“The Effect of Vitamin D3 on the Osteogenic Potential of Adipose Derived Stem Cells in Treatment of Induced Osteoporosis in Albino Rats”

(Histological and Immuno-histochemical Study)

Thesis Submitted to faculty of dentistry Ain Shams University, for Partial fulfillment of the requirements of Master Degree in Oral Biology

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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللهِ عَلَيْكَ عَظِيمًا﴾

صدق الله العظيم

سورة النساء آية (113)

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Dedication

To my parents who I really love and appreciate their efforts throughout my life,

To my supporting, Lovely husband ***Hany****,*

To my two little angels ***Abdullah*** *and* ***Taliah****,*

To my dear sister and brothers,

To the soul of my dear Grandparents,

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ABSTRACT

Background: Adipose derived stem cells therapy is an attractive putative option to treat various skeletal disorders especially osteoporosis which is one of the most common bone diseases in humans.

Aim: The primary aim of this work is to investigate the effect of vitamin D3 (Vit.D3) on the osteogenic differentiation potentiality of adipose derived stem cells (ADSCs) in treatment of induced osteoporosis in albino rats. The secondary aim is to improve the osteogenic differentiation potentiality of the ADSCs using available, low cost and safe agent as Vit.D3.

Methodology: Sixty adult male albino rats were used in this study and were divided into four main Groups: G.1 (Control gp.), EXP.G2 (Osteoporotic gp.), Exp.G3 (treated with ADSCs only) and Exp.G4 (treated with ADSCs + Vit.D3). At the end of the experimental period of each group, the mandibles were excised free and prepared for examination. The investigation of Vit.D3 effect on ADSCs was done using: Hematoxylin & Eosin for routine histological examination, Masson Tri-chrome stain for investigation of new bone formation, Anti-osteonectin antibodies as immuno-histochemical marker and morphometric study. All the collected data was tabulated and statically analyzed.

Results: Vitamin D3 successfully enhanced the osteogenic differentiation potentiality of the ADSCs by enhancing its differentiation into osteoprogenitor cells. This was represented by new active osteoblasts and young osteocytes detected by osteonectin immuno-histochemical marker. Masson Tri-chrome revealed higher new collagen formation and remodeling rate in Exp.G4 than in Exp.G3. Also, there was a significant increase in the number of osteoblasts, osteocytes, the thickness of the mandibular cortical plates and the surface area percentage of spongy bone trabeculae in Exp.G4 more than Exp.G3. Also, the number of osteoclasts revealed significant decrease in Exp.G4 compared to Exp.G3.

Conclusions/Significance: Vitamin D3 has a significant synergistic effect on the osteogenic differentiation potentiality of the ADSCs by enhancing its differentiation into osteoprogenitor cells and inhibition of its adipogenic differentiation. It also inhibit osteoclastogensis resulting in decrease in the resorptive activity. This finding will help to improve quality of life in osteoporotic patients by decreasing the susceptibility of fractures and by finding a more profound way of treatment and prevention of osteoporosis especially in elderly.

LIST OF ABBREVIATIONS

25(OH) D : OH= hydroxyl, D= Vitamin D.
ACOT2 : Acyl-CoA thioesterase 2.
ADSCs : Adipose derived stem cells.
Alox15 : Arachidonate 15-lipoxygenase.
ALP : Alkaline phosphatase activity.
ATP : Adinosine triphosphate.
BAT : Brown adipose tissue.
BMD : Bone mineral density.
BMP-2 : Bone morphogenetic protein-2
Coll I : Collagen I.
DMEM : Dulbecco's modified eagle media.
EDTA : Ethylene-Diamine-Tetra-Acetic acid
ERα : Estrogen receptor α.
ERβ : Estrogen receptor β.
EXP.G : Experimental group.
FACIT : Fibril associated collagens with interrupted triple helices.
FBS : Fetal bovine serum.
FFPE : Formalin fixed paraffin embedded tissues.
FGF : Fibroblast growth factor.
GC : Glucocorticoids.
H&E : Hematoxylin & eosin.
HA-TCP : Hydroxy apatite/ Tricalcium phosphate.

I.U.'S	: International unites.
IGF-1	: Insulin-like growth factor.
IL-1	: Interluken-1.
IL-6	: Interluken-6.
iPS	: Induced pluripotent stem.
iPSop	: Induced pluripotent stem –derived osteoprogenitors
ITAM	: Immunoreceptor tyrosine-based activation motif.
LPL	: Lipoprotein lipase
LRP5	: LDL receptor–related protein 5.
M-CSF	: Macrophage CSF.
Mpsl	: Methyl prednisolone
MSCs	: Mesenchymal stem cells.
NFAT	: Nuclear factor of activated T cells.
OPG	: Osteoprotegerin.
OSP.G	: Osteoporotic group.
P1NP	: Procollagen1 N-terminal extension peptide.
PBS	: Phosphate buffered saline.
PDGF	: Platelet derived growth factor.
PLA	: Processed lipoaspirate cells.
PPAR-γ	: Peroxisome proliferator-activated receptor γ.
PTH	: Parathyroid hormone.
RANK	: Receptor activator of nuclear factor κβ.
RANKL	: A ligand for receptor activator of nuclear factor κβ.

RDI	: Recommended daily intake.
RUNX2	: Runt-related transcription factor 2.
SCID	: Severe combined immunodeficiency mice.
SPARK	: Secreted protein acidic and rich in cysteine.
Stem.	: Stem cells (adipose derived stem cells).
SVF	: Stromal vascular fraction.
TGF-β	: Transforming growth factor.
TNF-α	: Tumor necrotic factor α.
TRACP5b	: Tartrate-resistant acid phosphatase 5b.
UCP1	: Unique uncoupling protein.
VDR	: Vitamin D receptor.
VEGF	: Vascular endothelial growth factor.
WAT	: White adipose tissue.