

## Biomolecular basis of the role of diabetes mellitus in osteoporosis and bone fractures

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### Abstract

Osteoporosis has become a serious health problem throughout the world which is associated with an increased risk of bone fractures and mortality among the people of middle to old ages. Diabetes is also a major health problem among the people of all age ranges and the sufferers due to this abnormality increasing day by day. The aim of this review is to summarize the possible mechanisms through which diabetes may induce osteoporosis. Diabetes mellitus generally exerts its effect on different parts of the body including bone cells specially the osteoblast and osteoclast, muscles, retina of the eyes, adipose tissue, endocrine system specially parathyroid hormone (PTH) and estrogen, cytokines, nervous system and digestive system. Diabetes negatively regulates osteoblast differentiation and function while positively regulates osteoclast differentiation and function through the regulation of different intermediate factors and thereby decreases bone formation while increases bone resorption. Some factors such as diabetic neuropathy, reactive oxygen species, Vitamin D, PTH have their effects on muscle cells. Diabetes decreases the muscle strength through regulating these factors in various ways and ultimately increases the risk of fall that may cause bone fractures.

**Key words:** Diabetes; Osteoporosis; Diabetic neuropathy; Muscle atrophy; Insulin; Receptor activator for nuclear factor  $\kappa$ -B ligand; Interleukin 6; Angiotensin II; Tumor necrosis factor; Advanced glycation end product

**Core tip:** The physical complications due to diabetes mellitus are not limited since there have been going research to elucidate the relation of other diseases with diabetes mellitus (DM). Osteoporosis is one of the complicated diseases of human that may be linked with DM through different networks in the body. In this review a precise relationship has been made between DM and osteoporosis through a broad range of biophysical pathways.

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### INTRODUCTION

Osteoporosis (OP) has become an alarming health problem through the entire world and about 200 million people in the world are under the threat of this deleterious health problem<sup>[1]</sup>. Although OP is often described as a silent disease because it is typically asymptomatic until a fracture occurs, the disease negatively and significantly impacts morbidity and mortality as it can lead to severe pain, deformity, disability, and death<sup>[2]</sup>. The signs of OP are deterioration of the microstructure of bone specifically at trabecular sites including vertebrae, ribs and hips, culmination in fragility fractures, pain and disability<sup>[2,3]</sup>. The occurrence of OP is prevalent among the aging women than the aging men although corticosteroid treatment, intake of excessive alcohol, cigarette smoking, low calcium intake and hypogonadism may be the secondary cause<sup>[1,2]</sup>.

Like osteoporosis, diabetes mellitus is a pandemic and a chronic metabolic disorder with substantial morbidity

ity and mortality, characterized by the presence of high blood glucose<sup>[2,4,5]</sup>. According to the report (September 2012) of the World Health Organization (WHO) about 374 million people in the world are under the threat of this deleterious health problem<sup>[6]</sup>. Under chronic condition DM adversely affects the different parts of the body including bone, nerve, muscles, retina of the eyes, cardiovascular system and nephron of kidney<sup>[4]</sup>. The effects of DM on bone cell are very complex and several investigations have been conducted to explore the exact mechanisms through which DM induces osteoporosis and bone fractures and all the investigations have come to the end with few findings<sup>[6]</sup>. The exact mechanism of diabetes mellitus (DM) induced osteoporosis is almost unknown but it is plausible that, patients with DM have increased rate of osteoporosis and bone fractures<sup>[3,7-10]</sup>. Hyperglycemia may induce osteoporosis and bone fractures through exerting its effects on bone cells and muscle cells through different possible pathways. This review has explained the possible molecular mechanisms through which DM may induce osteoporosis and bone fractures.

## EFFECT OF DIABETES MELLITUS ON BONE CELLS

The bone mainly comprise of three basic types of cells osteoblast, osteocyte and osteoclast<sup>[11]</sup>. Osteoblasts commonly called bone-forming cells which derived from the osteoblast progenitor cells, participate in mineralization and are unable to multiply<sup>[11]</sup>. Osteocytes are mature osteoblast which no longer secretes matrix, participates in nutrient/waste exchange *via* blood and unable to divide. Osteoclasts are cells that derive from the macrophage-monocyte cell lineage and participate in bone resorption<sup>[1,11]</sup>.

### Osteoblast

Osteoblast originates from the mesodermal progenitor cell and among the three basic types of bone cells it plays a crucial role in bone formation. Binding of different types of growth factors and hormones including bone morphogenetic protein (BMP), Wnt, transforming growth factor- $\beta$  (TGF- $\beta$ ), parathyroid hormone (PTH), platelet derived growth factors (PDGFs), fibroblast growth factors (FGF) with their receptors expressed on the cell surface of mesodermal progenitor cells (also known as mesenchymal stem cells) induce the activation of different types of transcription factors responsible for osteoblast differentiation, maturation and survival<sup>[1,12]</sup>.

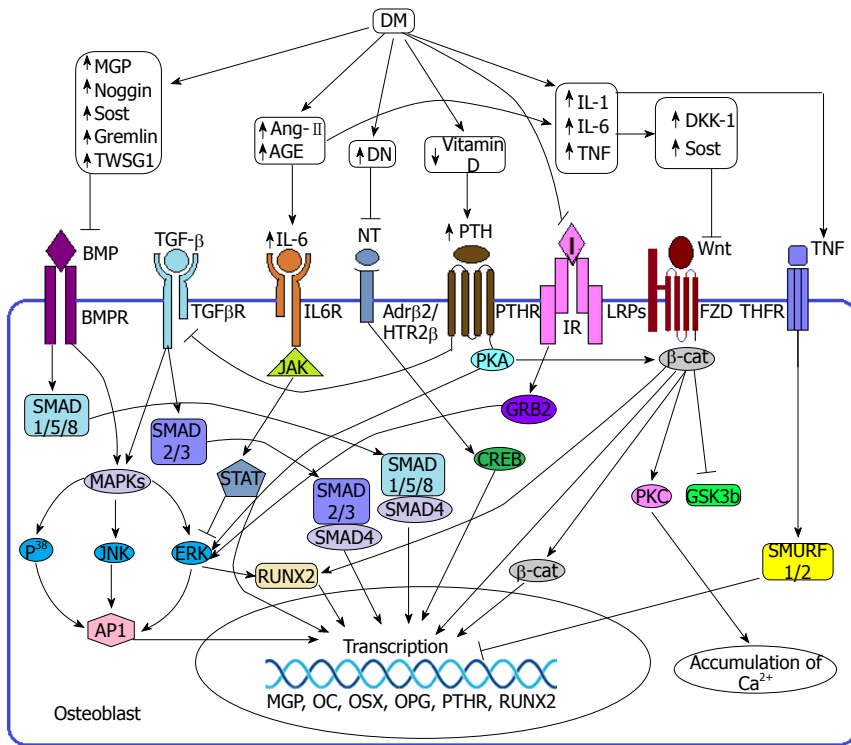
BMPs are the members of TGF- $\beta$  superfamily and known to be a potent inducer of osteoblast formation and thereby increase collagen synthesis and decrease collagenase-3 production<sup>[1,13]</sup>. There are several types of BMP proteins and among them BMP-2, BMP-4, BMP-5, BMP-6 and BMP-7 have strong capacity in osteogenesis<sup>[14]</sup>. BMP-2 and BMP-6 induce osteoblast formation and chondrocyte proliferation<sup>[14,15]</sup>. BMP-4 could participate in endochondral ossification<sup>[16,17]</sup> and BMP-7 induces the expression of markers including ALP activity and

