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Mammary tumor in mammals and the risk factors: comparative clinical pathological study

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Abstract

The samples of the study included three species of mammals, including: ten female dogs, five women and one Holstein Friesian cow. All of them had a lump on their mammary glands. The study aimed to determine the susceptibility of these species to mammary tumors. Case history and clinical findings were recorded for each individual from whom specimens were collected. The biopsies were subjected to H & E staining for histopathological examination as well as immunohistochemistry technique for BRCA1and Ki67 level determination. On histopathological slides, 8 out of 10 of dogs showed carcinoma, which was classified into adeno, lipo, solid or inflammatory, while the other two cases had benign adenoids. The cases of women revealed different patterns: one had ductal carcinoma in situ, another one with lobular carcinoma in situ, two cases had fibroadenoma and the last case showed a cyst. In the cow, the masses were founded in the teat of the left front quarter of the mammary gland with enlargement of local and sub scapular lymph nodes. In conclusion, female carnivores represented by female dogs were more sensitive to catch a mammary tumor than omnivores represented by women, while herbivores represented by a cow were the most resistant. Therefore, these data recommend performing more studies that focus on the variability among animal species in order to reduce the incidence of mammary tumors in mammals especially humans and dogs.

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Introduction

The incidence of mammary neoplasia among various species differs greatly. In women, breast cancer is one of the most frequent types of cancer globally and the cause of almost half a million deaths worldwide each year (McPherson *et al.*, 2000). Canine mammary tumors (CMTs) are the most common neoplasia in intact female dogs (*Canis familiaris*) (Egenvall *et al.,* 2005, Fidler *et al.,* 1967). They constitute about half of all tumors in female dogs and approximately half of them are malignant (Gilbertson *et al.,* 1983, Moulton *et al.,* 1970). In both women and dogs, mammary tumors develop with age and they rarely occur before 25 and 5 years of age, respectively (Cohen *et al.,* 1974). However, the studies discovered that both canine and human mammary tumors are hormone dependent (Cotran *et al.,* 1994, Schneider *et al.,* 1969). In contrast, mammary tumors are rare in cows, mares, ewes, and goats. In ruminants, the daily inspection of udders by palpation during milking operation would make the tumors to be detected soon. The morphological appearance of the bovine mammary gland is more similar to those of human beings than in rodents; therefore, bovine has been used for tumor studies. There are several factors that may affect the high rate of tumor development in women and low rate of tumor growth in ruminants. Besides diet, lifestyle, reproductive history and stress load, general physiology and metabolism might have an influence (Sonja *et al.,* 2013). On the other hand, the dairy cow's udder is subjected to a cyclic growth and influenced by hormones producing massive quantities of milk, this makes the udder prone to mastitis rather than tumors, which are very striking. Although the range of life span of a dairy cow is rather short (6-8 years), some animals also reach an age of up to 15 years without contracting cancer. For 8 years of bovine life is equivalent to 40 years of human life, many cows are allowed to live well beyond their "cancer age" (Povey *et al.,* 1969, Sweet *et al.,* 1940).

An effective marker for breast cancer diagnosis or prognosis has not yet been identified. Increasing amounts of evidence indicate that cancers are often heterogeneous, and the response to treatment depends on the subtype of breast cancer (Carey *et al.* 2007, Voduc *et al.,* 2010).

In dogs, for example, the breast cancer type 1 susceptibility protein (BRCA1) contributes to the risk of CMT in English springer spaniels (ESS) with 36% of this breed are affected by CMTs suggesting the use of dogs as a good model for investigating human breast cancer (Patricio *et al.,* 2009). In the same context, the morphological features of the bovine mammary gland are so similar to those in women especially at the molecular level (Raposo *et al.,* 2017). Consequently, determining the expression of BRCA1 and Ki-67 (proliferation marker) proteins by immuno-histochemistry in human and canine tumors would be helpful biomarkers for survival and choice of new targeted therapy (Rismanchi *et al.,* 2014). It is worth mentioning that Ki-67 nuclear antigen is actively expressed in cycling cells, but not after mitosis (Gerdes *et al.,* 1984), and this marker is frequently used to determine tumor cell proliferation (Raposo *et al.,* 2017).

This paper discusses the main features associated with the clinico-pathological characteristics, malignant transformation, and comparative aspects of different mammary gland tumors of female in three models including: canine, bovine and human.

