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Correlation between tear osmolarity and tear meniscus

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Abstract:	Purpose. To examine the relationship between tear meniscus height (TMH) and subjective meniscus grading (subjective TM) with tear osmolarity. Methods. Tear osmolarity measurements (using TearLab) and digital images of the tear meniscus were obtained in 177 consecutive patients undergoing an eye examination at our optometry clinic (Universidad de Santiago de Compostela, Spain) who fulfilled the study's inclusion criteria. Participants were also administered the McMonnies and Ocular Surface Disease Index (OSDI) questionnaires for the detection of dry eye disease. The lower tear meniscus was videotaped by a digital camera attached to a slit lamp in its central portion without fluorescein instillation. After the study, a masked observer extracted an image from each video, and measured the TMH using open source software (NIH ImageJ). Subsequently, the masked observer subjectively graded the appearance of each meniscus. For statistical analysis, subjects were stratified by age and by dry eye symptoms as indicated by their scores in the two questionnaires. Results. In the whole study population, a significant relationship was observed between osmolarity and TMH (-0.41, p<0.001) and osmolarity and subjective TM (r = 0.35, p<0.001). A cluster analysis revealed similar correlations when subjects were stratified by age or dry eye symptoms, these correlations being more pronounced in older and more symptomatic individuals. Objective TMH measurements and subjective meniscus quality were also correlated (r=-0.75, p<0.001). Conclusions. Osmolarity and both objective TMH measurements and subjective interpretation of the meniscus showed high correlation, especially in older symptomatic subjects.

SYNOPSIS

The aim of this study is to analyze the relationship between the tear meniscus height (TMH) (videotaped by a digital camera), symptoms analysis by OSDI and McMonnies questionnaires and tear film osmolarity, by using TearLab electric impedance osmometer (TearLab) on 177 subjects (from 18 to 65 years). Osmolarity and TMH showed high correlation between them, and this relationship was better in older symptomatic patients.

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ABSTRACT

Purpose. To examine the relationship between tear meniscus height (TMH) and subjective meniscus grading (subjective TM) with tear osmolarity.

Methods. Tear osmolarity measurements (using TearLab) and digital images of the tear meniscus were obtained in 177 consecutive patients undergoing an eye examination at our optometry clinic (Universidad de Santiago de Compostela, Spain) who fulfilled the study's inclusion criteria. Participants were also administered the McMonnies and Ocular Surface Disease Index (OSDI) questionnaires for the detection of dry eye disease.

The lower tear meniscus was videotaped by a digital camera attached to a slit lamp in its central portion without fluorescein instillation. After the study, a masked observer extracted an image from each video, and measured the TMH using open source software (NIH ImageJ). Subsequently, the masked observer subjectively graded the appearance of each meniscus. For statistical analysis, subjects were stratified by age and by dry eye symptoms as indicated by their scores in the two questionnaires.

Results. In the whole study population, a significant relationship was observed between osmolarity and TMH (-0.41, $p<0.001$) and osmolarity and subjective TM ($r = 0.35$, $p<0.001$). A cluster analysis revealed similar correlations when subjects were stratified by age or dry eye symptoms, these correlations being more pronounced in older and more symptomatic individuals. Objective TMH measurements and subjective meniscus quality were also correlated ($r=-0.75$, $p<0.001$).

Conclusions. Osmolarity and both objective TMH measurements and subjective interpretation of the meniscus showed high correlation, especially in older symptomatic subjects.

Keywords: Osmolarity; tear meniscus; TearLab Osmometer; ImageJ Software.

INTRODUCTION

Dry eye disease (DED) is a common condition that causes discomfort and visual distortions. The characteristic features of DED are changes in tear film composition and inflammation of the ocular surface.[1-4] An abnormally high tear film osmolarity induces inflammatory events that negatively affect ocular surface cells,[1-5] leading to symptoms such as eye irritation and blurred vision with impacts on quality of life.[4, 6-8] DED is also the main reason why individuals stop wearing contact lenses.[6]

There are a variety of diagnostic tools available for DED. Most methods are based on assessing symptoms and include questionnaires like the McMonnies or Ocular Surface Disease Index (OSDI), which have been adapted for the detection of DED.[9,10] Other tests commonly used are the Schirmer test, tear film break-up time (BUT), meibomian secretion scoring and sodium fluorescein and lissamine green staining of the cornea and conjunctiva.[11-13] However, many of these test are invasive or require eye manipulation which could destabilize the tear film.[14] The method proposed here, measuring tear meniscus height, provides a non-invasive indication of the total volume of the tear film.[15]

Tear meniscus height (TMH) is routinely measured by eye care practitioners through slit-lamp biomicroscopy.[11, 16] Reported mean TMH values for healthy eyes range from 0.14 to 0.46 mm,[17-30] depending on the technique employed[17, 21-25] and the different end points used.[19-22, 26-30] The most widely used method involves image capture of the meniscus using a camera attached to a slit-lamp and TMH measurements may be quantified by software assistance.[31, 32] ImageJ[33] is a free domain image analysis tool that quantifies features viewed in a picture.[34] This software helps interpret TMH data by enhancing resolution and hence the repeatability of measurements.[34]

In routine clinical practice, TMH is often measured using a graticule eyepiece attached to a biomicroscope.[19, 21, 24, 25, 27] However, using this method it is difficult to distinguish the upper limit of the tear meniscus during eye movement.[29] This has determined that many clinicians prefer subjective assessment of the tear meniscus (TM) appearance, or TM quality, over TMH measurements.[35] The main factors determined during TM assessment are the presence/absence of debris and/or foaming; its regularity (whether the top forms a regular or irregular line) and an estimate of height[36, 37] (Table 1).

