Title page .

Title of article: Clinical study of neurodegenerative treatment on glaucoma by insulin intravitreal injection.

Abstract

Purpose: Our trail was to study insulin intravitreal injection's (IIV) efficacity and safety to treat glaucoma neurodegeneration.

Method: Eleven subjects (11eyes) were recruited;10 patients treated in a double masked randomized sham controlled including 5 patients received one IIV injection 3UI and 5 patients received one injection of balanced salt solution(BSS) 3UI. A follow up during 168days was realized using Optical Coherence Tomography(OCT) and visual field(VF).The eleventh patient received two IIV injection without masking the injection content within one month between each injection and followed up for 877days.All the patients have a correct ocular pressure and no ocular treatment was stopped.

Results: The 5 patients who received IIV revealed a swift improvement of decibel(DB), and remains stable during the first month. The average improvement was 6.62DB during 168days.The 5 patients treated with one BSS injection showed no significant improvement regaining 1. 45DB.The last patient who received two injections showed increase from 7.54DB to 17.22DB with a functional amelioration of 9. 68DB.The OCT examination showed a structural improvement during the first month, then returned to the initial value. No complication was observed during and after the treatment.

Conclusion: Insulin shows not only efficacity and safety but more than that, the visual field(VF) of the patients became stable and show no deterioration in all the follow up, which confirmed that insulin act to improve the function rather than structure. it means insulin reconnect the stoma and improve the neurite outgrowth This treatment will change the evolution of this pathology and protect the glaucomatous patients against blindness.

Keywords: insulin – glaucoma – neurodegeneration disease – neurite outgrowth – visual field – average defect – nerve cells regeneration.

Introduction

Glaucoma is a progressive degenerative disease **(1)** and the first leading cause of blindness, (**1,2**). In adult population the prevalence of primary open glaucoma (POAG) cases was estimated approximately 2% in patients over 40 and 10% in 80 years old patients, with a 76 million cases number in 2020 and could reach 111.8 million in 2040 **(3),** this increase in glaucomatous patients in the word will therefore more important given the increase in aging .The first risk factor of glaucoma is the ocular hypertonia, which is due to an increase in resistance to the flow of aqueous humor through the meshwork trabecula.

All the antiglaucoma drugs currently avaible are going to have an action on lowering eye pressure which decreases the risk and the rate of disease progression, but currently there is no serious treatment for the neurodegenerative problems.

Anatomically and developmentally, the eye is an extension of the brain, Consequently, its share similar pathophysiological mechanisms as other degenerative disorders of the central nervous system (CNS) like the Alzheimer's disease **(4).**

According to previous studies insulin is approved as a safe and efficacy drug in clinical uses with no detectable clinical toxicity **(5)**.

Our approach is to use insulin in a clinical trial to treat neurodegeneration in glaucoma and solve this problem.

Materials and methods

1- subjects:

- **Inclusion factors**

Patients selected for this study suffer of an open angle glaucoma with strain eyes balanced under medical treatment or having previously under gone filtration surgery or laser SLT.

- **Exclusion factors**

  Mono-ophthalmic patients, diabetic retinopathy and high myopia, cataract, age related macular degeneration or another maculopathy, atrophic neuropathy from another origin, amblyopic eye.

2-Study design

This study was realized in an ophthalmology private clinical center located in Mostaganem, Algeria. In the confluence with the protocol of good clinical practice, and performed in adherence to the guide lines of the Helsinki declaration. Informed consent was taking from patients.

Eleven patients (seven men and four women) with average age of 62 years (±14), were recruited for this study, 10 patients are treated in a double masked sham controlled including 5 patients were received one injection of insulin rapid intravitreal 3 UI. The other 5 patients received 3 UI of BSS. with follow up of 168 days.

The last patient was a man aged 55 years old received two intravitreal injections of insulin 3UI in the left eye within one month between each injection in a prospective study, with follow up of 877days.

In every visit a complete exam was applicated, enclosing the OTC, standard visual field examination, ocular tension and optic nerve retinopathy.

3-Evaluated parameters:

After ophthalmic examination (VA, SL; fundus of the eye) and control of ocular pressure by the Glodman aplantion tonometer. all subjects were assessed with OCT Optovue exploration of RGC (average thickness, focal lost vision, global lost

vision, RNFL average thickness and visual field).

All the injections were conducted in the operating room after disinfection with the Betadine.

A ticket was placed to masque the reel continent of the injected substance.

The last patient treatment was not masked.

4- Statistical analyses:

Statistical analyses were performed using XL-Stat/Excel software. All the analyzes are based on individual eye in both sham and treated group .

Result

1-One injection Statistical interpretation

The function parameters are observed with the visual field (REODENSTOCK Field Analyzer device) by studying the indices average (DB, AD) and structural parameters using OCT (optovue oct device) by studying the indices (RGC, RNFL).

The average of all the patients was calculated and used in the statistical study.

