In The Name Of

God

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Thesis

Title:

Incidence of Microscopic Lumbar Intervertebral Disc Calcification and it's correlation to Endplate Degeneration Type and Disc Histopathologic Angiogenesis

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تقدیم به:

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آنان که هستي ام را معنا بخشیدند و والاترین و ناب ترین محبتها را بیدریغ نثارم داشتند و کلام را قدرت بیان وجودیشان نیست.

تقدیم به:

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"ماندانا"

که پیوسته صداقت و مهرش شوق زیستنم
بود؛ اوکه با قبول مشکلات
راه آموختن را برمن هموارکرد.

و

والدین گرامیش

که در این مسیر طولانی همواره پشتیبان
و مشوقم بودند.

تقدیم به:

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فرزندان عزیزم

که از خستگي این راه بي نصیب
نبودند و بردباري کودکانه شان در
این راه دلگرمي ام بود.

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و تمامي بيماران گمنامي كه علم جراحي
را بر بالينشان آموختم.

Contents:

Abstract:

STUDY DESIGN. this study measured the incidence of lumbar intervertebral disc nucleus polposus microscopic calcification and angiogenesis in adult patients undergoing discectomy and normal cadavers, and determined the endplate degeneration type radiologically.**OBJECTIVES**.the results were compared to determine the incidence of disc calcification in patients and cadavers, differences between two groups and relationship between disc microscopic calcification to disc degeneration type,histopathological angiogenesis, patients age, gender and duration of symptoms. **SUMMARY OF BACKGROUND DATA**. True incidence of microscopic calcification in normal or degenerated lumbar discs have not been fully defined nor linked to disc degeneration type and angiogenesis. Previous studies reported disc calcification incidence largely different and suggested that disc calcification occur in adult mainly in annulus and is usually asymptomatic. some studies demonstrated that angiogenesis and calcification related to each other in several another tissues.**METHODS**.the incidence of microscopic calcification in specimens of disc nucleus polposus obtained from two groups was measured: specimens obtained during surgery from 90 consecutive patients of 15 to 50 years old suffering from disc herniation in single level of L4-L5 or L5-S1 between 2005 and 2006,40 additional specimens of lumbar disc nucleus polposus obtained from normal cadavers of the same ages. Calcification determined microscopically by Von Kossa staining and angiogenesis by H/E and type of degeneration radiologically by Modic classification. **RESULTS**. prevalence of microscopic calcification was significantly higher in degenerated disc than normal cadaveric(54.4% versus7.5%) and in higher modic type than lower(III:95%, II:57.4% I:13%), also prevalence of angiogenesis was significantly higher in patients` than cadavers` discs(41% versus 7.5%) and in calcified than noncalcified discs (59.2% versus 19.5%)($P<0.001$).there was no relationship between disc calcification and patients gender ,level of discectomy and duration of symptoms.**CONCLUSION**.disc nucleus polposus microscopic calcification is a common event occur in adult patients suffering from lumbar disc herniation. diminished blood supply to nucleus polposus resulting in tissue breakdown and infiltration of macrophages and their interaction leads to up regulation of inflammatory cytokines,TNF-α,MMPs andVEGF that ultimately induce neovascularization and also induce calcification directly and indirectly. Mechanisms that link disc degeneration, angiogenesis and calcification remain a focus for further researches that may be useful in future medical treatments before surgical treatment of lumbar disc herniation.

Key words: disc herniation-disc angiogenesis-disc degeneration-disc calcification-nucleus polposus

Introduction:

Intervertebral disc herniation (HD) is one of the most common conditions to cause low back pain and/or sciatica and disability of working age group in developed countries [1 2].

The intervertebral disc, which consist of an outer fibrous annulus fibrosus (AF) surrounding an inner gelatinous nucleus pulposus (NP) is largely composed of the cartilage component including the proteoglycan aggrecan, and type II collagen [3].

Herniated disc is defined as a degenerated disc protruding into the spinal canal or neural foramina with resultant compression of nerve tissue [3].

Histologically HD is surrounded by granulation tissues characterized by inflammatory cell infiltration and new formed vessels [1 4]. It appears that both fibrotic and angiogenic reactions take place in disc herniation [5]. In several studies radiographic intradiscal calcification correlated significantly with disc degeneration [6]. the first case of children disc calcification reported in literature appears in 1924 by Baron [7] but the first

description of Adult intervertebral disc calcification is attributed to Von Lushka [8]. Calcification in an intervertebral disc may occur in three places: annulus fibrosus, fibrocartilaginous plate and centrally in the nucleus pulposus. The third consist of a deposit of amorphous calcium salts in degenerated tissue and is usually seen in adults, commonly in lumbar spine [9]. calcification within the intervertebral discs may resolve spontaneously especially in children or may persist permanently in Adult [7 10 11]. Disc calcification may be important. In a study radiographic intradiscal calcification correlated significantly with the morphological degree of degeneration [6]. Calcium containing crystal deposits disrupt disc extra cellular matrix [12] and were Likely to have dural adherence [13 14].