accelerated calcium mineralization which are required for osteoblast differentiation<sup>[14]</sup>. But BMP-3 has adverse effect on osteoblastogenesis<sup>[14]</sup>. BMP signaling has been identified as the major signaling molecules in the pre osteoblast because the binding of BMPs to its receptors (BMPRs) induce phosphorylation of SMADs proteins specially SMAD-1, SMAD-5 and SMAD-8 (Figure 1). SMADs then in turn directly activate the SMAD binding element (SBE) through the SMAD depended pathway and thereby induces the transcription of corresponding genes. On the non SMAD depended pathway BMPRs directly activate MAPK and then in turn activate the particular genes through inducing runt related transcription factor 2 (RUNX2) or activator protein 1 (AP-1)<sup>[14,18,19]</sup>.

Wnt is the member of highly conserved secreted glycoprotein family, rich in cystein residue and are divided into two classes: canonical Wnts (wnt1, wnt3a) and non-canonical Wnts (wnt5a). Binding of canonical Wnts with frizzled (FZD) and LDL receptor related proteins (LRPs) promotes: the phosphorylation and inactivation of *glycogen synthase kinase 3 beta* (GSK3b), prevents the degradation of  $\beta$ -catenin ( $\beta$ -cat) as well as subsequent translocation of  $\beta$ -cat in the nucleus for binding with the target genes (Figure 1). Binding of non-canonical wnts with FZD receptor promote the activation of heterotrimeric G proteins in order to enhance the deposition of intracellular calcium ion ( $\text{Ca}^{2+}$ ) through protein kinase C (PKC) mediated pathway or induce the formation of the cytoskeleton *via* Rho/c-Jun N-terminal kinase dependent mechanism<sup>[18,19]</sup>.

TGF- $\beta$  signaling is important for the regulation, proliferation and commitment to the osteoblastic lineage of MSC. Binding of TGF- $\beta$  with its receptor TGF $\beta$ R regulates the expression of target genes through two possible pathways: canonical and non-canonical. In the canonical or smad dependent pathway activated TGF $\beta$ R promotes the phosphorylation of R-SMADs (SMAD-2, 3) and thereby activate the target genes through SMAD-4 mediated signal transduction (Figure 1). In the non-canonical or non smad dependent pathway activated TGF $\beta$ R promotes the expression of responsive genes through MAPK, P38, ERK mediated signal transduction pathway<sup>[12,14]</sup>.

Immunohistochemical analysis revealed that the periosteum and bone are linked with the sympathetic, sensory and the glutaminergic nervous system specifically the growth plate and the metaphysic of long bones are more exposed to the neural network. Close contact of the nervous system with the bone cells, strongly implying a physiological role of neural signal on bone health<sup>[20,21]</sup>. In addition, osteoblast has been reported to express  $\beta$ -2 adrenergic receptors ( $\beta$ 2AR) and 5-hydroxytryptamine receptor (5HTR) for several neurotransmitters including serotonin and norepinephrine<sup>[21,22]</sup>. An *in vivo* experiment showed that 5HTR 2 $\beta$  facilitate osteoblast recruitment and proliferation and the absence of this receptor leads to osteopenia<sup>[22]</sup>. Binding of neurotransmitter with particular receptors activates the transcription factor CREB and ultimately induces the gene for osteoblast prolifera-



**Figure 1 Diabetes mellitus induced regulation of osteoblast.** During healthy condition bone morphogenetic protein (BMP), transforming growth factor  $\beta$  (TGF- $\beta$ ), Wnt, insulin and neurotransmitter signaling are mandatory to the osteoblast for its normal functioning and survival. Binding of BMP with its receptor (BMPR) activates the corresponding gene through (a) Smad dependent pathway: which requires the SMADs protein (SMAD 1/5/8) or (b) non smad dependent pathway: in which activates RUNX2 or AP-1 through MAPK-ERK mediated pathway. Wnt-Frizzled pathway positively regulates gene expression through  $\beta$ -catenin or RUNX2 mediated pathway and Calcium accumulation through PKC mediated pathway. TGF- $\beta$  is also a positive regulator of osteoblast function and exerts its effect on the respective gene through SMAD 2/3 depended pathway or MAPK-ERK mediated pathway. Peripheral nerve exposure to the osteoblast signals through the adrenergic receptor 2  $\beta$  (Adr $\beta$ 2) or 5HTR induced pathway. Binding of neurotransmitters on the Adr $\beta$ 2 or 5HTR receptor activates ERK or CREB to induce the expression of osteoblastic gene. Insulin is a beneficial factor of bone formation and it exerts its effect through GRB2-ERK mediated pathway. During diabetes mellitus (DM), hyperglycemia may induce the expression of several BMP inhibitors including MGP, Noggin, Sost, Gremlin, TWSG1 as well as several Wnt inhibitors including DKK-1, Sost. DM also induces the production of different proinflammatory cytokines including interleukin 6 (IL-6), IL-1, AT-2 and TNF which negatively regulates osteoblast functioning. Binding of IL-6 with its receptor IL-6 receptor (IL6R) sequesters ERK pathway as well as induce the gene to transcribe several inhibitors including MGP, OPG, OSX. DM induced DN limits the nerve signaling through damaging the peripheral nerves. TNF binding with TNFR induce SMURF1/2 and thereby inhibit the transcription process. DM also reduces the production of vitamin D which in turn induces the secretion of parathyroid hormone (PTH). PTH binding with PTH receptor (PTHR) inhibits TGF- $\beta$  signaling through inhibiting TGF $\beta$  receptor (TGF $\beta$ R) although PTHR activates  $\beta$ -Cat and ERK pathways. DM induced IR (type-2DM) or insulin deficiency (Type-1DM) also limits insulin mediated bone formation. TNT: Neurotransmitter; HTR2 $\beta$ : 5-hydroxytryptamine receptor 2  $\beta$ ; I: Insulin; IR: Insulin receptor; LRP: Low density lipoprotein receptor related protein; FZD: Frizzled; TNF: Tumor necrosis factor; TNFR: TNF receptor; JAK: Janus kinase; STAT: Signal transducers and activators of transcription; AP-1: Activator protein 1; ERK: Extracellular signal regulated kinase; MAPK: Mitogen activated protein kinase; RUNX2: Runt related transcription factor 2; PKA: Protein kinase A; PKC: Protein kinase C;  $\beta$ -cat:  $\beta$  catenin; GSK3b: Glycogen synthase kinase 3b; SMURF: SMAD ubiquitylation regulatory factor; MGP: Matrix gla protein; OC: Osteocalcin; OSX: Osterix; OPG: Osteoprotegerin; DKK-1: Dickkopf related protein 1; Sost: Sclerostin; TWSG1: Twisted gremlin; Ang-II: Angiotensin-II; AGE: Advance glycation end product; GRB2: Growth factor receptor bound protein.

tion through AP1 activation<sup>[18]</sup>(Figure 1).