1-1-Comparative study of the evolution of visual field in the treated group and the placebo group:

1-1-1Average DB:

- ***Insulin treatment***

The average DB was 13.17DB before treatment and reached 19.79DB at the 168 day of treatment, the average DB showed an increase of 6.62 DB **(Fig 1).**

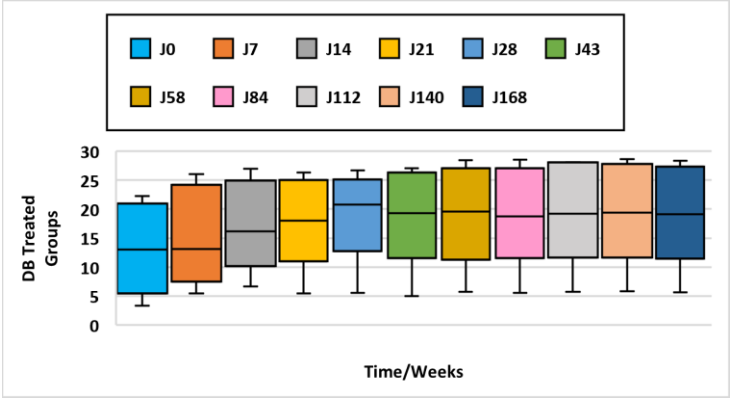

Figure 01 :The effect of insulin on DB in the treated groups during 168

- ***Placebo treatment***

The placebo group showed a non-significant improvement, before treatment it was 20.17DB while at the 168 day it attempts 21.62DB, showing an increase of 1.45DB **(Fig 2).**

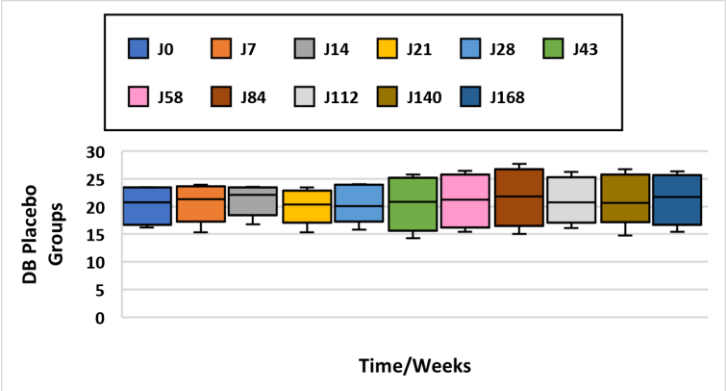

Figure 02 :The effect of insulin on DB in placebo groups during 168 days

1-1-2Average AD:

- ***Insulin treatment***

The average AD was -5.396 before treatment and reached -3.78 at the day 168, showing an increase of 1.616

(Fig 3).

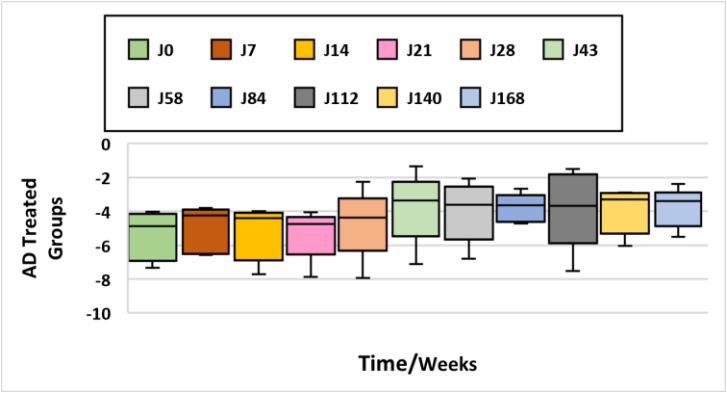

Figure 03 :The effect of insulin on AD in treated groups during 168 days

- ***Placebo treatment***

The placebo group showed a non-significant improvement, it was -3.49 before treatment while at the 168 day it attempted -3.46 **(Fig 4).**

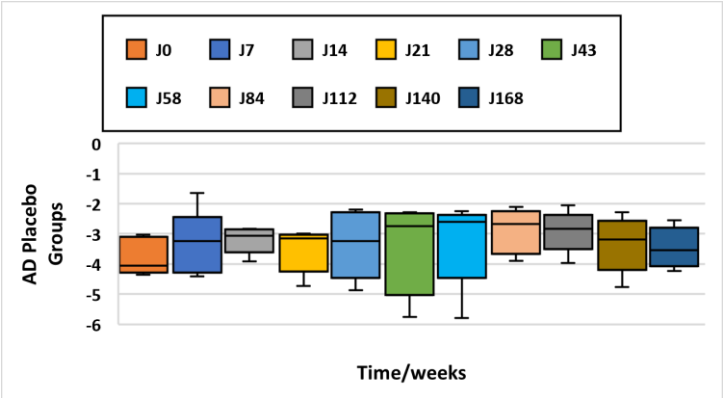

Figure 04: The effect of insulin on AD in placebo groups during 168 days

1-2-Comparative study of the evaluation of RGC and RNFL in the treated group and the placebo group:

1-2-1-RGC:

- ***Insulin treatment***

The average RGC was 76 µm before the treatment, it showed an increase until 21st day from the injection to attempt 90 µm and return after that to the initial value after 168 day (Fig 5).

