INTRODUCTION

Ameloblastomas are benign epithelial odontogenic tumors of the jaw bones that have the potential to grow into large size with bone deformity and are characterized by peculiar clinical behavior. They are classified as unicystic, multicystic and peripheral (extraosseous) types.

The unicystic ameloblastoma refers to the cystic lesion with ameloblastic epithelial lining accompanied by luminal, intraluminal or mural tumor...
proliferation\textsuperscript{3}. Multicystic/solid ameloblastoma is the most clinically significant odontogenic tumor which is often locally invasive\textsuperscript{4}. Microscopically, a variety of histopathologic types are shown with follicular and plexiform predominating\textsuperscript{4, 5}. The potential sources that take part in forming ameloblastoma include the stratified squamous epithelium of oral cavity, remnants of dental lamina, enamel organ of the developing tooth and the lining of odontogenic cysts particularly dentigerous cyst\textsuperscript{1}.

The participation of neural crest cells in the process of odontogenesis is recognized and the tooth is well known to be a derivative of the neural crest\textsuperscript{6}. During this process, the neural crest cells may impart few of their characteristics to oral epithelium via specific signal transductions\textsuperscript{7}. Therefore, the basal cell of oral epithelium may have the potential to proliferate and thus becoming a source for ameloblastoma\textsuperscript{8}. Moreover, it was demonstrated that the dental lamina has its origin from the neuroectoderm\textsuperscript{6} and ameloblastoma may originate from the dental lamina rests\textsuperscript{1}.

Neuroectodermal markers are numerous and include neuron-specific enolase (NSE), cluster of differentiation 99 (CD99), chromogranin, glial fibrillary acidic protein (GFAP) and synaptophysin\textsuperscript{9}.

Among these, synaptophysin is a 38 kDa membrane glycoprotein of presynaptic vesicles that in human is encoded by SYP gene\textsuperscript{10}. It presents in human neuroendocrine cells that is why it is used as a marker of neuroendocrine differentiation in tumors\textsuperscript{10, 11}.

Synaptophysin expressed in a variety of neoplasms of both neural and epithelial types. It was shown to be positive in ganglioneuroblastoma\textsuperscript{12}, medulloblastoma\textsuperscript{13} and neuroblastoma\textsuperscript{14}. In addition, synaptophysin immunostaining was detected in pancreatic neoplasms\textsuperscript{15}. Moreover, the occurrence of neuroendocrine differentiation in breast cancers was demonstrated by the expression of synaptophysin and other neuroendocrine markers\textsuperscript{16}.

In the literature, however, there are few reports regarding the expression of synaptophysin in ameloblastoma of the jaw. Therefore, this study was undertaken to evaluate the expression of synaptophysin in unicystic and multicystic ameloblastoma by immunohistochemistry and to verify the usefulness of synaptophysin as a neuroectodermal marker for ameloblastoma.

**MATERIALS AND METHODS**

**Selection of cases**

In the current study, 40 archival ameloblastoma cases were selected from Oral Pathology Department, Faculty of Dentistry, Tanta University after an acceptance from the head of department. These cases included unicystic and multicystic types (20 cases each). Unicystic ameloblastoma were histopathologically subdivided into luminal (8 cases) and mural type (12 cases). In multicystic ameloblastoma; follicular and plexiform subtypes were involved (11 and 9 cases respectively).

**Hematoxylin and eosin staining**

All samples were fixed in 10% formalin and routinely processed and embedded in paraffin. Serial sections cut at 4 μm were used for hematoxylin and eosin (H & E) staining.

**Immunohistochemical staining**

Other 4 μm-thick sections were cut and received on positive-charge slides for immunohistochemical staining. Tissue sections were deparaffinized in xylene and rehydrated in descending grades of ethanol. Blocking of endogenous peroxidase activity was done by methanol containing 0.3% H\textsubscript{2}O\textsubscript{2} for 30 minutes. Microwaving was performed for antigen retrieval using a citrate phosphate buffer (pH 6.0) and then the sections were incubated with the primary antibody at 4°C overnight. The immunohistochemical staining was carried out by monoclonal mouse anti-human synaptophysin (Dako) at dilution
of 1:100. After incubation with secondary antibody and for detection of the reaction, diaminobenzidine (DAB) was used. The sections were then counterstained by hematoxylin and dehydrated in ascending grades of ethanol. Finally, the slides were mounted and examined under light microscope. Negative control staining was performed by omitting primary antibody. Synaptophysin was considered positive if over than 10% of tumor cells showed strong or diffuse staining.

Statistical analysis

All data were collected, tabulated and statistically analyzed. A p value < 0.05 is required for assessing the significance.

RESULTS

In this study, more than 90% (37/40 cases) of ameloblastoma studied showed a positive reaction to synaptophysin (Table 1).

Synaptophysin immunoreactivity in unicystic ameloblastoma

Unicystic ameloblastoma was subclassified into two groups; luminal (8 cases) and mural (12 cases), (Table 1). In luminal variant, the tumor is confined to the luminal surface of the cyst. It consists of a fibrous cyst wall with a lining that contains ameloblastic epithelium and overlying loosely cohesive stellate reticulum-like cells (Fig. 1A). In mural ameloblastoma, the fibrous wall of the cyst

![Fig. (1) (A) and (B) Unicystic ameloblastoma; luminal type (A) exhibits a fibrous cyst wall with a lining consisting of ameloblast-like cells with reverse nuclear polarity and an overlying stellate reticulum-like cells. Mural ameloblastoma (B) showing a fibrous wall infiltrated by follicular ameloblastoma with a lining similar to luminal variant (H&E, (A) x40, (B) x20). (C) and (D) Synaptophysin immunostaining in luminal (C) and mural ameloblastoma (D). Both ameloblast and stellate reticulum-like cells in the lining and the ameloblastic follicle in connective tissue wall show positivity for synaptophysin. The expression is mostly membranous with sporadic cytoplasmic expression (IHC, (C) x40, (D) x20)
is infiltrated by ameloblastic epithelium in form of follicular or plexiform pattern (Fig. 1B).