Elevated secretion of PTH has been reported to sequester osteoblast differentiation and activation. Attachment of PTH with its receptor PTHR activates protein kinase A (PKA) and extracellular signal regulated kinase (ERK) and ultimately induces the expression of matrix gla protein (MGP) on osteoblast which is a potent inhibitor of BMP signaling<sup>[1,14]</sup>. PTH binding also drives internalization of PTHR-TGF $\beta$ R complex, which attenuates TGF- $\beta$  signaling in bone development<sup>[14]</sup>.

Several extracellular, intracellular and transcriptional BMP inhibitors such as matrix gla protein (MGP), Noggin, dickkopf-related protein 1 (DKK-1), Sclerostin, Gremlin, Ski, Smurf-1, Smurf-2, twisted gastrulation (Twsg1), Interleukin 6 (IL-6) and TNFs have been

identified in the down regulation of BMP and TGF- $\beta$  signaling pathways and ultimately suppress osteoblast function<sup>[1,13,14,23,24]</sup>. MGP is the member of mineral binding  $\gamma$ -carboxyglutamic acid containing protein family that directly and indirectly sequesters mineralization of bone cells. In the direct effect it acts as a part of a complex with  $\alpha$ -2-HS glycoprotein and in the indirect effect it inhibits the binding of BMP-2 with its receptor expressed on the osteoblast precursors<sup>[1]</sup>.

Diabetes mellitus (DM) not only induces the over-expression of DKK-1<sup>[25,26]</sup> Sclerostin<sup>[27,28]</sup> Gremlin<sup>[29,30]</sup> PTH<sup>[31]</sup> angiotensin II (Ang-II)<sup>[32]</sup> IL-6<sup>[33]</sup> and TNFs<sup>[33-35]</sup> but also sequesters the over expression of Vitamin D and neurotransmitters required for the normal growth of osteoblast. DM induced diabetic neuropathy is the com-

monest complication of non-traumatic lower limb amputations in diabetic patients. Although the exact pathogenesis of diabetic neuropathy remains unclear, there are emerging data from *in-vitro* and *in-vivo* clinical studies suggesting that hyperglycemia induced formation of advanced glycation end products (AGEs) may play a key role in the pathogenesis of diabetic neuropathy<sup>[36,37]</sup>. Under hyperglycemic conditions, concentrations of methylglyoxal, 3-deoxyglucosone and glyceraldehyde increase rapidly due to the increased breakdown of glucose. Elevated levels of methylglyoxal, 3-deoxyglucosone and glyceraldehyde lead to the formation of advanced AGEs which in turn modify nerve cell components as well as signal through the receptor for advanced glycation end product (RAGE) expressed on the nerve cells in order to produce different types of cytokines which may have roles on nerve damage<sup>[36-38]</sup>. AGEs have deleterious effect on nerve cells because they modify neuronal proteins including tubulin, neurofilament, laminin and actin through glycation and thereby sequester the nerve function (Figure 2)<sup>[36,37]</sup>.

Beyond the damage of peripheral nerve cells on osteoblast through diabetic neuropathy, DM induced AGEs and angiotensin- II also upregulate the expression of IL-6 that regulates osteoblastic genes required for their survival, differentiation and function<sup>[32,39,40]</sup> (Figure 1).

Reduced vitamin D levels in the body have been identified as a potential risk factor of osteoporosis and bone fractures. Deficiency of Vitamin D in the serum sequesters the intestine to absorb  $\text{Ca}^{2+}$  from diet and thereby signals the parathyroid gland to secrete elevated levels of PTH. Hyper secretion of PTH induces bone resorption and inhibit osteoblastogenesis in order to maintain the optimal level of calcium and phosphorus in the blood required for metabolic process and neuromuscular functions<sup>[41,42]</sup>. Through binding of PTH with its receptor PTH-1 expressed on osteoblast triggers intracellular signaling molecules such as PKA, mitogen activated protein kinase A (MAPK), cyclic AMP-responsive element binding protein, AP1 and RUNX2 and thereby induce the expression of the MGP responsive element<sup>[1]</sup> (Figure 1).

Bone marrow derived endothelial progenitor cells (EPCs) may have roles in angiogenesis during bone healing<sup>[3,43]</sup>. DM down regulates the expression of EPCs through different mechanisms and hereby decreases the rate of angiogenesis required for bone formation in the fracture sites<sup>[3,44,45]</sup>. Mesenchymal stem cells (MSC) derived from bone marrow act as a precursor of osteoblast formation<sup>[46-48]</sup>. Several labs based trials have come to the decision that, DM is responsible for the upregulation of peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), adipocyte fatty acid binding protein (aP2), TNF- $\alpha$  and consequently decrease the availability of MSC for osteoblast formation but increase the availability of MSC for adipocyte formation<sup>[3,4,34,35,48,49]</sup>. So it is intuitive that, in addition to direct interference with osteoblast formation DM also responsible for the deposition of lipid in the bone marrow and thereby leading to the expansion of marrow cavity as well as decreases the rate of blood flows to the

bone which is required for the transfer of nutrients<sup>[3,5]</sup>. The transformation of osteoblast to adipocyte makes the reduction of osteoblast number available for bone formation<sup>[3,50]</sup>. Advanced glycation end products (AGEs) have been identified as a biomarker for the increased risk of fractures because it decreases the synthesis of type I collagen and thereby decreases the bone strength. It is now well researched that DM is responsible for the over expression of AGE and have roles in bone rigidity<sup>[51-53]</sup>.

Several experimental studies implicated that, insulin has an anabolic effect on osteoblast development and it is intuitive that, insulin may exert its effect on osteoblast through IR-GRB2-ERK mediated pathway<sup>[4,54]</sup> (Figure 1). Beyond the synthesis of insulin pancreatic  $\beta$  cells also produce other osteoporotic factors including amylin and preptin. Amylin induces bone formation and sequesters bone resorption, preptin induces osteoblast differentiation and mineralization as well as reducing the apoptosis of osteoblast<sup>[4]</sup>. Osteocalcin is a peptide which positively regulates osteogenesis. DM limits the production of osteocalcin through the negative regulation of osteoblast by decreased synthesis of insulin, amylin and preptin. Testosterone is also an important factor of osteogenesis and it is obvious that, limited production of osteocalcin reduces the production of testosterone from the testes<sup>[4]</sup>.