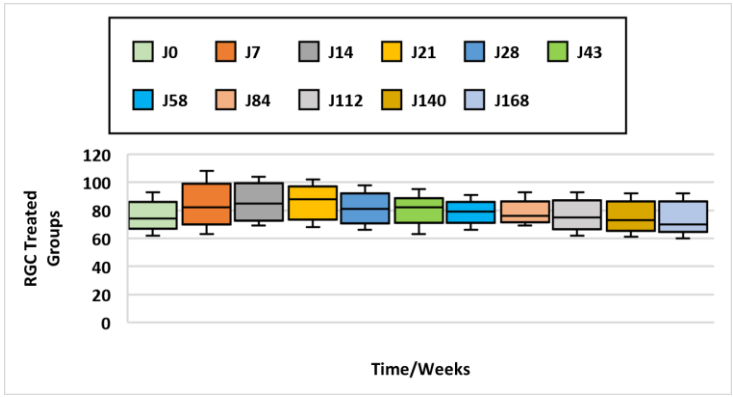

Figure 05 : The effect of insulin on RGC in treated groups during 168 days

- ***Placebo treatment***

The placebo group showed a non-significant improvement, it was 77.04µm before treatment and 75.72 µm after 168 days **(Fig 6).**

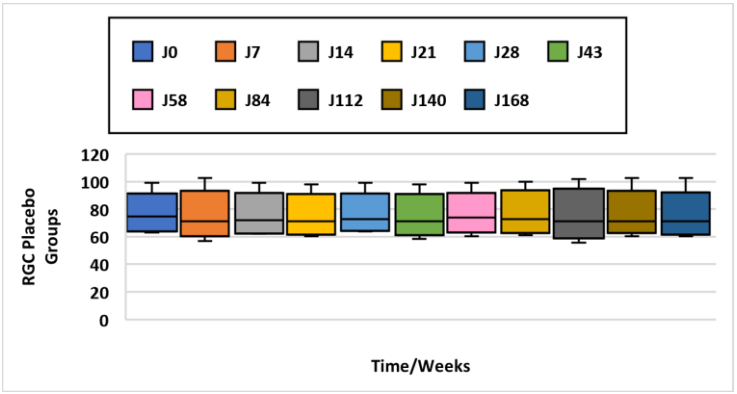

Figure 06 : The effect of insulin on RGC in placebo groups during 168 days

1-2-2-RNFL:

- ***Insulin treatment***

The average RNFL was 68.4 µm before treatment showing an increase of 80 µm on the 21th day ,returning to the initial value after 168 day **(Fig 7).**

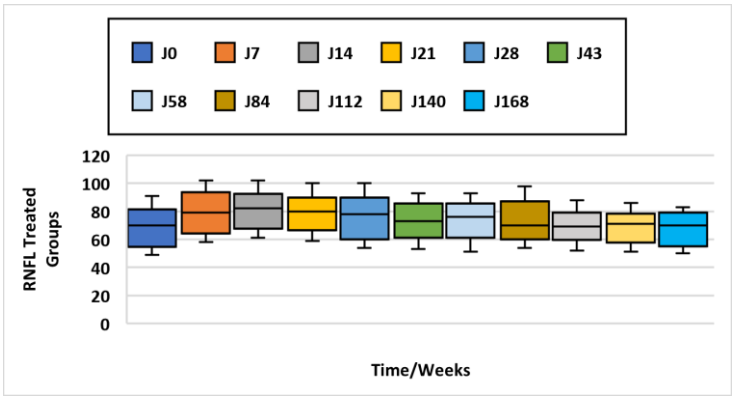

Figure 07 :The effect of insulin on RNFL in treated groups during 168 days

- ***Placebo treatment***

The placebo group was 74.7 µm before the treatment than decrease to 73.44 µm after 168 days **(Fig 8).**

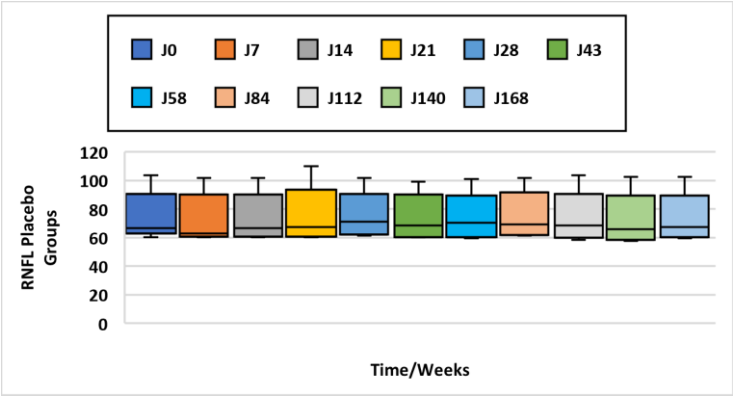

Figure08 : The effect of insulin on RNFL in placebo groups during 168day.

patient treated with 2 injections with rapid insulin with dose of 3UI.

The red area shows the points not seen by the patient in visual field and the blue area represent the points seen.

The Green sloping line going up from left to right across the two graphs shows amelioration and the yellow lines constitute the average decibel.

The first graph shows a field of vision on February 28,2020.

The first and the second injection was realized on March 8,2020; March30,2020 respectively.

The second graph shows a field of vision on July22,2022.

We conclude that there was an amelioration.