The true incidence of intervertebral disc calcification is unknown [7]. reported incidences for disc calcification is largely different such as rare[7], 3.1%[15], 14.7%[12](microscopic in annulus), 63% [16](microscopic), 65% [13] (radiological). The present study was designed to evaluate the incidence of microscopic calcification in normal cadaveric disc and degenerated herniated disc and determine its correlation with type of Disc degeneration. Previous studies showed that factors such as TGF-ß, TNF-α, VEGF, MMP-1, MMP-3 are associated with degeneration and angiogenesis in disc tissue [3 5 17], On the other hand other studies also showed this factors are associated with ectopic calcification in several other tissues such as vessel,

muscles[18] bone,[19] and ligamentum flavum[20]. So this study also designed to evaluate the incidence of disc angiogenesis and its probable relationship to disc calcification and degeneration type.

Materials and Methods

a) **Sampling**: studied population were patients of 15 to 50 years old with Lumbar Disc Herniation just in one level (L_4-L_5 or L_5-S_1) and for the first time were operated using Discectomy surgery. During the years of 2005-2006 samples of Nucleus Polposus part of disc were submitted from 90 of the consecutive above patients in the attending author service. Also for normal cadaveric disc specimens, samples were submitted from 40 normal 15 to 50 years old cadavers (without DH, Lumbar canal stenosis, known causes of disc calcification and systemic illness according to history) from L5-S1 disc nucleus polposus. Number of discs per patient or cadaver was 1. Care was taken to remove all granulation tissue and to sample only disc nucleus polposus. Specimens placed in 10% neutral buffered formalin then encoded and transported to laboratory for Histological study.

b) **Histological Study**: the current study is Histopathologic one, so all the samples were embedded in paraffin, sectioned by microtome, and stained with stains including hematoxylin and eosin (H/E) and Von Kossa, and then blindly reported in terms of existing microscopic calcification and microscopic angiogenesis (Fig. 1 & 2) by two skilled pathologists. H/E was used for angiogenesis and Von Kossa for calcification.

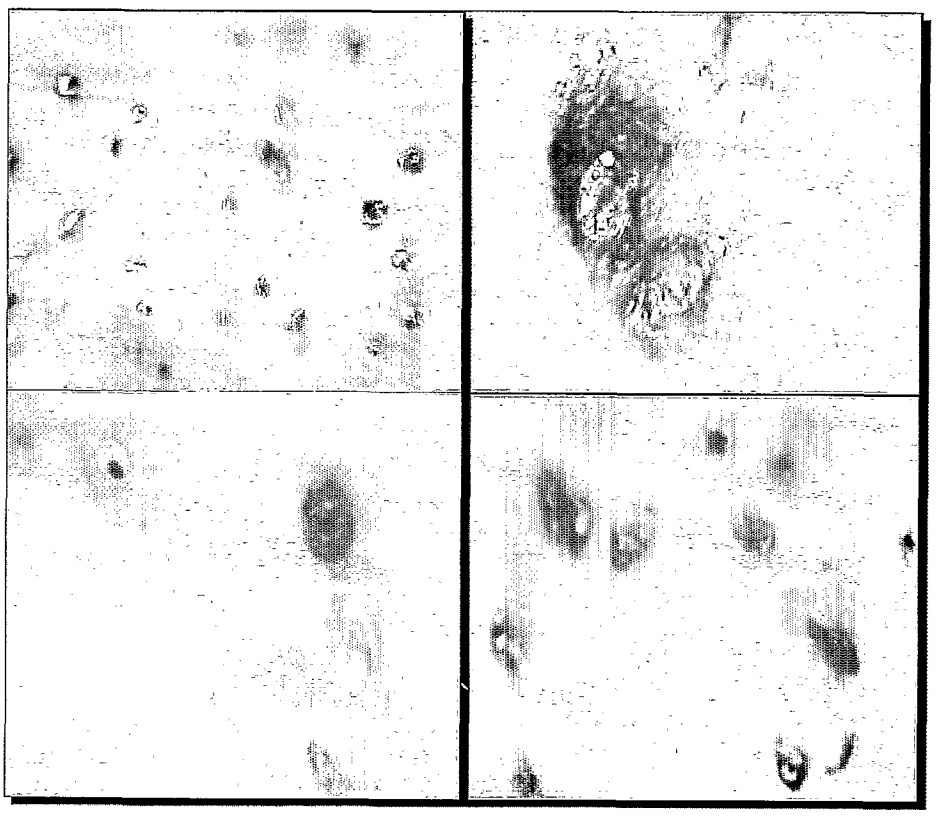

Figure1: Examples for microscopic calcification in disc samples. Micrographes showing various calcification shapes in nucleus polposus specimens under light microscopy.

