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Structural and Immunohistochemical Exploration of the Juxtaoral Organ of Chievitz

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ABSTRACT

Background: The juxtaoral organ of Chievitz (JOOC), also known as the buccotemporal organ, is an enigmatic bilateral structure located in the bucca of mammals. The morphological features of the JOOC were examined and described immunohistochemically, including its various neuroepithelial and stromal elements.

Methods: Five samples of JOOC obtained from elderly individuals were analysed, and all of them are well-preserved. To identify the types of epithelia in the JOOC and inform the markers that could correctly identify them, 36 antibodies were used on 36 samples of the JOOC to characterise the epithelial and neural elements. Chromic study and light microscopical investigations were made on the structural organisation.

Results: The JOOC presented a distinctive structure, characterised by neuroepithelial parenchyma surrounded by connective tissue and enclosed within a fibrous capsule. Strong immunohistochemical expression of high molecular weight cytokeratins, CD56, and PGP9.5 was demonstrated, indicating neural differentiation.

Conclusion: The JOOC is a benign neuroepithelial organ with specific immunohistochemical and histomorphological features. Given its limited tissue of origin and similar invasive patterns to other malignancies, accurately identifying its structure and markers is crucial to prevent misdiagnosis in clinical settings, particularly in distinguishing it from perineural invasion in carcinomas..

Keywords: *Neuroepithelial Juxtaoral Organ, Immunohistochemistry, Cytokeratins, Oral Pathology, Neural Differentiation, Clinical Diagnosis*

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1. INTRODUCTION

The JOOC was first described by Danish anatomist Hugo Wilhelm von Chievitz in 1875 in the buccal sulcus of human embryos [1,2]. It was first described as a transient structure that regresses prenatally in mammalian and reptilian embryos' early oral cavity development, and is found in the intraoral portion of the outer face between the buccinator and the temporal muscles at the level of the pterygomandibular raphe as a buccotemporal structure [3]. However, other studies have challenged this assumption, revealing that the JOOC continues into adulthood [4]. The JOOC is acknowledged as a permanent anatomical structure of significant importance for clinicians, surgeons, and pathologists, as its identification is crucial for accurate

diagnosis, surgical planning, and understanding of related pathological conditions [5].

The JOOC comprises nested epithelial parenchyma surrounded by a well-organised connective tissue stroma, containing abundant nerve bundles [6]. This structure has a typical length of 7-15 mm and a 1-2 mm diameter. While if the diameter is greater than 10 mm, hyperplasia or a submucosal tumor may be suspected [7,8].

Studies investigating the origin and functional significance of the mammalian JOOC have revealed it to be one of the most distinctive structures in mammals [1,3,9-13]. It has been described both as a vestigial structure from early embryonic stages and as a mechanoreceptor due to its rich nerve supply and developmental maturation [14]. However, JOOC has been reported to mimic diverse diseases, such as adenoid cystic carcinoma, mucoepidermoid carcinoma and thyroid carcinomas [1,7,15]. It has a remarkable pathological and anatomical importance in that it is likely to be misinterpreted as perineural invasion in patients with oral squamous cell carcinoma [1,16]. Such understanding is important to avoid misdiagnoses leading to unnecessary extensive surgical interventions [1,17,18].

In this study, the JOOC was analysed histologically and immunohistochemically, with a focus on characterising each of its distinct components. The purpose of this study is to discover unknown morphological aspects of the JOOC. The objective of this research is to provide a significant contribution by unravelling the immunohistochemical profile of the JOOC, a useful guide to the diagnosis of diseases, as well as in clinical practice of oral medicine and dentistry.

2. . MATERIALS AND METHODS

2.1. Tissue Origin and Preparation

Analysis of 5 well-preserved JOOC specimens was performed. Four of these samples were precisely taken from the bodies of three individuals aged 83, 84, and 86 years who voluntarily donated their bodies. Six adipose tissue samples were obtained from the infratemporal fossa. Out of these samples, three contained the entire JOOC, one had a partial JOOC, and the remaining two did not display any identifiable parts of the JOOC. The identification of the fifth JOOC specimen happened unexpectedly while analysing a surgical sample taken from a 62-year-old patient undergoing surgery for an oromaxillary tumour.

The extraction of the JOOC from human cadavers necessitates highly accurate techniques to attain the best outcomes. Initially, the facial skin around the buccal area is taken off, and dissection is performed on the parotid duct, parotid gland, and facial artery. The parotid gland is then gently repositioned towards the nasal side, as illustrated in Figure 1. Then, the buccinator muscle is dissected, and the masseter muscle is detached from the mandible and the zygomatic arch. Furthermore, the coronoid process of the mandible is incised, and the mandibular ramus is divided. Subsequently, the infratemporal fossa is uncovered, and the fibres of the temporal muscle are carefully extracted, as shown in Figure 2.

