



Epithelial Periostin Expression in Canine Mammary Tumors

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Abstract | Mammary neoplasms are a prevalent form of malignancy found in canines female . Periostin (POSTN) is a protein that performs crucial functions in the progression and spread of cancer cells, in addition to the advancement of tumors. The objective of this research was to evaluate the expression of PN in canine mammary gland tumors. The study material consists of 44 carcinomas and 6 benign tumors, together with 10 normal samples. Antibodies targeting the antigen POSTN have been used to conduct immunohistochemistry procedures. In normal biopsies, the expression of periostin was absent in the epithelial compartment. High levels of POSTN expression was detected in 93.2% of cancerous biopsies as well as in 16.7% of benignly diagnosed biopsies. With a noticeable difference between these two groups ($P \leq 0.01$). PN was found to be highly expressed in 92.1% of the newly diagnosed cases and 100% of the recurring cases. PN exhibited a higher level of expression in females (95%) compared to males (75%), with a statistically significant difference ($P \leq 0.01$). Additionally, PN expression was shown to be significantly higher in the advanced age group (10–15 years), with a 100% expression rate. Furthermore, there was a significant variation in periostin expression among different breeds ($P \leq 0.05$). The statistical analysis indicates that there is no significant variation in periostin expression across different cancer groups based on type ($P \leq 0.01$), stage ($P \leq 0.01$), and grade ($P \leq 0.01$) of cancer. In conclusion, periostin expression can be used to evaluate the existence of malignancies in dogs with mammary tumors.

Keywords | Periostin, Mammary tumors, Immunohistochemistry, Female canines, Cancerous biopsies, Neoplasms

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INTRODUCTION

Mammary neoplasms constitute around 40% of tumors and are frequently identified as cancerous lesions in female dogs (Santos *et al.*, 2013; Tarab and Al-Rekabi, 2022). Most breast tumors are believed to be malignant, and their occurrence becomes more common as patients get older, peaking between the ages of 9 and 11. Unspayed female dogs or those who are spayed at an advanced age are especially susceptible to some breast tumors because of

their hormonal reliance. Furthermore, studies have demonstrated that doing an ovariectomy at an early stage might decrease the possibility of acquiring mammary gland neoplasms (Sleekx *et al.*, 2011; Sorenmo *et al.*, 2011). The disease's clinical stage is assessed based on the tumor's size, the involvement of regional lymph nodes, and the existence of metastases (Sorenmo, 2003). The definitive diagnosis is determined through histopathological examination. In cases where there is uncertainty, an immunohistochemical examination (IHC) should be conducted alongside routine

ETHICAL APPROVAL

Permission to perform the research was acquired from the Aden Square Veterinary Hospital and Veterinary Clinics in Baghdad. Prior to each sample collection, complete authorization was obtained from the owners.

SAMPLE COLLECTION AND PREPARATION OF THE TISSUE FOR IMMUNOHISTOCHEMICAL DETECTION OF PERTIOSTIN IN PARAFFIN-EMBEDDED SECTIONS.

The study was approved by the Local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad, with Approval Number 553 P.G., dated March 10, 2024. This prospective study includes 50 pet dogs that have been chosen from cases received by veterinarian hospitals and clinics (with abnormal growth detected in the mammary glands and related abdominal region) in Baghdad city between October 2022 and June 2023. The selection criteria were a diagnosis of a mammary gland tumor in the removed tissue biopsies. Out of 50 biopsies, 4 (8%) were males and 46 (92%) were females. In addition, 10 apparently healthy dogs that underwent mastectomy were included for normal biopsies collection, which were used as controls (all of them were females).

Suspected mammary carcinoma tissues were isolated in small containers that contained 50 ml of 10% formalin to conserve the specimen until histopathological and immunohistochemistry procedures took place. The biopsies underwent fixation in a solution of neutral buffered formalin (10%), followed by dehydration using a series of ethanol solutions and clearing in xylenes. Subsequently, the paraffin embedding procedure was carried out, and the sections were stained with routine stains of hematoxylin and eosin stain. Examination for histological classification of mammary tumors in female dogs was performed according to (Goldschmidt *et al.*, 2011). Histological grading was assessed according to the Nottingham grading system (ELSTON *et al.*, 1991) and staging was assessed according to pathological staging.

PRINCIPLE OF THE TEST

The material containing the target antigen was treated with H₂O₂ for 5–10 minutes to neutralize any endogenous peroxidase activity. Next, the cytoplasmic antigen in the specimen will form a bond with the corresponding diluted rabbit primary antibody. The antibody is incubated with the polyExcel target binder for 10 minutes, followed by incubation with the polyExcel HRP-labelled polymer for another 10 minutes, as suggested. The staining process was finished within 5 to 10 minutes and then treated with 3,3-diaminobenzidine (DAB) substrate-chromogen, resulting in the formation of a brown-colored precipitate at the location of the antigen.

diagnostics. This involves using antibodies that target specific tumor cell antigens, which enables greater accuracy in prognosis and effective treatment (Dolka *et al.*, 2018; Khayoon and Al-Rekabi, 2020). Furthermore, there has been a developing curiosity about the tumor microenvironment in recent years due to its important effect on cancer development (Allen and Louise, 2011). The tumor stroma consists of several components, including the stroma of connective tissue (such as fibroblasts), cells that trigger inflammation, vascular system vessels, and specialized cells known as cancer-associated fibroblasts (CAFs). (CAFs) have an essential roles in the tumor's stromal environment and exhibit distinct functions and gene-expression profiles compared to regular fibroblasts (Pula *et al.*, 2013; Ratajczak-Wielgomas *et al.*, 2016; Obaid *et al.*, 2021). In addition, cancer-associated fibroblasts (CAFs) have a role in epithelial-mesenchymal transition (EMT), a significant event in tumor advancement, and contribute to the modification of extracellular matrix (ECM) proteins (Morra and Moch, 2011). (CAFs) are believed to contribute to the progression of breast cancer in female dogs by stimulating angiogenesis, facilitating the dissemination and attachment of cancerous cells, and increasing the process of EMT (Król *et al.*, 2012; Hussein *et al.*, 2019). Periostin (POSTN) is one of several markers found in cancer stroma cells.

