



MYOSITIS OSSIFICANS MIMICKING FEATURES OF OSTEOMYELITIS - A CASE REPORT AND REVIEW OF LITERATURE

Dental Science

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ABSTRACT

Myositis ossificans is a non-neoplastic, heterotrophic bone formation seen in muscles. Myositis ossificans has been classified into multiple categories including myositis ossificans traumatica, myositis ossificans progressive, idiopathic MO, and associated with paraplegia. Myositis ossificans traumatica is usually associated with history of trauma be it major or minor, or any kind of insult to the muscle tissue. Masticatory muscles are rarely associated with myositis ossificans, masseter being most commonly affected muscle. Trismus remains the main complaint with all the patients where myositis ossificans included the masticatory muscles hindering its range of motion. Multiple pathologies overlap the features of Myositis ossificans making its diagnosis difficult at initial phase. The case presented shows a surgical dilemma created due to overlapping clinical findings of an infectious process of hard and soft tissue. Myositis ossificans was an incidental histologic finding showing the pathognomonic zonal pattern. A review of literature on myositis ossificans affecting masticatory muscles has also been included.

KEYWORDS

Myositis Ossificans; Masticatory Muscles; Masseter; Osteomyelitis; Trismus

INTRODUCTION

Myositis ossificans (MO) is usually described as a non-neoplastic, heterotrophic type of bone formation in muscles or soft tissue. The reason it is called heterotrophic is because it occurs outside the usual site of ossification which is bone [1]. Myositis ossificans is though as a broad term which encircles four different clinical entities that includes: 1) MO traumatica (occurring due to trauma), 2) MO progressiva (an autosomal dominant variant which involves multiple sites), and 3) MO related with paraplegia and 4) Pseudomalignant MO (absence of any history or idiopathic) [2].

Myositis ossificans traumatica (MOT) is the type of MO most commonly seen, and is always associated with a history of trauma. The traumatic insult may be either macro-trauma or micro-trauma; single episode or multiple episodes. Majority of the reports of MOT are seen in orthopaedic literature due to the blunt trauma to the larger muscles like quadriceps femoris and brachialis anticus [3]. MOT has a very rare occurrence in head and neck region and in masticatory muscles, with the first case reported in English literature back in 1924 by Ivy and Eby involving masseter muscle [4]. In head and neck region sternocleidomastoid, and in masticatory muscles masseter are most commonly affected with MOT [5]. Masseter is most commonly involved owing to its location i.e. it externally covers major area of mandible making it prone for traumatic injuries; while other masticatory muscles are majorly affected due to iatrogenic injuries [6]. The most common clinical finding of MO seen in masticatory muscles is trismus [7]. We hereby report a case of Myositis Ossificans of left masseter muscle, which was an incidental finding of a patient who was provisionally diagnosed with infectious changes in the bone as well as soft tissue. Review of literature presenting Myositis ossificans associated to masticatory muscles is added.

CASE REPORT

A 61 year old male patient presented with chief complain of swelling on posterior aspect of left side of lower jaw associated with reduction in mouth opening since last 1 month. Patient gave history that before 2 months he experienced pain in lower left back tooth region for which he consulted at a dental clinic, where after clinical and radiological (Orthopantomogram) examination he was diagnosed with carious left mandibular second and third molar. Dentist prescribed oral medications and advised extraction of the carious teeth. Patient denied

the extraction and over the period of time experienced an increase in the pain intensity with aggravation of swelling in the associated region. He consulted a general surgeon for the swelling where he was given intravenous medications (antibiotics & analgesics). After the swelling subsided patient opted for extraction of the teeth. Few days after the extraction, patient started experiencing reduction in the mouth opening which aggravated with time and a week after that he experienced associated painful firm swelling in the region where the previous swelling was present. He consulted the dentist for the same who prescribed oral analgesics and advised mouth opening exercise. As none of the treatment resulted in any resolution of symptoms nor there was any improvement in the mouth opening, patient was referred to our department for further treatment. Patient did not have any significant medical history. Patient was though addicted to smoking since last 10 years. The vitals noted were under physiologic limit. Patient had got his pre-extraction orthopantomogram along with him which showed carious second and third mandibular molar of the left side along with periapical radiolucencies suggestive of periapical abscess.