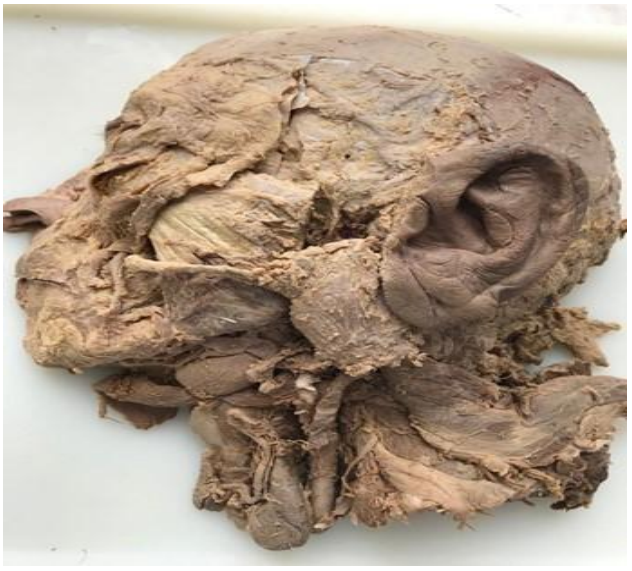

Figure 1: First step in the preparation of the juxtaoral organ. Dissection of the skin and moving of the parotid gland

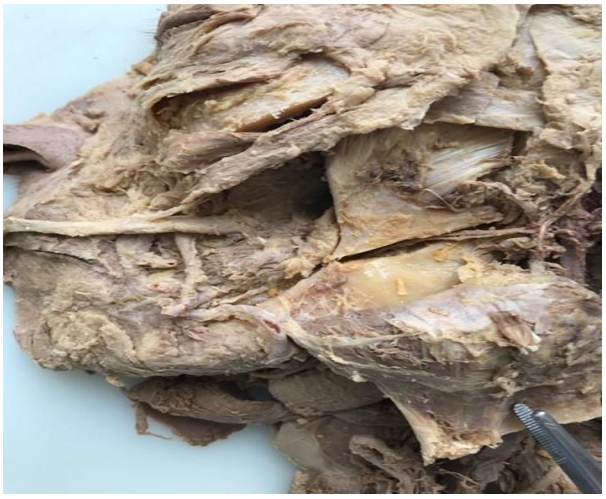

Figure 2: Mobilisation of the temporal muscle and splitting the ramus of mandible and exposing the infratemporal fossa

The JOOC in the adipose tissue lies medially to the pterygoid muscle, with muscle fibres connecting to its capsule, as shown in Figure 3. Diminutive branches originating from the buccal nerve penetrate the medial aspect of the JOOC capsule, which is adjacent to the mandible's ascending ramus. The buccal fat pad, where the JOOC is located, is then carefully isolated and collected.

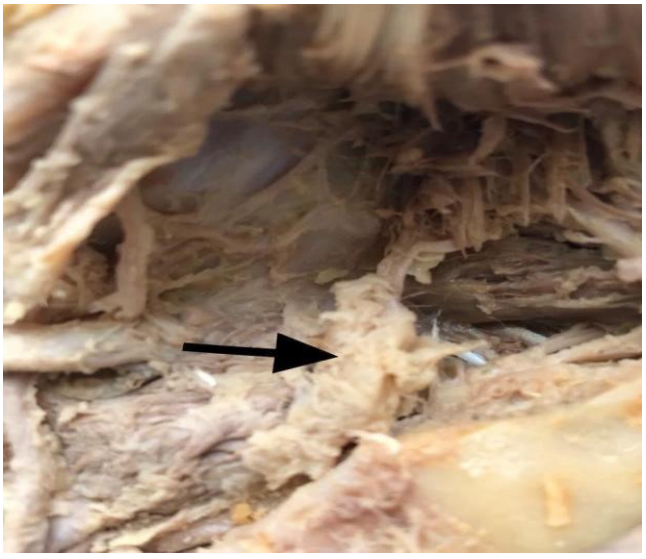

Figure 3: The juxtaoral organ surrounded by fat in the infratemporal fossa (arrow)

2.2. Microscopic and immunohistochemical examination:

The fatty tissue containing the juxtaoral organ was fixed in 4% buffered formalin to facilitate histological analysis. It was then sectioned into two or three levels and embedded in paraffin, as shown in Figure 4.