Tear fluid hyperosmolarity is a common feature to all types of DED. Normal tear film osmolarity is 302 ± 6.3 mOsm/l,[38-40] while a mean of 321 mOsm/l is reported for DED.[39] The most commonly used cut-off for a diagnosis of DED is 316 mOsm/l,[39, 41] which provides a sensitivity of 73%, and specificity of 90%.[41]

Hyperosmolarity has been described by the Tear Film and Ocular Surface Society Dry Eye Workshop as one of the main mechanisms of DED,[4] being the main cause of discomfort, ocular surface damage and inflammation.[1, 3, 38, 42] This has meant that tear film osmolarity measurement is considered the gold standard for DED diagnosis [2, 4-6, 40, 43, 44]

One of main dilemmas when trying to make a diagnosis DED is the poor correlation between dry eye tests,[23, 45] along with a lack of correlation between symptoms and objective signs.[46]

Osmolarity is defined as the number of dissolved solute particles in one kilogram of solution, regardless of particle shape, size, density, configuration or charge.[5] Hence a higher osmolarity will induce a greater amount of evaporation from the eye surface and thus reduce tear film volume. Given the tear meniscus holds some 75–90% of the total tear film volume[15] any reduction in TMH should indicate an increase in tear film osmolarity.

This study was designed to examine the relationship between TMH and osmolarity using the TearLab electric impedance osmometer to determine osmolarity and a new software tool to measure TMH. Subjective interpretation of TM appearance was also compared with objective TMH and osmolarity determinations. In addition, a cluster analysis was performed to examine the effects on the correlations observed of age, and of symptoms expressed as the scores obtained in two dry eye grading questionnaires (OSDI and McMonnies).

METHODS

Subjects and procedure

One hundred and seventy-seven (177) consecutive patients (78 male, 99 female) of mean age 25.92 ± 12.03 years (18 to 65 years) were recruited among subjects visiting the Optometry Clinic of the Optometry Faculty (Universidad de Santiago de Compostela, Spain) for an eye examination. Subjects were excluded if they had a history of conjunctival, scleral, or corneal disease, prior eye surgery, glaucoma, diabetes mellitus, a thyroid disorder or wore contact lenses. Qualifying subjects were administered two DED questionnaires (OSDI and McMonnies) and scheduled for another visit for tear osmolarity and meniscus height measurements. No participant was under any type of medication or used artificial tears at the time of the testing session. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Santiago de Compostela (Spain).

Only the right eye was examined because of induced excess tearing in the second eye and to avoid overstating the precision of statistical estimates.[47] All measurements were performed between 5 pm and 6 pm. Throughout the study, laboratory conditions of temperature, light and humidity were kept constant (temperature 20-23°C, relative humidity 50-60%).

DED questionnaires

The questionnaires at the screening visit were OSDI[6, 10, 48] and McMonnies grading DED questionnaires,[6, 9, 49, 50] which established the symptoms statement of the patient.

Ocular Surface Disease Index (OSDI)

The OSDI (Allergan Inc., Irvine, California)[51] is a 12-item self-administered questionnaire designed for rapid assessment of ocular surface symptoms related to chronic DED, severity and their effects on the patient. Total OSDI scores were calculated according to published guidelines[10, 51] and a score of 13 or more established as the cut-off for DED.[6, 10, 48]

$$\text{OSDI} = \frac{\text{sum of scores X 25}}{\text{number of questions answered}}$$

McMonnies

Subjects completed a modified McMonnies dry eye questionnaire to assess dryness symptom number, type and frequency.[52] This 12-item questionnaire elicits information about recent medical and medication history that could affect tear production. Symptoms included in the questionnaire are eye soreness, scratchiness, dryness, and grittiness (from the original McMonnies questionnaire) along with burning, stinging, foreign body sensation, and itchiness. As in prior studies, a cut-off of 14.5[49, 50] was used to differentiate between normality and mild to severe DED.

Tear meniscus height

Video capture and image selection

Meniscus videos were recorded by a Topcon DV-3 digital camera attached to the slit lamp and stored using Topcon IMAGEnet i-base at a spatial resolution of 1024x768 pixels in the RGB colour space. Subjects seated at the slit-lamp were instructed to look at a target positioned to induce primary eye gaze and a natural blink. The tear meniscus was acquired centrally (at the 6 o'clock position) (Figure 1) with magnification set at x500 (objective x40, ocular x12.5; without fluorescein). To avoid reflex tearing, a short moderate-illumination light beam (3 mm wide, 5 mm height) was used to prevent the light shining directly into the pupil during measurements.[19]

In total, 177 tear meniscus images extracted from recorded videos were examined. Images were selected from the recordings at the point when a fully-expanded stable meniscus was observed after the blink. A blinded observer then measured tear meniscus height by computer-assisted image analysis and outgrowth size was quantified using ImageJ software v1.47i (National Institutes of Health, Bethesda, MD; http://imagej.nih.gov/ij/).[33] Subsequent to TMH measurement, the observer assigned a subjective classification category to each central meniscus.

Objective measurement of tear meniscus height

The tear meniscus was measured according to defined criteria[19, 34] in its central portion (at 6 o’clock) as the distance between the darker edge of the lower eyelid and the upper limit of the tear meniscus using ImageJ. The TMH was marked using the *straight* tool, which allows the user to draw a line (Figure 1) in the middle of the illuminated area perpendicular to the eyelid margin, from the lowest meniscus limit to the highest one. Next, the height of the tear meniscus was calculated according to the software pixels, which were transformed into millimetres as 300 pixels = 1 millimetre. This conversion factor was previously calculated for our camera.

Subjective meniscus grading

The appearance of the meniscus was also graded according to the criteria described in Table 1, whereby grades 1 and 2 indicate a healthy or control meniscus and grades 3 and 4 represent an abnormal meniscus. The grading scale was based on that published by Khurana et al[36] and later modified by Garcia-Resua et al.[19] This evaluation was performed on the image obtained for the central tear meniscus using the slit lamp (Figure 1).

Osmolarity: TearLab™ osmometer

The TearLab™ Osmometer (TearLabTM Corp., San Diego, CA, USA) was used to measure the osmolarity of the tear film.[5, 53] The subject was seated with the chin tilted upward and eyes directed toward the ceiling. The instrument probe (housing the disposable microchip) is then placed on the lower tear meniscus until a beep is emitted indicating the tear sample has been collected. Only a 0.05 µl tear sample is needed and measurements are directly made on the tear meniscus using the probe, which takes up the sample through capillary action.[2, 12, 13] The TearLab quickly (in less than 10 seconds) converts the electrical impedance of the sample into osmolarity in mOsm/l, which is displayed on the device screen. Its measurement range is 275 to 400 mOsm/L.

