

Immunohistochemical Pattern of Pleomorphic Adenoma, Polymorphous Low Grade Adenocarcinoma and Adenoid Cystic Carcinoma in Minor Salivary Glands

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Abstract

Objective: To study the immunohistochemical pattern of CD 117, glial fibrillary acidic protein (GFAP), smooth muscle actin (SMA) and CD 43 in pleomorphic adenoma (PA), adenoid cystic carcinoma (AdCC) and polymorphous low grade adenocarcinoma (PLGA) of minor salivary glands.

Materials and Methods: Twenty cases of PA, 20 cases of AdCC and 10 cases of PLGA were retrieved from record files along with their paraffin blocks at Armed Forces Institute of Pathology, Pakistan. New histological diagnosis was made on freshly prepared H&E sections followed by application and analysis of immunostains.

Results: The mean age of the patients was 44 ± 15 (mean SD) (range; 17-86) years. There were 26 male and 24 female patients with a male to female ratio of 1.08:1. Fourteen cases of PA, 14 cases of AdCC and 6 cases of PLGA were positive for CD117. In case of GFAP, only 9 cases of AdCC and 3 cases of PLGA were positive; however, 16 cases of PA were also positive. Twelve cases of AdCC and 7 cases of PA were positive for SMA and half of the PLGA cases were also reactive. Nonetheless, the least expression was seen in case of CD 43, where only five cases of AdCC were positive. Six cases of PA and three cases of PLGA were also positive.

Conclusion: Our results suggest that the use of GFAP, SMA, CD 117 and CD 43 as an adjunct to histological examination is not helpful in differentiating PA, AdCC and PLGA from one another.

Key Words: Salivary Gland Neoplasm; Adenoma, Pleomorphic; CD 117 Antigen; Smooth Muscle Actin; Glial Fibrillary Acidic Protein; CD 43 Antigen, Immunohistochemistry

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2014; Vol. 11, No.1)

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Received: 2 July 2013

Accepted: 27 October 2013

INTRODUCTION

Salivary gland tumors make up 3-4% of all head and neck tumors [1]. Moreover, there is an overall increase in the incidence of salivary gland tumors worldwide, irrespective of the

etiologic factor [2-5]. Neoplasms of the minor salivary glands in contrast to the major salivary glands are rare, representing 10-15% of all salivary gland tumors [6]. Common malignant tumors of minor salivary glands include

adenoid cystic carcinoma, mucoepidermoid carcinoma and polymorphous low-grade adenocarcinoma, while pleomorphic adenoma is the most common benign tumor [1, 4, 7-9]. Parotid is the most common site of major salivary gland tumors, and the palate is the commonest site for minor salivary glands [1, 3, 5, 7]. Salivary gland tumors can express a wide array of morphological variety between different tumors and at times within a single tumor. Moreover, hybrid tumors, anaplasia and tendency for few benign tumors to transform into malignancy can lead to histopathological misinterpretation [10]. With passing time, Immunohistochemistry (IHC) has become an essential aid for pathologists and serves as an important adjunct for diagnosis [11-13]. The role of IHC is enhanced when the diagnosis of minor salivary gland tumors becomes problematic on routine H&E stain as a result of loss of characteristic histopathological features in an incisionally biopsied or fragmented biopsy sample. Multiple studies have been performed to investigate the role of immunomarkers in salivary gland tumors. A large number of studies have evaluated the role of CD 117 (c-kit) and smooth muscle actin (SMA) in differentiating AdCC from PLGA [13-17], while glial fibrillary acidic protein (GFAP) seems promising in differentiating between PA and PLGA [12, 18-20]. In salivary gland pathology, lot many antibodies have been applied to analyse different tumor cell types in order to establish differences among tumor types and highly variable results have been reported. In this study, we analysed the immunohistochemical pattern of pleomorphic adenoma, adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma that bear histological similarity on routine H&E stain. In such cases, it is critical to make a definite diagnosis as each of these tumors has an entirely different treatment protocol. We applied a panel of four immunomarkers including CD117, CD43, SMA and GFAP; these antibodies are the most commonly used

in worldwide literature; however, conflicting results were reported, this compelled us to explore their useful potential in the diagnostic efficacy of the mentioned tumors.

