Molecular events in the clinicopathological diagnosis of alveolar osteitis
Talaya Zahid, Sarah Ghafoor

Abstract
Alveolar osteitis (AO) is an extremely distressing outcome following extraction of a tooth. Its pathophysiology is poorly understood due to varied nature of presentation of the condition. However, a delay in the healing process of bone due to fibrinolysis is believed to be the underlying pathophysiology. This review highlights three major risk factors – trauma, bacterial accumulation due to poor oral hygiene, and smoking – in causing alveolar osteitis, and describes underlying related molecular events. Fibrinolysis results due to traumatic tooth extraction as well as due to accumulation of certain microorganisms which leads to the development of alveolar osteitis. Tumour necrosis factor-alpha (TNF-\( \alpha \)), Runt-related transcription factor 2 (Runx 2) and osteocalcin (OCN) can be used as molecular markers for evaluating alveolar osteitis. Assessment assays of such biomarkers can lead to a better understanding of the pathological process in providing a clearer picture to researchers and clinicians.

Keywords: Alveolar osteitis, Molecular markers, Delayed bone healing, TNF-\( \alpha \), Runx 2, OCN, Pathophysiology.

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Introduction
Alveolar osteitis (AO) is a leading cause of pain and discomfort following tooth extraction. It is described as postoperative pain inside and around the extraction site, which increases in severity at any time between the first\(^1\) and third day following extraction. It is accompanied by a partial or total disintegration of blood clot within the alveolar socket with or without halitosis.\(^2\) It resolves within 7-10 days.

AO has two recognised types: dry and granulomatous. The dry type is recognised when the clot fails to form within the extraction socket, whereas in the granulomatous type there is degradation of the clot with superimposed infection.\(^3\) A third type, known as marginal superficial AO, is also reported which is transient and resolves without any intervention. The classic triad of symptoms for the diagnosis of AO include clot-loss or necrosis, pain and fetor oris, termed “alveolar osteitis sicca dolorosa”, “localized alveolar osteitis”, “fibrinolytic alveolitis” or, in simple terms, as “dry socket”.\(^4\) Certain risk factors, like traumatic tooth extraction, poor oral hygiene and smoking, are correlated with the physiological, microbiological and molecular events leading to the development of AO. Dislodgement or loss of fibrin clot is commonly believed to be the major factor responsible. The molecular factors that initiate and lead to the development of AO needs further elaboration to better understand its diagnosis and treatment modality. This narrative review was planned to highlight the three major factors leading to AO, namely traumatic extraction, bacterial infection due to poor oral hygiene and smoking, and the molecular events that underlie the delayed healing process.

Role of trauma in alveolar osteitis (AO)
The process of fibrinolysis plays a pivotal role in the development of AO. Bone, like many other organs of the body, possesses fibrinolytic activity attributed to the presence of the plasminogen activators (PAs). These PAs are present in two varieties within the bone and are released following a traumatic exposure of the bone.\(^4\) These two activators are distinguished due to their physical properties according to in vitro experimentations. One is labile, which loses its activity when experimentally heated at a low potential of hydrogen (pH), and the other is stable which is firmly attached to the structural cell proteins and does not lose activity when exposed to high temperature and acidity.\(^5\) While the presence of stable and labile PAs in alveolar bone have been confirmed in studies, their role in determining the site-specific origin of AO is not established. The PAs are present in increased quantity within the alveolar bone, of which the stable type is released following trauma to the gingival and the mucosal tissue surrounding the extraction socket, leading to localised fibrinolysis. These PAs are also classified as tissue-type plasminogen activators (TPAs) and urokinase-type plasminogen activators (uPAs).\(^6\) The uPA is receptor bound and operates in a fibrin-independent manner, whereas TPA is fibrin-dependent and acts as an intravascular activation enzyme.\(^7\) These activators are a part of a complex enzyme system which converts plasminogen to plasmin as well as causes activation of collagenase. This results in degradation of fibrin and eventual regeneration of the extracellular matrix (Figure 1). This degradative activity of PAs is controlled by the production of plasminogen activator inhibitors (PAIs), which modulate the conversion of plasminogen by inhibiting the catalytic activity of PAs.\(^8\) PAIs
achieve this function by competing with the binding sites for vitronectin (VN), an extracellular component, on the domain 2 and 3 of the urokinase-type plasminogen activator receptor (uPAR) which is required for the binding of the receptor complex with the extracellular matrix, and hence, destabilises the cell–extracellular matrix (ECM) interactions (Figure 2). Therefore the plasminogen activator inhibitor type 1 (PAI-1), may at the same time, promote as well as inhibit cell adhesion.9 In AO, both types of PAs can cause hyper fibrinolysis, but it has been demonstrated that the granulation tissue associated with fibrinolysis presents with uPA and a hyper-production of PAI-1 in AO.4

