Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Assessment of glypican-3 immune expression in ameloblastoma, ameloblastic fibroma, ameloblastic carcinoma (pilot study)

Nayl Ahmed Al-ezzi Al-Malahy[1*], Shaymaa Omar Zayed[1] and Heba Ahmed Saleh[1]

Abstract

Background Odontogenic tumors a variety of benign and malignant lesions developed exclusively in the jaws. They are known to have variable clinical behaviors and different histological features. The development of odontogenic tumors is attributed to alterations in some genetic and biological molecules. The aim of this study is to assess Glypican-3 immune expression in different groups of odontogenic neoplasms as in Ameloblastoma, Ameloblastic Fibroma, and Ameloblastic Carcinoma, and compare it to dental follicle tissue as control group. Also correlate the marker expression with the known biological behavior of these tumors. This study included 40 cases were selected from oral and maxillofacial pathology department (ten cases in each group). Some sections are prepared to be stained with H&E stain and other sections with Glypican-3 immune stain. Histological examination and Histomorphometric analysis were done finally under light microscope. The area percents of Glypican-3 immune expression in all tumor sections were measured.

Results Level of expression of Glypican-3 revealed significant difference between the study groups (P-value < or = 0.05). The level of immune expression was highest in ameloblastic carcinoma group (M = 58.7) followed by ameloblastoma group (M = 33.9), then ameloblastic fibroma (M = 5.6) and lowest in the control group of dental follicle (M = 1.3).

Conclusions Glypican-3 immune expression demonstrated statistically significant difference among the study groups, indicating that it may have contributing role in tumor pathogenesis and its biological behavior.

Keywords Glypican-3, Odontogenic tumors, Ameloblastoma, Ameloblastic fibroma, Ameloblastic carcinoma

1 Background

Ameloblastoma (AB) is well known in literature as most common locally invasive odontogenic neoplasm with high rate of recurrence [1]. It commonly causes severe cortical expansion in the posterior area of the lower jaw and commonly arise in adult age. Radiographically AB commonly presented as multilocular radiolucent lesion in tooth bearing area and it may appear surrounding unerupted tooth in some cases. The long-standing lesions of AB are characterized by severe expansion of bones, displacement of the teeth, root resorption, paraesthesia and perforation of jaw bones. Histologically, the odontogenic epithelial tumor cells may arranged in follicular and plexiform patterns surrounded by fibrous stroma with other various subtypes. Surgical resection with wide safety area of the bone (beyond lesion radiographic margin) and reconstruction are reported to be the best choices of treatment of AB cases [2, 3].

Ameloblastic fibroma (AF) is considered as an uncommon, slowly-growing benign odontogenic neoplasm in the posterior area of the lower jaw. AF occurring at any

*Correspondence:
Nayl Ahmed Al-ezzi Al-Malahy
naylalmalahy@dentistry.cu.edu.eg
[1] Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt

age, but has a predilection to present in age group of 7–61 years with 80% in patients younger than 22 years [3]. AF mostly presented radiographically as unilocular radiolucent lesion, which may be associated with unerupted tooth. AF clinically exists as a well circumscribed swelling with slight cortical bone expansion and may cause displacement of the adjacent teeth. Histologically, it usually composed of islands and cords of odontogenic epithelial tumor cells immersed in ectomesenchyme (resemble the dental papilla) but without any actual hard dental tissue formation [4]. Simple surgical excision is the best treatment in the majority of AF cases [3].

Ameloblastic Carcinoma (ABC) is a malignant counterpart of Ameloblastoma with more aggressive behavior, and poorer prognosis [5]. It usually presented as fast growing, destructive clinical swelling with perforation of surrounding cortical bone and invasion into surrounding soft tissues. In most of the reported ABC cases, lesions presented with ill-defined radiolucency. ABC histologically appears as Ameloblastoma (AB) with malignant dysplastic features such as cellular pleomorphism, increase mitotic rate, basilar hyperplasia, nuclear hyperchromatism, perineural or vascular invasion and necrosis [3]. ABC frequently metastasis to cervical lymph node or lungs then this may be followed by bone or liver or brain metastasis [6]. Radical surgical excision is the favorable line of treatment of the ABC, which may be followed by radiotherapy in metastatic cases [3]. Jaw reconstruction in conjugation with prosthetic rehabilitation by using dental implants have a good outcome for the reestablish the mastication and esthetics after jaw resection in cases of AB& ABC [7].