Materials and methods

Three models of mammals (5 women, 10 female dogs and one Friesian cow) were included in the present study through a period over 1 year as follows:

1. Ten virgin non-spayed female dogs were diagnosed as CMTs at the Teaching Animal Hospital and in private veterinary clinics in Baghdad city. Case history and clinical findings, including: age, breed, body weight, spay history, occurrence of other diseases, gross description of lesions, the clinical stage of tumor at the time of surgery and surgical procedure of simple or regional mastectomy were recorded. In the TNM staging system, a "T" followed by a number shows the size of the tumor. In some cases, the size of the tumor cannot be determined (TX) or a tumor cannot be found (T0), the size of tumor was classified as T1 (<3 cm in greatest diameter), T2 (tumor >3 cm but <5 cm in greatest diameter), or T3 (tumor >5 cm in greatest diameter),

T4 tumor of any size, but has spread beyond the breast tissue to the chest wall and/or skin following the World Health Organization (WHO) guidelines as applied by Sorenmo *et al.*, 2009 .

2. Five women with breast masses referred the national cancer research center. Demographic, clinical finding, palpable breast masses and ultrasound screening were conducted for ambiguous breast lesions. The inclusion criterion for our study was any abnormality sings in the breast. Biopsies were taken with ultrasonographic [US] or surgical biopsies.

3. A cow case reported a mass in teat of the left front quarter of the mammary gland and another mass was close to the mammary gland of a 9-year-old Friesian cow, with enlargement in supra-mammary and prescapular lymph nodes.

Histopathology

Paraffin blocks and H&E slides (fixed at 10% buffered formalin) from cases were improved in this study and all available samples (mammary tumors, affected skin, subcutaneous and muscular tissues) underwent a histopathologic reviewing. Mammary tumors were diagnosed following the WHO's classification system (Sorenmo *et al.,* 2009). In each tumor, the histologic-malignant grade was established by scoring tubule formation, nuclear pleomorphism, and mitotic rate from 1 to 3 points, according to a grading system (Queiroga *et al.,* 2011). Microscopic examination of the skin was performed to study the possible neoplastic infiltration and other histological alterations.

Immunohistochemistry

The BRCA1 and Ki-67 protein expression was detected by immunohistochemical staining by using monoclonal primary antibodies specific for each. After sectioning the tissue biopsies, they were de-paraffinized and rehydrated. The streptavidin–biotin-complex peroxides method was used following a high temperature antigen unmasking protocol. Peroxidase activity, which may cause non-specific staining was blocked by incubation with 3% H_2O_2 for 10 min. The primary antibodies used were: a monoclonal mouse anti-human BRCA1 (clone GLK-2) and mouse monoclonal anti-human Ki-67 (clone MIB 1), both were purchased from Immunotech® and diluted to 1:50 and 1:25, respectively. The antibodies were incubated for 1 hour at room temperature. Then, after washing to remove access antibodies, the slides were incubated with anti-mouse biotinylated secondary antibody (Dako E04233, dilution 1:200) for 20 min at room temperature. Next, all the slides were incubated with streptavidin conjugated with peroxidase (Zymed P50242, 1:400) for 30 min at room temperature. All washes and dilutions were made in Tris–Buffered-Saline (TBS) (pH 7.4). The slides were developed with a chromogen solution containing 3-3 diaminobenzidine tetrachloride (Sigma Chemical Co. D5059) and H_2O_2 in TBS and finally counterstained in hematoxylin (Sigma GH5-2-16).

Interpretation of the BRCA1 immunohistochemical results was assessed as follows: BRCA1 expression had heterogenous intensity and its localization was within the cytoplasm and nucleus mainly. Cells were scored depending on the percentage of malignant cells as follows: tumor staining less than 10% were scored as 0 (negative),10-40% were scored as 1 (+), 40-70% were scored as 2 (++), more than 70% were scored as 3 (+++). In general, tumor cells were considered as being BRCA1 positive when more than 10% showed staining (Yang *et al.,* 2001).

The score of Ki-67 was calculated based on the average percentage of positively stained nuclei among the tumor cells. The scoring system was as follows: 0 for <5% positive cells, 1 for 5%–10% positive cells, 2 for 10%–20% positive cells, 3 for 20%–30% positive cells, 4 for 30%–50% positive cells, and 5 for >50% positive cells. Positive and negative control slides were also used. In each case, Ki-67 index was calculated as the mean of the proportion of positive nuclei in 8–10 representative fields (Allred *et al.,* 1998, Guoxin *et al.,* 2017).