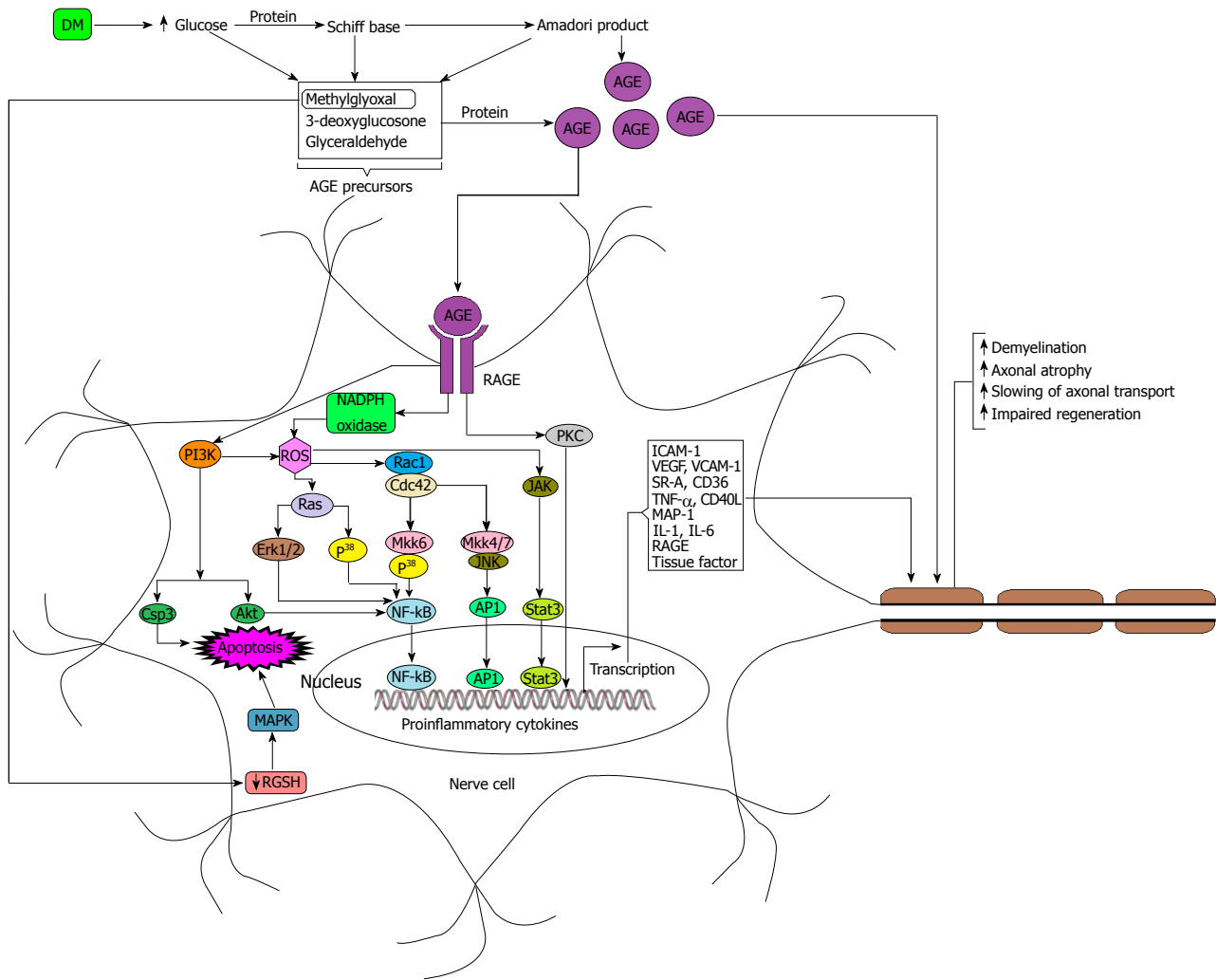
### Osteoclast

Osteoclasts are cells that derived from the monocyte-macrophage cell lineage and strongly participate in osteoclastogenesis. It is well documented that different types of mediators such as nuclear factor  $\kappa$ -B (NF- $\kappa$ B), receptor activator for nuclear factor  $\kappa$ -B ligand (RANKL), osteopontin (OPN), parathyroid hormone (PTH), macrophage colony stimulating factor (M-CSF), and angiotensin- II (AT- II) have prominent roles to induce osteoclastogenesis<sup>[1,13]</sup>.

In general osteoclast exerts its effects in osteoclastogenesis through three possible pathways (1) RANKL mediated; (2) M-CSF mediated; and (3) immunoreceptor tyrosine-based activation motifs (ITAMs). But in inflammatory condition osteoclastogenesis may take place through other pathways like MCP mediated, TNF mediated and IL-6 mediated<sup>[12,55]</sup>.

RANKL is a key factor derived from osteoblast and stromal cells, binds with the receptor expressed on the cell surface of monocyte-macrophage cell lineage and thereby triggers the differentiation of pre osteoclast to osteoclast through activating NF- $\kappa$ B and NFATc1<sup>[56]</sup>. RANKL inhibits the apoptosis of osteoclast through inducing the anti-apoptotic enzyme protein kinase B (PKB) (Figure 3). RANKL also responsible for the production of reactive oxygen species (ROS) including free radicals, oxygen ions and peroxides which are potent inducer of osteoclastogenesis<sup>[1,12,56-59]</sup>.

Binding of RANKL with its receptor RANK activates signal transduction pathways involving the adaptor protein TNF receptor-associated factor 6. Subsequently, several kinases such as p38 MAPK and JUN N-terminal



