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Research Article,

The Histological Analysis of Degenerative Spinal Conditions among Sudanese Patients

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Abstract:

Background:
Degenerative changes in the spine are common and occur in most people, these changes can develop into other conditions, such as bone spurs or oesteophytes, nerve compression, and can be cause pain. This study designed to study the histological changes of spinal conditions among Sudanese patients.

Materials and Methods:
This was prospective cross-sectional and laboratory based study, conducted at the research laboratory of the National Centre for Neurological Sciences (NCNS), Khartoum, Sudan during the period of from June 2022 to September 2022. It included all operated patients admitted to the National Center for Neurological Sciences and were diagnosed with degenerative spinal conditions, demographical data (gender, age, occupation, and duration of pain), clinical data (radiological diagnosis), and histological result.

Results:
In this study, the most common affected was male, the most common age groups in this study was 21 – 30 years, the most influential occupation in this study was workers, and histological result showed that, myxoid material extend to outside the cartilage.

Conclusion:
Myxoid material it is considered one of the causative factors of pain in patients with spinal degeneration.

Keywords: Degenerative, disc, conditions, pain, and myxoid material.

Introduction:

Spinal Degenerative involve the gradual loss of normal structure and function of the spine over time. Degenerative involves bony structures and intervertbral disc; they are usually caused by aging and most common in older adult but may also be the result of physical or mechanical stress, tumors, infections or arthritis. Possible degeneration spinal conditions may be degenerative disc disease, osteoarthritis, spinal stenosis, spinal disc herniation or slipped disc, and spinal tumors, and back pain related with these conditions that cause degeneration spine. (1)

These changes can develop into other conditions, such as bone spurs or oesteophytes, nerve compression. And can be cause debilitating pain and other symptoms if left untreated. The etiology of the degenerative changes may be mechanical or metabolic injury, such as spinal fractures, spinal surgery not related to degenerative disc disease or significant metabolic processes, such as mucoplysaccharidoses. (2)

Mucopolysaccharidoses, diabetes mellitus is considered specific causes of degenerative changes, mucoplysaccharidoses have a direct impact on cartilage and bone development resulting in advance degenerative changes in the spine. [3]

The intervertebral discs of patients with diabetes mellitus have decreased hexosamine content, deficiencies in proteoglycan synthesis and reduced concentrations of KS, which is a critical component of from homogentisic acid, which ostensibly impedes the normal metabolism of the disc matrix. [4]

Path-physiology of degenerative spinal condition causing spinal pain is the endless repetition of "the biological healing of mechanical damage" occurring over a lifetime, spinal pain by degenerative spinal disease occurs as a series of successive changes through the repetitive damage-healing process of various spinal structures includes the intervertebral disc rather than a temporary phenomenon of any given path physiologic change in one moment.

Degenerative spinal disease generally begins with degeneration of the intervertebral disc, and then herniation of the intervertebral disc resulting in subsequent radicular pain occurs when the nucleus pulpous with degeneration located in the intervertebral disc tears and penetrates the annulus fibrosus. Subsequently, disc space narrowing occurs and alters the spinal biomechanics, followed by degenerative changes to the vertebral endplate, vertebra itself, and facet joint. Finally, these changes lead to degenerative spine disease. [5]

The ability of connective tissue to retain water is diminished with aging as the content of glycocojugates particularly PGs aggregates of the ECM, there is as increase in glycoconjugates degradation and a decrease in synthesis that further contribute to decreased water content and connective tissue degeneration. [6]

PGs are present within the ECM of the intervertbral disc and on surface of its cells, and commonly referred to in the older literature as connective tissue mucins or mucopolyccaharides, these molecules are large glycoconjugates complex that are found in high concentrations within the ECM of connective tissues. The aggrecan is a major structural PGs in the ECM, it is large, possessing numerous GAGs chains, is responsible for maintaining tissue hydration through osmotic pressure provided by and CS and KS chains, the ability aggregate association with hyaluronan and expressed by chondrocyte in cartilage.