Glypicans (GPC) are a group of proteoglycans family that consists of six members (GPC-1 to 6). The GPC-3 gene encodes a 70-kDa surface protein, which is highly expressed in embryonic tissues [8]. GPC-3 immune expression is reported to be elevated in some malignancies as hepatocellular carcinoma, squamous cell carcinoma and other malignancies of salivary gland and odontogenic origin [8–10]. Previous documented studies reported the higher expression of GPC-3 in salivary gland neoplasms and also in some odontogenic tumors especially in those of aggressive or malignant nature [11, 12].

GPC-3 is widely used as marker for diagnosis and target for therapy of hepatocellular carcinoma [10]. Furthermore, GPC-3 acts as a negative regulator of Hedgehog (hh) signaling activity, by competing with patched PTCH1 for Sonic hedgehog (SHH) binding and components of the (SHH) pathway. The mutation of the GPC-3 gene on the X chromosome locus q26.1 become unable to control Hedgehog signaling during development, this leads to increase in cellular proliferation and risk of neoplastic development [13].

In an attempt to cover the gap of knowledge in studying the cytogenetics for this kind of oral neoplasms as they are rare entities, we performed the present study with a purpose to detect GPC-3 expression in variable odontogenic neoplasms and correlate it with their biological behavior. Investigating such type of relationships between glypican -3 immune expression &clinical behavior of odontogenic neoplasms is very important in introducing new clinical application (treatment line) of target immune therapy for such lesions instead of aggressive jaw resection strategy. This could finally change mode of treatment of such aggressive neoplasms an enhance quality of patient life after treatment.

2 Methods

This study included forty cases; which were divided into four groups as the follow: ten cases of AB, ten cases of ABC, ten cases of AF, and 10 cases of Dental follicle (DF) as a normal odontogenic tissue Table 1. The samples were taken from archival blocks between 2010 and 2021, from the Oral and Maxillofacial Pathology Department, Faculty of Dentistry. These selected ODTs cases were selected and rediagnosed according to histopathological criteria established by World Health Organization (WHO) [3].

The registered protocol for this study (included sample size) was approved by the Research Ethics Committee of our institute with (No. 22 9 21). This sample size for this pilot study was also approved by Medical Biostatistics Unit of our institute to be 40 cases (10 cases in each study group).

Anti-Glypican-3 Rabbit Monoclonal Antibody (GPC-3 Rabbit mAb) purchased from ABclonal (catalog no. A13988) was used for immunostaining following previously described guidelines [14]. The paraffin embedded specimens were cut into 4 μm-thick sections, mounted on slides, stained by routine Hematoxylin and Eosin (H & E) and examined under Leica DM500 binocular transmitted light microscope. Other histological sections were cut from each paraffin blocks and placed on positively charged slides (Opti plus slides) for immunostaining procedures. Ordinary light microscopy was used for microscopic examination of all stained sections of each group.

The GPC-3 immunostained sections were assessed by the computer image analyzer using the software Leica Qwin 500 image analyzer (Germany). The image analyzer was automatically calibrated to change the unit of measurement (pixels) into actual units of the micrometer. The area percent of GPC-3 antibody positive epithelial tumor cells was observed in a frame of measurement of 61,934 μm^2, and five fields were selected for cases with (×200)

Table 1 Demographic data of the study groups

Demographic data	Number of cases				Precentage			
	DF	AF	AB	ABC	DF (%)	AF (%)	AB (%)	ABC (%)
Site								
Ant. Max	3	0	0	0	30	0	0	0
Post. Max	0	0	0	0	0	0	0	0
Crossing Midline Max	0	0	0	1	0	0	0	10
Ant. Mand	1	0	0	0	10	0	0	0
Post. Mand	6	9	9	8	60	90	90	80
Crossing Midline Mand	0	1	1	1	0	10	10	10
Age								
< 20 y	5	0	0	0	50	0	0	0
20–40 y	5	4	6	2	50	40	60	20
40–60 y	0	0	1	4	0	0	10	40
> 60 y	0	0	3	4	0	0	30	40
Gender								
Male	7	8	6	10	70	80	60	100
Female	3	2	4	0	30	20	40	0

magnification. The most staining areas were measured after that the computer system converted the selected areas into a measurable green color.