Results and discussion

Table 1. Some clinical findings of the mammary tumors of dogs.

No. of case	Age	breed	Dog style	Density of mass	Onset of lesion (month)	Size (diameter in cm)	Histological type of cancer
1	8	German shepherd (GSP)	Military	Fibrosis	4	7	Inflammatory Carcinoma
2	11	Pointer(DSL)	Pet	Hard	7	10	Adenocarcinoma
3	12	German shepherd(GSP)	Military	hard	12	10	Carcinoma
4	9	Pomeranian	Pet	Fibrosis	6	4	Inflammatory Carcinoma
5	11	German shepherd(GSP)	Military	Hard	24	15	Adenocarcinoma
6	10	Belgian Malinois (MALI)	Pet	Fibrosis	9	10	Lipo- carcinoma
7	9	Rottweiler ROTT	Military	Multi lobules hard	6	7	Adenoma
8	8	Belgian Malinois (MALI)	Watchdog	Softy	6	5	Lipo - carcinoma
9	12	Jack Russell terrier (JT)	Military	Hard	3	8	Solid carcinoma
10	7	Cross breeds	Watchdog	Softy	15	5	Adenoma

Table 2. Some clinical findings of the mammary tumors of women.

Case	Density of mass	Onset of lesion (month)	Size (diameter in cm)	Histological type of cancer
1	High	3	2	Carcinoma in situ(ductal)
2	High	4	2.5	Carcinoma in situ (lobular)
3	Moderate	5	1.5	Fibroadenoma
4	Low	5	1	Cysts
5	Low	6	0.5	Fibroadenoma

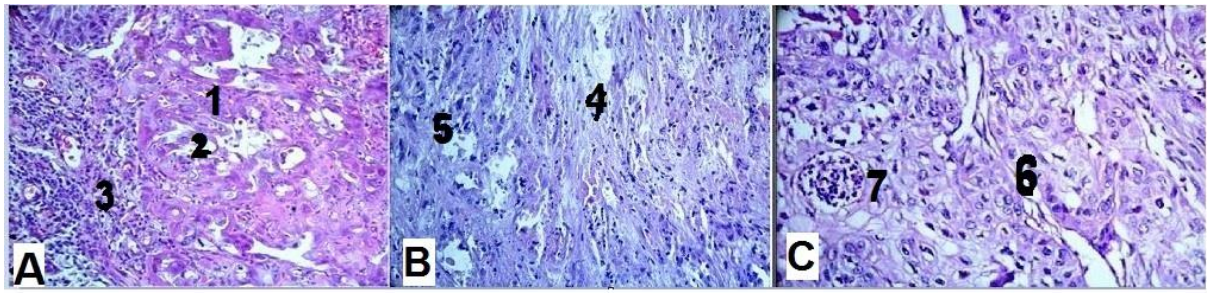

Fig. 1. Three sections of mammary tumors.

A: Inflammatory carcinoma in the mammary gland of a dog shows luminal projections of cancerous cells. 1: a lipid-rich material, 2: infiltrate of tumor cells in vascular dermal tissue, 3: heavy inflammatory cells (200x H&E).

B: Mammary gland of a cow shows metastatic fibrosarcoma. 4: dense fibrous stroma, 5: pleomorphic hyperchromatic nuclei of tumor cells embedded in a fibrous stroma. Apparently phagocytosed material has many densely staining nuclei and are surrounded by a net of light color fibers compactly cellular and consisted of interweaving bands of spindly cells (200x H&E).

C: Mammary gland of woman shows abnormal acini cells and pleomorphic lobular breast carcinoma. 6: hyperchromatic nuclei of tumor cells with numerous mitotic Fig.s and cytological evidence of malignancy had round to oval vesicular nuclei and variable nuclear size, 7: ductile lined by a single layer of flattened cells full of macrophages and degenerated cells (400x H&E).

