



IMPACT OF LOCAL INJECTION OF HUMAN GROWTH HORMONE VERSUS LOW-LEVEL LASER THERAPY ON MANDIBULAR CONDYLE IN ARTHRITIC GUINEA PIGS

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ABSTRACT

Objectives: This study aimed to compare the effect of intra-articular injection of human growth hormone (HGH) versus low-level laser therapy (LLLT) on mandibular condyle in arthritic guinea pigs.

Methods: A total of 30 male guinea pigs with average weight (250g -350g) had a temporomandibular joint (TMJ) arthritis induced by locally injecting complete freund's adjuvant (CFA) intra-articularly in the right side. Then they were divided into three groups; control, HGH and LLLT. The applications of both treatments were done every two days for two weeks, then all groups were euthanized. Arthritic TMJs were dissected and processed for histological, histomorphometric and statistical analysis..

Results: Histological evaluation showed increased thickness of articular cartilage and formation of new subchondral bone trabeculae and narrowing of marrow spaces in both study groups with statistical significant differences compared to control group.

Conclusion: Treatment of TMJ arthritis with HGH showed improvements in all parameters tested. HGH can be a promising modality to repair degenerative changes associated with CFA induced arthritis.

KEYWORDS: Temporomandibular joints, Arthritis, human growth hormone, low-level laser therapy.

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INTRODUCTION

Temporomandibular joint disorders refer to conditions that affect TMJs and masticatory muscles. Degenerative joint diseases (DJDs) are characterized by degeneration of the joint in addition to facial pain which result in disability.¹ Rheumatoid arthritis (RA) is a systemic heterogeneous autoimmune disease. Among several animal models,² guinea pigs are considered appropriate for studying various inflammatory and infectious diseases including arthritis.³

The HGH is a pleiotropic hormone secreted by the pituitary gland. The effect of HGH is produced through intermediate substances called somatomedins.⁴ Although it is known primarily as potent stimulant and regulator in somatic growth, it is involved also in bone and muscle mass regulation.^{5,6} HGH decreases the development of type I diabetes.⁴ HGH involvement in immune system regulation is thought to be via modulating several aspects such as thymic development, B cell responses and antibody production, natural killer cell and macrophage activity.⁷

The LLLT has excelled in treatment of DJDs due to its anti-inflammatory, analgesic and regenerative effects. LLLT might possess bio-stimulator properties that lead to blockage of some pro-inflammatory mediators, for example interleukin 1 beta (IL-1 β), Cyclooxygenase-2 (COX-2), prostaglandins E2 (PGE2) and Tumor necrosis factor- alpha (TNF α).⁸ In addition to modulation of tissue repair, matrix metalloproteinases (MMPs) activity reduction and analgesic properties via direct irradiation without thermal response.⁹

There is no curative treatment of RA.¹⁰ The proposed treatment should at least reduce or even prevent joint destruction and deformity in order to restore the joint function. This study aimed to compare the effect of HGH and LLLT in treatment of RA.

MATERIALS AND METHODS

This study was approved by the ethical committee, Faculty of Dentistry, Mansoura University, Egypt (NO 09030718). The sample size was calculated using G*Power 3.1.9.2. In a one-way ANOVA study, a sample sizes of 10 guinea pigs in each of the three groups (Control, HGH, LLLT) achieved 95% power to detect differences among the means versus the alternative of equal means using an F test (ANOVA: fixed effects, omnibus, one way) with a 0.05000 significance level to detect a hypothesized large effect size of .8. Thirty male guinea pigs with average weight (250 g – 350 g), were acclimatized under standardized conditions; temperature, humidity and housed in individual with free access to food.

Animals Grouping

Arthritis was induced by single intra-articular injection into the right TMJ with 0.125 ml of CFA (Sigma-Aldrich Chemie GmbH, Germany) and arthritis development was evaluated by a blind examiner 3 times a week.¹¹

The exact position of needle insertion was established by palpation of the condyle, while the mandible was manually moved from side to side.