The data recorded for the area percent for GPC-3 immune expression was statistically checked for normality by Kolmogorove- Smirnov test. Then the p-value of Kolmogorove- Smirnov test was recorded in the 4 study groups to be greater than 0.05, thus all the groups complied with the normal assumption ($p > 0.05$) and therefore proceeding with Paramentric test using one Way ANOVA Test to compare the date between the 4 groups. This was followed by Tukey Post Hoc for pairwise comparison to compare in-between the groups.

3 Results

3.1 Histopathological examination of H&E stained sections of different ODTs

All H&E stained sections were microscopic examined to confirm the diagnosis of all included cases in the study groups. Histological features of DF, AF, AB, &ABC were obviously detected in all cases during light microscopic examination according to the histological criteria previously described by the WHO [3] (Fig. 1).

3.2 Histopathological examination of Glypican-3-stained sections of ODTs

Cases of DF showed negative immunoreaction to GPC-3. weak immunostaining was only observed in scattered fibroblasts in few cases of DF (Fig. 2a). GPC-3 Immunohistochemical stained sections of AF cases revealed weak positive membranous immune expression in some of the neoplastic epithelial components of the tumor. Other neoplastic stromal cells showed obvious negative immune reaction of GPC-3 (Fig. 2b). In most of the cases of AB, the GPC-3 cytoplasmic & membranous immunoreactions were positively detected in both peripheral ameloblast like cells and central stellate reticulum like tumor cells (Fig. 2c). Moreover, most of ABC cases showed diffuse and intense positive GPC-3 cytoplasmic and membranous immune expression in all of malignant epithelial tumor cells. In addition, some fibroblasts showed cytoplasmic GPC-3 immune expression in Connective tissue stroma surrounding tumor cells in few cases of ABC (Fig. 2d).

The data of the area percent for GPC-3 immune expression was statistically checked to be normally distributed in the study groups using normality test. The p-value of Kolmogorove- Smirnov test was recorded in the 4 study groups to be greater than 0.05, this proves that all the groups complied with the normal assumption ($p > 0.05$).

Analysis of GPC-3 immune stained sections using one Way ANOVA Test (parametric test) revealed that the greatest mean value of area percent of GPC-3 immune expression was recorded in ABC group (58.77), followed by AB group (33.95), then AF group (5.65). The least mean value was obviously detected to be in the control group of DF (1.34). One way ANOVA test showed a statistically significant difference between all the study groups (Table 2 and Fig. 3).

By using Tukey post hoc Test for pairwise comparison this test revealed, a statistically significant difference was observed among AB group and ABC group on one