Periostin is an extracellular matrix glycoprotein that contains fascicles and is typically present in connective tissues with high collagen content. It is commonly found in the periosteum of the embryo, placental tissues, and valves of the heart, as well as in the periodontal ligaments. Additionally, it is upregulated in various adult tissues. Periostin is more abundant in tissues that are under stress or undergoing wound repair (Rios *et al.*, 2005; Hamilton, 2008). The expression of POSTN was reported in both cancer-associated fibroblasts (CAFs) and cancerous cells (Nuzzo *et al.*, 2016; EK Al-Hamdany, 2022). Moreover, Periostin has been shown to have a role in cancer cell survival, epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) breakdown, invasiveness, and distant metastatic processes (Nuzzo *et al.*, 2014; Muhammed *et al.*, 2023). Furthermore, Several types of research on cancer types, such as non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), malignant pleural mesothelioma (MPM), breast cancers (Ratajczak-Wielgomas *et al.*, 2016; Gonzalez-Gonzalez and Alonso, 2018; Ratajczak-Wielgomas *et al.*, 2020), have indicated that elevated Periostin levels are usually associated with tumour aggressiveness, cancer stage advancement, as well as poorer prognostic outcome, indicating that POSTN levels could be a promising predictive biomarker (Soltermann *et al.*, 2008; Dahinden *et al.*, 2010; Morra and Moch, 2011). Additionally, the expression of periostin in malignancies is strongly associated with undesirable predicted cancer factors and a worse prognosis (Hong *et al.*, 2013; Dawood and Mohammed, 2023).

Table 1: The percentage of positively (PR) stained cells and Staining Intensity of positively stained cells (SI) of Periostin in tissues of mammary gland tumor and normal biopsies of dogs.

percentage of positively stained cells of periostin	Staining Intensity score												Total No.
	NO score 0			Weak score 1			Moderate score 2			Strong score 3			
	M No.	B No	N No.	M No.	B No	N No.	M No.	B No.	N No.	M No.	B No.	N No.	
(Score 0) Negative staining	0	2	10	0	0	0	0	0	0	0	0	0	12
(Score 1) <10%	0	0	0	0	2	0	0	0	0	0	0	0	2
(Score 2) 10_<30%	0	0	0	3	1	0	0	0	0	0	0	0	4
(Score 3) 30_<50%	0	0	0	1	0	0	4	1	0	19	0	0	25
(Score 4) >50%	0	0	0	0	0	0	1	0	0	16	0	0	17
Total No.	0	2	10	4	3	0	5	1	0	35	0	0	60

M: Malignant; **B:** Benign; **N:** Normal biopsies .

IMMUNOHISTOCHEMICAL STAINING WAS PERFORMED USING

PRIMARY ANTIBODY: Rabbit polyclonal to periostin (Abcam), isotype: IgG, abcam (ab92460)

SECONDARY ANTIBODY: PolyExcel HRP/DAB Detection System Universal kit for rats and rabbit which contain:

- Polyexcel HRP: 50 ml of goat anti-rabbit Conjugate with HRP (Pathnsitu, USA).
- Polyexcel target binder :50 ml of rabbit anti- mouse (Pathnsitu, USA).

PROCEDURE

The paraffin-embedded samples were sliced into sections measuring 4 µm in thickness and then placed onto charge slides that had been coated with poly-lysine. The tissue slides were treated with xylene to remove the paraffin and then rehydrated using ethanol solutions of 95%, 80%, and 70%. Following this, the sections underwent antigen retrieval using citrate buffer (pH 9.0) by boiling in a microwave oven for 10 minutes. Then, endogenous peroxidase activity is blocked with hydrogen peroxide. Following that, for 1 hour, the sections were incubated with ready-to-use primary anti-periostin antibodies in a humidified chamber. After that, the sections were treated with poly Excel rabbit anti-mouse secondary antibody for 10 minutes. Next, the PolyExcel HRP of goat anti-rabbit conjugate with HRP (Pathnsitu, USA) was applied for 10 minutes. Subsequently, the protein was identified using the 3,3'-diaminobenzidine (DAB) substrate-chromogen, resulting in a brown coloration at the antigen site. Ultimately, the section was treated with Mayer's hematoxylin for 1 minute at room temperature, washed with water, dried using a series of ethanol and xylene solutions, and then coated with the appropriate DPX mounting medium and coverslip. The slide was assessed, and the stained cells were quantified with the assistance of a histopathologist using a light microscope. The postin immune reactivity in the epithelial compartment (inner luminal cells of normal mammary glands) or

cancer cells was determined based on the intensity and extent of staining. In general, the staining intensity was assessed using a scoring system ranging from 0 to 3 (0 = -ve stain, 1 = weakly +ve stain, 2 = moderately +ve staining, and 3 = strongly +ve stain). The extent of positive staining was estimated and scored on a scale of 0 to 4 (0 = -ve, 1 = <10% +ve cells, 2 = 10 to <30% +ve cells, 3 = 30 to <50% +ve cells, and 4 = >50% +ve cells). The sum of the intensity score and the extent of the staining score were used as the final staining scores (SS). SS of <4 were categorized in the low expression group, and cases with SS of 4 to 7 were categorized in the high expression group (Kim et al., 2017). Every instance of immunohistochemistry involves the inclusion of both positive and negative controls. In the negative control, the primary antibody is substituted with PBS.

STATISTICAL ANALYSIS

The SAS (2018) program was utilized to identify the impact of various variables on research parameters using statistical analysis. Chi-square test was used to examine the significance between percentages at probabilities of 0.05 and 0.01 in this investigation (Cary, 2018).

RESULTS AND DISCUSSION

PERIOSTIN SORE IN CMTS TISSUES AND NORMAL BIOPSIES OF DOGS

Table 1 shows the staining intensity of +ve-stained cells (SI) and the percentage of +ve-stained cells (PR) in cancer animals and controls. The final scores of periostin in samples obtained by the sum of both PR and SI in cancer were as follows: the ultimate count of ≥4 was considered high expression, and the ultimate count of <4 was considered low or low/-ve expression. High expression of periostin was observed in 93.2% (Figure 1 c, d, e, and f) of the malignant mammary carcinoma tissue and 16.7% of benign mammary gland tissue (Figure 1B), while all of the normal mammary gland tissues showed negative expression (Figure 1a). (Figure 2), with a significant difference between these groups (P≤0.01) (Table 2).