Immunostaining in unicystic ameloblastoma was observed in both luminal epithelial lining and ameloblastic nests in the fibrous wall of the cyst (Fig. 1C & D). In luminal variant, synaptophysin was seen in 87.5% (7/8 cases). Its expression was predominantly membranous with sporadic cytoplasmic expression (Fig. 1C) and the expression was recognized in both ameloblast-like cells and the overlying stellate reticulum-like cells.

Mural type exhibited a positive reaction to synaptophysin in 91.6% (11/12 cases). Its expression was detected in both luminal epithelial lining and the infiltrating ameloblastic follicles in the fibrous wall of the cyst (Fig. 1D). The expression was mostly membranous with cytoplasmic expression in some cells of both lining and ameloblastic nests.

**Synaptophysin immunoreactivity in multicystic ameloblastoma**

Multicystic ameloblastoma was subdivided into: follicular (11 cases) and plexiform (9 cases). Follicular subtype showed nests which consisted of outer columnar ameloblast-like cells and inner stellate reticulum-like cells (Fig. 2A), whereas the plexiform type exhibited a proliferation of the tumor cells with a reticular pattern (Fig. 2B).

All cases of follicular ameloblastoma demonstrated a positive immunostaining to synaptophysin in 91.6% (11/12 cases). Its expression was detected in both luminal epithelial lining and the infiltrating ameloblastic follicles in the fibrous wall of the cyst (Fig. 1D). The expression was mostly membranous with cytoplasmic expression in some cells of both lining and ameloblastic nests.

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synaptophysin (11/11). The expression was seen in both outer ameloblast-like cells and the inner stellate reticulum-like cells in a membranous and cytoplasmic distribution (Fig. 2C). Immunoreactivity for synaptophysin was shown in 88.8% (8/9 cases) of plexiform ameloblastoma. The expression was detected in all tumor cells with prominent membranous pattern (Fig. 2D). The number of cases studied and the percentage of synaptophysin positive cases were shown in Table 1. The results were significant with p value < 0.05.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Immunoreactivity of synaptophysin in ameloblastoma</th>
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<tr>
<td>Ameloblastomas</td>
<td>Number of cases</td>
</tr>
<tr>
<td>Unicystic (luminal)</td>
<td>8</td>
</tr>
<tr>
<td>Unicystic (mural)</td>
<td>12</td>
</tr>
<tr>
<td>Multicystic (follicular)</td>
<td>11</td>
</tr>
<tr>
<td>Multicystic (plexiform)</td>
<td>9</td>
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DISCUSSION

Ameloblastoma is one of the benign odontogenic epithelial tumors that exhibits a locally aggressive behavior with the classic view about the neural crest origin of the cells giving this tumor. It is originated from dental lamina remnants, the enamel organ in development, epithelial lining of odontogenic cysts or from the cells of the basal layer of oral mucosa.

Synaptophysin is an integral membrane glycoprotein that occurs in presynaptic vesicles of neurons and expressed in several neoplasms of both neural and epithelial types. There were few reports regarding the expression of synaptophysin in ameloblastoma. This study aimed to evaluate the expression of synaptophysin in ameloblastoma both unicystic and multicystic types.

In this study, synaptophysin expression was detected in more than 90% of ameloblastoma cases including unicystic and multicystic variants. The expression was revealed in ameloblast and stellate reticulum-like cells in predominantly membranous pattern with sporadic cytoplasmic expression.

A previous study showed almost similar findings in unicystic ameloblastoma in which both luminal and infiltrating nests exhibited positivity for CD56 (a protein associated with nervous system development). Other reports demonstrated the expression of several neuroectodermal markers in ameloblastoma. In one study done on thirty two cases of ameloblastoma, immunoreactivity to CD99, synaptophysin, S100 and NSE was seen giving a link between neuroectoderm and ameloblastoma. Moreover, another study using NSE, synaptophysin and CD99 demonstrated a potential for basal cells of the oral ectoderm particularly that overlying the bone of the jaw to give rise to odontogenic cysts and neoplasms.

It has been suggested that ameloblastoma arise from remnants of dental lamina. Therefore, the results of this study and others may be explained by the thought that dental lamina originated from the neuroectoderm. In addition, the odontogenic epithelium of ameloblastoma may have the potential to express neuroectodermal signals after years from completing odontogenesis process. Accordingly, synaptophysin was shown to be positive in almost all cases of ameloblastoma in the current study.

On the other hand, it was noted that few cases of ameloblastoma in this study did not show immunoreactivity to synaptophysin. This finding may be attributed to the decalcification process and probably to the small size of the specimens as well. This feature showed that although synaptophysin has been detected in a high percentage of ameloblastoma both unicystic and multicystic types, this reaction can be avoided and a few cases of ameloblastoma may be negative for synaptophysin.
In conclusion, the expression of synaptophysin in ameloblastoma supports the relationship between tumorigenesis of this neoplasm and neural crest cells. Moreover, synaptophysin may be a valuable neuroectodermal marker of ameloblastoma. Additional studies with a wide sample range and more advanced methodology are recommended to support these findings.

REFERENCES

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