Annals of Diagnostic Pathology 40 (2019) 105–135

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

The dilemma of extensive unilocular radiolucent lesions of the jaws - value of immunohistochemistry as a diagnostic marker and prognostic Indicator

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ARTICLE INFO

Keywords:
Immunohistochemistry (IH)
Histopathological Examination (HPE)
Unicystic Lesions
Dentigerous Cysts
Unicystic Ameloblastoma
Calretinin
Ki-67
Proliferating Cell Nuclear Antigen (PCNA)
Proliferation Index (PI)
Labelling Index (LI)

ABSTRACT

Introduction: Large Unilocular radiolucent lesions of the jaws often present a dilemma to both, the Oral Pathologist and the Maxillofacial surgeon with regards to their accurate diagnosis as well as their most appropriate treatment modality. A precise identification as to whether the lesion is a cyst or a tumor is imperative before any treatment is instituted. Once the correct diagnosis and likely prognosis are established, a management protocol can be planned which will completely eliminate the lesion, while at the same time, ensure least possible morbidity for the patient, such as pathological jaw fractures, persisting neurological deficits, esthetic deformity, functional debility, recurrence/persistence of the lesion, etc.

Aim & objectives: To establish the value of Immunohistochemistry (IH) as a Diagnostic marker and Prognostic indicator for extensive Unilocular radiolucent lesions of the jaws. To assess its role as an adjunct to Histopathological Examination (HPE) in distinguishing Odontogenic tumours from the cysts, by identifying the former using IH Tumor Markers; and in aiding in selection of the most appropriate and effective treatment option for each of such ambiguous lesions, based on their prognosis as indicated by the expression of lH Cell Proliferation Markers.

Materials and method: Thirty cases of large Unilocular Radiolucent lesions of the jaws (Maxilla/Mandible) were managed over a period of three years. Histopathological examination (HPE) and Immunohistochemical (IH) analysis were carried out of the biopsy specimens in all the cases. Calretinin, an Immunohistochemical Tumor marker, was used to distinguish between Odontogenic cysts and tumours. Ki-67 and Proliferating Cell Nuclear Antigen (PCNA), Immunohistochemical Cell Proliferation markers, provided information on the aggressive potential of the lesions.

On the basis of the above information, an appropriate management protocol was established for each of these different lesions. Nerve sparing enucleation and curettage was employed for the established cases of Odontogenic Cysts; Enucleation and curettage, peripheral ostectomy, followed by chemical cauterization was employed for the Unicystic Ameloblastomas and other Odontogenic tumours with a low Ki-67 and PCNA Proliferation Index (PI)/Labelling index (LI $\leq$ 3); Marginal resection was carried out for the tumours with a higher Labelling Index (LI $>$ 3 $\leq$5), and Segmental resection (including partial/complete Maxillectomy, Hemimandibulectomy with/without disarticulation) for the aggressive pathologies with high Labelling Index (LI $>$ 5).

Results: Of the thirty cases of large Unilocular radiolucent lesions of the Maxilla and Mandible, thirteen were diagnosed as Dentigerous cysts, one as Dentigerous cyst showing Ameloblastomatous transformation; two as Unicystic Ameloblastomas, one as the Mural variant of Unicystic Ameloblastoma; four as Follicular Ameloblastomas, two as Plexiform Ameloblastomas; four as Acanthomatous Ameloblastomas; one as Ameloblastic Fibroma and two as Adenomatoid Odontogenic Tumours. The predictive and prognostic indication of the Immunohistochemical markers correlated well with the post treatment findings.

Conclusion: In cases of extensive Unilocular lesions of the jaws, where ambiguity often exists in both diagnosis and appropriate treatment plan to be employed, Immunohistochemistry can serve as an invaluable tool in establishing the precise diagnosis, guiding the treatment plan, as well as indicating the likely prognosis of these lesions.

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https://doi.org/10.1016/j.anndiagpath.2019.04.007

1. Introduction

The most common cause of a large unilocular lytic lesion of the jaws is the Odontogenic cyst, and when seen in association with an impacted tooth, it is most likely to be a Dentigerous cyst [1]. This is the most common non-inflammatory Odontogenic cyst which develops from the epithelial remnants of the dental follicle as a result of fluid accumulation between the follicular epithelium and crown of a developing or

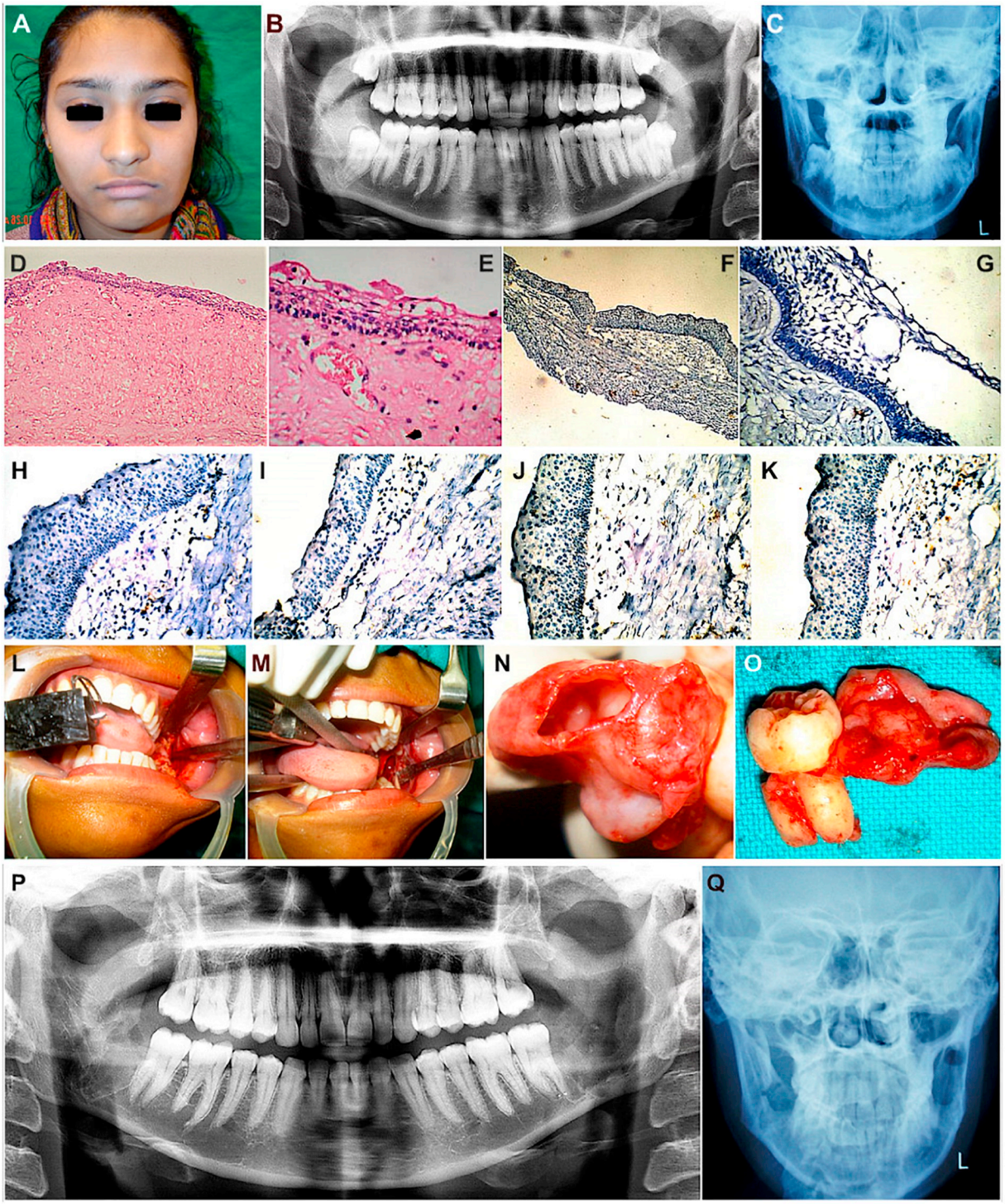

Fig. 1. (A) 24 yr old female patient who reported with pain & swelling of the left angle of the mandible, accompanied by Trismus. (B, C) Radiographs revealed a single large unilocular radiolucency in the left angle and ramus region of the mandible, associated with crown of impacted lower left third molar too (38). The lower right third molar (48) was also impacted. (D, E) Histopathological examination (magnification X50, X100) confirmed the diagnosis of Dentigerous cyst and showed Cyst lining composed of stratified squamous epithelium. (F, G) A negative Calretinin expression, further helped to rule out the possibility of Unicystic Ameloblastoma. (H–I) Negative expression of Ki-67. (J, K) Negative expression of PCNA. (L-O) Removal of 38 and Enucleation of the cyst adherent to its crown, followed by curettage of the bony defect. Impacted 48 was extracted as well. (N, O) Gross appearance of the cystic lesion closely adherent to the crown of the impacted tooth 38, characteristic of the Dentigerous cyst. (P, Q) Post-op radiographs.

Table 1
Clinical and radiographic findings, histopathological and immunohistochemical findings, confirmatory diagnosis and treatment protocol employed in the 30 cases of large Unilocular radiolucent lesions of the jaws.

Dentigerous cysts								
1.	24 yr/F (Fig. 1)	Pain & swelling of Lt angle of mandible, Trismus	Large unilocular radiolucency associated with crown of impacted 38.	Fibrous Connective tissue capsule with a cyst lining composed of stratified squamous epithelium.	Negative	0	0	Removal of 38 and Enucleation of the cyst adherent to its crown, followed by curettage of the bony defect.
2.	54 yr/M	Partially edentulous with diffuse swelling & pain in upper right quadrant.	Single unilocular radiolucency surrounding unerupted 13	Fibrous capsule with a hyperplastic epithelial lining, both of which contained a dense chronic inflammatory infiltrate.	Negative	0	0	Enucleation & extirpation of the lesion along with the tooth enclosed within.
3.	48 yr/M	Pain and swelling in 38 region.	Unilocular radiolucency associated with the crown of impacted 38.	Cyst lining composed of stratified squamous epithelium.	Negative	0	0	Enucleation and curettage followed by chemical cauterization with Carnoy's solution
4.	12 yr /F	Missing teeth 23 and 24. Bony hard, non-tender, expansile swelling in left maxilla	Unilocular radiolucency in Lt Maxilla with retained teeth appearing to float within the cyst cavity	Fibrous capsule with a hyperplastic stratified squamous epithelial lining, with a dense chronic inflammatory infiltrate in the sub epithelial connective tissue zone.	-ve	-ve	-ve	Enucleation and curettage with removal of the tooth involved, via an intraoral buccal vestibular approach.
5.	10 yr/M	Multiple missing teeth [23, 24, 25), retained deciduous lateral incisors. Bony hard, expansile swelling of left maxilla	Unilocular radiolucent lesion of Lt Maxilla with tooth bud 23 within.	Stratified squamous epithelial lining with a fibrous connective tissue capsule, characteristic of Dentigerous cyst.	-ve	-ve	-ve	Enucleation & curettage via intraoral upper vestibular approach.
6.	10 yr/M (Fig. 2)	Slowly growing, bony hard swelling in the left parasymphysis region of the mandible, in association with retained deciduous canine	Impacted 33 which was enclosed within a large unilocular radiolucent lesion extending almost to the inferior border of the mandible.	Cyst cavity with a fibrous capsule and a hyperplastic epithelial lining, both of which contained a dense chronic inflammatory infiltrate.	-ve	-ve	-ve	Enucleation and curettage after de-roofing of the cystic lesion via an intraoral approach. Fresh autologous Platelet Rich Fibrin (PRF) was placed into the bony cavity to hasten bone fill and reossification.
7.	11 yr/F	Swelling and pain over the right upper cheek. Retained deciduous canine 23.	Larger unilocular radiolucent lesion enclosing displaced 23. Lesion protruding into the maxillary antrum, occupying half of it.	Stratified squamous epithelium lining the cystic cavity, surrounded by a fibrous tissue capsule.	-ve	-ve	-ve	Enucleation and curettage of lesion. 23 within, removed along with the pathology. Autologous PRF placed prior to closure of mucoperiosteal flap.
8.	30 yr/M	Slowly enlarging bony swelling over the left angle region of the mandible, missing 38.	Large well defined monocular radiolucent lesion in left angle of mandible, enveloping an impacted third molar tooth.	Cystic lining composed of stratified squamous epithelium, surrounded by a connective tissue capsule with chronic inflammatory cell infiltrate.	-ve	-ve	-ve	Enucleation and curettage via an intraoral buccal vestibular approach, followed by placement of PRF within the bony cavity to enhance reossification of the resultant defect.
9.	19 yr/F	Pain and swelling in the right retromolar region, associated with an unerupted third molar tooth 48.	Single unilocular radiolucent lesion in the right angle of the mandible, surrounding an impacted and inferiorly displaced third molar tooth.	Cystic cavity lined by stratified squamous epithelium and surrounded by connective tissue with a dense inflammatory infiltrate.	-ve	-ve	-ve	Enucleation and curettage of the entire cystic lesion along with the tooth within; followed by placement of PRF in the resulting defect for a quicker bone fill.
10.	12 yr/M	Slowly enlarging swelling in left infraorbital region, associated with retained deciduous upper canine and missing 23.	Large Unilocular radiolucency in the upper anterior region, with displaced tooth 23 within it. Displacement of roots of adjacent teeth by the pathology, which also had invaded the left antrum, noted.	Stratified squamous epithelium lining cystic space and surrounded by a fibrous capsule.	-ve	-ve	-ve	Enucleation and curettage of the entire lesion via intraoral vestibular approach, extirpation of the antral lining om the involved side. PRF placed prior to closure of mucoperiosteal flap.
11.	18 yr/F	Missing lower permanent canine, retained deciduous canines. Bony swelling in symphysis region, with expansion of the labial cortical plate.	Large oval unilocular radiolucency in symphysis region of mandible crossing the midline, and containing impacted and inferiorly displaced 33 and 43 within.	Stratified squamous epithelial lining and a connective tissue capsule contain = g chronic inflammatory cells.	-ve	-ve	-ve	Removal of a labial cortical window of bone via an intraoral vestibular approach, deroofing of the cystic lesion followed by its enucleation along with removal of the two impacted canines within the cyst. Fresh, autologous PRF placed within the bony defect to hasten subsequent reossification.

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Table 1 (*continued*)

Dentigerous cysts								
12.	24 yr/F	Painful expansion of left angle region of mandible.	Impacted and inferiorly displaced 48, surrounded by a large unilocular radiolucency involving the right angle.	Cyst lining composed of stratified squamous epithelium, surrounded by a fibrous connective tissue capsule.	-ve	-ve	-ve	Enucleation and curettage along with removal of the tooth involved.
13.	19 yr/F	Bony swelling of chin, missing 43	Medium sized radiolucency enveloping horizontally impacted 43	Cyst lining composed of stratified squamous epithelium, surrounded by a fibrous connective tissue capsule.	-ve	-ve	-ve	Enucleation and removal of 43, followed by Curettage of bony defect.
Dentigerous cysts with evidence of ameloblastomatous transformation								
14.	10 yr/M (Fig. 3)	Tender, slowly enlarging, bony hard swelling over the left cheek region.	Large, well demarcated unilocular radiolucency with radiopaque borders, filling up the entire left maxillary antrum with the tooth bud 26 within the lesion. NCCT confirmed the extent of the lesion.	Fibrous capsule with a hyperplastic stratified squamous epithelial lining, with a dense chronic inflammatory infiltrate in sub epithelial zone. Few areas showing the typical tall columnar basal cells with reverse palisading of the nuclei, indicative of ameloblastomatous change.	Weak positivity	2.1	1.7	Enucleation & curettage via an intraoral approach; followed by Chemical cauterisation with Carnoy's solution.
Unicystic Ameloblastomas								
15.	55 yr/M (Fig. 5)	Persistent oozing of a foul smelling fluid from the right lower molar region, for past three years.	Large unilocular radiolucency hollowing out the entire right angle and ramus region of the mandible, with horizontally impacted and displaced tooth 48 lying within. Expansion of buccal and lingual cortical plates, widening of anterior border of the ramus, expansion and bowing of the inferior border of the mandible in the angle region.	A fibrous capsule and Stratified squamous epithelial lining of varying thickness lining a cystic cavity. Dense inflammatory infiltrate in the subepithelial zone.	Positivity exhibited by the epithelial cells.	1.8	1.6	Enucleation and curettage of the Cystic lesion along with removal of the tooth 48 within. Vigorous curettage and peripheral ostectomy carried out with vulcanite trimmers. Chemical cauterization of the bony walls with Carnoy's solution. Placement of PRF prior to watertight closure.
16.	23 yr/M (Fig. 4)	Pain and swelling in the region of left angle of mandible.	An extensive unilocular radiolucency involving the left angle and ramus of the mandible extending posteriorly almost up to the tip of the coronoid process. A horizontally impacted third molar lay within the radiolucent space	Hyperplastic cystic lining composed of stratified squamous epithelium of varying thickness. In some location, tall columnar cells were evident with reverse palisading of nuclei.	Positivity expressed by epithelial cells at various locations.	0.9	0.5	Enucleation and curettage with removal of the impacted 38, followed by Chemical cauterization of the walls of the bony cavity with Carnoy's solution. Fresh autologous Platelet Rich Fibrin was placed within the residual cavity to encourage subsequent bone fill.
Follicular variant of ameloblastoma								
17.	39/F (Fig. 8)	Exophytic gingival swelling with expansion of the alveolar ridge and hard palate in 21 to 15 region. Case was misdiagnosed elsewhere and treated conservatively for pyogenic granuloma. Recurrence of the lesion, which rapidly increased in size.	Large Unilocular radiolucency involving Rt Maxilla, causing destruction of the alveolar process and the anterolateral wall of the maxillary antrum	Richly cellular odontogenic epithelial tumor follicles, set in a dense fibrovascular connective tissue stroma	Diffuse and strong expression of Calretinin by the tumor cells	7.3	6.6	Partial maxillectomy (Rt)including the alveolar ridge and anterior hard palate from 22 to 16 region, with removal of antral lining.