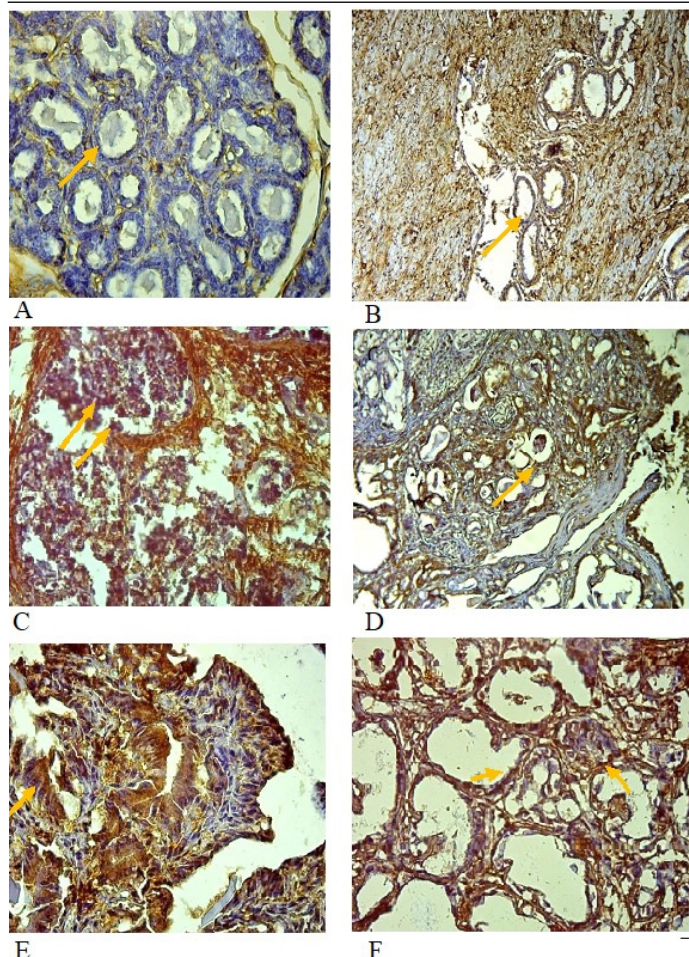


Figure 1: Immunohistochemical section of the epithelial compartment in canines with mammary tumors shows.

***A:** negative expression in the normal mammary gland ; **B:** low expression in benign tumor ; **C:** high expression in ductal carcinoma ; **D:** high expression in invasive Mammary carcinoma ; **E:** high expression in invasive papillary carcinoma ; **F:** high expression in intra ductal papillary carcinoma **A,C,E,F:** 40X, **B:** 4X, **D:** 10X.

PERIOSTIN SCORE IN CMTS IN NEWLY DIAGNOSED AND RECURRENT TUMORS

The percentage of +ve (PR) stained cells and the staining intensity of +ve stained cells (SI) are described in Table 3. High expression of periostin was found in 35 patients (92.1%) with newly diagnosed cancers, while low or negative expression was found in 3 (7.9%). While all of the recurrent cancer patients showed high expression (100%) (Table 4) (Figure 3). Chi-square test was applied to compare the final periostin score, indicating a statistically significant disparity between the two groups ($P \leq 0.01$).

PERIOSTIN EXPRESSION IN CANCER GROUP IN RELATION TO SEX, AGE AND BREED OF DOGS

The tissue expression of periostin in relation to the sex, age, and breeds of dogs presented in (Tables 5, 6 and 7), respectively, showed significantly higher expression in females

(95%) than males (75%), and in the advanced (10–15 year) age group (100%), with a significant difference in periostin expression among breeds ($P \leq 0.05$).

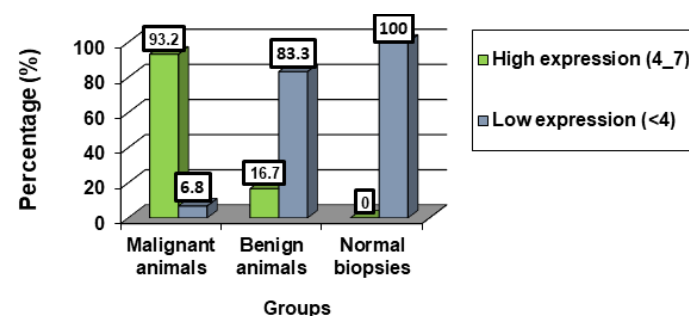


Figure 2: Expression of periostin in mammary gland tumor tissues and normal biopsies of dogs.

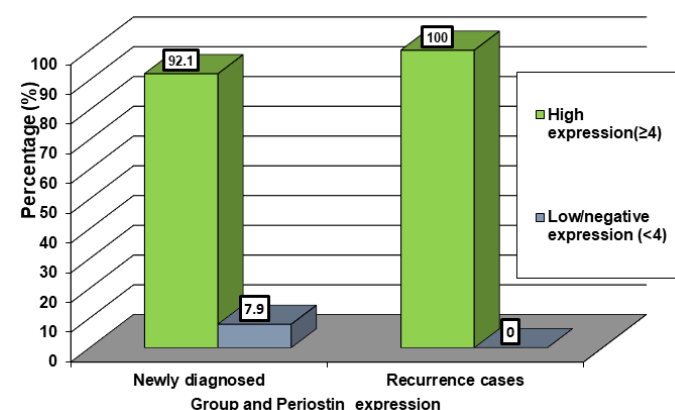


Figure 3: Tissues Expression of periostin in newly diagnosed and recurrent canine mammary carcinoma.

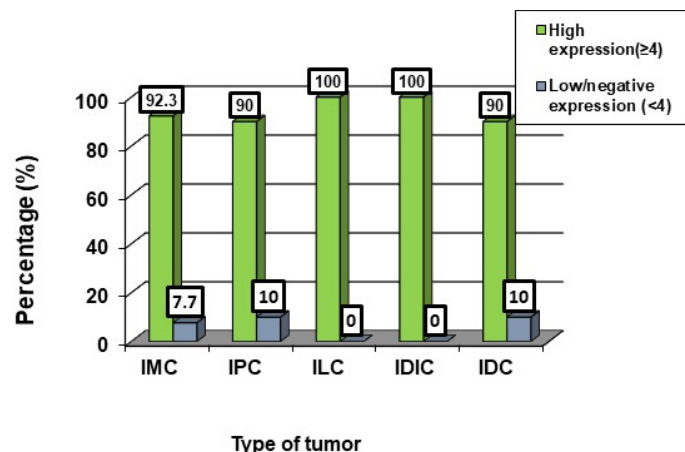


Figure 4: Expression of periostin in mammary gland cancer in dogs according to type of tumor.

SCORE OF PERIOSTIN ACCORDING TO THE TYPE OF CANCER

The (PR) stained cells and (SI) in mammary carcinoma and the final score according to the types of cancers presented in (Table 8 and 9) (Figure 4). Statistical analysis shows no significant difference in periostin expression ($P \leq 0.01$).

Table 2: Final score of periostin expression in mammary gland tumor tissues and normal biopsies of dogs.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Malignant	44	3(6.8%)	41(93.2%)	32.818 **
	Benign	6	5(83.3%)	1(16.7%)	2.667 NS
	Control	10	10(100%)	0	7.028 **
Chi-Square (χ^2)		--	4.871 *	31.094 **	---

* (P≤0.05), ** (P≤0.01).