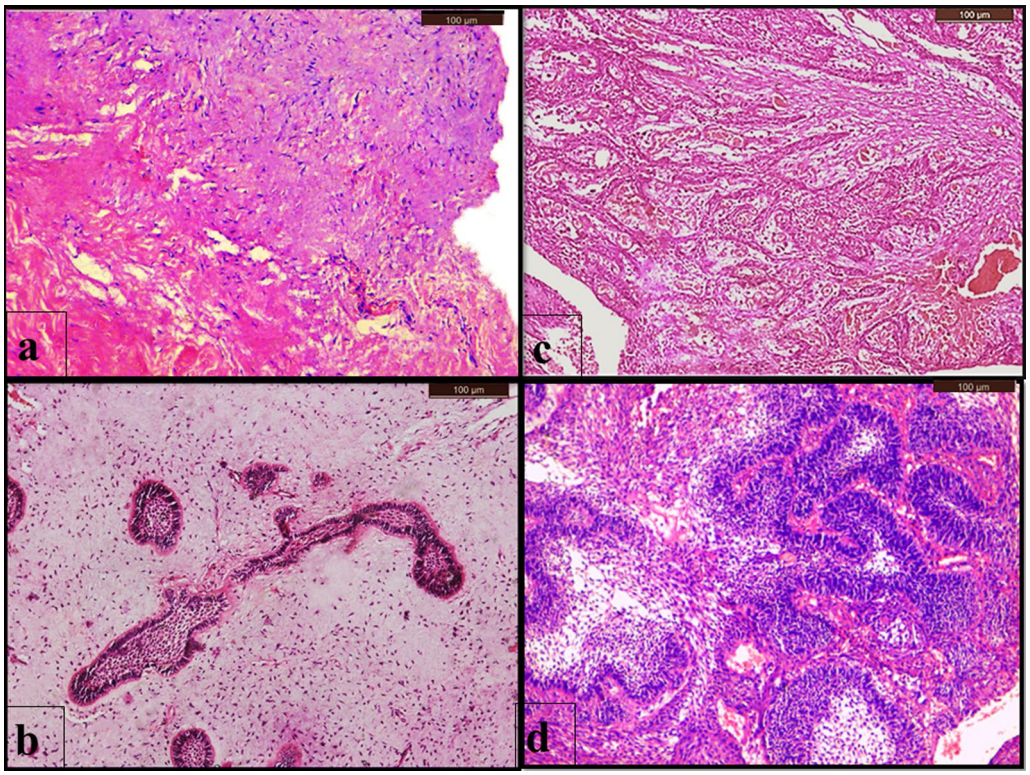

Fig. 1 photomicrographs of H&E stained histological sections of ODTs, **a** DF presented with loosely arranged collagenous stroma. **b** AF showing branching cords of bland odontogenic epithelium surrounded by primitive mesenchymal stroma. **c** AB (hemangioplexiform pattern) showing anastomosing cords of odontogenic epithelium with in fibrous stroma. **d** ABC showing follicles and plexus of odontogenic epithelium with obvious dysplastic signs

side and all other groups on the other side ($p < 0.05$). On the contrary, no statistically significant difference was detected between group DF and group AF ($p = 0.720$) (Table 2).

4 Discussion

ODTs are a variety of jaw lesions with different nature, as they derived from tooth-developing apparatus during abnormal process of odontogenesis. ODTs have variable clinical and histological presenting feature, thus they usually associated with different clinical or biological behavior [15]. AB was selected in our study for being the most commonly occurring benign but locally aggressive ODT of epithelial origin with high recurrence rate. AF cases were selected in our study as it is indolent, benign, odontogenic neoplasm with documented non-aggressive biological behavior. ABC was also chosen in this study because it is the most common malignant ODT and it is well-known with its aggressive and destructive behavior with tendency to metastasis [1].

Some documented studies in the literature have been reported the role of GPC-3 in various neoplasms and reported the link between its expression and the aggressiveness of such lesions e.g., hepatocellular carcinoma, oral squamous cell carcinoma, malignant salivary gland tumors, odontogenic cysts, lung squamous cell carcinoma, and melanoma [12, 16]. Therefore, the novelty of our study is to assess the expression of GPC-3 in odontogenic neoplasms with variable nature and correlate its expression with their clinical behavior. Investigate such a relationship between level of GPC-3 expression and aggressiveness of such odontogenic neoplasms would alter the aggressive mode of treatment (jaw resection) given to such oral tumors to become more conservative and secure quality of the patient life.

According to our immunohistochemical finding, the GPC-3 immune expression was observed as cytoplasmic and membranous pattern in odontogenic tumor cells. This is owing to nature of glypicans (as heparan sulfate proteoglycans), which linked glycosyl-phosphatidylinositol (GPI) anchor to the cytoplasmic surface of the cell membrane.

In our study, intense predominantly membranous and cytoplasmic immune staining pattern of GPC-3 was observed in malignant (ABC) or aggressive (AB) odontogenic neoplastic cells than benign odontogenic neoplastic