On local examination patient had maximum inter-incisal mouth opening of 6mm (Figure 1). A single diffuse extra oral swelling over the left ramus region of mandible extending superio-inferiorly from ala-tragus line to inferior border of mandible and antero-posteriorly from anterior border ramus to its posterior border, measuring around 5*5 centimeters in dimension was present. On palpation the swelling was firm, mildly tender, non-compressible and non-fluctuant in nature with no signs of overlying skin abnormalities or any signs of draining sinus tract. No temporomandibular joint deformity was noted, and the lymph nodes were non palpable. Thorough intraoral examination was not possible due to reduced mouth opening though a palpable swelling over the region of anterior border of ramus was seen, with normal appearing overlying mucosa and no signs of any sinus tract formation or fistula was noted. Owing to the clinical presentation the provisional diagnosis of submasseteric space infection was attained.

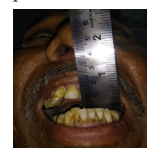


Figure 1. Preoperative Mouth Opening – 6 mm.

Intraoral and extraoral aspiration was negative. Orthopantomogram was advised which showed loss of trabecular pattern apical to the extraction socket (Figure 2). The findings were not conclusive so for further investigation computed tomographic (CT) scans were advised along with Ultrasonography (USG). USG findings showed swelling in intramuscular plane which was hypo echoic in pattern along with few internal cystic areas with possibility of organized hematoma with fibrosis (Figure 3). CT findings revealed mild bony irregularities involving the alveolar surface at the site of tooth extraction along with cortical breach over the outer cortex of body and angle of mandible (Figure 4) with mild increase in the bulk of masseter. Few mildly enlarged sub centimeter sized lymph nodes noted at level IA, IB, II. Findings were in favor of infective etiology and acute osteomyelitis. Blood and urine investigation did not show any abnormality and all the variables were under the physiologic range.

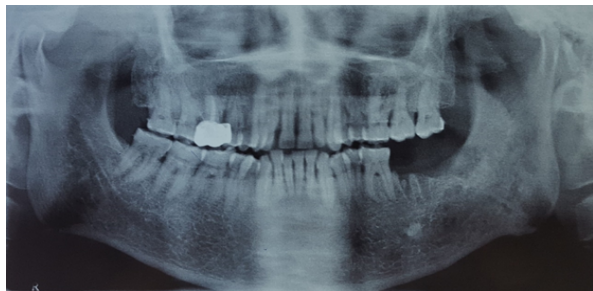


Figure 2. Preoperative Orthopantomogram Showing Loss Of Trabeculation In Periapical Region Of Extracted Left Mandibular Second And Third Molar

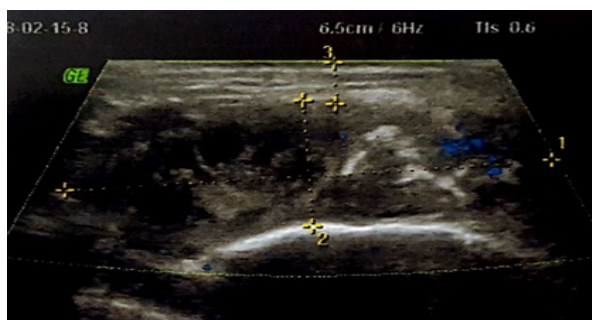


Figure 3. Ultrasonography showing the hypoechoic submasseteric hematoma.

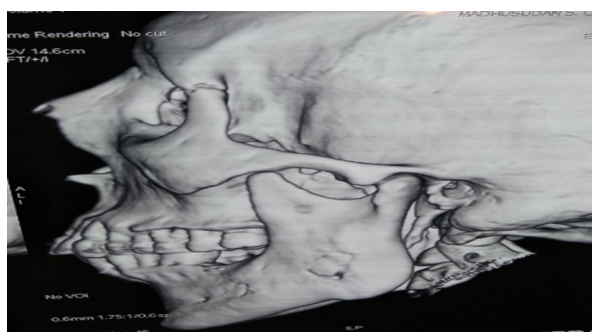


Figure 4. 3-dimensional Computed Tomography Image Showing Cortical Perforation At Region Of Left Mandibular Second And Third Molar And At Masseteric Region.