Moreover, not a single study has ever been carried out in our setup that has looked for a pattern of immunomarkers in SGT. Thus, there is a need for lot more research in the respective subject to reach a definite diagnosis and in turn to deliver a right therapy protocol to the patient.

MATERIALS AND METHODS

The study design is retrospective descriptive in which a total of 50 cases were retrieved from record files along with their paraffin embedded sections. These included 20 cases of PA, 20 cases of AdCC and 10 cases of PLGA. The data on gender, age and the site of involvement were extracted from the clinical histories in each case. Histological features of all the selected cases were reviewed from freshly prepared H&E sections and the new diagnosis regardless of the previous diagnosis was made by one consultant histopathologist. In cases that there was difference with the original diagnosis, the case was also reviewed by a second histopathologist to confirm the revised diagnosis. For each tumor type, 4 μ m thick tissue sections with microtome were taken and placed on charged slides (vectabond). Immunohistochemical staining was performed by mouse monoclonal antibodies manufactured by Thermo Fisher Scientific company and the streptavidin biotin technique was applied. The clone designation of immunomarkers was as follows:

- CD 117 (T595)
- GFAP (Astro5+Astro6)
- SMA (α sm-1)
- CD 43 (84-3C1)

For SMA, CD 43 and GFAP, microwave heat-induced epitope retrieval was performed using citrate buffer (pH 6.0). For CD 117, pressure cooker induced epitope retrieval was performed as advised by the manufacturer. All

incubations were done in the humidity chamber. Positive controls for every marker were also stained as advised by the manufacturer.

1. Skin for CD 117
2. Brain for GFAP
3. Bowel wall for SMA
4. Tonsil for CD 43

The immunoreactivity was considered positive if greater than 10% of the tumor cells were stained. Positivity was graded according to the percentage of tumor cells stained as weak (10-25%), moderate (25-50%) and strong (50-100%). This criterion is being used by Andreadis et al. and Woo et al.; and was taken as reference for the present study [11, 21].

RESULTS

The mean age of the patients was 44 ± 15 (mean SD) (range, 17-86) years. There were 26 male and 24 female patients with a male to female of 1.08:1. The most common site of tumor presentation was the palate making up 58% of the cases followed by the maxillary ridge (14%) and the tongue (6%). The detailed results of positive or negative expression of immunomarkers in the three tumor types are summarized in Table 1.

Photomicrographs of all immunostains in the three tumor types are shown in Figure 1-3.

DISCUSSION

Various studies have been conducted worldwide to analyze the pattern of different immunomarkers in salivary gland tumors that pose diagnostic difficulty because of histologic similarities. In the present study, regarding the expression of CD 117, in most of the positive cases, of either the PA or tubular type of AdCC, the inner luminal cells were diffusely reactive with CD 117 and only in a few cases, diffuse staining was observed with no discrimination between the luminal and abluminal cells. The diffuse staining pattern was more pronounced in cases of AdCC with solid or cribriform histological subtypes.

However, no such pattern was appreciated by PLGA, probably because of its diverse histological morphology.

As per results, we can make out that CD 117 expression was almost the same in AdCC and PA, thus practically of no use in distinguishing the two tumors based on the percentage of positive cases.

Table 1. Expression of Immunomarkers in the Three Tumor Types

Immunoreactivity and Staining Intensity				
Tumors	Negative (0-10% cells)	Weak (10-25% cells)	Moderate (25-50% cells)	Strong (>50% cells)
Ckit Expression				
PA	6	-	1	13
AdCC	6	-	1	13
PLGA	4	1	2	3
GFAP Expression				
PA	4	-	3	13
AdCC	11	4	2	3
PLGA	7	1	-	2
SMA Expression				
PA	13	2	4	1
AdCC	8	2	5	5
PLGA	5	1	2	2
CD 43 Expression				
PA	14	1	-	5
AdCC	15	-	4	1
PLGA	7	-	2	1