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Table 1 (*continued*)

Dentigerous cysts								
18.	35 yr/F (Fig. 9)	Pain and slowly enlarging bony hard swelling of left side of mandible.	Extensive destruction of the left ramus and angle of the mandible, by a Unilocular radiolucent lesion	Round epithelial follicles with reverse palisading of the peripheral Ameloblastic cells confirming Follicular variety of Ameloblastoma.	Diffuse Calretinin positivity	5.8	5.6	Radical resection of the left ramus and angle was carried out, together with a 1.5 cm margin of clinically apparently sound bone. The resection included the condyle, that is, disarticulation was carried out. Immediate reconstruction using a Recon plate with a condylar element was carried out.
19.	63ys/M (Fig. 10)	Swelling and pain in the right body of mandible	Extensive hollowing out of the right body of mandible by a Unilocular radiolucency.	Epithelial follicles with reverse palisading of peripheral tall columnar cells and central polyhedral cells.	Strong positivity, in the central stellate reticulum like cells and cells lining areas of cystic degeneration within the follicles.	5.8	4.9	Segmental resection was carried out jaw continuity maintained using a Recon plate. However, 6 months later, there was infection and exposure of the implant. Which had to be removed and secondary reconstruction of the defect was done using the Free fibular bone graft.
20.	40 yr/F (Fig. 7)	Vague pain and diffuse slowly enlarging swelling of left side of lower jaw.	Extensive unilocular radiolucent lesion involving and hollowing out most of the left angle, ramus and part of body of the mandible.	Numerous epithelial tumor follicles, set in a dense fibrovascular connective tissue stroma. Multiple areas of cystic degeneration within the follicles.	+ve	6.9	6.4	Hemimandibulectomy with disarticulation.
Plexiform variant of ameloblastoma								
21.	39 yr/M (Fig. 15)	Vague pain in the right side of Mandible, with H/O multiple extractions	Large Unilocular radiolucency involving right body of the mandible, missing teeth of the lower right quadrant.	Anastomosing cords and strands of odontogenic epithelial cells	Strong Calretinin positivity	7.9	7.2	Segmental resection of Rt body of mandible,leaving behind the upper part of the ramus with the condyle and coronoid process; and maintaining the jaw continuity using an angled reconstruction plate.
22.	34 yr/F	Swelling and pain in right body of mandible.	Large unilocular radiolucency in right body of mandible causing displacement as well resorption of roots of adjacent teeth.	Anastomosing cords and strands of odontogenic epithelial.	Strong positivity	5.3	4.6	Hemimandibulectomy, with secondary reconstruction planned using Free fibular graft
Acanthomatous variant of ameloblastoma								
23.	34 yr/F (Fig. 12)	Pain and swelling of left side of Mandible	Large unilocular radiolucency involving right body of mandible in region of first molar, causing displacement of roots of adjacent teeth.	Epithelial follicles exhibiting reverse palisading of the peripheral cells and Acanthomatous differentiation and squamous metaplasia of central cells.	Positivity expressed throughout the epithelial follicles.	6.6	6.3	Segmental resection of right body of mandible and immediate reconstruction using iliac crest bone graft.
24.	37 yr/F (Fig. 14)	Slowly growing, bony hard swelling of left side of face.	Large Unilocular radiolucency involving entire left Ramus of mandible.	Epithelial follicles/islands, with peripheral layer of tall columnar Ameloblastic cells with reverse palisading of their nuclei and the central stellate reticulum-like cells, in this case, undergoing squamous metaplasia, a characteristic of the Acanthomatous variant. There were areas of keratin formation	Strong expression in the epithelial lining of Microcysts and the areas of squamous metaplasia	6.7	6.6	Resection with disarticulation. Planned for Secondary reconstruction using Free fibular graft, at a later date.

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Table 1 (*continued*)

Dentigerous cysts								
25.	40 yr/F (Fig. 13)	Slowly enlarging bony swelling of the left side of the lower jaw.	Extensive involvement of the entire Lt ramus and angle and part of body of the mandible by a large Unilocular radiolucency.	Epithelial follicles with peripheral layer of tall columnar Ameloblastic cells and the central stellate reticulum-like cells undergoing squamous metaplasia, characteristic of Acanthomatous Ameloblastoma.	Prominent Calretinin staining of the areas of Acanthomatous differentiation	6.9	6.4	Hemimandibulectomy with disarticulation. Immediate reconstruction using a free fibular bone graft
26.	39 yr/F (Fig. 11)	Diffuse swelling and pain over the region of left angle region of mandible,	Unilocular radiolucent lesion with osteolytic destruction and hollowing out of the left ramus, angle and part of body of the mandible.	Epithelial tumor follicles, with evidence of Acanthomatous differentiation at the central regions of many of the follicles.	+ve	7	6.5	Mandibular resection with disarticulation. Immediate stabilization carried out using a Titanium Reconstruction plate, with secondary reconstruction to follow at a later date.
Adenoameloblastoma (Adenomatoid Odontogenic Tumor)								
27.	24 yr/M	Localized bony hard swelling in the left infraorbital region with obliteration of the nasolabial sulcus.	Well defined Unilocular radiolucency in association with an impacted 23	Numerous whorls of glandular or duct-like structures, with lumen of varying size and lined by cuboidal/columnar ameloblast-like cells and polygonal cells in between the ductal structures.	Occasional positivity expressed by few of peripheral cells lining the whorls.	-ve	-ve	Lesion enucleated in toto along with the tooth within, followed by curettage of the bony walls, which was then cauterized with Carnoy's Solution.
28.	23 yr/M (Fig. 17)	Localized bony hard swelling in the right maxillary canine region with obliteration of the nasolabial sulcus	Unilocular radiolucency in association with an impacted 12	Biopsy specimen comprised of numerous whorls of glandular or duct-like structures, with lumen of varying size and lined by cuboidal/columnar ameloblast-like cells and polygonal cells in between the ductal structures.	Negative	-ve	-ve	Lesion was enucleated in toto along with the tooth within and it shelled out quite easily from the surrounding bony cavity which was then cauterized with Carnoy's Solution.
Ameloblastic Fibroma								
29.	39 yr/M (Fig. 16)	Bony expansion of the right Body of mandible	Extensive hollowing out of Rt body of mandible by a monocular radiolucency, with spheroid expansion of the buccal cortical plate and intact lingual cortex.	Islands of odontogenic epithelium which were surrounded by a, richly cellular ectomesenchymal stroma resembling the dental papilla	Both, epithelial as well as mesenchymal components were negative for Calretinin	3.8	3.4	Marginal resection, with complete removal of the tumor together with 1 cm of normal appearing bone all around, preserving the integrity of the inferior border of the mandible. Followed by primarily reconstruction using a iliac crest bone graft and reinforcement of the inferior border using a recon plate.
Mural variety of Unicystic Ameloblastoma								
30.	17 yr/M (Fig. 6)	Slowly expanding, large bony swelling over the right side of the mandible	Single very large unilocular radiolucency involving the entire ramus, angle and body of the mandible on the right, which was seen associated with impacted second and third molar teeth. There was considerable expansion of the buccal and lingual cortical plates	Cystic lining in some areas was composed of innocuous looking stratified squamous *epi* resembling a Dentigerous cyst. In other areas on the same slide, however, the epithelium seemed to be proliferating into the underlying connective tissue wall of the cyst giving a distinct impression of the mural variety of UA	Strong positivity	6.6	5.2	Hemimandibulectomy planned, followed by Secondary reconstruction of the mandible at a later date.

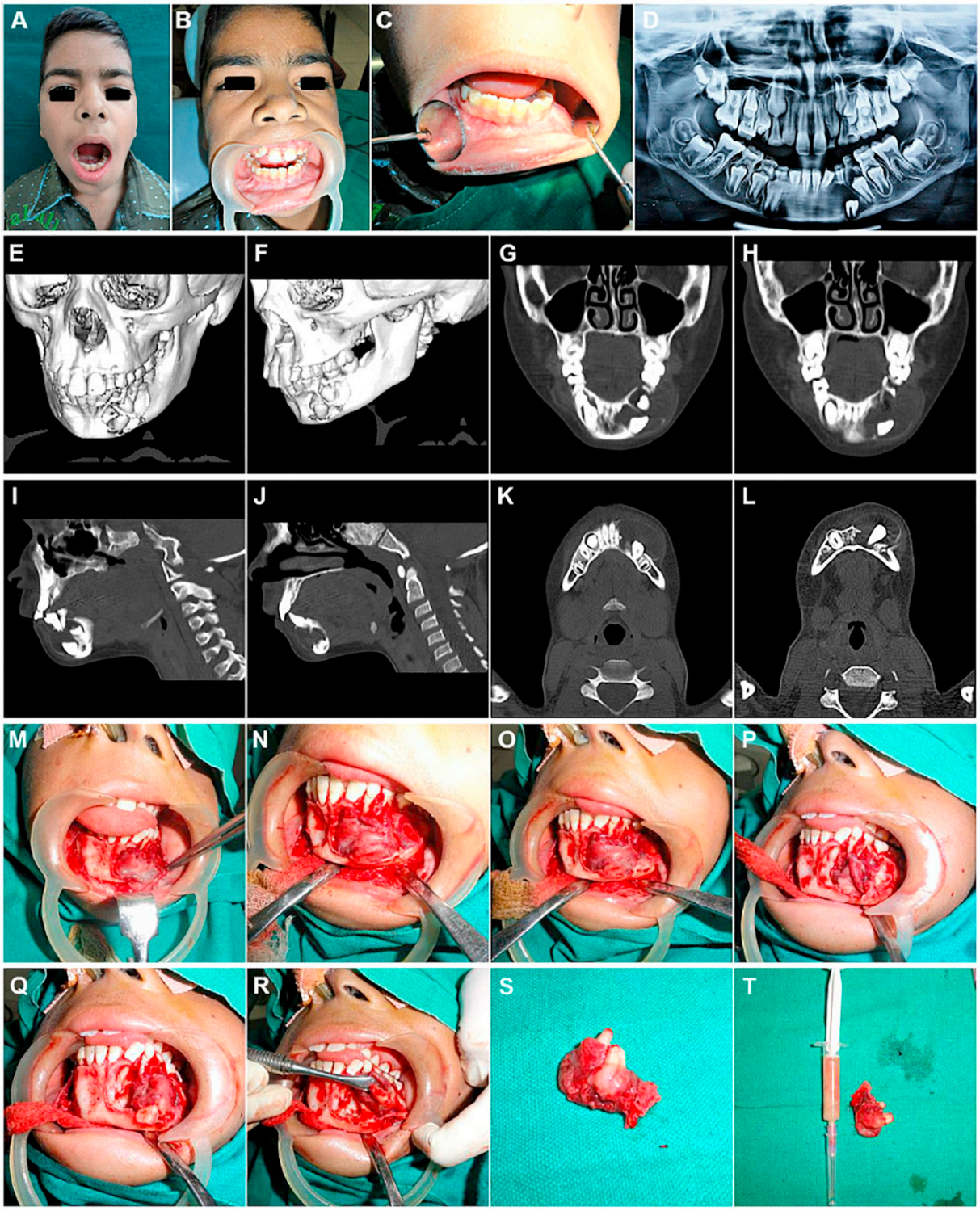

Fig. 2. (A-C) 10 yr old male patient with a slowly growing, bony hard swelling in the left parasymphysis region of the mandible, with expansion of the labial cortical plate and in association with retained deciduous canine. (D-L) Radiographs and NCCT revealed impacted lower left canine tooth (33) which was enclosed within a large unilocular radiolucent lesion extending almost to the inferior border of the mandible. (M-V) After confirmation of Dentigerous cyst by incisional biopsy, the patient was taken up for enucleation and curettage after deroofing of the cystic lesion via an intraoral approach. The lesion shelled out easily from the underlying bony cavity which was thereafter curetted thoroughly. (V, X) Fresh autologous Platelet Rich Fibrin (PRF) was placed into the bony cavity to hasten bone fill and reossification, followed by closure. (Y-AA′) H&E stained sections of the incisional biopsy showed a cyst cavity with a fibrous capsule and a hyperplastic epithelial lining, both of which contained a dense chronic inflammatory infiltrate. (AB′, AC′) IH findings of negative reactivity for Calretinin and Ki-67, confirmed the diagnosis of a Dentigerous cyst and not a Unicystic A.

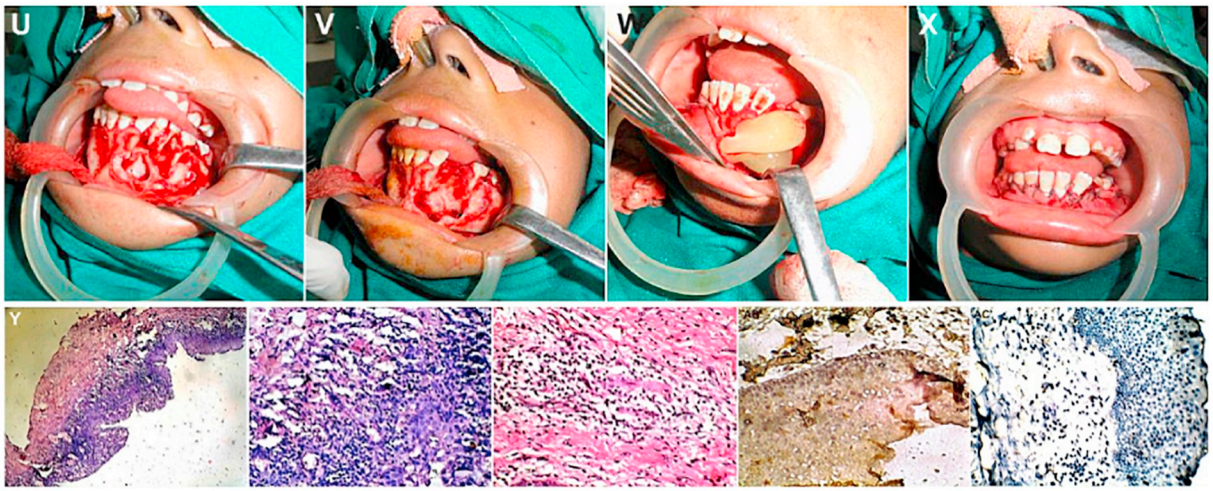

Fig. 2. (*continued*)

unerupted tooth [2].

Unless noticed on radiographs as an incidental finding, these cysts often remain undetected. They are capable of enlarging unobtrusively and asymptomatically, attaining quite large sizes, causing considerable hollowing out of the jaws. It is usually only when they are associated with pain, infection, inflammation or a visible bony expansion, that they are observed, investigated and diagnosed. A cause of concern is that these cysts may also transform into an Odontogenic tumor such as Ameloblastoma and its variants [3], or even into Mucoepidermoid carcinoma [4] or Squamous cell carcinoma [5,6]. Accurate and precise diagnosis is crucial in order to institute the correct surgical treatment modality and manage the pathology adequately [7].

When Odontogenic tumours present as a Unilocular radiolucency, they exhibit marked clinical and radiographic similarities to the Odontogenic cysts, sometimes with even overlapping histological features [8]. However, they vary considerably from the latter in their biological behavior, in terms of invasiveness, aggressiveness, tendency for recurrence and malignant transformation; and hence require vastly different therapies and treatment modalities [9]. In this way, what may appear as an innocuous Unicystic lesion, may in fact be an aggressive and locally destructive tumor, adding to its enigma and diagnostic dilemma for the Oral pathologist and a conundrum for the Maxillofacial surgeon as to its most appropriate management modality [10].

Histopathological (HPE) and Immunohistochemical (IH) examination of biopsy specimens play an extremely important role, both, in the diagnosis and prognosis of these lesions. IH is an evolving science, and can be defined as the technique of detection & localization of specific cellular proteins or antigens in biological tissue specimens, by using labeled antibodies and visualizing the immunological complexes that are formed as a result of these Antigen-Antibody (Ag-Ab) recognition and interactions [11].

IH can be used in two possible ways [12]. One is in *Tumor Immunodiagnosis*, the basis of which is that certain cells & tissues express certain unique proteins or antigenic determinants (also called '*Tumor markers*'), which are specific to that particular cell or tissue type, thus serving as a diagnostic indicator. 'Calretinin' is an intracellular anti-apoptotic protein, specific to ameloblastomatous cells [13], that serves as a diagnostic marker, to correctly differentiate Odontogenic cysts from the tumours [14,15].

IH can also be used to assess the proliferative activity of tumor cells by assessing the expression of various Cell Cycle associated antigens like 'Ki-67' [16], (Proliferating Cell Nuclear Antigen) 'PCNA', (Argyrophilic Nucleolar Organizer Regions) 'AgNORs' etc., which are known as '*Proliferation markers*' [17]. They thus serve as a prognostic indicator, and give us an idea about the nature of the tumor in terms of the likely clinical course, biological behavior, aggressive potential and probable

Table 2
An evaluation and comparison of the Immunohistochemical expression of tumor marker and proliferation markers among the 30 cases of large Unilocular radiolucent lesions of the jaws.