Surgical curettage along with tissue biopsy under general anesthesia was planned. Extra oral submandibular approach was opted, as access through intraoral approach was difficult due to negligible mouth opening. After layer wise dissection, reaching the submasseteric plane fibrous masses were observed in the muscle tissue along with fibrous adhesion of the muscle to the bone periosteum. The fibrous masses and adhesions were detached followed by thorough curettage of the surrounding soft tissue and of bone. Mouth opening achieved after that was 52 mm (Figure 5). The fibrous masses were sent for histopathology examination (Figure 6). Layer wise closure was done. Post operatively patient was started with aggressive physiotherapy for mouth opening.



Figure 5. Intraoperative Mouth Opening Of 52 Mm.



Figure 6. Excision Of The Fibrous Mass.

Histopathology report stated that H & E stained section showed connective tissue comprising of nodular fasciitis with short fascicles or haphazard fibroblasts that are uniform with faint eosinophilic cytoplasm and tapering processes. Muscular tissue along with chronic inflammatory infiltrate, fat cells and osteoid tissue are seen. There were three distinct zones noted: 1) a central portion in which there is fibroblastic proliferation which is markedly cellular (Figure 7); 2) intermediate portion where there is collagen with proliferating spindle cells, with early trabeculation of ossifying areas (Figure 8); 3) peripheral zone of osteoid trabeculae rimmed by osteoblast (Figure 9). The overall histologic picture of zonal pattern was suggestive of myositis ossificans (Figure 10). Patient continued with aggressive physiotherapy thereafter. The healing was uneventful and 1 year follow up showed patients mouth opening of 43 mm (Figure 11).

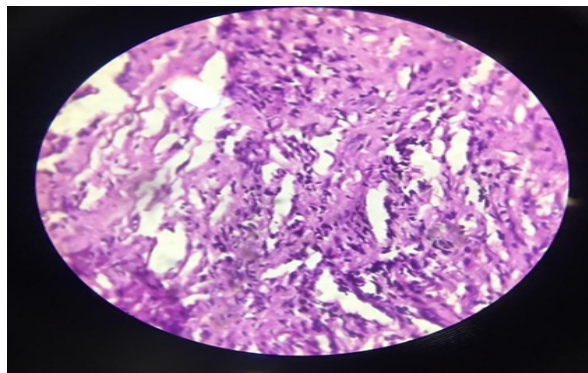


Figure 7. Histopathologic Section Showing The Central Zone Of Fibroblastic Activity With Marked Cellularity.

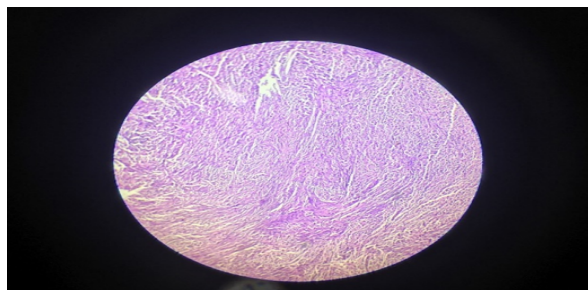


Figure 8. Histopathologic Section Showing Intermediate Portion Where There Is Collagen With Proliferating Spindle Cells, With Early Trabeculation Of Ossifying Areas.

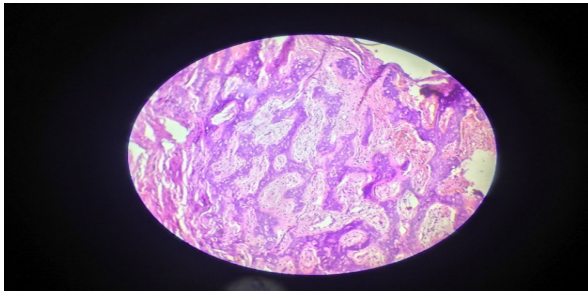


Figure 9. Histopathologic Section Showing Peripheral Zone Of Osteoid Trabeculae Rimmed By Osteoblast.

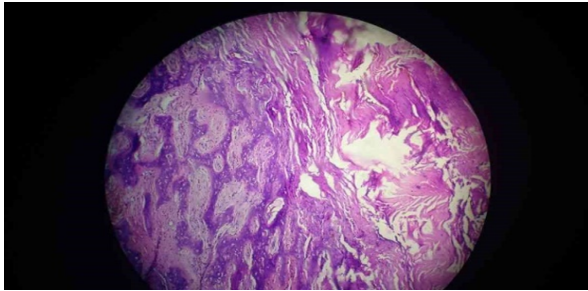


Figure 10. Pathognomonic "Zonal Pattern" Of Myositis Ossificans.