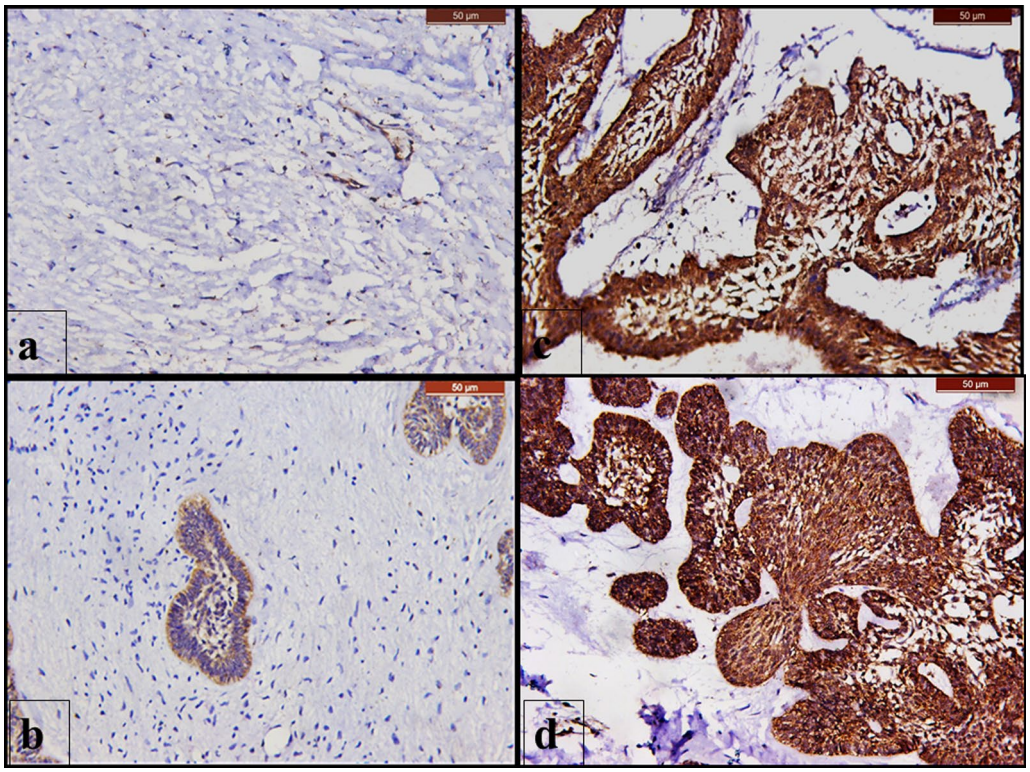

Fig. 2 Photomicrographs of GPC-3 immune expression in different sections of ODTs **a** DF negative GPC-3 immune expression was observed in DF. **b** Weak positive immunoreaction of GPC-3 was shown in some epithelial tumor cells of AF. **c** Obvious positive cytoplasmic GPC-3 expression was presented in all epithelial cells of AB. **d** Intense cytoplasmic and nuclear immune expression of GPC-3 were detected in the malignant tumor cells of ABC

Table 2 Describing area percent of GPC-3 for the 4 study groups

	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	F	p value
				Lower Bound	Upper Bound				
AB	33.95[b]	7.21607	3.22712	24.99	42.90	25.22	41.12	86.277	0.000
AF	5.65[a]	1.10591	0.49458	4.28	7.02	4.48	6.96		
ABC	58.77[c]	10.62718	4.75262	45.57	71.96	40.23	67.33		
DF	1.34[a]	0.50613	0.22635	0.71	1.97	0.79	1.94		

*Significant at $p < 0.05$

Means with different superscript letters are significantly different (Tukey's post hoc test)

cells (AF) and normal odontogenic tissue (DF). We reported that the difference in the mean values of GPC-3 expression was high and statistically significant between the study groups. The greatest area mean of GPC-3 immune expression was recorded in the malignant ODT (ABC), followed by the locally aggressive ODT(AB), then the benign ODT (AF), whereas the lowest area percent was recorded in the DF (as normal odontogenic tissue).

