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METHODS

A glymphatic system and new etiopathogenic hypothesis on glaucoma patients who underwent osteopathic manipulative treatment: A pilot study

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Purpose: Malfunctioning of the lymphatic or glymphatic system in the brain plays an important role in central neurodegenerative pathologies with a buildup of neurotoxins. Recent studies have shown functional links between aqueous humor and cerebrospinal fluid via the glymphatic system, offering new perspectives and unifying theories on the vascular, biomechanical, and biochemical causes of chronic and primary open-angle glaucoma (POAG). The aim of this randomized pilot study is to compare the variations in intraocular pressure (IOP) between cases of compensated POAG under pharmacological therapy and glaucoma patients undergoing osteopathic manipulative treatment (OMT), considering that this manipulation can influence IOP.
Materials and methods: A total of 40 patients, all covered by the Helsinki Convention, were randomly divided into two groups: treated group (TG) and control group (CG), each with 20 cases. These patients were compensated glaucoma sufferers, who do not require changes in therapy or operations and as a result affect their eye pressure. IOPs were measured both before and after OMT, which are scheduled into four sessions at an interval of 7.3 and 150 days, and compared with CG.
Results: The average IOP of both the groups was compared and found to have a statistically inconclusive reduction in the right eye (RE) ($p = 0.0561$) and a significant effect in the left eye (LE) ($p = 0.0073$). The difference in reduction of IOP between the groups was observed 10 months after the first session or 5 months after the last session, and it can be observed during a checkup 13 months after the beginning of the study, or rather 8 months in the absence of treatment, with a highly statistically significant ($p = 0.000434$).
Conclusion: The results show that OMT can affect IOP after each session and that the pressure is significantly lower even months after the final treatment session.

Keywords: glaucoma, glymphatic system, osteopathic manipulative treatment (OMT)

Introduction and aims

The of primary open-angle glaucoma (POAG) are still unclear. The recent discovery of a lymphatic system in the brain and eye (1–5), called the glymphatic system, appears to shed new light on the etiopathogenic causes of this disease, bringing together various different hypotheses: vascular, biomechanical, and biochemical (6).

Intraocular pressure (IOP) is the principal risk factor for glaucoma and the main factor that can be rectified by therapy (7). According to Flammer (8, 9), vascular dysregulation is in reality a rectifiable factor, but one which is difficult to identify on the spot.

In normal-tension glaucoma (NTG), damage to the visual field occurs while damage to the visual field may be absent in cases of high ocular pressure (10). In patients with NTG, the cerebrospinal fluid (CSF) pressure, which is equivalent to the intracranial pressure, appears to be lower (10–13), while in patients with high IOP who present no functional damage, the CSF pressure is higher (14, 15). Other studies substantiate the preceding hypotheses explaining how the

influence of translaminar pressure has a determining role in POAG (16–18). Conversely, a retrospective study of CSF pressure in NTG puts the preceding hypotheses in doubt, suggesting changes in the investigative methodology, with the aim of proving the validity of the new theories concerning translaminar pressure (19).

Recent systematic studies have shown that blood flow may be lower in other parts of the body and that this reduction in blood flow to the eye is a precursor to glaucomatous damage. This presupposes that hemodynamic changes can be, at least in part, a primary factor in patients with glaucoma (8, 9). This further explains the effect of osteopathic manipulative treatment (OMT) in areas, such as cervical-cranial, aiming at an overall improvement in lymphatic drainage and vascular perfusion inside the cranium (20–26).

In the osteopathy literature, it is found that several techniques have been proposed for the eyes and orbital areas and demonstrated the influence of OMT on IOP (27–35).

The aim of this work is to identify the effect and duration of osteopathic treatment on ocular pressure, in patients with POAG, who were stable and under medication.

Materials and methods

In this study, 40 patients (80 eyes), all of the Caucasian descent, were examined for 13 months. The patients were randomized into two groups: TG and CG (20 per group), with Excel 2010. As glaucomatous disease often strikes where the damage and progression do not manifest symmetrically, we chose to evaluate the effect of OMT on both eyes to determine the effect of OMT in either of the eyes.