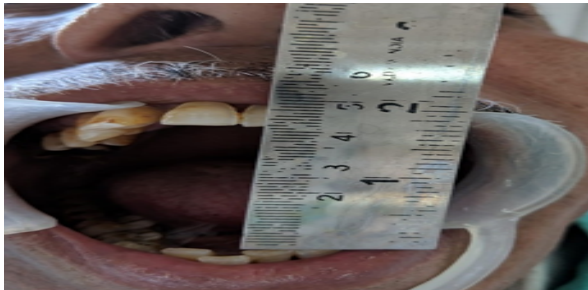


Figure 11. 1 Year Follow Up Showing Mouth Opening Of 43 Mm.

DISCUSSION

Myositis ossificans (MO) in its literal sense is an inflammatory ossification inside the muscle tissue. Though this terminology is not accepted by many researchers owing to the variations seen in the particular pathology. Ackerman argued with the assigned nomenclature as being poorly designated term established by customs for a group of lesions appearing in the soft tissues that may or may not be associated with periosteum [19]. He further added that there might not be any association of inflammation and in some instances he marked no presence of bone or muscle tissue [8]. Recently, Kransdorf

et al defined MO as a benign, solitary, self-limiting, ossifying soft-tissue mass typically occurring within skeletal muscle [9].

MO has been classified into various groups, mainly depending on its etiology and the disease course. Lewis in 1923 classified MO into three forms: traumatic, nontraumatic, and neurotic [10]. In the following year Noble classified it into myositis (fibrous) ossificans progressiva, traumatic MO circumscripta, and MO circumscripta without history of trauma. The later one is considered as pseudomalignant type and it also includes idiopathic forms seen in association with burns, polio, paraplegia, or infection. MO Progressiva is an autosomal dominant disease seen with distinctive features of symmetric congenital malformation of hands and feet along with progressive soft tissue heterotrophic calcification which is generalized. The patients with MOP have poor prognosis of survival [11].

Myositis Ossificans traumatica (MOT) carries various names such as myositis ossificans circumscripta, heterotrophic ossificans, localized myositis ossificans, fibrodysplasia ossificans circumscripta or traumatic myositis ossificans. It is majorly reported in the orthopedic literature and most commonly seen in larger muscles like quadriceps femoris and brachialis anticus [3]. MOT occurring in masticatory muscles is though a rare finding. (Table I) shows an electronic search done in PubMed and Google Scholar for the words "myositis ossificans in masticatory muscles", where 44 reported cases of myositis ossificans from 43 articles were recorded in the time period of 1/01/2001 to 31/08/2019. Results obtained with Myositis ossificans progressive and cases with other histologic diagnosis were excluded. Majority of the reviews report masseter to be the most affected masticatory muscle, followed by medial pterygoid, lateral pterygoid and finally the temporalis. In our review of literature of MOT cases in masticatory muscles reported from the year 2000 till date showed higher occurrence of MOT in medial pterygoid and temporalis than masseter. Temporalis was mainly involved in cases where multiple sites were involved. Male to female ratio of MOT occurrence has shown male dominance with sex ratio of M3:F1 [12], M2.5:F1 [7], M1.42:F1 [13]. Our review of literature showed male to female ratio to be M 1.15: F1. Average age of occurrence reported in literature has been in the range of 30 – 40 years, with the average age recorded in our review of literature being 35.75 years. Multiple etiologies have been reported to cause MOT in the masticatory muscles. Our review of literature shows that out of 44 cases reported, 22 were due to history of trauma, 5 were due to history of extractions, 4 were due to injections usage, 5 were due to history of prior infection, 3 were idiopathic, 1 was due to chemo-radiotherapy, and for 3 cases we could not find the relevant data. The cases which reported infection coupled with third molar extraction had almost similar sequel of incidence in their history as with our case. Majority of patients present with the preliminary complain of restricted mouth opening which may or may not be associated with pain or swelling all the time. Our review of literature showed almost all cases associated with trismus, out of which 7 had associated complain of pain and 5 cases had associated complain of swelling.