Statistical analysis

After confirming their normal distribution using the Kolmogorov-Smirnov test, numerical data (osmolarity and TMH) were compared using parametric tests. Non-parametric tests were used for McMonnies scores, OSDI scores and subjective TM grades. Numerical variables are provided as means and their standard deviation (SD) whereas non-parametric variables are expressed as medians and interquartile ranges (IQR).

To examine the effects of age and DED symptoms on correlations between variables, a cluster analysis was also performed on three data sets:

- *Whole study population:* 177 subjects (n = 177).
- *Subjects stratified by age:* 25 years or younger (n = 88); 25 to 45 years (n = 67); and 45 years or older (n = 22).
- *Subjects stratified by symptoms:* a score indicating DED in both questionnaires (n = 104); a score indicating DED in one questionnaire (n = 40); and a score indicating the absence of DED in both questionnaires (n = 33).

Relationships were assessed through Pearson's correlation for parametric data and through Spearman ρ for nonparametric data. Correlations between variables was described as weak (0.2 – 0.4), moderate (0.4 – 0.6), fairly good (0.61 – 0.8), or strong (0.8 – 1.0).[50, 54]

All statistical tests were performed using the software package SPSS v. 19.0 for Windows (SPSS Inc., Chicago, IL). Significance was set at $p \leq 0.05$.

RESULTS

Whole study population

Descriptive statistics for the whole study population (n = 177) are provided in Table 2.

In our subjective TM evaluation, most subjects showed an intact (n=109) or slightly diminished (n= 45) meniscus. An abnormal TM was detected in 29 subjects, in whom the meniscus was described as markedly diminished or discontinuous, and in 2 subjects lacking a meniscus.

Figure 2 shows the correlation between osmolarity and both the objective and subjective TM data recorded for the study group. Osmolarity was moderately negatively correlated with TMH ($r = -0.41$, $p < 0.001$) and weakly positively correlated with subjective TM quality ($r = 0.35$, $p < 0.001$). As expected, strong negative correlation was observed between TMH and tear meniscus quality ($r = -0.75$, $p < 0.001$) indicating that the worse the subjective meniscus quality, the smaller the objective TMH measurement.

Stratification by age

Descriptive statistics for the subjects stratified into three age groups are provided in Table 3. As for the whole study population, osmolarity was correlated with both subjective meniscus quality and objective TMH for each age range. In all age groups, osmolarity was inversely related to TMH, and correlation was greater with age. Thus, correlation was weak for the younger subject group ($r = -0.28$, $p = 0.01$) (Figure 3A) and moderate for the older groups (< 45 years, $r = -0.40$, $p=0.001$, and > 45 years, $r = -0.52$, $p = 0.014$, (Figures 4A and 5A)).

Correlation between osmolarity and TM quality was weak to fairly good depending on age (from r = 0.27 for ≤ 20 years to r = 0.63 for ≥ 45 years, all $p < 0.012$) (Figures 3B, 4B and 5C).

When examining TMH and TM quality, significant correlation was observed ($p < 0.001$ in all cases) ranging from fairly good (r = - 0.69 for subjects ≤ 20 years old to r = - 0.75 for subjects 20 to 45 years of age) (Figures 3C and 4C) to strong (r = - 0.90 for subjects ≥ 45 years) (Figure 5C) such that greater objective and subjective tear meniscus data were better correlated in older subjects.

Stratification by symptoms

Descriptive statistics for the subjects stratified according to their questionnaire scores are provided in Table 4.

Figures 6-8 illustrate correlations among the variables examined detected in each of the three symptom groups. Once again, TMH versus osmolarity and TMH versus TM quality were negatively correlated while positive correlation was detected between osmolarity and TM quality.

For non-symptomatic subjects (scores in both questionnaires indicated no DED), correlation between osmolarity and meniscus variables was very weak for both the objective (TMH) (r = - 0.19, p = 0.049) (Figure 6A) and subjective (TM quality) (r = 0.08, p = 0.4) (Figure 6B) assessments. In subjects with mild symptoms (scores in one questionnaire indicated DED) this relationship was moderate in both comparisons (r = - 0.48, p = 0.002 for osmolarity vs TMH; r = 0.51, $p < 0.001$ for osmolarity vs TM quality) (Figures 7A and 7B). Finally, when only strongly symptomatic subjects were considered (scores in both questionnaires indicated DED), osmolarity correlations with both the objective (r = - 0.52, p = 0.002, for osmolarity vs TMH, Figure 8A) and subjective (r = 0.64, p<0.001 for osmolarity vs TM quality, Figure 8B) meniscus variables were slightly improved over those observed in the subjects with mild DED symptoms. Correlations between objective TMH and subjective TM quality measurements were fairly good in non-symptomatic subjects (r = - 0.60, p < 0.001) (Figure 6C), and strong in those with mild or severe symptoms (r = - 0.81, p < 0.001 or r = - 0.90, p < 0.001 respectively, Figures 7C and 8C). Thus, correlations became stronger with increasing symptoms.

DISCUSSION

In this study we detected an expected statistically significant relationship between osmolarity and TMH (see Figure 2) such that higher osmolarity values (indicating poorer tear film quality) give rise to lower TMH values. This trend was also observed in the cluster analysis (Figures 3 to 8), whereby osmolarity and TMH showed improved correlation when one questionnaire indicated DED and even better correlation when both questionnaires identified dry eye symptoms. This suggests that correlations between these tear film tests are more pronounced in symptomatic subjects. In addition, when the study population was stratified by age, better correlation between the two variables was noted in the older subjects. In agreement with this finding, Glasson et al.[50] detected a lower TMH in symptomatic intolerant contact lens wearers than control subjects. These authors also noted that TMH correlated negatively with osmolarity (Pearson’s correlation for meniscus height [mm] vs osmolarity [mOsm/kg] was r = -0.566; p = 0.014 and r = -0.438; p = 0.047 for two subject groups).[50] On the contrary, Nichols et al.[46] detected no correlation between tear meniscus height and symptoms of dry eye. However, TMH was measured using the variable beam height on the slit-lamp biomicroscope, which is a less precise method and much more influenced by subjective errors and interpretation than the image analysis method used here.

