

Research Article

Comparison of P53 Marker Expression Between Dentigerous Cyst and Calcifying Odontogenic Cyst

Samira Mostafazadeh¹ , Babak Ebrahimi², Fariba Abdal^{3,*} 

¹Department of Oral and Maxillofacial Pathology, Urmia University of Medical Sciences, Urmia, Iran

²Faculty of Dentistry, Urmia University of Medical Sciences, Urmia, Iran

³Department of Oral and Maxillofacial Pathology, Ilam University of Medical Sciences, Ilam, Iran

Abstract

Background: Calcifying odontogenic cyst is a developmental cyst that often appears in jaw cysts, and dentigerous cyst is the most common developmental cyst of the jaw, which has the ability to turn into a neoplasm. p53 is increased in some odontogenic cysts and tumors. The aim of this study is to compare the expression of P53 marker in dentigerous cyst and calcifying odontogenic cyst by immunohistochemical method. **Methods:** In this descriptive-analytic cross-sectional study, the studied population is the sample of odontogenic dentigerous cysts and calcifying odontogenic cysts in the pathology center of urmia. First, all the files in the archive of the department from 2000 to 2021 were examined and the cases of cysts were examined separately and in terms of the expression of P53 marker in dentigerous cysts and calcifying odontogenic cysts by immunohistochemical method. **Results:** The results showed that the overall mean expression of p-53 marker in dentigerous cyst group was 33.45 with a standard deviation of 11.86%, among which the lowest and highest expression of the said marker was 12% and 54%, respectively. The overall average expression of p-53 marker in the calcifying odontogenic cyst group is 59.55 with a standard deviation of 13.73%, among which the lowest and highest expression of the said marker was 30% and 87%, respectively. There is a statistically significant difference between dentigerous cyst patients and odontogenic calcifying cyst patients in terms of p-53 marker expression, and the said marker is more expressed in patients in the odontogenic calcifying cyst group compared to dentigerous cyst patients ($p < 0.001$). **Conclusions:** It seems that this protein (p53) can probably be a suitable marker to confirm the ability to predict invasion in premalignant oral lesions such as cysts in odontogenic calcification patients.

Keywords

P53 Marker, Denttiigerous Cyst, Calcifying Odontogenic Cyst, Immunohistochemical

1. Introduction

Calcifying odontogenic cyst is a developmental cyst that appears in approximately one percent of jaw cysts [1]. This cyst was first described by Rywkind in 1932 [2]. The cal-

cifying odontogenic cyst has significant variation in clinical features, histopathology and also biological behavior, and several classifications have been proposed for this lesion [1,

*Corresponding author: dr.faribaabdal@yahoo.com (Fariba Abdal)

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3, 4]. In most cases, a cyst appears to be benign, while in some cases, it does not have any characteristics of a cyst, which may be tumoral [5]. In 2005, the World Health Organization described cystic calcifying odontogenic cyst type as a low-grade malignancy and changed its name to calcifying cystic odontogenic tumor and introduced its solid type as dentinogenic gum cell tumor, which is a very aggressive nature. has [6, 7].

Dentigerous cyst is the most common facial developmental cyst [8], which occurs in most cases in the second and third decade of life and is mostly related to the third molar of the mandible and the canine of the maxilla [9, 10]. This cyst is clinically asymptomatic, but it has the potential to become massively enlarged and cause cortical expansion and erosion [11, 12]. The dentigerous cyst is reduced through the hydrostatic pressure caused by the accumulation of fluid between the enamel epithelium, and an unerupted tooth crown is created [13, 14]. This cyst sometimes turns into squamous cell carcinoma, mucoepidermoid carcinoma or ameloblastoma [15].

