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Review Article

Dentistry Section

Role of High Mobility Group Box 1 and Receptor for Advanced Glycation End Products in the Periodontal Disease Pathogenesis- A Review

R RAMYA[1], DEVAPRIYA APPUKUTTAN[2], SANGEETHA SUBRAMANIAN[3], PSG PRAKASH[4]

ABSTRACT

Periodontitis is a multifactorial chronic inflammatory disease associated with increased secretion of several proinflammatory mediators such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-17 (IL-17), Tumour Necrosis Factor-α (TNF-α), Prostaglandins (PGs) in response to increased bacterial load on the tooth surface and within the gingival sulcus leading to the destruction of both hard and soft tissue of the periodontium. The Receptor for Advanced Glycation End products (RAGE) binds multiple ligands, has a transmembrane configuration and is a part of the immunoglobulin receptor family. In healthy individuals RAGE is expressed at lower levels when compared to those with periodontitis and diabetes mellitus. Binding of RAGE to its ligands plays a key role in the inflammatory reactions that lead to disruption of a multitude of cellular processes. One such RAGE ligand involved in inducing hyperinflammatory phenotype is High Mobility Group Box 1 (HMGB1). HMGB1 is a chromatin binding protein predominantly localised in the nucleus and is associated with multiple functions such as Deoxyribonucleic Acid (DNA) repair, nuclear homeostasis, genome stability. In the presence of stressors, it migrates extracellularly and behaves like a Damage Associated Molecular Pattern (DAMP), has chemokine and cytokine activity. Extracellular HMGB1 binds to Toll Like Receptors (TLR)- 2,4 and RAGE, the HMGB1-RAGE interaction in particular has been associated with hyper inflammatory responses and currently hypothesised to be a bigger player in the pathogenesis of periodontitis as well as diabetes associated periodontitis. Therefore, understanding thoroughly the molecular mechanisms driving the tissue destruction in periodontal disease will effectively enable novel therapeutic drug based interventions based on interrupting the HMGB1-RAGE axis which in turn could mitigate the inflammation mediated tissue destruction.

Keywords: Diabetes mellitus, Inflammation, Pathogenesis, Periodontitis, Proinflammatory mediators, Toll like receptors

INTRODUCTION

Periodontitis is a multifactorial chronic inflammatory disease associated with the production of the proinflammatory cytokines such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-17 (IL-17), Tumour Necrosis Factor-α (TNF-α) in response to increased bacterial load on the tooth surface and within the gingival sulcus leading to the destruction of both the hard and the soft tissue of the periodontium [1]. Several systemic diseases like diabetes mellitus, rheumatoid arthritis, atherosclerosis, stroke, pneumonia, alzheimer's disease etc., have been associated with periodontitis. The most compelling evidence for association has been linked between periodontitis and Diabetes Mellitus (DM) indicating a two-way relationship. Periodontitis has been recognised as the sixth complication of DM [2]. The exaggerated periodontal destruction observed is attributed to the hyperglycaemic state and the associated hyper-inflammatory response in DM. Likewise, the inflammatory state associated with periodontitis is associated with poor glycaemic control [3].

The Receptor for Advanced Glycation End-products (RAGE) binds multiple ligands, has a transmembrane configuration and is a part of the immunoglobulin receptor family. It is expressed on the surface of several cell types, including endothelial cells, monocytes, smooth muscle cells, and fibroblasts. RAGE is over-expressed in inflammatory diseases like diabetes mellitus and periodontitis when compared to healthy individuals [4]. RAGE- ligand binding has been implicated to play a crucial role in mediating inflammatory responses that result in the disruption of a multitude of cellular processes. High Mobility Group Box 1 (HMGB1) binds to RAGE and induces hyper-inflammatory phenotype and lately the HMGB1-RAGE axis has been hypothesised to be a bigger player in the pathogenesis of both periodontitis and diabetes associated periodontitis [5]. Therefore, understanding the molecular mechanisms driving the tissue destruction in periodontitis will enable novel therapeutic drug based interventions based on blocking the HMGB1-RAGE axis that could possibly mitigate the inflammation mediated tissue destruction in diabetes associated periodontitis [5].