Several authors have related higher osmolarity values to tear film abnormalities, which lead to symptoms and discomfort.[14, 38-40, 55] Moreover, a lower tear meniscus height seems to induce similar symptoms in patients with different forms of DED,[20, 56, 57] thus we would expect higher osmolarity values in individuals with lower meniscus heights.

When the tear meniscus was subjectively graded, significant correlation was also found between a higher osmolarity and worse meniscus quality. This relationship was significant and gained in strength as subject age and dry eye symptoms increased.

Finally, when we compared objective TMH measurements with subjective interpretations of meniscus quality, good to strong correlation was observed between subjective meniscus assessment and TMH (Figures 2 to 8). This determined that for lower TMH values measured using software assistance, an experienced observer graded the tear meniscus as worse, and again stronger correlation was shown in older and more symptomatic subjects. This finding is consistent with the results of a previous study in which we measured TMH using a graticule eyepiece.[19] Accordingly, based on objective TMH measurements, a meniscus picture scale could be designed to help clinicians grade the tear meniscus.

Few studies have addressed the relationship between osmolarity and tear meniscus height,[25] and even fewer have considered the subjective interpretation of the tear meniscus.

Using the TearLab osmometer, tear osmolarity can be easily and quickly measured. However, the method has two important shortcomings; methods based on the electric impedance principle are less repeatable than freezing point-based techniques[41] and each TearLab measurement is quite expensive. The mean osmolarity value obtained here was 305, which is similar to values reported for normal subjects.[39, 41]

To enhance repeatability and improve meniscus measurement and capture, in this study we combined digital video capture with software image analysis assistance, as in an earlier study.[34] Through the use of capture techniques, images can be objectively and accurately interpreted, and detailed measurements can be made later. Here we used the ImageJ (National Institutes of Health, Bethesda, MD)[33] package to measure TMH in its central portion (at 6 o'clock) as the distance between the darker edge of the lower eyelid and the upper limit of the tear meniscus.[19, 21, 22, 27, 28, 34]

According to our findings, we propose the use of TMH photographs to design a grading scale for clinical guidance. In effect, grading scales for anterior eye variables (e.g., bulbar redness) have significantly improved the monitoring of ocular physiology changes, and are proving more stable and sensitive than verbal descriptions.[58, 59]

Our results indicate better correlation between tear film variables in symptomatic patients and older subjects, whose tear film characteristics are worse than in younger individuals.[60] We observed that older persons obtained worse scores in the OSDI and McMonnies questionnaires (Table 3). In addition, older subjects showed worse dry eye symptoms (the mean age of subjects classed as having DED by the two questionnaires was 42.03 years, while those whose scores in neither or one questionnaire indicated DED were 20.81 and 25.93 years old, respectively (Table 4)).

Our study also revealed that TM quality (both objective and subjective measurements) and osmolarity were better correlated in symptomatic subjects, especially those whose scores in both questionnaires indicated they had dry eye disease. Our findings also confirm the assumption made by Tomlinson et al.[23] that the potential for correlation is greater with a wider range of values offered by the inclusion of a dry eye group, and that normal subjects, however, may yield significance if a large enough range of parameter values is included.

Moreover, it seems that the use of inexpensive questionnaires and subjective TM grading (as opposed to expensive osmolarity testing and video recording) may be a valid protocol for dry eye screening purposes, especially for older more symptomatic subjects. To identify the aetiology of the DED, however, several tests are needed including osmolarity measurement, since hyperosmolarity is known to play an important role in the pathogenesis of dry eye.

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FIGURE LEGENDS

Figure 1. Example of how tear meniscus height (TMH) is measured by the ImageJ software.

Figure 2. Correlation between osmolarity and tear meniscus variables in the whole study population. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM **, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Figure 3. Correlation between osmolarity and tear meniscus variables in subjects aged 20 years or younger. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Figure 4. Correlation between osmolarity and tear meniscus variables in subjects aged 20 to 45 years. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Figure 5. Correlation between osmolarity and tear meniscus variables in subjects aged 45 years or older. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM **. **Pearson's test; **Spearman ρ test.*

Figure 6. Correlation between osmolarity and tear meniscus variables in subjects whose scores in two questionnaires indicated the absence of dry eye. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Figure 7. Correlation between osmolarity and tear meniscus variables in subjects whose scores in one questionnaire indicated dry eye. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Figure 8. Correlation between osmolarity and tear meniscus variables in subjects whose scores in two questionnaires indicated dry eye. A) Correlation between osmolarity and TMH*, B) Correlation between osmolarity and subjective TM**, C) Correlation between TMH and subjective TM**. **Pearson's test; **Spearman ρ test.*

Table

<table>
<tr><th>Grade</th><th>Group</th><th colspan="2">Description</th></tr>
<tr><td>1</td><td>Intact</td><td>Meniscus of variable height and regular shape. Absence of debris</td><td rowspan="2">Grades 1 and 2 represent healthy meniscus</td></tr>
<tr><td>2</td><td>Slightly diminished</td><td>Regular, but less visible. Absence of debris</td></tr>
<tr><td>3</td><td>Markedly diminished or discontinuous</td><td>Diminished meniscus of irregular shape. Presence of debris</td><td rowspan="2">Grades 3 and 4 represent abnormal meniscus</td></tr>
<tr><td>4</td><td>Absent</td><td>Invisible meniscus</td></tr>
</table>

Table 1. Subjective classification of the meniscus scheme.