TABLE I. Review of literature " Myositis Ossificans of Masticatory Muscles"

AUTHORS	YEAR	NUMBER OF CASES	ETIOLOGY	CHIEF COMPLAIN	MUSCLE INVOLVED	GENDER	AGE	RECURRENT OF TRISMUS POST-SURGERY
Ramakrishnan P et al [27]	2019	1	EPILEPSY	TRISMUS	MEDIAL PTERYGOID	FEMALE	35	Absent
Karaali S. and Emekli U [13]	2018	1	NOT MENTIONED	TRISMUS	MEDIAL PTERYGOID	FEMALE	30	Not recorded
Kayal L et al [28]	2018	1	NON	TRISMUS	MASSETER	MALE	36	Absent
Cavalheiro BG et al [29]	2016	1	TRAUMA	TRISMUS AND PAIN	MULTIPLE MUSCLES	MALE	71	Absent
Bedi RS et al [30]	2016	1	TRAUMA	TRISMUS	MASSETER	FEMALE	20	Absent
Fité-Trepat L et al [7]	2016	1	INFECTION	PAIN+SWELLING+TRISMUS	MASSETER	FEMALE	49	Absent
Demirkol M and Tutar E [31]	2015	1	TRAUMA	PAIN	MASSETER	FEMALE	64	Absent
Mashiko T et al [32]	2015	1	TRAUMA	TRISMUS	MASSETER	MALE	36	Absent

Becker OE et al [33]	2015	1	TRAUMA	TRISMUS	TEMPORALIS	MALE	17	Absent
Fallahi-Motlagh M et al [34]	2015	1	TRAUMA	TRISMUS	MEDIAL PTERYGOID	MALE	46	Absent
Jiang Q et al [35]	2015	1	TRAUMA	TRISMUS	LATERAL AND MEDIAL PTERYGOID	FEMALE	42	Absent
Torres AM [6]	2015	1	TRAUMA	TRISMUS	MEDIAL PTERYGOID	FEMALE	36	Present
Reddy SPD et al [36]	2014	1	TRAUMA	TRISMUS AND SWELLING	TEMPORALIS AND MEDIAL PTERYGOID	MALE	21	Absent
Almedia LE et al [37]	2014	1	TRAUMA	TRISMUS	LATERAL PTERYGOID	FEMALE	12	Present
Kumar N et al [38]	2014	1	TRAUMA		MASSETER	MALE	26	Absent
Spinzia A et al [39]	2014	1	TRAUMA	TRISMUS	LATERAL PTERYGOID	MALE	30	Absent
Kamalapur MG et al [40]	2014	1	TRAUMA	TRISMUS + PAIN	LATERAL PTERYGOID +MEDIAL PTERYGOID +TEMPORALIS	FEMALE	20	Absent
Deliverska E [41]	2013	1	TRAUMA	TRISMUS	MASSETER	MALE	55	Absent
Piombio P et al [42]	2013	1	TRAUMA	TRISMUS	MASSETER	FEMALE	62	Absent
Ahmed K et al [43]	2014	1	TRAUMA	TRISMUS	MASSETER	MALE	20	Absent
Fry RR et al [44]	2014	1	TRAUMA	TRISMUS	TEMPORALIS	MALE	25	Absent
Boffano P et al [45]	2014	1	TRAUMA	TRISMUS	MEDIAL PTERYGOID	FEMALE	37	Absent
Schiff MJ ; Meara DJ [5]	2013	1	TRAUMA	TRISMUS	TEMPORALIS	FEMALE	41	Absent
Nemoto H et al [46]	2012	1	TRAUMA	TRISMUS	BILATERAL MASSETER+ TEMPORAL+ LATERAL PTERYGOID	MALE	39	Absent
Jayade B et al [47]	2013	1	TRAUMA	TRISMUS	MEDIAL PTERYGOID +LATERAL PTERYGOID +TEMPORALIS	FEMALE	25	Absent
Choudhary A et al [12]	2012	1	LA INJECTION	TRISMUS	MEDIAL PTERYGOID	MALE	31	Absent
Godhi SS et al [48]	2011	1	THIRD MOLAR EXTRACTION	TRISMUS	BILATERAL TEMPORALIS+LATERAL PTERYGOID	MALE	21	Present
Guarda Nardini L [49]	2011	1	INFECTION	TRISMUS + PAIN	TEMPORALIS	MALE	50	Absent
Siddiqi KM et al [50]	2011	1	INFECTION	TRISMUS	MEDIAL + LATERAL PTERYGOID	MALE	19	Absent
Thangavelu A et al [1]	2011	1	INFECTION	TRISMUS	MEDIAL PTERYGOID	FEMALE	36	Absent
Trautmann F et al [51]	2010	1	THIRD MOLAR EXTRACTION	TRISMUS	MEDIAL PRETYGOID	MALE	33	Absent
Ramieri V et al [52]	2010	1	IDIOPATHIC	TRISMUS	MEDIAL PTERYGOID	MALE	34	Absent
Kruse AL et al [53]	2009	1	CHEMORADIC THERAPY	TRISMUS	BILATERAL MASSETER	FEMALE	35	Absent
Seehra, J., & Lloyd, R [54]	2009	1	INFECTION	PAIN+SWELLING+TRISMUS	TEMPORALIS+PTERYGOID	FEMALE	23	Present
Conner GA &	2009	1	THIRD	TRISMUS	MEDIAL	FEMALE	18	Present

