



# Association of Cytokine TNF- $\alpha$ in Development of Osteoarthritis: A Comprehensive Study

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## Abstract

Osteo-Arthritis (OA) is a disease of joints affecting the normal functions of joint and causes physical disability. Many factors are responsible for development of osteoarthritis including over age, obesity, gender, drug abuse, over load on joints and genetic factors. OA causes health problems and impairs the quality of life with increased economic burden across the world. Apart from the pro-inflammatory role of cytokine Interleukin-1 (IL-1) in osteoarthritis, Tumor necrosis factor-alpha (TNF- $\alpha$ ) is also involved in the progression of osteoarthritis. Members of TNF family are secreted from lymphocytes and natural killer cells but in OA patients it is also secreted from chondrocyte cells to influence the catabolic processes in Extra Cellular Matrix (ECM) by inducing the activity of matrix metalloproteinases (MMPs). In promoter region, most of the Single nucleotide polymorphisms (SNPs) of TNF- $\alpha$  located on -863, -857, -308, and -238. These SNPs are involved in various diseases including OA, rheumatoid Arthritis, systemic lupus erythematosus etc. Significance association of SNP -G308A of the TNF- $\alpha$  gene in OA has been observed in various studies. Aim of this mini review is to conclude the fundamental roles of TNF- $\alpha$  cytokine in patients with OA.

**Keywords:** Cytokine, Gene Variants, Osteoarthritis, Pathophysiology, TNF- $\alpha$

## 1. Introduction

Osteoarthritis is age related joint disease that adversely affects the normal daily activities of life in elderly population. It is marked by continuous breakdown of articular cartilage and other components of joints<sup>1</sup>. It also leads to development of osteophyte. OA may affect almost all joints (hip, hand, knee etc) whereas in an obese person knee OA is most common. Females have slightly higher prevalence of developing OA as compared to males, females aged fifty and above suffer mostly from hand OA<sup>17</sup>. Among pro-inflammatory cytokines, both IL-1 $\beta$  and TNF- $\alpha$  have predominant roles in destruction of healthy cartilage by inducing some proteases<sup>20</sup>. Two important cells of joint viz. chondrocyte and synovial cell secrete these pro-inflammatory cytokines in extracellular spaces, where they induce inflammatory response<sup>18</sup>. Anti-inflammatory cytokines such as IL-4, IL-10, IL-13 inhibit the action of pro-inflammatory cytokines<sup>30</sup>. Developing OA is marked by over expression of degradative enzymes and pro-inflammatory cytokines. Most common enzymes in synovial fluid are Matrix Metallo-Proteinases (MMPs), aggrecanases<sup>37</sup>. It can be very difficult to understand about concentrations of pro-inflammatory cytokines because some patients show high concentrations whereas in others show low concentrations. Due to this reason, cytokines remain controversial.

Over the years, many studies have revealed that the genetic susceptibility strongly contributing in pathophysiology of OA development. High levels of pro-inflammatory cytokines in synovial fluid of OA patients indicate the breakdown of cartilage and progression of disease<sup>18</sup>. Members of TNF-family are predominantly synthesised by immune cells (B and T lymphocytes), NK-cells. It has been also reported that these cytokines concentration also high in synovial fluid in OA patients (O'Rourke *et al.*, 2008). TNF- $\alpha$  encoding gene is present at most polymorphic region of DNA, where the major histocompatibility complex type-III (MHC-III) gene is located<sup>12</sup>. Number of gene variants of TNF- $\alpha$  increase the susceptibility in OA. Aim of this study to explore the impact of TNF- $\alpha$  cytokine and their gene variants in development of OA.