HMGB1 is a non histone nuclear protein localised within the nucleus and a non Advanced Glycation End products (AGE) ligand of RAGE [4]. In disease state, it is actively secreted extracellularly from macrophages and monocytes following stimulation by Lipopolysaccharides (LPS), TNF-α, oxidative stress or other stimuli [5]. Once secreted, HMGB1 acts as a Damage Associated Molecular Pattern (DAMP)/Alarmin, and it plays an important role in the pathogenesis of inflammatory chronic conditions such as diabetes, septic shock, rheumatoid arthritis, and atherosclerotic lesions. It also activates other immune cells and act as a multifunctional proinflammatory cytokine [6]. Interaction of HMGB1 and Toll-Like Receptor (TLR) 2, TLR4 and TLR9 leads to increased production of inflammatory cytokines indicating its crucial role in the destruction of both the hard and soft tissues of the periodontium [7].

Morimoto-Yamashita Y et al., found that during the initial stages of gingival inflammation, HMGB1 is continuously secreted from the gingival epithelial cells [4]. Additionally, HMGB1 via a positive feedback loop stimulates overt inflammation in periodontitis patients, and this has been demonstrated by Bonaldi T et al., [8]. They observed that HMGB1 stimulated the release of proinflammatory mediators like Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), IL-8, TNF-α, Monocyte Chemoattractant Protein-1 (MCP-1), which in turn favour more secretion of HMGB1 in a positive feedback loop. According to Ito Y et al., in 2012 [6], the secreted

HMGB1 increases the expression of cell adhesion molecules on the endothelial cells, thereby resulting in migration of neutrophils and other immune cells which further perpetuate the inflammatory responses. As inflammation proceeds the macrophages release more cytokines, chemokines and HMGB1 and these mediators promote osteoclastogenesis and bone resorption. Thus, it has been evidenced that HMGB1 possibly initiates, amplifies and then prolongs the inflammation in periodontal tissues [4,8].

Increased expression of HMGB1 has been demonstrated in the Gingival Crevicular Fluid (GCF) of chronic periodontitis patients and their levels correlated with the severity of periodontitis [7,9]. Ping Xie P et al., observed that gingival tissues from chronic periodontitis patients showed an increased expression of HMGB1 and the levels were associated with increased concentration of IL-1β, IL-6, IL-8 [9]. Overall, these studies suggest that the continued release of HMGB1 can act as an important amplification signal involved in upregulation of inflammatory cytokines for progressive periodontal destruction [6-9].

Zhu L et al., reported that HMGB1-RAGE binding activates nuclear factor-κB (NF-κB) with release of proinflammatory cytokines, which further upregulate RAGE expression and secretion of HMGB1 sustaining the inflammatory phenotype [10]. Chang PC et al., and Claudino M et al., demonstrated over-expression of RAGE in experimental models of diabetes associated periodontitis when compared to non diabetic controls and chronic periodontitis [11,12]. Literature evidences are suggestive that HMGB1 and RAGE are both over-expressed and collectively involved in the pathogenesis of periodontal disease [6-12]. Till recently the focus was on RAGE-AGE axis, that mediates the periodontal destruction in diabetes, however currently HMGB1 is gaining importance because of its role in the progression of several inflammatory, autoimmune diseases and in tumour progression. Lately, multitude studies have suggested that the HMGB1–RAGE axis is significant in the pathogenesis of periodontal disease, therefore the present review aims to discuss in detail on the same [4].

HIGH MOBILITY GROUP BOX 1 (HMGB1)

High mobility group proteins research was pioneered by the efforts of Ernest Johns of the Chester Beatty Research Institute in London along with his colleagues Graham Goodwin and Clive Sanders in 1973 [13]. They isolated two groups of proteins from the calf thymus chromatin [26]. The first group of proteins were named “high-mobility group” proteins, or HMG proteins, because of their ability to migrate rapidly in the polyacrylamide gel electrophoresis and they demonstrated high solubility in 10% trichloroacetic acid. This group was further categorised into HMG-1 and HMG-2, and had more than 55% acidic or basic amino acids. The second group of proteins showed slow mobility and were referred to as “low mobility group” proteins. The ground-breaking work by these researchers laid the foundation for the extensive research on these proteins and several HMG proteins have been identified till date [13,14].

The most abundant, highly expressed and evolutionarily conserved proteins among the HMG family of proteins are the HMGB proteins. Previously referred to as HMG1, amphoterin, p30. The HMGBs are a four membered family localised in the nucleus and consisting of HMGB1, HMGB2, HMGB3, and HMGB4 [14]. HMGB1 seems to be the most migratory among them and shuttles between the nucleus and the cytoplasm. The translocation is regulated by changes in its Nuclear Localisation Signal (NLS), which is influenced by cell type, tissue, and stress. The normal nuclear-cytoplasmic HMGB1 ratio is around 30:1. Following various stresses such as infection, sepsis, cell injury, bacterial products, oxidative stress, HMGB1 translocates from the nucleus to the cytoplasm, including mitochondria and lysosomes [13,14].