2-Two injections results

The two graphs represent the evolution of decibel level over a period of 877days in a

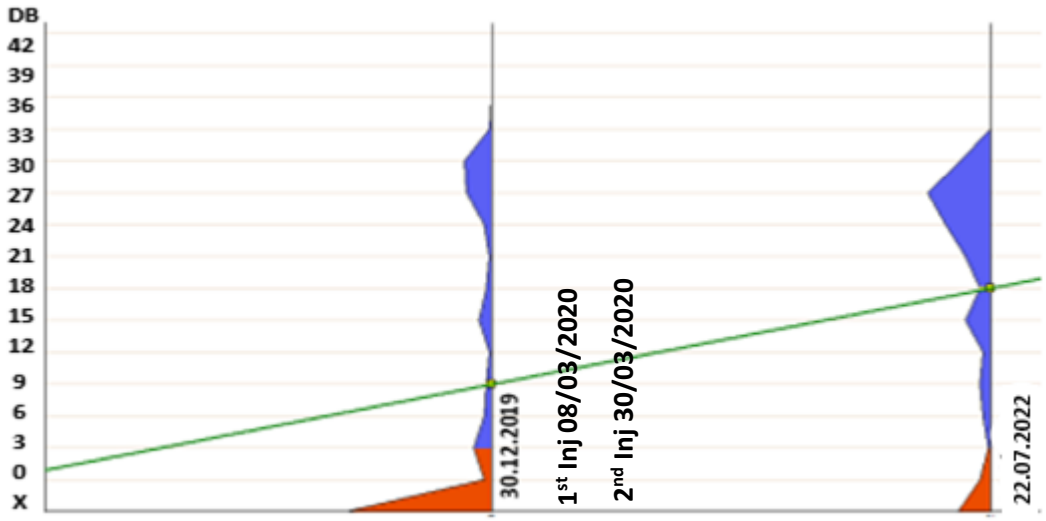

Figure09: Decibel level graphs presentation-dB level, with follow-up of 877days.

- ***Red area:*** ***the point not seen by the patient in visual field***
- ***Blue area:*** ***the point seen by the patient in visual field***
- **Green sloping line: going up from left to right across the two graphs shows amelioration.**
- **Yellow lines: The average decibel**

- *1-1-The visual function field (VF) shows decibel (dB) examinations for left eye* **(fig 10).**