## 2. Role of TNF- $\alpha$ in Pathophysiology of Osteoarthritis

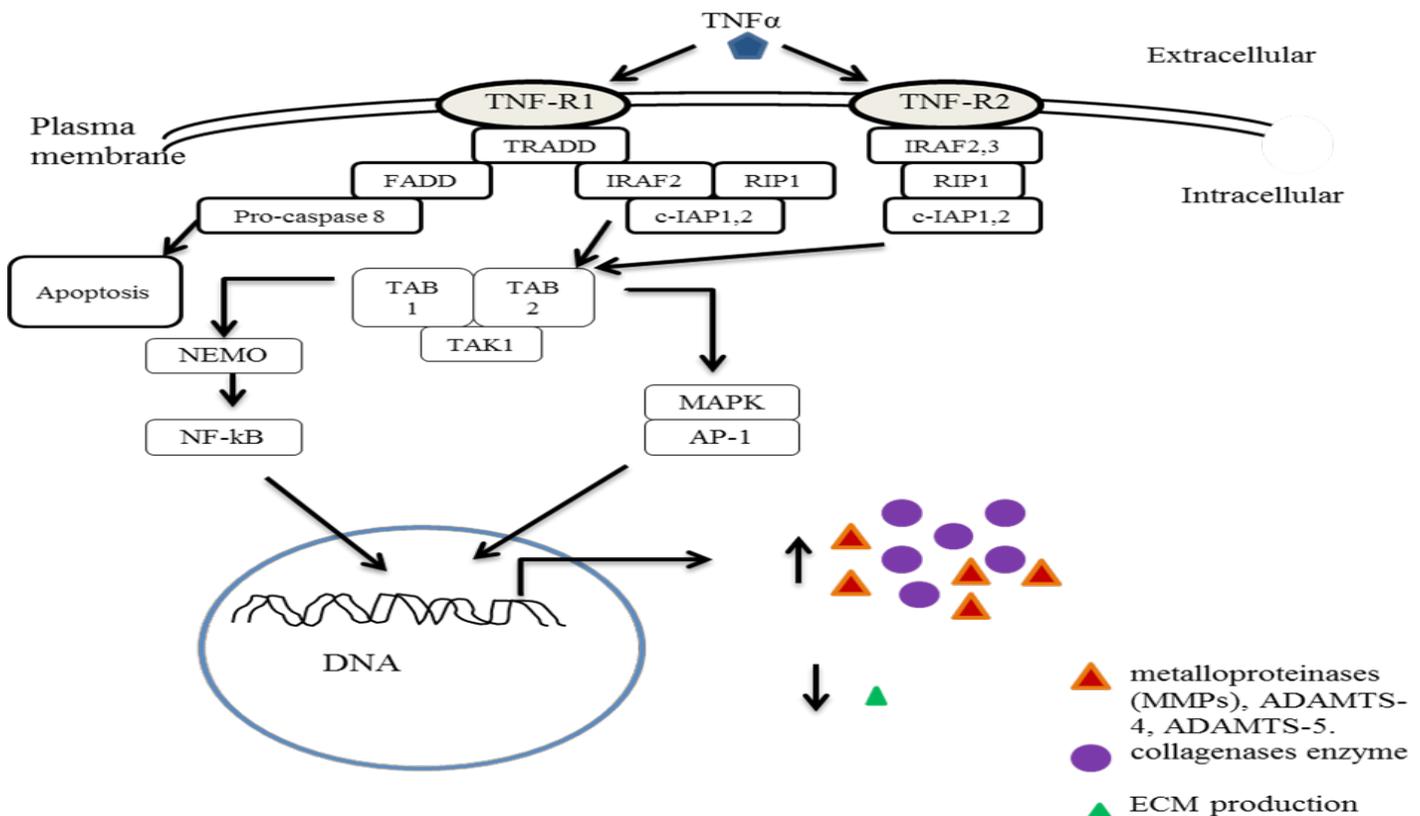
TNF- $\alpha$  is a pro-inflammatory cytokine which plays a role in pathophysiology of OA<sup>34</sup>. It is a 17 kDa secreted protein by activated chondrocyte, macrophage and other cells which affects the synthesis of other cytokines such as IL-1 and IL-8<sup>9</sup>. TNF- $\alpha$  along with IL-1 $\beta$  involved in etiology of the disease. It is one of the 19 members of Tumor Necrosis Factor (TNF)

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superfamily<sup>5</sup>. TNF- $\alpha$  is primarily composed of three identical transmembrane proteins Type II (mTNF $\alpha$ ) and later TACE/ADAM17 metalloproteinase cause maturation of mTNF $\alpha$  into soluble free TNF- $\alpha$  (sTNF- $\alpha$ ), which is secreted out by the cell<sup>11</sup>. Both IL-1 $\beta$  and TNF- $\alpha$  are secreted from same cell of the joint.