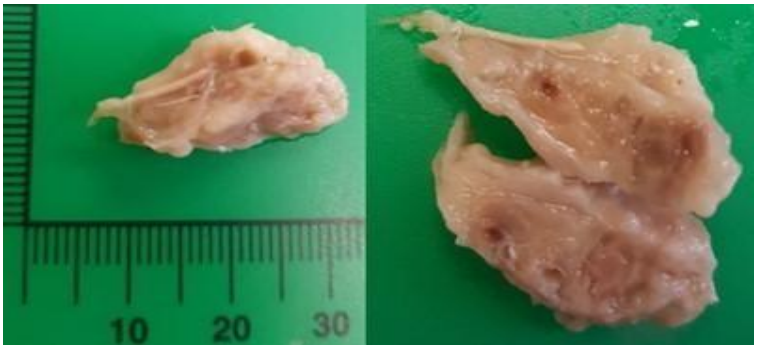

Figure 4: The extracted buccal fatty tissue with branches of the buccal nerve that enter the JOO embedded in adipose tissue.

From each paraffin block, a total of 30 slides were prepared. Every third slide, measuring 3µm in thickness, was specifically designated for H&E staining and traditional special stains, such as silver and Masson-Goldner Trichrome, all suitable for examination under light microscopy.

For the immunohistochemical study, certified antibodies commonly employed in our surgical pathology department for routine immunohistochemistry evaluation were utilised. A standard immunohistochemical stain protocol involving a primary antibody, polymer detection system, and DAB chromogenic staining [19].

The staining results were juxtaposed with positive and negative control tissue slides to ensure a comprehensive analysis. The internal positive control was considered if it was present in the examined sections. The remaining two sections (1µm thick) underwent careful immunohistochemical scrutiny, using the structures identified in the adjacent H&E slides for a detailed examination. To delve into the stromal and parenchymal components of the JOOC, a wide range of 36 antibodies was utilised, focusing on different cell structures and differentiation antigens. This thorough panel covered epithelial, neuroendocrine, and neurogenic features markers, along with myogenic, vascular, organ-specific, and cell-specific indicators. For more information, refer to Table 1 for specifics.

Table 1:Markers Array and antibody specificity (1-6)

1. Cytokeratins and epithelial markers			
CK5-6/14	CK20	p40/p63	EMA
CK7	CK19		
	Pan-Cytokeratin - (CKMNF116)		
2. Neuroendocrine and neurogenic markers			
Chromogranin	MBP	CD56	Sox-10
Synaptophysin	GFAP	Islet-1	
INSM-1	PGP9.5	S100	
3. Vascular markers			
CD31	CD34	ERG	
4. Myogenic markers			

Actin		Smoothlin	Myosin
5. Tissue and organ specific markers			
TTF-1	Satb-2	OCT-4	Calretinin
PDX-1	GATA-3	PAX-8	CD99
CDX-2	NKX3.1	Dog-1	
6. Tissue matrix markers			
Collagen IV		Glut-1	Ki-67

3. RESULTS

3.1. Topographic Anatomy and Histomorphologic Presentation

The JOOCs, which range in length from 7 to 15 mm and in diameter from 1 to 2 mm, were found to be compact, encapsulated fusiform structures found bilaterally. They were situated within the buccal fat pad, resting adjacent to the medial surface of the ascending ramus of the mandible, nestled medially to the pterygoid muscle at the angle of the mandible. Small branches from the buccal nerve, a sensory division of the trigeminal nerve, innervated the JOOC.

In the light microscopy, the JOOC appeared as an elongated neuroepithelial structure encased in an outer fibrous capsule measuring 50-75µm thick, comprised of fibroblasts and dense collagenous connective tissue (fig. 5). Blood and lymphatic capillaries and small nerves stemming from the buccal nerve passed through the capsule (fig. 6). The neuroepithelial parenchyma of this organ was surrounded by a stromal matrix of loose connective tissue containing CD34-positive fibroblasts (fig. 5 & 7).

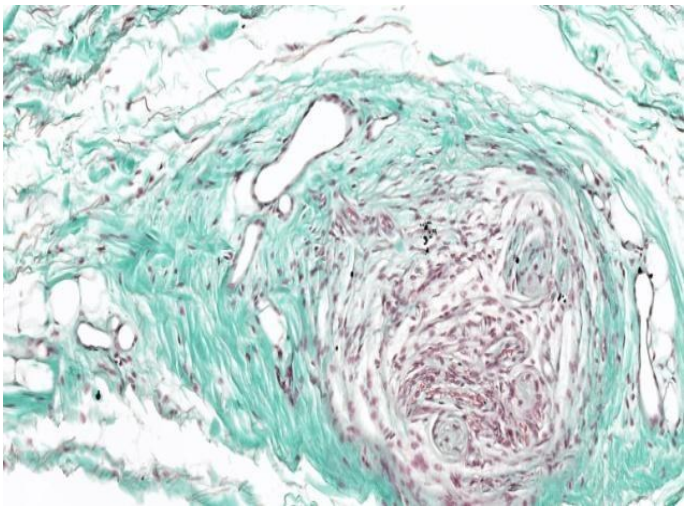

Figure 5:Masson-Goldner-Trichrome stain highlighting the external fibrous capsule of the JOOC. In the center, a stromal matrix is composed of loose connective tissue.

