(20%) وقد اصيبت بعض الكلاب باكثر من نوع من السرطان،كما وقد سجلت حالات لاورام حميدة واخرى تحولات نسيجية فقط غير سرطانية.

Introduction

Cancers in dogs occur naturally, so it is one of the best cancer model for human and cancer studies, because these tumor are various, infect the aged and obese, otherwise dogs live in the same environment so exposed to same carcinogens. Dogs are greater resembling humans biologically, such as (telomere and telomerase actions) in addition to common spontaneous epithelial cancer [1, 2], many anatomic and clinical resemble are seamed such as type and subtypes of cancer between the two species, and same treatment schemes [3].

Cancer is the chief cause of death in dogs over 10 years in aged , with 50% of older dogs developing the disease and approximately one in four dogs eventually dying from it. [4]

The primary studies to natural dog tumors could be for developing the knowledge about human cancers [5]. All breeds, both sex and all ages are able to infect with cancer but there are variables. Multiple cancers are common to find in some breeds, in other side, there is specific cancer for specific breed. Histiocytic cancer covers a broad range of clinical appearances, from benign cutaneous histiocytoma to highly aggressive histiocytic sarcoma (HS). HS is a multisystem disease with tumors appearing at the same time like the skin or subcutis, or other organs. This is a virulent and fatal disorder of dendritic cell origin [6].

Cancer incidence of dogs is rising. Invert of other animals, the good health care of pets lead to early correct diagnosis of cancer, more cure because owners are ready to pay for diagnostic tests and treatment. Veterinary epidemiologists get better their ability to track canine cancer. Scientists are more work to know which breeds are at an increased risk of each type of cancer. This allows veterinarians to check dogs at risk breeds, leading to earlier identifying and more effective treatment. Veterinarians and searchers can work together to design studies using dog populations to benefit cancer in humans and animals [7].

In Iraq there were limited studies about cancer in dogs such as surgical and histopathological studies, in mammary glands, testicular cancer and other sites [8,9].

The aim at this study is to assessment the incidence of tumors in dogs which arrived to teaching veterinary hospital in Baghdad during a year, clinically and pathologically study, estimate the certain relevant risk factors and determine the types of cancer through cross sectional analysis.

Materials and Methods

The case history, clinical signs and tumor tissue samples collected from twenty dogs arrived to Veterinary Hospital in Adan Square in Baghdad from January 2016 to January 2017. Samples collected with agreement of owners. The disease developed spontaneously in uncastrated male and unsterilized female. Data included the vaccination plan and general clinical evaluation such as: breed, age, sex, weight, clinical signs and body temperature, date of discovering the mass, location in body. Gross descriptions of tumor color, shape, diameter measurement, time of surgery. Follow-up to check recurrence metastasis, or death and necropsy results if performed, and the presumptive diagnose was based on clinical history and physical examination. Relationships between variables were evaluated,

the significant differences between animals and statistical data was analyzed in SPSS program to find frequency, percentages of each variable and the relation between them. For histological examination, after collected tissue, immediately fixed in formalin 10% and then have processed step by step according to Humason1979[10] as the following:

1 - **Washing specimens in distilled water for 6–8 hours, or overnight**

2 - Dehydration and Clearing:-

70% alcohol 1–2 hours
95% alcohol 1–2 hours
100% alcohol (first time) 1/2–1 hour
100% alcohol (second time) 1/2–1 hour
Xylene (first time) 1/2–1 hour
Xylene (second time) 1/2–1 hour

3 - ***Paraffin Infiltration***

Use two to three changes of paraffin, 1/2–1 hour each. Under ideal circumstances.

4 - Embedding in paraffin.

5- Sectioning by rotary microtome.

6 - Staining by typical hematoxylin and eosin stain.

7- Mounting by DPX (mixture of distyrene, a plasticizer, dissolved in xylem).

Then, observation and reading cross sectional slides of cases and classified them by professional pathologist from college of Vet. Med. in Baghdad University according to the World Health Organization International Histological Classification of Tumors of Domestic Animals[11].

Result:

Table 1- Clinical finding to some variables

Place	**No.**	**%**	**Breed**	**No.**	**%**
Baghdad	17	85	Belgian	4	20
			Terrier	6	30
Diwania	1	5	Pointer (GSP)	2	10
Babel	1	5	German Shepherd	5	25
Wasett	1	5	Labrador Retriever	1	5
Total	20	100			
Gender	n.	%	Cross breed	2	10
Male	8	40	**Total**	20	100
Female	12	60	**Site of tumor**	n.	%
Total	20	100	Mammary gland	5	25
Life style			Uterus	2	10
Police and military	14	70.	Face	1	5

Pet dog	6	30	Abdomen	1	5
Total	20	100	Rectum anus	2	10
Age		100	Mouth (a papillary Exophytic mass)	1	5
<1 Year	1	5	Tail	1	5
>5-10 Years	7	35			
>10 -15 Years	9	45	Limb	5	25
>15 - 20 Years	3	15	Testes	1	5
			peritoneum	1	`5
Total	**20**	**100**	**Total**	**20**	**100**

The results in (table 1) showed that most of the cases were from Baghdad (85%), and the females were (60%) higher than males (40%). Otherwise there were fife types of breeds (Belgian, terrier, pointer GSP, German shepherd GSD and Labrador retriever) were affected and diagnosed as tumors. Terrier breed was the highest frequency (30%) and Labrador retriever breed was lower rate (5%). The mammary glands and limbs more effected positions (25% each one) followed by both the uterus and rectum (10%). the highest rate of age groups were showed over ten years to fifteen (45%), followed by the group 5 to 10 years (35%) and the least was less than one year (5%).