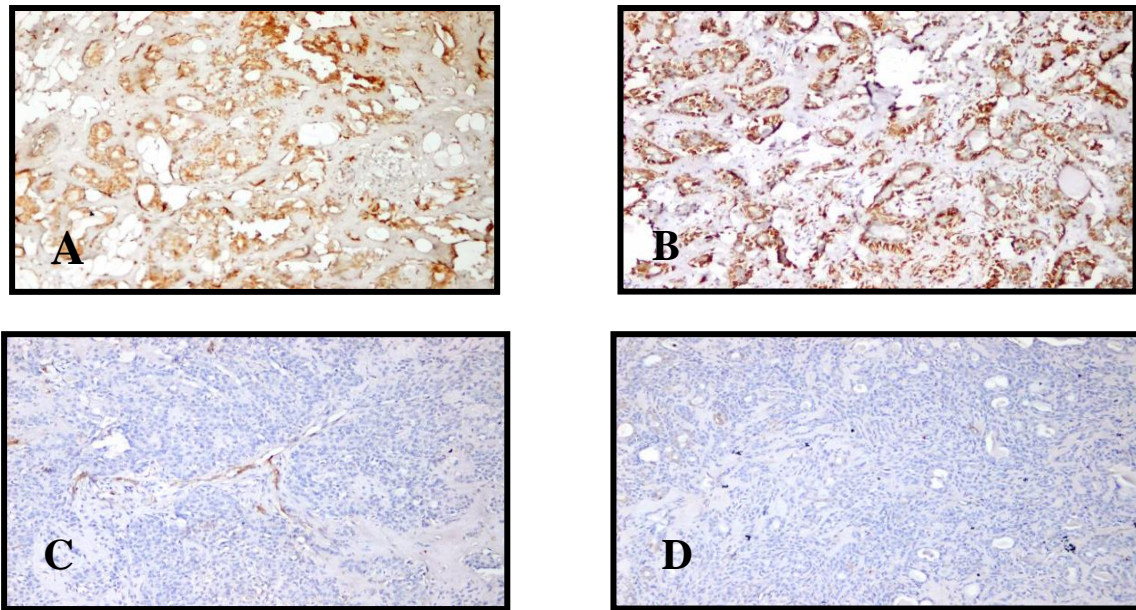


Fig 1. Pattern of immunomarkers in PA A) Moderately positive expression of CD 117 B) Strong positive expression of GFAP C) Negative expression of SMA with positive internal control of blood vessels D) Negative expression of CD43