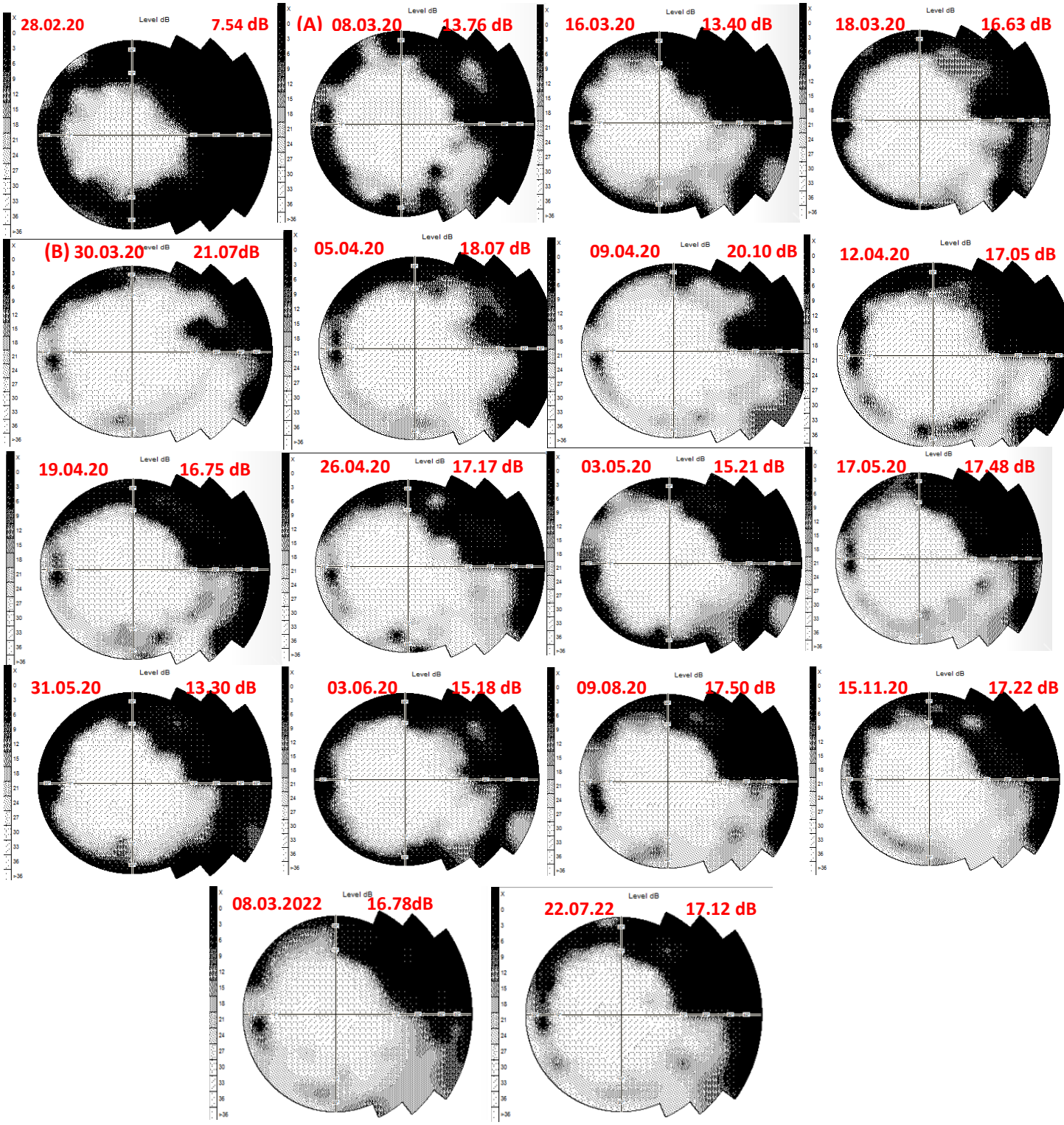

Figure10: Visual Field changes after 2 injections of 3UI of rapid-acting insulin at one-month intervals with a follow-up of 877days.

(A) Date of the first injection
(B) Date of the first injection

1-2-Statistical interpretation for two injections study:

1-2-1-Average DB: Insulin treatment with 2-injections:

The average dB was 7.54 dB before treatment and 13.76 dB in the first injection. It reached 21.07dB in the second injection, showing an increase of 9.68 dB during 877days **(Fig 11).**

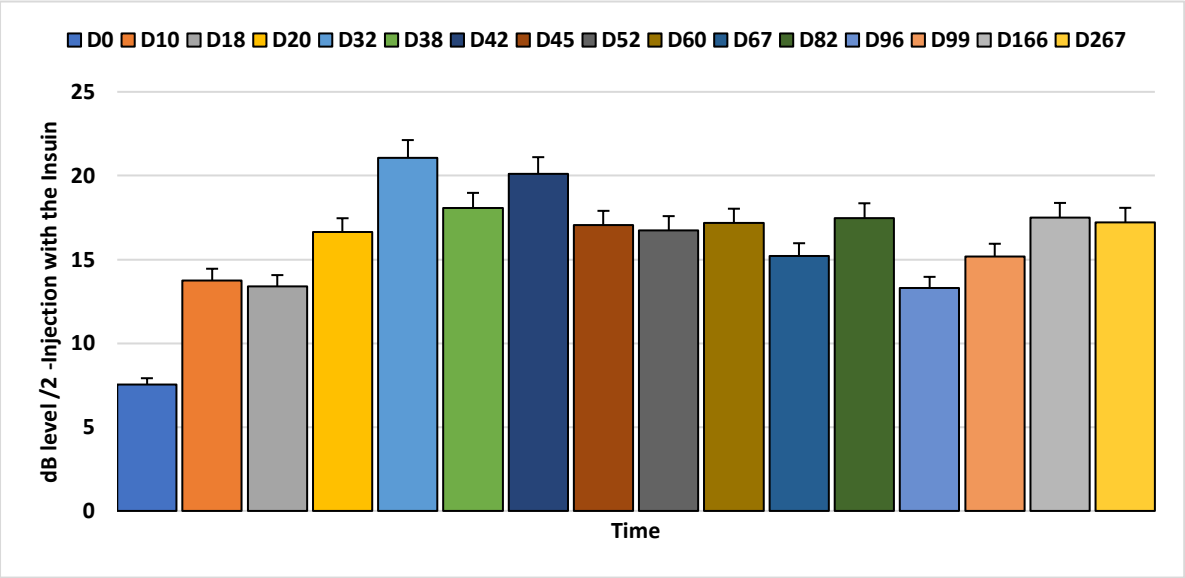

Figure 11: The effect of 2-injection with insulin on the dB levels in one patient during 877days.

1-2-2-Average AD: Insulin treatment

The average AD was -7.33 before treatment and was -6.06 in the first injections. It attempted to -3.44 at the second injection, showing an increase of 3.09 dB during 877days **(Fig 12).**