S. no.	Diagnosis of the Unilocular radiolucent lesion	Number of cases	Calretinin expression	KI-67 LI (Average LI)	PCNA LI (Average LI)
1.	Dentigerous Cyst	13	-ve	-ve	-ve
2.	Dentigerous Cyst with evidence of Ameloblastic transformation	01	Weakly +ve	2.1 (2.1)	1.7 (1.7)
3.	Unicystic Ameloblastoma	02	Weakly +ve; Weakly +ve	1.8; 0.9 (2.25)	1.6; 0.5 (1.05)
2.	Follicular Ameloblastoma	04	+ve; +ve; +ve	7.3; 5.8; 5.8; 6.9 (6.45)	6.6; 4.9; 5.6; 6.4 (5.9)
3.	Plexiform Ameloblastoma	02	+ve; +ve	7.9; 5.3 (6.6)	7.2; 4.6 (5.9)
4.	Acanthomatous Ameloblastoma	04	+ve;+ve; +ve; +ve	6.7; 6.9; 6.6, 7.0 (6.8)	6.6; 6.4; 6.3, 6.5 (6.45)
5.	Adenoameloblastoma/Adenomatoid Odontogenic Tumor (AOT)	02	-ve; +ve	-ve; −ve (0)	-ve; −ve (0)
6.	Ameloblastic Fibroma	01	+ve	3.8 (3.8)	3.4 (3.4)
7.	Mural variety of Unicystic Ameloblastoma	01	+ve	6.6 (6.6)	5.2 (5.2)

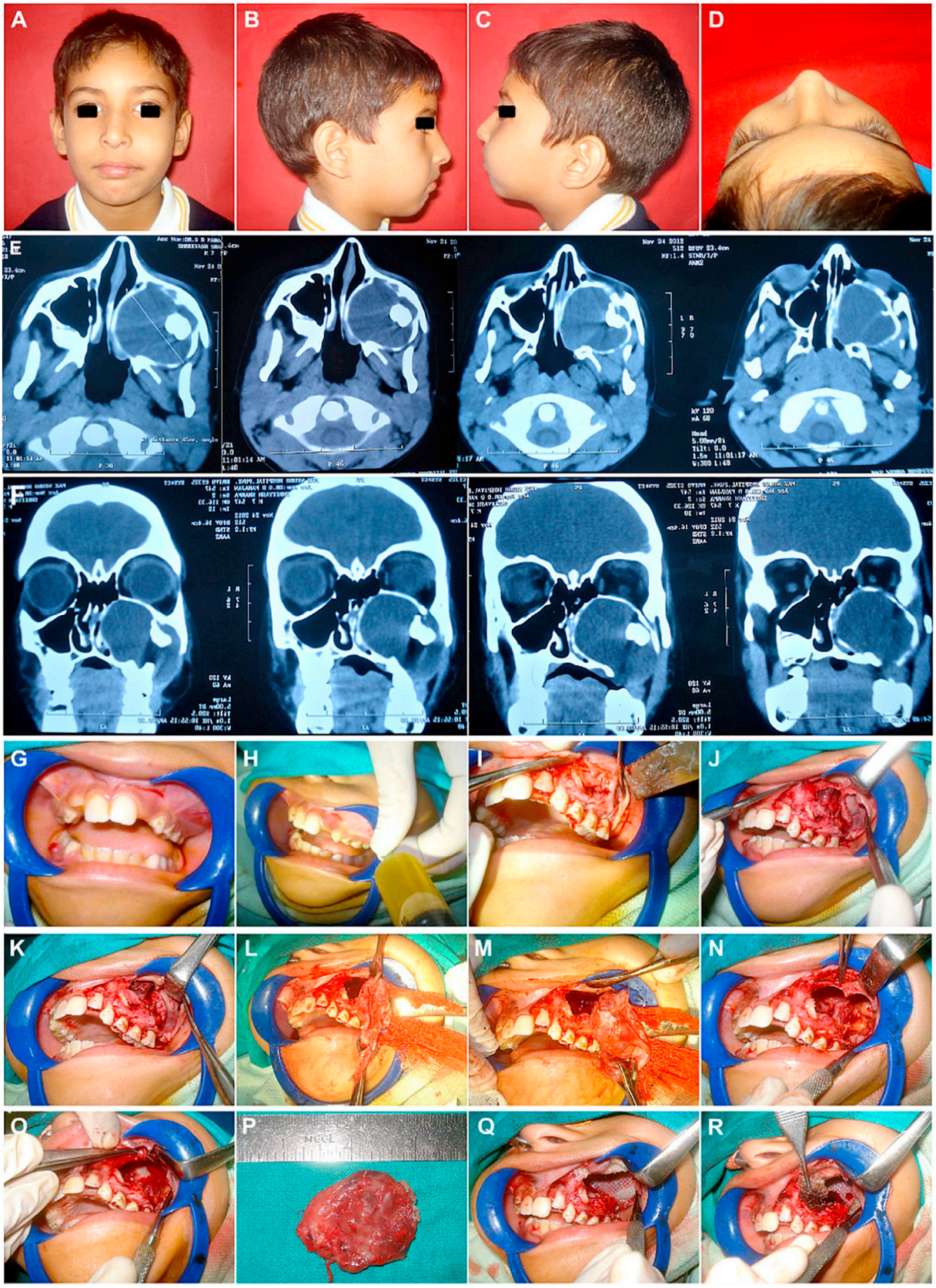

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Fig. 3. (A-D) 10-year-old patient with a slowly expanding bony swelling in the left infraorbital and cheek region, causing obliteration of the nasolabial fold. (E, F) Large unilocular expansile lesion, filling up the entire left maxillary antrum and extending laterally by perforation of the anterolateral wall, and medially into the nasal cavity. Orbital floor (roof of the antrum) appears intact. A displaced second molar tooth germ was seen within the large lytic lesion, giving the impression of a Dentigerous cyst. (G) Expansion of the buccal cortical plate evident in left molar region. (H) Aspiration of clear straw coloured cystic fluid from within the cystic lesion. (I–K) Mucoperiosteal flap raised exposing the thinned out cortical bone with perforations, revealing the cystic lining of the lesion within. (L-N) Cyst enucleated in toto along with its entire lining. (O) The entire antral lining was carefully extirpated as well, as the lesion was an IH proven case of Dentigerous cyst with evidence of Ameloblastomatous change. Infraorbital Nerve carefully dissected and preserved. (P) Enucleated pathological specimen. (Q-S) Chemical curettage of the bony defect and walls of the maxillary antrum carried out using Carnoy's solution, after carefully isolating the Infraorbital Nerve and the adjacent soft tissues using Vaseline gauze. (T, U) Gross appearance of the cystic lesion with the unerupted second molar tooth germ within and the clear cystic fluid aspirate typical of a Dentigerous cyst. (W-Z) Histopathological examination of H&E stained sections of the incisional biopsy, revealed Fibrous capsule with a hyperplastic epithelial lining, both of which contained a dense chronic inflammatory infiltrate. Few areas showing the typical tall columnar basal cells with reverse palisading of the nuclei, indicative of ameloblastomatous change. (AA′-AD′) Immunohistochemical examination of the biopsy specimen revealed Calretinin positivity expressed by the epithelial cells, suggestive of their ameloblastomatous transformation. (AE′-AH′) A positive, but low Ki-67 Labelling Index of 2.1 expressed by the cells in the epithelial zone. (AI′-AL′) A low PCNA Proliferative Index of 1.7, indicative of a lesion with a low invasive potential.

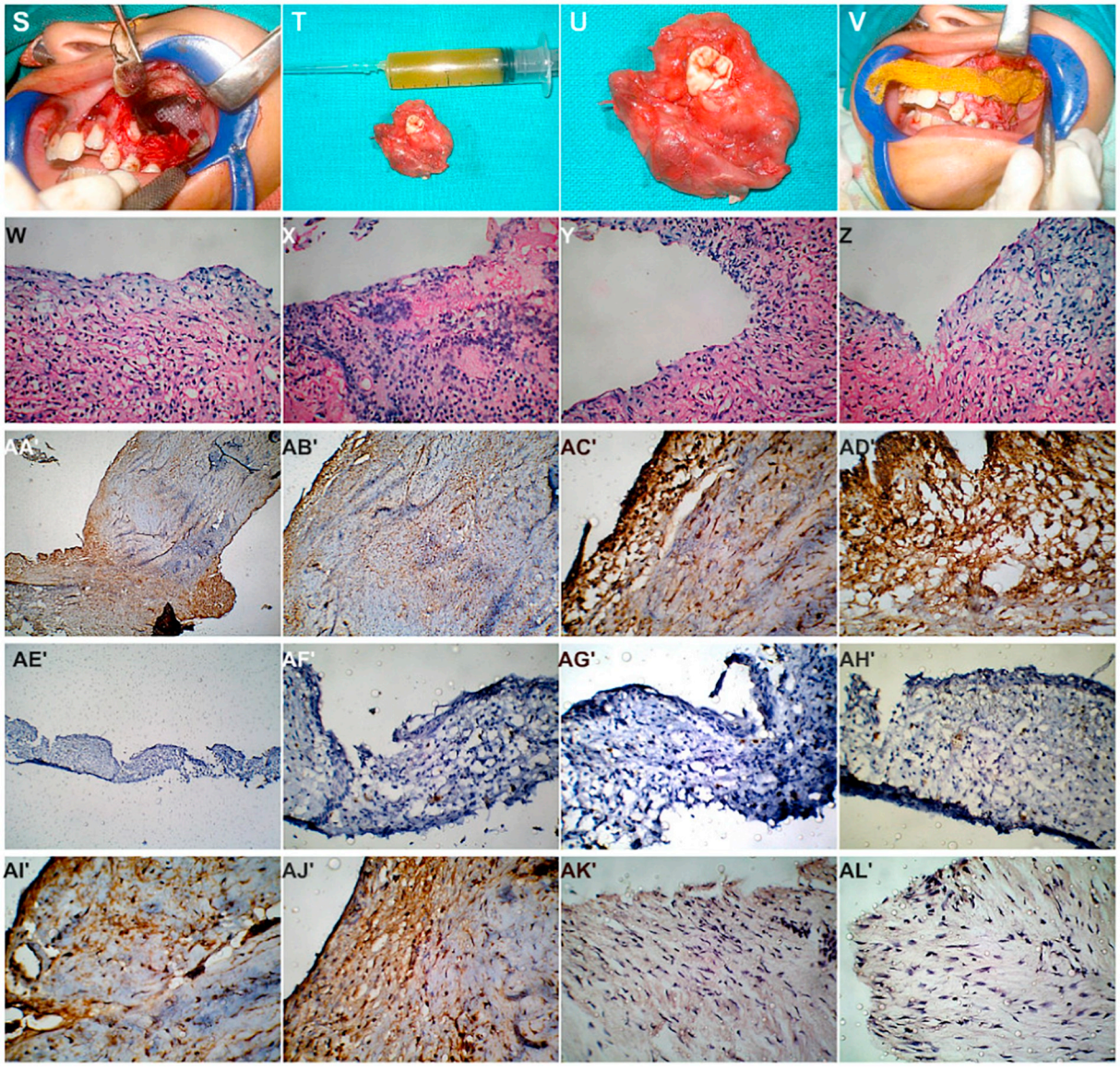

Fig. 3. (*continued*)

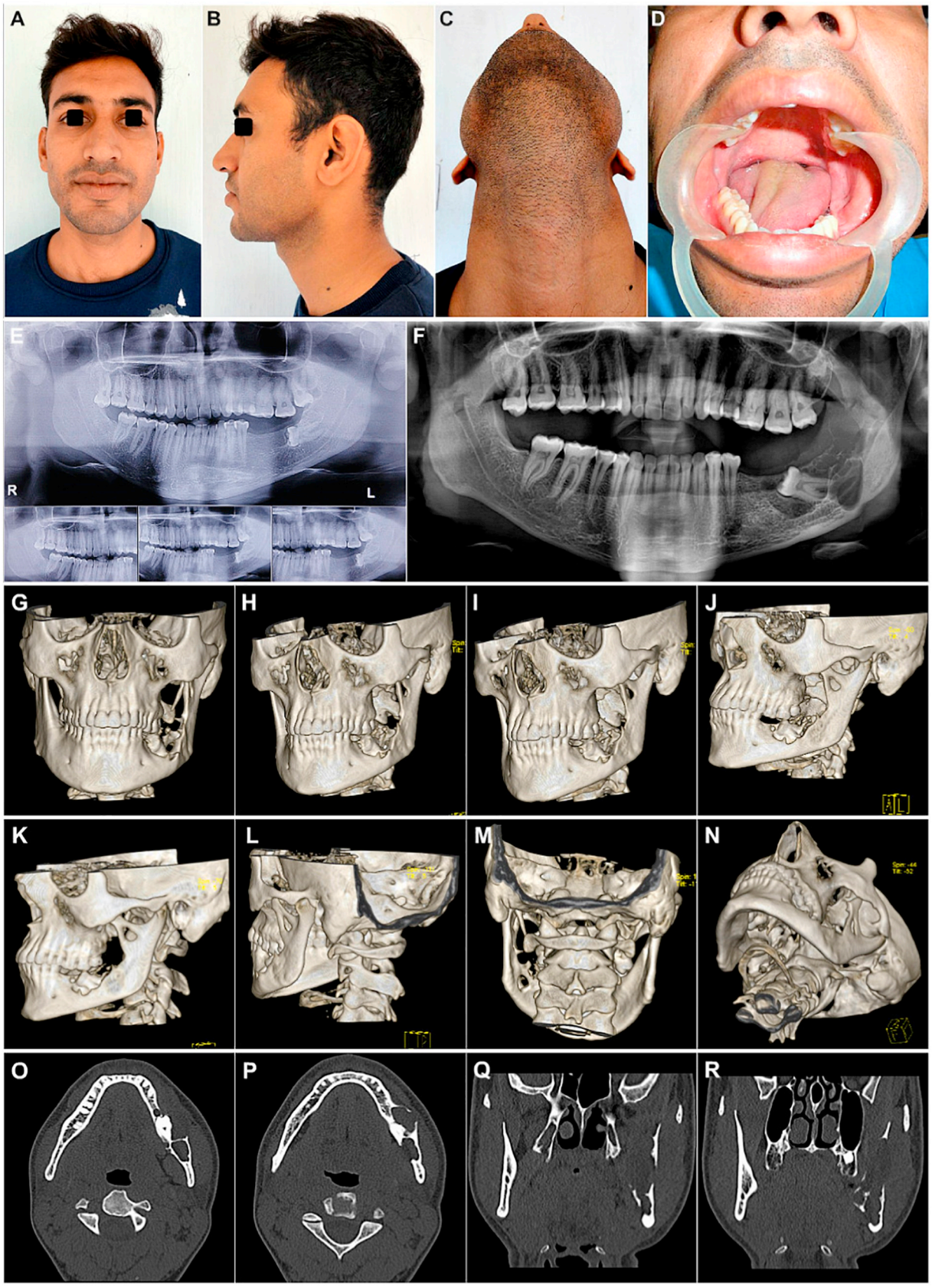

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Fig. 4. (A-D) 23 yr old male patient who reported with complaints of pain and swelling in the region of left angle of mandible. On intraoral examination, all three molars were missing on the left side. (E) OPG taken at 13 yrs. of age. History revealed that 10 years the patient had been managed by marsupialisation of a cystic lesion in the same region, and removal of lower left first and second molars (teeth 36 and 37) which were involved. (F-R) Present Radiographs revealed an extensive unilocular radiolucency involving the left angle and ramus of the mandible extending posteriorly almost up to the tip of the coronoid process. A horizontally impacted third molar lay within the radiolucent space. (S—U) Histopathological examination of an incisional biopsy revealed features of a Dentigerous cyst with a hyperplastic stratified squamous epithelial lining of varying thickness. (V, W) IH examination revealed positive expression of Calretinin by the epithelial cells lining the cyst forming a brownish band-like appearance. This was suggestive of a Unicystic Ameloblastoma with no luminal or mural proliferation of the tumor cells. (X, Y) There was positive Ki-67 expression by few of the basal cells of the epithelial lining of the cyst. The Ki-67 Labelling Index was very low LI = 0.9, indicating a tumor of insignificant aggressive or invasive potential. (Z) PCNA labelling index was low as well LI = 0.5 (AA′-AF′) Conservative treatment of Enucleation and curettage with removal of the impacted 38 was carried out. (AG′) This was followed by Chemical cauterization of the walls of the bony cavity with Carnoy's solution. (AH′ –AL′) Fresh autologous Platelet Rich Fibrin was placed within the residual cavity to encourage subsequent bone fill, followed by closure.

Fig. 4. (*continued*)

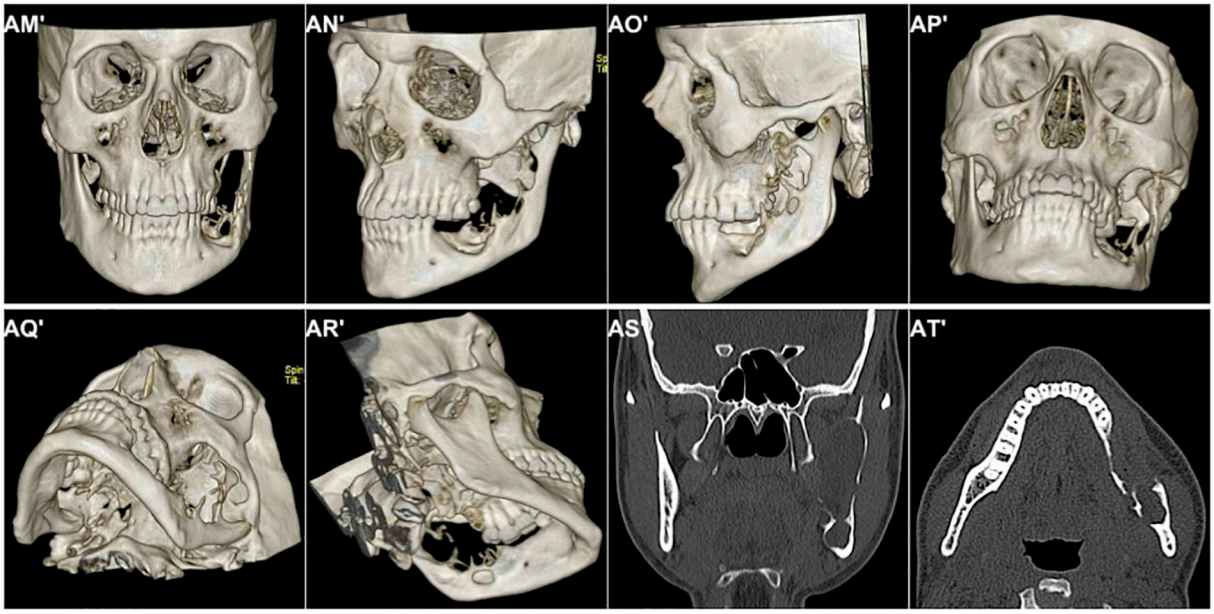

Fig. 4. (*continued*)

prognosis, including likelihood of recurrence, and so on [11,18].