Table 2 - Frequency of types of cancer and tissue changes according body location and histological features

Types of primary cancer and tissue changes	No.	%	Site in body	Histological features
Sequmas cell carcinoma	7	35	Skin in different site of the body like head ,face ,leg and abdomen	Irregular masses of epidermal cells surrounded large amount of keratin (horn pearls) that surrounding by concentric layer of squamous hyperchromatic cells , tumor cells extended into dermal layer
Mammary cell carcinoma	1	5	Mammary glands	Pleomorphic , hyperchromatic nuclei of tumor cells in the stroma , large squamatous metaplasia of ductal epithelial cells , fibrosis and inflammatory cells infiltration in the interstitial mammary cell
Adenocarcinoma	4	20	, Intestine (anus). Mammary glands	Dysplasia .basophilic pleomorphic form. Several layers of nuclei and mitotic figures, signet ring . mucosal lakes
Papillary adenocarcinoma	2	10	Mammary glands	Pleomorphic, hyperchromatic nuclei of tumor cells that form papillary projection into the glandular acini and proliferation of

				epithelial cells of acini. inflammatory cells infiltration in the interstitial area
Leiomyosarcoma	1	5	Intestine(anus)	Elongated cells ,irregular direction, hyperchromatic and vesicular, mitotic figure in the muscular layer ,fibrosis, inflammatory cells infiltration
Carcinoid tumor	1	5	Intestine	Compact solid group s surrounded by thin basement membrane tumor cells expressed pleomorphic ,hyperchromatic in the sub mucosa
Interstitial (Leydig) cell tumors	1	5	Testes	Non-specific findings, recapitulates the evolution of Leydig cells; Growth patterns
Oral squamous papilloma	1	5	Mouth cavity	benign proliferation of the stratified squamous epithelium cell
Simple cystic endometrial hyperplasia	1	5	uterus	glands were cystically dilated and with occasional out pouching surrounded by abundant densely cellular stroma and gave a "Swiss Cheese" appearance
adenomyosis	1	5	uterus	cervical adenomyosis infiltrated by many neutrophils
Total	20	100		

(Table- 2) showed the most type of cancer was Sequmas cell carcinoma (35%), all of them in skin mainly in forelimbs followed by Adenocarcinoma (20%) in mammary glands and intestine. and benign oral squamous papilloma (5%) . There were tissues disturbing like Simple cystic endometrial hyperplasia and adenomyosis in uterus (5%) each one

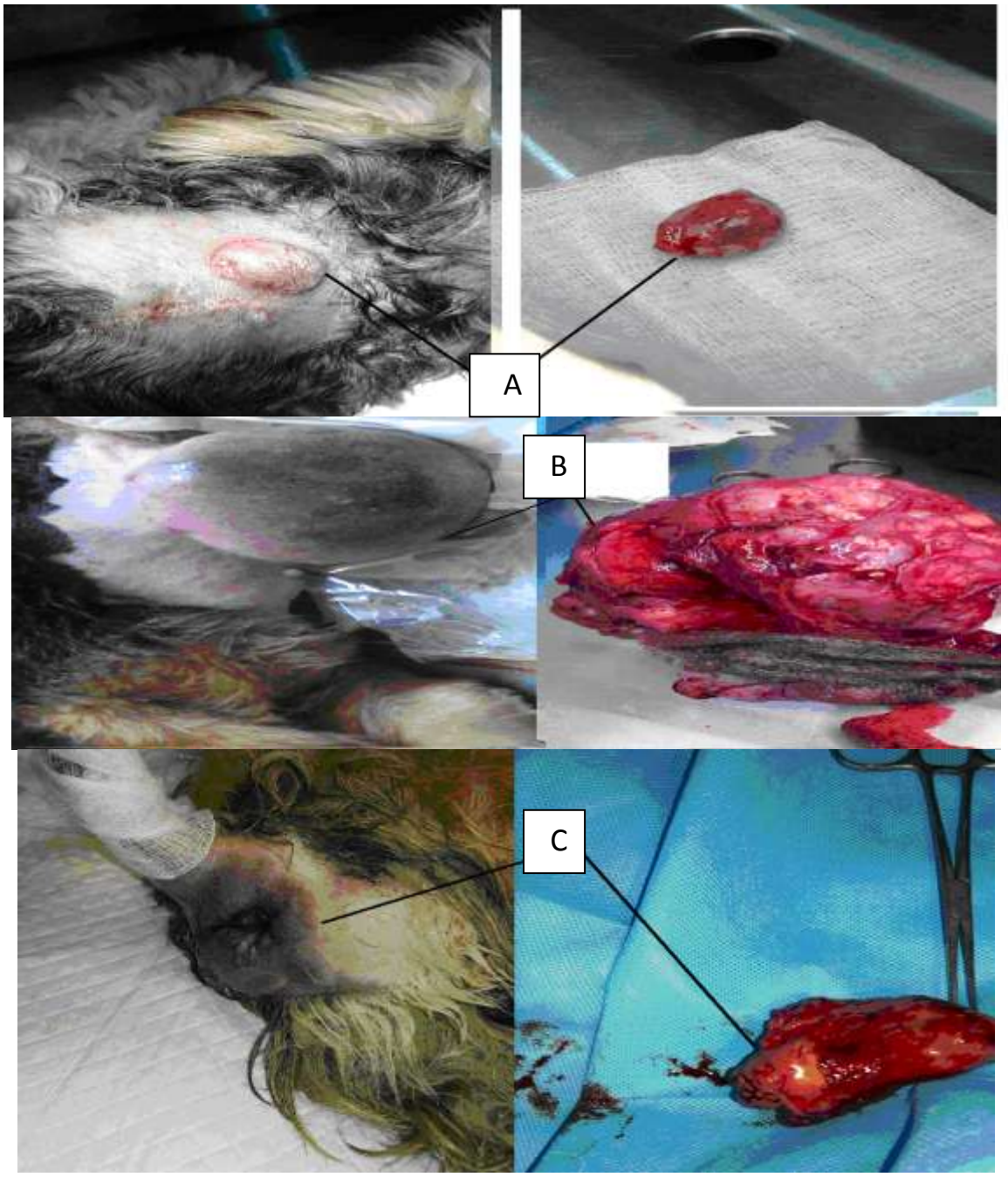

Figure1- The lesions (tumor masses) showed photographs of three gross lesions of three cases with three types of cancer as flowing:
A - Female terrier pet dog soft oval darkness, firmness, painful, in right chin abdomen mammary gland mass (5cm in diameter) in abdominal region (Squamous cell carcinoma)
B - Female German shepherd worker dog large over size darkness ,firmness ,warmth, edematous mammary gland mass too advanced (15cm in diameter (adenocarcinoma)
C - Male Labrador retriever, worker dog, rectum anus darkness mass tumor 5cm in diameter (Leiomyosarcoma)

Histological examination

Histological examination appeared all the collected samples tissues, as shown in Table- 2 , seven types of tumors were recognized malignent and benign tumors as adenocarcinoma, Leiomyosarcoma in intestine lesions, and adenocarcinoma, carcinoma, and papillary adenocarcinoma in mammary glands lesions and squamous cell carcinoma in skin lesions.