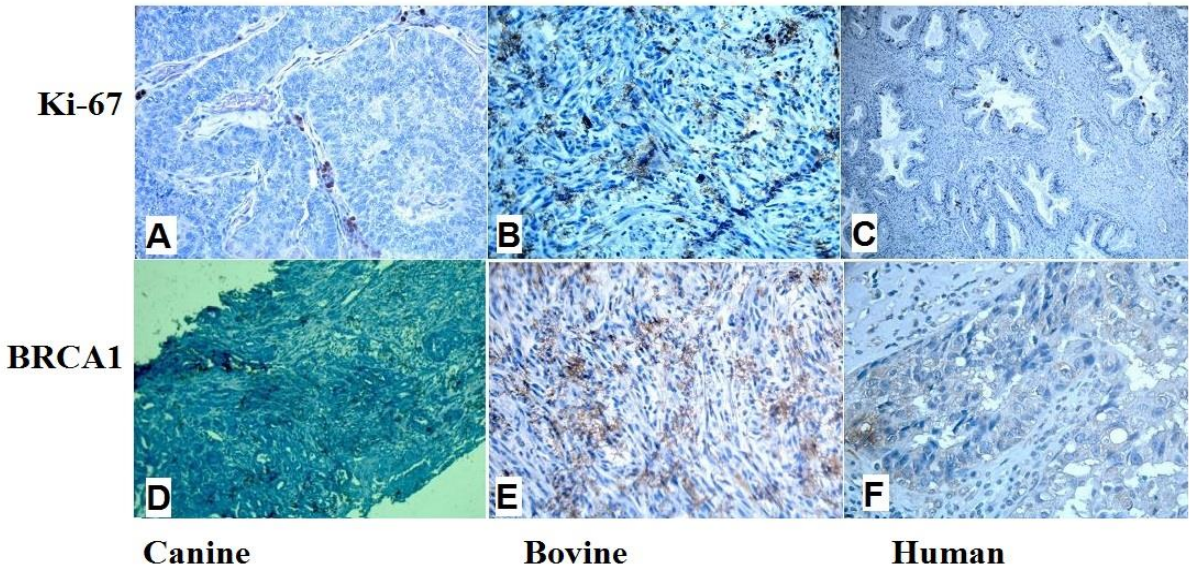

Fig. 2. Immunohistochemical staining with Ki-67and BRCA1 antibodies.

A, B and C: Ki-67 in malignant mammary gland of dog, cow and woman, respectively. A; score 0 negative, B: score 2 moderate, C: score 1 weak.

D, E and F: BRCA1 staining in malignant mammary gland of dog, cow and woman, respectively. D: score 0, E: score 1 (+) and F: score 1 (+) mostly the cytoplasm of cells was stained not nucleus.

This study showed variance tendency and aggressive mammary gland tumors in females of three models of mammals: herbivores, carnivores and omnivores representing bovine, canine and human, respectively. Canine and bovine are the suitable types of mammals to study the mammary neoplasia in human (Sonja *et al.,* 2013, Alabbody *et al.,* 2018). However, experimental models exemplified by genetically engineered mice (GEM) have been useful for investigating the role of steroid hormones, hormone receptors, and other growth factors in the pathogenesis of breast cancer. Yet rodents differ considerably in mammary gland development and types of breast cancer compared to women. Furthermore, many mammary gland cancers in mice are viral- or toxin-induced, so that the validity of these models has been questioned. Old world primates are closer models for women with similar breast development and endocrine profiles. Although the low prevalence of spontaneous breast cancer in macaques, as well as higher costs and humane issues of maintaining them in the laboratory setting make them unsuitable for most studies (Munson *et al.*, 2007).

In this study, the main sign of physical examination of mammary tumors of intact female dogs was appearance of a lump or mass (usually painless). The lump was detected easily by gently palpating the dog's mammary glands. The results showed that ages of dogs were between 7-12 years, with a mean of 7.9 ± 2 years. The military dogs were more frequent 5/10, whereas 3/10 were pet dog and 2/10 were Watchdogs. The density of tumor on palpation was hard in 5/10 dogs, fibrous in 3/10 and soft and spongy in 2/10 animals. The onset of lesions depended on the aggressiveness of tumor, which lasted for 4 - 24 months. The 4th and 5th glands were the most common tumor glands, 4/10 and 3/10, respectively, which were close to the groin. Occasionally, the edema of the proximal portion of the limb caused lameness. Although not definitive, there were certain features that distinguished benign mammary tumors. Generally, these masses were small, smooth, distinct, and slow growing. Usually they were moveable under the skin. On the other hand, malignant tumors were fast growing, involved multiple glands with or without mammary nodules, firm, warm, with edema, erythema and thickness, and indefinite boundaries (irregular shape) too.

They were not movable and sticking together with the overlying skin or underlying muscle. Sometimes, a malignant tumor caused skin ulceration and bleeding. The histopathological examination revealed that 8/10 of tumors were carcinoma, such as adeno, lipo, solid or inflammatory carcinoma, and two cases were benign adenoids. These animals survived after surgical treatment, especially those with grade I and II. There was one case of metastatic carcinoma spread to the lung, which in turn diagnosed by X-ray examination (Table 1).