The details of the Immunodiagnostic procedures employed, management protocols implemented thereafter and follow up findings have been presented in this Study.

2. Aim

To establish the value of Immunohistochemistry as a Diagnostic marker and Prognostic indicator for extensive Unilocular radiolucent lesions of the jaws. To assess its role as an adjunct to Histopathological Examination (HPE) in distinguishing Odontogenic tumours from the cysts, by identifying the former using IH Tumor Markers. To assess its role in aiding in selection of the most appropriate, optimal and effective treatment option for each of such ambiguous lesions, based on their prognosis as indicated by the Proliferation Index (PI)/Labelling Index (LI) values of the lH Cell Proliferation Markers.

3. Objectives

a) To carry out a Histopathological (HPE) study and correlate it with an Immunohistochemical (IH) analysis of 30 cases of large Unilocular radiolucent lesions of the jaws (Maxilla/Mandible), and to examine and assess the IH expression of –
   i) Calretinin a '*Tumor Marker*', to identify and distinguish the Odontogenic tumor from the cyst, and to arrive at a confirmatory diagnosis;
   ii) '*Cell Proliferation Markers*', namely Ki-67 and Proliferating Cell Nuclear Antigen (PCNA), to evaluate the nature and predict the clinical behavior of these lesions, based on their Labelling Index values.

b) To establish a correlation between the Histopathological variant of the Cyst/Tumor and its Immunohistochemical Proliferation/ Labelling Index, so as to predict the pathology's aggressive potential and potential for recurrence.

c) To establish a management protocol for each of the large Unicystic jaw pathologies (Cysts and Tumours), based on their prognosis as indicated by the Immunohistochemical Proliferation Index (PI)/ Labelling Index (LI) value.

d) To evaluate the early and late post-operative complication rates –
   i) Jaw fracture
   ii) Infection
   iii) Neurological deficits
   iv) Jaw deformity
   v) Functional debility (Impaired Masticatory efficiency, Deranged Occlusion, Reduced Interincisal Mouth opening)
   vi) Recurrence of the lesion

4. Material and methods

Thirty patients of age groups ranging from 10 to 80 years, with large Unicystic lesions involving either the Maxilla or Mandible, were managed over a period of three years. Inclusion criteria included patients with a single Unilocular Radiolucent Lesion involving either the maxilla or the mandible. Patients with Multilocular radiolucent lesions or with multiple radiolucent lesions of the jaws were excluded from this Study.

Histopathological examination (HPE) and Immunohistochemical (IH) analyses were carried out on tissue samples from the lesions at the biopsy stage itself, to establish a confirmatory diagnosis and also assess the clinical nature of the lesions, in order to formulate an ideal treatment plan for each individual case.

HPE was performed using Hematoxylin-Eosin stained sections observed under magnifications of 10×, 50× and 100×. IH studies were carried out, by staining for Calretinin, Ki-67 and Proliferating Cell Nuclear antigen (PCNA). Calretinin is an intracellular antiapoptotic protein, exclusively expressed in all types and variants of Ameloblastoma and none of the Odontogenic cysts. Hence, it served as a valuable specific and confirmative diagnostic marker for variants of Ameloblastoma, distinguishing them clearly from other Odontogenic Cysts and pathologies. IH studies of the tissue samples were also carried out to detect expression of Ki −67 Ag and Proliferating Cell Nuclear Antigen (PCNA) by the cells of the lesions. Both of these are Cell proliferation markers, expressed in various stages of the Cell cycle in actively dividing cells, thus giving an idea of the aggressive potential of the lesions.

IH examination was performed in all 30 cases using the Avidin-Biotin Immunoperoxidase (ABI) Complex method, using Diamino-

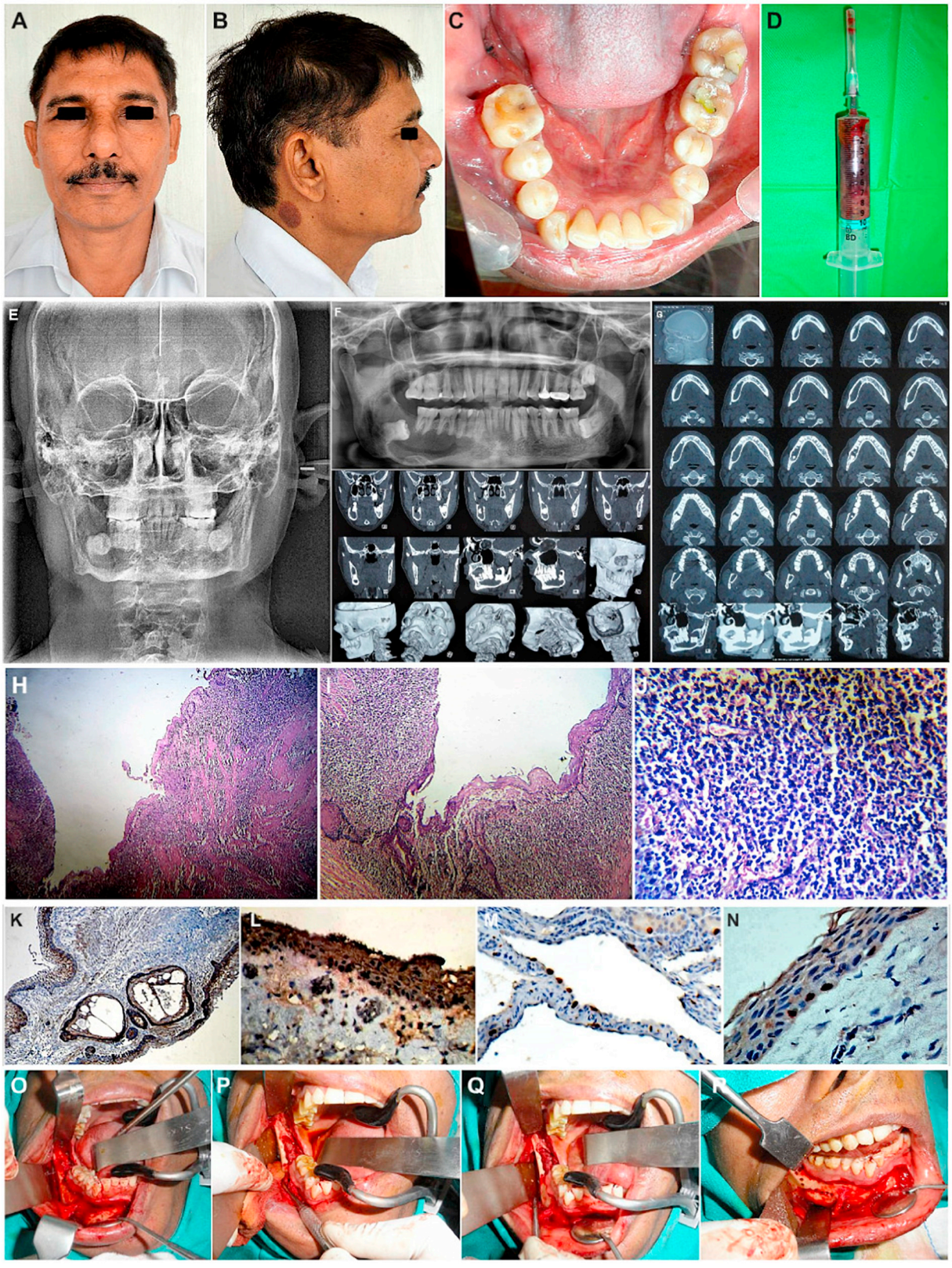

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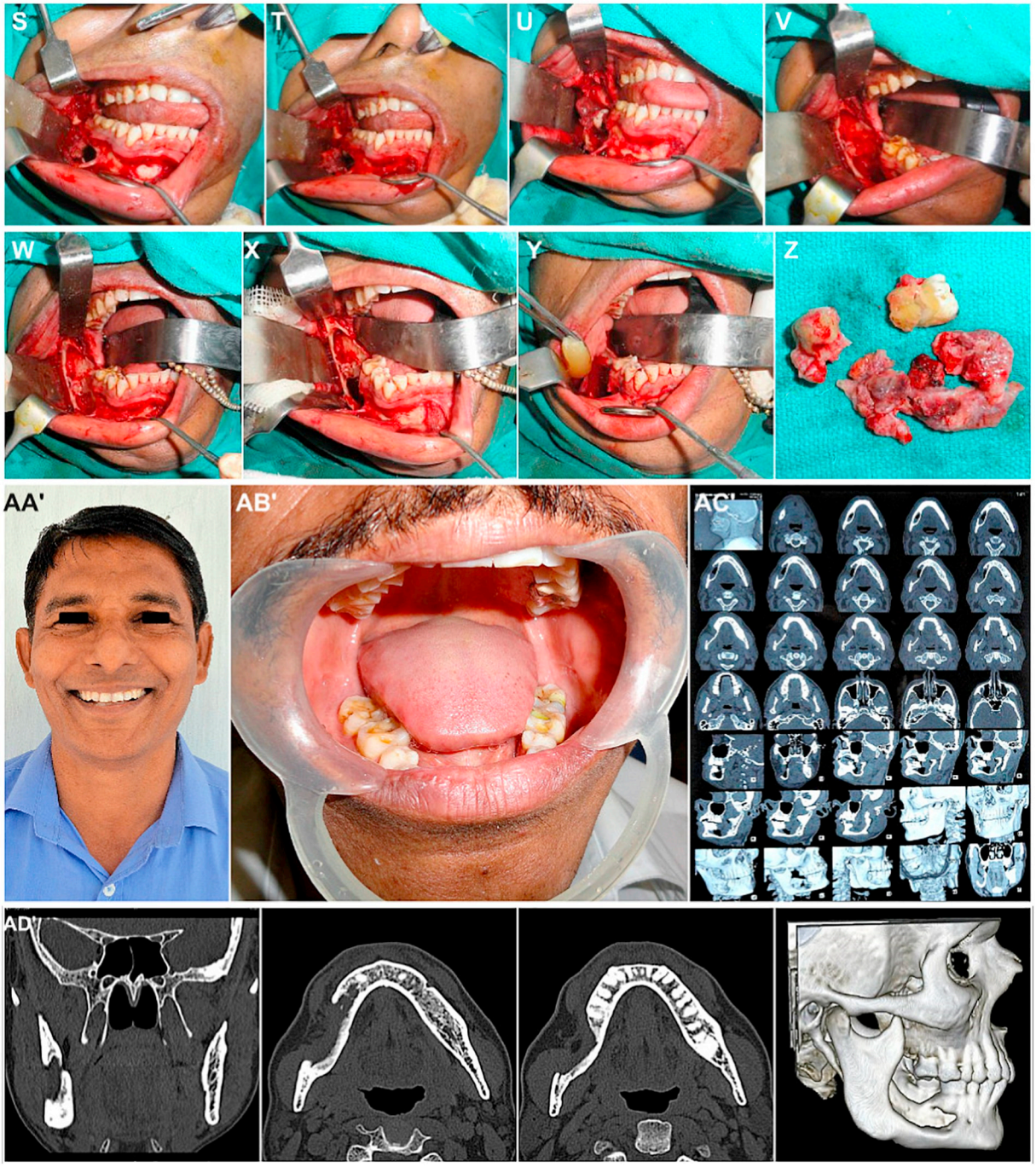
Fig. 5. (A, B) 55 yr old patient with the complaint of persistent oozing of a foul smelling liquid from the right lower molar region. (C) Intraoral examination revealed inflamed and tender right retromolar region, with missing lower right first, second and third molar teeth (46, 47 and 48). (D) Aspiration using a wide bore needle revealed foul smelling purulent material admixed with blood. (E, F) Radiographs (Paranasal Sinus PNS view skull and Orthopantomogram OPG) revealed a large unilocular radiolucency involving the right angle and ramus region of the mandible, with the horizontally impacted and displaced tooth 48 lying within. 38 was also impacted, however uninvolved with any evident pathology. (G) NCCT showed extensive destruction and hollowing out of the angle and ramus region with the impacted third molar. There was expansion of the buccal and lingual cortical plates as well as widening of the anterior border of the ramus in addition to a expansion and bowing of the inferior border of the mandible on the right side. The posterior border of the ramus appeared intact. (H-J) Histopathological examination of an incisional biopsy revealed a cystic lining comprising of stratified squamous epithelium of varying thickness with a dense inflammatory infiltrate in the subepithelial zones and fibrous capsule, suggestive of a Dentigerous cyst. At a few locations, the epithelial cells appeared tall, columnar with reverse palisading of their nuclei. (K, L) Immunohistochemical evaluation revealed a strong uptake of Calretinin by the epithelial cell, clearly indicative of an Ameloblastomatous transformation or a Unicystic Ameloblastoma. (M, N) Labelling Indices of Ki-67 and PCNA were 1.8 and 1.6 respectively, indicative of a low aggressive potential. (O-T) Lesion was uncovered by removing buccal cortical plate of bone. (U, V) Pathology along with Cystic lining enucleated and involved tooth 48 removed, followed by thorough curettage. (W) Peripheral ostectomy carried out, removing 2 mm of bone all along the margins and walls of the bony cavity. (X) Chemical cauterization of the cavity walls using Carnoy's solution. (Y) Autologous Platelet Rich Fibrin placed within the cavity followed by watertight closure. (Z) Enucleated cystic pathology and the tooth within. (AA′, AB′) Post-operative photographs showing a good healing of the operated site. (AC′-AE′) Post-operative NCCT with 3-D reconstruction showing the large residual bony defect. (AF′) A comparison of the OPGs taken immediately following surgery and at one and 3 months postoperatively, showing a good progress in reossification and bone fill of the bony cavity, especially at the inferior border of the mandible.



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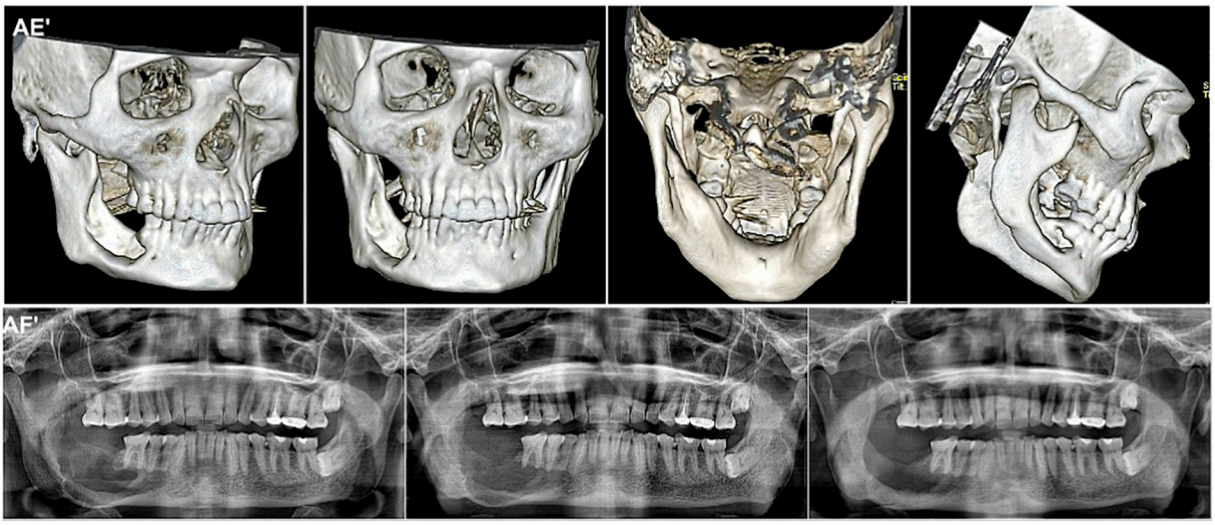


Fig. 5. (*continued*)

benzidine (DAB) as the chromogen/dye for visualization. Positivity was expressed as brown nuclear staining of the cells.

4.1. Steps in IH staining

The slides with the tissue specimen were incubated overnight at 4^0C with the Primary Monoclonal Antibody raised against the three Human Antigens of interest that were to be detected, namely, the Calretinin, Ki −67 Ag and Proliferating Cell Nuclear Antigen (PCNA).