The average mean age of patients was 71.2 years (range 34–83), all diagnosed with stable and medicated POAG. Patients with increased eye pressure, reduction in the visual field or visual acuity, undergoing topical treatment temporarily, or those requiring an anti-glaucoma operation or extraction of a cataract were excluded from this study.

The average age of the patients in TG was 70.5 years (range 34–87), while that in CG was 71.8 years (range 51–95).

In the TG patients, the average defect (AD) was −5.55 dB in the right eye (RE) and −7.06 dB in the left eye (LE), while in the CG patients, the AD was −6.51 dB in RE and −4.65 dB in LE.

The inclusion and exclusion criteria are given in **Table 1**.

Every patient had a preceding follow-up of a minimum of 3 years and a maximum of 10, with at least three visual field tests, at least one for each year. Four osteopathic treatments were performed at regular intervals for all patients, from 1 week to 5 months (see **Table 2**). The CG was checked at the same interval to measure the ocular pressure.

Informed consent was obtained for all patients, as set out in the Helsinki Declaration. Every member of the TG provided a detailed personal medical history and underwent a general health check to exclude local or general pathologies that could falsify or compromise the OMT (following the inclusion and exclusion criteria). From the second examination onward, they completed a quality survey questionnaire in order to suspend the OMT in case of the appearance of eventual side effects due to the treatment (**Table 3**) (36).

In the TG, the ocular pressure was measured immediately before and after the OMT to detect any variation. Ocular pressure was measured for all 40 patients in a seated position, using a Goldmann pressure apparatus. Ocular pressure was measured in the CG (one single measurement) with no osteopathic treatment, at the same interval as the TG patients.

The visual field test was carried out with a Humphrey apparatus, program 30\2, threshold test, not earlier than 3 months from the beginning of the study and not later than 3 months from the end.

The OMT was not directed exclusively at the cranial-cervical area; rather it had the aim of favoring the systemic and local circulation and of acting on the somatic dysfunction (SD). SD is defined as an expression of a compromised or

TABLE 1 | Inclusion and exclusion criteria.

Inclusion criteria
Clinical diagnosis of bilateral primary open-angle glaucoma (40 patients)
POAG, divided into two randomized groups.
Follow-up of at least 3 years before the start of the study
Reliable visual field tests (at least three), one per year
Last visual field test before the experiment not earlier than 3 months from the beginning of the study
Glaucoma under pharmacological control
Pachymetry readings fall in the normal range
Patients are cooperative
Signing of the informed consent form
Exclusion criteria
Other types of glaucoma
Any kind of ocular or systemic anomaly or pathology which would render the osteopathic manipulation unreliable, impracticable, or impossible to evaluate
Change in pressure reduction therapy during the course of the study
Anti-glaucoma operations or cataract operations during the course of the study
Contraindications to the OMT
Patients with low compliance

TABLE 2 | Methods and timescales for the osteopathic treatment and tonometry testing.

- Complete medical history of the patient
- Treatment to increase neurolymphatic drainage and reduce neurotoxicity: favoring the drainage of the cervical lymphatic ducts by means of manipulation of the cranial and periorbital sutures, and visceral manipulation. Average duration is 50 min.
- Timescale for the osteopathic treatments:
First treatment TIME ZERO,
Second treatment after a week,
Third treatment after a month,
Fourth and last treatment after 5 months from the first
- Tonometry before and after every osteopathic treatment and at 10 and 13 months from the beginning of the study
- Survey of the appreciation of the treatment

TABLE 3 | Questionnaire on the appreciation of the treatment.

Have you noticed any changes in the pain or discomfort you were experiencing?
Have you noticed any new pains emerge since your last treatment?
Have you noticed any changes in your daily/work activities?
Have you noticed any changes in your sporting activity?
Have you noticed any changes in your digestion?
Have you noticed any changes in your bowel movements?
Have you noticed any changes in your sleep patterns?
Drowsiness? Waking up during the night? Hours of sleep? Morning waking times?
Have you experienced episodes of emotional stress, anxiety, or feeling unwell lately?
Have you injured yourself recently?

altered function of somatic structures – skeletal, arthrodial, and myofascial structures – and their related vascular, lymphatic, and neural components. It is considered a principal reversible and functional factor that influences homeostasis and can be the cause of many pathologies even in areas well away from where the dysfunction is located and whose normalization is considered essential to restore normal mobility and functioning of the entire somatic system (body).