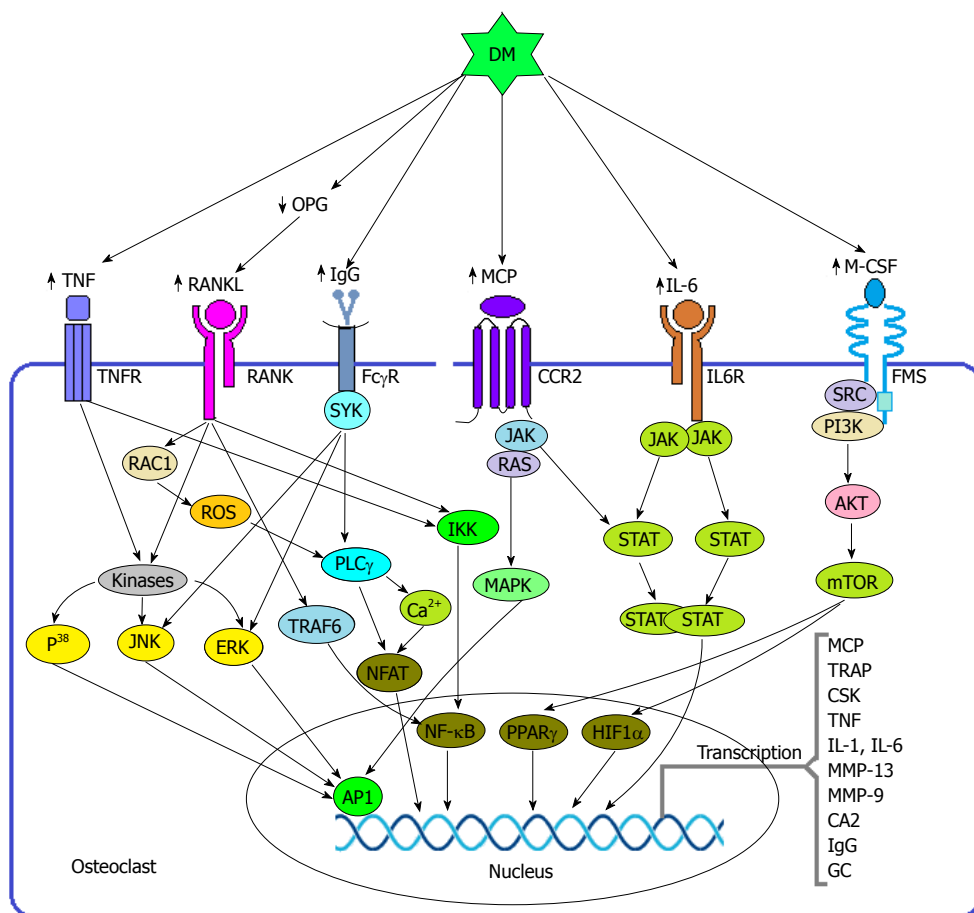
**Figure 2 Diabetes mellitus induced Peripheral nerve damage.** During hyperglycemic condition concentrations of methylglyoxal, 3-deoxyglucosone and glycerlaldehyde increase rapidly due to the increased breakdown of glucose. Elevated levels of methylglyoxal, 3-deoxyglucosone and glycerlaldehyde lead to the formation of advance glycation end products (AGEs) which in turn modify nerve cell components as well as signal through the receptor for advance glycation end product (RAGE) expressed on the nerve cells in order to produce different types of cytokines which may have roles on nerve damage. RAGE induced nicotinamide adenine diphosphate hydrogen (NADPH) oxidase is the major source of reactive oxygen species (ROS) and ROS plays a crucial role to activate nuclear factor kappa B (NF-κB) through Ras-Erk, Rac1-Mkk6 depended pathway. ROS also activates AP-1 and Stat-3 through Rac1-Mkk4/7, JAK-Stat mediated pathway respectively. RAGE may induce apoptosis through PI3K-Csp3 depended pathway as well as activates NF-κB through PI3K-Akt mediated pathway although PI3K may participates in ROS production. Diabetes mellitus (DM) induced methylglyoxal may directly participates in apoptosis through MAPK mediated pathway. Activated NF-κB, AP-1 and Stat3 act congruously to transcribe the genes of proinflammatory cytokines and other factors which are responsible for the destruction of peripheral nerve cells. AGE also participate directly on the modification of axon and thereby reduce the potentiality of signal transduction. DM: Diabetes mellitus; AGE: Advance glycation end product; JAK: Janus kinase; RAGE: Receptor advance glycation end product; ROS: Reactive oxygen species.

kinase 1 are activated, which in turn induce the transcription *via* the various hetero and homodimers of the AP1 family of proteins including FOS, FOSB, FOS-related antigen 1 (FRA1), FRA2, JUN, JUNB and JUND (Figure 3). AP1 regulates the differentiation, proliferation and apoptosis, of various cell types<sup>[12]</sup>.

RANKL is necessary for osteoclastogenesis but an experiment conducted on mouse model showed that, M-CSF acts as a positive catalyst in RANKL activation because the addition of M-CSF requires less time to do a particular resorption process than the RANKL alone<sup>[60]</sup>. Osteoprotegerin (OPG) is a prominent factor for osteoclast activation because the affinity of OPG for RANKL prevents the binding of RANKL with its receptor RANK and thereby decrease the RANKL-RANK medi-

ated pathway of osteoclast multiplication, survival and bone resorption<sup>[1]</sup>.

According to the immunoreceptor tyrosine-based activation motifs (ITAMs), binding of immune complex like immunoglobulin G (IgG) with its receptor FcγR activates spleen tyrosine kinase (SYK), which in turn induces NFATC1 through the activation of phospholipase Cγ (PLCγ) (Figure 3). NFATC1 is an important transcription factor that transcribes the genes that encode calcitonin receptor, tartrate-resistant acid phosphatase, matrix metalloproteinase 13 and cathepsin K. All these factors enable the acidification and degradation of the bony matrix<sup>[12]</sup>. DM is thought to be a potent inducer of IgG because an experiment conducted on mouse model showed that non-obese diabetic mice spontaneously produce natural



**Figure 3 Diabetes mellitus induced regulation of osteoclast.** During normal physiology several osteoblastogenic modulators including RANKL, M-CSF, monocyte chemoattractant protein (MCP), and immunoglobulin G (IgG) binds with their receptors expressed on osteoclast and activates different signal transduction pathway to transcribe the particular gene. Binding of RANKL with RANK triggers several possible pathways to induce the corresponding element. It may induce transcription factor NF-κB through TRAF or IκB kinase (IKK) mediated pathway as well as induces nuclear factor of activated T cells (NFAT) through reactive oxygen species (ROS)-phospholipase Cγ (PLCγ) mediated pathway. RANK also may induce AP-1 through triggering the kinase enzymes. Macrophage colony stimulating factor (M-CSF) activates transcription factors peroxisome proliferator activated receptor γ (PPARγ) and hypoxia inducible factor 1 α (HIF1α) through PI3K-AKT mediated pathway. MCP activates AP-1 signaling through RAS-MAPK mediated pathway which requires the assistance of JAK. IgG also signals through the Fc receptor γ chain (FcγR) to activate NFAT via the induction of PLCγ as well as activates AP-1 through the kinase enzyme systems and both of the pathways require the activation of SYK. During the state of DM, it induces the upregulation of osteoclastogenic factors stated above and thereby induce the differentiation and activity of osteoclast. In addition to the above factors, DM also induces the synthesis of some proinflammatory cytokines which also favor the bone resorption by osteoclast. Interleukin 6 (IL-6) exerts its effect through JAK-STAT mediated pathway although MCP activated JAK may contribute to the activation of STAT to some extent. TNF also activates NF-κB and AP-1 through IKK and Kinase system respectively. CCR2: CC chemokine receptor 2; mTOR: Mammalian target of rapamycin; OPG: Osteoprotegerin; ERK: Extracellular signal regulated kinase; JNK: JUN N terminal kinase; TRAP: Tartrate resistant acid phosphatase; CSK: Cathepsin K; MMP: Matrix metalloproteinase; CA2: Carbonic anhydrase 2; GC: Glucocorticoid.

IgG autoantibodies<sup>[61]</sup>.