In the intestine in different sites section , shows hyperplasia and dysplasia of epithelial layer that showed hyperchromatic pleomorphic nuclei with mitosis with neutrophils in filtration in the stromal tissue (adenocarcinoma Figure- 2) in other section it was reported dysplasia with basophilic pleomorphic apparently form several layers of nuclei , and mitotic figures were seen (adenocarcinoma Figure- 3).

As well as the villi lining by mucus secretion cells form large cystic like structure with severe inflammatory cells infiltration particularly neutrophils in submucosa (Figure-4). In other sections tumor cells produced mucin in their cytoplasm that pushed their nuclei to one site form what called signet ring as well as mucin appear in the lumen of mucosal glands that compressed the epithelial lining ,cystic structure filled by mucin lining tumor cells this pattern called mucosal lakes, fibrosis and inflammatory cells infiltrated in the stromal layer were seen (adenocarcinoma Figure- 5,6) . While another section in the intestine shows elongated cells, irregular direction, hyperchromatic and vesicular with few mitotic figure in the muscular layer (Leiomyosarcoma Figure- 7). On the other hand another section in the intestine showed compact solid group surrounded by thin basement membrane tumor cells expressed pleomorphic ,hyperchromatic in the sub mucosa (carcinoid tumor Figure-8).

Sections in the mammary gland were showed pleomorphic, hyperchromatic nuclei of tumor cells in the stroma, and inflammatory cells infiltration in the interstitial. one of them appeared mass or cord of tumor cells , large squamatous metaplasia of ductal epithelial cells as well as fibrosis (mammary cell carcinoma Figure- 9),in the other section , mammary gland showed tumor cells that form papillary projection into the glandular acini (papillary adenocarcinoma Figure-10).

One section in the skin showed hyperplasic of the stratified squamous epithelium and congested blood vessels and inflammatory cells particularly mononuclear cells infiltration in the dermal layer (Figure-11). Another section was showed irregular masses of epidermal cells surrounding large amount of keratin that form what called horn pearls which surrounded by concentric layer of hyperchromatic squamous cells , and the tumor cells extended into derma (squamous cell carcinoma Figure- 12)

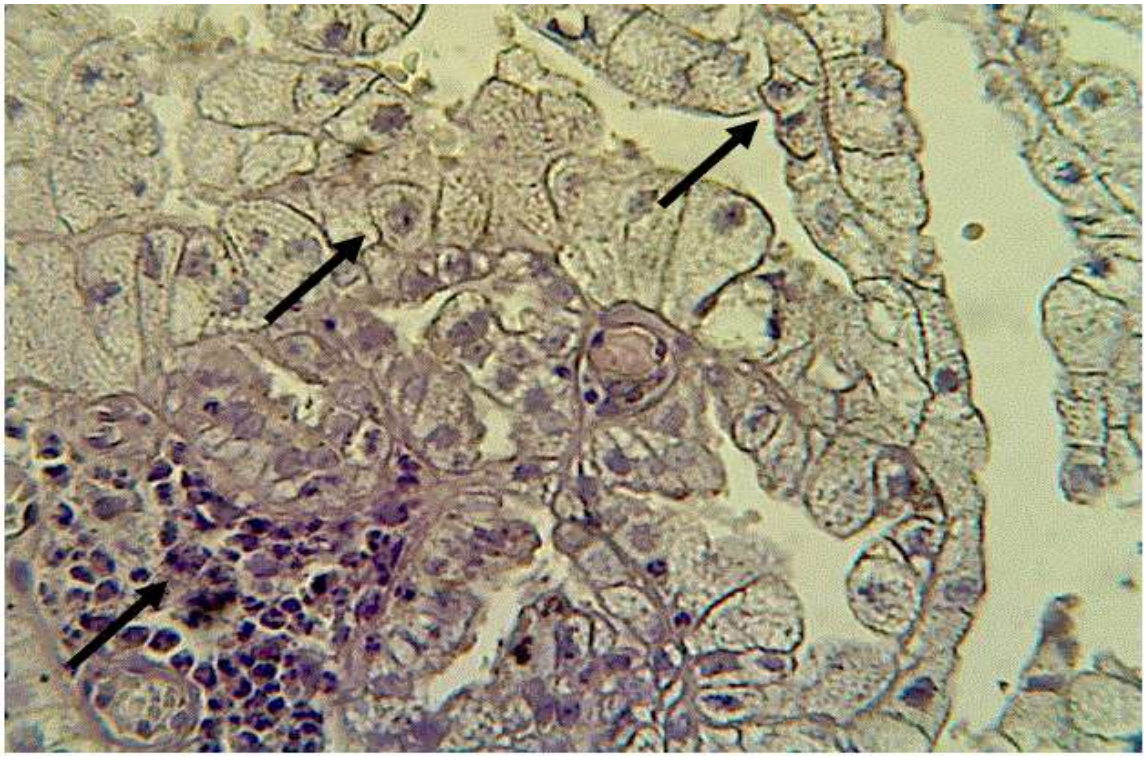

Figure 2-Section in the intestine shows hyperplasia and dysplasia of epithelial layer that showed hyperchromatic pleomorphic nuclei **(upper arrow)** with mitosis (**middle arrow)** with neutrophils infiltration in the stromal tissue**(lower arrow)** adenocarcinoma (H&E stain 400X)