Table 3: The percentage of positively (PR) stained cells, Staining Intensity of positively stained cells (SI) of periostin in tissues of newly diagnosed and recurrent canine mammary carcinoma.

percentage of positively stained cells ofIL-8	staining Intensity score						Total No.
	Weak score1		Moderate score2		Strong score3		
	Newly No.	Recurrence No.	Newly No.	Recurrence No.	Newly No.	Recurrence No.	
(Score 0) Negative staining	0	0	0	0	0	0	0
(Score 1) <10%	0	0	0	0	0	0	0
(Score 2) 10_<30%	3	0	0	0	0	0	3
(Score 3) 30_<50%	1	0	4	0	15	4	24
Score 4) >50%	0	0	1	0	14	2	17
Total No.	4	0	5	0	29	6	44

Table 4: Final score of periostin expression in canine mammary carcinoma tissues of Newly and Recurrence cases.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Newly diagnosed	38	3(7.9%)	35(92.1%)	26.947 **
	Recurrence cases	6	0	6(100%)	4.496 *
Chi-Square (χ^2)		--	1.275 NS	20.512 **	---

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

Table 5: Expression of periostin in mammary gland tumor according to sex.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Male	4	1(25%)	3(75%)	1.00 NS
	Female	40	2(5%)	38(95%)	32.40 **
Chi-Square (χ^2)		--	0.333 NS	29.878 **	---

** (P≤0.01), NS: Non-Significant.

Table 6: Expression of periostin in mammary gland tumor dogs according to age.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	≤ 5	8	2(25%)	6(75%)	2.00 NS
	5_10	22	1(4.6)	21(95.4%)	18.181 **
	10_15	14	0	14(100%)	12.251 **
Chi-Square (χ^2)		--	0.333 NS	8.331 **	--

** (P≤0.01), NS: Non-Significant.

PERIOSTIN SCORE IN RELATION TO MALIGNANCY GRADE

Periostin was highly expressed in (83.3%) of Grade I, (95.2%) of Grade II, and 100% of Grade III, as shown in

(Tables 10 and 11) (Figure 5). Statistical analysis showed no significant difference (P≤0.01). The marker showed positive staining in the cytoplasmic tumor cells.

Table 7: Expression of periostin in mammary gland tumor dogs according to breed.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	German shepherd	14	3(21.5%)	11(78.5%)	4.571 *
	Pointer	7	0	7(100%)	4.398 *
	Belgian (Malinois)	5	0	5(100%)	4.215 *
	Terrier	7	0	7(100%)	4.398 *
	Chihuahua	3	0	3(100%)	1.278 NS
	Cross breed	3	0	3(100%)	1.278 NS
	Boxer	3	0	3(100%)	1.278 NS
	Husky	2	0	2(100%)	1.278 NS
Chi-Square (χ^2)		--	1.219 NS	4.892 *	--

* (P≤0.05), NS: Non-Significant.

Table 8: The percentage of positively (PR) stained cells, Staining Intensity of positively stained cells (SI) of periostin in mammary gland cancer according to type of tumor.

percentage of positively stained cells of periostin	staining Intensity score															Total No.
	Weak score1					Moderate score2					Strong score3					
	IMC	IPC	ILC	IDPC	IDC	IMC	IPC	ILC	IDPC	IDC	IMC	IPC	ILC	IDPC	IDC	
(Score 0)Negative staining	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(Score 1) <10%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(Score 2) 10_<30%	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	3
(Score 3) 30_<50%	1	0	0	0	0	0	1	0	0	3	4	4	5	0	5	23
Score 4) >50%	0	0	0	0	0	0	0	0	0	1	7	4	0	5	1	18
Total No.	2	1	0	0	1	0	1	0	0	4	11	8	5	5	6	44

***IMC:** Invasive mammary carcinoma; **IDC:** Invasive ductal carcinoma; **IPC:** Invasive Papillary carcinoma; **ILC:** Invasive lobular carcinoma; **IDPC:** Intra ductal Papillary carcinoma.

Table 9: Expression of periostin in mammary gland tumor dogs according to type of tumor.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Invasive mammary carcinoma	13	1(7.7)	12(92.3%)	9.307 **
	Invasive ductal carcinoma	10	1(10%)	9(90%)	6.400 **
	Invasive Papillary carcinoma	10	1(10%)	9(90%)	6.400 **
	Invasive lobular carcinoma	6	0	6(100%)	4.497 *
	Intra ductal Papillary carcinoma	5	0	5(100%)	4.215 *
Chi-Square (χ^2)		--	0.602 NS	3.756 NS	--

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

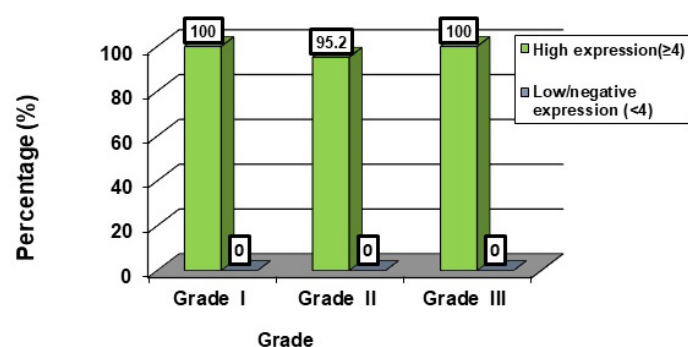


Figure 5: Expression of periostin in mammary gland cancer in dogs according to Grade of tumor.

SCORING OF PERIOSTIN ACCORDING TO THE STAGES OF CANCER

High expression of periostin was seen in all stages of cancer, and Chi-square showed no significant difference (P≤0.01) (Table 12). (Table 13), (Figure 6).

The tumor microenvironment includes the entirety of the surroundings of tumor cells, except the tumor cells themselves. It has become increasingly recognized as an important component in cancer's progression and advancement. The tumor microenvironment is characterized by a high

Table 10: The percentage of positively (PR) stained cells, Staining Intensity of positively stained cells (SI) of periostin in mammary gland cancer according to grade of tumor.

percentage of positively stained cells of periostin	staining Intensity score									Total No.
	Score 0 \ Weak score1			Moderate score2			Strong score3			
	G I	G II	G III	G I	G II	G III	G I	G II	G III	
(Score 0) Negative staining	0	0		0	0		0	0	0	0
(Score 1) <10%	0	0	0	0	0	0	0	0	0	0
(Score 2) 10_<30%	2	1	0	0	0	0	0	0	0	3
(Score 3) 30_<50%	0	1	0	1	3	0	4	10	8	26
Score 4) >50%	0	0	0	0	1	0	5	5	3	15
Total No.	2	2	0	1	4	0	9	15	11	44

G I: Grade I; **G II:** Grade II; **G III:** Grade III.