In the nucleus, it is engaged in various DNA-related functions such as DNA replication, repair, recombination, transcription, and genomic stability. HMGB1 is secreted actively extracellularly from the immune cells (such as macrophages, monocytes, and dendritic cells) and endothelial cells under stressful conditions and functions like a DAMP [13,14]. Extracellularly it mediates inflammation, immune responses, cell growth, cell proliferation, cell death and further possesses antimicrobial, cell proliferative, and mitotic action. The functions are mediated not only by receptors mediated signalling, the redox state and the structure of the proteins [15].

HMGB1 Structure

The primary structure of Human HMGB1 comprises of 215 amino acids and DNA binding domains i.e., HMG A box and HMG B box with 9-79 aa and 95-163 aa, respectively at the C-terminus [14]. The proinflammatory and anti-inflammatory actions are associated with the B and A box respectively. The former interacts with the C-terminal acidic tail upregulating the anti-inflammatory effect is increased. The C terminus has a large number of acidic amino acid residues, that are assumed to protect the safeguards the HMG A and B-box whilst migrating from the nucleus. Furthermore, it also regulates the binding or bending of DNA by interacting with the DNA-binding N-terminal domain. Over-expression of HMGB1 without the C-terminal tail suppresses the expression of several reporter genes in the cell [14].

The secondary structure of the HMGB boxes are composed of 3 alpha helices and 2 loops forming an L-shape with angle of 800. The A box has a greater alpha helix composition and is therefore more positively charged than the B box. The tertiary structure is maintained by the interaction of HMGB1 domains with the C terminus. Different purification procedures and extraction methods may affect and hamper the function of HMGB1 proteins because the structure of HMGB1 may vary depending on the method used to extract it [14].

HMGB1 Function

Cytosolic HMGB1: In the cytoplasm HMGB1 functions as a positive regulator of autophagy by Beclin-1 binding [16].

Membrane HMGB1: Membrane associated HMGB1 has been associated with outgrowth of neuronal processes, activation of platelets, maturation of erythroid cells, cell adhesion and non specific immune responses. The generation of Neutrophil Extracellular Traps (NETs) by activated neutrophils is an important innate immune mechanism for fighting pathogenic microorganisms [14]. HMGB1 migrates to the membrane after neutrophil activation and is then released, causing NET formation. Membrane HMGB1 is possibly involved in cell-cell and cell-matrix interactions. In the presence of infection or injury or inflammatory stressors HMGB1 membrane expression is increased on the cell surface of myeloid dendritic precursors predominantly, and on the surface of gamma/delta T-cells and Cell of Differentiation 4 (CD4) cells. Subsequently, the secreted HMGB1 acts in an autocrine/ paracrine manner to modulate immune responses and favours polarisation of the T-cells towards Th1 phenotype [17].

Extracellular HMGB1: Immune cells can actively secrete HMGB1 or it can be released passively by dead, dying, or wounded cells. Once in the extracellular environment, HMGB1 induces cell migration, proliferation, differentiation, stimulates antimicrobial defence, immune and inflammatory response and also favours tissue regeneration. HMGB1 has been linked to cell differentiation into a variety of cell types, including T-cells, cancer cells, and stem cells. HMGB1 is also involved in cell differentiation outside of the cell [14].

a. **Inflammatory response:** Extracellular HMGB1 can preferentially bind various receptors to activate macrophages, monocytes, neutrophils, eosinophils, astrocytes, fibroblasts, keratinocytes, dendritic cells, natural killer cells, T-cell and endothelial cells.

b. **Cell migration:** Many physiological and pathological processes involve cell migration, which is an important mechanism in the

formation and maintenance of multicellular organisms. Cell migration is mediated by chemokine-receptor interactions. HMGB1 has been shown in a number of studies to operate as a possible chemotactic factor formed from host cells that promote the migration of a variety of cell types [14].

c. **Tissue regeneration:** HMGB1 has been demonstrated to enhances periodontal remodelling and repair [18].