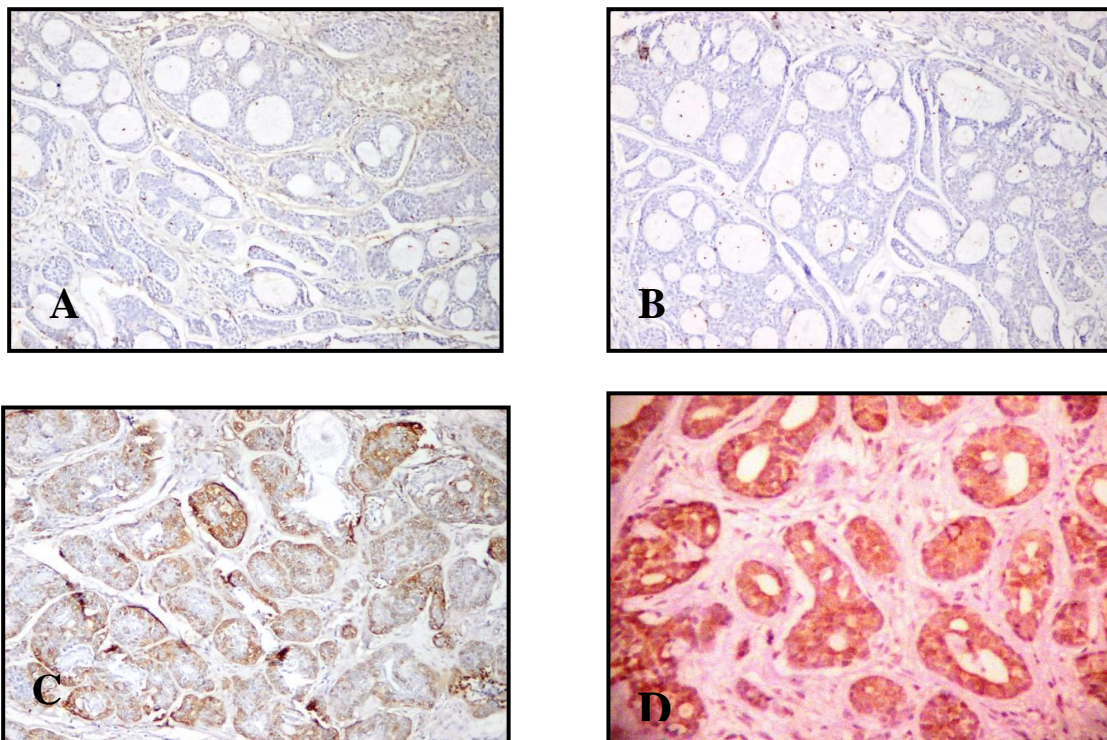


Fig 2. Pattern of immunomarkers in AdCC A) Negative expression of GFAP B) Negative expression of CD 43 C) Positive expression of SMA in myoepithelial cells D) Positive expression of CD 117 in both epithelial and myoepithelial cells

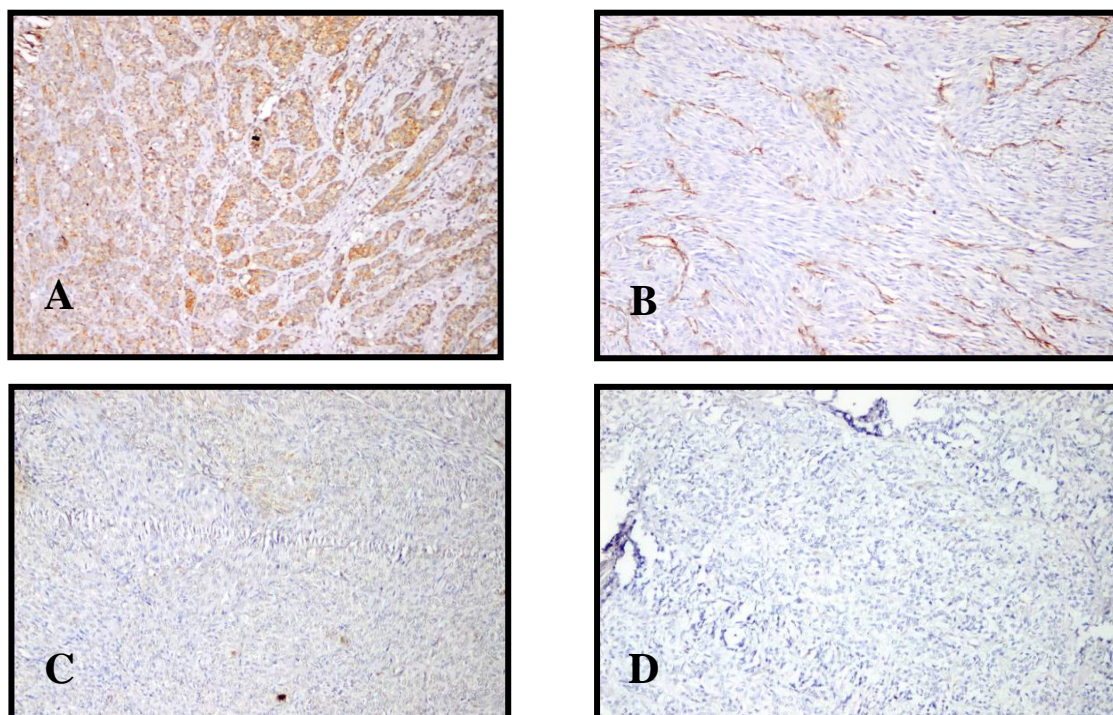


Fig3. Pattern of immunomarkers in PLGA A) Strong positive expression of CD117 B) Negative SMA expression in tumour cells with strong positive internal control of blood vessels C) Negative expression of CD 43 D) Negative expression of GFAP

This result of ours is analogous to studies conducted in Greece and America. In year 2006, a study carried out by Andreadis et al. in Greece showed that all of the PAs (n=20) were moderately positive where 25-50% of the tumor cells showed reactivity with c-kit. Similarly, in case of AdCC, only 3 cases were negative while 11 out of the total 14 cases showed more than 50% of the neoplastic cells were immunoreactive [21]. Similarly, an American study led by Chandan et al. in 2004, focused on the immunolocalization of CD 117 in cell block preparations of AdCC and PA. Their findings revealed c-kit expression in 100% (15/15) of pleomorphic adenoma and adenoid cystic carcinoma (10/10) specimens. However, the percentage of positive cells was variable as 60% of PA's and 80% of AdCC being scored as 3+ (> 50% cells positive), respectively. The rest of the cases had less than 50% tumor cell positivity [22].