Table 11: Expression of periostin in mammary gland tumor dogs according to grade of tumor.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Grade I	12	2(16.7%)	10(83.3%)	5.333 *
	Grade II	21	1(4.8%)	20(95.2%)	17.19 **
	Grade III	11	0	11(100%)	8.935 **
Chi-Square (χ^2)		--	0.333 NS	4.488 NS	--

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

Table 12: The percentage of positively (PR) stained cells, Staining Intensity of positively stained cells (SI) of periostin in mammary gland cancer according to stage of tumor.

percentage of positively stained cells of periostin	staining Intensity score															Total No.
	Weak score1					Moderate score2					Strong score3					
	S0	S I	S II	S III	S IV	S0	S I	S II	S III	S IV	S0	S I	S II	S III	S IV	
(Score 0)Negative staining	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(Score 1)<10%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(Score 2) 10_<30%	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	3
(Score 3)30_<50%	0	0	0	0	1	3	1	0	1	1	4	4	1	5	4	24
Score 4)>50%	0	0	0	0	0	0	0	0	0	1	2	1	4	6	3	17
Total No.	1	1	1	0	1	3	1	0	1	2	6	5	5	11	7	44

***S0:** Stage 0; **S I:** Stage I; **S II:** Stage II; **S III:** Stage III; **S IV:** Stage IV.

level of complexity, comprising various non-tumor cells (e.g., fibroblasts, endothelial cells, infiltrating leukocytes) as well as a diverse array of proteins and soluble substances; these include extracellular matrix (ECM) proteins, hormones, growth factors, as well as cytokines (Lu *et al.*, 2012; Gonzalez-Gonzalez and Alonso, 2018; Rasoul, 2023). The interactions between microenvironment components and tumor cells are very complicated (Gonzalez-Gonzalez and Alonso, 2018; Kudo and Kii, 2018). In addition, cancerous cells can modify the micro environment by altering the characteristics of the surrounding tissue and vice versa (Lu *et al.*, 2012).

Moreover, the invasiveness and migratory characteristics of cancer cells have been linked to structural alterations in the extracellular matrix (ECM) assisted by the binding of inte-

grins. These integrins, frequently modified in cancer cells, facilitate cellular interactions with the extracellular matrix (ECM), resulting in modifications in cellular behaviour that contribute to the progression of different medical conditions (Maroteaux *et al.*, 2018; Alshaikhli *et al.*, 2023). One protein found in the extracellular matrix (ECM) is Periostin (Ratajczak-Wielgomas *et al.*, 2015).

POSTN is a non-structural protein that promotes the production and cross-linking of type 1 collagen, inducing fibrosis (Kudo, 2011). Additionally, it stimulates cell migration by binding to integrin, initiating the proliferative and migratory mechanisms of fibroblastic cells (Conway *et al.*, 2014). Moreover, POSTN is synthesized by cancer-associated fibroblasts (CAFs), which enhances tissue regeneration in normal conditions, and its expression appears

Table 13: Expression of periostin in canine mammary gland cancer according to stage of tumor.

Marker	Groups	Number of animals	Score		Chi-Square (χ^2)
			Low expression (<4)	High expression (4_7)	
Periostin	Stage 0	9	1(11.1%)	8(88.9%)	5.443 *
	Stage I	6	1(16.7%)	5(83.3%)	2.667 NS
	Stage II	5	1(20%)	4(80%)	1.800 NS
	Stage III	13	0	13(100%)	10.761 **
	Stage IV	11	0	11(100%)	8.974 **
Chi-Square (χ^2)		---	0.251 NS	7.170 NS	---

* ($P\leq0.05$), ** ($P\leq0.01$); NS: Non-Significant

to be increased in many diseases, including inflammatory conditions and malignancies (Bornstein and Sage, 2002).

Generally, PN is a protein that is currently being used in scientific studies in veterinary medicine. It has been found to have significant levels in the stroma of squamous cell carcinoma, as well as in the heart and periodontal ligament of dogs (Zhu et al., 2014; Janus et al., 2016).

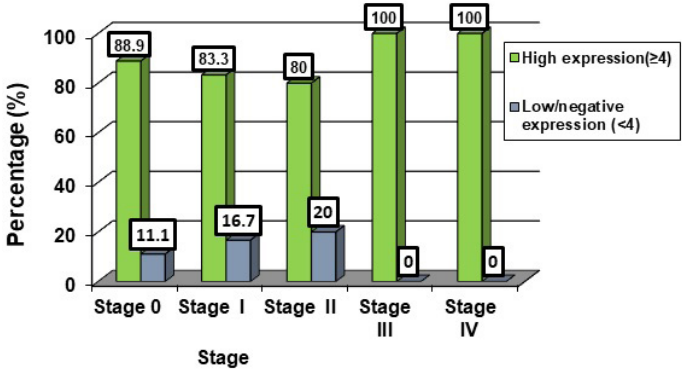


Figure 6: Expression of periostin in canine mammary gland cancer according to stage.

In addition, there was an apparent increase in the expression of POSTN in the skin fibroblasts of dogs with chronic inflammation, which leads to the assumption that POSTN may have a role in the underlying mechanism of atopic dermatitis in dogs (Mineshige et al., 2015). (Mineshige et al., 2018) reported that there is a belief that fibroblasts produce POSTN, which may have a role in the progression of canine squamous cell carcinoma.

Research published by (Puglisi et al., 2008) showed that Periostin was absent in normal breast cancer. In our research, we did not detect the expression of PN within the cytoplasm of the epithelial compartment in normal biopsies. However, Researchers found that the expression of Periostin is minimal or cannot be detected in normal breast epithelium. Furthermore, based on its established mesenchymal expression pattern, it is exclusively expressed in the stroma (Kharraishvili et al., 2011). It is still unclear if periostin is secreted mainly by breast cancer cells, if it is also produced by cancer-associated stromal cells, or may-

be both. Multiple investigations have shown that periostin overexpression mostly occurs in the stroma associated with cancer (Puglisi et al., 2008).

However, the findings are in direct opposition to the results presented by (Shao et al., 2004). The causes for this diversity are not obvious, although they might be due to variations in research methods, scoring systems, and the levels at which protein expression is considered significant.

