

**Histological and Immunohistochemical
Evaluation of the Effect of Local exogenous
Application of VEGF /N-acetyl D- glucosamine
on bone Healing
(Experimental Study in rats)**

A thesis Submitted to the Council College of Dentistry
Baghdad University

**In Partial fulfillment of Requirements for the Degree
Doctor of Philosophy in Oral Histology and Biology**

By

Dr. Enas fadhil kadhim

B.D.S.; M.Sc. oral histology

Supervised by

Prof. Dr. Athraa Y.Alhijazi

B.D.S.; M.Sc.; Ph. D

Abstract

Background: Bones are rigid organs that constitute part of the endoskeleton of vertebrates. They support and protect the various organs of the body, produce red and white blood cells and store minerals. Bone tissue has been shown to contain numerous cell-to-cell signaling peptides called growth factors. These growth factors are thought to have important regulating effects for bone remodeling and bone healing. Several growth factors are expressed during fracture repair. Of these, vascular endothelial growth factor (VEGF), is of particular interest because of its ability to induce neovascularization (angiogenesis). The responses to exogenous VEGF observed that a slow-release formulation of VEGF, applied locally at the site of bone damage, may prove to be an effective therapy to promote human bone repair.

N-acetyl glucosamine is a monosaccharide, which are the derivatives of carbohydrates, it helps the body in making several other chemical agents which are used in the formation of bones, muscles and cartilages.

N-acetylglucosamine is very effective in providing support for the skeletal system of the humans, and may provide strength to the ligaments and joints, and the skin remains fresh and glowing.

Aims of study

1. To identify role of local exogenous application of vascular endothelial growth factor and application of N- acetyl- D glucose amine as a biomaterial in bone healing.
2. To analyze the expression of Vascular endothelial growth factor, tissue non specific ALP and bone matrix protein (collagen-1) by

immunohistochemistry in created bone defect after application with different biomaterials in a rat model.

Materials and Methods

In this experimental study fifty four male Albino rats, weighting (300-400) gram, aged (6-8) months were used and maintained under control conditions of temperature, drinking and food consumption. The animals were subjected for a surgical operation of medial sides of both tibial bone (right side was considered as experimental site ,while left be the control one). The animals were divided into following groups according to the applicable growth factors.

A. Contol group the bone defect treated with 1 μ m of normal saline and its number represented the all number of the following experimental groups as the left side of each animal considered to be the control.

B. Experimental group includes

- **Group I contains (18) rats**, the bone defect treated with 1 μ ml of Vascular endothelial growth factor.
- **Group II contains (18) rats** the bone defect treated with 1 μ ml of N-acetyl D-glucosamine.
- **Group III contains (18) rats**, the bone defect treated with 1 μ m combination of (VEGF& N-acetyl D- glucosamine).

Every single group composed of 18 rats that studied in three periods 3,7,10 days (6 rats for each period).

Histologic and immunohistochemical evaluation for VEGF, alkaline phosphatase and collagen type 1 were carried for all animals.

Results:

A. For Histological findings

An early apposition of osteoid tissue was detected in VEGF group. Osteoblast, osteocyte and osteoclast cells count were demonstrated to be with high mean value in VEGF group followed by N- acetyl D- glucosamine group in periods 3,7,10 days post operative of bone defect. Inflammatory phase as first step in bone healing was detected in all study groups at 3 day, and combination group records highest value in the mean of inflammatory cell count in comparisum to others. Finally the study demonstrated that exogenous VEGF/ N-acetyl D-glucosamine has a direct effect on osteoblastic activity.

B. For Immunohistochemical findings

The present result showed positive expression of VEGF by bone marrow stromal cells, adipocytes, mesenchymal stem cells, precursor endothelial cells, and bone cells include osteoblasts and active osteocytes in different periods in all groups but in different score. Therefore, our primarily data provide evidence that VEGF activity is essential for appropriate bone formation and mineralization in response to injury. At day 3, bone marrow stromal cells in combination group records high VEGF expression, VEGF group is the second. At 7 and 10 day VEGF group records a highest VEGF expression in comparisum to other groups.

The data showed a high expression for ALP during the formative stages of bone in all groups except combination group shows the lowest count for positive cells that expressed ALP.

The present results showed that at day 3, bone marrow stromal cells in VEGF and N-acetyl D-glucosamine group records high collagen type I expression.

Conclusion: the study demonstrated that low application of VEGF / N-acetyl D-glucosamine could be an effective therapeutic for bone injuries, these data are promising for a possible future clinical usage.