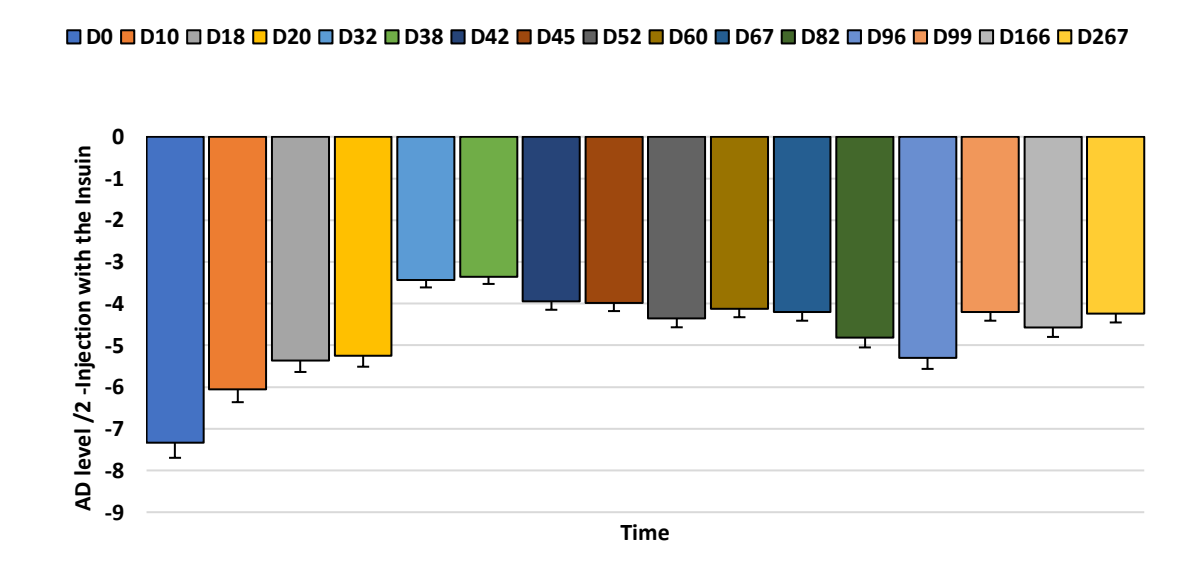

Figure 12: The effect of 2-injection with insulin on the AD levels in one patient during 877days.

Discussion

Insulin – receptors and signaling pathways

Glaucoma and Alzheimer disease share many identical symptoms and characteristics as disease that affecting different locations of the brain **(6-7)** it has been mentioned as an Ocular Alzheimer's disease **(8).**

Glaucoma present with many common characteristics including increased cell death, abundant neurofibrillary tangles, consequent dystrophic neuritis, impaired deposits of APP(Amyloid protein precursor) , which are resulting from many pathological pathways such as mTOR; Beta amyloid aggregation, Tau hyperphosphorylation and IDE (insulin degrading enzyme) downregulation, increased expression of apoptotic genes, disturbing energy metabolism and mitochondrial dysfunction with elevated OS (oxidative stress) causing DNA damage. **(9).**

Insulin is a potent neuroprotective agent that acts mainly against apoptosis, beta amyloid toxicity, oxidative stress, and other ischemia **(10).** On the other hand, the Insulin and IGF (Insulin-like growth factor) have essential roles in growth and development **(11).** It has been shown that antiapoptotic effects of insulin depends on the PI3K pathway to stimulate neurite formation. (**12).**

Insulin is part of a family of peptides including insulin-like growth factors I/II (IGF-I/II) and relaxin **(13).** However, the expression of the insulin receptor is present in the retina **[14,15].**

The insulin Receptor and IGF-1R (Insulin-like growth factor-1 receptor) belong to a family of transmembrane receptor tyrosine kinases permit the kinase of the receptors to phosphorylate these proteins on tyrosine residues **(16).** Most of insulin responses are mediated by IRS-1 and IRS-2.

Insulin and its receptor are generally expressed throughout the brain and have been postulated to play a crucial role in synaptic plasticity. Although structural remodeling of dendritic spines is associated with stable expression of synaptic plasticity. **(17).**

IR signaling plays an important role in neuronal proliferation during development, this was confirmed with a study that shown that IRS-2 mediated the effects of insulin on brain growth **(18)**, outgrowth, maturation, and axons reconstruction **(19).** Moreover, insulin had an increasing effect on the protein expression of the dendritic scaffolding protein post-synaptic density-95 (PSD-95) in hippocampal CA1 region by PI3K/mTOR activation pathway. The process activates extracellular signal-related kinase/mitogen-activated protein kinase (ERK/MAPK) and PI3K/Akt (protein kinase B) pathways inhibiting GSK-3b. These pathways are involved in inhibition of apoptosis, cell growth (and proliferation) and reduction in OS [**20–21].**

The insulin receptor tyrosine-kinase substrate P58/53 IRSp53 co-expressed with the IR in the synapse-rich molecular layer and in granule cell layer of the cerebellum, like-wise, in the cultured hippocampal neuron's synapses **(22)** The neurite formation depends on insulin and IGF2 presence which are important for NGF (nerve growth factor), there for this effect depend to the presence of the astrocytes.

Ser/Ther kinase Akt are important promote neuronal survival, Akt has been revelated as a primordial mediator of several aspects of neurite outgrowth, implicating elongation, branching and caliber. Moreover, activated Akt targets and inactivates pro-apoptotic proteins such as Bad, caspase 9, and

glycogen synthase kinase 3 beta (GSK3β). Activated Akt also protects against neuronal hypoxia by NO-induced (nitric oxide) and stop apoptosis by preventing the transcriptional activity of p53 **(23)**

Vascular dysfunction of neurovascular contribute to the pathogenesis of glaucoma **[24-25].** This is sustained by a balance between the vasodilator action of NO and vasoconstrictor action of endothelin-1 [**26–27**], Which is regulate by insulin in vascular endothelium via PI3K and MAPK signaling **(28)** . The PI3K dependent signaling cause an endothelial dysfunction by an imbalance between NO secretion and the hyperphosphorylation of caspase 9 cause endothelial cell apoptosis **(29).**

Insulin has antiapoptotic effects by mTOR activation which suggesting that p70SK protein (one of the downstream targets of PI3K/Akt/mTOR pathway, may be one of the mechanisms through which insulin prevent apoptosis **(30)**

This activation modulates mitochondrial electron transport chain function, and inhibits also FOXO1/HMOX1 and conserves the NAD+/NADH ratio, which regulates the SIRT1/PGC1α pathway for mitochondrial biogenesis and function **(31).**

All of this show that enhancing mitochondrial function may be an important therapeutic benefit to targeting insulin signaling in glaucoma pathogenesis.