(Borecka et al., 2020) discovered that the presence of PN in cancer-associated fibroblasts (CAFs) in more than 90% of mammary cancerous tissues and 25% of samples have been diagnosed as an adenoma in canine females. This finding is consistent with the observation made by (Ratajczak-Wielgomas et al., 2015), who found that POSTIN was highly expressed within CAFs in invasive ductal carcinomas (IDC) among women, which is similar to our results that revealed high PN expression in 93.2% of the mammary carcinomas as compared to 16.7% of the benign tumors, with a significant difference observed among the groups ($P\leq0.01$). The findings indicate that POSTN may have a role in the development of cancer in the mammary gland in dogs, comparable to its involvement in breast cancer in humans. The reason for this is that periostin stimulates enhanced cell viability, formation of new blood vessels, invasiveness, metastatic pathways, and epithelial-mesenchymal transition of cancerous cells through attaching to integrins. Integrins, which frequently exhibit modifications in cancer cells, induce cell-ECM interaction and modify intracellular activities that contribute to the development of different conditions (Maroteaux et al., 2018).

Additionally, (Ratajczak-Wielgomas et al., 2020) found that there was a significant increase in POSTN expression in invasive ductal carcinoma (IDC) compared to non-invasive ductal carcinoma and fibrocystic changes in women. Our study also revealed significant variations in the levels of POSTN expression between malignant breast cancer samples and benign mammary tumor samples ($P\leq0.01$). Moreover, the periostin staining was more intense and diffuse in mammary carcinoma compared to the faint staining of the marker in the benign ones. The findings suggest that

POSTN may have a role in the development of cancer in the mammary gland in dogs, similar to its role in women.

Our results indicate that periostin is highly expressed in both newly diagnosed (92.1%) cases and recurrent ones (100%), with statistical differences among the high-expression group ($P \leq 0.01$). The examination of survival databases for breast cancer patients revealed that an increased expression of periostin is indicative of a reduced probability of survival and a considerably elevated risk of recurrence, particularly in cases of initial breast cancer (Nakazawa *et al.*, 2018).

There were statistically significant correlations between the PN expression in cancer cells and the sex of animals. (75%) of the male dogs presented in our study seem to have high postin expression as compared to females (95%) ($P \leq 0.01$). Breast tumors are predominantly seen in females. However, (Puglisi *et al.*, 2008) demonstrated a substantial correlation between periostin and the expression of estrogen and progesterone receptors in breast cancer. This finding presents an explanation for the overexpression of periostin in males.

There was a considerable association between periostin expression and the age and breed of the dog. However, (Borecka *et al.*, 2020) were unable to identify any association between the expression of POSTN and the age and breed of the dogs.

Evaluation of the histological grade of cancers is a crucial prognostic indicator that significantly influences subsequent prognosis and the determination of treatment (Case *et al.*, 2017). In the conducted research, we obtained a high percentage of periostin expression in all the presented grades of malignancy ($P \leq 0.01$) as well as stages of the cancers ($P \leq 0.01$). In a study conducted by (Borecka *et al.*, 2020), he found postin expression in 77 carcinomas of canine mammary gland tumors, which was quantified through IHC. The results show that high levels of periostin expression were detected in 100% of grade 3 cancers, 96.3% of grade 2 cancers, and 88% of grade 1 cancers.

It is important to mention that numerous reports indicate the similarity (such as in epidemiology, clinical and morphological characteristics, and prognostic factors) between mammary malignancies in female dogs and breast cancer in women. These reports suggest that mammary cancer in female dogs could serve as a potential model for human cancers. Hence, it may be concluded that several proteins, such as POSTN, carry out homologous roles in both the animal and human bodies (Vascellari *et al.*, 2016; Abdelmegeed and Mohammed, 2018). Based on the information provided, it may be concluded that POSTN may have a role in the development of mammary cancers in female dogs.

CONCLUSIONS AND RECOMMENDATIONS

This study showed an increase in periostin tissue expression in canine mammary cancers as compared to benign tumors as well as normal biopsies, so that POSTIN could be used as a diagnostic marker. Future studies should focus on elucidating the role of periostin in canine mammary tumorigenesis better to understand its implications for tumor progression and metastasis. In addition, studying canine mammary tumors can have a substantial influence on diagnostic and treatment options for human breast cancer. By identifying similarities between these two conditions, scientists may discover new indicators of disease, create specific treatments, provide mechanisms for translating research findings into clinical practice, and develop healthcare strategies, eventually enhancing the results for individuals with breast cancer. Finally, we highly recommend assaying a large number of patients at different ages, grades, and stages of cancer, then testing the studied marker (periostin) in follow-up treatment.

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NOVELTY STATEMENT

This study presents novel insights into the role of periostin (POSTN) expression as a potential biomarker for the evaluation of malignancies in canine mammary tumors. By conducting immunohistochemical analysis, we demonstrated a significant association between elevated periostin expression and mammary carcinoma, with distinct patterns observed in benign tumors and normal tissue samples. Furthermore, our findings reveal significant correlations between periostin expression and various clinicopathological factors such as sex, age, and breed of the dogs, shedding light on the potential utility of periostin as a diagnostic and prognostic marker in veterinary oncology. Overall, this research contributes novel evidence to the understanding of the tumor microenvironment in canine mammary neoplasms and highlights periostin as a promising target for further investigation in canine cancer research.

AUTHOR'S CONTRIBUTION

These authors each contributed equally.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