Arthritic animals were allocated randomly into 3 equal groups. The right TMJs were injected with normal saline in **control group (CG)** and HGH (Somatropin 4 IU, Sedico Pharmaceutical Co., Egypt) in **HGH group (HGHG)**, while exposed to LLLT (Quicklase, UK) in **LLLT group (LLLTG)**. All animals were euthanized after 8 days from the last of saline, HGH or laser session via overdose of xylazine and ketamine.

HGH injection

After 28 days from induction of arthritis, anesthesia was induced by injection of ketamine (75mg/kg body weight) and xylazine (25mg/kg body weight) intra-peritoneally. TMJ area was shaved and disinfected with 10% povidone iodine,

then 1mL of 2mg/kg body weight of HGH was injected in the right TMJ every 2 days for twelve days (6 injections).¹² While CG was injected using the same protocol but with saline.

LLLT

GaAAs laser with 1064nm λ , 6W output power (Quicklase, UK) was used. Animals right TMJs received an application of 3 J/cm² at a single point, 12 seconds duration, seven sessions and forty eight hour intervals.⁹

Histological & statistical analysis

TMJ sections were fixed, decalcified then processed and stained with routine H & E stain. Masson's trichrome and Toluidine blue stains were used for collagen and glycosaminoglycan detection respectively, followed by histomorphometric analysis.

Ten different standardized fields were analyzed, by a blind examiner, for each group. The percentage of collagen in newly formed subchondral bone, disk thickness, upper and lower compartments, marrow spaces and thickness of cartilage, represented by distance from the superior border of the mandibular cartilage to boundary with the zone of endochondral ossification, were measured, followed by statistical analysis.

Qualitative data were initially tested for normality using Shapiro-Wilk test (Statistical Package for Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA). The data was described using mean and standard deviation. One way Analysis of variance (ANOVA) followed by Tukey were used for comparing data. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

TMJ represents unique properties compared to other joints, and is frequently affected by RA¹³, which leads to arthritic changes in all components of the joint, that may reach up to complete loss of

the condyle and exposure of subchondral bone. CFA arthritic experimental model has been successfully used, and mandated in certain strain of animals as guinea pigs for arthritic induction.¹⁴

Clinically the injected TMJs showed signs of inflammation, but there was no weight loss in guinea pigs.

The CG revealed, in H & E stained sections, articular cartilage thinning, irregularities and severe atrophy in some parts. In addition to subchondral bone (sb) destruction resulting in exposure of articular cartilaginous region with an increase in marrow spaces and osteoclastic activities represented by Hawship's lacunae Figure (Fig.1, A). These results were in agreement with those of Lemos et al.,¹⁵ Xu et al.,¹⁶ who observed destructive changes in CFA-injected TMJ.

While H & E sections of HGHG, showed increased cartilage thickness and subchondral bone formation accompanied by narrowing of marrow cavities. Among the important findings were the reduction of disk thickness, the chondrocytes with normal architecture and some degenerative changes signs. (Fig.1, A1). Concomitantly Feizbakhsh et al.,¹⁷ reported a significant higher cartilage thickness in rabbit's condyle due to HGH stimulatory effect.

The significant increase in cartilage and subchondral bone may be due to ability of HGH to stimulate mitosis, and to increase size and specific differentiation of cells such as bone forming cells.¹⁸ Livne et al.,¹⁸ demonstrated that addition of HGH on mice chondrocytes may increase the anabolic activities of cell proliferation and sulfated proteoglycan synthesis. Lewinson et al.,¹⁹ proved that HGH stimulates endochondral bone formation. In addition, the local administration of HGH may urge more specific effects and less systemic side effects.¹⁷

H & E stained sections of LLLTG showed chondrocytes with almost normal architecture and thickness, regenerated cartilage, and newly formed

subchondral bone trabeculae with narrowing of the intervening marrow cavities. (Fig.1, A2). These findings, may be due to LLLT dose-specific, anti-inflammatory effect.²⁰ Additionally LLLT up-regulates several cellular processes including oxidative phosphorylation that control biological function normalization at the cellular level.²¹ concomitantly Carvalho et al.,⁸ and Lemos et al.,¹⁵ demonstrated significant action of LLLT in treatment of arthritic rodent TMJ.