d. **Angiogenesis:** Angiogenesis is the formation of new vascular system from pre-existing vessels, and it is involved in a number of physiological and pathologic processes such as inflammation, wound healing, and tumour growth. Extracellular HMGB1 activates the Mitogen-Activated Protein Kinase-Extracellular signal-Regulated Kinase (MAPK/ERK1/2) pathway, resulting in potent angiogenesis. When HMGB1 and its receptor RAGE are activated, NF-kB is amplified, which promotes inflammation and angiogenesis by overexpressing leukocyte adhesion molecules and the generation of proinflammatory cytokines and factors favouring angiogenesis. Furthermore, TLR4 is essential for HMGB1-mediated neovascularisation. Angiogenesis is also induced by HMGB1 in combination with heparin. HMGB1 enhances neovascularisation by stimulating integrin-dependent homing of endothelial progenitor cells in ischaemic areas [4].

e. **Bacterial killing:** The first line of defence against invading microorganisms may be aided by HMGB1. NET is a major bacterial killing mechanism. Interestingly, HMGB1 stimulates NET formation via interactions with TLR4, implying that it may aid in bacterial death via NET. Exogenous HMGB1, on the other hand, has the ability to reduce neutrophil-mediated bacterial death following binding to RAGE. Thus, bacterial death mediated by HMGB1 is receptor-dependent [17].

f. **Proliferation:** Smooth muscle cells, T-cells, mesangioblasts, cardiac stem cells, and cancer cells all respond to HMGB1 by activating cell proliferation signals. HMGB1 acts solely as a proliferation signal for T-cells in the presence of inadequate doses of anti CD3 antibody and RAGE. Reduced HMGB1 promotes cancer cell proliferation whereas oxidised HMGB1 induces cell death. The redox state of HMGB1 regulates the survival and death of tumour cells [14].

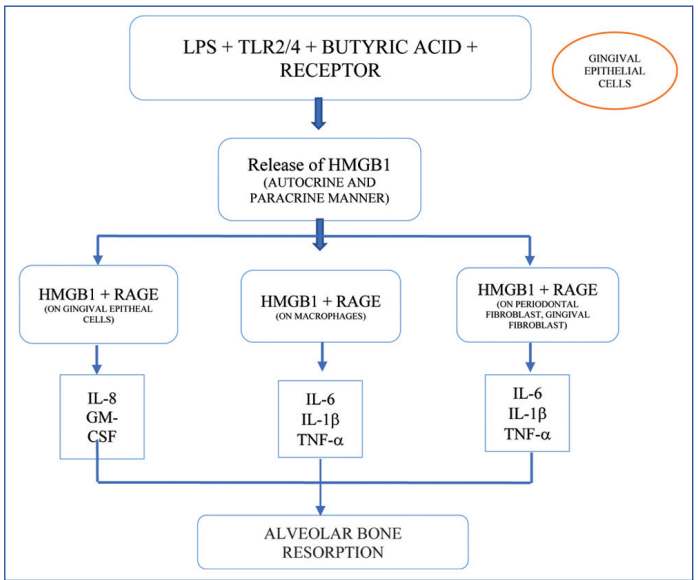
g. **Cell death:** Extracellular HMGB1 in excess is cytotoxic, resulting in cell death (apoptosis and necrosis) as well as tissue damage. In glioblastoma cells, HMGB1 can cause a unique type of cell death that lacks the hallmarks of apoptosis, autophagy, or conventional necrosis. Exogenous HMGB1 can enter host mitochondria via an endocytosis-independent process, leading to the development of vacuolated large mitochondria and fast DNA depletion [16]. Without the involvement of TLR2, TLR4, or RAGE signalling, exogenous HMGB1 localises to mitochondria and induces the formation of enlarged mitochondria. However, c-Jun N-terminal kinase (JNK) activation by reactive oxygen species is required for this process [14].

h. **Immune response:** The immune system is composed of two components: innate and adaptive responses, which eliminate cancer cells and foreign substances to defend the body from pathogens and other chemicals [4,14]. B-cells and T-cells, which are antigen-specific and respond within four to seven days, are essential for the adaptive immune response. HMGB1 is involved in the regulation of innate and adaptive immune responses through direct effects or cell-cell interactions [18].

HMGB1 Role in the Periodontium

Lipopolysaccharides, Reactive Oxygen Species (ROS), proinflammatory cytokines and other noxious stimuli activate the cells of the periodontium and release HMGB1 that in turn is implicated in the persistence or exacerbation of periodontitis. It also plays a role in osteoclastogenesis [4]. During early inflammation, gingival epithelial cells release a variety of cytokines and chemokines, and TNF-α promotes cytoplasmic migration of HMGB1 from the nucleus. The released HMGB1 induces more translocation in an autocrine manner, as well as GM-CSF production from the gingival epithelial cells which induces immune cell differentiation and activation [18]. Macrophages produce more cytokines, chemokines, and HMGB1 as inflammation progresses due to the continual extracellular release of HMGB1. Furthermore, IL-1β that is secreted encourages osteoclastogenesis and bone resorption. The HMGB1 secretion loop thereby initiates, exacerbates, and prolongs periodontal inflammation [18].