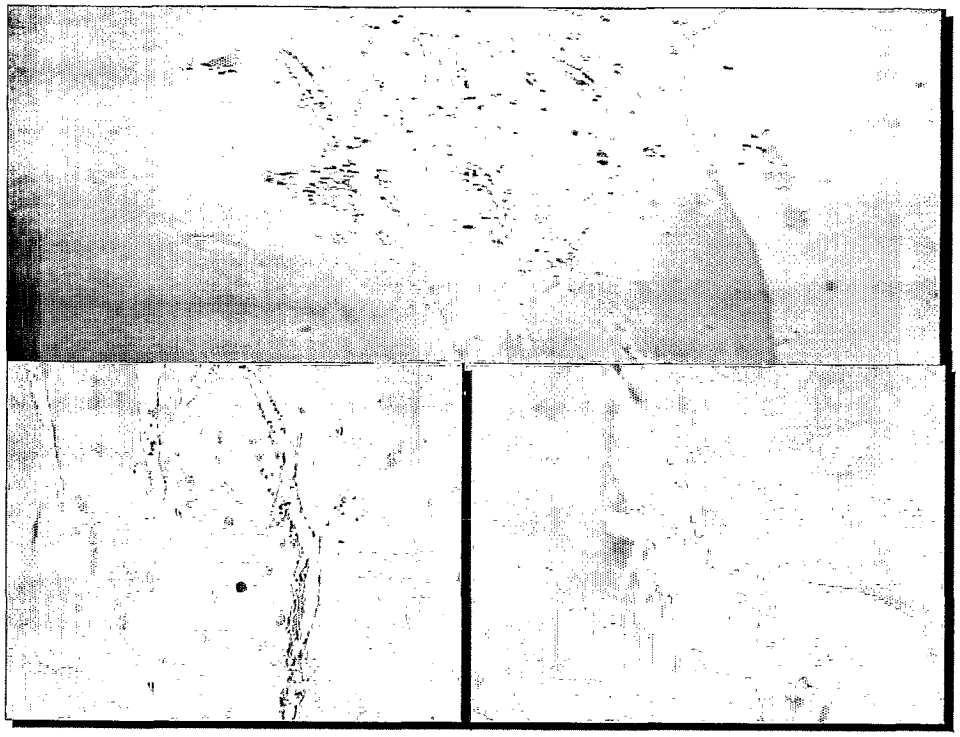

Figure2: Examples for microscopic angiogenesis in disc samples. Micrographes showing various patterns of disc nucleus polposus angiogenesis under light microscopy.

c) **Radiological Study**: Magnetic resonance images were obtained with a GE 1.5 T unit (General Electric Medical Systems, Signa, Exita). The lumbosacral MRI of all patients were studied by a skilled radiologist to investigate the type of degeneration. The modic classification[21] was used for degeneration type. Kokkonen showed a strong positive correlation between endplate degeneration seen on MRI and disc degeneration[22]. According to modic classification three main type of endplate changes have been verified based on the signal intensity of adjacent bone marrow: type I changes are characterized by a decreased

signal on T_1 weighted (T_1W) images an increased signal on T_2 weighted (T_2W) sequences, correspond to edema at histology. Type II changes are characterized by increased signal relative to normal bone marrow on T_1W, a slight increased signal or iso intensity on T_2W sequence, and fatty marrow at histology. Type III changes are characterized by a decreased signal on both T_1 and T_2 sequences, and correspond to dense sclerosis[21 22].

d) **Statistical Analysis**: Data analysis was done using SPSS version 11.5, and the chi-square test was used to assess differences in proportions. P values < 0.05 were considered statistically significant.

Results

The mean age of studied patients was 38.37 years (SD ±8.59), ranging from 17 to 50 and the median age was 39 years. 31 of them were female and 59 male, 60 suffered L4/L5 HD and 30 L5/S1.

Frequency of microscopic calcification in disc sample of patients was 54.4% (49 from 90) and of cadavers was 7.5% (3 from 40), that in patients was significantly more than of the cadavers. ($\chi^2 = 25.43$, $df = 1$, $P < 0.001$)

Calcification frequency in disc sample of patients with Disc herniation that according to MRI were in Modic group type I, was 13%(3 from 23), in type II was 57.4%(27 from 47), and in type III was 95%(19 from 20), in addition to those patients that their disc sample was lacked microscopic calcification, Modic of type I was significantly higher ($\chi^2 = 20.13$, $df = 2$, $P < 0.001$).