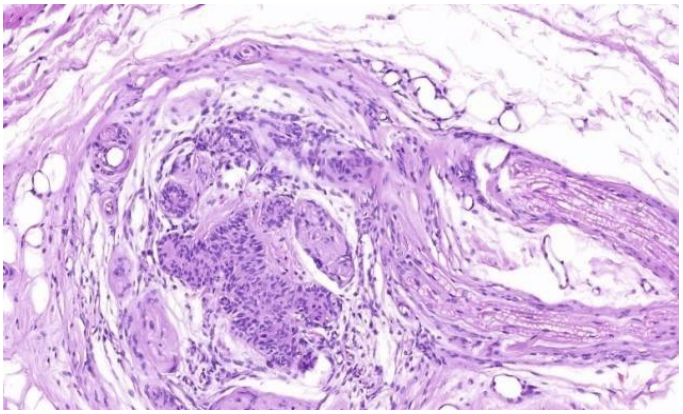

Figure 6: Microphotography (H&E) showing the complete cross-section of the JOOC with nerve fibers penetrating the external capsule and the parenchymal neuroepithelial structures in the center.

Additionally, the neuroepithelial parenchyma of this organ was comfortably nestled within a loose connective tissue stromal matrix. The core of the parenchyma contained both epithelial and neural elements embedded in the stromal matrix. The epithelial part, mainly found in the central area of the organ, displayed a unique organisation, forming small nests or branched cords, as depicted in Figure 8.

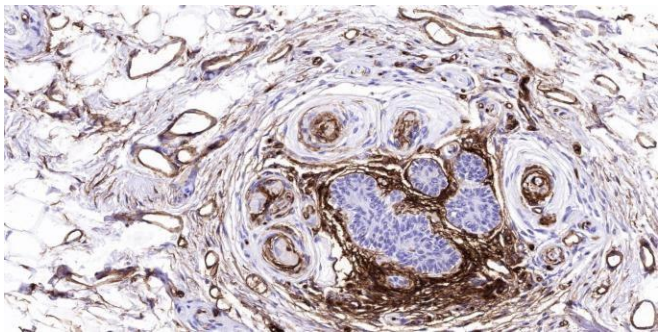

Figure 7: CD34 immunohistochemical stain highlighting the stromal fibroblasts of the JOOC. CD34 also labels the endothelial cells of the blood vessels present in the section.

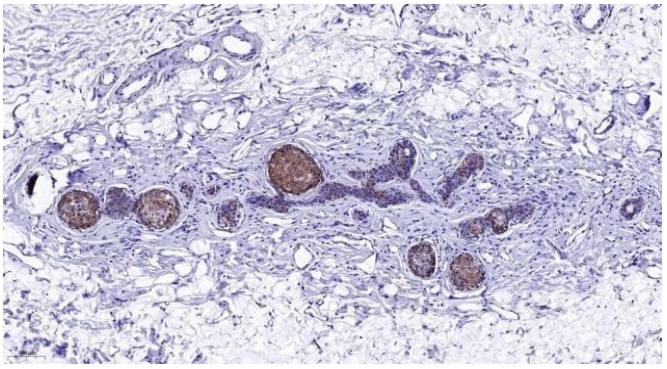

Figure 8: Longitudinal section of JOOC with a central epithelial cord and nests composed of squamoid differentiated epithelial cells labeled by CK5/14

Furthermore, the epithelial cells exhibited a distinctive distribution pattern, with cuboidal basaloid cells arranged in a

palisading nuclear pattern at the periphery. In contrast, the central region contained large polygonal and oval squamoid differentiated epithelial cells (fig. 9). After examining all specimens, it was found that the epithelial cells did not show keratinisation or tubular differentiation. However, signs of involution were observed in one specimen, along with microcalcification within the central part of the epithelial nests (fig. 10).

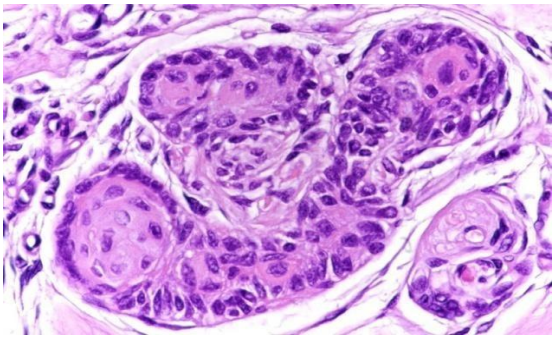

Figure 9: High magnification of the epithelial component showing basaloid arranged cells in the periphery and central squamoid differentiated cells.

