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Research Article

Quantification of the Water Content of Human Intervertebral Discs in Various Regions and Conditions

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Abstract

Background: Intervertebral disc (IVD) water is a basic factor for modeling tissue growth and nutritional diffusion and its amount is directly related to the development of degenerative processes. ***Objective***: direct assessment of human IVD water contents in different disc regions in correlation to age and disc degeneration processes. ***Methods***: 103 IVD samples that were taken from 30 cadavers underwent a lyophilization process to assess their water contents. The results were tabulated and tested statistically in relation to different parameters, including gender, age, spinal level and degeneration grading. ***Results***: This study reveals that the water content was significantly less in the group whose age was more than the median age (83 years) when compared with the group whose age was less than the median age. Additionally, the study shows that the water content is dramatically and significantly reduced in individuals who have degenerative changes. ***Conclusions***: The water content of the intervertebral disc directly correlates with age and the degeneration process, decreasing as age and degeneration progress.

Keywords: Annulus Fibrosus, Intervertebral disc, Lyophilization, Nucleus Pulposus, Water content.

القياس الكمي للمحتوى المائي للأقراص البينفقرية البشرية في مختلف المناطق والظروف

الخلاصة

الخلفية: المحتوى المائي للأقراص البينفقرية (IVD) هو عامل أساسي لنمذجة نمو الأنسجة والانتشار الغذائي ويرتبط كميته ارتباطا مباشرا بتطور العمليات التنكسية. **الهدف**: التقييم المباشر للمحتوى المائي للاقراص البينفقرية البشرية في مناطق القرص المختلفة فيما يتعلق بالعمر وعمليات تنكس القرص. **الطريقة**: خضعت 103 عينة من IVD مأخوذة من 30 جثة لعملية التجفيد لتقييم محتوياتها المائية. تم جدولة النتائج واختبارها إحصائيا فيما يتعلق بمعايير مختلفة، بما في ذلك الجنس والعمر ومستوى العمود الفقري ودرجات التنكس. **النتائج**: كشفت هذه الدراسة أن محتوى الماء كان أقل بشكل كبير في المجموعة التي كان عمرها أكثر من متوسط العمر (83 سنة) بالمقارنة مع المجموعة التي كان عمرها أقل من متوسط العمر. بالإضافة إلى ذلك، تظهر الدراسة أن محتوى الماء ينخفض بشكل كبير وكبير لدى الأفراد الذين يعانون من تغيرات تنكسية. **الاستنتاجات**: يرتبط المحتوى المائي للقرص الفقري ارتباطا مباشرا بالعمر وعملية التنكس ، ويتناقص مع تقدم العمر وعمليات التنكس الفقري.

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INTRODUCTION

Intervertebral discs play a significant role in the normal functioning of the human spine. IVDs are fibrocartilaginous cushions acting as the fundamental joints between adjacent vertebrae in the vertebral column. They allow the vertebral column to be flexible. They also provide shock absorption in the vertebral column, helping to prevent vertebral crushing. IVD comprises three main parts: a *nucleus pulposus*, an annulus fibrosus and two vertebral endplates that fix the IVDs to adjoining vertebrae [1]. The nucleus pulposus (NP), a gelatinous structure at the center of the IVD, contributes significantly to the spine's strength and flexibility. NP consists of 66–86% water and the remaining is predominantly type 2 collagen fibers and proteoglycans [2]. Proteoglycan comprises a "core protein" with one or more covalently bound glycosaminoglycan (GAG) chain(s). They include aggrecan and versican that join to form hyaluronic acid.