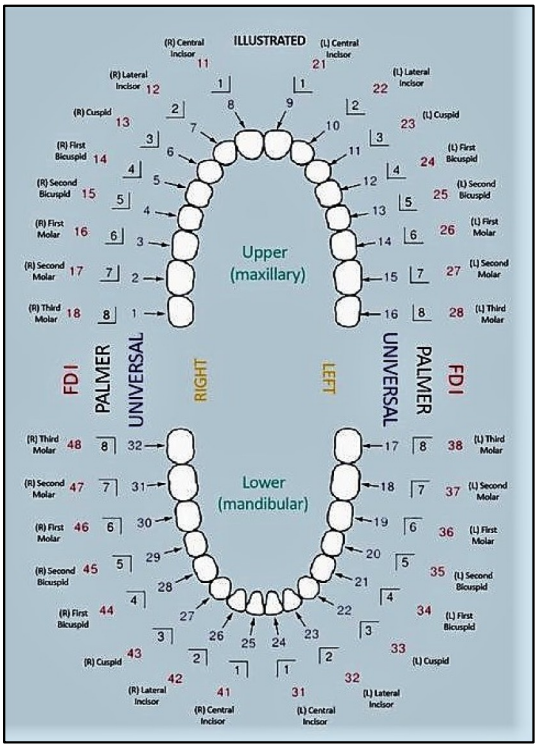
Primary Mouse Monoclonal antibodies were used against Ki-67 (Clone MIB-1; 1:100 dilution, Dako, Carpinteria, CA, USA) and PCNA (Clone PC10; dilution 1:100, Dako, Carpinteria, CA, USA). For Calretinin, optimally and precisely prediluted Rabbit Anti- Calretinin polyclonal primary antibody (Biogenex laboratories Inc., USA), was employed. The slide was then washed in phosphate buffered saline and incubated with the Secondary antibody, which is biotinylated, that is, conjugated with Biotin (Vitamin H/B7) and with the streptavidin/peroxidase complex for 30 min each (LSAB þ-labeled streptavidin-biotin, Dako).

Thus the secondary Ab was the labeled Ab, unlike the Primary Ab which was unlabeled. An egg white protein called Avidin has a very strong affinity for Biotin, so an Avidin- Horseradish Phosphate (HRP) Complex was used as the reporter molecule which forms the ABI complex which can be visualized or developed using DAB as the chromogen which is turned brown by the HRP. Thus, the nuclei expressing Ki 67 Ag and PCNA Ag turn brown. The Ki-67 and PCNA Labelling Index (LI) or the Index of Positivity/Proliferation Index (PI) was calculated by counting the number of positive cells in five histopathological fields, dividing by the total number of cells in that field and multiplying by 100.

On one end of the spectrum was a negative Calretinin, Ki-67 and PCNA expression of the tissue samples from the monocystic lesion, which ruled out Odontogenic tumours and was confirmatory of the Histopathological diagnosis of an Odontogenic cyst. On the other end of the spectrum was positivity for all the three IH markers with a high Labelling Index (LI)/Proliferation Index(PI), indicative of an Odontogenic tumor with high aggressiveness and recurrence potential.

4.2. Surgical protocol employed

Nerve sparing enucleation and curettage was carried out for all the cases confirmed as Odontogenic cysts, with a negative expression of Ki-67/PCNA (Labelling Index = 0). Enucleation and curettage, peripheral ostectomy, followed by chemical cauterization using Carnoy's solution was employed for the Unicystic Ameloblastomas and other Odontogenic tumours with a low Ki-67 and PCNA Proliferation Index (PI)/Labelling index (LI) values ≤ 3. In all the cases diagnosed as Odontogenic tumours exhibiting a LI > 03 (LI > 3 ≤5), a more aggressive protocol of marginal resection including 1 cm of apparently healthy bone all along the margins of the lesion was carried out. All the aggressive lesions and pathologies with Labelling Index exceeding 5 (LI > 5), were managed by segmental resection of the jaw (including partial/complete Maxillectomy, Hemimandibulectomy with/without disarticulation) with primary or secondary reconstruction. Teeth depiction is as shown (as per the Universal system of Tooth numbering).



Universal system of tooth numbering

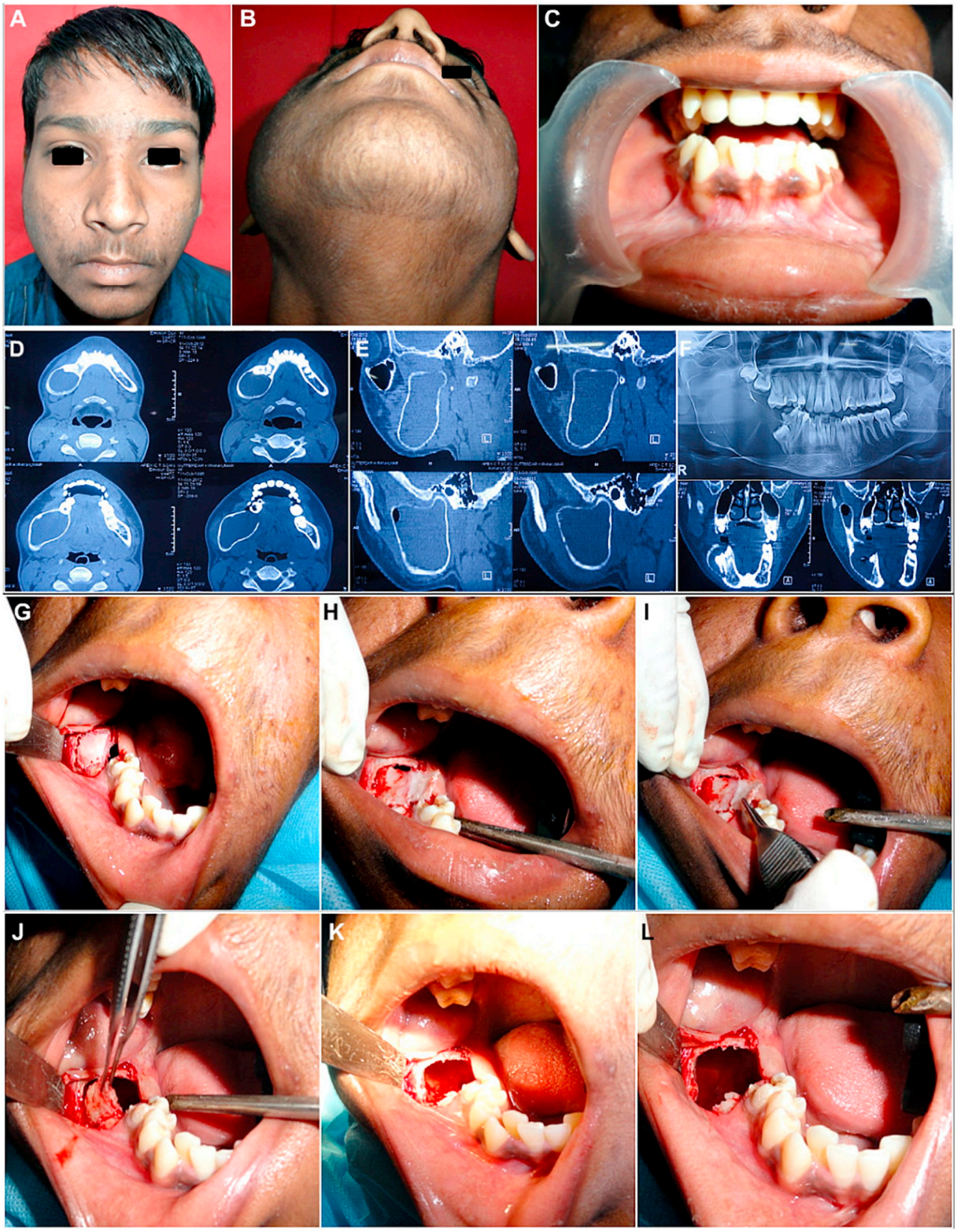


Fig. 6. (A-C) 17 yr old male patient with a large swelling over the right side of the mandible. There was considerable expansion of the buccal and lingual cortical plates. (D-F) Radiographs and CT scans revealed a single very large unilocular radiolucency involving the entire ramus, angle and body of the mandible on the right, which was found associated with an impacted second molar tooth, the third molar being missing. (G-I) An incisional biopsy was carried out, an almost eggshell thin plate of bone peeled away quite easily from an underlying thick cystic lining. (J-L) On removal of the thinned out window of cortical plate, a large cave-like cavity was seen hollowing out the jaw. (M-O) On histopathological examination, the cystic lining in some areas was composed of innocuous looking stratified squamous epithelium, resembling a Dentigerous cyst. In other areas, however, the epithelium was seen to be proliferating into the underlying connective tissue wall of the cyst giving a distinct impression of the mural variety of Unicystic Ameloblastoma. (P-R) Strong Calretinin positivity expressed in all histopathological fields, confirming the diagnosis of Ameloblastoma. (V, W, X) High Ki-67 LI of 6.6 and PCNA LI of 5.2, indicative of the high aggressive potential of the mural variety of Unicystic Ameloblastoma, as compared to the simple Unicystic and luminal types, and the need for them to be treated as the classical intraosseous Ameloblastoma.

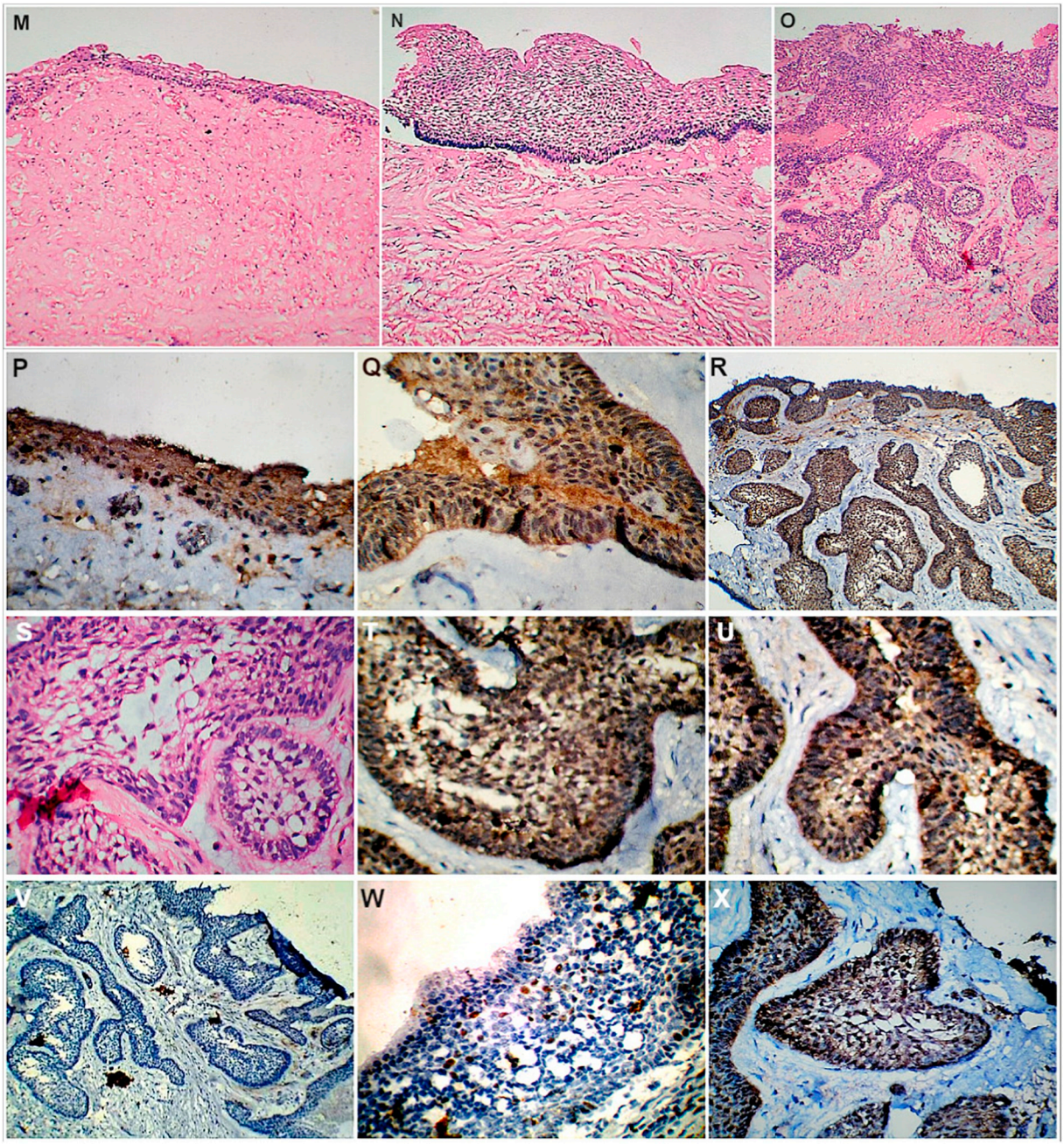


Fig. 6. (*continued*)

5. Results

Each of the thirty cases of large Unilocular radiolucent lesions of the jaws was identified and classified based on its Clinical and Radiographic features and a confirmatory diagnosis was then arrived at by correlating the HPE and IH analysis of biopsy specimens (Table 1). A correlation was also drawn between the Labelling Index values of the IH proliferation markers Ki-67 and PCNA, with the histological typing of the pathologies (Table 2), which also dictated the treatment plan for each case.

The expression of Ki-67 and PCNA were strongest (Average values of LI 6.8 and 6.45 respectively) in the squamous arcades of cells and areas of squamous metaplasia in the central areas of epithelial islands of the Acanthomatous variety of Ameloblastoma, confirming them to be

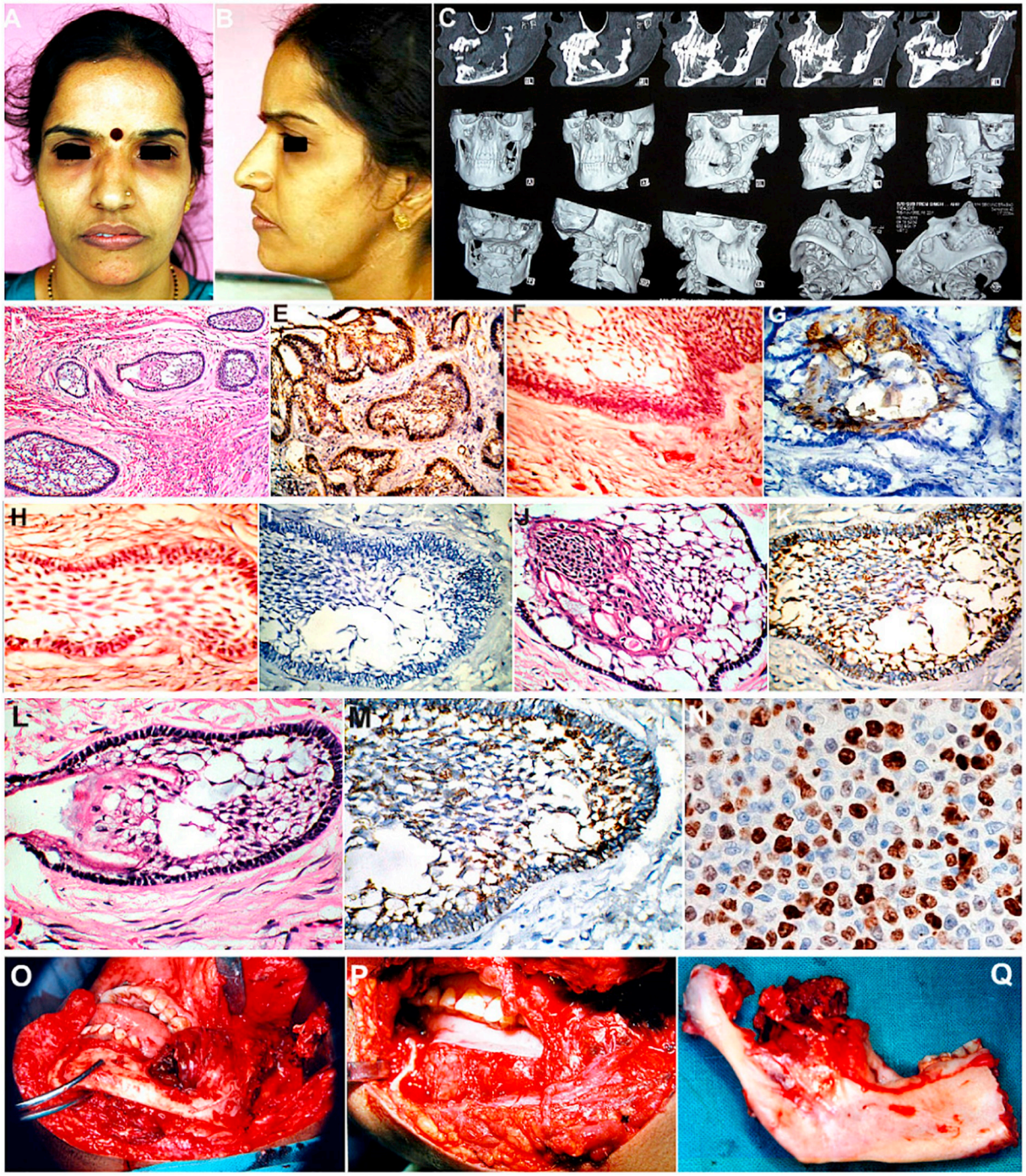


Fig. 7. (A- C) 40-year-old female patient with an extensive unilocular radiolucent lesion involving and hollowing out most of the left angle, ramus and part of body of the mandible. (D-G) Incisional biopsy confirmatory of Follicular Ameloblastoma. H&E stained sections compared with corresponding histopathological fields of tissue sections showing strong Calretinin positivity exhibited by the central and peripheral cells of the follicles as well as by cells lining the areas of cystic degeneration within the follicles. (H, I) Certain areas showed negative uptake of Calretinin. (J, K) High PCNA Proliferative Index of 6.4 exhibited by both the peripheral basal cells as well as the central polyhedral cells. (L-N) A high Ki-67 Labelling Index of 6.9 (Magnification X100, X150, X250). (O-Q) Tumor exposed followed by Hemimandibulectomy.