TNF- $\alpha$  is mainly synthesized in synovial chondrocyte cells. Elevated level of TNF- $\alpha$  was detected in synovial membrane, cartilage tissue, sub-chondral bone layer<sup>7</sup>. TNF- $\alpha$  has affinity to interact with two receptor isotypes (TNF-R1 and TNF-R2). These receptors are expressed on membrane of about all nucleated cells<sup>22</sup>. The receptor TNF-R1 can be stimulated by the soluble as well as membrane forms ligand whereas receptor TNF-R2 only recognises membrane form of ligand. Thus, the receptor TNF-R1 has higher impact on cartilage breakdown than of receptor TNF-R2<sup>22</sup>. Elevated expression of TNF-R1 has also observed in Fibroblast-like synoviocyte<sup>25</sup>. Structurally TNF-R1 and TNF-R2 are unrelated with their carboxyl terminal domain. TNF-R1 has associated with Death Domain (DD) in their carboxyl terminal which lack in TNF-R2<sup>11</sup>. Insight the intracellular dissimilarity between these two receptors, they relay different signal from outside of the cell to the nucleus. Signal transduction through TNF-R1 is involved by formation of two complexes. The complex-1 is inherent

in the expression of proteins responsible for prevention of apoptosis and secretion of inflammatory cytokine whereas second complex is directly involving in signal transduction and fragmentation of cell<sup>35</sup>. The binding of TNF- $\alpha$  to TNF-R1 leads to interaction of carboxyl terminus (Death Domain) with adapter proteins such as TRADD, TRAF, c-IAP1, c-IAP2 and RIP1<sup>6</sup>. This interaction results in proteasomal degradation of RIP1 protein and interaction with other proteins such as TAB1, TAB2 and TAK. Subsequently, the phosphorylation of IKK protein takes place which is associated with NEMO protein. IKK is responsible of activation of most important transcription factor NF- $\kappa$ B<sup>21</sup>. During activation of complex-1 other pathway also triggered involving Janus Kinase (JNK), Extracellular Regulated Kinase (ERK) and Mitogen Activated Protein Kinase (MAPK)<sup>14,38</sup>. Activation of complex-2 through TNF-R1 results in endocytosis of receptor and interaction with FADD and subsequently activation of pro-caspases-8 into activated caspases-8. This pathway finished with cell death<sup>28</sup>. After the formation of complex of mTNF- $\alpha$  and TNF-R2, interaction of adopter proteins such as TRAF2, TRAF3, RIP1, c-IAP1 and c-IAP2 with receptor occur and proteosomal degradation of RIP1 takes place. End responses through this signal are the activation of transcription factor NF- $\kappa$ B and AP1<sup>24</sup> (Figure 1).



**Figure 1.** Intra cellular signaling pathway of TNF- $\alpha$  in OA

The final responses are the production of metalloproteinases (MMPs), ADAMTS4, ADAMTS-5, collagenases and inhibition of Extra Cellular Matrix (ECM) components production. Tumor necrosis factor- $\alpha$  receptor1 (TNF- $\alpha$ R1), Tumor necrosis factor- $\alpha$  receptor2 (TNF- $\alpha$ R2), Tumor necrosis factor receptor type 1 associated death domain protein (TRADD); Fas-Associated protein with Death Domain (FADD); TNF receptor-associated factor 6 (IRAF6); c-IAP also called BIRC3 (Baculovirus IAP repeat-containing protein3). Receptor-interacting protein kinase 1 (RIP1); TAB1 is also known as mitogen-activated protein kinase kinase kinase 7 inactivating protein 1 (MAP3K7IP1) and TAB2 is also known as mitogen-activated protein kinase kinase kinase 7 inactivating protein 2 (MAP3K7IP2); TAK is a mitogen-activated protein kinase kinase kinase 7 (MAP3K7); NF- $\kappa$ B essential modulator/NF- $\kappa$ B inhibitor kinase1,2 (NEMO/IKK1,2); mitogen-activated protein kinase (MAPK); basal transcription activating protein called activator protein-1 (AP-1).