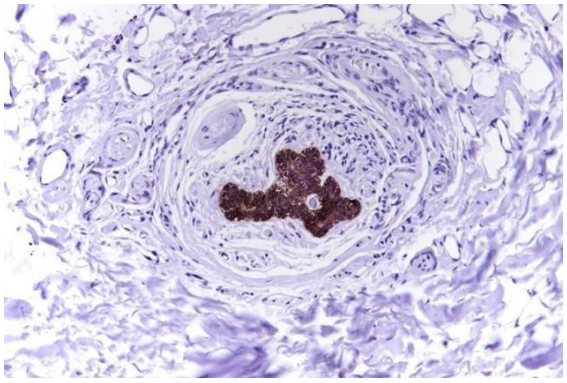

Figure 10: Cross-section of the JOOC showing an epithelial core of squamoid differentiated epithelial cells. All cells show strong expression of the cytokeratins 5&14 (CK5/14). Note the calcification in the central epithelial area.

During the examination of five specimens, a noteworthy discovery was made. In one specific case, an uncommon occurrence was observed in which the JOOC was duplicated unilaterally. The two JOOCs, varying in size, were prominently situated next to each other (Figure 11). Noting that most specimens were obtained from elderly donors aged between 62 and 86 years old, varying degrees of focal signs of involution were observed.

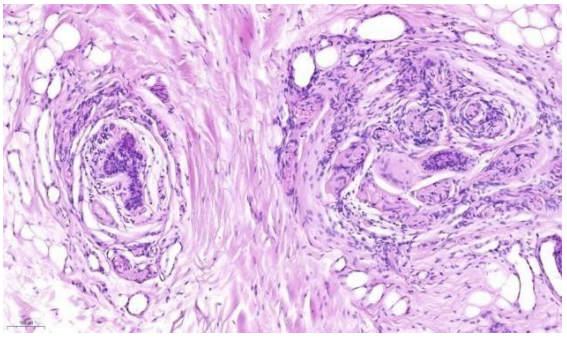

Figure 11: Cross-section of the buccal fat containing two separately encapsulated JOOC.

3.2. Immunohistochemical Profile

The primary aim of this extensive immunohistochemical exploration was to pinpoint any unusual antigen expression

patterns that might be present. The documented atypical antigen expression across diverse tissues and tumour varieties underscored its significance as a pivotal domain for differentiation investigations. An array of 36 specific antibodies holding abilities to target markers of clinical and differentiation significance was employed and classified from weak to strong expression (Table 2).

Table 2: Positive expression and marker targets

Antibody	Expression in the epithelial components
1. Cytokeratins and epithelial markers	
Pan-Cytokeratin (CKMNF116)	Strong expression
CK5/14	Strong expression
p40/p63	Moderate expression
CK19	Moderate expression
2. Neuroendocrine and neurogenic markers	
CD56	Weak to moderate expression
3. Vascular markers	
All negative	
CD34	Negative
4. Myogenic markers	
All negative	
5. Tissue and organ-specific markers	
All negative	
6. Tissue matrix markers	
Ki-67	<1%

At the central part of the epithelial nests, the large polygonal and oval squamoid cells (Fig. 9) exhibited high molecular weight Cytokeratins 5-6/14 expression. These Cytokeratins, commonly found in stratified squamous epithelium, are illustrated in Figures 8 and 10. Additionally, during the study, it was noted that focal moderate expression of type 1 cytokeratin (CK19) was mainly observed in the peripheral cells. CK19 is present in both simple and complex epithelia and is the smallest human cytokeratin. Cytokeratins 7 and 20 and the epithelial membrane antigen (EMA) were absent in the samples examined.

Moreover, an interesting finding was observed related to the moderate nuclear expression of p40 and p63, both of which are known for their crucial roles in regulating the differentiation of stratified epithelia. Moreover, the expression of the neural cell adhesion molecule CD56 on the cell membrane is another characteristic of the epithelial cells. Notably, the epithelial cells in the JOOC show minimal proliferative activity, with a Ki-67 proliferation index of less than 1%, and no mitotic figures were detected. On the other hand, CD34-positive fibroblasts were identified, as illustrated in Figures 5 & 8, surrounding the neuroepithelial parenchyma of this organ, situated within a loose connective tissue stromal matrix.

None of the cells in the JOOC tested positive for myogenic markers, including actin, myosin, and smoothelin, as well as for neurogenic and melanocytic markers such as Sox-10 and S100. Moreover, they did not exhibit any expression of endothelial markers CD31, CD34, and ERG, nor for various other organ and tissue-specific transcription factors like TTF-1, PDX-1, CDX-2, Satb-2, NKX3.1, GATA-3, Oct-4, and PAX-8. Similarly, no expression is observed for other tissue-specific markers, such as Dog-1 and Calretinin (as mentioned in Table 2).