In our study the obvious dominating immune expression of GPC-3 was seen in ABC and AB in comparison with AF and DF. Through this finding we can suggest the contributing role of GPC-3 in the aggressiveness and tumorigenesis of ODTs. Our immunohistochemical results were previously confirmed by. Mendes et al. [12], who stated that immune expression of GPC-3 can differentiate between aggressive and non-aggressive types of ODTs. As they previously observed that the aggressive cases of keratocystic odontogenic tumor and AB exhibited elevated significant expression of GPC-3

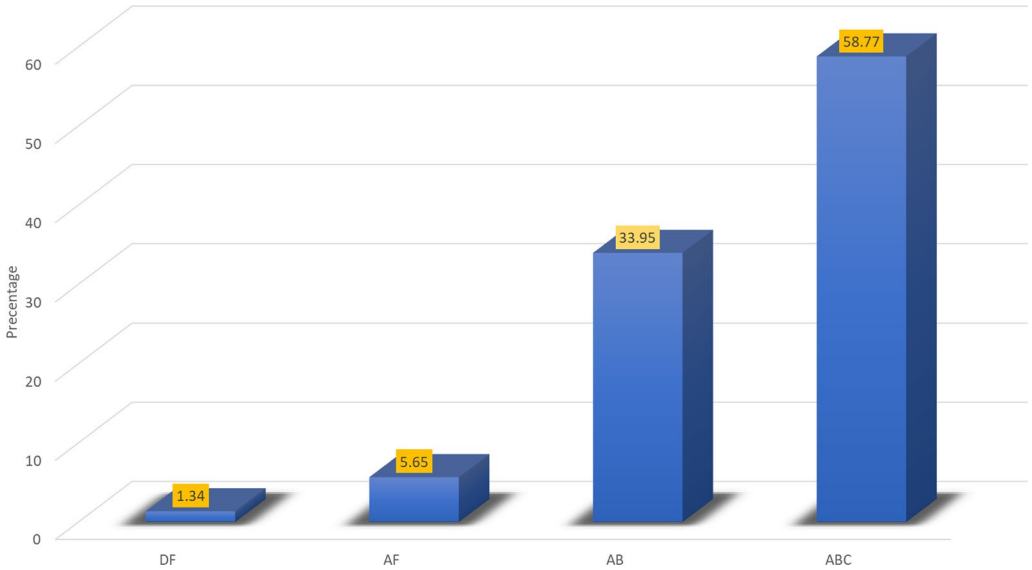

Fig. 3 Mean area percent of GPC-3 between the study groups

compared to non-aggressive ODTs as adenomatoid odontogenic tumor.

Chaturvedi et al. [17] also reported a high immuno-expression of GPC-3 in the aggressive cases of AB, and odontogenic keratocyst (OKC) but the absence of GPC-3 immuno-expression in non-aggressive odontogenic tumors as (adenomatoid odontogenic tumors and calcifying cystic odontogenic tumors). Finally, they also concluded the association between GPC-3 elevated immune expression and the aggressiveness & recurrence of such tumors [17].

Our presented finding showed a low expression of GPC-3 in AF group and the absence of expression in DF groups compared to AB and ABC. This observation also highlighted the correlation between GPC-3 and aggressiveness of some ODTs (explored for the first time in this study). Moreover, our reported finding is supported by earlier study done by Mendes et al. [12], who suggested that lower immune expression of GPC-3 in the non-aggressive cases of unicystic AB compared to the aggressive cases of conventional AB.

In addition, the overexpression of GPC-3 has been documented in different types of malignant neoplasms, which confirmed the role of GPC-3 in developing malignancy. Andisheh et al. (2019) also demonstrated in their study on different salivary gland neoplasms, the role of GPC-3 in invasion and development of malignancy of other category of oral tumors. As they clearly reported the elevated GPC-3 expression in salivary glands carcinomas (as mucoepidermoid carcinoma and acinic cell carcinoma) in comparison to low GPC-3 expression in the benign salivary gland neoplasms (as pleomorphic adenoma) [11]. Their reported finding was on the same line with our observed and reported results of GPC-3 expression.

Andisheh et al. (2020) studied the expression of GPC-3 in oral squamous cell carcinoma (OSCC) and suggested that, the overexpression of GPC-3, confirming its role in carcinogenesis of such cases. They also documented that the immune expression of GPC-3 was significantly higher in samples with larger tumor size. In contrast, there was no significant relation between expression of GPC-3 and tumor staging, grading and metastasis [9].

As regard dental follicle, GPC-3 expression was observed to be almost negative except for some scattered fibroblast. This immune expression noted in some fibroblasts was previously explained by Matsuo & Kimura (2013) who revealed that FGF receptors are proved to be regulated by heparan sulfate proteoglycans, thus GPC-3 is acting as a co-receptor for fibroblast growth factor (FGF)-9 [18, 19].