Beyond the roles of RANKL and M-CSF in osteoclastogenesis, on the state of hyperglycemia a group of proinflammatory cytokines is activated including TNF, IL-1 and IL-6 and these cytokines have profound effects on the differentiation and activation of osteoclast<sup>[12,62-64]</sup>. Although osteoclast differentiation and activation is primarily dependent on the presence of M-CSF and RANKL, osteoclastogenesis is enhanced in the presence of TNF, IL-1 or IL-6. This is partly a consequence of the induction of RANKL in target cells, but these pro-inflammatory cytokines also responsible for the differentiation and activation of osteoclasts from the preosteoclast. In addition, under normal concentrations of RANKL, TNF can induce the differentiation of monocytes and macrophages to preosteoclasts. The osteoclastogenic ac-

tivity of TNF is mediated by p55 TNF receptor and may be partly counteracted by the activation of the p75 TNF receptor<sup>[12]</sup>.

IL-6 is thought to be the most abundant and effective cytokines in blood because: (1) the concentration of IL-6 and IL-6 receptor (IL-6R) is higher than the other cytokines; (2) IL-6 mediates the production of other cytokines related to osteoclastogenesis like glucocorticoid (Figure 3); and (3) Estrogen deficiency exerts its effects in osteoclastogenesis via IL-6 mediated pathway as well as IL-6 is a potent inducer of IgG production<sup>[12,61,65]</sup>.

DM not only induces the overexpression of RANKL<sup>[3,66]</sup> M-CSF<sup>[3,66]</sup> NF-κB<sup>[67]</sup> and OPN<sup>[34,68]</sup> but also stimulates the over expression of several proinflammatory stimulus such as IL-6, MCP, IgG and TNFs which are so important for the maturation and activation of osteoclast. The

DM may induce monocyte to secrete IL-6 through ROS, PKC, MAPK, and NF- $\kappa$ B mediated pathways<sup>[69,70]</sup>.

Estrogen deficiency stimulates osteoclast formation not only by decreasing the OPG production but also by increasing the production of TNF- $\alpha$ , RANKL and osteoclast precursors through stimulating the T cells<sup>[71,72]</sup>. There is striking evidence on behalf of this regard that, estrogen levels are significantly lower in DM patients<sup>[73]</sup>. Adiponectin is another factor secreted by the adipose tissue and there has been increasing evidence suggest that, adiponectin stimulates the differentiation and mineralization of osteoblast but directly inhibits osteoclast activity and bone resorption<sup>[74]</sup>. Some *in situ* studies have shown that adiponectin percentage is lower in individuals with DM than the individuals without DM<sup>[75]</sup>.

Intracellular ROS mediated oxidative stress plays a crucial role in bone health because ROS promotes RANKL mediated osteoclast differentiation and function. Patients with type 2 DM have shown elevated level of mitochondrial ROS and thus supporting the point that, DM may have another role in ROS mediated osteolysis and bone fractures<sup>[76,77]</sup>. As mentioned before, diabetic neuropathy is a cause of increased production of IL-6, TNF and some other factors, so it is intuitive that, diabetic neuropathy may have a positive role in osteoclast functioning<sup>[12,38,64]</sup>.

## EFFECT OF DIABETES MELLITUS ON MUSCLE CELLS

Muscle atrophy is a physiological condition which associated with the depression of protein synthesis as well as an increase in protein degradation<sup>[78]</sup>. There are some other evidences showed that, DM is associated with diabetic neuropathy mediated muscle atrophy or directly triggers muscle atrophy through TNF- $\alpha$ , NF- $\kappa$ B mediated pathway and thereby induces muscle weakness<sup>[68,79-81]</sup>. Weakness of the muscle is a risk factor of bone fractures because an individual with weak muscles is more likely to fall down than a normal individual. In addition to muscle weakness, diabetic polyneuropathy also induces bone resorption through osteolysis<sup>[82,83]</sup>.

DM is directly associated with muscle atrophy through an increased activity of the ubiquitin proteasome system (UPS) although other pathways may involve in this process<sup>[78]</sup>. There are several inducers of UPS including glucose<sup>[33]</sup> TNF- $\alpha$ <sup>[84,85]</sup> Ang-II<sup>[86]</sup> IL-6, Glucocorticoid (GC)<sup>[85]</sup> and most of them exert their effects on myogenesis responsive gene through NF- $\kappa$ B mediated pathway<sup>[68,78]</sup>.

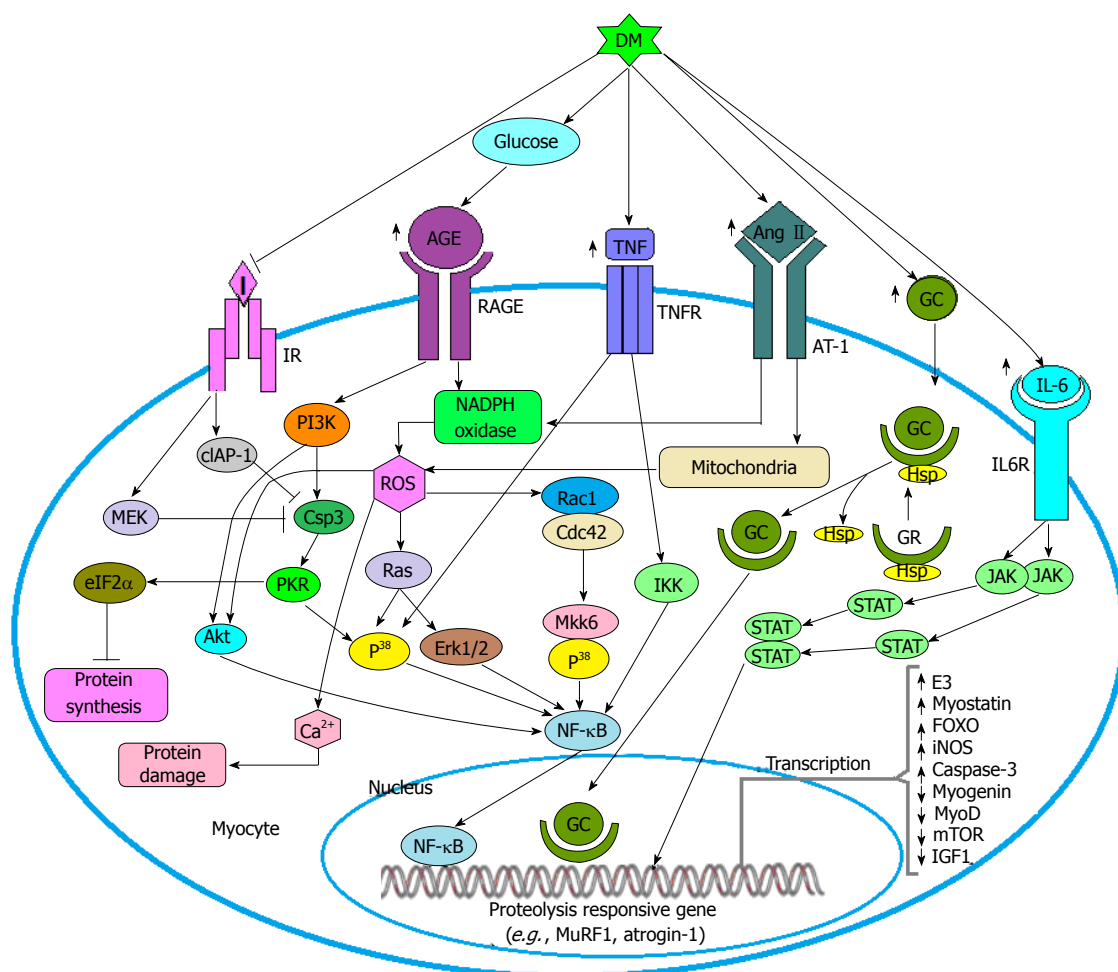
High extracellular glucose concentrations is a potential precursor of AGE formation and several evidences have shown that, AGE may induce the formation of ROS through NADPH oxidase and PI3K/Akt mediated pathway and ultimately activates the transcription factor NF- $\kappa$ B<sup>[37,38,78]</sup>. AGE may induce PKR through caspase-3 mediated pathway and activated PKR then in turn induces NF- $\kappa$ B through P<sup>38</sup> MAPK mediated pathway as well