Within the NP, aggrecan is primarily responsible for water retention. The NP also has a small number of cells that produce extra-cellular matrix products and sustain the NP's integrity [3]. The *annulus fibrosus* (AF) comprises "lamellae," or concentrical layers, of collagen fibers. Every lamellar layer has an alternating orientation of fibers, allowing for efficient resistance to multi-directional movements. The annulus fibrosus encompasses an inner and an outer part. These parts differ principally in their collagen constitution. The outer part consists mainly of type-1 fibers, whereas the inner part mainly contains type-II fibers [4]. The aqueous and proteoglycan constituents of the disc significantly influence its extracellular matrix integrity and strength. In healthy discs, like those found in young adults, the negatively charged branched GAG sulfate chains and carboxylic groups have a hydrophilic property that makes the disc matrix pressurized by attracting water through osmosis [5]. Hydrostatic pressure in the NP directly influences the disc water volume, which in turn enhances the disc's capacity to evenly distribute compressive loading onto the adjacent vertebrae and function as a shock absorber [6]. The loss of tissue hydration caused by aging and/or degeneration will also impair a disc's cellular performance and viability. As we age, the percentage of PG and water decreases [7]. Furthermore, the inverse relationship between disc hydration and spinal stress concentrations suggests that increased loading gradients in the disc will lead to a reduction in water and GAG during aging and degeneration, a phenomenon evident in the load-bearing lumbar spine. Water and GAG concentrations tend to vary with the degree of disc degeneration, with the greater loss being proportionally associated with severe disc degeneration [8]. The aim of our study is to quantify the mean water concentrations in human intervertebral discs according to disc location and characterize the influences of gender, age, vertebral region, spinal level, and the degree of degeneration in samples of the Iraqi population.

METHODS

Study design

The investigation was carried out in the Department of Human Anatomy at Mustansiriyah Medical College in Baghdad, Iraq, employing a histological cross-sectional design. It was approved by the local ethical committee of Al-Mustansiriyah Medical College, and informed consent was obtained from the relatives of all participants. For the research, 103 IVD samples were collected from 30 cadavers (17 males and 13 females). The mean duration between the day of demise and the inclusion of cadavers as subjects in this research was inconsistent, but typically spanned from one to three days.

Evaluation method

Macroscopic analyses were performed on cadaveric discs by utilizing the mid-sagittal section of every hemi-sectioned spine. A modified grading scheme was employed to classify the dissected discs in accordance with the standards outlined by Agorastides and colleagues [9] (Figure 1).

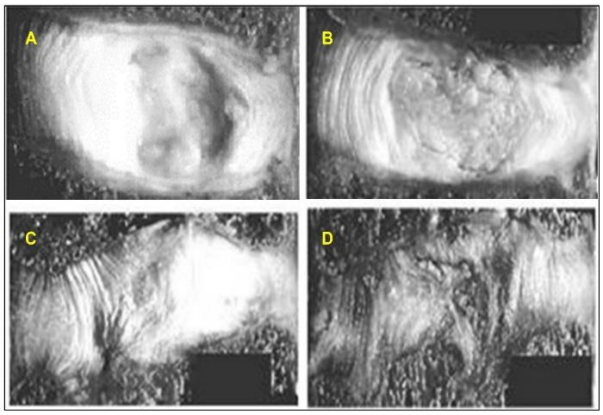

Figure 1: Stages of disc degeneration grading system using sagittal plane discogram. Grade 1 non-degenerated disc (A), Grade 2 mature non-degenerated disc (B), Grade 3 degenerated disc (C), and Grade 4 severely degenerated disc (D).

Grade I (non-degenerated disc): The NP and AF are distinguishable; the IVD is reflective and white. No indications of structural disruption in AF are present. Grade-II (mature non-degenerative disc): The distinction between NP and AF is diminished. IVD has a creamy hue, NP is more fibrous but remains flexible, and no indications of structural disruption were observed. Grade III (degenerated disc): It is challenging to differentiate between NP and AF; NP is desiccated, fibrous, and frequently discolored. AF and NP osteophytes exhibit fissures along the anterior and/or posterior periphery of the vertebral body. A grade-IV degenerated disc is characterized by the absence of differentiation between individual regions of the disc, discoloration, fibrousness, and rupture of both the AF and NP, frequent presence of osteophytes, and constricted IVDs. A rectangular cutting implement was utilized to section each of the 30 human intervertebral discs into three to four samples. The water quantification techniques employed in this study were derived from the modified approaches proposed by Birch [10]. Precisely 100 mg of moist tissue was precisely weighed from three distinct regions of each IVD specimen using a digital balance: the anterior annulus fibrosus (AAF), nucleus pulposus (NP), and posterior annulus fibrosus (PAF). Assuming a constant weight, tissues were desiccated under vacuum conditions after being frozen at -70°C. We then recalculated the dry weight of the lyophilized tissues by reweighing them. The water content was determined by calculating the percentage of the saturated weight.

