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# Salivary Tumour Necrosis Factor - α Key Inflammatory Mediators in Periodontal Disease

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## Abstract

Oral health is indispensable to overall health and oral diseases especially periodontitis is well known for its high prevalence and rapid morbidity. Function of the host immune response is the most crucial feature in periodontitis. And this is the reason for both disease progression and severity. Search for a biomarker for periodontitis has turned on researches working out in finding many newer molecules (cytokines), that can help the clinician in deciding regarding the patient's status. This review shortly covers the multiple facets of crosstalk engaged by salivary Tumour Necrosis Factor-α key inflammatory mediators in periodontal disease.

**Keywords:** Genetics, Immunoregulatory Cytokines, Salivary TNF, TNF-α

## 1. Introduction

Periodontitis is a persistent microbial infectious ailment which causes inflammation mediated obliteration of periodontal ligament and alveolar bone that supports our teeth. Pathogenesis of the periodontal disease depends on both bacterial and host immune factors. The progression and severity of the disease is also determined mainly by role of host immune responses. The presence of the infectious agent is crucial to initiate the host responses and the following inflammatory reactions in chronic periodontitis, stimulation of various cytokines against bacterial antigens play crucial role in immune response of the patient[1,2]. TNF is an immunoregulatory cytokine which is central to the pathogenesis of inflammatory condition. It is one of the important of proinflammatory cytokines which is a "bine edged sword" in that it produces both bacterial and tissue destruction.

Cytokines are polypeptides produced by various cell types (but primarily by activated lymphocytes and macrophages) that modulate the function of other cell types. The main cytokine molecules that mediate inflammation are IL-1, TNF (α and β). The mechanism involved in this process of these cytokines is associated with tissue destruction that stimulates bone resorption and induction of tissue degrading proteases. TNF-α is a pro inflammatory cytokine which is produced mainly by macrophage[3–5]. It can also be created by various other cell types such as CD4 lymphocytes, NK cells, neutrophils, mast cells and neurons. There are two types of TNF, they are TNF-α (cachectin) and TNF-β (lymphotoxin). TNF-α has stimulus to proliferation, differentiation and activation of osteoclast. It also induces the production of MMP from mesenchymal cells and may be linked to destruction of connective tissue. This article discusses the role of salivary TNF-α in periodontal disease.

## 2. History

In end of 19th century Dr. William B. Coley acknowledged the hypothesis of an antitumoral response of immune system[6]. In 1968, Dr. Gale A. Grayen found a cytotoxic factor generated by lymphocytes and called it lymphotoxins. In 1975 L loyd J. Old found one more cytotoxic factor generated by the macrophage and called as tumor necrosis factor. In 1984 DNAs encoding Lymphotoxin and TNF was cloned and appeared to be similar. Then TNF is renamed as TNF-α, lymphotoxin is

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renamed as TNF-β. In 1985 Bruce et al., discovered the cache tin which was actually TNF.

## 3. Organization

TNF-α is created as a 233 amino acid long type II trans membrane protein organised in established homotrimer. STNF/soluble TNF is released through proteolytic cleavage of membrane integrated form by enzyme called TNF-α converting enzyme[7]. This veiled form of TNF-α has triangular pyramid shape weighs about 17KDa. Both forms, secreted and membrane bound have similar and distinct biological activities. TNF protomers are made of two antiparallel pleated sheets and antiparallel β strands that form Jelly roll β-structure.

## 4. TNF Induced Signalling Pathways

### 4.1 TNF Receptors

TNF-α binds to two types of receptors TNF - R1 and TNF - R2. TNF - R1 (55KDa) is articulated in tissues is fully activated by both forms of TNF, but TNF - R2 (wt75KDa) is seen typically in cells of immune system and retort to membrane bound forms of TNF. TNF - R1 is the main receptor for cellular effects in most cell types. TNF - R1 is a death domain containing receptor with an extracellular domain, a transmembrane domain and a intracellular domain. TNF - R2 does not have death domain but it can mediate cell death through TNF - R1 indirectly. TNF - R1 forms a homotrimer to take the TRPD (TNFR associated death domain) this TRPD serve as platform to recruit the downstream adaptor protein (RIP, TRAF2 and FADD) to generate distinct signalling pathways like NF-kB (Nuclear factor kappa - light chain- enhancer of activated B cells) pathway, MAPK pathway and cytotoxic pathway.

### 4.2 NF-Kb Activation by TNF α

TRADD recruit TRAF2 and RIP. TRAF2 (TNF receptor associated factor 2) recruit IKK (Inhibition of KB Kinase) which enables the RIP (receptor interacting proteins) activate IKb (which usually bind to NF-Kb and inhibits its translocation). This leads to NFkB translocate into nucleus and mediate transcription of the proteins which are concerned in the cell survival, multiplication, provocative response and anti apoptotic factors. NF-Kb also induce antioxidant MnSOD (Mitochondria – localised manganese superoxide dismutase) which is having an apoptotic effect and also having necrotic cell death.