The high levels of insulin in brain extracts, was consolidated by the detection of insulin secretion in neuronal cultures **(32)** and the presence of insulin immunoreaction inside the Golgi and the rough endoplasmic reticulum in the brain **(33)** Which was detected using insulin antisera, in the retinal layers including the ganglion cell layer, and this was approved in human and mouse retina, as well as optic nerve glial cells **(34)**

There is evidence that the insulin had a central origin, firstly, C-peptide was found in the brain from human cadavers, and the highest content were specifically in the hypothalamus **(35).** Secondly, the synaptic vesicles within nerve-endings store the insulin in the adult rat's brain. **(36)**

Furthermore, insulin mRNA was located in the CA1 and CA3 regions of the hippocampus, precisely in the dentate gyrus, and in the granule cell layer of the olfactory bulbs of the neonatal rabbit brain **(37)**

Usually, insulin protect against beta amyloid; in the case of Insulin resistance which is closely associated with reduced responses to insulin signaling in IR, IRS-1, PI3K and IGF1 in IGF1R, IRS-2, PI3K signaling pathways, the Aβ (Beta amyloid) metabolism is affected as a consequence of Tau hyper phosphorylation **(38),** that has been implicated in neurofibrillary endo threads and tangles (NFT) formation in both AD (Alzheimer disease) and glaucoma **(39).**

Glucose hypometabolism results in both reduced glucose transporter expression and the decreased O-GlcNAcylation of tau. They proposed that hypometabolism in the brain reduces the O-GlcNAcylation of tau that conversely increases its phosphorylation, which induces the NFTs (neurofibrillary tangle). **(40-41)**

Accordingly, impaired cerebral glucose metabolism, mainly thiamine metabolism and insulin resistance, could promote Aβ accumulation and tau hyper phosphorylation.

The reduced brain insulin/PI3K/Akt pathway leads to the overactivation of

GSK-3β calpain-1 and downregulation of O-GlcNAcylation, which promoted altered tau hyperphosphorylation and neurodegeneration **(42)**

By contrast, serine phosphorylation of IRS-1/2 by C-Jun N-terminal Kinase (JNK1) and other kinases down regulates insulin-stimulated tyrosine phosphorylation, and that corresponds with insulin resistance **(43).** Likewise, ubiquitin-mediated degradation of IRS-1/2 also aid to insulin resistance formation **(44) (fig 13).**

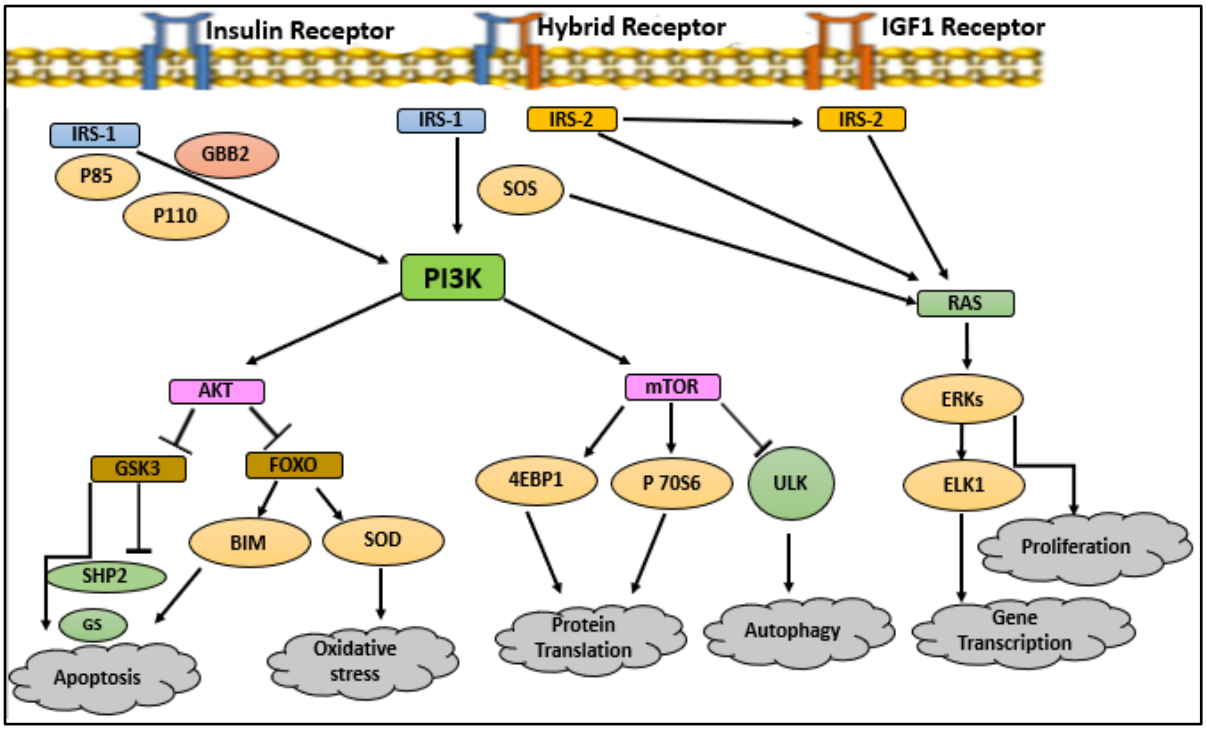

Figure 13 : Insulin pathways signaling

On the other hand, the presence of the GLUT was noticed in the brain likewise: GLUT-1; GLUT-2; GLUT-3 and GLUT-4; with different level and distribution than the peripheral (Table)