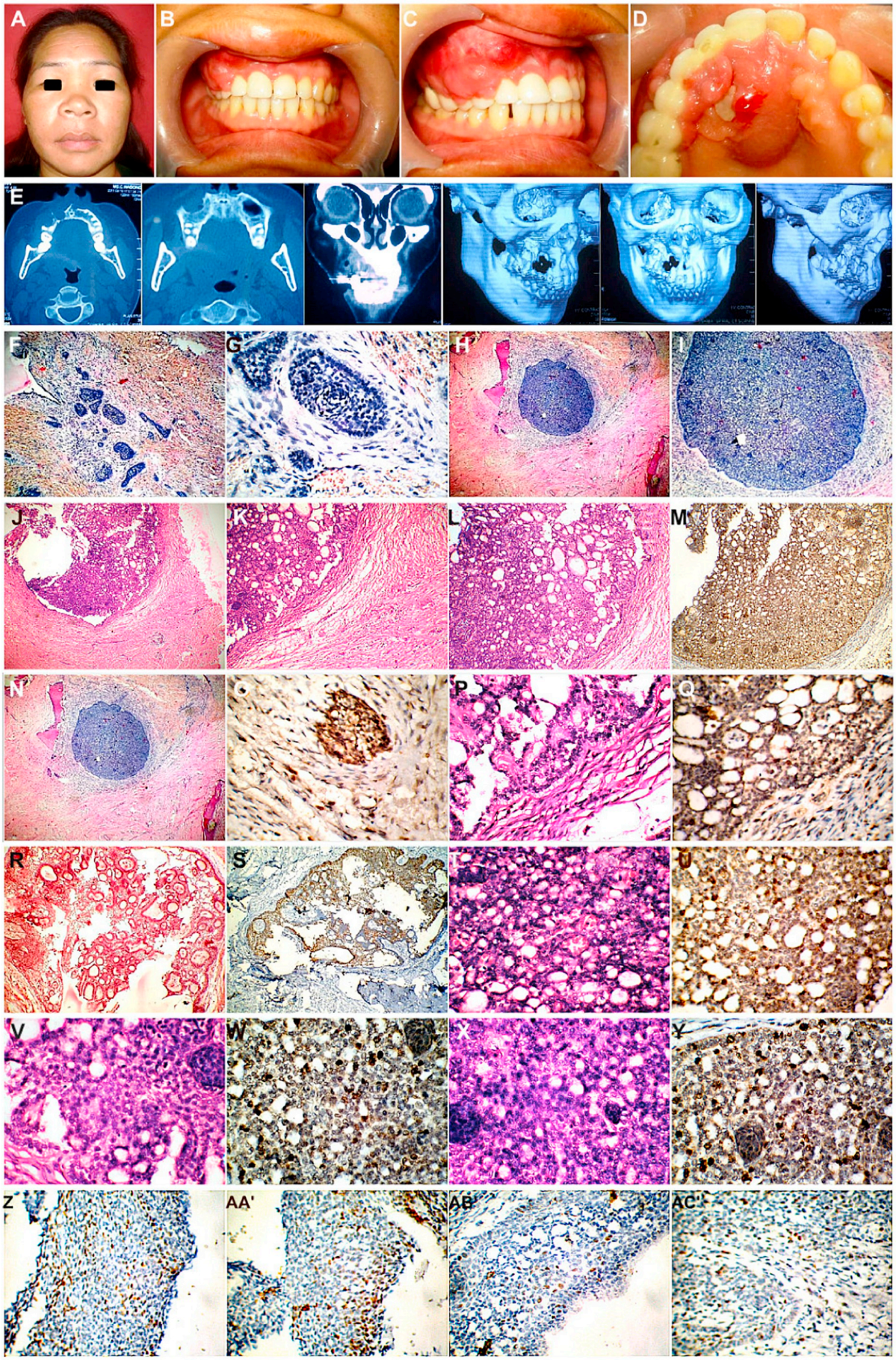


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Fig. 8. (A-D) 39 yr old patient with an Exophytic gingival swelling with expansion of both, the alveolar ridge as well as the hard palate extending from the left upper central incisor to right second premolar tooth (21 to 15) region. (E) NCCT revealed a large Unilocular radiolucency involving Rt Maxilla, causing destruction of the alveolar process and the anterolateral wall of the maxillary antrum. (F-K) Histopathological examination confirmed the diagnosis as the Follicular variety of Ameloblastoma and revealed richly cellular odontogenic epithelial tumor follicles, set in a dense fibrovascular connective tissue stroma. (L-U) Hematoxylin & Eosin stained sections of tumor tissue compared with corresponding fields of Calretinin stained sections, revealing strong and diffuse Calretinin positivity expressed by the tumor cells in all the histopathological fields. (V—Y) H&E sections compared with corresponding fields depicting PCNA positivity. High PCNA LI of 6.6. (*Z*-AE′) Ki-67 LI of this tumor was very high at 7.3, the large number of cells taking up the brown nuclear stain can clearly be visualized. (AF′-AK′) Partial maxillectomy (Rt) including the alveolar ridge and anterior hard palate from 22 to 16 region, with removal of antral lining. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

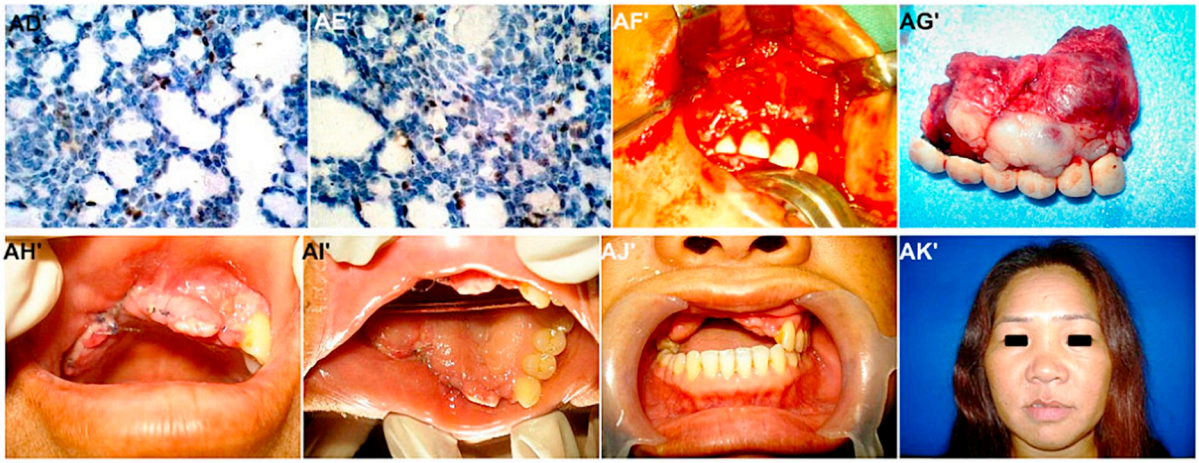

Fig. 8. (*continued*)

the most aggressive type. This was followed by the Plexiform variety (Average LI of 6.6 and 5.9 for Ki-67 and PCNA respectively), thereafter by the Follicular variety of Ameloblastoma (Average LI of 6.45 and 5.9), and the positivity was seen more in the peripheral or basal cell layer of the epithelial strands, follicles or islands, so it is probably these outer cells which account for the growth activity and progression of the neoplasm and form its proliferative front. These tumours were managed by segmental jaw resection followed by primary or secondary reconstruction.

The mural variety of Unicystic Ameloblastoma exhibited a high LI value of 6.6 and 5.2 for Ki-67 and PCNA, and had to be treated like a solid multicystic Ameloblastoma.

Last of all the were the Unicystic Ameloblastomas, with an average LI of 2.25 and 1.05 for Ki-67 and PCNA respectively, and could be successfully treated by enucleation and curettage, peripheral ostectomy of the bony margins, followed by chemical cauterization.

The Ameloblastic Fibroma, although negative for Calretinin, showed a moderate expression of both Ki-67 and PCNA (3.8 and 3.4 respectively) and was managed by marginal resection, preserving continuity of inferior border of the mandible.

The cases of Adenomatoid Odontogenic Tumor (Adenoameloblastoma) showed a negative expression of both the Proliferation markers, and hence were managed conservatively by Enucleation, Curettage, Peripheral ostectomy and Chemical cauterization with Carnoy's solution.

There were no significant immediate post-operative complications noted in any of the cases, other than moderate oedema and swelling in three cases. There was nil incidence of late complications such as recurrence in any of the cases managed. Neurological deficits such as Inferior alveolar nerve and Infraorbital nerve paraesthesia was noted in the cases of marginal/segmental resection. No pathological fracture took place in any of the cases.

6. Discussion

An Immunodiagnostic study of biopsy tissue samples in all the thirty cases of large Unilocular radiolucent lesions of the jaws, was carried out using Calretinin, Ki-67 and PCNA, and the results were corroborated with the Histopathological findings. This helped in arriving at an accurate diagnosis with a precise distinction between cyst and tumor. It also aided in formulating the most appropriate management modality for each case, based on the prognostic evidence provided by the IH Cell Proliferation Markers.

Calretinin is an intracellular antiapoptotic protein [19], exclusively and consistently expressed in all types and variants of Ameloblastoma and none of the Odontogenic cysts [2]. Hence, in this Study, where there often was a confusion as to whether a unilocular radiolucency was a Dentigerous cyst, a Unicystic Ameloblastoma or a Keratocystic Odontogenic Tumor, Calretinin served as a valuable, reliable, specific and confirmative diagnostic marker for Ameloblastomatous tissue,

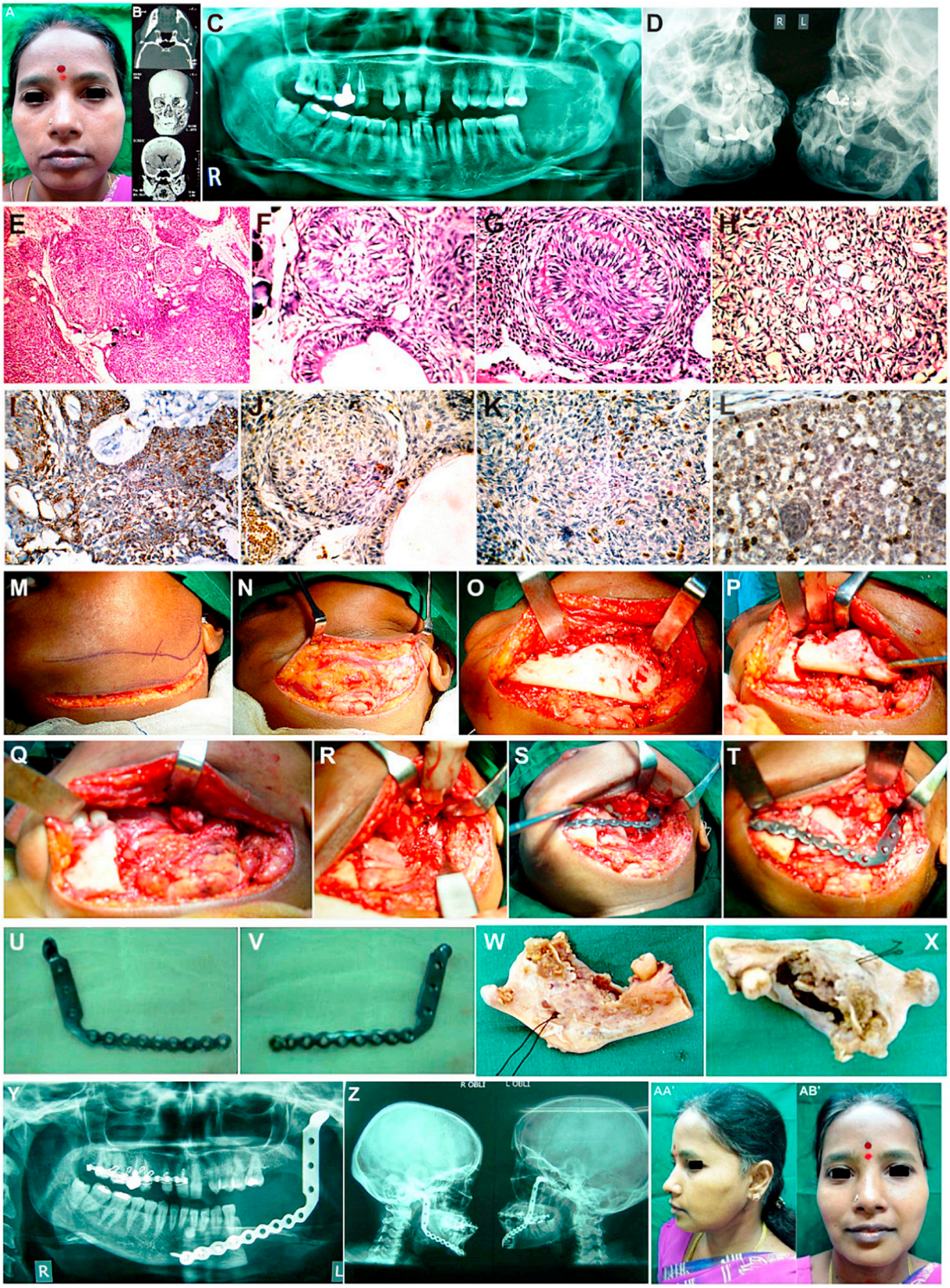

Fig. 9. (A-D) 35-year-old female patient with extensive destruction of the left ramus and angle of the mandible caused by a single, large Unilocular radiolucent lesion. (*E*-H) Incisional biopsy revealed round epithelial follicles with reverse palisading of the peripheral Ameloblastic cells confirming it to be of the Follicular variety of Ameloblastoma. (I, J) Diffuse Calretinin positivity exhibited by both the peripheral as well as central cell of the follicles. (K) High Ki-67 LI of 5.8. (L) High PCNA LI of 5.6. Both, the rich cellularity of the tumor in all histopathological fields as well as high Proliferative indices for both Ki-67 and PCNA, were indicative of its aggressive and invasive nature. (M-R) Resection of the left ramus and angle of the mandible with disarticulation was carried out, with removal of a 1.5 cm margin of apparently sound bone. (S—V) Immediate reconstruction was carried out using a Reconstruction plate with a condylar element. (W-X) Excised specimen. (Y-AB) The native articular disc had been retained (visible in panel R as white and glistening; and the metal condylar replacement articulated against this native disc, with excellent functional as well as cosmetic results post operatively.

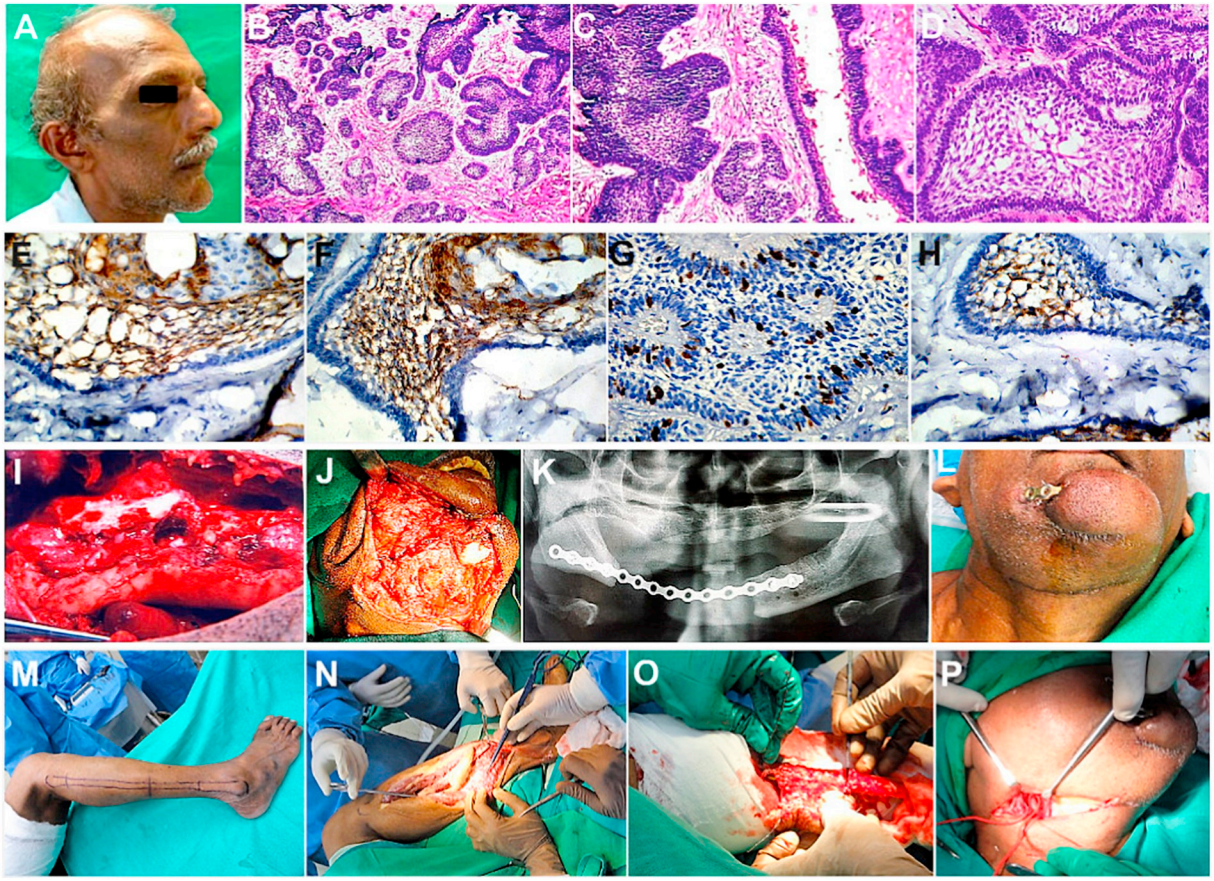

Fig. 10. (A-D) 69 yr old patient with a large radiolucent lesion involving the right body of mandible, diagnosed as the Follicular variant of Ameloblastoma. (E, F) Strong Calretinin positivity expressed by cells of the follicular islands, more in the central stellate reticulum like cells and cells lining areas of cystic degeneration within the follicles. (G) Ki-67 positivity expressed mainly by the outer/peripheral/basal Ameloblastic cells lining the epithelial islands with only occasional positivity in the central stellate reticulum-like cells, thus indicating that it is probably these Ameloblastic cells that are the proliferating cellular component and form the advancing front and reflect the growth activity of the tumor, accounting for its progression. Ki-67 LI = 5.8. (H) PCNA Labelling Index = 4.9. (I-L) Segmental resection was carried out jaw continuity maintained using a Recon plate. (L) Four months later, there was infection and exposure of the implant. (M-P) Reconstruction plate removed and secondary reconstruction of the defect carried out using the Free fibular bone graft.

distinguishing and differentiating them clearly from Odontogenic Cysts and other pathologies [20].