In periodontitis patients, elevated HMGB1 has been identified in Gingival Crevicular Fluid (GCF) and positive correlation has been demonstrated with the periodontal clinical measures [7,9]. The combination of HMGB1 and IL-1β amplifies the proinflammatory characteristics of IL-1β as cellular activation induced by the complex is substantially higher than that induced by equal quantities of IL-1β alone. Thus, IL-1β activation is involved in HMGB1-induced oral inflammatory diseases [4,14,18]. The above findings are suggestive that HMGB1 has a very minor proinflammatory capabilities on its own, but its position as a carrier protein for cytokines or other mediators are capable of eliciting cell activation makes it more potent. Butyric acid produced in the periodontal pocket has been shown to induce apoptosis in murine and human T/B-cells, as well as fibroblasts isolated from patients with periodontitis. It induces necrosis of gingival epithelial cells to release HMGB1 [Table/Fig-1] [4,14,18,19].



[Table/Fig-1]: Role of HMGB1 in the progression of periodontitis [4,14,18,19].

The Human Gingival Fibroblast (HGF) secretes HMGB1 in response to LPS from two main periodontal pathogens, *P. gingivalis* and *A. actinomycetemcomitans*. During apoptosis and necrosis, these cells are a major source of HMGB1. It was previously thought that HMGB1 may be released passively after necrosis but not after apoptosis, because HMGB1 stays adherent to the chromatin inside the nucleus even at the late stage of apoptosis, also known as secondary necrosis [20]. Recent research has shown that HMGB1 release by apoptotic cells varies depending on cell type, and that the increased HMGB1 release was reduced by the addition of carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-fmk), a powerful caspase-2 and caspase-3 inhibitor, strongly implying that HMGB1 is secreted when HGF undergoes apoptosis [21,22].

The secretion of HMGB1 by periodontal tissues is hypothesised to boost proinflammatory cytokine production and prolong periodontitis. HMGB1 enhanced the synthesis of TNF-α, IL-1, IL-6, IL-11, and

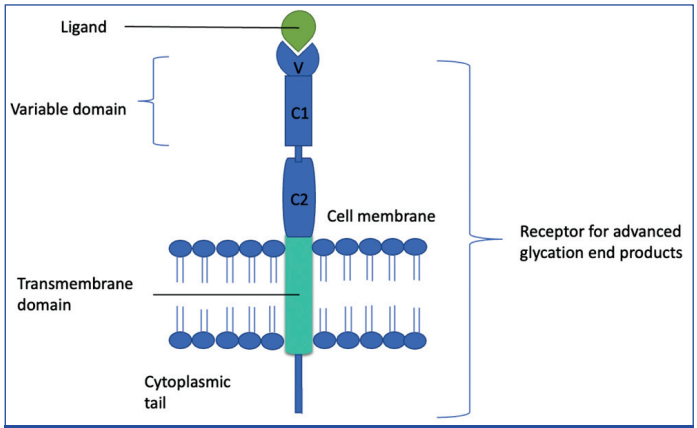
IL-17 mRNA in immortalised human Periodontal Ligament Cells (PDLC) [23]. It also increased TLR4 and TLR2 expression in human polymer dispersed liquid crystals, and anti TLR2 and anti TLR4 antibodies reduced HMGB1-induced osteoclastogenic cytokine expression and secretion, as well as RANKL expression. According to Park JS et al., RAGE plays only a minor role in macrophage activation by HMGB1, while TLR2 and TLR4 signalling increases TNF-α, IL-1 and IL-6 production in cultured mouse neutrophils and macrophages [24]. Liu L et al., observed the presence of HMGB1 and TLR4-positive cells around periapical lesions surrounding the apical foramen, indicating osteoclast activation [25].

Fibroblasts in the Periodontal Ligament Fibers (PDLF) have been hypothesised to participate significantly in the process of wound healing and regeneration in the periodontium. In-vitro experiments have revealed that the HMGB1 protein increases PDLF proliferation and migration and enhanced expression of osteopontin, Runx2, osteocalcin, bone morphogenetic protein and alkaline phosphatase [23,26]. HMGB1 helped stabilise the organisational and the operating integrity of the periodontium after periodontal injury, such as orthodontic tooth movement, but not periodontal regeneration. The inhibition of HMGB1 has been linked to the advancement of periodontitis disease in studies. In a mouse periodontitis model, glycyrrhizin binds to HMGB1 specifically and reduces cytokine activity, limiting periodontal progression [27].