### 4.3 MAPK activation by TNF

Of the 3 major MAPK (Mitogen activated protein kinase) cascades TNF induce strong commencement of JNK (JUN N-terminal kinase), response of the p38-MAPK and minimal activation of the ERKs (Extracellular signal regulated kinase). TRF2/RAC activates JNK which induce kinase of MLK2 (Mixed lineage kinase), TAK1 (Transforming growth factor β – activated kinase 1), ME KK1 and ASK (Apoptosis signal regulating kinase). There are two MAP2Ks (JNKK1 and JNKK2) that stimulate JNK. Then JNK translocate to the nucleus and activates the transcription factors (JUN and ATF2) (Activating transcription factor 2). JNK pathway is involved in cell proliferation pathway has a function in cell proliferation cell differentiation and it is normally pro-apoptotic.

### 4.4 TNF Induce Cytotoxicity

TNF induce apoptosis in variety of cell types. TNF provoke cell death plays simply a small function compared to its function in the inflammatory process. TRAPD binds FAPD which then recruits cystein proteases caspase 8. This caspase 8 trigger the activation of executer caspase 3-7 and endonuclease and results in loss of the cell protein, disintegration of the DNA and ultimately apoptotis cell death (this is called extrinsic apoptotic pathway)

It also induce intrinsic apoptotic pathway by caspase 8 which activates BCL-2 interact domains that cause loss of the mitochondrial membrane potential and release of cytochrome C and S mac commencing from the mitochondria to cytosol.

### 4.5 Biological action of TNF – α

TNF-α induce metalloproteinases M-GM-CSF and IL 6. Primary function of the TNF-α is organisation of immune cells. TNF, as endogenous pyrogen is capable of causing fever, apoptotic cell death, cachexia, inflammation and inhibit tumorigenesis.

### 4.6 TNF in GCF

TNF can be estimated from GCF and can be detected by immunoadsorption, magnetic separation technology. The levels estimated by this technique revealed higher levels compared to paper strips which were previously used[8].

## 4.7 Salivary TNF α in Pathogenesis of Periodontal Disease

Two large groups of microorganisms namely A. A and P. gingivalisis capable to induce the monocytes plus macrophages which in turn produces TNF-α[9]. TNF Alpha shares many of the cellular action of IL-1 β. TNF α increases neutrophil activity and mediate cell time turnover by inducing MMP secretion[10]. It also stimulates the osteoclast development and limits the tissue repair by induction of apoptosis in fibroblast. Study done by Geve et al., stated that the levels of TNF-α is higher than the healthy periodontium.

## 4.8 Proinflammatory Effects of TNF-α

TNF alpha stimulate the endothelial cell to express the selectins that facilitate leukocyte trapping, activation of macrophage, IL-beta production and stimulation of PGE2 by macrophage and gingival fibroblast.

## 4.9 TNF-α Action in Bone Resorption

TNF alpha causes bone resorption through its skill to induce IL-1 and GM-CSF (Granulocyte macrophage colony stimulating factor) that inhibits collagen production, collagenases and arouse osteoclast differentiation in the presence of M-CSF.

## 4.10 Action of TNF-α in Inflammation

Mobilization of unstimulated neutrophils from the marrow into the circulation is controlled by TNF, IL1, CSF. Aggregation and priming of PMNs, recruitment of the neutrophils keen on the area of inflammation are controlled by TNF-α. TNF-α induces a stop sign through P38 MAPK pathway and causes firm neutrophil adhesion and inhibit the neutrophil polarization and chemotaxis near chemoattractant.

## 4.11 Action of TNF-α on Endothelial Cells

TNF-α act on endothelial cells that cause endothelial discontinuity. It increases surface thrombogeneicity of endothelium and also induce synthesis of other cytokines and growth factors. It stimulates the production of P selectin and E selectin on their luminal space which causes rolling and increased expression of ICAM (Intercellular adhesion molecule).

## 4.12 Action of TNF-α on Lymphocytes and Monocytes

TNF alpha increases the secretion of prostacyclin, increases making of the ELAM-1 (Endothelial leukocyte adhesion molecule 1) induction plus secretion of IL-8, extravasation of lymphocytes and monocyte, which induce formation of granuloma.

## 4.13 Action of TNF-α on Immune Cells

Cytokines release in peripheral blood when exceeds can lead to the negative changes in the immune responses. TNF-α induces ICAM-1 binding of T cells to Antigen Presenting cells. It has co mitogenic action for thymocytes and induces IL-2 recepter expression. It as well augment β Cell proliferation, surface immmunoglobulins plus the antibody production. TNF Alpha also acts as mediator of Ig E dependent cutaneous reaction.

## 4.14 Action of TNF-α on Connective Tissue Elements

TNF-α stimulate alkaline phosphatase activity in osteoblast which induce ostesclast to resorb bone. It stimulate the chondrocyte to increase cartilage turnover and also induce collagenase in cartiage. TNF αinhibits proteoglycan synthesis.