Proliferating cells progress along the so-called “Cell cycle”, where they can occupy any of the functional states [21]. When cells are in the active phase of the Cell Cycle, they express certain proteins like Ki-67, PCNA, AgNORs (Silver staining Nucleolar Organizer Regions) etc., which can be detected by IH means using Poly- or Monoclonal antibodies [22]. ***Ki-67 Antigen*** is one such nuclear protein which is essential for maintaining the Cell Cycle and is expressed by all proliferating cells in all the active phases of the cell cycle, like the ‘S' or the Synthesis phase, which is the DNA synthesis and replication phase, ‘G2’ which is the post synthetic gap, during which there is intense biochemical activity and spindle protein assembly, ‘M' or the Mitotic phase, in which karyokinesis and cytokinesis occurs, and ‘G1’ or the post-mitotic gap; except for the ‘G0’ or Resting phase [11]. It can be detected using MIB1 (Mindbomb Homolog 1) murine monoclonal antibody and thus, in any given cell population, the cells which are positive for Ki-67 are the ones that are actively proliferating, so it is an extremely reliable and reproducible cell proliferation marker [23].

***PCNA* (*Proliferating Cell Nuclear Antigen*)** is a cofactor of DNA Polymerase delta, which actively participates in DNA replication and repair. Hence PCNA is an important Cell cycle associated Antigen, which serves as a very useful Cell proliferation marker, which is typically expressed by those cells which are in the G1 and S phases of the cell cycle, when the DNA synthesis occurs [24].

The Biological behavior (in terms of aggressiveness, invasiveness, tendency for recurrence and malignant transformation) and Probable prognosis of Odontogenic tumours is rather difficult to predict on the basis of Clinical, Radiographic and Histological assessment alone [25]. This is where the value of Prognostic proliferative IH markers steps in,

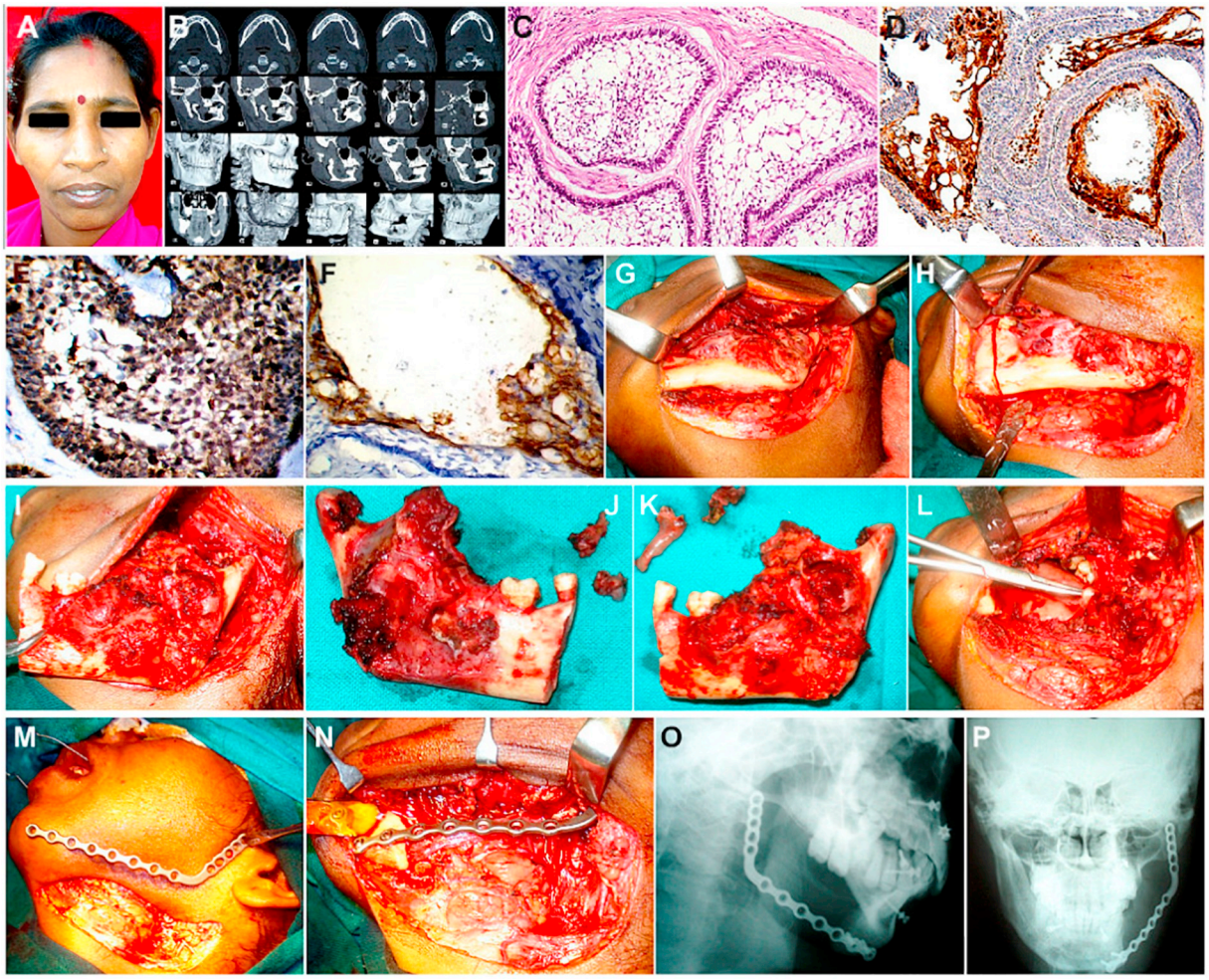

Fig. 11. (A, B) 39-year-old female patient with a Unilocular radiolucent lesion with osteolytic destruction and hollowing out of the left ramus, angle and part of body of the mandible. (C) HPE (H&E) sections of the biopsy specimen was confirmatory of the Follicular variant of Ameloblastoma, with evidence of Acanthomatous change/differentiation at the center of many of the follicles. (D) Strong positivity for Calretinin was expressed by the cells of the tumor follicle, particularly by the tall columnar cells lining the follicles, with moderate positivity in the areas of cystic degeneration within. (E) A high Ki-67 Labelling Index of 7. (F) A high Labelling Index of PCNA of 6.5, prognostic of the high aggressive potential of the tumor. (I-L) Mandibular resection with disarticulation carried out. (M-P) Immediate stabilization carried out using a Titanium Reconstruction plate, with secondary reconstruction to follow at a later date.

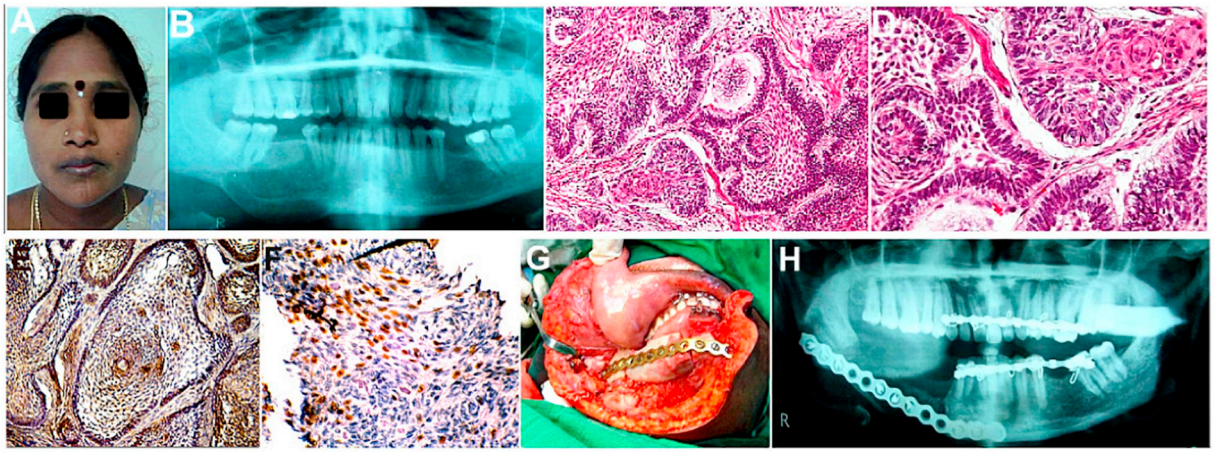

Fig. 12. (A, B) 34 yr old patient with a large unilocular radiolucent lesion involving the right body of mandible. (C, D) Histopathological examination of biopsy specimen confirmed Acanthomatous Ameloblastoma. (E) Immunohistochemical evaluation showed positivity for Calretinin throughout the epithelial follicles, by the outer peripheral basal cells of the epithelial follicles as well as the inner cells which had undergone squamous metaplasia. (F) A high Ki-67 Labelling Index value of 6.6, was indicative of an aggressive tumor. (G, H) Segmental resection of body of mandible with continuity defect was carried out followed by immediate reconstruction using an Iliac crest block graft stabilized and fixed in place using a Titanium reconstruction plate.

Fig. 13. (A, B) 40-year-old female patient with a large radiolucent lesion involving the entire ramus, angle and part of mandibular body. (C) Incisional biopsy was confirmatory of Acanthomatous variety of Ameloblastoma. (D) IH examination revealed prominent Calretinin staining, especially in the regions of Acanthomatous differentiation. (E, F) The peripheral and the central tumor cells showed a very high Ki-67 labelling index of 6.9, and PCNA labelling Index of 6.4, indicating a high proliferative activity and confirming the tumor to be an aggressive one. (G-L) Resection with disarticulation carried out with stabilization of the mandible with Maxillomandibular fixation to prevent contracture that could lead to mandibular deviation post operatively. (M-N) Secondary reconstruction carried out 4 months later using Free fibular graft.

because it makes logical sense that proliferative activity of constituent tumor cells is directly proportional or correlates well with the aggressiveness of the tumor [26].

There often exists ambiguity in the diagnosis and management of large lytic lesions involving the jaws [27]. The fact that there are often overlapping histopathological features between Odontogenic cysts and tumours, further compounds the problem of distinguishing one from the other [9]. As their clinical behaviour, aggressive and invasive potential and tendency for recurrence are quite different, they require appropriately different treatment and management modalities for a successful outcome [28].

In this Study, IH analysis was carried out at the biopsy stage using Calretinin (Tumor Marker) so to arrive at a precise confirmatory diagnosis. Also, an evaluation of the Labelling Indices of Ki-67 and PCNA (Cell Proliferation Markers) gave valuable information about the nature of each type of pathology, and guided in formulating an Ideal treatment plan for each individual case.

Enucleation is the procedure of choice for removal of most cysts and other benign pathology of the oral and maxillofacial region. It is suitable for those pathologies which are surrounded by a capsule, which is

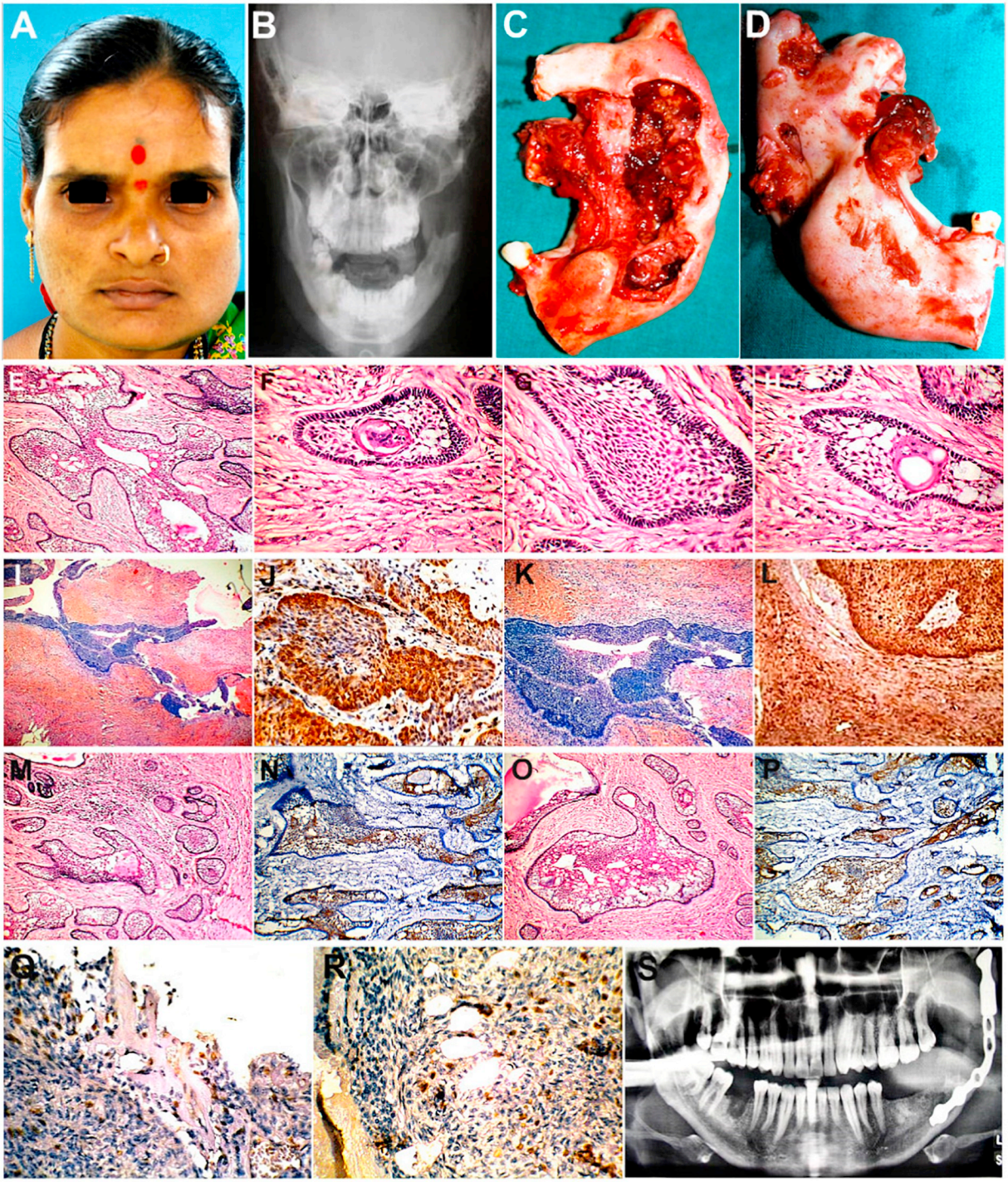

Fig. 14. (A-D) 37 yr old patient with a large unilocular radiolucent lesion involving the left ramus of mandible treated by resection with disarticulation, after Histopathological and Immunohistochemical evaluation of incisional biopsy specimen proving the lesion to be an aggressive Acanthomatous variety of Ameloblastoma. (*E*-H) Hematoxylin % Eosin stained sections of the tissue specimen (X50, X100) showed epithelial follicles/islands, with peripheral layer of tall columnar Ameloblastic cells with reverse palisading of their nuclei and the central stellate reticulum-like cells, in this case, undergoing squamous metaplasia, a characteristic of the Acanthomatous variant. There were central areas of keratin formation. (I-L) IH staining was done for Calretinin which demonstrated a very strong uptake and expression, particularly in the areas of squamous metaplasia within the epithelial islands. (M-P) Intense immunopositivity for Ki-67 was observed within the epithelial islands and the cells lining them. High Ki-67 Labelling index of 6.7, confirming it to be an aggressive tumor. (Q, R) A high PCNA positivity with LI of 6.6. (S) An immediate reconstruction with a Titanium Reconstruction plate was carried out following the resection and patient planned for Secondary reconstruction at a later date.