Anti HMGB1 antibodies have been examined in a variety of illnesses, including sepsis and cerebral infarction. In a mouse periodontitis model, anti HMGB1 antibody reduced activity of Myeloperoxidase (MPO), migration of neutrophils, and resorption of bone in a dose-dependent manner. Periodontal inflammation can be treated more quickly by reducing HMGB1. The antibody inhibited TNF-induced IL-1 production in human gingival epithelial cells and TNF-induced GM-CSF synthesis in THP-1 (human leukaemia monocytic cell line) cells in-vitro [18].

RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (RAGE)

RAGE was discovered in 1992 in bovine lung and given the name RAGE because of its ability to act as an AGE receptor [5]. RAGE is also a signalling receptor that has an impact on gene expression and cellular properties. It is a type 1 transmembrane glycoprotein that belongs to the Ig superfamily of receptors due to the presence of several Ig-like domains in its extracellular region [Table/Fig-2] [5].



[Table/Fig-2]: Schematic representation of RAGE [5].

The ectodomains are positively charged and constitutes of a VC1 domain that is ligand binding followed by the C2 domain, there is a transmembrane domain and a cytoplasmic domain which is important for intracellular signalling [28,29]. The gene coding RAGE is present on the 6th chromosome in the region coding for Major Histocompatibility Complex (MHC) class III, that constitutes genes associated with adaptive and innate immune responses. It has exons 11 in number, including a high level of DNA and protein conservation across mammalian species, and undergoes significant alternative RNA splicing, creating over 20 distinct mRNA transcripts; however, the full form RAGE isoform is the most common [5,28].

RAGE is a Pattern Recognition Receptor (PRR) that is upregulated in chronic inflammation, and is a hallmark of disease states. It engages with DAMPs present endogenously, for instance glycated end products, high mobility box-1 calcium binding calgranulin proteins, β amyloid peptide, and several other amyloid proteins. Further they also bind macrophage adhesion ligand-1 complement proteins phospholipid derivatives, LPS, transport protein transthyretin, heparin sulphate, and proteins produced during stressful conditions [29].

RAGE Ligand Signaling

RAGE promotes MAP/ERK 1/2, p38, SAPK/JNK, signal transducer and activator of transcription 3, Ak strain transforming, and Ras homologous GTPases, among other intracellular signalling pathways {Ras-related C3 botulinum toxin substrate 1(Rac1), Cdc42}. Transcription factors such as NF-kB, Early Growth Response protein 1 (EGR-1), and SP-1 are activated downstream as a result of this activation. Following this activation, there is increased expression of proinflammatory genes such as Vascular Cell Adhesion Molecule-1 (VCAM-1), IL-1, IL-6 and several other immune modulators [30,31]. Intracellular or cytoplasmic domain of RAGE is required to mediate these signalling processes.

Jules J et al., demonstrated the presence of a shortened version of RAGE i.e. Receptor for Advanced Glycation End products truncated Intracellular Domain (RAGEDICD), that is produced by alternative splicing and lacks the majority of the Intracellular Domain (ICD). This unique isoform has been found in the lungs, kidneys, brain, and heart of both humans and mice. Through the MAP kinase pathway, RAGEDICD (RAGE cytoplasmic) expression inhibits RAGE-ligand signalling in C6 glioma cells. It further downregulated important cell tumorigenic pathways involved in cell adhesion, maintaining cell viability, cell migration, and tissue invasion, as well as tumourigenesis. Therefore, such RAGE isoforms producing by alternate splicing may be used as newer therapies for regulating RAGE signalling, and function [32].