The loss of PGs in degenerate disc has a major effect on the discs load-bearing behavior, the osmotic pressure of the disc falls and the disc is less able to maintain hydration under load, degenerate discs have a lower water content than do normal age-matched discs, and when loaded they lose height and fluid more rapidly, and the discs tend to bulge. [7]

Materials and Methods:

This study was prospective cross-sectional and laboratory based study, done at the National Centre For Neurological Science, Khartoum state, Sudan during the period from June 2022 to September 2022. The study included all degeneration spine patients (21) operated at National Centre for Neurological Science during the period of this study. The diagnosis was confirm radiologically as having degeneration spinal conditions (disc prolapsed, oestophytes, tumors, chiari malformation and arachnoid cyst). All operated patients had a thorough examination in NCNS for degenerative spinal conditions were evaluated and full history according to degenerative spinal conditions protocols were taken by neurosurgeon. The tissue samples were contains bony and soft part have been taken from surgical room in NCNS by neurosurgeon and kept in sterile container that containing 10% NBF and then processed for histological techniques. The demographical data (gender, age, occupation, and duration of pain), clinical data (radiological diagnosis), and histological results (histological changes) were analyzed using Statistical Package for Social Science (SPSS) program.

Ethical approval was obtained from the NCNS; personal data were collected from registry data base office at NCNS by using standardized non-self-questionnaire as well as hospital and medical record. This study was approved by the ethical committee of Scientific Research Deanship Al-Neelain University.

Tissue preparation:

In this study, from each condition, two parts of tissue samples have been collected. One part contains bony tissue and the other part contains soft tissue. All samples (bony and soft part) were

placed in sterile container that containing 10% NBF for a minimum 48 hours to allow completion of the fixation process, formalin was used to protect tissue samples from extrinsic damage and preserve tissue and cells. and then decalcification for (bony part) was done using 10% Nitric acid (HNO_3), this process was performed to complete removal of calcium salts from tissue and make them amenable for sectioning, the volume of solution was at least 10 times the estimated volume of the sample. The samples were left in this solution for 24 hours, and then were used end point of decalcification such as chemical method to check removal of calcium salts from tissues.

Tissues Processing:

Automatic tissue processor is comprised of a number of glass beakers for fill different processing chemicals. It is one of the important machines required in histopathology for processing organ. The machine is started in the evening so that the process is complete in the morning, and embedding is done. The blocks were subjected to trimming process (5micron), and then thin slices (2-4 micron) from tissue was obtained using microtome machine. Bony part was stained with H**&**E stain and soft tissue was stained with H**&**E and combination AB-PAS stains.

Results:

Demographical results

A total of 21 operated patients were diagnosed radiologically as having degeneration spinal conditions. In the current study, male constituted 71.4 % and female which represented 28.6% of the patients, the most affected age groups in this study was 21 – 30 years in 28.6%, followed by the age group more than 60 years in 23.8% of the patients, the most influential occupation in this study was workers in 33%, followed by house wife in 23.8 % of the patient.

Concerning to duration, this is study showed the duration 1 month that constituted 4.8 %, the duration 2 month and 10 years represented 9.5% for each, the duration 3 month and 5 month represented 19% for each, the duration 11 month, 1 years, 7 years, 8 years, and 15 years represented 4.8 % for each, the duration 2 years that constituted 14.2 % .

In the present study, spinal conditions showed that, disc prolapsed condition represented the highest frequency 38.1% while oesteophytes, tumor, arachnoid cyst and chiari malformation represented 23.8% , 23.8% , 4.8% , 9.5% respectively. (Table 1, 2, 3, 4, 5)

Table (1): Distribution of degenerative spinal conditions within the gender:

Gender	Frequency	Percentage %
Male	15	71.4 %
Female	6	28.6 %
Total	21	100%

Table (2): Distribution of degenerative spinal conditions within age groups:

Age groups	Frequency	Percentage %
10-20 Years	2	9.5 %
21-30 Years	6	28.6 %
31-40 Years	2	9.5 %
41-50 Years	3	14.3 %
51-60 Years	3	14.3 %
> 60 Years	5	23.8 %
Total	21	100 %

Table (3): Distribution of degenerative spinal conditions within occupations:

Occupations	Frequency	Percentage %
Employee	4	19 %
Worker	7	33.3%
House Wife	5	23.8 %
Students	3	14.3 %
Farmer	1	4.8 %
Driver	1	4.8 %
Total	21	100%

Table (4): Distribution of degenerative spinal conditions within durations of pain:

Duration of pain	Frequency	Percentage %
1 month	1	4.8 %
2 month	2	9.5 %
3 month	4	19 %
5 month	4	19 %
11 month	1	4.8 %
1 Year	1	4.8 %
2 Year	3	14.2 %
7 Year	1	4.8 %
8 Year	1	4.8 %
10 Year	2	9.5 %
15 Year	1	4.8 %
Total	21	100 %

Table (5): Distribution of degenerative spinal conditions:

Spinal Conditions	Frequency	Percentage %
Disc Prolapsed	8	38.1 %
Oesteophyte	5	23.8 %
Tumor	5	23.8 %
Arachnoid Cyst	1	4.8 %
Chiari malformation	2	9.5 %
Total	21	100 %

Histological results

Histological changes of bony and soft parts (Haematoxylin & Eosin stain):

Regarding the disc prolapsed with H&E stain, the histological findings of dense compact bone showed focal hematopoiesis, and dense fibrosis.
In the present study, the soft tissue samples revealed multiple lobules of degenerative cartilage with a lot of myxoid material extend to outside the cartilage.
Regarding the oesteophytes with H&E stain, the histological findings of irregular bone trabeculae showed dense fibrosis. In soft tissue the myxoid material (extra cartilage).

The degenerative spinal condition associated tumor, H&E stain revealed irregular thin bone trabeculae covered by cartilage cap, dense fibrosis (compact bone), and hemorrhage in marrow space. The soft tissue showed degenerated cartilage, myxoid material (extra cartilage), and lobules of sclerosed cartilage.
The histological findings of chiari malformation with H&E stain, showed minimal hematopoiesis in compact bone. The soft tissue samples revealed lobules of sclerosed cartilage.
Regarding the arachnoid cyst with H&E stain, the histological findings of dense compact bone showed dense fibrosis with active hematopoiesis, H&E of soft tissue revealed lobules of sclerosed cartilage and fibrosis.
In this study, histological features associated with degenerative spinal conditions, that have been recognized in disc prolapsed was large cleft, while hyper-cellularity, clusters formation, and micro fissure was detected in tumors, the chiari malformation showed large cleft and fragment of fibrous tissues, the micro fissure was seen in arachnoid cyst. (Figure 1, 2, 3, 4, 5, 6)

Histological changes of soft parts (combination Alcian Blue-PAS stains):

Strong diffuse AB-PAS stain was detected in myxoid materials of disc prolapsed. Moreover, oesteophytes showed strong focal AB-PAS stain in myxoid materials. In additional that, tumors showed strong diffuse AB-PAS stain in myxoid materials. While chairi malformation showed strong diffuse AB-PAS stain in myxoid materials and lobules of sclerosed cartilage. Negative stain of AB-PAS stain showed in arachnoid cyst. (Figure 7, 8, 9, 10)

In the present study, myxoid material showed that, Strong positive (diffuse) constituted the highest frequency 71.4%, while strong positive (focal) and negative stain which represented 23.8% , 4.8 % respectively.
The cross-tabulation degenerative spinal conditions with myxoid materials, the combination AB-PAS stain with myxoid material showed that, strong positive (diffuse) in disc prolapsed in 100% (8 patients), strong positive (focal) in oesteophytes in 100% (5 patients), strong positive (diffuse) in tumors in 100% (5 patients), arachnoid cyst was negative results (1 patient), and strong positive (diffuse) in chiari malformation in 100% (2 patients). (Table 6, 7)