**Poor oral hygiene and role of bacteria in alveolar osteitis (AO)**

Poor oral hygiene has been implicated in the development of AO due to accumulation of certain pathogenic microorganisms with fibrinolytic properties. Fibrinogen, the precursor of fibrin, is also implicated in the defence mechanism against microorganism. Deposition of a fibrin network following any injury protects the exposed area from invasion of microorganism.8 Certain microorganisms of oro-nasal origin have been demonstrated to possess the fibrinolytic property which causes dissolution of the blood clot and cause ease of invasion of the microorganisms into the origin site. These include the Staphylococcus (S.) aureus and the beta (β) haemolytic species of the streptococcus that were isolated from the nose and throat region of the patients with epistaxis.10 Bacteroides gingivalis and treponema denticola, associated with periodontal disease and acute necrotising ulcerative gingivitis, have also demonstrated fibrinolytic activity.11,12 In another study, the presence of fibrin and fibrinogen degrading enzymes by microorganisms in blood collected from the alveoli of surgically-extracted mandibular third molars, plaque of advanced periodontal disease cases, necrotic tooth pulp and from the angle of mouth of patients with angular cheilitis has been documented.13 The organisms with apparent fibrin and fibrinogen degrading activity are several (Table). Fibrinolytic

### Table: Area of isolation and bacterial strain with fibrinolytic activity.

<table>
<thead>
<tr>
<th>Area of isolation</th>
<th>Strains with fibrinolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar blood after extraction of mandibular 3rd molar</td>
<td>Actinomyces viscosus, Peptostreptococcus intermedius, Propionibacterium acnes</td>
</tr>
<tr>
<td>Subgingival plaque of teeth with pocket depth of more than 6mm</td>
<td>Actinomyces viscosus, Propionibacterium acnes, Fusobacterium gondii, Fusobacterium nucleatum, Clostridium ochreolum, Peptococcus variabilis, Peptococcus micros, Bacteroides species</td>
</tr>
<tr>
<td>Root canal of necrotic teeth with intact crown</td>
<td>Propionibacterium acnes, Fusobacterium species, Bacteroides species</td>
</tr>
</tbody>
</table>
microorganisms interact with the plasminogen in general through two mechanisms. In the first mechanism, the microorganism can either produce plasminogen receptors on their surface which activate plasminogen either by complex formation or by proteolysis. In the second mechanism, the plasminogen is immobilised after being recruited on the cell surface receptors present on bacteria, which is then converted to plasmin by enzyme activators derived from the host. The cell surface-bound plasmin is then used by bacteria to cause degradation of host tissue, ECM and basal membrane. This degradative process helps the bacteria to invade the host tissue and delay the repair process.6