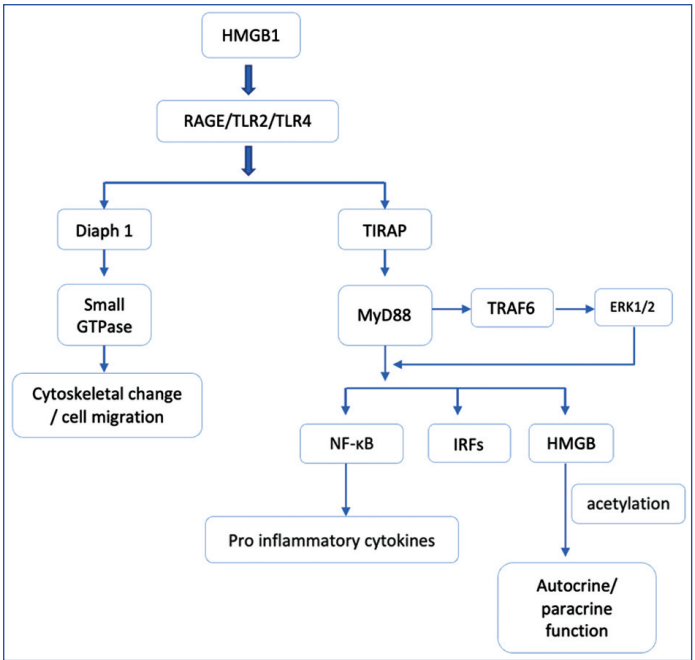
RAGE appears to trigger signalling by interacting with adaptor proteins like diaphanous-1/mDia1, ERK1/2, Protein Kinase C, Toll-Interleukin-1 Receptor (TIR) domain-containing adaptor protein (TIRAP), and Dedicator of Cytokinesis (DOCK7). mDia1 is the only protein that binds directly to the cytoplasmic domain of the RAGE and modulates intracellular signalling by activating MAP kinase and Rac-1/Cdc42. It has been identified that the amino acids R366 and Q367 in the RAGE binds to the mDia1 FH1 domain in the crystal structure of RAGE/mDia1. Protein kinase C (PKC) phosphorylates the RAGE cytoplasmic domain at S391 and influences downstream kinase activity [33].

Rage Gene Polymorphism

In serum and tissue, RAGE and its ligands have been shown to be potential biomarkers for disease onset and severity. The primary polymorphisms in the RAGE gene are a Single-Nucleotide Polymorphism (SNP) generating a protein coding alteration in the V domain (Gly82Ser) and two promoter variants (–374T/A and –429T/C). When stimulated with S100A12, cells bearing the 82S allele of the RAGE have a higher binding affinity to S100A12 in transfected cells in-vitro than cells expressing the 82G allele; additionally, when stimulated with S100A12, cells bearing the 82S allele produce inflammatory mediators at a higher rate than cells expressing the 82G allele, including TNF-α and IL-6 [34].

HMGB1-RAGE-sRAGE AXIS

The role of HMGB1-RAGE axis provided a new link in the pathogenesis of diabetes-related periodontitis [Table/Fig-3] [5,28-31].



[Table/Fig-3]: HMGB1 signal transduction pathway: HMGB1 binds with RAGE/TLR2/TLR4 and activates Diaph 1 and TIRAP. myD88 activates TRAF6, translocates NF-kB, triggers inflammatory responses [5,28-31].

In diabetic mice, inhibiting HMGB1 with sRAGE prevented bone loss associated with periodontitis, and periodontitis patients had lower levels of sRAGE and cleaved RAGE [28-35]. However, because other RAGE ligands may also bind to this receptor, sRAGE used to neutralise RAGE receptors may not completely block HMGB1. Because it binds to the protein directly and reduces its proinflammatory effects, metformin, the first-line therapy for type 2 diabetes, is also an HMGB1 inhibitor [35]. Morimoto-Yamashita Y et al., discovered that HMGB1 is mostly found in the nucleus of epithelial cells in healthy gingival tissues, but it is also found in the perinuclear region and the cytoplasm of inflamed gingival epithelial cells [4]. HMGB1 enhanced periodontal ligament cell proliferation and migration, according to Wolf M et al., [26]. Increased levels of alkaline phosphatase specific activity and osteopontin expression were indicated by HMGB1 upregulating osteogenic differentiation of periodontal ligament cells. HMGB1 can also act as a carrier protein for cytokines that can activate cells and cause them to produce more cytokines. HMGB1, a chemotactic factor for osteoblasts and osteoclasts that modulates endochondral ossification and is actively secreted by chondrocytes, is a chemotactic factor for osteoblasts and osteoclasts that modulates endochondral ossification. TLR2/4 dependent signalling pathways trigger the activation of NFB, and HMGB1 stimulates osteoblast migration [28].