Moreover, none of the cells tested positive for various neuroendocrine markers, including chromogranin, synaptophysin, Insm1, and Islet-1. It's crucial to note that Insm-1 and Islet-1 are transcription factors recognised for their role in governing the growth and specialisation of neuroendocrine cells, sympathetic neurons, and neuroblasts. Hence, the JOOC parenchyma does not exhibit histomorphological or immunohistochemical signs indicative of glandular, neuroendocrine, myoepithelial, or other organ-specific differentiation. On the flip side, a few small sensory nerves that originate from the buccal/trigeminal nerve penetrate the external fibrous capsule of the JOOC (fig. 6). To emphasise the neural aspect and the nerve fibres in the sections, immunohistochemical staining was performed using antibodies against the protein gene product 9.5 (PGP 9.5) and CD56. PGP 9.5, a neuron-specific protein found in cells of the central and peripheral nervous systems, was observed (Figs. 12 and 13).

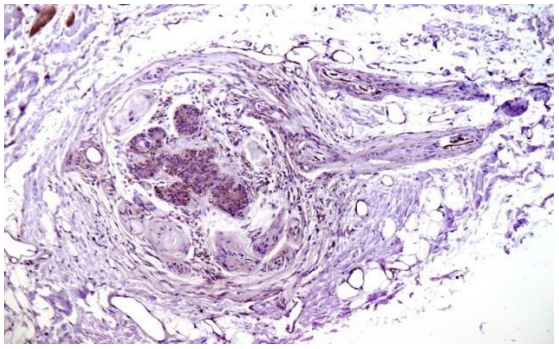

Figure 12: CD56 immunohistochemical stain. CD56 labels the extra- and inracapsular nerve fibers of the JOOC. In the central part the epithelial cells also exhibit positivity to CD56.

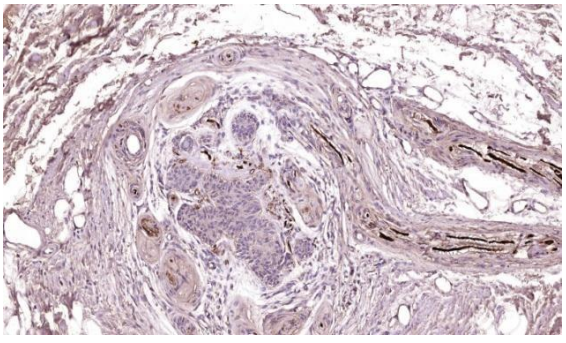

Figure 13: PGP 9.5 immunohistochemical stain highlighting the nerve fibers entering the external capsule of the JOOC and branching through the stroma with contact to the Pacinian corpuscles-like structures.

Within the stromal area protected by the capsule, specific nerve fibres end in unique globular formations, resembling Pacinian corpuscles commonly found in the skin, as shown in figures 14 and 15. They are surrounded by layers resembling onion skin, lamellar sheets of fibroblasts, and a thin outer capsule-like layer (figure 16). Moreover, a couple of delicate neural fibres reach out to directly contact the epithelial clusters or infiltrate them (Figure 14). The lamellar fibroblasts in our samples were marked with collagen IV (Figure 17). In the JOOC, the structures resembling Pacinian corpuscles are smaller and denser in the skin. The Pacinian corpuscle-like structures appear smaller and more tightly packed than those typically seen in the skin. During the study, there was no direct connection between large nerve fibres and epithelial clusters, a key indicator of neural invasion in cancerous growths, as shown in Figures 16 and 18. In addition, in all five JOOC

specimens examined, no lymphoid stroma or inflammatory reaction was observed, a contrast often seen in conjunction with neoplastic invasion (Figure 18). Unlike muscle spindles, no intrafusal muscle fibres or other myosin or actin-positive muscle fibres were detected (Figure 19).

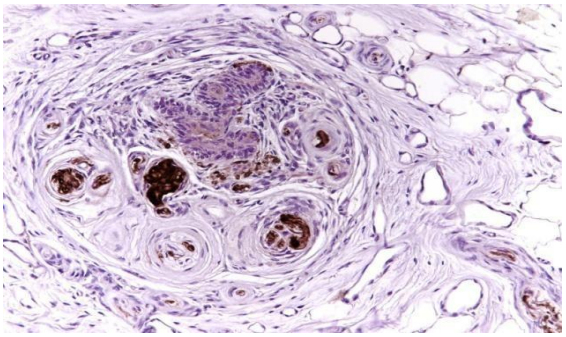

Figure 14:S100highlighting central dendrites within the Pacinian corpuscles-like structures. Nerve fibers end within Pacinian corpuscles-like structures.