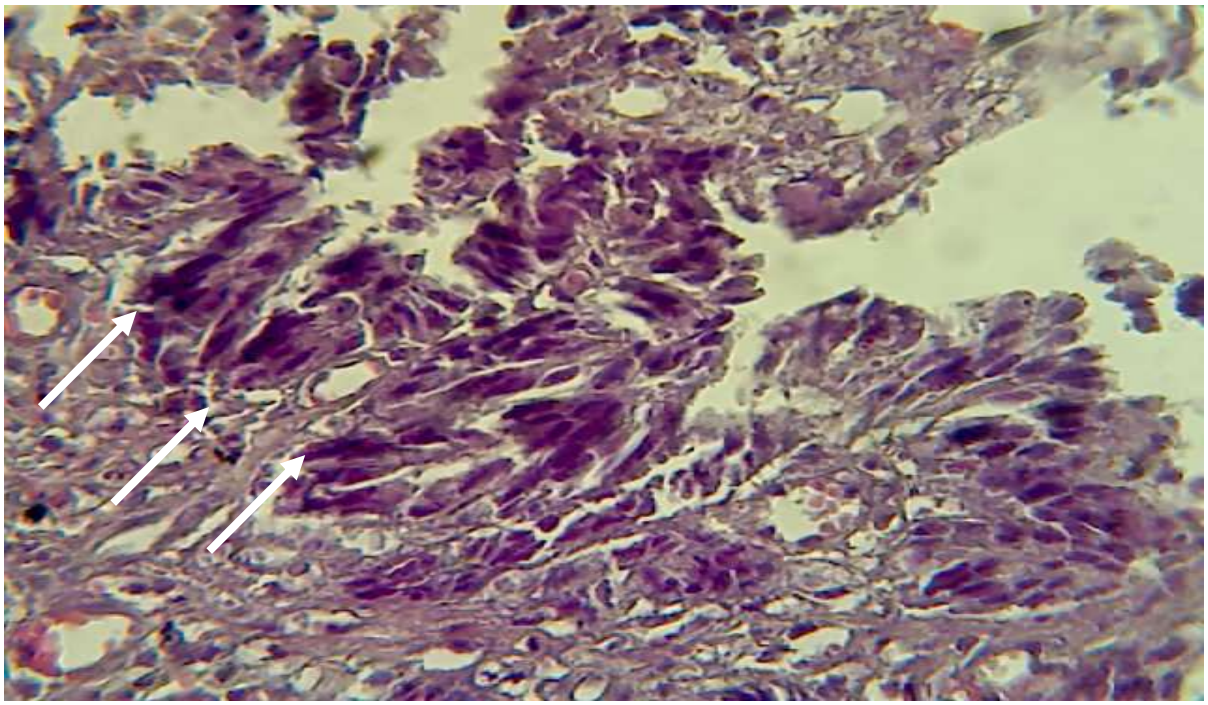

Figure 3- Section in the intestine shows dysplasia with basophilic pleomorphic apparently form several layers of nuclei .mitotic figures were seen **(white arrows)**
adenocarcinoma (H&E stain 400X)

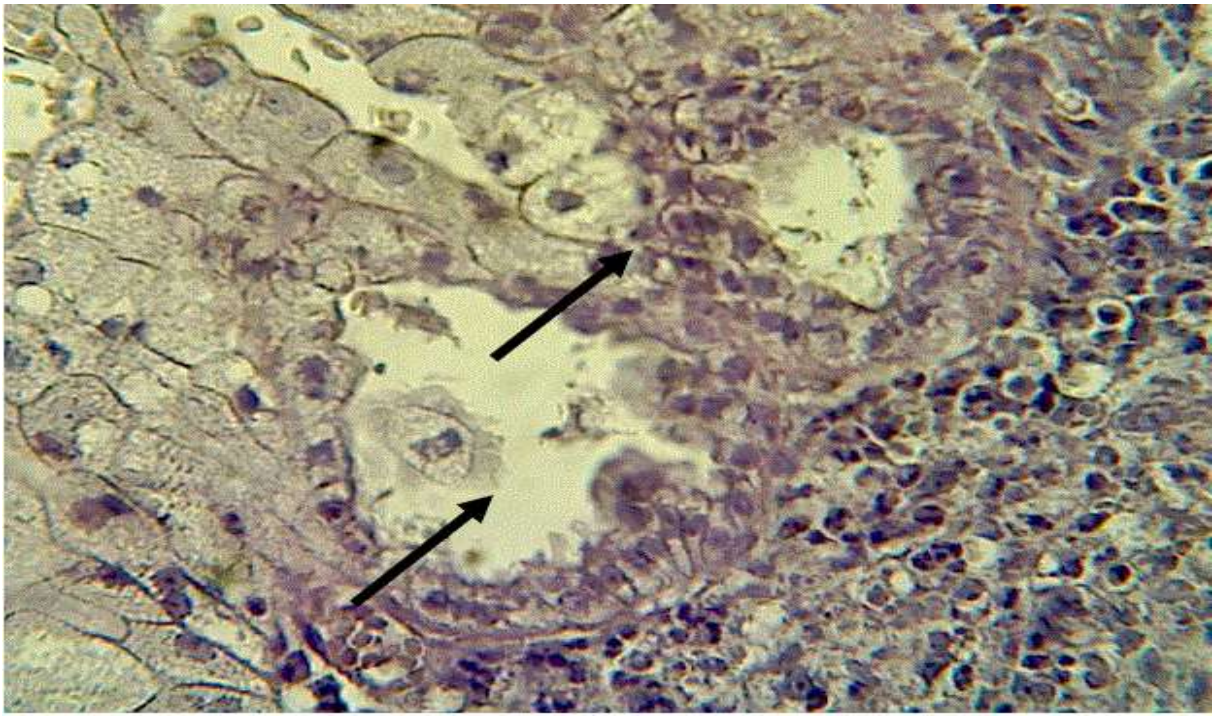

Figure 4- Section in the intestine shows dysplasia with basophilic pleomorphic apparently form several layers of nuclei.mitotic figures were seen **(upper arrow)** as well as the villi lining by mucus secretion cells form ;large cystic like structure with severe inflammatory cells infiltration particularly neutrophils in submucosa **(lower arrow)** adenocarcinoma (H&E stain 400X)