P53 protein is the product of a tumor suppressor gene called TP53 [5], which is expressed in the G1 phase of the cell cycle to allow repair of possible DNA damage [16]. If the DNA is subject to irreversible damage, the cell will proceed to apoptosis and as a result, the division of the damaged cell will be prevented [4]. The half-life of P53 is short and about 20 minutes [5]. Under normal conditions, this protein is continuously produced and in the nucleus of the cell, it is connected to MDM2 protein (mouse double minute 2) until this complex is transported to the cytoplasm, where it is degraded by proteosomes. This process keeps the cellular concentration of P53 low [16]. Therefore, increasing the concentration of this protein depends on inhibiting its degradation, under stress, certain types of protein are released from the nucleus to the nucleoplasm and prevent the exit of P53 to the cytoplasm and Subsequently, it prevents its decomposition and this protein accumulates in the nucleus [17]. Considering that the production amount of P53 is low and its half-life is short, it is possible to detect it in conditions where the protein is expressed in high amounts or accumulated inside mutated cells [18, 19].

Immunohistochemical studies regarding the expression of P53 in odontogenic cysts have obtained conflicting results [16]. A number of studies have confirmed the presence of this protein [20, 21] and some others have rejected it [22]. Therefore, this study will be conducted in order to find a relationship between cystic changes and P53 expression, taking into account the difference in the clinical behavior of these two cysts, which are common developmental cysts, until the expression level of this protein in the said cysts is determined and with Compare each other.

2. Material and Method

The sample size is based on the study of Setara Shojaei et al.

[23] and considering the statistical power of 80%, the second type error of 20%, and the confidence level of 85%. According to the mentioned study, the parameters including the mean and standard deviation of P53 expression in calcifying odontogenic cyst groups were 36.03 ± 17.28 and the same mean in dentigerous cyst was 25.51 ± 11.76 . The sample size for each group was estimated to be 11 samples, and due to conducting the study in the form of separated groups and considering the drop in the number of samples, in order to increase the power of the study and detect differences, we took the sample size of 20 samples in each group from the beginning.

The present study was carried out using an analytical method. The studied population is the samples of odontogenic cysts, dentigerous cells and calcifying odontogenic cysts in the pathology department of the specialized pathology laboratory. First, all the files in the archive of the department from 2012 to 2022 were examined and the cases of cysts were separated. The H&E slides of these cases were studied and the histopathological diagnosis will be confirmed based on the existing criteria. All cases of non-inflammatory cysts were selected.

Considering things such as the presence of sufficient tissue in the paraffin block, the thickness and sufficient amount of epithelium in the sample stained with H&E, the presence of minimal bleeding, and the presence of information related to background variables (age, sex, and location of the lesion), Selected cases were prepared for immunohistochemical staining.

From the paraffin blocks of each sample, two sections with a thickness of 5 microns were prepared and placed on a glass slide prepared with polylysine. The standard method of STREPTAVIDIN-IMMUNOPEROXIDASE BIOTIN was used for staining.

First, the prepared sections will be deparaffinized by xylene and dehydrated using diluted alcohol solutions. Endogenous peroxidase activity will be inhibited by incubating the samples with 2% peroxydehydrogen in methanol for thirty minutes. For antigen retrieval, the samples were microwaved for 15 minutes in 0.01 M citrate buffer and pH 6, at a high degree.

After that, washing was done for three consecutive times for 5 minutes each time with tris buffer sulfate. To avoid non-specific reactions, the sections were incubated with 10% serum for 10 minutes. Then the reaction with p53 antibody (prediluted, Copenhagen, Dako, Denmark) was performed for one hour at room temperature.

In the next step, the samples will be washed with PBS in three times of 5 minutes. The reaction with the secondary antibody will be done at room temperature for 30 minutes and washing with PBS was done again. After that, the samples were incubated with DAB for 5-10 minutes and stained with Mayer's hematoxylin as a counterstain.

The positive control for p53 marker was a positive sample of squamous cell carcinoma of the tongue and the negative

control was by substituting non-reactive mouse serum instead of the primary antibody. Sections stained with p53 protein were observed under a light microscope and brown colored cells were considered as positive. Cell counting was done with x400 magnification and the ratio of stained cells to total cells was measured. Based on this, the amount of stained cells is 0-5% as negative, 25-5% as +, 25-50% was considered as ++, more than 50% as +++.