Somatic dysfunction is identified through palpation of various structures where the compromised functionality of the tissues has its origin. Connective tissue alterations bring about an individual reaction manifested in changes in T.A.R.T. – the texture of the tissue (T), structural asymmetry (A), restricted motion (R), and tenderness (T) (37).

The OMT process is divided into four phases:

- Recording of patients' medical history
- Osteopathic examination
- OMT
- Exit test

Patients' medical history

Patients are invited to sit at a desk where their personal medical history is recorded. Furthermore, a carefully cataloged record is made of any eventual pain, previous health conditions, previous operations, any serious accidents, current medication regimes, and any irregular bodily function parameters regarding fatigue, sleep patterns, digestion, bowel movement, and urination (38).

Osteopathic examination

The patients in the TG underwent the following osteopathic examinations for the initial evaluation (entry test) and after the OMT (exit test).

Diaphragm mobility test

All the patients underwent the diaphragm breathing test to determine any breathing imbalances in the diaphragm movement or any mechanical restrictions which could influence the correct exchange of the fluids (blood and lymph) (39, 40) or the circulation of the CSF (41). The tests were carried out by palpation and manual examination of the following musculoskeletal structures: rib margins, costoxifoid angle, sternum, ribs, and clavicles. A manual examination of thoracic expansion was also performed to determine the range of diaphragm breathing movement (38).

Spinal column and ribcage mobility test

These tests are carried out with the patient seated and prone and are used to identify the SD. Any eventual SD detected in the spinal column or the ribcage was identified by mobilization and palpation of the vertebral segments (38).

Abdominal palpation

Abdominal palpation is carried out to identify any eventual correspondence between the autonomous nervous system, a dysfunctional vertebral tract known as a "facilitated segment" and the viscera that are innervated from this region. A facilitated segment is diagnosed using the T.A.R.T. model and by vertebral mobilization, which shows a regular and rhythmic lateral inclination of the vertebral transverse processes, on the side of the body where the organ is located (38, 42, 43).

Craniosacral test

These tests are carried out with the patient in the supine position and are aimed at evaluating the craniosacral system and any eventual alterations in the expression of this movement. The approach to the cranium was analogous to the evaluation of the other areas of the body, such as testing the mobility and asymmetry of the cranial and sacral bones.

By means of palpation, the tension vector is identified and traced to its origin, distinguishing the skin, fascial tissue, bone, and pachymeninges (dura mater) layers.

In one of these layers, the origin of the tension vector can be found, at which point the evaluation of the SD through the mobility of the revealed structure can proceed (38, 42).

Once the SD is identified on the three levels – musculoskeletal, visceral, or craniosacral – the OMT begins. The treatment carried out at every session did not represent a series of previously chosen techniques, but it was based exclusively on the clinical evidence gathered in the initial tests by means of osteopathic palpation. This practice is known as "blackbox" (44). At the end of the treatment, exit tests were performed (equivalent to the entry tests), particularly in the

area where any eventual SD was discovered. On average, the osteopathic manipulation lasted for 50 min and was carried out with patients either lying on their sides or in supine or prone positions.

Results and statistical analysis

The appreciation survey questionnaire and the recording of the side effects, particularly in relation to the OMT, revealed a unanimously favorable reaction in the treated patients. None of the patients reported side effects or negative reactions from the manipulation throughout the entire period of the treatment, nor for months afterward.

The statistical tests performed include the following:

The Mann-Whitney test, which involves a comparison between treated and non-treated patients.

The Wilcoxon test, which involves the comparison in a longitudinal sense.

A comparison test from a group of three individual treatments, before the osteopathic manipulation, at a distance of 5 months and a distance of 13 months.