Disarrangement of collagen fibers with matrix defects or cavitation and loss of metachromasia were observed in CG (Fig.1, B&C). These results were in agreement with Lemos et al..¹⁵ who reported an increase loss of metachromasia and even loss of arrangement of collagen fiber and extracellular matrix (ECM) destruction in arthritic non-treated group.²²

Statistical analysis of values recorded from histomorphometrical analysis among the 3 groups revealed that, the HGHG showed a statistically significant difference when compared to the CG in all measurements except for lower compartment measurements. While when HGHG were compared

to LLLTG, there was a statistically significant difference in all measurements except collagen average area, subchondral bone and upper compartment. Comparing the LLLTG with the CG there was a statistically significant difference in all measurements except disc thickness and upper compartment. The P value was (P= 0.05). **Table (1)**

Regenerated collagen fibers with almost normal arrangement observed in the HGHG may be attributed to increase of collagen synthesis by HGH.²³ In addition the cartilage thickness increase with slight loss of the metachromatism were also reported. (Fig 1, B1& C1).²³ Similarly and in accordance with this study results (Fig 1, B2& C2), Lemos et al.,²⁴ showed positive effects of LLLT via slight reduction in metachromasia and promoting arrangement of the collagen fibers. Additionally, Melis et al.,²⁵ revealed that LLLT not only increases the vascularization but also increases the fibroblast formation.

From the presented results both HGH and LLLT can accelerate the process of tissue repair and reduce the joint morphological changes. The superior regenerative effect of HGH over LLLT on

TABLE (1): Comparison between the three studied groups in relation to collagen average area, Marrow cavities percentage, cartilage thickness, subchondral bone trabeculae, upper and lower compartment and disk thickness using One Way ANOVA test followed by Tukey post hoc test.

parameter	control	HGH	LLLT	P value
Collagen Surface area	3.2822 ^A ±0.00763	8.7428 ^A ±1.71289	5.7880 ^A ±1.04612	.001*
Cartilage	50.4150 ^A ±1.66923	112.8430 ^A ±28.049	72.7280 ^A ±6.43870	.001*
Marrow cavities	14.5330 ^{AB} ±1.78791	9.6680 ^A ±1.26487	10.9450 ^B ±2.76587	.001*
Lower compartment	14.3770 ^A ±.93299	12.5584 ^{AB} ±0.49827	15.6681 ^B ±2.40464	.001*
Upper compartment	19.4170 ^{AB} ±1.52259	15.5420 ^A ±2.0438	16.5420 ^B ±2.61201	.001*
Disc thickness	30.2002 ^A ±1.76613	62.9730 ^A ±1.91515	47.7794 ^A ±5.61275	.001*
Subchondral bone	3.3100 ^{AB} ±0.86111	8.6500 ^A ±1.76147	9.1660 ^B ±1.89454	.001*

Data were expressed as mean±SD

SD:standard deviation P:Probability

N.B: Same superscript letters in a row indicate significant difference * (P < .05).

the cartilage thickness might be due to the ability of HGH, to stimulate progenitor cell proliferation, cartilage differentiation and mineralization of extracellular matrix, in addition to Growth factor-1(IGF-1) local production which in turn stimulates bone and growth plate.^{26,27}

Within the limitations of the current study, the results of HGH injection in guinea pigs' arthritic TMJ may successfully relieve many of RA features compared to LLLT. Further investigations are needed to study insulin growth factor (IGF) role before this step can be extrapolated to a human model.