	Mean / Median	SD / IQR	Minimum	Maximum
McMonnies	6.00	3.00 – 13.00	0.00	33.00
OSDI	8.33	4.17 – 17.71	0.00	64.58
Osmolarity	305.68	14.37	275.00	357.00
TMH	0.20	0.06	0.06	0.36
Subjective TM	1.00	1.00 – 2.00	1.00	4.00

Table 2. Descriptive statistics. DED questionaires (McMonnies and OSDI) and subjective TM are dimensionless parameters. Osmolarity is indicated in mOsm/l and TMH is indicated in mm. Mean and SD for parametric variables; median and IQR for non-parametric variables. SD = Standard deviation. IQR = Interquartile range. n = 177

Age range	Lower or equal than 20 years n = 88		Between 20 and 45 years n = 67		Higher or equal than 45 years n = 22	
	Mean / Median	SD / IQR	Mean / Median	SD / IQR	Mean / Median	SD / IQR
Age	18.93	0.85	30.83	6.39	54.18	6.27
McMonnies	4.50	3.00 – 7.00	6.00	4.00 – 12.00	20.00	15.00 – 27.25
OSDI	8.33	4.17 – 12.50	10.42	4.17 – 18.75	31.25	16.66 – 43.23
Osmolarity	303.93	15.76	304.80	12.01	315.41	11.55
TMH	0.21	0.05	0.20	0.051	0.18	0.08
Subjective TM	1.00	1.00 - 2.00	1.00	1.00 – 2.00	2.00	1.00 – 3.00

Table 3. Descriptive statistics for the age range stratified groups. DED questionaires (McMonnies and OSDI) and subjective TM are dimensionless parameters. Age is indicated in years, Osmolarity in mOsm/l and TMH is indicated in mm. Mean and SD for parametric variables; median and IQR for non-parametric variables. SD = Standard deviation. IQR = Interquartile range

	Fail no questionnaire n = 104		Fail one questionnaire n = 40		Fail two questionnaire n = 33	
	Mean / Median	**SD / IQR**	**Mean / Median**	**SD / IQR**	**Mean / Median**	**SD / IQR**
Age	20.81	3.64	25.93	12.60	42.03	14.37
McMonnies	4.00	3.00 – 6.00	10.00	6.00 – 13.00	17.00	15.00 – 25.50
OSDI	6.25	2.08 – 8.33	16.67	14.58 – 22.92	29.17	19.79 – 40.63
Osmolarity	302.26	14.17	308.60	14.75	312.92	11.04
TMH	0.21	0.05	0.20	0.06	0.15	0.07
Subjective TM	1.00	1.00 – 2.00	2.00	1.00 – 2.00	2.00	1.00 – 3.00

Table 4. Descriptive statistics for the symptom assessment stratified groups. DED questionaires (McMonnies and OSDI) and subjective TM are dimensionless parameters. Age is indicated in years, Osmolarity in mOsm/l and TMH is indicated in mm. Mean and SD for parametric variables; median and IQR for non-parametric variables. SD = Standard deviation. IQR = Interquartile range.

Figure

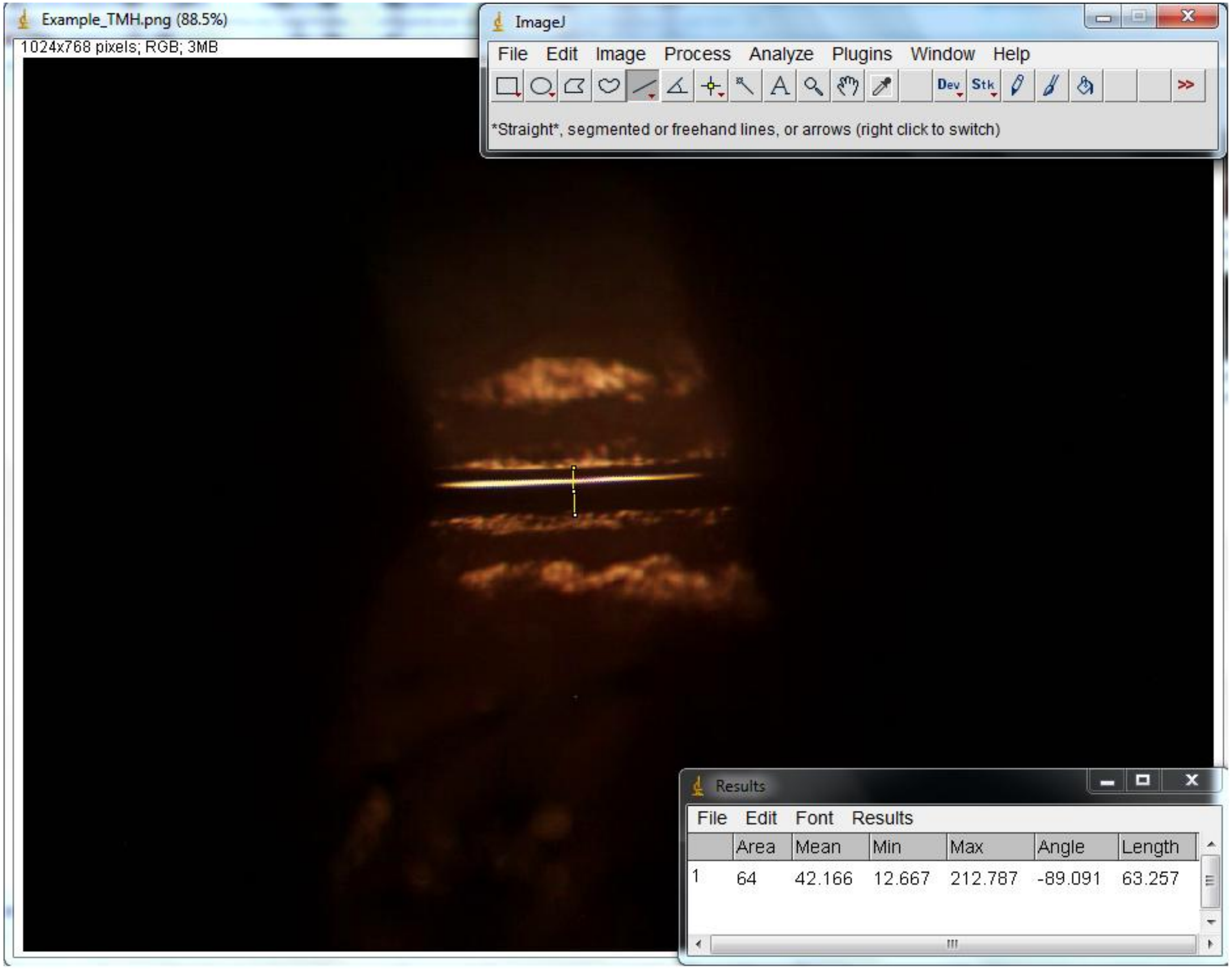
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Figure 2