Our previous findings support the recommendations of Ho and Kim [16] and also Shimizu et al. [20] who proved the absence of GPC-3 expression in normal tissue and its higher expression in malignant neoplasms. They finally suggest using of GPC-3 as target immunotherapy, hopefully to increase the survival rate and improve patient life quality in case of malignancy especially progressive hepatocellular carcinoma patients [16, 20]. At the end, we must clearly mention the limitation of this studythe small number of sample size which is attributed to the rarity of these neoplasms and also excluding the cases with incomplete data from our work could limit the number of included cases in our study.

5 Conclusion

Finally, we should state that the expression of GPC-3 in different ODTs is related to their natures (as benign, locally aggressive or malignant lesion) and may also affect their clinical behaviors (aggressive with high recurrent rate or non-aggressive indolent lesions). Expression of GPC-3 is higher and statistically significant in malignant (ABC) than in locally aggressive neoplasm (AB) and benign indolent nature (AF). Investigating such type of relationships could open gate for proper use of immune target therapy for such lesions, which could save quality of patient life by such very conservative line of treatment (instead of aggressive surgical jaw resections). Further studies are recommended to investigate GPC-3 expression on other different types of odontogenic neoplasms and by other investigatory techniques as insitu hybridization or PCR to deeply investigate these rare types of oral tumors from different cytogenetic aspects.

Abbreviations

AB	Ameloblastoma
ABC	Ameloblastic carcinoma
AF	Ameloblastic Fibroma
CT	Connective Tissue
DAB	Di-Amino Benzine
DF	Dental Follicle
GPC-3	Glypican-3
H&E	Hematoxylin and Eosin
ODTs	Odontogenic Tumors
WHO	World Health Organization

Acknowledgements

I would like to thank all the, oral and maxillofacial pathologists and pathology lab technicians in faculty of dentistry, Cairo University for helping us to complete this work.

Author contributions

Conceptualization: HS& SO, methodology: NA Formal analysis and investigation: NA& HS writing—original draft preparation: NA. Writing—review and editing: HS, supervision, validation and final editing: SO, all authors commented on previous versions of the manuscript, All authors read and approved the final manuscript.

Funding

This work is self-funded by the authors, and no funding is obtained for this study.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available until this work is accepted in the journal but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study followed the ethical standards of the Research Ethical Committee, Faculty of Dentistry, Cairo University. This study required the utilization of removed neoplasms from patients in the last years and stored them for research purpose under the institution's ethical standards. Therefore, the Research Ethics Committee of our institute approved this study with (approval No. 22 9 21).

Consent for publication

Not applicable.

Patient consent

We have the ethical committee's approval to use these preserved tissues. All our research cases were obtained from a bank of paraffin blocks in the oral and maxillofacial pathology department, as these excised tissues are stored and kept in certain conditions approved by the ethical committee in our institute.

Competing interests

The authors declare no competing interests.

Received: 7 January 2024 Accepted: 29 February 2024
Published online: 11 March 2024