Molecular markers of alveolar osteitis (AO)
The histological analysis of AO is more or less similar in most of the animal models studied so far with the exception of a few differences.14,15 Evaluation of molecular markers for AO can be a fast and practical method to validate the results of histological events in the repair of AO. This can be done by comparing the timeline of the histological events with the expression of molecular factors during that phase of healing. Hence, it provides a visual picture of the histological events with the ability to quantify the molecular events. The markers selected were those related to the healing process of bone such as, osteocalcin (OCN), alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx 2), collagen type I (COL-I), vascular endothelial growth factor (VEGF) and tumour necrosis factor-alpha (TNF-α). A positive correlation in the expression of Runx 2 and OCN has been reported that indicates improved healing of bone. Expression of TNF-α was elevated in samples where there was a delay in the healing process. For example, in samples with induced AO which did not undergo any treatment, either curettage and irrigation or placement of intra-alveolar medicament, revealed highest levels of TNF-α compared to those samples which underwent normal healing or those which were followed by a treatment modality. Runx 2, OCN and TNF-α have significant correlation regarding improved healing of bone and can be used as indicators in evaluating new bone formation and inflammatory infiltrate in dry socket in rats.16

Tumour Necrosis Factor-Alpha
Role of TNF-α in causing chronic inflammatory diseases, like rheumatoid arthritis (RA), has been well documented. It acts as a pro-inflammatory cytokine leading to the destruction of joint.17 Evidence from the studies of the healing of post-extraction sockets and induced AO reveals

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**Figure-2:** Function of urokinase – type plasminogen activator receptor.

**Figure-3:** Downstream inhibition of (BMP) pathway.
an increase in expression of TNF-α in cases where there is a delay in healing process. This increase in its expression has been correlated to the decrease in bone formation as well as causing the intense pain associated with this condition.

**Tumour Necrosis Factor-Alpha as an activator of bone resorption and inhibitor of bone formation**

Tumour Necrosis Factor-Alpha (TNF-α) levels increases after extraction of a tooth, but it appears that in animal models of AO, its levels remain elevated for a longer duration compared to those undergoing normal healing. This prolonged increase in its production leads to a greater inflammatory infiltrate. One reason is attributed to the microbial products that activate the resident and inflammatory cells leading to an activation of a cascade of inflammatory mediators. In the models of AO, which underwent a treatment either of curettage and irrigation or placement of intra-alveolar medicament, a reduction in the density of the inflammatory infiltrate has been reported. This correlated with a greater bone neoformation in these animal models. TNF-α is a potent stimulator of bone resorption and inhibitor of new bone formation.

TNF-α has been determined as a negative regulator of bone neoformation in mice with a deficient TNF receptor (TNFR) which leads to an increase in production of cartilage, bone and bone marrow. Significantly, the expression of Bone morphogenetic protein (BMP) 2, 4 and 7 is increased during endochondral bone formation in TNF receptor-1 (TNFR-1)-deficient mice. This indicates a negative effect of TNF-α on bone formation. This suggests a downstream inhibition of BMP signalling via activation of Stress-activated protein kinases / Jun amino-terminal kinases (SAPK/JNK) pathway, leading to phosphorylation of proteins such as SMAD (Sma and Mad proteins from Caenorhabditis elegans and Drosophila, respectively) 1, 5, 8 and transcription of Id-1 (Inhibitor of DNA binding) gene (Figure 3).

**Tumour Necrosis Factor-Alpha and pain related to alveolar osteitis (AO)**

Pain in AO is severe in relation to the local inflammatory changes occurring in the tissue. The severity of the pain has been attributed to the central origin of pain compared to the peripherally mediated origin. TNF-α has been indicated in mediating this painful condition and may act directly on nociceptive neurons to increase pain sensitivity. Peripheral pain receptors which transmit signals to the central nervous system (CNS) contain an abundance of TNFR-1 and TNF receptor-2 (TNFR-2) receptors. Blocking these receptors through TNF-α neutralisation leads to a reduction of noception which is independent of reduction of inflammation which indicates a neuronal site of action of TNF-α. However, TNF-α cannot be taken as the sole mediator of pain related to the inflammation observed in AO since many other inflammatory cytokines are released at the site of extraction which may lead to the increased intensity of pain related to the condition. A study showed a novel model of transgenic mice that contained genes for over-expression of TNF-α in odontoblasts of teeth and osteocytes of bone over a targeted area of nociceptive neurons of the trigeminal ganglia. This resulted in a mouse model mimicking the painful condition of pulpitis and osteitis.