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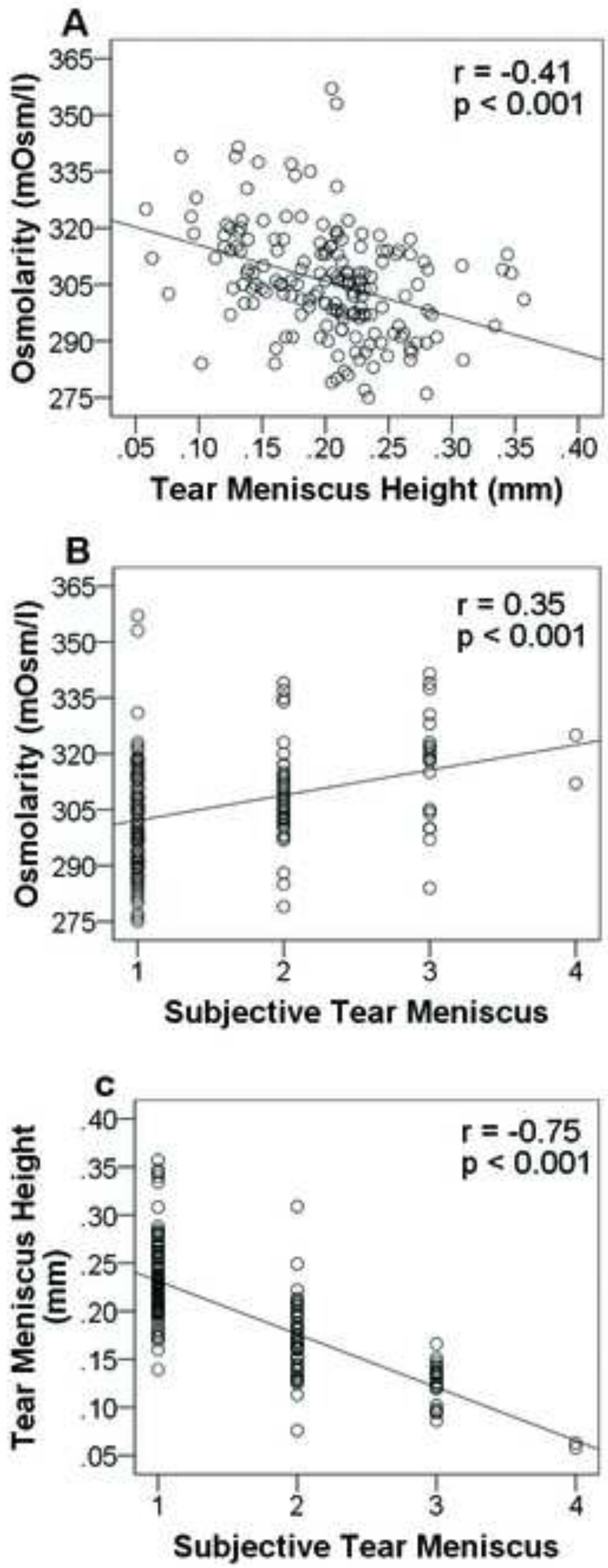

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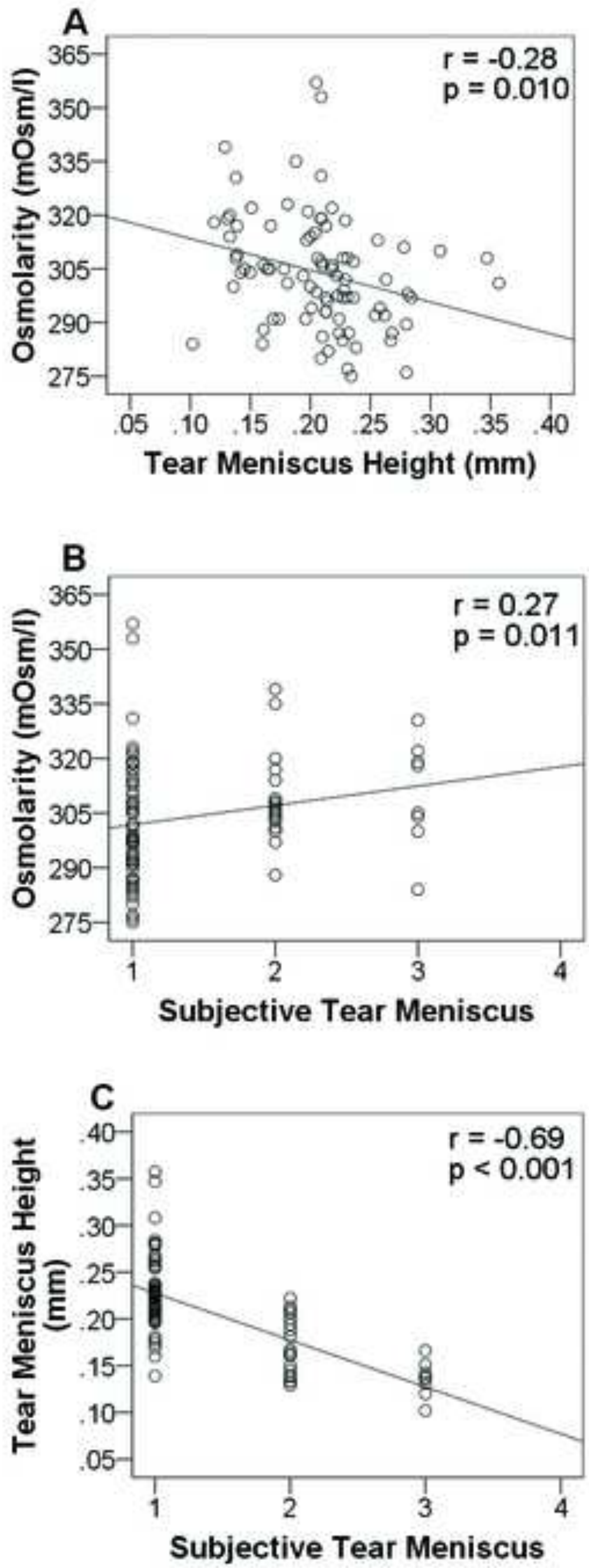