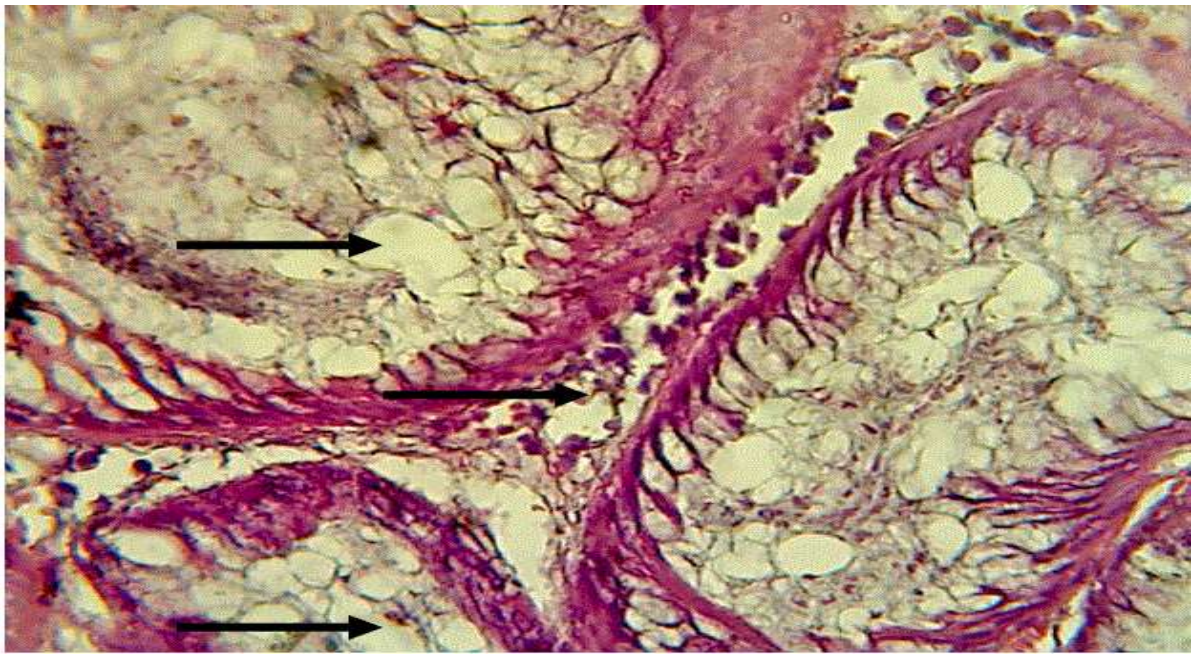

Figure 5- Section in the intestine shows pleomorphic , hyperchromatic nuclei of tumor cells that produced mucin in their cytoplasm that push their nuclei to one site form what called signet ring**(lower arrow)** as well as mucin appear in the lumen of mucosal glands **(upper arrow)** and inflammatory cells infiltrated in the stromal layer **(middle arrow)** adenocarcinoma (H&E stain 400X)

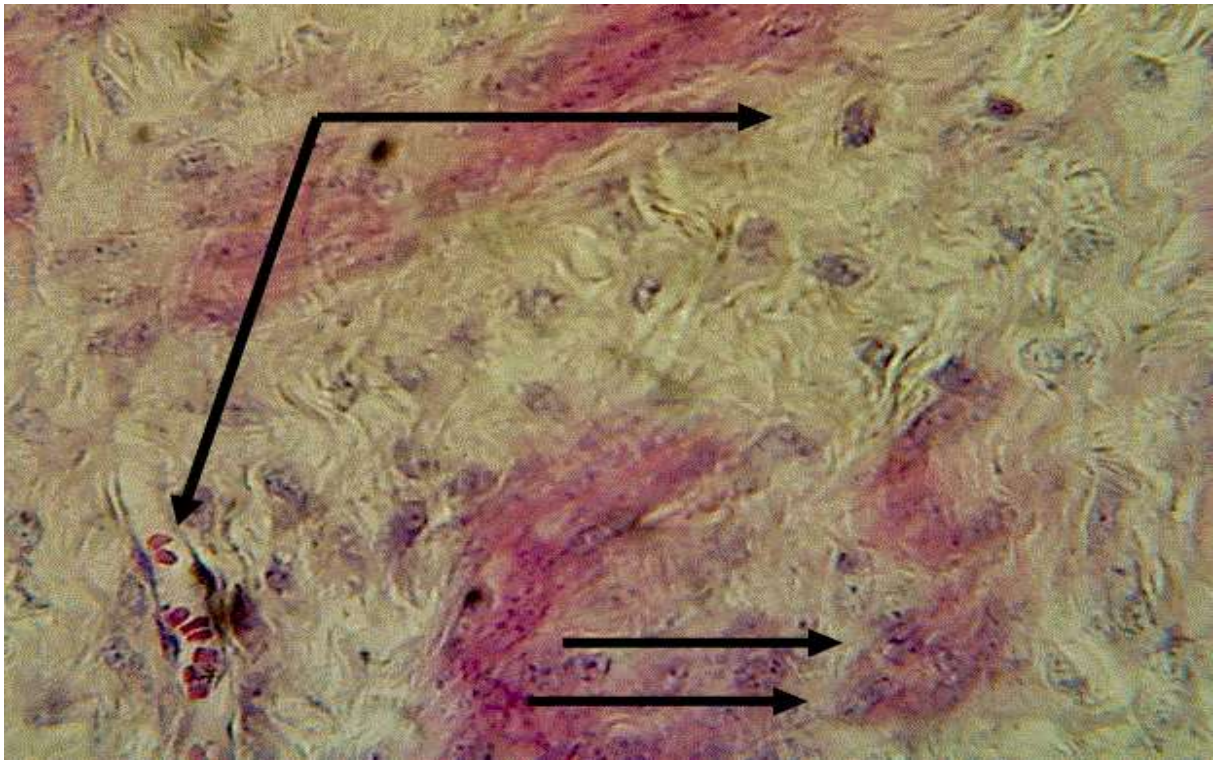

Figure 6-Section in the intestine shows elongated cells ,irregular direction, hyperchromatic and vesicular **(cross arrows)** with few mitotic figure (**parallel arrows)** in the muscular layer (Leiomyosarcoma) (H&E stain400X)