The data was coded in spss software version 20 and analyzed at a significant level of 0.05 using standard statistical tests. Quantitative demographic data with average and standard deviation and qualitative demographic data with frequency and percentage in the form of tables and in cases where necessary; It was presented as a diagram for better understanding. The normality of quantitative data was evaluated using the Kolmogro-Smirnoff test, and according to the distribution of normality and non-normality of quantitative variables, standard parametric and non-parametric tests were selected and applied. To compare the average occurrence of the gene under investigation in the groups, if the distribution was normal, the t-test was used, and otherwise, the non-parametric Mann-Whitney test was used. In comparison

of these averages according to age groups, if normal, analysis of variance test was used, otherwise Kruskal-Wallis test was used. Chi-score test is used to compare the frequency of clinical information according to gender, and to evaluate the relationship between quantitative variables in the groups under investigation, Pearson's correlation coefficient was used if it was normal, and Spearman's correlation coefficient was used otherwise.

3. Results

In the present study, the overall average expression of p-53 marker in the calcifying odontogenic cyst group was 59.55 with a standard deviation of 13.73%, among which the lowest and highest expression of the said marker was 30% and 87%, respectively. the overall average expression of p-53 marker in the dentigerous cyst group was 33.45 with a standard deviation of 11.86%, among which the lowest and highest expression of the said marker was 12 and 54%, respectively.

Other data are collected in [Tables 1 to 6](#).

Table 1. Distribution of frequency and percentage of gender between the two groups.

Cyst type	men		women		total number (percent)
	number	percent	number	percent	
Dentigerous cyst	8	40	12	60	20 (100)
calcifying odontogenic cyst	10	50	10	50	20 (100)

Dentigerous cyst and calcifying group Odontogenic cysts have been somewhat uniform in terms of gender distribution.

Table 2. Overall average expression percentage of p-53 marker in people with odontogenic cysts under investigation In the present study.

	overall average	Standard deviation	lowest	highest
The percentage of p53 marker expression	46.50	18.30	12	87

The overall average expression of p-53 marker in people with odontogenic cysts under investigation is 46.50 with a standard deviation of 18.30%, among which the lowest and highest expression of the said marker was 12% and 87%, respectively.

Table 3. Overall mean percentage of p-53 marker expression in the group.

	overall average	Standard deviation	lowest	highest
The percentage of p53 marker expression	33.45	11.86	12	54

The overall average expression of p-53 marker in the dentigerous cyst group was 33.45 with a standard deviation of 11.86%, among which the lowest and highest expression of the said marker was 12 and 54%, respectively.

Table 4. The overall average expression percentage of p-53 marker in the calcifying odontogenic cyst group.

	overall average	Standard deviation	lowest	highest
The percentage of p53 marker expression	59.55	13.73	30	87

The overall mean expression of p-53 marker in the calcifying odontogenic cyst group was 59.55 with a standard deviation of 13.73%, among which the lowest and highest expression of the said marker was 30% and 87%, respectively.

Table 5. Comparison of p-53 marker expression percentage by gender in the present study.

	men		women		P-value
	overall average	Standard deviation	overall average	Standard deviation	
The percentage of p53 marker expression	47.22	18.61	45.91	18.47	0.001<

The data in the above table compares the mean expression percentage of p-53 marker by gender in the present study by using the t-test, and its data shows that there is a statistical

difference between the mean expression percentage of p-53 marker in men and women. There is significance and the said marker is more expressed in men than in women ($p < 0.001$).

Table 6. Comparison of the expression percentage of p-53 marker according to the groups under investigation in the present study.

	dentigerous cyst		calcifying odontogenic cyst		P-value
	overall average	Standard deviation	overall average	Standard deviation	
The percentage of p53 marker expression	33.45	11.86	59.55	13.73	0.001<

Explanation: The data in the above table are compared using the t-test to compare the average expression percentage p-53 marker in two groups under investigation in this study and its data shows that there is a statistically significant difference between dentigerous cyst patients and odontogenic calcifying cyst patients in terms of p-53 marker expression

and the said marker in Patients in the calcifying odontogenic cyst group are more expressed compared to patients in the dentigerous cyst group ($p < 0.001$). (Tables 1-5)

Microscopic views of H&E and IHC staining (DC&COC) are presented in Figures 1 and 2.