Dunny M [55]			MOLAR EXTRACTIO N+ MULTIPLE INJECTIONS		PTERYGOID TEMPORALI S			
Rattan V et al [56]	2008	1	LA INJECTION	TRISMUS	MEDIAL PRETYGOID	MALE	45	Absent
Manzano D et al [57]	2007	1	INFECTION+ THIRD MOLAR EXTRACTIO N	TRISMUS	TEMPORALI S	MALE	51	Absent
Yano H et al [58]	2005	2	TRAUMA	TRISMUS	MASSETER	2 MALE PATIENTS	18, 34	Absent
Uematsu Y et al [59]	2005	1	THIRD MOLAR EXTRACTIO N	MASS	TEMPORALI S	FEMALE	38	Absent
Aoki T et al [60]	2002	1	MULTIPLE ALCOHOL INJECTION	PAIN+SWEL LING+TRISM US	MASSETER+ LATERAL PTERYGOID	MALE	44	Present
Kim DD et al [61]	2002	1	IDIOPATHIC	TRISMUS	BILATERAL LATERAL PTERYGOID	FEMALE	30	Present
Saka B et al [62]	2002	1	MULTIPLE INJECTION	TRISMUS	TEMPORALI S	MALE	33	Absent
Mevio E et al [63]	2001	1	MULTIPLE EXTRACTIO NS	TRISMUS	TEMPORALI S	FEMALE	55	Absent

Multiple theories for pathogenesis of MOT have been postulated to give the exact insight into the process of its development and occurrence. Carey proposed four theories to explain the probable pathogenesis: one being proliferation of bony fragments been displaced into surrounding soft tissue; second due to detachment of periosteal fragments into surrounding tissue along with the proliferation of osteoprogenitor cells; third being the migration of subperiosteal osteoprogenitor cells into surrounding tissue occurring due to periosteal perforations due to trauma; and last being the metaplasia of extrasosseous tissue which are exposed to bone morphogenic proteins (BMP), which are derived by the lysis of the bone fragments which got displaced into the surrounding tissue [14]. The last theory is though the most accepted of all. Kan demonstrated the cellular mechanism of the heterotrophic bone formation was the sequelae of deregulation of the stem cells pertaining to the local tissue injury and the associated inflammation [15]. Recent studies though state that the extra skeletal bone formation is due to the result of the process called as endothelial mesenchymal transition, in which due to the muscle injury cytokines like BMP 2 and BMP 4 are released, which act on the on vascular endothelial cells of skeletal muscle and cause them to undergo endothelial-mesenchymal transition. These endothelially derived stem cells differentiate into chondrocytes and osteoblasts when exposed in the inflammatory condition; further stimulating the chondrocytes to form bone through endochondral ossification [16]. It is difficult to identify the exact etiology and probable pathogenesis for MOT in our case, as both low grade infection and third molar extraction can lead to the loss of periosteal integrity and exposure of osteoprogenitor cells to the surrounding tissue in inflammatory environment which might have triggered the heterotrophic bone formation.

The diagnosis of MOT should be based on the accurate patient history coupled with the radiographic and microscopic finding. Shirkhoda et al described 4 stages of MO where he stated the progression of the lesion which showed parallel radiographic and histologic findings [17].