After HMGB1 treatment, the migration rate of osteoblasts increases 2.3-fold, and it has a synergistic effect on osteoclastogenesis with Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) [36]. The production of RANKL, IL6, and TNF- by osteoblasts promotes inflammatory bone loss. In-vitro and in-vivo, RANKL causes HMGB1 release, which is needed for RANKL-induced osteoclastogenesis [37]. Systemic infusion of an anti HMGB1 neutralising antibody significantly reduced periodontal inflammation and bone resorption. HMGB1 expression was increased in the gingiva and periodontal ligament in experimental periodontitis. In cases of periodontitis, the level of HMGB1 in gingival crevicular fluid has shown a positive connection with probing pocket depth and clinical attachment loss. *P. gingivalis* causes bone loss by inhibiting osteoblast differentiation and osteogenesis and stimulating osteoclastogenesis and osteoclast activation. *P. gingivalis* suppresses osteoblast differentiation and osteogenesis while stimulating osteoclastogenesis and osteoclast activation, resulting in bone loss. It also inhibits innate immune responses by using an NDK homologue that interferes with extracellular ATP-mediated P2X7 activation. As a result, *P. gingivalis* evades host immunological responses by suppressing inflammasome activation and HMGB1 secretion [30].

Schmidt AM et al., and Lalla E et al., observed higher expression of AGEs in tissue biopsies from diabetic periodontitis patients, implying that AGEs are generated as a result of chronically elevated serum glucose levels in diabetics [38,39]. Katz J et al., studied RAGE expression by cells in periodontal lesions and discovered a difference in RAGE mRNA expression between diabetic and non diabetic patients, demonstrating the robustness of elevated AGE or RAGE in diabetic gingival tissues compared to non diabetic gingival tissues [40]. Xie P et al., examined the GCF and gingival tissues of patients with chronic periodontitis, and peri-implantitis in clinical trials, and reported that HMGB1 levels were elevated and that it plays a crucial role in periodontal tissue destruction [9]. Nogueira AV et al., used LPS and IL-1β to stimulate (mPDLF) in-vitro and detected a significant increase in the protein expression of HMGB1 in mPDLF and increase in HMGB1 mRNA in gingival tissues of LPS induced periodontitis model [20].

Pei X et al., found upregulated expression of HMGB1 and RAGE in the periodontal tissues of rats with diabetes and periodontitis associated with severe periodontal tissue destruction and increased expression of TNF-α suggesting that the HMGB1/RAGE signal axis is important for diabetes associated periodontal destruction and an important link in the inflammatory pathway [41]. Liu L et al., found increased apoptosis of bone-lining cells and osteoblasts in diabetic rats in an animal model [25]. As a result, diabetes is linked to an inflammatory response that persists, increased cell loss, increased alveolar bone resorption, and decreased new bone formation. Increased apoptosis of bone-lining cells and PDL fibroblasts may impact the latter. Diabetes enhances the death of osteoblast cells in-vivo, according to Alikhani M et al., [42], and this leads to diabetes-mediated reduction of new bone production.

Epidemiological investigations have shown that periodontitis is related to diabetes and liver disease [43]. The liver is a critical organ for glucose metabolism as well as an important immunological organ that regulates inflammation and metabolic diseases.

RAGE deposition has previously been discovered in gingival biopsies of diabetic and non diabetic periodontal diseases. In non diabetic patients, RAGE deposition tends to rise linearly with chronological age [31]. RAGE levels were higher in diabetic patients than in non diabetic patients. As a result, higher RAGE levels in gingival tissues may indicate a risk for the emergence of periodontal disease rather than being pathogenesis drivers [44,45].

Individual susceptibility to periodontal disease has received a great deal of attention lately. If RAGE is a risk factor for the onset of periodontal disease, there are two important factors to consider. Firstly, increased risk may be related to the individual's rate of RAGE formation from multiple sources; however, reduced risk may be related to the individual's ability to limit AGE formation as well or to the efficacy of RAGE removal by scavenger processes, such as the glyoxalase-1 receptor or macrophage scavenger receptor [46].

CONCLUSION(S)

The role of HMGB1-RAGE axis delivers a good understanding about the pathogenesis of periodontitis and diabetes associated periodontitis. HMGB1 can possibly be used as a diagnostic biomarker. Identifying the role of HMGB1 and RAGE as key mediators of inflammation can enable novel therapeutic drug based interventions based on blocking the HMGB1-RAGE axis that could possibly mitigate the inflammation mediated tissue destruction in diabetes associated periodontitis

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PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Periodontics, SRM Dental College, Chennai, Tamil Nadu, India.
2. Professor, Department of Periodontics, SRM Dental College, Chennai, Tamil Nadu, India.
3. Professor, Department of Periodontics, SRM Dental College, Chennai, Tamil Nadu, India.
4. Professor, Department of Periodontics, SRM Dental College, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. R Ramya,
C-2/4, Coast Guard Quarters, GM Pettai Road, Chennai, Tamil Nadu, India.
E-mail: ramya7raj@gmail.com

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