Table (6): Distribution of degenerative spinal conditions within the myxoid material:

Appearance	Frequency	Percentage %
Strong positive (diffuse)	15	71.4 %
Strong positive (focal)	5	23.8 %
Negative stain	1	4.8 %
Total	21	100 %

Table (7): The cross-tabulation degenerative spinal conditions with myxoid material:

Spinal Conditions	Myxoid material			Total
	Strong positive (diffuse)	Strong positive (focal)	Negative stain	
Disc prolapsed.	8	0	0	8
Oesteophyte.	0	5	0	5
Tumor.	5	0	0	5
Arachnoid cyst.	0	0	1	1
Chiari malformation.	2	0	0	2
Total	15	5	1	21

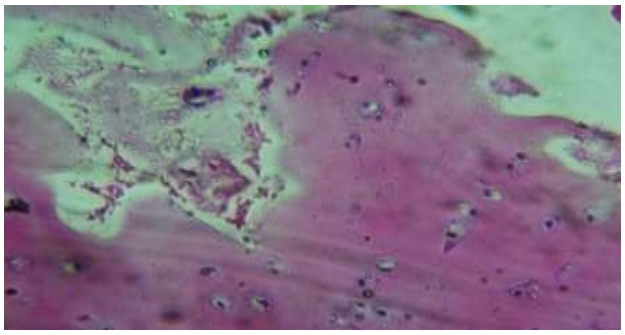

Figure (1): Disc prolapsed result with haematoxylin & eosin stain.

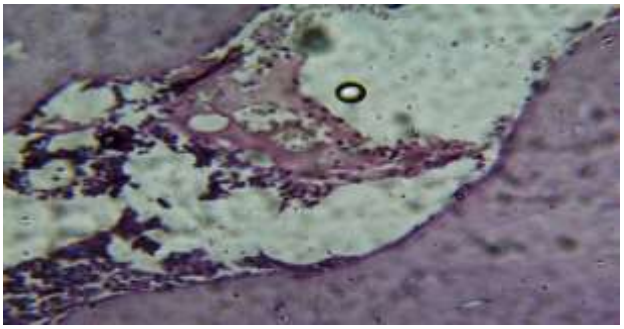

Figure (2): Oestophyte result with Haematoxylin & Eosin stain.

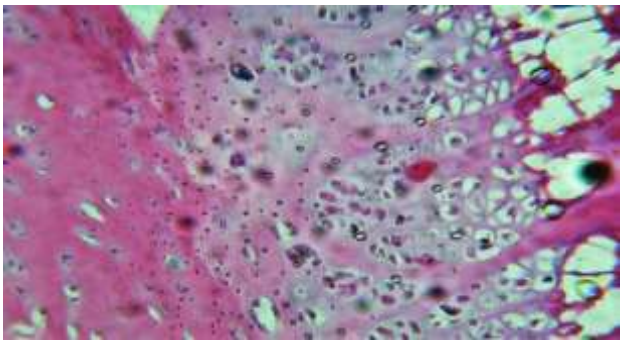

Figure (3): Tumor result with Haematoxylin & Eosin stain.

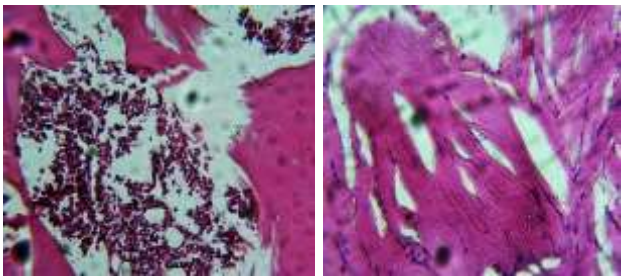

Figure (4): Chiari malformation result with haematoxylin & eosin stain.