The initial phase comprised of capillary and cell proliferation surrounding the wound, as there is no calcification in this phase the radiographic findings are not evident. The second stage comprised of incipient bone formation, which occurs during 1-2 weeks. Then comes the intermediate phase at 4 weeks followed by the late phase at 6 weeks. The mature lesion appears as a radiopaque rim with central radiolucent areas. These findings exactly parallels the histologically pathognomonic zonal pattern which seen microscopically in the mature lesion. Plain radiographs can be of diagnostic value if the lesion is in mature phase. Overlap of other cranial bones and vertebra limits its diagnostic usage in MOT of masticatory muscles. Panoramic image

of our case did not show any signs of radiopaque mass of flecks, on the contrary loss of bony trabeculation and sparse radiolucent areas around the extraction socket was found, which could be suspected due to presence of chronic periapical infection. Ultrasonography can be utilized for the cases which are in initial phase, and no ossification has taken place [18]. Our ultrasonographic findings did match with the initial phase of MO i.e. of hematoma formation along with fibrosis in the muscular layer, but the findings were not completely diagnostic. Computed tomography is considered the gold standard for the diagnosis of MO as it delineate the pathognomonic zonal character, which is apparent in the mature stages. For the initial stage the findings appears as a soft-tissue swelling or a low-attenuation soft-tissue mass without associated calcifications [8]. These initial findings did match with our case, though the other more significant findings of bony irregularities involving the alveolar surface at the site of tooth extraction along with cortical breach over the outer cortex of body and angle of mandible coupled with history of facial space infection drove our diagnosis towards osteomyelitis. Other radiologic modalities like MRI (which shows findings of low signal intensity rim), bone scintigraphy have also been used for diagnostic purpose [8].

Ackerman described the zonal phenomena in histologic picture of MO [19]. He described 3 distinct zones:

Zone 1: central region of the lesion which shows hi cellular activity
Zone 2: intermediate zone, which shows immature osteoid formation may be observed
Zone 3: the outer rim of mature bone with high collagenous fibrous stroma.

This zonal pattern seen with MOT is the most differentiating feature of MOT from osteosarcoma. Though a long standing case of MOT which shows complete calcification might lack this characteristic of zonal pattern [1]. MOT in its initial stage may mimic features of soft tissue carcinoma both clinically and on radiograph, hence the histologic examination is of utmost importance. Other important differential diagnosis of MOT include nodular fasciitis, osteoma, osteochondroma, chondromas, and hemangiomas [7]. The active process of osteomyelitis with soft tissue edema and a periosteal reaction may mimic the initial phase of MOT creating a diagnostic dilemma [20].

The treatment of MOT ranges from medical therapy to surgical resection of the calcified mass, although surgical resection of the mass is opted by majority of operators. Non-surgical options have been used with the rationale that surgical procedure may cause more trauma of the soft tissue, making it more prone for the recurrence of MOT. Non-surgical options that have used include using injection of magnesium

sulfate and oral administration of magnesium lactate [21], low dose radiation [22], bisphosphonates [23], indomethacin [24], and warfarin [25], and steroid injections [26]. Surgical resection of the calcified mass remains the sole treatment for apparent ossified masses on radiograph. Dilemma occurs in the cases of MOT in its initial stage as ours. As the occurrence of MOT is very rare when compared to infective osteomyelitis of mandible, along with the history of infection and the CT scan findings of bony changes and soft tissue swelling, we opted for surgical exploration and curettage of the lesion. Fibrous masses were seen inside the masseter muscle on exploration whose histologic findings diagnosed it be MOT of masseter muscle. Another dilemma about MOT is the decision on when to operate and in which phase of MOT. Radiographically apparent lesion or ossified masses replacing the muscle tissue are usually in their late phase i.e. more than 6 weeks, for which surgery remains the optimal treatment option. Due to overlapping features of initial phase of MOT with multiple pathologies, it is difficult to diagnose and accordingly treat the case. Once diagnosed, a thorough follow up is mandatory owing to chances of reoccurrence of MO even after the treatment. Our review of literature showed 7 articles showing reduction in mouth opening even after intervention suggestive of reoccurrence.

CONCLUSION:

Myositis ossificans is a rare entity seen in the maxillofacial region. The cases of MO seen in masticatory muscles usually are usually a sequelae of previous trauma, extraction of molars, infection, and local anesthetic injection. The initial phase of lesion is difficult to diagnose due to overlapping features of other condition. The zonal pattern seen in the histologic section remains the gold standard for the exact diagnosis. Surgical excision remains the main stay treatment, but should be done once the lesion has matured, as intervention during initial phase has higher chances for reoccurrence. A thorough long term follow up is mandatory to identify the reoccurrence at its initial stage.

Ethical Approval: Not Applicable

Conflict of interest: None

Acknowledgment: None

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