Thus, in accordance with these studies; we can conclude that CD 117 is not a useful marker in the differential diagnosis of AdCC and PA. Moreover, regarding the expression of CD 117 in differentiating AdCC from PLGA, again varying results are reported. In a study conducted by Andreadis et al., seven out of 14 cases (50%) of PLGA were entirely negative for the marker and in the rest of the positive cases; none showed more than 50% positivity [21]. In 2002, in a similar study performed by Penner et al., 100% of AdCC (9/9) were positive in which most of the cases (7/9) had more than 50% cells positive with CD 117. However, 57% of PLGA (8/14) were reactive for the particular immunomarker, but none of the cases showed more than 50% positivity [14]. Likewise, in year 2007 in a study carried out by Epivatianos et al., 83% of AdCC (10/12) and 41% of PLGA (5/12) were positive. Here again, the major difference lies in the number

of positive tumor cells, in all positive cases of AdCC more than 50% and in all positive PLGA cases less than 50% of the tumor cells were positive [23].

Thus, while considering the reaction of CD 117, the number of positive cases of PLGA is less than that of AdCC in most of the studies mentioned above, but a strong inference cannot be made from their results as none of the studies showed a significant p value.

However, Mino et al. established an opposing result of CD 117 expression in 2003 by differentiating AdCC from other salivary gland tumors. Their results showed 62/64 (94%) of AdCC and only 19% (3/16) of pleomorphic adenoma were positive ($p=0.005$). While 25% (2/8) of PLGA were positive, but calculating its significance value with the results of AdCC revealed a p value = 0.1, this may be attributed to a very small number of PLGA cases [15].

Unlike the CD 117 staining pattern, the opposite was seen in case of GFAP, as the majority cases of PA showed diffuse staining of both luminal and abluminal cells with minority cases showing positive staining of abluminal cells only. This shows affinity of this marker for the myoepithelial differentiation and again scanty positivity with no definite pattern was observed in case of PLGA also, this may explain lack of myoepithelial differentiation in histogenesis of PLGA.

Although the results of GFAP expression of our study seem to differentiate these tumors by looking at the number of positive cases, as it was expressed in most of PA cases (80%) in contrast to 45% AdCC and 30% PLGA. These results were statistically not significant enough to make such a conclusion.

However, an American study conducted by Curran et al. in 2007 concluded very convincing results in differentiating PA from PLGA, with GFAP showing intra lesional positivity in all cases of PA ($n=21$) while entirely negative in all cases of PLGA ($n=30$), it was proved by a highly significant p value of < 0.001 [20]. Likewise, in another study carried out by Dei-

himi et al., who looked for the GFAP staining in PA and mucoepidermoid carcinoma, their results of GFAP reactivity showed that the abluminal cells were diffusely positive for the marker in all cases of PA ($n=25$) and secondly it highlighted the chondromyxoid areas of PA [24]. This finding is similar to our study, as in half of the cases of PA there were areas of chondromyxoid change and all these areas were diffusely and strongly highlighted by GFAP. Similar findings were established in 1990 by Anderson et al., who also confirmed GFAP positivity in PA, whereas cases of PLGA were uniformly non-reactive [12].

The role of GFAP in distinguishing PA from AdCC is not well documented. An American study performed in year 2007 by Shah et al., detected GFAP immunolocalization in cell block preparations of PA and AdCC. Their results showed 100% cases of PA ($n=10$) were positive for the marker and all cases of AdCC ($n=8$) were negative for GFAP. Tissue follow-up confirmed the diagnosis of PA and AdCC in all cases. Subsequent tissue follow-up in these cases revealed 4 cases of AdCC negative and 4 cases of PA positive for GFAP. So if we calculate the significance value by summing up the results of tissue sections of all the cases, it turns to be very significant ($p=0.0027$) [25].