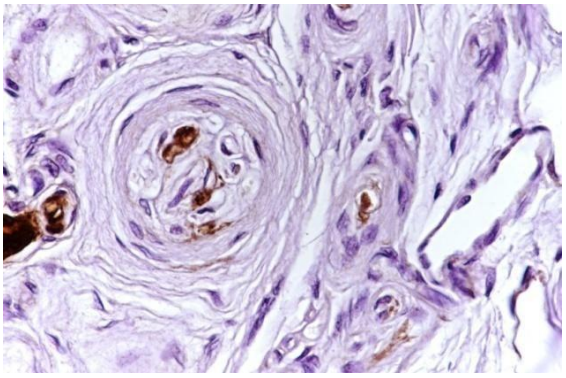

Figure 15:S100 immunohistochemical stain highlighting dendrites in the center of the Pacinian corpuscles-like structures.

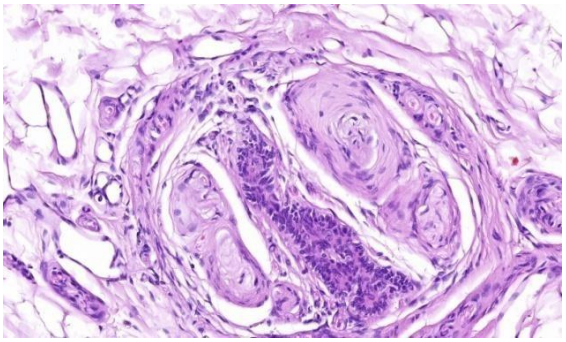

Figure 16:Microscopic cross-section of the JOOC showing multiple Pacinian corpuscles-like structures composed of lamellar sheets of fibroblasts. In the central area of the JOOC an epithelial cord surrounded by loose connective tissue.

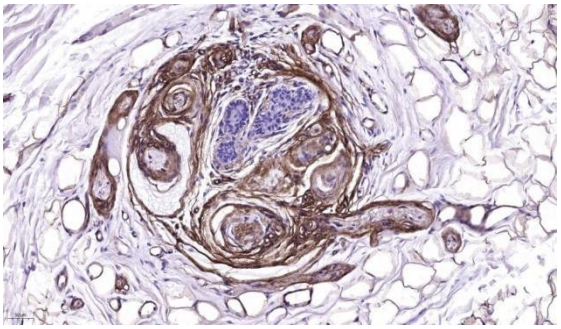

Figure 17: Collagen IV immunohistochemical stain highlighting the fibroblasts forming the lamellar sheets of the Pacinian corpuscles-like structures. Collagen IV stains also the perineural matrix and connective tissue of the stroma.

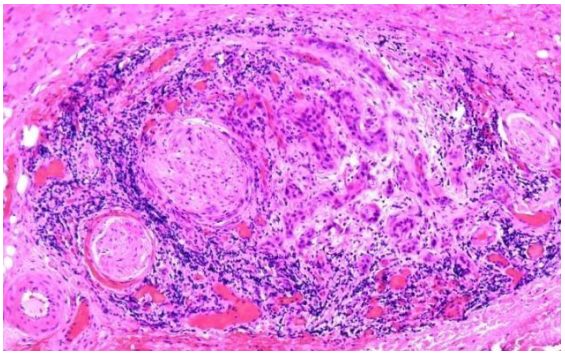

Figure 18:Perineural invasion by squamous cell carcinoma in the oromaxillary region. Tumor cells show direct contact with the relatively large nerve fibers. Tumor cords invade the adjacent tissues. The stroma shows moderate lymphocytic inflammatory reaction.

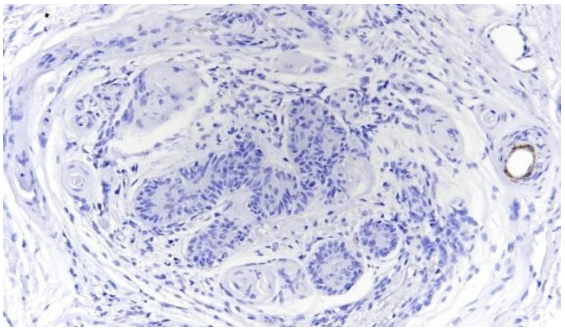

Figure 19: Myosin immunohistochemical stain. No muscle structures within the JOOC. Note positive internal control with myosin highlighting the arteriole's muscle layer in the capsule's right side.

4. DISCUSSION

The immunohistochemical profile of JOOC is crucial as it provides vital insights into its differentiation potential, clinical relevance, and functional characteristics within the oral and maxillofacial region. Despite earlier beliefs of regression before birth [3], recent studies indicate that the JOOC persists into adulthood, confirming its status as a permanent anatomical structure of significant importance [2,4]. The JOOC's anatomical characteristics, including shape, size, and location among the masticatory muscles, along with its innervation pattern of neuroreceptors, indicate a mechanoreceptive role. This function includes receiving mechanosensory signals from different structures in the oromaxillary area during actions like swallowing, sucking, and chewing [20]. While no documented cases of malignant tumours originate from the JOOC, accurately characterising the immunophenotype of JOOC components is essential. This helps distinguish primary

carcinomas of the oral mucosa from the epithelial component of the JOOC. Nevertheless, the absence of clear phenotypic characteristics currently obstructs such improvements.