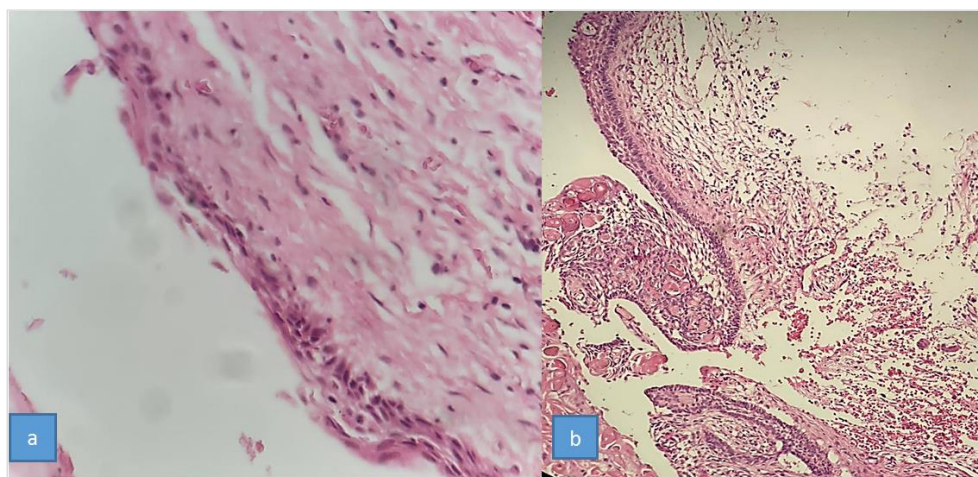


Figure 1. Hematoxylin eosin (H&E) in the groups: a (dentigerous cyst), b (calcifying odontogenic cyst) $\times 10$ magnification.

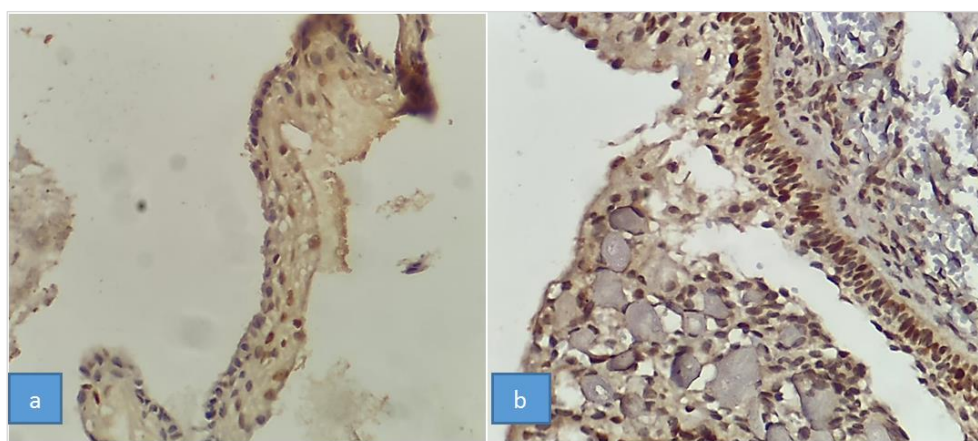


Figure 2. P53 Immunohistochemistry staining (IHC) in the groups: a (dentigerous cyst), b (calcifying odontogenic cyst) $\times 10$ magnification.

4. Discussion

Odontogenic cysts and tumors are an important part of jaw and oral lesions that arise from remnants of odontogenic epithelium in different stages of odontogenesis [24], in the present study, 20 people have dentigerous cysts and 20 people have calcifying odontogenic cysts. The expression of p-53 marker has been investigated and compared, and the results of the present study in terms of the number of samples compared to other comparable studies such as Shojaei et al.'s study [23] and Khalili et al.'s study [25] The sample is higher, so it has the required validity. These cases are considered a strength in the generalizability of the results of this study.

In the present study, 45% of men and 22% of women in both dentigerous cyst and calcifying odontogenic cyst groups have been examined and compared in terms of p-53 marker expression. This means that both dentigerous cyst and calcifying odontogenic cyst groups were the same in terms of gender distribution (Table 1). Therefore, the gender variable as a potential confounding variable cannot bias the results.