The mean ocular pressure in both TG and CG was compared throughout the treatment cycle and showed a statistically significant lowering in both eyes (RE: $p < 0.0561$, LE: $p < 0.0073$). The reduction of the ocular pressure in the TG compared to the CG was maintained at 10 months from the first treatment or rather after 5 months from the last, and was shown to be present even at checkup after 13 months from the beginning of the study or rather 8 months in the absence of treatment ($p < 0.000434$).

	TIME 0		TIME 1 WEEK		TIME 1 MONTH		TIME 5 MONTHS		10 MONTHS	13 MONTHS
RE	1_0	1_1	2_0	2_1	3_0	3_1	4_0	4_1	5_0	6_0
tratt1	17	15	18	17	18	16	17	16	16	18
tratt2	13	10	12	10	15	13	13	10	12	10
tratt3	19	16	13	13	16	15	15	14	12	10
tratt4	17	16	16	14	15	13	17	16	16	16
tratt5	16	15	15	14	15	14	12	12	12	11
tratt6	20	18	18	18	15	15	15	10	17	15
tratt7	21	15	21	16	16	16	24	23	21	19
tratt8	18	16	17	16	18	17	16	16	16	17
tratt9	15	12	16	13	14	10	16	11	12	14
tratt10	15	10	17	15	20	16	16	15	16	16
tratt11	16	12	15	12	14	12	12	12	14	14
tratt12	18	17	17	16	14	13	18	14	15	14
tratt13	17	15	15	15	15	13	13	14	16	14
tratt14	16	14	19	17	18	17	17	15	15	15
tratt15	20	18	19	17	18	18	19	18	19	16
tratt16	12	12	14	12	15	13	14	10	12	11
tratt17	15	13	15	14	15	15	15	14	16	17
tratt18	15	13	16	14	16	18	14	13	13	27
tratt19	18	12	17	12	19	18	20	15	18	14
tratt20	17	14	16	14	16	15	16	14	15	17

TZERO IOP BEFORE OMT	1_O	TZERO IOP AFTER OMT	1_1
T7 DAYS IOP BEFORE OMT	2_O	T7 DAYS IOP AFTER OMT	2_1
T1 MONTH IOP BEFORE OMT	3_O	T1 MONTH IOP AFTER OMT	3_1
T5 MONTHS IOP BEFORE OMT	4_O	T5 MONTHS IOP AFTER OMT	4_1
T10 MONTHS IOP BEFORE OMT	5_O	T13 MONTHS IOP BEFORE OMT	6_O

	TIME 0		TIME 1 WEEK		TIME 1 MONTH		TIME 5 MONTHS		10 MONTHS	13 MONTHS
LE	1_0	1_1	2_0	2_1	3_0	3_1	4_0	4_1	5_0	6_0
tratt1	17	15	16	16	16	15	16	16	15	16
tratt2	18	17	18	17	16	16	15	14	16	13
tratt3	18	17	13	12	17	15	15	12	12	10
tratt4	17	16	16	14	15	13	15	14	15	14
tratt5	17	15	14	13	15	13	14	14	12	12
tratt6	20	18	20	20	15	15	15	11	17	16
tratt7	17	15	18	18	16	16	22	20	21	20
tratt8	18	17	17	17	18	16	16	16	17	18
tratt9	16	10	15	13	13	11	15	10	12	13
tratt10	17	15	16	15	20	18	16	15	15	16
tratt11	15	15	16	12	15	12	12	13	13	18
tratt12	19	16	17	16	17	16	17	13	15	14
tratt13	18	16	16	14	15	13	13	14	15	13
tratt14	20	15	18	17	20	18	18	16	16	16
tratt15	19	18	18	17	20	18	18	17	19	19
tratt16	12	12	15	12	14	11	15	12	12	12
tratt17	15	12	16	14	15	14	16	14	17	17
tratt18	16	13	16	13	18	15	14	10	13	20
tratt19	16	12	18	13	18	18	19	16	18	14
tratt20	17	15	16	15	19	18	16	15	17	18