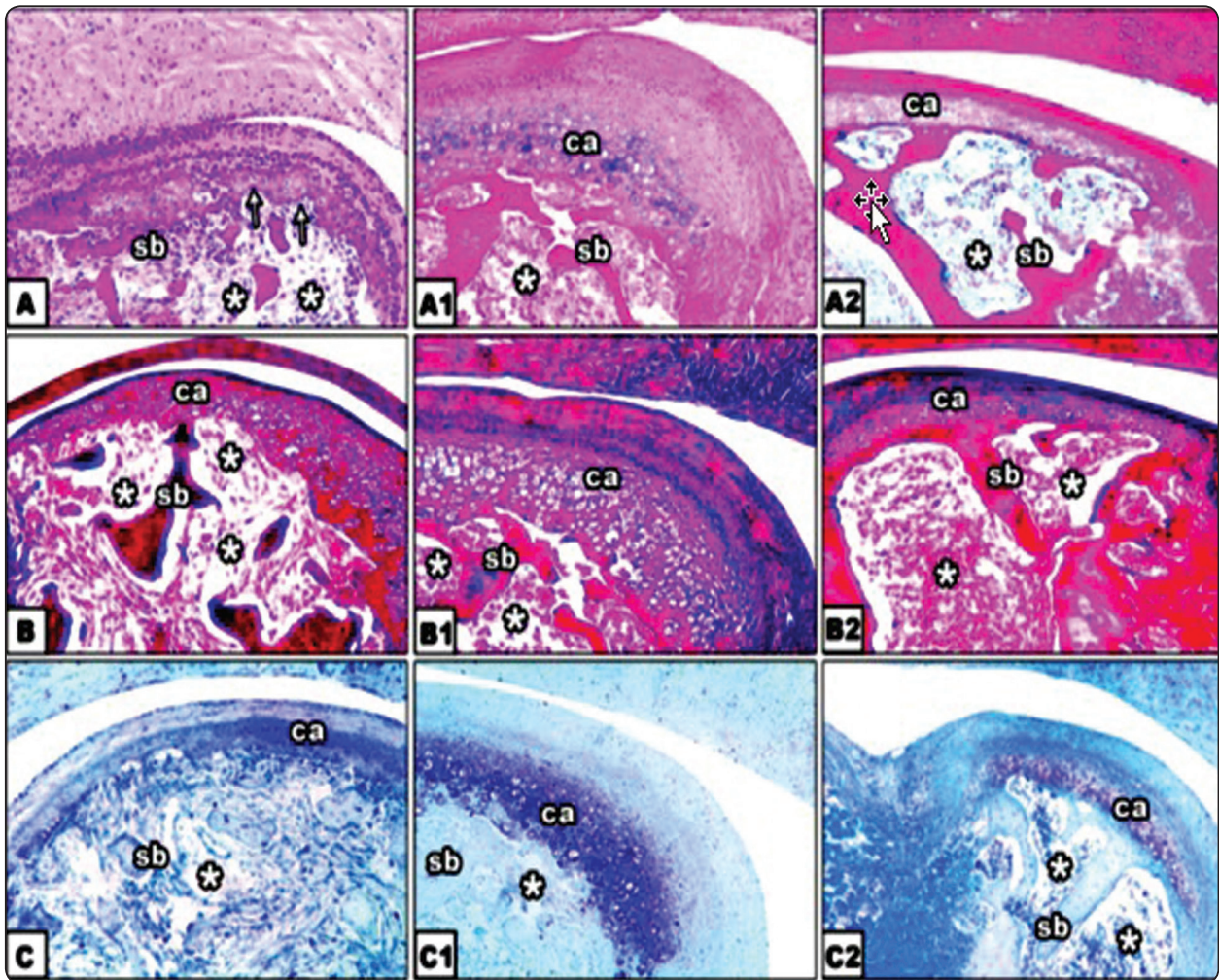


Fig. (1) : Coronal section of the TMJ in (A) control group showing thinning and irregularity of some parts of the articular cartilage, severe atrophy in other parts of the cartilage and destruction of the subchondral bone (sb). (A1) Coronal section of the TMJ in HGH group showing increase in the thickness of regenerated articular cartilage layer (ca). New regenerated subchondral bone trabeculae (sb) (H&E X 100). (A2) Coronal section of the TMJ in LLLT group showing slight increase in the thickness of regenerated articular cartilage layer (sb) while decrease in marrow spaces (*) (H&E X 100). (B) Coronal section of TMJ in control group showing marked decrease in articular cartilage (ca) thickness and disarrangement of collagen with matrix defects or cavitations. (Masson's Trichrome X100). (B1, B2) Coronal section of the condyle in HGH and LLLT group respectively showing regenerated collagen in some areas of regenerated cartilage (Masson's trichrome X 100). (C) Coronal section of TMJ in control group showed sever loss of the metachromatism in degenerated cartilage. (C1, C2) Coronal section of TMJ in HGH & LLLT respectively showing slight loss of the metachromatism of both cartilage & bone matrix. (Toluidine blue X 100).

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