## 4.16 Action of TNF-α on Fibroblast

TNF-α upregulates the production of the myofibroblast marker α-SMA (α smooth muscle acting) and ED-ASN which is expressed by myofibroblasts during the process of wound healing. TNF alpha was able to inhibit TGF – β1- stimulate Smad phosphorylation. TNF-α also inhibits the TGF β1, stimulates Type I collagen and fibronectin production in dermal fibroblast.

## 4.17 Salivary TNF-α in Type 2 Diabetes

An association between the TNF and the development of diabetes has also been described. TNF-α inhibit the insulin transduction and contribute to insulin resistance in diabetes mellitus. In diabetes advanced glycation end products is produced that can connect to macrophage receptors and induces a sequence of cytokine (IL, TNF-α) up-regulation. This leads to elevation of expression of MMP in diabetic periodontal tissue which causes collagen destruction.

In 2001 Yoshihiro et al reported that TNF-α produced by periodontal inflammation synergistically affect insulin resistance in type II DM and also anti infective treatment for periodontal disease is effective in improving metabolic control in DM. In 2014 Prima et al., reported that salivary TNF alpha is significantly higher in type II DM with chronic periodontitis group compared to healthy group.

### 4.18 Salivary TNF-α in Smokers

Smoking reduces the basic host response in bacterial clearance and neutralizing of illness. Prima et al during 2014 reported that TNF-α level in smokers in the company of chronic periodontits higher compare to healthy periodontium and non smokers.

## 5. Genetics

It has been hypothesised that there may be genetic predisposition in some individuals to produce large quantities of TNF during inflammation. The principal mechanism of TNF mediated lethality is through increased susceptibility to the complication of cerebral malaria and this is implicated in other diseases like Rheumatoid arthritis[11], multiple sclerosis, meningitis and acute respiratory disorder[12]. TNF-α gene is located within CL III region of MHC complex on short arm of chromosome 6. There is a base transition polymorphism in the promoter region that are mainly G to A transition in the position – 376, - 380, -238, +589, +489., and 587[13]. Solitary nucleotide polymorphism on the promoter region of TNF gene is studied for susceptibility for tumor risk.

Individuals having an AA/GA genotype at TNF-308 had higher TNF expression levels and amplified cancer risk includes non hodgkins lymphoma, hepatocellular carcinoma, gastric cancer, ulcerative colitis, associated colorectal cancer and non small cell lungetumour.

In 1998, Kernman and Digiuine reported an increased TNF α308n2 allelic frequency in caucasian patients in the company of chronic periodontitis in the healthy individuals. This difference was related with severity of the disease. In 1998, Gallbraith et al., demonstrated 308 n2 allele is a risk factor for the sternness of adult chronic periodontitis and its presence is used to detectthe most severe case of the periodontal disease. In 2003 Lin et al reported an amplified occurrence for the TNF α308n2 allele of Chinese patients with chronic periodontitis.

### 5.1 Theraupeutic uses of TNF-α

Anti TNF α therapies (monoclonal antibodies) used in the treatment of septic shock[14], rheumatoid arthritis, local application of recombinant TNF used to isolate cancer bearing organ an extremities have met success in tumor regression. TNF blockers either applied topically or by injection through carrier such as collagen alginate or hyaluronan offers new potential for tooth migration, eruption and treatment of pericoronitis[15]. In China Geng et al., reported that salivary TNF-α and IL6 in patients with chronic periodontitis were higher than healthy individuals. In United States Rai et al., reported that salivary TNF-α were significantly higher in patients with periodontitis than control group. High cost and activation of latent TB is the disadvantage of systemic TNF blockers[16]. The saliva TNF α analysis can be utilised as diagnostic tool and as prognostic biomarker.

Ng et al evaluated the potential salivary biomarker correlation with bone resorption. TNF-α also reviewed and estimated in this study[17]. Frogde et al., reported that mean salivary TNF alpha considerably higher in the individual with periodontal disease. Hemsen et al., evaluated the function of TNF alpha as proinflammatory cytokines which regulates transcription of MMPs that cause tissue destruction in chronic periodontitis.

### 5.2 Salivary TNF Alpha in Other Oral Disease

Increased level of IL6, TNF alpha is seen in individuals with cancer and premalignant lesion namely as oral lichen planus with oral submucous fibrosis. Brailo et al., found that increased level of salivary TNF-α in patients with oral leukoplakiaas compared to healthy controls. Hahberger et al., reported that TNF-α and its dependant cytokines are increased in saliva during oral carcinogenesis and they have capacity to stimulate the events related to early invasion of oral cancer. Batoal et al., reported that salivary level of TNF α may plays role in pathogenesis of recurrent apthous stomatitis became salivary TNF-α was higher in recurrent apthous stomatitis patients when compared to healthy controls.

Future investigations will be directed towards study of other components of the cytokine network and their possible interrelations with chronic and aggressive periodontitis. High titres of the salivary TNF-α were there in diseased compared to healthy individual. Whenever there is an interference with normal host defence

mechanism, increased level of TNF stimulates destructive effects within the host.

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