There were not any significantly association between the existence of calcification in patients' disc sample and gender: Female(61.3%,19 from 31)& Male(50.8%,30 from 59) ($\chi^2 = 0.89$, $P = 0.35$) and level of disc herniation: L5/S(50%,15 from 30)& L4/L5(56.7%,34 from 60) ($\chi^2 = 0.36$, $P = 0.55$) and duration of symptoms (radiculopathy) in three symptoms duration groups of: 1-3 months(63.3%,19 from30)& 4-6 months(41.1%,12 from29)& more than 6 months(58.1%,18 from 31) ($\chi^2 = 3.12$, $P = 0.21$).

Also correlation of microscopic calcification with age become 0.332 calculating Eta coefficient.

Microscopic angiogenesis frequency (histopathologic) in patients' disc sample was 41% (37 from 90) and in cadavers' was

7.5 %(3 from 40), that significantly higher in patients' disc sample than of cadavers' ($\chi^2 = 14.69$, $df = 1$, $P < 0.001$).

Also the statistical analysis showed that there is a meaningful Association between microscopic calcification and histopathological angiogenesis. Frequency of microscopic angiogenesis in disc sample of the patients with calcification was 59.2%(29 from 49) and in those without calcification was 19.5%(8 from 41) ($\chi^2 = 25.51$, $df = 1$, $P < 0.001$).

Discussion

Our results demonstrate that lumbar intervertebral disc microscopic calcification has significantly more prevalence in degenerated herniated discs than normal discs (54.4% versus 7.5%), especially in higher type of degeneration (13% in Modic type I, 57.4% in Modic type II and 95% in Modic type III). In addition lumbar intervertebral disc microscopic angiogenesis has more prevalence in calcified herniated disc specimens (59.2% in calcified and 19.5% in noncalcified herniated disc specimens), and there is no correlation between herniated

lumbar disc microscopic calcification and patient's gender and level of HD (L_4-L_5 or L_5-S_1) and duration of symptoms.

Previous reports suggest that disc calcification in adult occur mainly in annulus and is usually asymptomatic[7 9], but our study demonstrate that nucleus polposus calcification is a common event occur in adult patient suffering from HD.

Long term changes related to intervertebral disc calcification may include Scoliosis, loss of vertebral body height, osteophyte formation and disc space narrowing [7].Calcium containing crystal deposits not only disrupt disc extra cellular matrix but may also accelerate preexisting degenerative changes via an elevation in matrix metalloproteinase [12]. Calcified discs were Likely to have dural adherence and it should be noted that the majority of interadural penetrations occurred in calcified discs[13 14].

the aetiology of disc calcification remain uncertain[11]. some recognized causes of calcification in intervertebral discs including hyperparathyroidism, haemochromatosis, ochronosis, pseudogout, gout, hypercalcemic states and degenerative diseases [9 10 11]. The relationship between crystals and disc degeneration merit further investigations[12]. A previous study (Feinberg et al.1990) showed that hyroxyapatite (HAP) were generally associated with histological signs of disc degeneration[23 24]. Whether degeneration initiates crystal deposition or vice versa still remains a focus of debate[23].

At birth, the human disc has some vascular supply within both the cartilage endplates and the annulus fibrosus, but these vessels soon recede [8]. With increasing age water is lost from the matrix and the proteoglycan content also changes and diminishes, the cartilage and endplates undergoes thinning, altered cell density, formation of fissures, and sclerosis of the subchondoral bone. These changes are similar to those seen in degenerative disc disease[8 22]. Diminished blood supply on the endplate, resulting in the tissue breakdown beginning in the nucleus polposus and starting in the second life decade[25]. Histologically the granulation tissue of HD is characterized by marked infiltration of macrophages[1]. The interaction of macrophages with disc tissue leads to the production of tumor necrosis factor α (TNF-α) resulting in upregulation of expression of MMP-3 and MMP-7 as well as vascular endothelial growth factor (VEGF) that may potently induce new vessel formation[1 3]. Therefore interactions between VEGF and MMPs may tend to promote matrix degradation and neo-vascularization in HD [3] . Other factors such as fibroblast growth factor, transforming growth factor ß (TGF- ß) and pleiotrophin may play a role in neovasculization of damaged disc tissue[5 26 27]. In several tissues such as ligamentum flavum, vessels, muscles and bone inflammatory cytokines, VEGF and MMPs can promote ectopic calcification[18 20] . In patients with rheumatic heart disease and atherosclerosis, VEGF expression and