References

1. Al-aroomy L, Wali M, Alwadeai M, El Desouky E, Amer H (2022) Odontogenic tumors: a retrospective study in Egyptian population using WHO 2017 classification. Medicina Oral Patologia Oral y Cirugia Bucal 27(3):e198. https://doi.org/10.4317/medoral.24661
2. Jordan RC, Speight PM (2009) Current concepts of odontogenic tumors. Diagn Histopathol 15(6):303–310. https://doi.org/10.1016/j.mpdhp.2009.03.002
3. El-Naggar AK. WHO classification of head and neck tumors. International Agency; 2017.
4. Carroll C, Gill M, Bowden E, O'Connell JE, Shukla R, Sweet C (2019) Ameloblastic Fibroma of the mandible reconstructed with autogenous parietal bone: report of a case and literature review. Case Rep Dent. https://doi.org/10.1155/2019/5149219
5. Kruse AL, Zwahlen RA, Grätz KW (2009) New classification of maxillary ameloblastic carcinoma based on an evidence-based literature review over the last 60 years. Head Neck Oncol 1(1):1–7. https://doi.org/10.1186/1758-3284-1-31
6. Braimah R, Uguru C, Ndukwe K (2017) Ameloblastic carcinoma of the jaws: Review of the literature. J Dent Allied Sci 6(2):70–73. https://doi.org/10.4103/jdas.jdas_4_17
7. Raoufi-Danner S, Carl S, Jahan A (2018) Oral rehabilitation of patients with ameloblastoma of the mandible. Clinical results in three patients with different bone reconstruction techniques. Open Dent J 1:1. https://doi.org/10.2174/1874210601812011107
8. Capurro MI, Xu P, Shi W, Li F, Jia A, Filmus J (2008) Glypican-3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. Dev Cell 14(5):700–711. https://doi.org/10.1016/j.devcel.2008.03.006
9. Andisheh-Tadbir A, Goharian AS, Ranjbar MA (2020) Glypican-3 expression in patients with oral squamous cell carcinoma. J Dent 21(2):141. https://doi.org/10.30476/DENTJODS.2019.84541.1089
10. Feng M, Ho M (2014) Glypican-3 antibodies: a new therapeutic target for liver cancer. FEBS Lett 588(2):377–382. https://doi.org/10.1016/j.febslet.2013.10.002
11. Andisheh-Tadbir A, Ashraf MJ, Gudarzi A, Zare R (2019) Evaluation of Glypican-3 expression in benign and malignant salivary gland tumors. J Oral Biol Craniofac Res 9(1):63–66. https://doi.org/10.1016/j.jobcr.2018.09.002
12. Mendes RB, Dias RB, Figueiredo AL, Gurgel CA, Santana Filho M, Melo LA, Trierveiler M, Cury PR, Leonardi R, Dos Santos JN (2017) Glypican-3 distinguishes aggressive from non-aggressive odontogenic tumors: a preliminary study. J Oral Pathol Med 46(4):297–300. https://doi.org/10.1111/jop.12501
13. Chen CP (2012) Prenatal findings and the genetic diagnosis of fetal overgrowth disorders: Simpson-Golabi-Behmel syndrome, Sotos syndrome, and Beckwith-Wiedemann syndrome. Taiwan J Obstet Gynecol 51(2):186–191. https://doi.org/10.1016/j.tjog.2012.04.004
14. Saleh HA, Makawi DM, Rashad AE (2023) Immunohistochemical assessment of the potential behavior of glandular odontogenic cyst and inflammatory periodontal cyst using E-cadherin and N-cadherin.

Beni-Suef Univ J Basic Appl Sci 12(1):97. https://doi.org/10.1186/s43088-023-00439-9
15. Santosh AB, Jones TJ (2014) The epithelial-mesenchymal interactions: insights into physiological and pathological aspects of oral tissues. Oncol Rev 8(1):239. https://doi.org/10.4081/oncol.2014.239
16. Ho M, Kim H (2011) Glypican-3: a new target for cancer immunotherapy. Eur J Cancer 47(3):333–338. https://doi.org/10.1016/j.ejca.2010.10.024
17. Chaturvedi TP, Gupta K, Agrawal R, Kumar PN, Gupta J (2022) Immunohistochemical expression of Ki-67 and Glypican-3 to distinguish aggressive from nonaggressive benign odontogenic tumors. J Cancer Res Ther 18(Suppl 2):S205–S209
18. Ng A, Wong M, Viviano B, Erlich JM, Alba G, Pflederer C, Jay PY, Saunders S (2009) Loss of glypican-3 function causes growth factor-dependent defects in cardiac and coronary vascular development. Dev Biol 335(1):208–215. https://doi.org/10.1016/j.ydbio.2009.08.029
19. Matsuo I, Kimura-Yoshida C (2013) Extracellular modulation of Fibroblast Growth Factor signaling through heparan sulfate proteoglycans in mammalian development. Curr Opin Genet Dev 23(4):399–407. https://doi.org/10.1016/j.gde.2013.02.004
20. Shimizu Y, Suzuki T, Yoshikawa T, Endo I, Nakatsura T (2019) Next-generation cancer immunotherapy targeting glypican-3. Front Oncol 9:248. https://doi.org/10.3389/fonc.2019.00248

Publisher's Note