This case also indicates the validity of the results of the present study, away from potential confounding variables that may underreport or overreport the results.

The findings showed that in the present study, the overall average expression of the p-53 marker in the subjects with odontogenic cysts under investigation was 46.50 with a standard deviation of 18.30%, among which the lowest and highest expression of the said marker was 12% and 87%, respectively (Table 2). Also, the findings indicated that in the current study, the overall average expression of the p-53 marker in the dentigerous cyst group was 33.45 with a standard deviation of 11.86%, among which the lowest and highest expression of the said marker was 12% and 54%, respectively (Table 3). In the present study, the overall mean expression of p-53 marker in the calcifying odontogenic cyst group was 59.55 with a standard deviation of 13.73%, among which the lowest and highest expression of the said marker was 30% and 87%, respectively (Table 4).

In previous studies such as the study of Gurgel et al. [26], Gaballah et al. [16] and several other studies [6, 7] in this regard, the expression of p53 marker was reported between 41

and 91 percent. Perhaps one of the reasons for this variation in the occurrence of the mentioned marker is the number of sample sizes, different study design types, and differences in measurement tools. One of the other reasons for justifying these conflicting results in the complex biology of P53 is access to antibody, the effect of tissue preparation and the protocol used for immunohistochemistry, such as fixation methods, antigen retrieval method from paraffin blocks, and the type of antibody. Both the stated reasons are effective in the accuracy of the results and the variation in the percentage of occurrence of p53 marker in different cysts.

The findings showed that there is a statistically significant difference between the mean expression percentage of p-53 marker in women and men, and the said marker is expressed more in men than in women (Table 5). This finding was previously reported in the study of Abou-Bakr et al. [27] as well as the study of Zydrón et al. [28].

The findings also showed that there is a statistically significant difference between dentigerous cyst patients and calcifying odontogenic cyst patients in terms of p-53 marker expression, and the said marker is more in patients in the calcifying odontogenic cyst group compared to patients in the dentigerous cyst group. It is stated (Table 6). This finding was consistent with the study by Shujaei et al. [23]. These results indicate that the occurrence of p53 marker in these two groups can indicate the biological behavior of these two cysts. One of the other reasons for the occurrence of this difference in the two groups and the lower incidence of p53 marker in the dentition in the dentigerous cyst compared to the calcifying odontogenic cyst may be the fact that, on the other hand, the main trigger for the formation of dentigerous cysts is not clearly known; But often an inflammatory infiltration is observed in the capsule of the cyst, which causes epithelial cells to multiply early. This problem (response to inflammatory stimulation) may cause the growth process of the cyst, but the proliferation of cells may be non-continuous and only in short periods of time, which probably causes a decrease in the expression of P53 in dentigerous cysts. Therefore, the statement Less p53 in dentigerous cysts could be due to cellular stress produced by inflammatory stimuli, although this cyst.

It has an evolutionary origin [29] Similar to the present study, in the study of Piattelli et al. [22] and Gadball et al. [30], a lower incidence of p53 marker was reported in dentigerous cyst compared to odontogenic calcifying cyst. P53 gene mutation can be associated with increased cell proliferation [26]. The high expression of this protein in the epithelial lining of odontogenic calcifying cyst may mean more proliferative activity and as a result, more aggressive biological behavior of this lesion compared to dentigerous cyst [31].

5. Conclusion

The findings indicate that there is a statistically significant difference between dentigerous cyst patients and odontogenic calcifying cyst patients in terms of p-53 marker expression

and the said marker in patients in the odontogenic calcifying cyst group compared to the dentigerous cyst group patients It is further stated; So this protein can probably be a suitable marker to confirm the ability to predict invasion in oral premalignant lesions such as cysts occurring in odontogenic calcification patients.

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Author Contributions

Samira Mostafazadeh: Conceptualization, Project administration, Supervision, Writing – original draft

Babak Ebrahimi: Data curation, Formal Analysis, Writing – review & editing

Fariba Abdal: Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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