In dogs, mammary gland tumors are frequent and tend to be sporadic as in humans. Mammary tumors represent approximately 52% of all tumors in female dogs, and 50% of them are malignant (Megalhaes *et al.,* 2012), which has been shown to be caused by genetic mutations or promoted by hormones. Cancers in women and domestic dogs have similar expression profiles (Munson *et al.,* 2007). Moreover, CMTs have epidemiologic, clinical, morphologic, and prognostic features comparable to those of human breast cancer (Chrisp *et al.,* 1980, Frese *et al.,* 1985). The high incidence of CMTs may be due to the long period of exposure to the estrogen hormone during the heat period without mating, this can jeopardize mammary gland to a big risk factor. Usually, the estrus cycle lasts for 12-21 days in bitches. It has been found that the incidence of CMTs is low (0.05%) in female dogs spayed before their first estrus cycle, while it increases to 8% or 26% if spayed after the first or second cycle, respectively. If the dog is spayed later than the second estrus cycle, the risk for malignant tumors is the same as in intact bitches (Schneider *et al.,* 1969).

In this study, dogs possessed various sizes of masses ranged between 5-10cm in diameter. Studies found that the size of tumor is helpful to determine its stage and is strongly related to prognosis (chances for survival). In general, the smaller tumor tends to be of better prognosis (Frese *et al.,* 1985). Concerning breeds, certain kinds are predisposed to mammary tumors more than others, especially German shepherd 3/10, Belgian Malinois 2/10, and the rest were one of the Pointer, Terrier, Pomeranian, and Rottweiler. Some breeds were observed to develop CMTs at a younger age, e.g. English Springer Spaniel (ESS) whose median age of onset had been shown to be 6.9 years of age (Egenvall *et al.,* 2005).

In human, the five cases of breast lumps were taken from women in their forties decade. The histopathological assessment showed that one of the cases had ductal carcinoma in situ, the second had lobular carcinoma in situ, another case had a cyst, and two cases had fibroadenoma. The malignant lesions were painless, dense or solid upon palpation, hypoechoic was found on an ultrasound scan with ill-defined borders, sunray appearance, irregular edges and sometimes tender, soft, or rounded lesions. They could be painful, swelling of all or part of a breast was observed, skin irritation or dimpling sometimes looked like an orange peel, nipple retraction, redness, scaling or thickening of the nipple or breast skin, liquid material nipple discharge, sometimes masses spread to lymph nodes under the arm or around the collar bone, which looked as lump in these areas. Tumor size, tissue texture, breast and the surrounding area description were estimated by the pathologist or radiologist based on the largest diameter of the resected specimen or on ultrasound examination. However, size evaluation is inexact, and pathologists tend to round the tumor size to the nearest centimeter or half-centimeter (Table 2).

Breast cancer is often familiar; however, several genes are known to confer increased risk for hereditary breast cancer in humans including BRCA1. A similar hereditary pattern has not been described for mammary tumors in dogs who could not interpret the positive results in this study. It has been suggested that the origin of CMTs and breast cancer of human is multifactorial and depends on an interaction between multiple major and minor genes and environmental factors. Dogs have a history of inbreeding, which might lead to slow levels of genetic variation and accumulation of the worst genetic traits. Canine mammary tumors have been found to more homogeneous within a single breed. This should allow for an easier identification of risk factors within a

breed (Egenvall *et al.,* 2005, Cotran *et al.,* 1994, Dorn *et al.,* 1968, Walsh *et al.,* 2007, Sutte *et al.,* 2004, Lindblad *et al.,* 2005).