Statistical analysis

Statistical analyses were performed using SPSS "Statistical Package for the Social Sciences (SPSS Inc.,

Chicago, IL, USA)" version 26 for Windows. Continuous data were represented as mean±SD. For the normally distributed variables, inter- and intra-group comparisons were executed using ANOVA and *t*-tests. A less than 0.05 *p*-values were regarded as a statistically significant [11,12].

RESULTS

This analysis utilized 103 IVD samples in total (63 lumbar and 40 thoracic). The water content exhibits substantial variation across distinct regions of the disc. The average water content of NP was 7% higher than the combined water content of the two annuli; the hydration values for NP, AAF, and PAF were 73.51±8.5%, 65.91±9.2%, and 67.02±9.2%, respectively. The statistical analysis of variance and covariance (ANOVA) conducted on the disc regions indicated the existence of a significant disparity in water content (*p*-value= 0.004) (Figure 2).

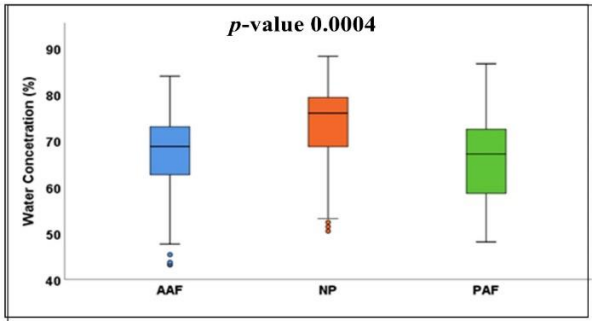

Figure 1: Mean water concentration in different disc regions (*p*-value= 0.004). Values were expressed as mean±SD. AAF: Anterior Annulus Fibrosus; NP: Nucleus Pulposus; PAF: Posterior Annulus Fibrosus.

Two categories of cases were formed on the basis of the median age, which was 83 years. Student tests and one-way ANOVA indicate that there is a statistically significant difference in water concentration across all disc regions based on age, with water concentration decreasing as age increases (Table 1).

Table 1: Mean water concentration in different disc regions according to age

Regions	Age		*p*-value
	≤ Median	> Median	
AAF (%)	70.17±6.6	64.58±8.7	0.000
NP (%)	76.92±6.3	69.75±9.1	0.000
PAF (%)	69.99±7.9	61.41±8.5	0.000

Values were expressed as mean±SD. AAF: Anterior Annulus Fibrosus; NP: Nucleus Pulposus; PAF: Posterior Annulus Fibrosus.

Samples were divided into three categories according to the degree of degeneration: grade 2, which denoted mild degeneration; grade 3, which represented moderate degeneration; and grade 4, which represented severe degeneration. Testing the hypothesis demonstrates that the water concentration in all disc regions is inversely proportional to the grade of degeneration (Table 2).

Table 2: Mean water concentration in different disc regions vs grade of degeneration

Region	Grade of degeneration			*p*-value
	Grade 2	Grade 3	Grade 4	
AAF (%)	65.14±4.4	60.48±8.2	53.51±7.6	0.000
NP (%)	67.41±4.8	62.14±9.5	61.06±8.3	0.009
PAF (%)	64.04±8.6	59.37±8.3	49.98±4.9	0.000

Values were expressed as mean±SD. AAF: Anterior Annulus Fibrosus; NP: Nucleus Pulposus; PAF: Posterior Annulus Fibrosus.

This finding establishes a significant distinction in water content across all disc regions. As shown in Tables 3, 4, and 5, hypothesis testing fails to confirm any significant differences in water content among disc regions with respect to gender, vertebral region, and spinal level.

Table 3: mean water concentration in different disc regions according to gender

Disc type	Gender		*p*-value
	Males	Females	
AAF (%)	67.38±8.3	67.33±8.8	0.671
NP (%)	74.71±8.3	72.35±7.9	0.116
PAF (%)	69.83±8.7	67.68±9.8	0.124

Values were expressed as mean±SD. AAF: Anterior Annulus Fibrosus; NP: Nucleus Pulposus; PAF: Posterior Annulus Fibrosus.