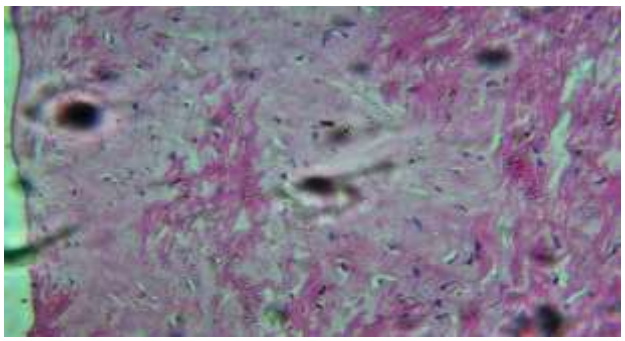

Figure (5): Arachnoid cyst result with haematoxylin & eosin stain.

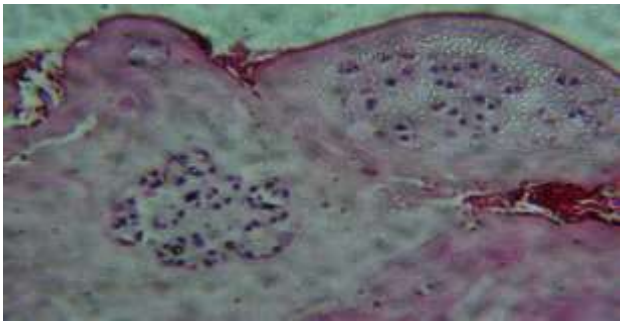

Figure (6): Histological features with haematoxylin & eosin stain.

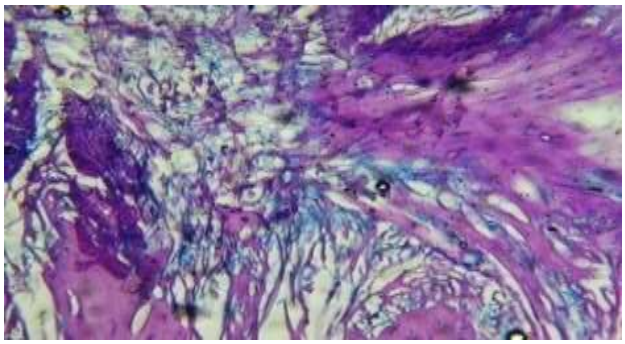

Figure (7): Disc prolapsed result with combination Alcian blue-PAS stain.

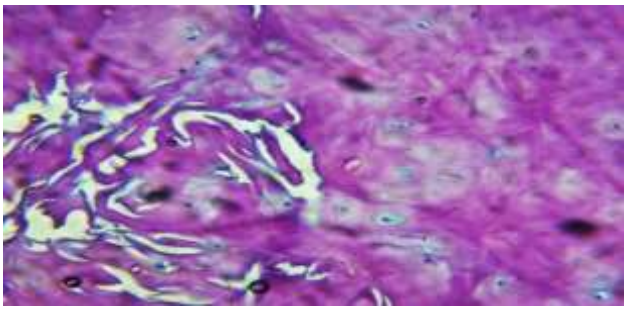

Figure (8): Oestophyte result with combination Alcian blue-PAS stain.

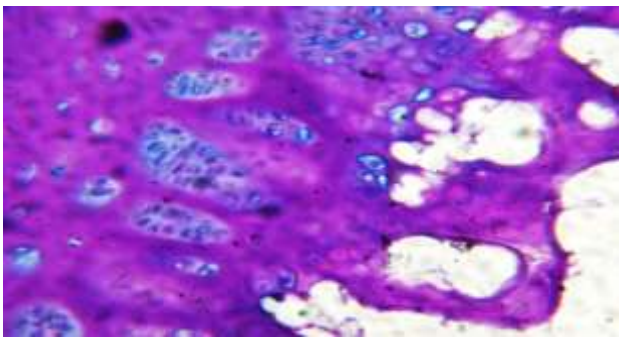

Figure (9): Tumor specimen result combination Alcian blue-PAS stain.