The JOOC is located in the soft tissue above the mandibular angle in the buccotemporal space, serving as a vestigial neuroepithelial structure. While the function of the epithelial part in the JOOC's operation remains uncertain, performing immunohistochemical staining for both epithelial and neural components can help shed light on this function. Our study thoroughly analysed five preserved human samples of the JOOC, uncovering a consistent structure consisting of neuroepithelial parenchyma surrounded by connective tissue stroma and enclosed in a fibrous capsule. The neural fibres that enter the JOOC capsule originate from the buccal nerve, a sensory branch of the mandibular nerve derived from the trigeminal nerve. These fibres divide within the stroma and form multiple neuroreceptors similar to Pacinian corpuscles found in the skin. As evidenced by Ide et al.'s (2006) study, the potential for tumours arising from the neural component of the JOOC exists in the JOOC's anatomical region [21]. The presence of epithelial structures within the JOOC parenchyma is an intriguing discovery. The histomorphology and immune profile of this epithelium exhibit features commonly seen in non-keratinising squamous epithelium without clear indications of additional epithelial, myoepithelial, neuroendocrine, or organ-specific differentiation. However, Ide et al. (2003) highlighted that limited documentation of primary benign or malignant tumours originating from this epithelium [22].

The study did not identify any specific immunohistochemical marker profile or abnormal marker expression indicating the JOOC epithelium. As a result, these markers cannot be considered characteristic of primary carcinomas originating from this organ. However, it's important to note that the perioral organ has unique features that help differentiate it from malignant entities. With well-defined, non-keratinised squamous cell nests, it contrasts with metastatic squamous cell carcinoma, characterised by atypical squamous cells and keratin pearl-like formations. Due to its location, the JOOC occupies a rather privileged position within the infratemporal fossa; to be more precise, it resides within the mandibular angle. Wang et al.'s (2019) research mentioned that any deviation from this size and shape, particularly the irregular size and shape, should raise suspicion for pathology. Furthermore, JOOC generally has benign cytopathologic features; most samples contain no signs of dysplasia, keratinisation or increased growth [23]. Furthermore, there is no invasion into the capsule or adjacent tissue, and no undermining of the adjacent cortex, which suggests a benign disease. The lack of an inflammatory stromal response, which is associated with tumorigenesis, adds to the evidence that JOOC is a benign neoplasm.

It is demonstrated that the JOOC epithelial cells exhibit membranous expression of the neural cell adhesion molecule CD56, which may indicate their potential for further neural differentiation. Bommanavar et al.'s (2017) study elaborated that CD56 is often found in neural tissues, suggesting a neurosensory function of the JOOC [16]. While CD56 expression is common in the odontogenic epithelium and certain odontogenic tumours in the oromaxillary region, it's important to note that its focal expression is also frequently detected in squamous cell carcinoma. However, the study by Kawai et al. (2009) mentioned that CD56 expression is not limited to this specific tissue or tumour type alone (Figure 12) [23]. Therefore, considering the histomorphology and immunoprofile, the epithelial component appears similar to the stratified epithelium of the buccal mucosa or an abnormal immunohistochemical profile.

This study offers valuable insights by clarifying the immunohistochemical profile of the JOOC, revealing a unique pattern characterised by the presence of high molecular weight cytokeratins. On the other hand, the detection of low-molecular-weight cytokeratins or specific tissue transcription factors suggests the presence of neoplastic tissue other than squamous cell carcinoma. The JOOC exhibits a unique cytokeratin expression pattern, with high-molecular-weight cytokeratins 5, 6, and 14 showing strong positivity. This pattern resembles stratified squamous epithelium, suggesting a differentiation towards the squamous epithelial lineage in the JOOC. Moreover, the moderate presence of CK19, primarily in peripheral cells, suggests a varied potential for epithelial differentiation within this organ. Notably, the specimens under investigation lacked expression of CK7, CK20, and epithelial membrane antigen (EMA). This identification is important as it sets the JOOC apart from other epithelial tissues, emphasising its distinct immunophenotypic profile. Additionally, the low rate of cell proliferation seen here is a key differentiator between JOOC epithelial cells and carcinoma cells with perineural invasion. Typically, carcinoma cells with perineural invasion exhibit a Ki-67 proliferation index exceeding 10% and display easily visible mitotic figures in malignant cells. Moreover, identifying structures that resemble Pacinian corpuscles, like epithelial cords adjacent to Pacinian corpuscles-like formations in samples from the buccotemporal/oromaxillary region, is a distinctive feature indicating the presence of the JOOC [15,24]. Furthermore, the moderate nuclear expression of p40 and p63, which are essential regulators of stratified epithelial differentiation, provides additional evidence supporting the stratified epithelial lineage of the JOOC..

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