DISCUSSION

Three essential components comprise human intervertebral discs: the nucleus pulposus, the annulus fibrosus, and the cartilaginous endplate. The annulus fibrosis is the rigid collagenous portion of the intervertebral disc [12,13], whereas the nucleus pulposus is the central portion composed of a highly hydrated gel-like substance.

Table 4: Mean water concentration in different disc regions according to vertebral region

Disc type	Vertebral region		*p*-value
	Thoracic region	Lumbar region	
AAF (%)	67.60±7.5	69.59±8.6	0.253
NP (%)	73.59±6.9	73.52±9.9	0.969
PAF (%)	65.67±7.8	66.18±10.87	0.778

Values were expressed as mean±SD. AAF: Anterior Annulus Fibrosus; NP: Nucleus Pulposus; PAF: Posterior Annulus Fibrosus.

Intervertebral discs consist primarily of three histological components: water, fibrillar collagen, and proteoglycans. The quantities of these components exhibit variability among the intervertebral discs. Proteoglycan and water content are generally greater in the nucleus pulposus compared to the annulus fibrosis. This disparity in composition accounts for the substantially higher water content observed in the nucleus pulposus [14,15]. The findings of this research indicate that the water content was notably diminished in the cohort consisting of participants aged 83 years or older, in comparison to the cohort comprising participants aged below the median age. A study conducted by Urban and Robert (2003) unveiled that mild indications of disc degeneration are observed in approximately 20% of adolescents.

Table 5: Mean water concentration in different disc regions according to spinal level

Vertebral level	Disc type		
	AAF	NP	RAF
T8-T9	52.10±7.7	72.10±8.3	63.80±7.9
T9-T10	64.88±6.2	69.50±8.4	64.57±9.5
T10-T11	61.40±8.8	73.78±5.8	63.73±7.1
T11-T12	69.33±5.4	74.27±5.1	65.66±5.1
T12-L1	67.32±6.7	76.47±7.1	68.42±8.1
L1-L2	70.72±8.9	72.17±10.5	68.25±10.5
L2-L3	69.80±7.9	73.82±10.7	70.05±7.4
L3-L4	69.47±7.5	75.38±8.9	66.37±9.0
L4-L5	71.12±8.2	72.60±10.2	58.67±14.0
L5-S1	65.14±14.0	71.06±12.4	60.97±17.7
p-value	0.062	0.722	0.279

Values were expressed as mean±SD.

Furthermore, this degeneration exhibits a significant progressive trend with age, particularly among males, culminating in severe degeneration in approximately 10% of discs aged 50 years and 60% of discs aged 70 years [16]. Indeed, dividing notochord cells are present in the nucleus pulposus during fetal and childhood. These cells secrete proteoglycans and the extracellular matrix, which is subject to high osmotic pressure and facilitates the transport of water into the intervertebral discs to regulate hydration status and swelling pressure [17]. Conversely, as age progresses, the quantity of aggregating proteoglycans (aggrecan) tends to diminish; consequently, the water content also diminishes. Further, the research demonstrates that individuals undergoing degenerative changes exhibit a substantial and remarkable reduction in water content. Degenerative processes have been observed to decrease proteoglycan levels, and the absence of proteoglycan is regarded as an early indicator of degeneration [18]. As stated previously, proteoglycan loss decreases the osmotic pressure of the nucleus pulposus, edema pressure, and hydration status; this accounts for the substantial decrease in water level that occurs during the progression of intervertebral disc degeneration. Our research findings indicate that no statistically significant variation in water content was observed between the sex categories. This research is consistent with the findings of Menon *et al*. (2023) [13] and diverges from those of Kenawy *et al*. (2023) [14] and Mosley *et al*. 2019 [19]. The intervertebral disc biochemical profiles of males and females appear to be identical; however, their responses to inflammation and injury differ [15]. The study revealed that there was no statistically significant variation in water contents across the vertebral region and spinal level.

Conclusion

The water content of intervertebral discs is directly proportional to both age and the degeneration process; as both age and degeneration progress decrease water content.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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