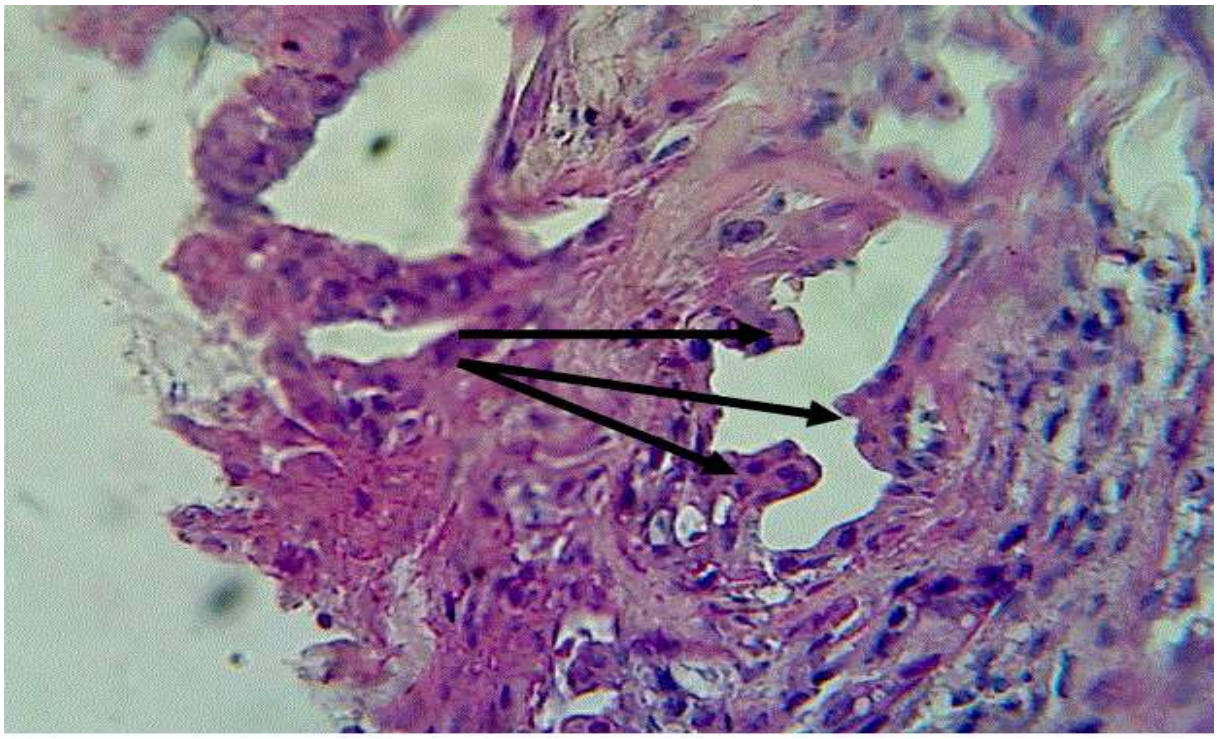

Figure7 - Mammary gland section shows pleomorphic , hyperchromatic nuclei of tumor cells that form papillary projection into the glandular acini and proliferation of epithelial cells of acini**(triple arrows)** in addition inflammatory cells infiltration in the interstitial ,papillary adenocarcinoma (H&E stain 400X).

Figure 8- Section in the mammary gland shows mass or cord of pleomorphic , hyperchromatic nuclei of tumor cells in the stroma **(middle arrow)** in addition to large squamatous metaplasia of ductal epithelial cells **(right arrow**) as well as fibrosis and inflammatory cells infiltration in the interstitial **(left arrow)** , mammary cell carcinoma (H&E stain 400X)