In accordance with the above findings indicating the role of TNF-α in mediating the inflammatory and nociceptive response, it has been assumed that the resulting condition of AO and its pain which fails to subside with the prescription of simple analgesics may be a result of over-expression of TNF-α in AO that leads to pain through peripheral innervation of nociceptors to CNS.

**Runx 2**

Runx 2 has been described as a master regulator of osteoblastogenesis during the embryonic stage of bone development as well as throughout life and causes differentiation of the pre-osteoblasts to osteoblasts, leading to new bone formation. Runx 2 binds to the osteoblast-specific elements and upregulates the skeletal genes that promotes the differentiation of osteoblast lineage. The pathway to osteoblast differentiation is complicated as it involves an interplay of Wingless-type integration site family pathway (Wnt) as well as Notch and fibroblast growth factor (FGF) signalling. However, in
simplified terms, BMP regulates the expression of Runx 2 and ALP by first binding to the BMP receptors. BMP receptors are of two types, Bone morphogenetic receptor-1 (BMPR-1) and Bone morphogenetic receptor-2 (BMPR-2). These receptors are of the serine–threonine kinase type which, on binding with BMP, leads to the formation of an activated quaternary complex which eventually activates the intracellular SMAD proteins via phosphorylation. Once activated, the SMAD proteins cause either a direct activation of the osteoblast differentiation gene within the nucleus or they do so via Runx 2 activation, which translocates within the nucleus and activate the differentiation genes (Figure 4).26 In cases of induced AO, there is a significant decrease in production of Runx 2. This decrease correlates with the decreased and delayed bone-formation observed in animal models of AO.16

Role of smoking in alveolar osteitis (AO)
Smoking has been considered one of the key factors in the development of AO. However, the incidence reports are widely variable in linking smoking as a direct variable to causing the condition. This is due to the fact that not one factor is responsible for the development of AO, and multiple factors contribute to the development of this clinical condition. Recent evidence suggests a link between tobacco smoking and a delay in the healing process of bone, by disruption of the Bone morphogenetic protein (BMP) pathway leading to a decrease in expression of Runx 2 and consequently bone formation.27 Other studies report that nicotine significantly down-regulates the expression of BMP 2, 4 and 6 in osteoblastic cells during bone regeneration in experimental animals.28 Another study links a sustained level of BMP-2 messenger ribonucleic acid (mRNA) for a longer period in control groups compared to those where smoking lead to an abrupt decrease on day 10.29 Hence, it can be concluded that although not a direct mediator of AO, the products in tobacco smoking may lead to a delay in the healing process of bone which can be linked to a higher incidence of development of AO in smokers compared to non-smokers.30

Discussion
The current management of AO requires frequent visits of the patient to the dentist due to the temporary relief of pain provided by prescribing either centrally acting drug, such as acetaminophen or peripherally acting drug, such as flurbiprofen, which are most frequently prescribed. Another treatment modality is the placement of eugenol dressing within the socket, which contains eugenol and lidocaine as active ingredients.31 Eugenol dressing provides effective relief in pain, but it is again a temporary measure and requires frequent change of the dressings. Recent study has reported that a significant decrease in postoperative inflammation and pain following the extraction of third molars occurs in patients who receive preoperative administration of 8mg dexamethasone injection in the deltoide muscle one hour prior to the surgical procedure.32 Though it is a palliative relief, it can be suggested that the treatment of AO be actively started by the dentist via prescribing of dexamethasone or similar corticosteroids, as these drugs reduce inflammation and molecular markers, leading to improved healing.33

Conclusion
Alveolar osteitis is a pathophysiological process involving delayed alveolar healing following extraction of a tooth. The events of delayed alveolar healing can be linked to certain risk factors, including trauma, bacterial accumulation due to poor oral hygiene, and smoking. Molecular events form the basis of these risk factors. OCN, Runx 2 and TNF-α can be used as markers to understand the process of bone healing in AO. Assessment assays of such biomarkers can lead to a better understanding of the pathological process in providing a clearer picture to researchers and clinicians.

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References