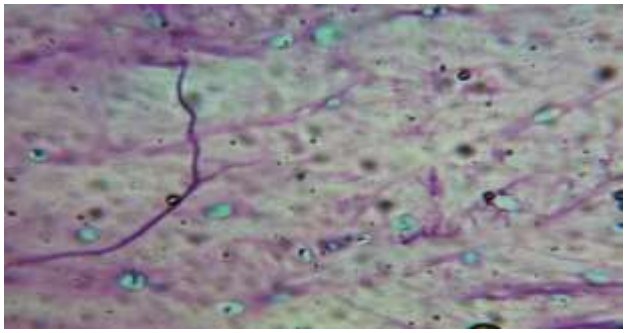

Figure (10): Chiari malformation result with combination Alcian blue-PAS stain.

Discussion:

Disc degeneration is a common musculoskeletal condition, it can progress to disc herniation, spinal canal stenosis and conjunction with facets joints arthrosis, and the factors initialing and influencing the progression of disc remain unclear. Nevertheless, there is a general agreement that spinal mechanical stress accelerates the progression of disc degeneration. [8] Young men are more susceptible to disc degeneration than are young women, most likely due to increased mechanical stress and physical injury, disc degeneration becomes apparent in men in the second decade of life, at almost a decade earlier than in women. The severity of age matched disc degeneration is also generally greater in young and middle-aged men. [9]

Human discs undergo age related degenerative changes that contribute to some of the most common causes of impairment and disability for middle aged and older persons that included neck and back pain, with increasing age they undergo striking alterations in volume, shape, structure, and composition that decrease motion and alter mechanical properties of the spine. [10] Disc degeneration are seen in the age group 11-16 years, about 20% of people in their teens have discs with mild degeneration, degeneration increases steely with age, particularly in males, so that around 10% of 50- year old discs and 60% of 70 year old discs are severely degenerate. [7]

Occupation load affects the risk of disc degeneration of the spine. Accidents back injuries and vehicle driving is associated with as increased risk of disc degeneration, the risk factors of the disc degeneration that included back accidents, recurrent minor trauma, heavy manual materials-handling and postural loading, heavy physical exercise, whole-body vibration, sedentary work, car driving, and overweight. Many studies have indicated that work-related physical stress is associated with an increased risk of disc degeneration. [11]

Proteoglycans are found in different forms in within different types of ECM and connective tissues, PGs in the nucleus pulpous of disc, contain GAGs chains of chondrotin sulfate and keratin sulfate attached to a polypeptide with covalent bounds, because of the decrease of PGs, the disc starts to dehydrate and recurrent damage occurs. As a result of this, the disc enters a degenerate process and herniated disc. [12]

The loss of proteoglycans in degenerate discs has a major effect on the disc load-bearing behavior, with loss of proteoglycans, the osmotic pressure of the disc falls [13] and the disc is less able to maintain hydration under load. With increasing age, water is lost from the matrix, and the proteoglycan content also changes and diminishes, and when load they lose height and fluid more rapidly, and the disc tend to bulge. [14]

When degeneration occurs, the nucleus pulpous becomes less gelatinous and more fibrous, and cracks and fissures eventually form. More blood vessels begin to grow into the disc from the outer areas of the annulus. There is an increase in cell proliferation and cell clusters formation as well as an increase in cell death. The cartilage undergoes thinning, altered cell density, and formation of fissures. These changes are to those seen in degenerative disc. [15]

Cells clusters are commonly seen in areas adjacent to the newly formed blood vessels or tissues clefts within aging or degenerate discs. [16, 17] In these areas, cells may easily access to nutrient supply and undergo proliferation. However, because the tissues clefts would expectedly alter the mechanical environments of the adjacent tissues, the cells within these areas would exhibit alter cell phenotype. [18]