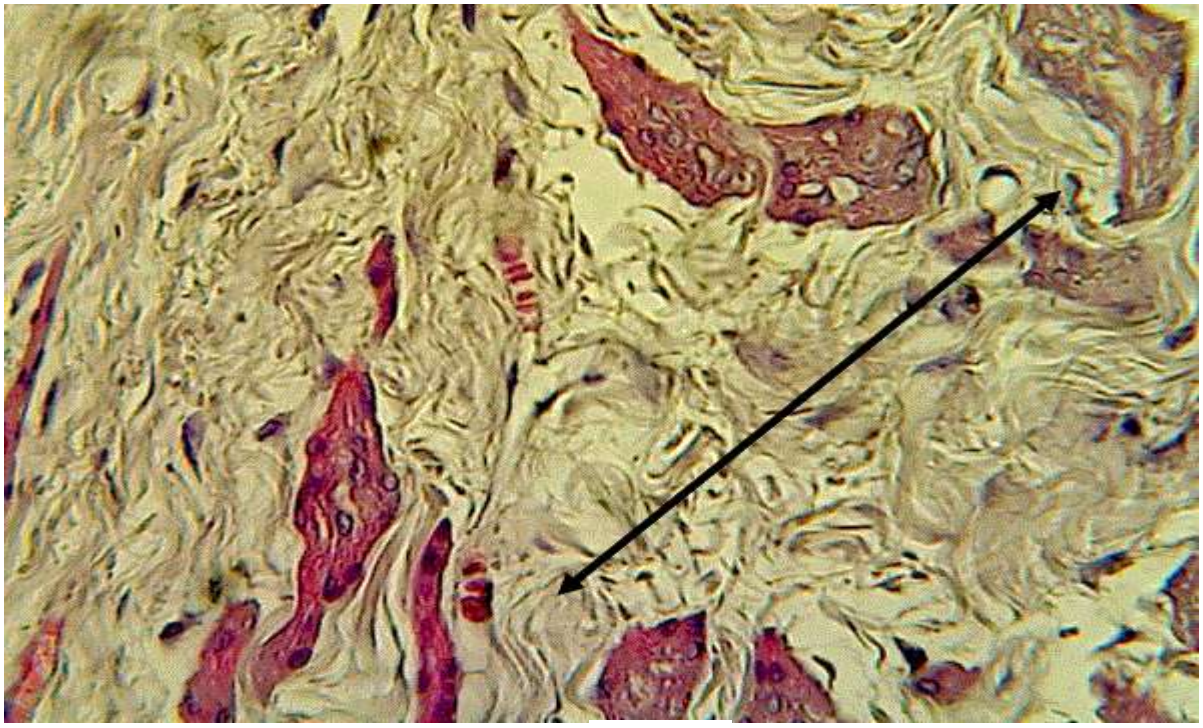

Figure 9- Section in the intestine shows compact solid group surrounded by thin basement membrane tumor cells expressed pleomorphic, hyperchromatic in the sub mucosa **(the straight)**, carcinoid tumor (H&E stain 400X)

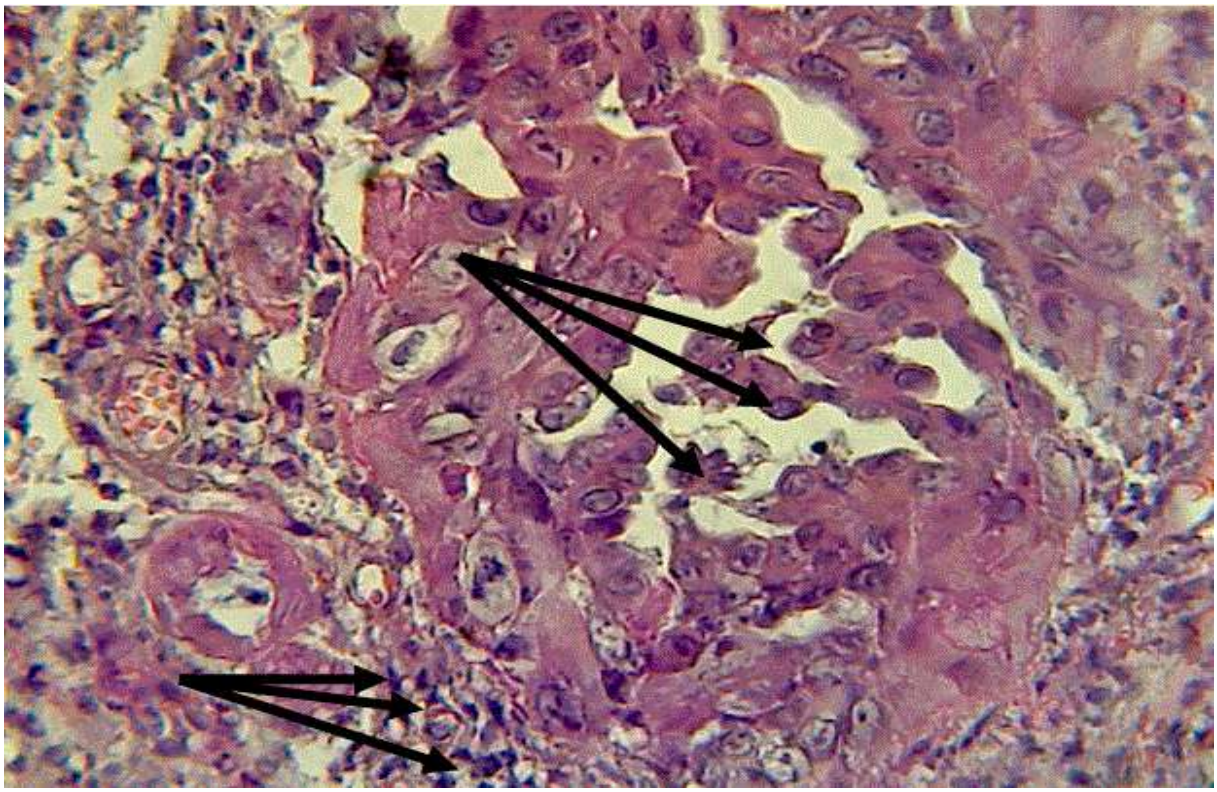

Figure 10- Section in the mammary gland shows pleomorphic , hyperchromatic nuclei of tumor cells that form papillary projection into the glandular acini **(upper arrows)** in addition inflammatory cells infiltration in the interstitial **(lower arrows**), papillary adenocarcinoma (H&E stain 400X).