- Abdelmegeed SM, Mohammed S (2018). Canine mammary tumors as a model for human disease. *Oncol. Lett.*, 15(6): 8195-8205 <https://doi.org/10.3892/ol.2018.8411>
- Allen M, Louise Jones J (2011). Jekyll and Hyde: the role of the microenvironment on the progression of cancer. *J. pathol.*, 223(2): 163-177. <https://doi.org/10.1016/j.jhytol.2011.04.039>
- Alshaikhli RJ, Alkazaz, AA, Aljanabi AA (2023). Association of rs965513 Polymorphism Near FOXE1 Gene with Papillary Thyroid Carcinoma in a sample of Iraqi patients. *Iraqi J. Sci.*, 64(12): 6257-6265. <https://doi.org/10.24996/ijs.2023.64.12.14>
- Borecka P, Ciaputa R, Janus I, Bubak J, Piotrowska A, Ratajczak-Wielgomas K, Nowak M (2020). Expression of Periostin in Mammary Cancer Cells of Female Dogs. *in vivo*, 34(6): 3255-3262. <https://doi.org/10.21873/invivo.12162>
- Bornstein P, Sage EH (2002). Matricellular proteins: extracellular modulators of cell function. *Current opin. cell biol.*, 14(5): 608-616. [https://doi.org/10.1016/S0955-0674\(02\)00361-7](https://doi.org/10.1016/S0955-0674(02)00361-7)
- Case A, Brisson BK, Durham AC, Rosen S, Monslow J, Buza E, Volk SW. (2017). Identification of prognostic collagen signatures and potential therapeutic stromal targets in canine mammary gland carcinoma. *PloS one.*, 12(7): e0180448. <https://doi.org/10.1371/journal.pone.0180448>
- Conway SJ, Izuhara K, Kudo Y, Litvin J, Markwald R, Ouyang G, Kudo A (2014). The role of periostin in tissue remodeling across health and disease. *Cell. Mol. Life Sci.*, 71: 1279-1288. <https://doi.org/10.1007/s00018-013-1494-y>
- Dahinden C, Ingold B, Wild P, Boysen G, Luu V D, Montani M, Schraml P (2010). Mining tissue microarray data to uncover combinations of biomarker expression patterns that improve intermediate staging and grading of clear cell renal cell cancer. *Clin. Cancer Res.*, 16(1): 88-98. <https://doi.org/10.1158/1078-0432.CCR-09-0260>
- Dawood HH, Mohammed RK (2023). The Correlation Study between TP53 Gene Expression and Acute Myeloid Leukemia in Iraq. *Iraqi J. Sci.*, 64(11): 5615-5623. <https://doi.org/10.24996/ijs.2023.64.11.14>
- Dolka I, Czopowicz M, Gruk-Jurka A, Wojtkowska A, Sapierzyński R, Jurka P (2018). Diagnostic efficacy of smear cytology and Robinson's cytological grading of canine mammary tumors with respect to histopathology, cytomorphometry, metastases and overall survival. *PLoS One.*, 13(1): e0191595. <https://doi.org/10.1371/journal.pone.0191595>
- EK AL-Hamdany (2022). Identification and Characterization of Canine Mammary Tumors Stem Cells: A Review. *Al-Anbar J. Vet. Sci.*, 15 (1): 66-72. <https://doi.org/10.37940/AJVS.2021.15.1.9>
- Elston CW, Ellis IO (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*, 19(5): 403-410. <https://doi.org/10.1111/j.1365-2559.1991.tb00229.x>
- Goldschmidt M, Peña L, Rasotto R, Zappulli V (2011). Classification and grading of canine mammary tumors. *Vet. Pathol.*, 48(1): 117-131. <https://doi.org/10.1177/0300985810393258>
- Gonzalez-Gonzalez L, Alonso J (2018). Periostin: a matricellular protein with multiple functions in cancer development and progression. *Front. Oncol.*, 8: 225. <https://doi.org/10.3389/fonc.2018.00225>
- Hamilton DW (2008). Functional role of periostin in development and wound repair: implications for connective tissue disease. *J. cell commun. signaling*, 2: 9-17. <https://doi.org/10.1007/s12079-008-0023-5>
- Hong LZ, Wei XW, Chen JF, Shi Y (2013). Overexpression of periostin predicts poor prognosis in non-small cell lung cancer. *Oncol. Lett.*, 6(6): 1595-1603. <https://doi.org/10.3892/ol.2013.1590>
- Hussein SI, Shubber SS, Yaseen NY (2019). Bio-distribution of Gold Nanoparticles in Tumor Mass and Different Organs in Implanted Mice with Mammary Adenocarcinoma AM3 (in vivo study). *The Iraqi J. Vet. Med.*, 43(2): 17-22. <https://doi.org/10.30539/iraqijvm.v43i2.525>
- Janus I, Kandefor-Gola M, Ciaputa R, Noszczyk-Nowak A, Pasławska U, Tursi M, Nowak M (2016). The immunohistochemical evaluation of selected markers in the left atrium of dogs with end-stage dilated cardiomyopathy and myxomatous mitral valve disease—a preliminary study. *Irish Vet. J.*, 69, 1-9. <https://doi.org/10.1186/s13620-016-0077-2>
- Kharaishvili G, Cizkova M, Bouchalova K, Mgebrishvili G, Kolar Z, Bouchal, J (2011). Collagen triple helix repeat containing 1 protein, periostin and versican in primary and metastatic breast cancer: an immunohistochemical study. *J. clin. Pathol.*, 64(11): 977-982 <https://doi.org/10.1136/jclinpath-2011-200106>
- Khayoon HA, Al-Rekabi FM (2020). Cytotoxic Effect of Resveratrol on Colorectal Cancer Cell Line. (2020). *The Iraqi J. Vet. Med.*, 44(1): 68-74. <https://doi.org/10.30539/ijvm.v44i1.939>
- Kim GE, Lee JS, Park MH, Yoon JH (2017). Epithelial periostin expression is correlated with poor survival in patients with invasive breast carcinoma. *PLoS One.*, 12(11): e0187635.
- Król M, Pawłowski KM, Szyszko K, Maciejewski H, Dolka I, Manuali E, Motyl T (2012). The gene expression profiles of canine mammary cancer cells grown with carcinoma-associated fibroblasts (CAFs) as a co-culture in vitro. *BMC Vet. Res.*, 8: 1-22. <https://doi.org/10.1186/1746-6148-8-35>
- Kudo A (2011). Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. *Cell. Mol. life Sci.*, 68: 3201-3207. <https://doi.org/10.1007/s00018-011-0784-5>
- Kudo A, Kii I (2018). Periostin function in communication with extracellular matrices. *J. cell. commun. signaling*, 12: 301-308. <https://doi.org/10.1007/s12079-017-0422-6>
- Lu P, Weaver VM, Werb Z (2012). The extracellular matrix: a dynamic niche in cancer progression. *J. cell biol.*, 196(4): 395-406. <https://doi.org/10.1083/jcb.201102147>
- Maroteaux L, Roumier A, Doly S, Diaz SL, Belmer A (2018). Encyclopedia of Signaling Molecules. *Encycl. Signaling Mol.*, 2458-2481. https://doi.org/10.1007/978-3-319-67199-4_286
- Mineshige T, Kamiie J, Sugahara G, Yasuno K, Aihara N,