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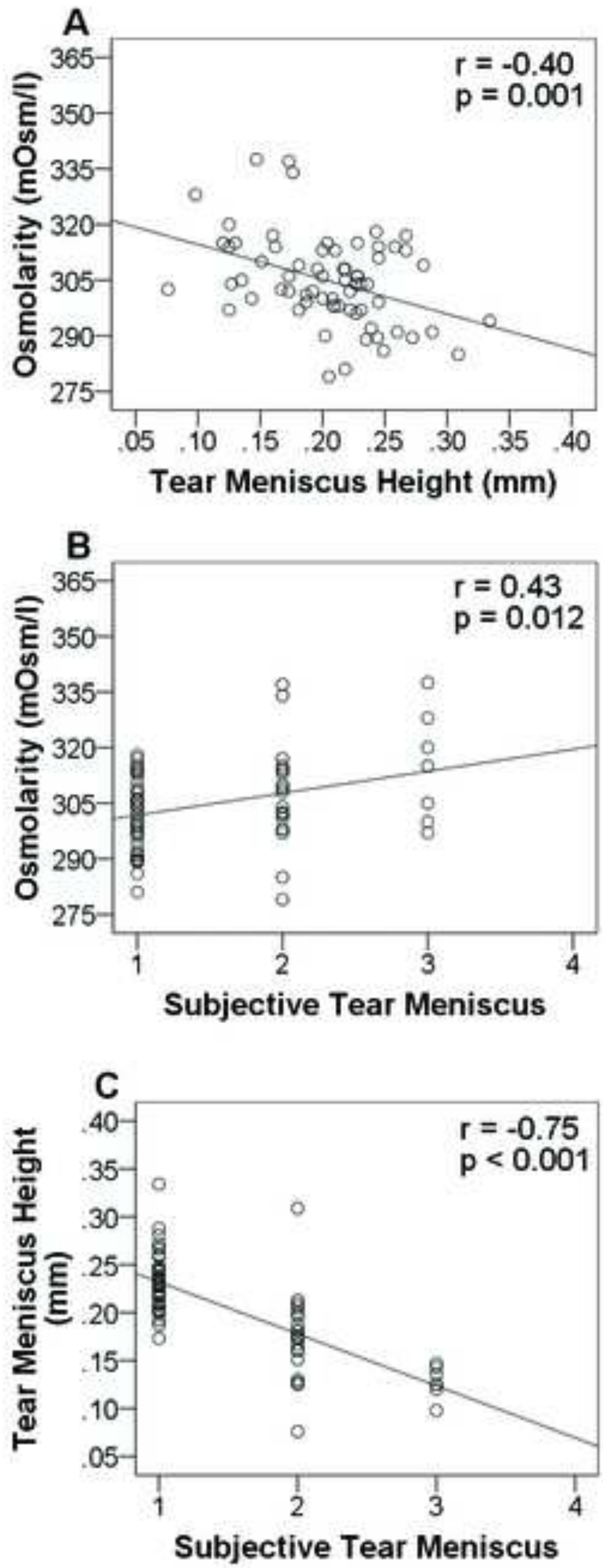

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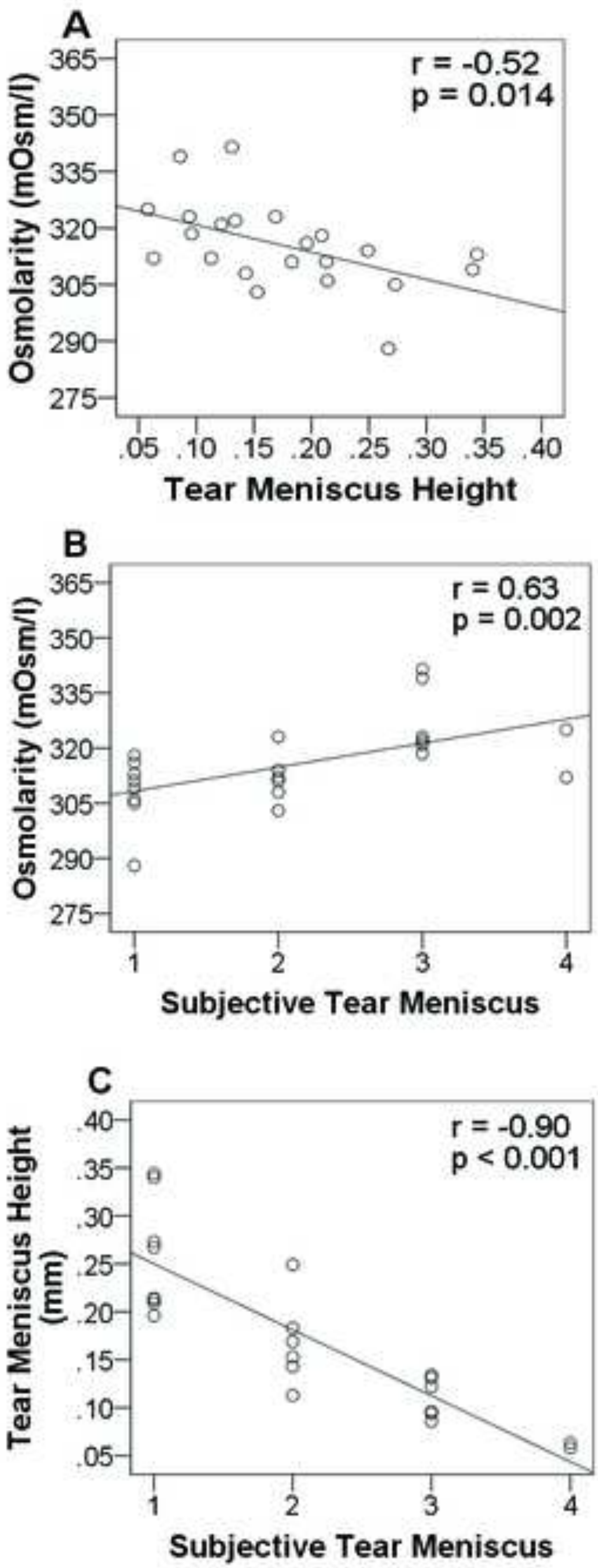