Moreover, it has been shown that the chondrocytic cell proliferating, as well as the number and the size of cell clusters adjacent to tear and clefts, increase with age in human disc. The increase in cell proliferation is so commonly seen in degenerative discs therefore it has been take as an indicator of disc degeneration. [19, 20] Many investigations have showed that the proliferation activity of chondrocyte cells augments in the process of disc aging and degeneration. Have reported that proliferating cells are often seen in

human degenerative disc especially in areas where cell clusters. [19]

It is now clear that herniated- induced pressure on the nerve root alone be the cause more than 70% of normal, asymptomatic people have disc prolapsed pressurizing the nerve roots but no pain. [21]

The reason movement and good posture are so important is because discs help to support the pressure of body, the constant pressure pushes the nucleus of the disc against the outer wall and over time, weakens it, the discs absorb water from their surrounding and if they are under constant pressure (compression), they cannot absorb water. Additionally, as the discs lose water, the walls of the disc can dry out and weaken, making them less able to keep the nucleus inside, rather like the weakened walls of dam which holds back water, if the disc herniated, the nucleus can press against one of the nerves and this pressure can cause pain and the material of the nucleus causes a chemical irritation to the nerve and pain. [22]

When there is an injury to the disc, the body has a natural "inflammatory "response to heal an injury. Inflammation is a good thing, but if pain persists, the inflammation can be a source of pain in itself, this is why often taken anti-inflammatory to dampen down the inflammation, herniation can be caused by an injury such as falling or a collision, where the impact pushes the nucleus violently against the disc wall causing it to rupture (herniated), or more commonly, where a disc wall has been weakened over time, a twisting movement, poor bending posture or improper lifting can force the nucleus against the disc wall which is unable to contain it. The body will repair itself, provided the conditions are right and the injury is not too severe. However, if the pain persists, the spinal segment is not moving and over time it can become stiff and immobile and prevent the healing mechanism from working normally.

Most disc herniations or slip discs get better without surgery. In fact, many herniated discs do not even cause pain, many research studies show herniated discs are found in many people who have no pain associated with the finding, the human body removes any foreign objects by activating special cells called macrophages and monocytes that attack and destroy foreign material. [23]

To the body, a herniated disc, which is the nucleus pulpous in the wrong location, is considered a foreign object, in addition, the largest disc herniation tend to have the most water content, with time, the disc piece will dehydrate, over time, the disc herniation is removed, and shrinks back so it does not irritate the nerves or the tissues, the body also heals back the tear in the annulus, and re-ties the annular fibers, that healed annulus is now associated with a scar, and though it helps hold the nucleus back into its position, it is a weaker area, and has small but increased change of re rupture, the disc will continue with the natural dehydration process and will bulge over time. [24]

Most of the time, the body does remove the disc herniation on its own, this is the reason why many people will have disc herniations or bulges on MRI without any pain, disc herniation can regress or disappear spontaneously without surgical intervention; greater than 90% of people with disc herniations do get better after 6-8 weeks and no surgery needed, surgery is a last resort to treat a herniated disc when the pain is so severe and unresolved, or if the nerve pain is causing weakness in the leg, then surgery can be carried out to remove the part of the disc pushing on the nerve. [25]

Conclusion:

This study concluded that, histological analysis for degenerative spinal condition is valid instrument for evaluating disc degeneration in human sections, and degenerative changes was found to be in male more than female due mechanical stress among male, the highest patients with degeneration disc was in workers and this might be due physical stress among workers, myxoid material (strong diffuse AB-PAS stain) was found in 71.4 % of samples, and it is considered one of the causative factors of pain in patients with spinal degeneration.

Abbreviations:

NCNS: National Centre for Neurological Sciences.
AB-PAS: Alcian Blue- Periodic Acid Schiff.
KS: Keratin Sulfate.
ECM: Extracellular Matrix.
GAGs: Glycosaminoglycans.
PGs: Proteoglycans.
CS: Chondrotin Sulfate.
NBF: Neutral Buffer Formalin.

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Conflict of interest
The authors declare that there are no conflicts of interest.

Author Contribution
All authors similarly contributed to this manuscript, covered wrote, corrected and authorized this manuscript.

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