- Kawarai S,Shirota K (2015). Expression of periostin in normal, atopic, and nonatopic chronically inflamed canine skin. *Vet. Pathol.*, 52(6): 1118-1126. <https://doi.org/10.1177/0300985815574007>
- Mineshige T, Ogihara K, Kamiie J, Sugahara G, Chambers J K, Uchida K Shirota K (2018). Increased expression of the stromal fibroblast-secreted periostin in canine squamous cell carcinomas. *J. Vet. Med. Sci.*, 80(3): 473-479. <https://doi.org/10.1292/jvms.17-0647>
- Morra L, Moch H (2011). Periostin expression and epithelial-mesenchymal transition in cancer: a review and an update. *Virchows Arch.*, 459: 465-475. <https://doi.org/10.1007/s00428-011-1151-5>
- Muhammed SO, Utba NM, Raof YS (2023). Gene Polymorphism of Human Lymphotoxin Alpha in Iraqi Breast Cancer Women. *Iraqi J. Sci.*, 5564-5571. <https://doi.org/10.24996/ij.s.2023.64.11.9>
- Nakazawa Y, Taniyama Y, Sanada F, Morishita R, Nakamori S, Morimoto K, Yang J (2018). Periostin blockade overcomes chemoresistance via restricting the expansion of mesenchymal tumor subpopulations in breast cancer. *Sci. rep.*, 8(1): 4013. <https://doi.org/10.1038/s41598-018-22340-7>
- Nuzzo PV, Buzzatti G, Ricci F, Rubagotti A, Argellati F, Zinoli L, Boccardo F (2014). Periostin: a novel prognostic and therapeutic target for genitourinary cancer. *Clin. Genitourin. cancer*, 12(5): 301-311. <https://doi.org/10.1016/j.clgc.2014.02.005>
- Nuzzo PV, Rubagotti A, Zinoli L, Salvi S, Boccardo S, Boccardo F (2016). The prognostic value of stromal and epithelial periostin expression in human breast cancer: correlation with clinical pathological features and mortality outcome. *BMC cancer.*, 16:1-13. <https://doi.org/10.1186/s12885-016-2139-y>
- Obaid QA, Khudair KK, Al-Shammari AM (2021). 2-Deoxyglucose Glycolysis Inhibitor Augment Oncolytic Virotherapy to Induce Oxidative Stress and Apoptosis in Breast Cancer (Part III). *The Iraqi J. Vet. Med.*, 45(2): 26-32. <https://doi.org/10.30539/ijvm.v45i2.1257>
- Puglisi F, Puppini C, Pegolo E, Andreetta C, Pascoletti G, D'Aurizio F, Di Loreto C (2008). Expression of periostin in human breast cancer. *J. clin. Pathol.*, 61(4): 494-498. <https://doi.org/10.1136/jcp.2007.052506>
- Pula B, Witkiewicz W, Dziegiel P, Podhorska-Okolow M (2013). Significance of podoplanin expression in cancer-associated fibroblasts :a comprehensive review. *Int. j. oncol.*, 42(6): 1849-1857. <https://doi.org/10.3892/ijo.2013.1887>
- Rasoul LM (2023). The Anti-cancer Impact of Genetically Engineered Newcastle Disease Virus Expressing GFP Gene Against U87-MG Cell Line. *Iraqi J. Sci.*, 64(12): 6204-6214. <https://doi.org/10.24996/ij.s.2023.64.12.10>
- Ratajczak-Wielgomas K, Dziegiel P (2015). The role of periostin in neoplastic processes. *Folia histochem. Cytobiol.*, 53(2): 120-132. <https://doi.org/10.5603/FHC.a2015.0014>
- Ratajczak-Wielgomas K, Grzegorzolka J, Piotrowska A, Gomulkiewicz A, Witkiewicz W, Dziegiel P (2016). Periostin expression in cancer-associated fibroblasts of invasive ductal breast carcinoma. *Oncol. Rep.*, 36(5): 2745-2754. <https://doi.org/10.3892/or.2016.5095>
- Ratajczak-Wielgomas K, Kmiecik A, Grzegorzolka J, Piotrowska A, Gomulkiewicz A, Partynska A (2020). Prognostic significance of stromal periostin expression in non-small cell lung cancer. *Int. J. Mol. Sci.*, 21:7025. <https://doi.org/10.3390/ijms21197025>
- Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, Conway SJ (2005). Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol. Cell. Biol.*, 25(24): 11131-11144. <https://doi.org/10.1128/MCB.25.24.11131-11144.2005>
- Santos AA, Lopes CC, Ribeiro JR, Martins LR, Santos JC, Amorim IF, Matos AJ (2013). Identification of prognostic factors in canine mammary malignant tumours: a multivariable survival study. *BMC Vet. Res.*, 9: 1-11. <https://doi.org/10.1186/1746-6148-9-1>
- Cary (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. N.C. USA
- Shao R, Bao S, Bai X, Blanchette C, Anderson RM, Dang T, Wang XF (2004). Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular endothelial growth factor receptor 2 expression. *Mol. Cell. Biol.*, 24(9):3992-4003. <https://doi.org/10.1128/MCB.24.9.3992-4003.2004>
- Sleeckx N, De Rooster H, EJVK, Van Ginneken C, Van Brantegem L (2011). Canine mammary tumours, an overview. *Reproduction in domestic animals= Zuchthygiene*, 46(6): 1112-1131. <https://doi.org/10.1111/j.1439-0531.2011.01816.x>
- Soltermann A, Tischler V, Arbogast S, Braun J, Probst-Hensch N, Weder W, Kristiansen G (2008). Prognostic significance of epithelial-mesenchymal and mesenchymal-epithelial transition protein expression in non-small cell lung cancer. *Clin. Cancer Res.*, 14(22): 7430-7437. <https://doi.org/10.1158/1078-0432.CCR-08-0935>
- Sorenmo K (2003). Canine mammary gland tumors. *Veterinary Clinics: Small Anim. Pract.*, 33(3): 573-596. [https://doi.org/10.1016/S0195-5616\(03\)00020-2](https://doi.org/10.1016/S0195-5616(03)00020-2)
- Sorenmo, KU, Rasotto R, Zappulli V, Goldschmidt MH (2011). Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. *Vet. Pathol.*, 48(1): 85-97. <https://doi.org/10.1177/0300985810389480>
- Tarab MK, AL-Rekabi FM (2022). Therapeutic Trial of Modified Levofloxacin (MOLVX) Against Induced Colorectal Aberrant Crypt Foci (Precancerous Tissue) in Mice. *The Iraqi J. Vet. Med.*, 46(2): 53-65. <https://doi.org/10.30539/ijvm.v46i2.1554>
- Vascellari M, Capello K, Carminato A, Zanardello C, Baioni E, Mutinelli F (2016). Incidence of mammary tumors in the canine population living in the Veneto region (Northeastern Italy): Risk factors and similarities to human breast cancer. *Prev. Vet. Med.*, 126: 183-189. <https://doi.org/10.1016/j.prevetmed.2016.02.008>
- Zhu X, Wang Y, Liu Y, Huang GTJ, Zhang C (2014). Immunohistochemical and histochemical analysis of newly formed tissues in root canal space transplanted with dental pulp stem cells plus platelet-rich plasma. *J. Endod.*, 40(10): 1573-1578. <https://doi.org/10.1016/j.joen.2014.05.010>