The role of neoplastic myoepithelium in the histogenesis of various salivary gland tumors has been explored worldwide; however, controversy still exists. The role of SMA in differentiating AdCC from PLGA has been reported in the medical literature. An American study conducted by Prasad et al. established the myoepithelial differentiation in all cases of AdCC ($n=13$) included in their study, in contrast to which none of the PLGA ($n=26$) showed positive reactivity to SMA. Their results showed a very significant statistical p value ($p<0.0001$). The staining in case of AdCC was more pronounced in the outer layer of the tubular structures of tubular subtype as compared to cribriform and solid subtypes

[26]. These findings are parallel to the present findings in AdCC, in which we found the same pattern in positive cases; however, the percentage of positive cases is lower as compared to the other studies. Any good reason for this reduced reactivity cannot be explained on behalf of the different technique used for immunohistochemistry, as in all cases the positive internal control of blood vessels is present. Recently, another American study reported in 2008 by Prasad et al. configured the same conclusion as diffuse expression of SMA in 20 AdCC versus one PLGA ($P < 0.0001$) was noted [27]. Another study with nearly similar results was published in 2007, by Epivatianos et al. [23], where 100% of the cases of AdCC (12/12) were positive and only 25% of the cases of PLGA (3/12) were positive for SMA, although not very significant statistically ($p = 0.9$). Overview of all the studies mentioned above including the present study conclude that SMA expression was much lower in case of PLGA as compared to AdCC, this may represent the decreased role of myoepithelial cells in the histogenesis of PLGA.

On the other hand, in case of pleomorphic adenoma, myoepithelial cells make a good amount of tumor cells, which in turn impart positive reaction to myoepithelial markers including SMA. A very convincing study carried out in 1997 by Saveria et al. [28] showed that 94% of the cases were positive for SMA. But the results of our study are contrary to this study, as eight out of 20 cases were entirely negative, ten had less than 50% of the tumor cells positive and only two cases had more than 50% tumor cell positivity (all the cases had positive internal control). However, Dehimi et al. also concluded somewhat similar results to the present study, in which 12 out of 25 were negative, 12 showed weak staining, while only one case demonstrated 25-50% of tumor cell positivity. This negative or weak staining in case of PA may be attributed to the fact that neoplastic myoepithelial cells lose some of the features of myoepithelial cells

such as myofilaments and hemidesmosomes, so it is possible that incomplete expression of smooth muscle actin in PA is related to the stage and level of myoepithelial differentiation [24]. The same justification may also prove reduced SMA expression in cases of PA included in our study.

CD 43 expression can be seen in a number of neoplasms, primarily of hematopoietic origin. Evidence also suggests a role for CD 43 in epithelial neoplasms, such as colon adenocarcinoma and small cell lung carcinoma. Its role in salivary gland tumor pathology is very scanty and has only been analysed in America by Woo et al. and Seethala et al. in two separate studies [11,29]. In 2008, Seethala et al. described CD 43 expression by immunohistochemistry and mRNA in situ hybridization in adenoid cystic carcinomas. Their results showed 36%, that is 9 out of 20 AdCCs positive for the immunomarker [29]. However, more convincing results were shown in a study carried out in 2006 by Woo et al., in which CD 43 was used as an ancillary marker to distinguish between AdCC and PLGA. Immunoreactivity was detected in 100% of AdCCs ($n=12$), ranging from weak to strong staining, while only one case of PLGA was positive (7%) ($p=0.006$). This expression of CD 43 in AdCC may be explained by the fact that it is a recognized ligand of intercellular adhesion molecule-1 (ICAM-1) and the interaction between the two led to the unique capacity of AdCC to undergo distant metastasis and thus positive immuno expression [11].

Variable results are seen in my study, all the three tumor types showed very little reactivity with CD43, as 15 out of 20 AdCC, nine out of 20 PA and three out of 10 PLGA were entirely negative for this marker; therefore, it does not support its role in the differential diagnosis of such histologically mimicking tumors.

CONCLUSION

In conclusion, the expression of the immunomarkers was not restricted to any specif-

ic tumor type and the histopathological features on routine H&E are the gold standard for diagnosing tumors with somewhat equivocal histological features. Moreover, differences of results worldwide may be attributed to the technique sensitivity of IHC leading to false positive and false negative results

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