The catabolic effect of TNF- $\alpha$  leads to cartilage breakdown and also induces the sensory neurons through the TNF- $\alpha$  receptor 1, 2 (TNFR1 and TNFR2). TNF- $\alpha$  induced neuropathic pain may be treated by application of anti-inflammatory medications. Ibuprofen and celecoxib are known to reduce the pain through their anti-inflammatory properties<sup>32</sup>. It causes initiation of events of cascade in inflammatory reactions with other pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 etc<sup>3</sup>. Both TNF- $\alpha$  and IL-1 $\beta$  are involved synergistically in many cases during course of OA. These cytokines are sharing some cytosolic events and trigger the catabolic phenomenon and inflammation during development of OA<sup>16</sup> TNF- $\alpha$  is affecting the function of chondrocyte, blocking the production of extracellular components such as collagen type 2, proteoglycan<sup>15</sup>. Activated chondrocytes are increasing the expression of extracellular components degradative enzymes include MMPs and ADAMTS-4<sup>29</sup>. Chondrocyte apoptosis and migration of CPCs (Chondrogenic progenitor cells) will result in reduction of any chance of reappearing of normal articular cartilage<sup>10</sup>. Both TNF- $\alpha$  and IL-1 $\beta$  also disrupt the normal functioning of mitochondria and causes reduction in ATP synthesis hence, less energetic chondrocyte cell will also lose the mitochondrial membrane permeability<sup>23</sup>.

### 3. Gene Variants of TNF- $\alpha$ in Osteoarthritis

Gene for TNF $\alpha$  cytokine is located in core region of major histocompatibility complex. This gene encodes one of the most important proinflammatory cytokine is known as TNF $\alpha$ . This cytokine plays important role in cartilage damage of healthy joints especially in hand and knee. A study on Han Chinese population articulated that allele 'A' of TNF- $\alpha$  -308 variant has

an impact on risk of OA however, rs361525 SNP of TNF- $\alpha$  -238 has no impact on same population with OA<sup>4</sup>. HLA (Human leucocyte antigen)-class II/III region and TNF- $\alpha$  gene, where some SNPs such as rs7775228 and rs10947262 were found to be associated with knee OA<sup>27,33</sup>.

Recent study in Finnish women population was suggested that TNF $\alpha$  gene variants play crucial function in etiological process of hand OA. Both haplotype and minor alleles positioned at -1031 and -863 of the TNF $\alpha$  were involved independently in risk of hand OA<sup>31</sup>. Another study has been done in Egyptian female with severity of early onset knee OA suggested that TNF- $\alpha$  -G308A polymorphism involved in susceptibility to disease progression<sup>2</sup>.

Human Leucocyte Antigen (HLA) class-II and class-III consist of some variants of TNF- $\alpha$  gene which include rs7775228 and rs10947262. It has been observed that these variants are strongly associated with possibility to develop knee OA<sup>27,33</sup>. As per high level of expression of TNF- $\alpha$  with -308A allele and low expression of -308G allele, meta-analysis concluded that AA and AG genotypes influence the rate of risk of OA, whereas individuals with GG genotype might have lesser impact on developing of OA<sup>19</sup>. Among other variants of TNF- $\alpha$ , the minor allele of the "-308" locus is affecting the TNF- $\alpha$  protein expression under various stimuli<sup>36</sup>. Results of some previous studies unclear the association of gene variants of TNF- $\alpha$  with OA<sup>13</sup>. It was found that TNF- $\alpha$  can perform function independently in OA or in association with other cytokines such as IL-1 $\beta$ , IL-6 etc<sup>18</sup>. The locus "-1082" polymorphism of IL-10 inhibits the synthesis and production of TNF- $\alpha$ . IL-10 is an anti-inflammatory cytokine might be opposing the function of TNF- $\alpha$  in OA<sup>8</sup>.

### 4. Conclusion

OA is considered as one of the most prevalent joint disorder in elderly population worldwide. It commonly affects knee and hand joints but other joints also affected with limited function. It affects the daily activity of an individual who are susceptible to OA. Among other risk factors, proinflammatory cytokines such as IL-1, TNF- $\alpha$  are involved in progression of disease. During onset of OA, elevated levels of these cytokines observed in synovial fluid.

The TNF- $\alpha$  along with IL-1 $\beta$  plays important role in cartilage destruction in joints and leads to progression of OA. Remodelling of joint bone also influenced by these cytokines. After binding of TNF- $\alpha$  with their receptors, it activates proinflammatory signal cascade through different cytosolic kinases and transcription factors.

In continue, proinflammatory cytokines directly force the expression of proteolytic enzymes such as collagenases and others in the synovial fluid of joints. These enzymes are

responsible for breakdown of healthy cartilage and extra cellular matrix components of joint and leads to narrowing of joint space<sup>37</sup>. This will result in friction of two bone terminus and inflammation.

The gene variants of TNF- $\alpha$  are located in MHC protein encoding region. Among other variants of TNF- $\alpha$ , the locus “-308” has been involved in susceptibility of disease<sup>4</sup> that reported in many studies over decay.

## 5. References

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