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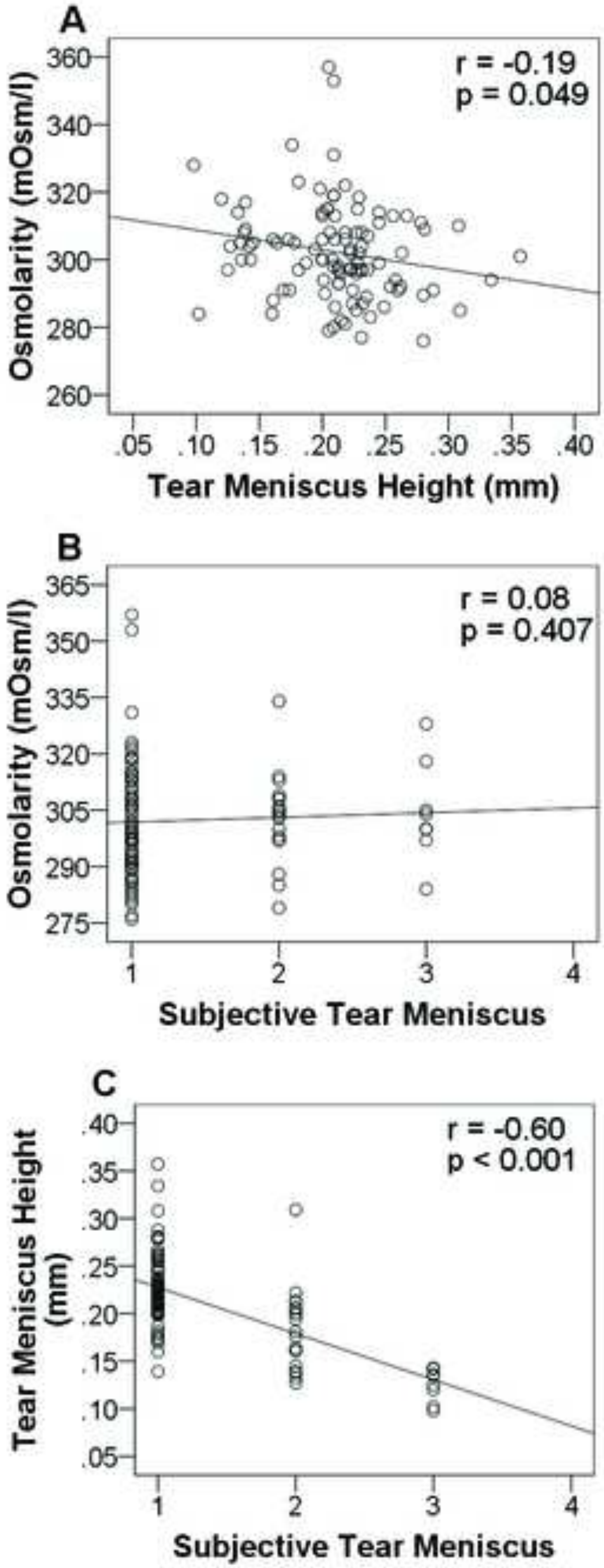

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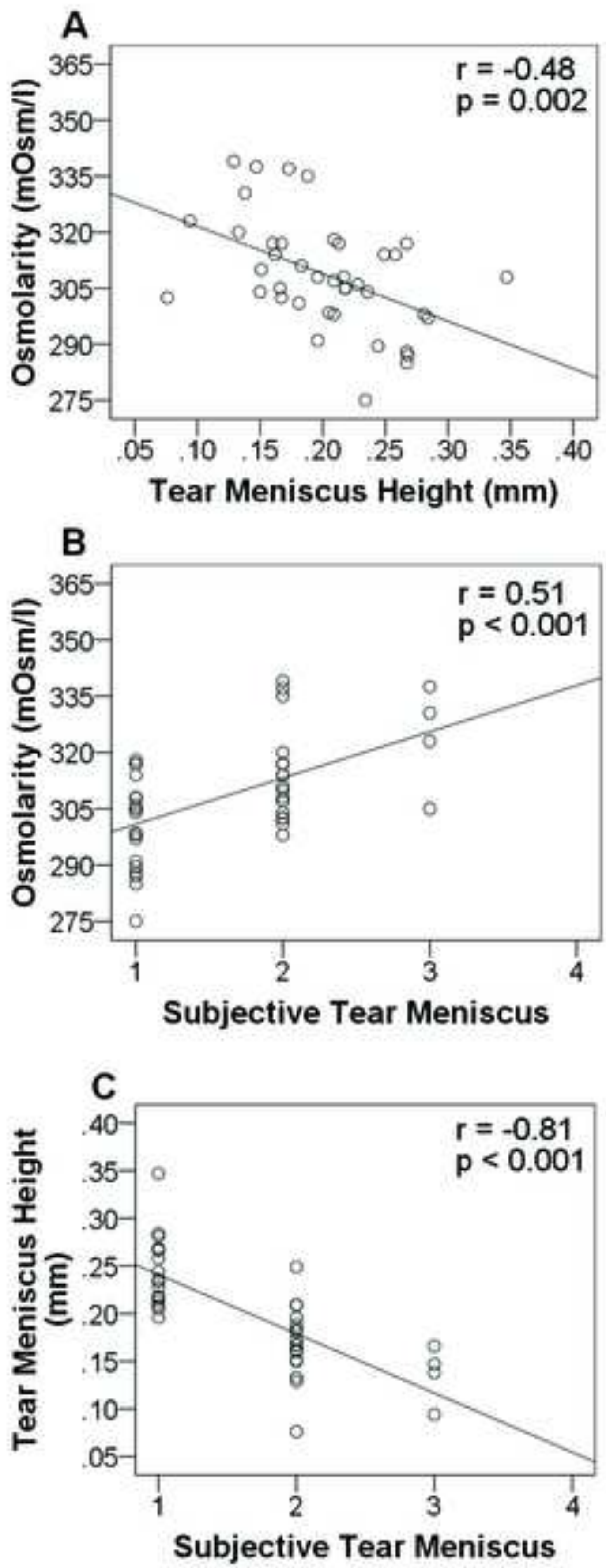

Figure 8

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