Table: the different distribution of Glut-transporters between the central and peripheral region

Glucose transport Isoforms	location	abundance in central	abundance in periphery
GLUT-1	ubiquitous	+++	+
GLUT-2	hypothalamus	++	+++
GLUT-3	cerebellum, striatum , cortex,hippocampus	+++	+
GLUT-4	olgactory bulb , hyppocampus (dentate gyrus) And hypothalamus cerebellum	+	+++
GLUT-8	hypothalamus, dentate gyrus , amygdata And primary olfactory cortex	++	-

The inflammatory responses are closely associated with the development of insulin resistance in peripheral and central tissues and the presence of TNFα producing hypothalamic inflammation **(45).** This inflammation interacts with the processing and deposit of β- amyloid peptide (Aβ), with low amount of insulin producing anti-inflammatory effect. JNK inhibitors stimulate the neuroinflammatory response, while JNK activation promotes neuroinflammation and facilitates insulin resistance **(46) (fig14)**

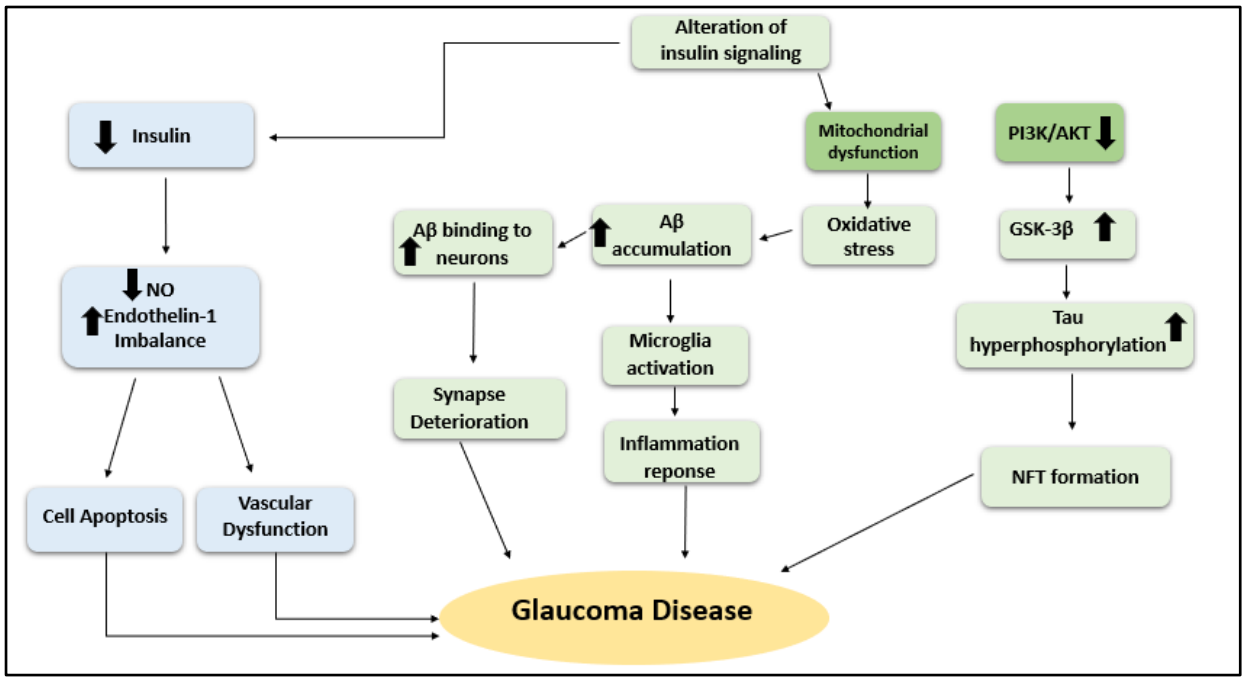

Figure14: relationship between alterations of insulin signaling and glaucoma disease pathogenesis.

Conclusion

In this trial we investigated the insulin effects to promote the visual function; this regeneration is visible on visual field. as we saw in discussion how the insulin promotes the neurite out growth, but also the increase in visual function stays stable during this study (24 months) the use of tow injections shows better results. These results are confirmed returning to different theoretical and experimental studies that we have mentioned it in the discussion. it explains that insulin promote the regeneration of synaptic and dendrites, by activating the different insulin signaling pathways.

Other studies will be carried out for the generalization of this treatment in all glaucomatous patients. this treatment will change the dramatic evolution that this pathology can have to the glaucomatous patient.

Acknowledgments:

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