TZERO IOP BEFORE OMT	1_O	TZERO IOP AFTER OMT	1_1
T7 DAYS IOP BEFORE OMT	2_O	T7 DAYS IOP AFTER OMT	2_1
T1 MONTH IOP BEFORE OMT	3_O	T1 MONTH IOP AFTER OMT	3_1
T5 MONTHS IOP BEFORE OMT	4_O	T5 MONTHS IOP AFTER OMT	4_1
T10 MONTHS IOP BEFORE OMT	5_O	T13 MONTHS IOP BEFORE OMT	6_O

RE	1_0	2_0	3_0	4_0	5_0	6_0
ctrl1	18	18	17	20	18	21
ctrl2	17	17	17	19	18	18
ctrl3	16	18	18	18	19	20
ctrl4	21	21	22	19	21	17
ctrl5	16	17	15	18	18	16
ctrl6	21	20	17	19	18	19
ctrl7	17	19	18	18	19	18
ctrl8	16	18	17	18	16	14
ctrl9	17	18	17	16	16	20
ctrl10	19	17	19	18	17	15
ctrl11	19	18	20	17	18	20
ctrl12	18	19	20	19	21	19
ctrl13	18	19	20	18	20	20
ctrl14	19	18	19	18	18	17
ctrl15	19	18	19	18	19	18
ctrl16	20	19	18	18	21	21
ctrl17	20	21	22	22	18	17
ctrl18	19	19	18	18	17	17
ctrl19	20	17	18	19	18	20
ctrl20	18	17	18	16	14	19

TZERO IOP	1_O	T5 MONTHS IOP	4_O
T7 DAYS IOP	2_O	T10 MONTHS IOP	5_O
T1 MONTH IOP	3_O	T13 MONTHS IOP	6_O

LE	1_0	2_0	3_0	4_0	5_0	6_0
ctrl1	18	18	19	19	19	20
ctrl2	18	18	18	19	18	19
ctrl3	18	18	19	17	20	19
ctrl4	20	19	18	19	20	19
ctrl5	17	16	16	18	17	16
ctrl6	18	18	18	20	18	17
ctrl7	18	18	19	19	18	19
ctrl8	17	19	18	17	19	15
ctrl9	19	19	18	17	16	20
ctrl10	17	17	17	18	18	15
ctrl11	18	19	20	19	17	19
ctrl12	18	18	19	18	20	20
ctrl13	18	18	19	18	19	21
ctrl14	18	20	17	20	17	18
ctrl15	20	18	19	18	19	19
ctrl16	19	18	19	19	19	19
ctrl17	21	20	18	19	18	18
ctrl18	18	19	19	17	18	16
ctrl19	19	19	18	19	19	20
ctrl20	19	18	20	18	20	19

TZERO IOP BEFORE OMT	1_O	T5 MONTHS IOP BEFORE OMT	4_O
T7 DAYS IOP BEFORE OMT	2_O	T10 MONTHS IOP BEFORE OMT	5_O
T1 MONTH IOP BEFORE OMT	3_O	T13 MONTHS IOP BEFORE OMT	6_O

The fluctuations in pressure in CG maintained a random evolution while still remaining within the normal range.

The visual field tests, carried out over 14 months, showed up neither significant differences between the two groups nor significant differences in the AD.

Discussion

The inspiration for this pilot study of integrated medicine arose from the growing interest primarily on the part of neurobiologists and secondarily ophthalmologists, in the circulation of the CSF in connection with eye fluids. Recent studies have shown how the CSF enters the optic nerve of rodents by way of the glymphatic system and suggested that further research on humans could take us to the same conclusion (4, 5).

Wostyn et al. explained how the stasis of the glymphatic system, in the region of the lamina cribrosa of the optic nerve, could influence the structure of the axons of the ganglion cells developing glaucoma (45).

According to these new models, the CSF is secreted not only from the choroid plexus located inside the cerebral ventricles but also inside the arterial paravascular spaces that are made up of glia cells, called Virchow and Robin Spaces (VRS) (46). VRS are made up of astrocyte pedicels that are wrapped around the capillaries of the cerebral parenchyma, and these astrocyte sheaths present numerous acquaporine-4 canals, thus facilitating the passage of the CSF into the interstices and its intermixing with the interstitial fluid (IF) (46–48).