On the other hand, within three decades, a veterinary author reported the first case of tumor mass in the mammary gland of a cow. The masses rose in the teat of the left front quarter of the mammary gland and close to the mammary gland, with enlargement in supra-mammary and prescapular lymph nodes. Other clinical signs appeared on the cow were loss of appetite, fever, lameness and desperation. This case report concerns masses which originated within or very close to the mammary gland and spread to several distant organs. The sole case of bovine mammary tumor (fibrosarcoma) in this study confirms the previous studies regarding rare occurrence and the incidence of mammary cancer in cattle is not common. This condition is of clinical importance, and by using translational research there is a chance that understanding the processes occurring in mammary gland cells of bovine species will eventually provide clinical benefits for the disease and treatment of other species of mammal's mammary neoplasia (Sonja *et al.,* 2013, Pandey *et al.,* 1984). It is worth mentioning that the enzootic bovine leukosis (EBL) has been diagnosed by the presence of the tumors and/or general lymph node enlargement. Approximately 5% of animals infected with EBL grow B-cell lymphoma or lymphosarcoma in various lymph nodes and organs after a long latent period when cancer cells penetrate many organs including udder and its skin as secondary metastatic tumors (Povey *et al.,* 1969). The cow of this study may be infected with EBL especially the lymph nodes were also enlarged. The EBL was recorded in Iraq in 1994 (Hasso *et al.,* 1994) and some studies were done in Baghdad and other governorates (Amjeed 1994, Yousif 1997, Khudhair *et al.,* 2016, Alabbody 2017). In 1997 Yousif reported that the seropositivety was 8.4% in imported cattle (Friesian) but null in local animals using ELISA assay (Yousif 1997). Differential diagnoses for masses in the udder included mastitis, abscess, hematoma, and other conditions that may lead to misdiagnosis and removal of animals from herds without proper examination. Consequently, here the question arises as to whether the primary tumor did arise within the interstitial tissue of the mammary gland, or in a subcutaneous location cranial to the gland, from which it could have invaded the mammary tissue. Nevertheless, the incidence of mammary neoplasia in ruminants is still almost non-existing (Sonja *et al.,* 2013). Ford and colleagues had reported only 41 cases of mammary gland neoplasia in cattle from 1902 until 1989 (Ford *et al.,* 1989). Additionally, other cases had also been reported from 1992 to 2012 (Murphy *et al.,* 1992, Ohfuji 2012). In a spontaneous case of secretory mammary carcinoma in a cow, the neoplasm was highly aggressive and malignant (Ford *et al.,* 1989). In a study, out of 606 reported neoplasms the most common were squamous cell carcinomas of the eyelid and vulva, whereas there were no mammary malignancies detected (Bastianello 1982a).

Epidemiological data support a role for hyperestrinism in the genesis of human breast cancer, in which more than 60% of breast cancers in women are estrogen receptor positive. Possibly, the low rate of mammary carcinoma in cattle could be partially due to the high rate of pregnancy in this species. This is because increased parity shortens exposure to estrogen, and the high lactation demands give protection against mammary carcinoma. Concerning humans, pregnancy at a young age reduces woman's lifetime risk of breast cancer by up to 50%. Although breast cancer in women is a complex disease, for which the pathogenesis and risk factors are only partially understood. Similar mammary cancer occurs in many other mammalian species and comparing cancers across these species could provide invaluable insight into key factors that are common to the pathogenesis of this important disease. However, the cow case in this study augments the few cases of bovine mammary tumors that have been reported and assists in the future discernment of the biological behavior of this rare neoplasm (Murphy *et al.,* 1992, Enger *et al.,* 1997).

Mammary gland morphogenesis involves the regulatory function of several signaling pathways, i.e. growth factors and hormones.

During the tumorigenic process the signaling is deregulated, thus allowing the mammary epithelium to expand, proliferate, and invade neighboring tissue (hyperplasia). In humans, there are roughly 1.5 million cases and 500,000 deaths from breast cancer just in 2012 (World Cancer Report WHO 2014). There are several factors associated with increased risk of breast cancer, including age of menarche, first child, onset of menopause, diet, level of exercise, obesity, alcohol consumption, presence of benign breast disease, exposure to radiation, and family history (McPherson *et al.,* 2002, Alabbody *et al.,* 2018). Furthermore, the species differ in diet regimes and in reproductive strategies. Diet low in fat and high in vegetable fibers is beneficial in protecting against breast cancer. Fruit and vegetable intake have been hypothesized to reduce the risk of breast cancer in humans. On the contrary, carnivores including dogs, tend to eat a lot of meat and tend to be obese; these could be two major risk factors for development of mammary neoplasia in addition to the hormonal factors (Perez *et al.,* 2000).

Conclusions

Translational research using different mammary gland cell populations from several species may lead to comparative studies and may help reduce the risk and contribute to discovering preventive methods and new therapeutic targets to treat breast cancer. By these studies there is a chance that understanding the processes occurring in mammary gland cells of bovine and canine species will eventually provide clinical benefits for prevention, diagnosis and treatment of human breast cancer patients. Comparing the pathology of mammary cancers in domesticated and human, including the hormonal, dietary, and geographic environments in which they evolved, could help distinguish critical from incidental risk factors.

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