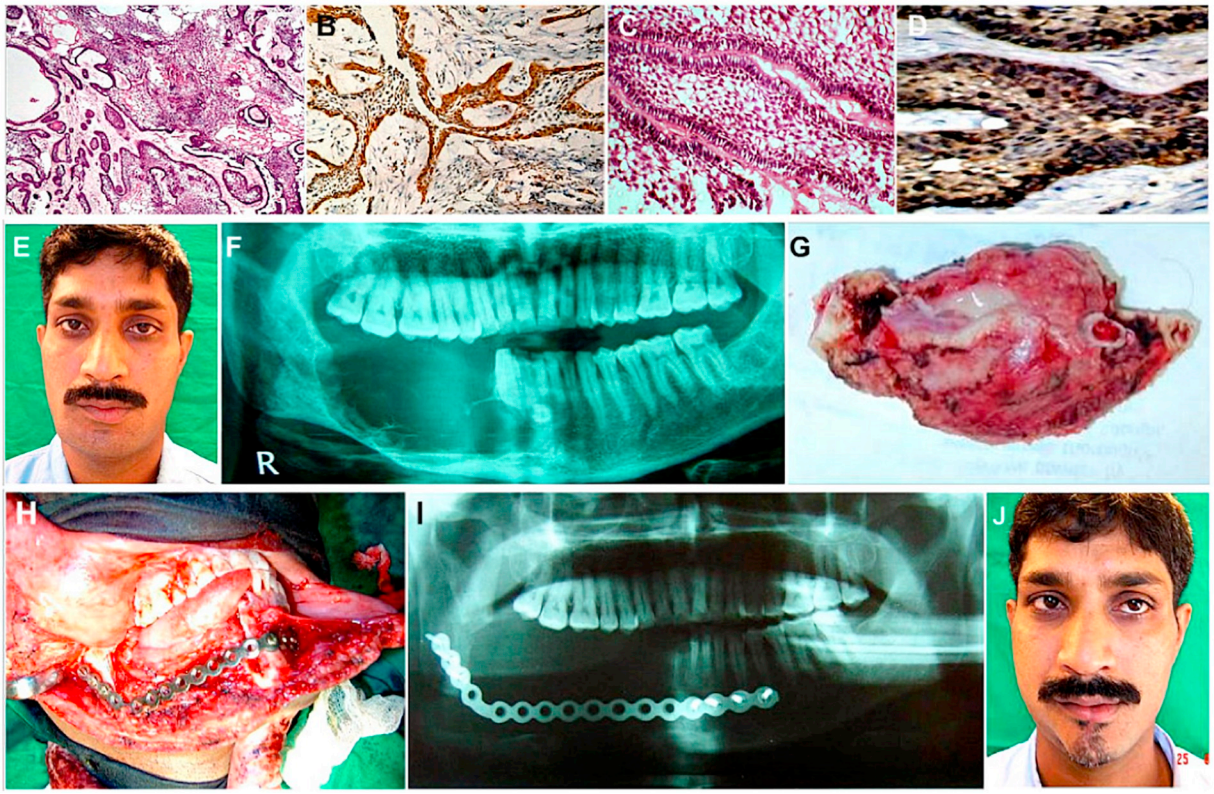

Fig. 15. (A, C) Histopathological examination of biopsy specimen confirmatory of Plexiform variety of Ameloblastoma. (B) Strong Calretinin positivity expressed by the anastomosing cords and strands of odontogenic epithelial cells. (D) High Ki-67 Labelling Index of 7.9. (E) 39 yr old male patient with complaint of vague pain in the right side of Mandible, with H/O multiple extractions. (F) Large Unilocular radiolucency involving right body of the mandible, missing teeth of the lower right quadrant. (G-J) Segmental resection of Rt body of mandible, leaving behind the upper part of the ramus with the condyle and coronoid process; and maintaining jaw continuity using an angled reconstruction plate. Planned for subsequent secondary definitive reconstruction at a later date.

anatomically distinct and cleave easily from the surrounding tissue, and are thus amenable to this type of therapy. Physical or chemical curettage may be added to the enucleation procedure in certain pathologies that require additional removal of surrounding bone suspected to have microscopic invaginations of the lesion, or satellite cysts/tumours, to help ensure their complete removal and to decrease the incidence of persistence and recurrence of the lesion. Curettage may be completed with a sharp curette or a round diamond/tungsten carbide bur with copious cool irrigation to remove 1 to 2 mm of bone containing any pathological remnants.

Meticulous technique in the procedure of enucleation and curettage is particularly important in the surgical management of lesions that tend to have high recurrence or persistence rates, in which case, the addition of Carnoy's solution to curettage or peripheral ostectomy has been shown to be even more effective in decreasing the recurrence rate, than are the enucleation procedures alone [29]. This procedure was employed, in this study, for all the cases of Dentigerous cysts showing histopathological evidence of ameloblastomatous change, and also in those cases diagnosed as Unicystic Ameloblastoma of the luminal and intraluminal subtype. It was also employed for the two cases of Adenoameloblastoma, which showed a nil expression of Proliferation markers Ki-67 and PCNA. Routine radiographic follow-up was carried out for a period of one to two years, to rule out recurrence [30].

The enucleation and curettage approach is contraindicated in solid locally aggressive benign and malignant lesions [31]. Solid benign aggressive lesions such as Ameloblastoma and its variants such as the Follicular, Acanthomatous, Plexiform varieties, have an extremely high recurrence rate and require resection with at least 1-cm margins in the mandible and partial/total maxillectomy [32,33].

Mural varieties and Solid Ameloblastoma too not amenable to enucleation and curettage (mechanical and chemical), as this treatment results in an unacceptably high recurrence rate [34] and all such cases in this study were addressed with an extended surgical resection, ranging from marginal resection, maintaining the continuity of the jaw to segmental resection and even Hemimandibulectomy and maxillectomy for the immunohistochemically proven aggressive tumours **(**Table 1**).**

Malignant lesions require an even more aggressive composite resection, with or without Radical Neck Dissection [35]. None of the cases in this study of 30 patients with large Unilocular radiolucent lesions of the jaws were diagnosed with malignancy. Hence this treatment modality was not employed.

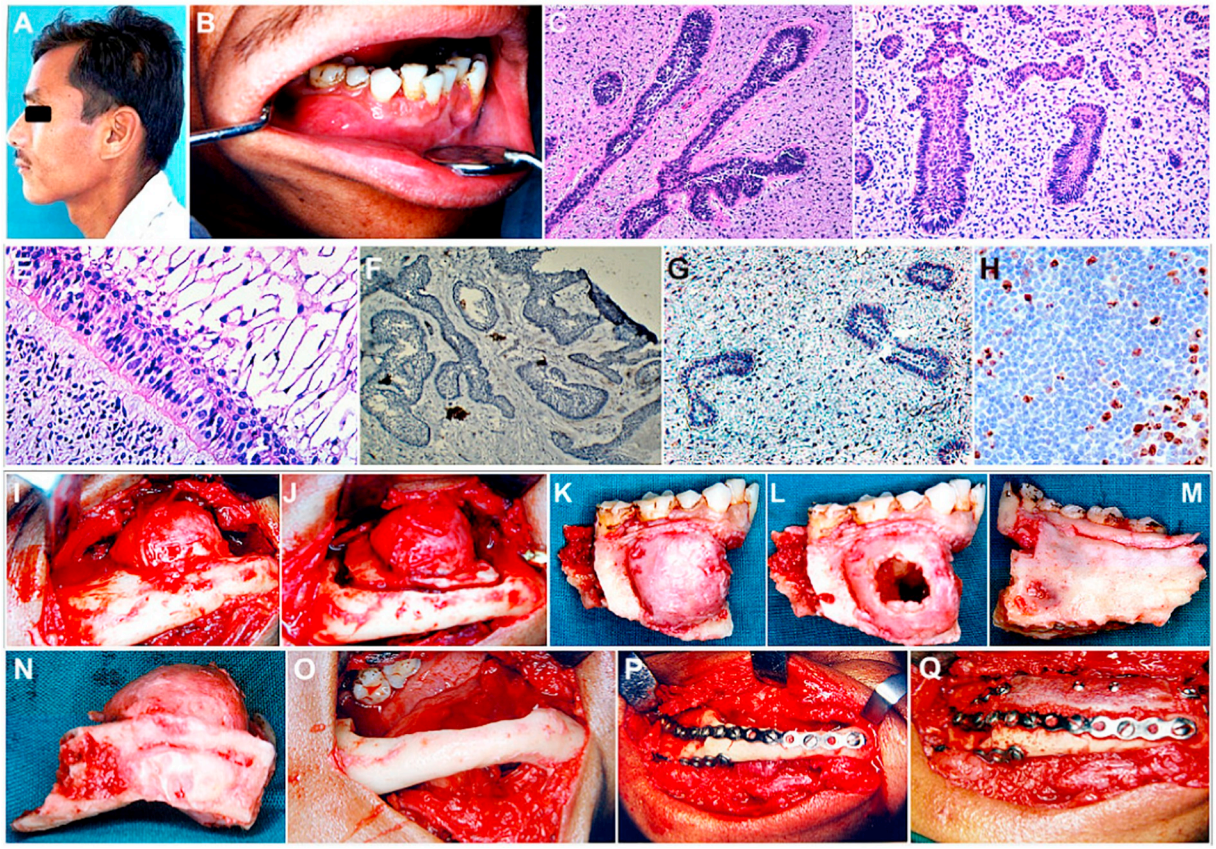

Fig. 16. (A, B) 39 yr old male patient with a spheroid bony expansion of the right body of mandible. (C-E) Incisional biopsy revealed islands of odontogenic epithelium which were surrounded by a, richly cellular ectomesenchymal stroma resembling the dental papilla, indicative of Ameloblastic fibroma. (F, G) Both, the epithelial as well as mesenchymal components of this tumor were negative for Calretinin. (H) Ki-67 Labelling Index was 3.8. (I—O) Marginal resection, with complete removal of the tumor together with 1 cm of normal appearing bone all around, preserving the integrity of the inferior border of the mandible. (P, Q) This was followed by primary reconstruction using an iliac crest bone graft and reinforcement of the inferior border using a reconstruction plate.

7. Conclusion

Immunohistochemistry can be of immense value as a Diagnostic marker as well as a Prognostic Indicator, especially when dealing with ambiguous pathologies such as extensive Unilocular radiolucent lesions of the jaws. It serves as an important predictor as to the aggressiveness of the lesion and its potential for recurrence, thus guiding the clinician to choose the most ideal treatment plan and an appropriate surgical procedure. A higher Immunohistochemical Labelling Index (LI) is indicative of a greater invasive, destructive and aggressive potential of the lesion with a higher tendency for recurrence, thus meriting a more radical or aggressive approach to its elimination; while a lesion with a low LI can be managed successfully by a relatively conservative approach.

Nevertheless, Immunohistochemistry is not always infallible as it is an intricate and technique sensitive lab procedure after all, where mistakes can occur in antigen retrieval and tissue processing, yielding false positive or negative results. It must be viewed as an adjunct to, rather than a substitute for Light microscopy.

Compliance with ethical standards

Disclosure of potential conflicts of interest

The author of this article has not received any research grant, remuneration, or speaker honorarium from any company or committee whatsoever, and neither owns any stock in any company. The author declares that she does not have any conflict of interest.

Research involving human participants and/or animals

All procedures performed on the patients (human participants) involved were in accordance with the ethical standards of the institution and/or national research committee, as well as with the 1964 Helsinki declaration and its later amendments and comparable ethical standards.

Ethical approval

This article does not contain any new studies with human participants or animals performed by the author.

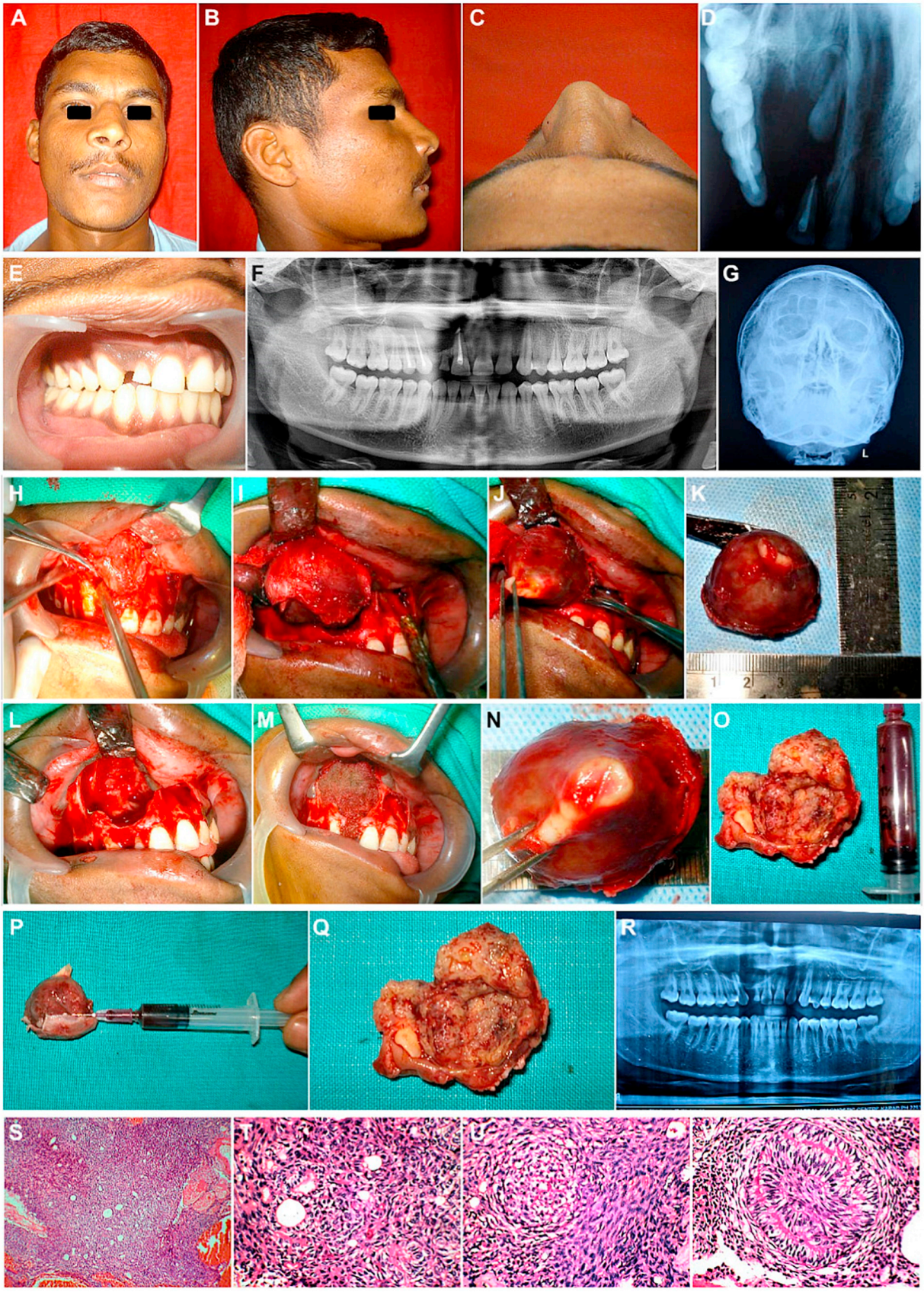

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Fig. 17. (A-D) 23 yr old male patient with a slow growing bony hard swelling in the right infraorbital region in relation with an unerupted lateral incisor tooth. (*E*-F) Radiographs revealed a large unilocular radiolucency engulfing the displaced permanent right lateral incisor tooth, giving the appearance of a Dentigerous cyst. (H−O) Following incisional biopsy, the cystic lesion was enucleated in-toto via an intraoral vestibular approach, followed by curettage and cauterization with Carnoy's solution. (N-Q) Gross specimen revealed a well encapsulated lesion with the impacted tooth within. Aspirate revealed thick cystic fluid admixed with blood. (S−V) Histopathological examination of the biopsy specimen revealed solid areas of cells in the form of duct-like patterns, as well as whorls and rosettes, interspersed with areas of calcification, confirming the diagnosis of Adenomatoid Odontogenic Tumor (AOT) or Adenoameloblastoma. (W, X) Cellular multinodular proliferation of cells in the form of nests and rosettes. (Y-AD′) The glandular or duct-like structures, with lumen of varying size were lined by Cuboidal to columnar ameloblast-like cells and polygonal cells in between the ductal structures. (AE′, AF′) Negative expression of Calretinin in any of the histopathological fields. (AG′, AH′) Negative immunoreactivity for both Ki-67 as well as PCNA by the tumor cells, indicative of their indolent nature low growth potential.

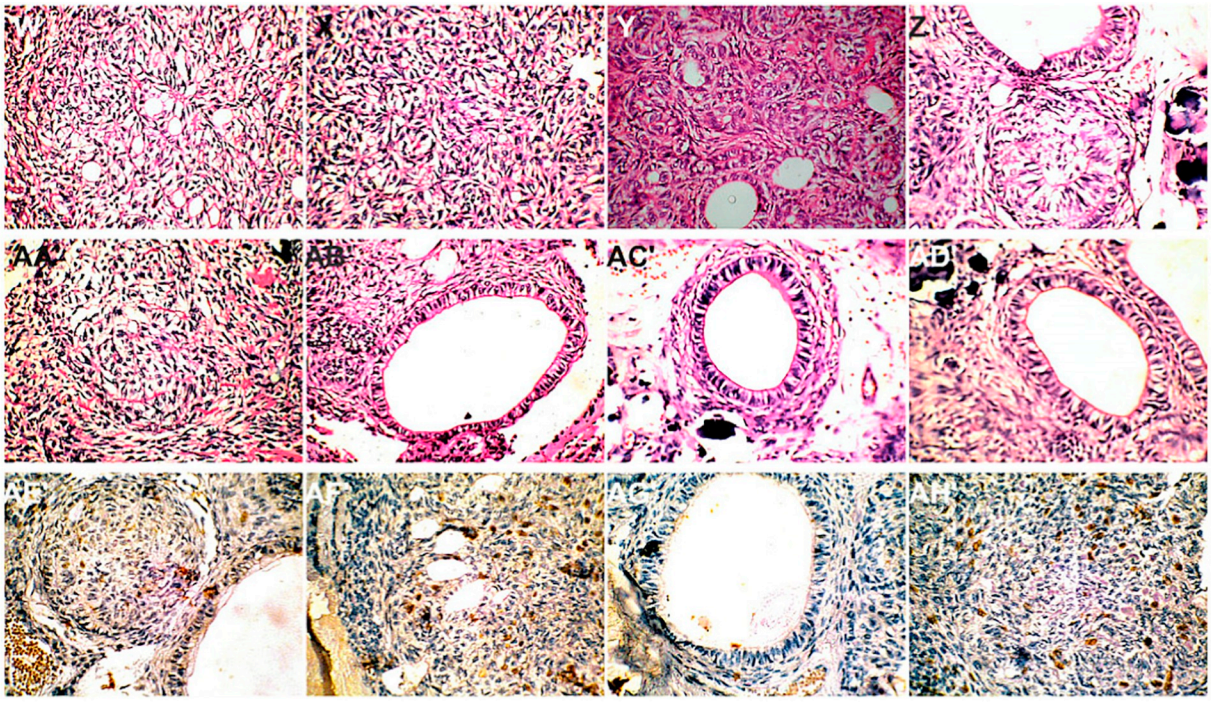

Fig. 17. (*continued*)

Informed consent

Informed consent was obtained from all the individual participants in this study.

Funding

This study was not funded by any organization/society.

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