Cerebrospinal fluid and interstitial fluid seem to mingle in the interstitial spaces, which then drain off interstitial solutes and catabolytes through lymphatic ducts present in the dura mater located in correspondence with cranial sinuses. This system of ducts flows together in the cervical lymph ducts and exits through the right lymph duct and the thoracic duct into the subclavian veins (1–3, 47, 48). The fluids inside this system of perivascular canals are driven by arterial pulsation and diaphragmatic breathing (39–41, 48, 49).

Various studies carried out *in vivo* on rodents and recently on humans show that the function of catabolyte clearance attributed to the glymphatic system takes place mainly during deep sleep and is almost absent during waking hours (2, 50–53). This function takes place via an expansion of the interstitial space, facilitating the ingress of CSF to the brain and its interchange with the IF. An eventual malfunction of the glymphatic system and the consequent deficit in the elimination of catabolytes can influence the homeostasis of the CNS, favoring the development of central neurodegenerative disease due to a buildup of neurotoxins (54–58).

In 2006, Flammer and Pache attempted to bring glaucoma into a wider medical discussion and debated the systemic peculiarities revealed in POAG. These systemic alterations include cardiovascular system, autonomous nervous system, and immune system, as well as endocrinological, psychological, and sleep disturbances (59).

Every patient at every osteopathy session was treated according to whatever clinical evidence was discovered during the osteopathic evaluation test and ascertained from their relative medical histories.

This "blackbox" practice can be described as an individually tailored treatment for every patient and is performed based on the problems and medical conditions of each individual (37, 60).

The results are obtained from the analysis of the initial tests and a comparison with the final tests.

In the TG, the osteopathic tests showed frequent SD of the diaphragm and those organs below the diaphragm, the ribcage, the occipital area, and the cervical tract C1–C2. At the end of the session, exercises were recommended (60) to encourage correct diaphragmatic breathing, especially in sedentary patients who had restricted ribcage mobility, often associated with shallow and irregular breathing.

Current knowledge in the medical field identifies the thoracic diaphragm as a principal factor for lymphatic and CSF circulation (39–41). A diaphragm is a transverse membrane which creates two distinct zones, whose correct functioning depends on the maintenance and balancing of the pressures within the two zones it divides.

Osteopathy recognizes other structures as diaphragm, including the pelvic floor, the thoracic outlet, the buccal floor, the diaphragm of the hypophysis, and the tentorium cerebelli. These structures are considered to be general-purpose pumps which permit the expansion, distribution, transmission, and regulation of the fluids (blood, CSF, and lymph) to the peripheries (37, 60).

Recently, osteopathic medicine has proposed manipulations which seem to influence the flow of CSF for conditions such as chronic fatigue syndrome (61), where this flow appears to be reduced. OMT also appears to induce changes during sleep in healthy patients (62). With these considerations, the improvement or resolution of visceral or structural problems could be extended throughout the body in addition to the cranial and cervical regions to include functional areas of the lymphatic system (37, 60).

Support for the efficiency of osteopathic treatments in neurodegenerative pathologies, such as glaucoma, could be attributed to the positive influence that these treatments would have not only on the IOP but also on the cerebral vascular perfusion, also enabling to influencing venous, lymphatic, and CSF circulation by facilitating its drainage (20–35).

With regard to future projects, we have planned a follow-up of at least 3 years and the use of Angio-OCT for measuring eventual variations in the vascularization of the retina and the optic nerve subsequent to OMT.

Conclusion

A reduction in pressure after the first treatment, which was persistent and statistically significant at every manipulation session carried out, shows that in some selected cases, it is possible to influence ocular pressure by means of osteopathic treatments without interfering with in-place pharmacological regimes.

Therefore, this places before the panorama of scientific research a possible starting point for future research and investigation into POAG in both the ophthalmological and osteopathic spheres. However, the small sample and an insufficiently long follow-up do not permit us to evaluate the eventual progression and the stabilization of the disease (visual field) and cannot provide conclusive data on the duration of the pressure-reducing effects of OMT.

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