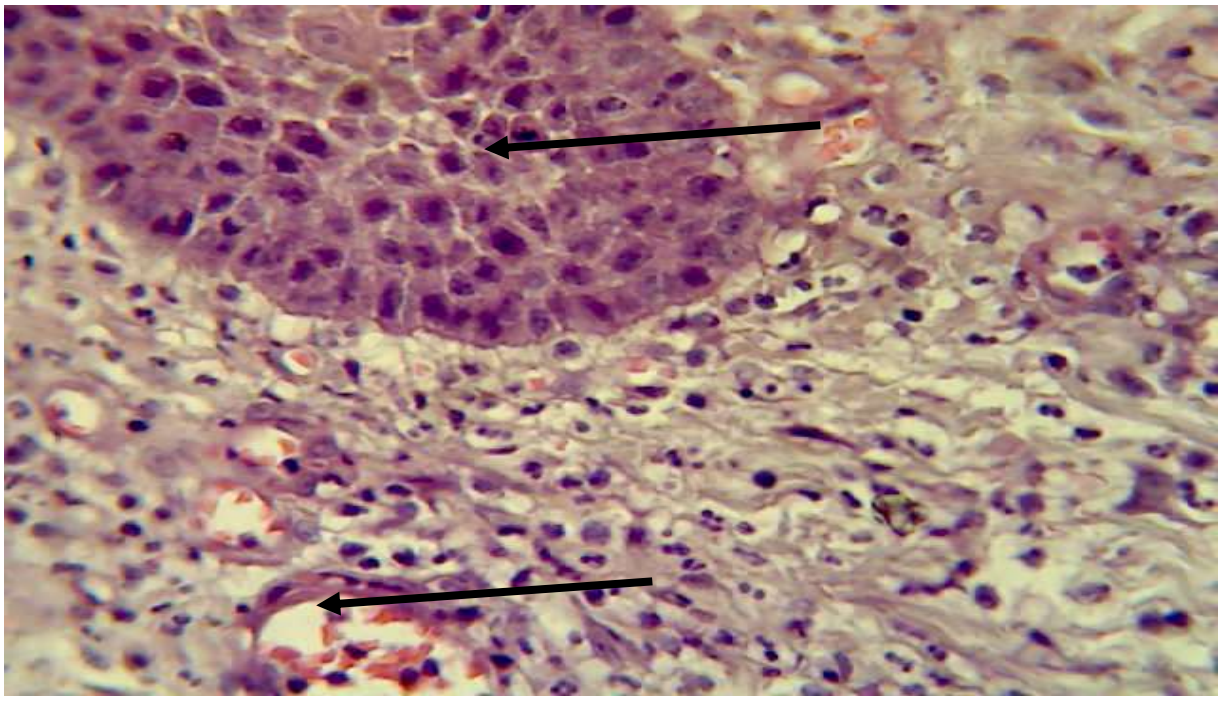

Figure 11-Section in the skin shows hyperplasic of the stratified squamous epithelium **(upper arrow)**, congested blood vessels and inflammatory cells particularly mononuclear cells infiltration in the dermal layer (**lower arrow)**, (H&E stain 400X).

Figure 12-Section in the skin shows irregular masses of epidermal cells surrounded large amount of keratin that form what called horn pearls which surrounding by concentric layer of squamous hyperchromatic squamous cells (**upper arrows**) ,the tumor cells extended into dermal layer (**lower arrow**), squamous cell carcinoma (H&E stain 400X).

Discussion

The results showed high rate incidence of tumors in dogs, most of the cases in Baghdad 85% and only three cases from nearby provinces (Babel, Diwania and Wastte) 5% each one, this lead to nearness these provinces to Baghdad and facility of medical intensive care in capital hospital , not all cases in these cities take chance of treatment but most cases in Baghdad take this chance. The study age groups showed that more than ten years to fifteen were the highest rate (45%) followed by the age

group (5 to 10) years (35%) and the least was less than one year(5%) . this result is agreeing with other studies, like Miller,et.al.2013 when the peak incidence of the cancer in dogs is between 8 and 14 years of age and anther like Jefferson,et.al. 2015 had been reported more frequent between 6 and 14 years of age [12, 13].

In this study the incidence of tumor was in females (60%) higher than males (40%). That agree with other studies which appeared the anatomy and physiology of the reproductive tract of females and chronic action of progesterone hormone and their influence on the enlargement of the mammary gland make them prone to different diseases including cancer through their reproductive life (estrus, pregnancy, lactation). The prolonged of progesterone duration and other hormones is the main cause for cancer specially reproductive organ cancer [14,15]

In this study there were fife types of breeds: (Belgian, Terrier, Pointer GSP, German Shepherd GSD and Labrador Retriever). Terrier breed was recorded the highest frequency (30%) and Labrador breed was lower rate(5%). Most of the studies seem to agree on at-risk breeds, study in United Kingdom (UK Kennel Club) recognized breeds reports cancer incidence is the highest in some types like the Irish water spaniel, followed by Rottweiler, Bull terrier, Welsh terrier, Giant schnauzer and other types [16]. While anther study was found the highest number of the cancer cases was diagnosed in Terrier breed dogs (30%) and German Shepherds (25%) [17] which confirmed our findings . In this study mainly dogs were working with military forces (army and police) (70%), and just (30%) were pet dogs , this may expose them to chemical material like (TNT, C4, drugs and other carcinogenic materials) which was highly threat to public health and life of dogs.[18] Generally in the United Kingdom, cutaneous histiocytoma is the most common canine tumor type was reported, followed by lipoma, adenoma, soft tissue sarcomas, mast cell tumor, and lymphomas, which includes both malignant and benign tumors.[4]. A study from the Danish Veterinary Cancer Registry reported that the frequency of benign and malignant tumors is similar in their country, with the most commonly reported malignant neoplasms being adenocarcinomas (21%), followed by mast cell tumors (19%) and lymphomas (17%) [19]. Dermatopathies are the most frequent conditions 18 types were found, such as mammary neoplasia (23.7%), subcutaneous lipoma (21.6%), papillomatosis 3%) and mastocytoma (6.2%).[13] In this study the most type of cancer was sequmas cell carcinoma (35%) all of them in skin mainly in forelimbs which agree with these studies, followed by adenocarcinoma (20%) in mammary glands and intestine. and benign oral squamous papilloma (5%)There were tissue disturbing like simple cystic endometrial hyperplasia and adenomyosis all of them in uterus (5%) each one. The most infected sites in this study were mammary glands and limbs (25% each one) followed by both the intestine and uterus (10% each one)**,** This is due to the hormonal imbalance and its troubles in the breast and uterus and the environmental contaminants that the dog exposed to, especially the worker dog with the security forces [19]. Statistical analysis did not reveal any significant differences between the particular parts of the body

Conclusions

This is the first describing study on canine tumor in Baghdad provide phenotypical and histological data.

Tumor in dogs should be treated (surgical treatment) as soon as possible after it is first observed, that expected may be the best treatment. We recommend sterilized females and castrated male very necessary when it is not possible to get in normal reproductive cycle to avoid the risk of increasing the sex hormones and its side effects.

The information on this search provides an overview of the epidemiological state of dog's cancer in this area. It is important to perform epidemiological studies in other cities of the country and include external consultation like clinics, because our data were obtained from canines that came to internal consultation in veterinary hospital in Baghdad only. We recommend advance studies about cancer in dogs and relationship between clinical, histopathological, with immunohistochemistry features that, benefit both oncology and comparative medicine. Canine cancers may provide an appropriate animal model for studies of cancer pathogenesis, being an easily accessible material for research.

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