as activates eIF2 $\alpha$  which would depress protein synthesis by decreasing translational efficiency<sup>[78]</sup>(Figure 4).

Several studies have implicated that TNF- $\alpha$  is a prominent cytokine in cachexia induced muscle atrophy<sup>[84]</sup> as well as a potent inducer of insulin resistance<sup>[87]</sup>. Binding of TNF- $\alpha$  with its receptor expressed on myocyte activates nuclear transcription factor NF- $\kappa$ B through P<sup>38</sup> MAPK or IKK mediated pathway and activated NF- $\kappa$ B then in turn induces the transcription of inducible nitric oxide synthase (iNOS) as well as transcribes the gene MuRF-1 responsible for muscle wasting<sup>[84]</sup>(Figure 4).

Ang-II which is the major peptide of the renin-angiotensin system has been implicated as a modulator of muscle wasting<sup>[88]</sup>. Ang-II exerts its effect on muscle atrophy not only through the generation of ROS but also through the activation of IL-6 and Glucocorticoid as well as through disrupting insulin signaling in muscle cells. It is experimentally determined that, ROS has a significant role in the reduction of muscle strength<sup>[89,90]</sup>. There are two sources of Ang-II induced ROS production (1) NADPH oxidase; and (2) Mitochondria, but NADPH oxidase is thought to be prominent between the two sources. ROS may contribute to muscle wasting activity through three mechanisms (1) by increasing the absorption of Ca<sup>2+</sup> in order to activate calcium-activated proteases; (2) by stimulating the UPS through activating caspase-3; and (3) by up-regulating atrogen-1 and MuRF-1 in muscle to activate the proteasome system through transcribing E3 ligases<sup>[86]</sup>(Figure 4).

Ang-II induced glucocorticoid (GC) plays an important role in muscle wasting because several *in-vivo* and *in-vitro* studies have shown that, addition of different types of GC antagonist of experimental model reduce the rate of muscle wasting<sup>[86,91,92]</sup>. GC exerts its effect on muscle through two ways (1) through sequestering the anabolic action, and (2) through inducing the catabolic action<sup>[91]</sup>. On behalf of the anti-anabolic action firstly, GC inhibits the transport of amino acids into the muscle and thereby limits the protein synthesis<sup>[91]</sup>. Secondly, GC sequesters the stimulatory effects of insulin and insulin like growth factor 1 (IGF-1)<sup>[91,92]</sup>. Thirdly, GC negatively regulates the synthesis of MyoD, an important transcription factor that regulates the differentiation and development of muscle cells as well as required for regeneration and self-renewal of skeletal muscle cells<sup>[92]</sup>. Fourthly, mechanistic target of rapamycin (mTOR) is a kinase protein which regulates the translation of muscle protein. GC inhibits the activity of mTOR through enhancing the transcription of REDD1, a repressor of mTOR function<sup>[91]</sup>. Finally, GC inhibits myogenesis through the downregulation of myogenin, an important a transcription factor required for differentiation of satellite cells into myofibrils<sup>[91,92]</sup>. On behalf of the catabolic activity firstly, GC stimulates the synthesis of several components (*e.g.*, E3) required for UPS through the upregulation of the respective genes including MuRF-1 and atrogen-1<sup>[91,92]</sup>. Secondly, GC induces the overexpression of myostatin a growth regulator which inhibits the development of muscle mass through



**Figure 4 Diabetes mellitus induced regulation of skeletal muscle.** Diabetes mellitus (DM) induced elevated blood glucose is the major source of advanced glycation end product (AGE) which binds with its receptor advanced glycation end product (RAGE) to activate the signal cascade into myocyte. RAGE activation enhances the generation of reactive oxygen species (ROS) through the activation of nicotinamide adenine dinucleotide hydrogen (NADPH) oxidase. Ang-II also induces the production of ROS not only by activating NADPH oxidase but also by inducing the mitochondria. ROS may exert its effects on nuclear factor kappa B (NF-κB) through Rac1-Mkk6 and Ras mediated pathway or accelerate the damage of muscle protein through Ca<sup>2+</sup> dependent pathway. Beyond the generation of ROS, RAGE also activates PI3K which in turn activates NF-κB through Csp3- PKR and Akt mediated pathway. Activated PKR may induce the activation of eIF2α that inhibits protein synthesis. DM induced proinflammatory cytokines interleukin 6 (IL-6) activates the gene through JAK-STAT signaling pathway and TNF activates the factor NF-κB via IKK or MAPK38 induced pathway. Ang- II induced GC also has a role in muscle atrophy and GC exerts its effect through GC-GCR complex mediated pathway. Insulin signaling is also important for muscle growth because it sequesters the activity of Csp3 through inducing the production of cIAP-1 and MEK which are potential inhibitors of Csp3. Type 1 DM reduces the production of insulin and type 2 DM makes the cell insulin resistant, so due to the deficiency of insulin it limits the functioning of cIAP-1 and MEK.

downregulating the proliferation and differentiation of satellite cells<sup>[91,93,94]</sup>. Thirdly, GC induces the breakdown of myofibrillar protein through the upregulation of caspase-3<sup>[89]</sup>. Finally, Forkhead Box O-1 (FOXO-1) is a transcription factor that induces UPS through the upregulation of genes including atrogin-1/MAFbx and MuRF1. Several lab based experiments have come to the decision that, GC induces the production of FOXO-1 through stimulating the respective genes<sup>[91,92]</sup>(Figure 4).

IL-6 is a proinflammatory cytokine which has been implicated as a potential factor of muscle atrophy<sup>[86,95,96]</sup>. Ang-II induced IL-6 upregulates the transcription of serum amyloid A (SAA) and both of the factors (IL-6 and SAA) act synergistically to trigger muscle atrophy<sup>[93]</sup>. An *in-vitro* study has shown that, IL-6 exerts its effect on muscle wasting through JAK/STAT mediated pathway<sup>[97]</sup>(Figure 4).

Insulin deficiency (ID) and insulin resistance (IR) are the hallmark of type-1 and type-2 DM respectively. IR has been implicated as a potential inducer of overall protein degradation as well as caspase-3 mediated actin cleavage. Elevated level of intracellular insulin inhibits caspase-3 protein through MEK and cIAP-1 mediated pathway but during IR or ID condition insufficiency of insulin cannot exert its inhibitory effect on caspase-3<sup>[98]</sup>(Figure 4).

DM induced diabetic retinopathy may be another risk factor of bone fractures because diabetic retinopathy is a leading cause of vision loss and blindness and consequently augments the rate of stumble mediated bone fractures<sup>[99]</sup>. Abnormal movement caused by polyneuropathy and heart failure caused by diabetic cardiovascular complications also promotes the rate of fall<sup>[4,5]</sup>.

Beyond the role of vitamin D in osteolysis, it is intuitively



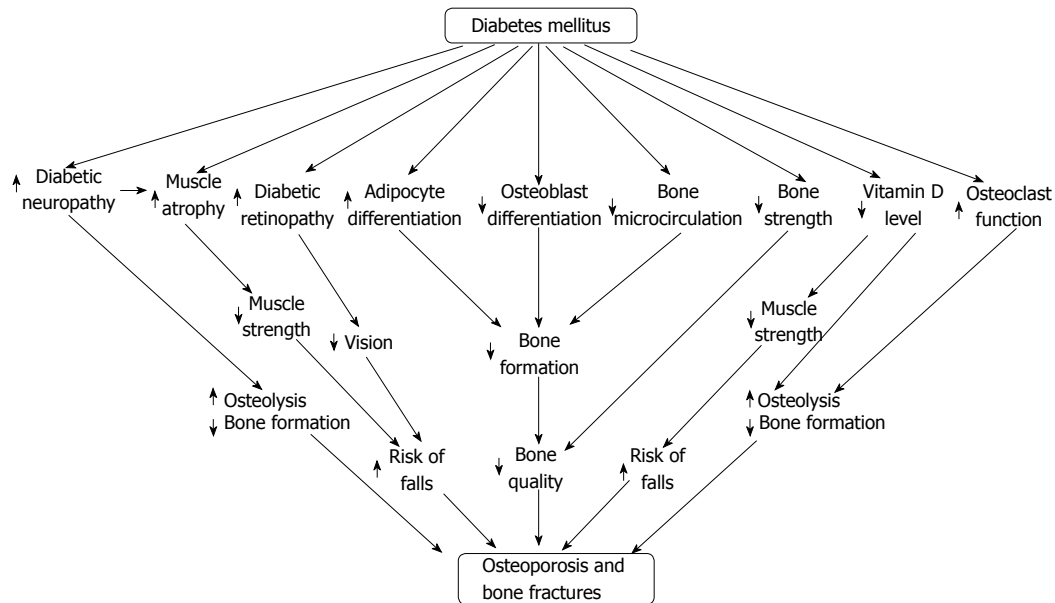


Figure 5 Possible pathways of diabetes mellitus induced osteoporosis.

tive that, vitamin D exerts a range of effects in skeletal muscle cells. Muscle activity specially the power stroke is a  $Ca^{2+}$  depended process and due to the lack of  $Ca^{2+}$  the system will be shut down. An inadequacy of vitamin D can turn down the availability of calcium and phosphorus and thereby postpones the activity of muscles<sup>[100]</sup>. Some *in vitro* and *in vivo* trial have shown that, vitamin D levels are significantly lower in patients with DM<sup>[101,102]</sup>.

## DISCUSSION

Diabetes mellitus may be an obvious cause of osteoporosis and bone fractures due to its broad range of effects on different mediators of the human body. It mainly regulates the bone cells (specifically osteoblast and osteoclast) and the muscles to exert its effects to facilitate osteoporosis as well as reduction of muscle strength<sup>[3,98]</sup>(Figure 5). DM negatively regulates the normal functioning of osteoblast but positively regulates the osteoclast functioning in order to facilitate the process of osteoporosis<sup>[1,98]</sup>. DM reduces the availability of MSC to produce osteoblast but simultaneously increases the availability of MSC for adipocyte formation<sup>[1]</sup>. Due to the continual differentiation and deposition of adipocytes into the bone marrow increase the bone marrow cavity to make the bone fragile as well as decrease the bone microcirculation<sup>[1,99]</sup>. The limitation of osteoblast functioning, over production of adipocytes and fluent functioning of osteoclasts all these effects negatively regulate the bone formation but positively regulate the bone resorption and ultimately cause osteoporosis. DM induced diabetic neuropathy acts as a prominent factor in osteoporosis and muscle atrophy. Diabetic neuropathy inhibits bone formation through sequestering osteoblast formation and function but facilitates osteolysis through inducing the osteoclast generation and function as well as reduc-

ing muscle strength through inducing the expression of different cytokines and ROS<sup>[75,98]</sup>. DM directly causes muscle atrophy through different mediators or indirectly causes muscle atrophy through diabetic neuropathy. DM induced diabetic retinopathy is another risk factor that may cause bone fractures through reducing eye sight<sup>[98]</sup>. Reduced muscle strength as well as reduced eye sight may cause elevated rate of fall down or stumble that may cause bone fractures. Vitamin D is an essential factor of bone and muscle activities because deficiency of vitamin D stimulates the production of PTH which is a negative regulator of osteoblast functioning but a positive regulator of osteoclast functioning which then in turn reduce bone formation and increase bone resorption respectively<sup>[53,54]</sup>. DM induced Vitamin D deficiency also causes the reduction of muscle strength because it lowers the rate of  $Ca^{2+}$  absorption by the intestine and thereby reduces the activity of muscle which may be a risk factor of bone fractures through increasing the rate of fall<sup>[55,98]</sup>.

## CONCLUSION

Diabetes mellitus exerts its diabolical effects on bone, neuron and muscle cells through a broad spectrum of mechanisms. It declines the production of various stimuli required for normal homeostasis of the above cells and accelerates the synthesis of several cytokines and other factors which may directly destroy the target cells or indirectly antagonize the signaling pathways of the stimulus. As human body is a network of different pathways so any imbalance on any part of the pathway may tends the body vulnerable to different threats. DM is the potent source of excess glucose in the blood which is the principal key to create a lot of abnormalities in the body including Osteoporosis and bone fractures, cardiovascular disease, diabetic nephropathy, diabetic neuropathy, Dia-

betic retinopathy and muscle atrophy. Other fatal diseases like HIV and cancer may be linked with hyperglycemia and several investigations have been running to elucidate the mystery of DM induced mechanism. Although several drugs are available to treat osteoporosis, regular physical exercise would be a better way to get rid of from this type of life threatening disease.

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